

# T. Pradeep Publications 2014

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# **1. Journal Covers**



Water 0.00 \_1.00 0.25 0.75 Ag<sub>68</sub>(SR)<sub>34</sub> 0.50 0.50 m/z 0.75 0.25 100 1.00 പ്പം വലം 0.50 0.75 1.00 00 0.25

**Cover Picture** Atanu Ghosh and Thalappil Pradeep Synthesis of Atomically Precise Silver Clusters

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# **COVER PICTURE**

The cover picture shows a schematic phase diagram of the three-component system used for the experiments. Silver and gold clusters have been synthesized by using the miscibility principle of solvents, without phase transfer agents. Three solvents, water, methanol, and toluene/chloroform have been used, which make miscible or immiscible phases depending on the composition. Separate regions of the phase diagram make different clusters, shown by diffreent colors, with the same reactant compositions. The mass spectrum of the characterized product,  $(Ag_{68}SR_{34})$ , is shown. Details are discussed in the article by A. Ghosh and T. Pradeep on p. 5271ff. For more on the story behind the cover research, see the Cover Profile.







Read the full text of the article at 10.1002/ejic.201402587

# Synthesis of Atomically Precise Silver Clusters by Using the Miscibility Principle





भारत सरकार विज्ञान और प्रौद्योगिकी मंत्रालय विज्ञान और प्रौद्योगिकी विभाग

GOVERNMENT OF INDIA Ministry of Science and Technology Department of Science and Technology Technology Bhavan, New Mehrauli Road New Delhi - 110016



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Invited for the cover of this issue is Thalappil Pradeep at the Indian Institute of Technology Madras, India. The cover image shows a schematic phase diagram of the three-component solvent system used for preparing monolayer-protected silver clusters. The different clusters obtained by keeping the reactants the same but adjusting the solvent system by moving to different regions of the phase diagram are shown in different colors.

#### In one word, how would you describe your research?

Novel. This work introduces a novel method for the synthesis of monolayer-protected clusters. The well-known concept of the "principle of miscibility of solvents" in general physical chemistry has been invoked judiciously to make various silver cluster cores without use of phase transfer reagents (PTRs).

#### What is the most significant result of this study?

This study has enabled the preparation of specific clusters suitable for applications by tailored variation of the solvent composition without using PTRs.

#### What prompted you to investigate this topic?

The ongoing thrust in the development of new atomically precise monolayer-protected clusters, owing to their diverse applications in various fields of importance, has prompted us to come up with an easy and efficient strategy to synthesize many new clusters. Although various processes exist to synthesize such clusters, the presence of a PTR in all of these methods is a drawback, because the PTR stays in the final product tenaciously as an impurity. The problem of PTR impurities has been circumvented by introducing several techniques that do not require the use of a PTR; however, those techniques have not been suitable for synthesizing a large variety of clusters, especially silver clusters. At this juncture, we envisaged that mixing different solvents in certain compositions could be an alternative to the use of a PTR and such a method could result in different atomically precise clusters at different solvent compositions. One of the solvents used in our study is water, because it is easy to reduce metal ions in the presence of sodium borohydride as the reducing agent: The metal ion of interest gets transferred into the appropriate organic solvent(s), and subsequent reduction by nascent hydrogen formed in a protic solvent practically drives the formation of clusters. The cluster again gets phase-separated at the newly formed phase boundary.

# Is your current research mainly fundamental or rather applied?

The aim of the present work has been the use of a fundamental concept for cluster synthesis. It is fundamentally important and

provides the materials that can be implemented in useful applications.





# A piece of paper ...

... that is impregnated with multi-walled or single-walled carbon nanotubes generates ions from diverse analytes at voltages as low as 3 V, as T. Pradeep et al. show in their Communication on page 5936 ff. This miniaturized ion source is held in front of a massspectrometer inlet to collect the mass spectrum. Common pesticides from the surface of an orange, active molecules from tablets, and a variety of analytes, such as amino acids, can been characterized.

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# **2. Books** Only cover page is attached

# **Aquananotechnology: Global Prospects**

DavidE.Reisner,T.Pradeep,CRCPress,New York,837Pages

# AQUANANOTECHNOLOGY GLOBAL PROSPECTS



# DAVID E. REISNER • T. PRADEEP



CRC Press Taylor & Francis Group

# **3. Journal Papers**

## **Journal Papers Published in 2014**

- Luminescent AgAu alloy clusters derived from Ag nanoparticles: Manifestations of tunable Aul-Cul metallophilic interactions, Kumaranchira R. Krishnadas, Thumu Udayabhaskararao, Susobhan Choudhury, Nirmal Goswami, Samir Kumar Pal and T. Pradeep, *Eur. J. Inorg. Chem.*, 5 (2014) 908-916 (DOI: 10.1002/ejic.201301424).
- Development of ultralow energy (1-10 eV) ion scattering spectrometry coupled with reflection absorption infrared spectroscopy and temperature programmed desorption for the investigation of molecular solids, Soumabha Bag, Radha Gobinda Bhuin, Rabin Rajan J. Methikkalam, Luke Kephart, Jeff Walker, Kevin Kuchta, Dave Martin, Jian Wei and T. Pradeep, *Rev. Sci. Instrum., 85 (2014) 014103 (DOI:* 10.1063/1.4848895).
- Supramolecular functionalization and concomitant enhancement in properties of Au<sub>25</sub> clusters, Ammu Mathew, Ganapati Natarajan, Lauri Lehtovaara, Hannu Hakkinen, Ravva Mahesh Kumar, Venkatesan Subramanian, Abdul Jaleel and T. Pradeep, ACS Nano, 8 (2014) 139-152 (DOI: 10.1021/nn406219x).
- Sequential electrochemical unzipping of SWNTs to graphene ribbons revealed by *in-situ* Raman spectroscopy and imaging, Robin John, Dhanraj B. Shinde, Lili Liu, Feng Ding, Zhiping Xu, Cherianath Vijayan, Vijayamohanan K. Pillai and T. Pradeep, ACS Nano, 8 (2014) 234-242 (DOI: 10.1021/nn403289g).
- Mixed-monolayer-protected Au<sub>25</sub> clusters with bulky calix[4]arene functionalities, Jukka Hassinen, Petri Pulkkinen, Elina O. Kalenius, T. Pradeep, Heikki Tenhu, Hannu J. Häkkinen, and Robin H. A. Ras, *J. Phys. Chem. Lett., 5 (2014) 585–589 (DOI: 10.1021/jz500052h).*
- 6. Molecular ionization from carbon nanotube paper, R. Narayanan, D. Sarkar, R. G. Cooks and T. Pradeep, Angew. Chem. Int. Ed., 53 (2014) 5936 –5940 (DOI: 10.1002/anie.201311053).
- 7. Noble metal clusters: Applications in energy, environment and biology, Ammu Mathew and T. Pradeep, *Part. Part. Syst. Charact., 31 (2014) 1017-1053 (DOI: 10.1002/ppsc.201400033).*
- Manifestation of the difference in reactivity of silver clusters in contrast to its ions and nanoparticles: The growth of metal tipped Te nanowires, Anirban Som, Akshaya Kumar Samal, Thumu Udayabhaskararao, M. S. Bootharaju and T. Pradeep, *Chem. Mater., 26 (2014) 3049–3056 (DOI:* 10.1021/cm403288w).
- Emergence of metallicity in silver clusters in the 150 atom regime: A study of differently sized silver clusters, Indranath Chakraborty, Jayanthi Erusappan, Anuradha Govindarajan, K. S. Sugi, Thumu Udayabhaskararao, Atanu Ghosh and T. Pradeep, Nanoscale, 6 (2014) 8024–8031 (DOI: 10.1039/c4nr00679h).
- 10. Blue emitting undecaplatinum cluster, Indranath Chakraborty, Radha Gobinda Bhuin, Shridevi Bhat and T. Pradeep, *Nanoscale, 6 (2014) 8561-8564 (DOI: 10.1039/C4NR02778G).*
- 11. Coalescence of atomically precise clusters on graphenic surfaces, Atanu Ghosh, T. Pradeep and Jaydeb Chakrabarti, J. Phys. Chem. C, 118 (2014) 13959–13964 (DOI: 10.1021/jp503001s).
- 12. Synthesis of atomically precise silver clusters using the miscibility principle, Atanu Ghosh and T. Pradeep, *Eur. J. Inorg. Chem., 2014 (2014) 5271-5275 (DOI: 10.1002/ejic.201402587).*
- 13. Spatiotemporal mapping of three dimensional rotational dynamics of single ultrasmall gold nanorods, Kamalesh Chaudhari and T. Pradeep, *Scientific Reports (Nature), 4 (2014) 5948 (DOI: 10.1038/srep05948).*

- 14. Using ambient ion beams to write nanostructured patterns for surface enhanced Raman spectroscopy, Anyin Li, Zane Baird, Soumabha Bag, Depanjan Sarkar, Anupama Prabhath, T. Pradeep and R. Graham Cooks, Angew. Chem. Int. Ed., 53 (2014) 12528-12531 (DOI: 10.1002/anie.201406660).
- 15. Ag<sub>11</sub>(SG)<sub>7</sub>: A new cluster identified by mass spectrometry and optical spectroscopy, Ananya Baksi, M. S. Bootharaju, Xi Chen, Hannu Hakkinen and T. Pradeep, J. Phys. Chem. C, 118 (2014) 21722–21729 (DOI: 10.1021/jp508124b).
- 16. Controlled synthesis and characterization of the elusive thiolated Ag<sub>55</sub> cluster, Indranath Chakraborty, Shrabani Mahata, Anuradha Mitra, Goutam De and T. Pradeep, *Dalton Trans., 43 (2014) 17904–17907* (*DOI: 10.1039/c4dt02476a*).
- 17. Reversible formation of Ag<sub>44</sub> from selenolates, Indranath Chakraborty and T. Pradeep, *Nanoscale*, 6 (2014) 14190-14194 (DOI: 10.1039/C4NR03267E).
- 18. Isolation and tandem mass spectrometric identification of a stable monolayer protected silverpalladium alloy cluster, Sreya Sarkar, Indranath Chakraborty, Manoj Kumar Panwar and T. Pradeep, J. Phys. Chem. Lett., 5 (2014) 3757–3762 (DOI: 10.1021/jz5019509).
- 19. Approaching sensitivity of tens of ions using atomically precise cluster-nanofiber composites, Atanu Ghosh, Vedhakkani Jeseentharani, Mohd Azhardin Ganayee, Rani Hemalatha, Kamalesh Chaudhari, Cherianath Vijayan and T. Pradeep, *Anal. Chem., 86 (2014) 10996–11001 (DOI: 10.1021/ac502779r).*
- 20. Antimicrobial silver: An unprecedented anion effect, J. R. Swathy, M. Udhaya Sankar, Amrita Chaudhary, Sahaja Aigal, S. Anshup and T. Pradeep, *Scientific Reports (Nature), 4 (2014) 7161 (DOI: 10.1038/srep07161).*
- 21. Optical rotation by plasmonic circular dichroism of isolated gold nanorod aggregates, Kamalesh Chaudhari and T. Pradeep, *Appl. Phys. Lett.*, 105 (2014) 203105 (DOI: 10.1063/1.4902318).
- 22. Simple and efficient separation of atomically precise noble metal clusters, Atanu Ghosh, Jukka Hassinen, Petri Pulkkinen, Heikki Tenhu, Robin H. A. Ras, and Thalappil Pradeep, *Anal. Chem., 86 (2014) 12185–12190 (DOI: 10.1021/ac503165t)*.
- 23. Vacuum ultraviolet photoabsorption of interstellar icy thiols, Radha Gobinda Bhuin, Bhalamurugan Sivaraman, J.-I. Lo, Raja Sekhar B. N., Bing-Ming Cheng, T. Pradeep, and Nigel Mason, *J. Chem. Phys.*, 141 (2014). 231101 (DOI: 10.1063/1.4903840).
- 24. Identification of effective substrates for the direct analysis of lipids from cell lines using desorption electrospray ionization mass spectrometry, Amitava Srimany, Jayashree Balasubramanyam, Subramanian Krishnakumar, Sailaja Elchuri and T. Pradeep, *Rapid Commun. Mass Spectrom, (2014)* (Just accepted).
- 25. Size evolution of protein protected gold clusters in solution: A combined SAXS-MS investigation, Ananya Baksi, Anuradha Mitra, Jyoti Sarita Mohanty, Haiwon Lee, Goutam De and T. Pradeep, J. Phys. Chem. C, (2014) (Just accepted).

Some of the papers listed here may appear in 2015.



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## Luminescent AgAu Alloy Clusters Derived from Ag Nanoparticles – Manifestations of Tunable Au<sup>I</sup>–Cu<sup>I</sup> **Metallophilic Interactions**

Kumaranchira R. Krishnadas,<sup>[a]</sup> Thumu Udayabhaskararao,<sup>[a]</sup> Susobhan Choudhury,<sup>[b]</sup> Nirmal Goswami,<sup>[b]</sup> Samir Kumar Pal,<sup>[b]</sup> and Thalappil Pradeep\*<sup>[a]</sup>

Keywords: Clusters / Nanoparticles / Luminescence / Closed-shell ions / Metallophilic interactions / Silver / Gold

Luminescent AgAu alloy quantum clusters are synthesized by a simple method that utilizes the galvanic reduction of polydisperse plasmonic silver nanoparticles. The clusters are characterized by ultraviolet-visible (UV/Vis) absorption spectroscopy, photoluminescence (PL) spectroscopy, X-ray photoelectron spectroscopy (XPS), transmission electron microscopy (TEM), and matrix-assisted laser desorption ionization mass spectrometry (MALDI MS). Selective and tunable quenching of cluster luminescence by Cu<sup>II</sup> ions is observed and depends highly on the solvent as well as the protecting ligands. Metal-ion selectivity is exclusively caused by metallophilic interactions with the cluster core, and the tunability depends on the nature of the protecting ligands as well as

#### Introduction

Inherent molecule-like properties and synergistic effects owing to the presence of heteroatoms make dimetallic quantum clusters fascinating materials in modern cluster science. Doping with other metals has been shown to enhance the chemical stability<sup>[1]</sup> as well as tune the electronic structure of quantum clusters.<sup>[2]</sup> The presence of a heteroatom in the core is expected to result in unusual chiroptical and magnetic properties in dimetallic clusters. Despite their promising applications, the synthesis of such dimetallic quantum clusters with atomically precise composition is a big challenge. Among these clusters, Au-Ag<sup>[3]</sup> and Au-Pd<sup>[4]</sup> systems are common systems, and Au-Cu,<sup>[5]</sup> Au-Pt,<sup>[6]</sup> and Ag-Ni<sup>[7]</sup> clusters have also been reported. The simultaneous

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solvent effects. Detailed XPS and time-resolved luminescence measurements reveal that the tunability of luminescence quenching is achieved by the systematic variation of the metallophilic interactions between the Au<sup>I</sup> ions of the alloy cluster and Cu<sup>I</sup> ions formed by the reduction of Cu<sup>II</sup> ions by the cluster core. This is the first report of tunable metallophilic interactions between monolayer-protected quantum clusters and a closed-shell metal ion. We hope that these results will draw more attention to the field of quantum clustermetal ion interactions and provide useful insights into the stability of these clusters, origin of their intense luminescence, mechanisms of metal-ion sensing, and also help in the development of methods for tuning their properties.

reduction of individual precursors<sup>[3-7]</sup> and galvanic reduction of presynthesized quantum clusters<sup>[10,11]</sup> are some of the methods utilized for the synthesis of dimetallic quantum clusters. Although galvanic reduction is extensively utilized for the synthesis of dimetallic nanocrystals with controlled shapes and compositions,<sup>[8]</sup> it is rarely utilized for the synthesis of atomically precise quantum clusters. Murray et al. have shown that it is possible to make dimetallic clusters from silver clusters<sup>[9]</sup> by this method, and galvanic reduction has recently been utilized to synthesize atomically precise Ag-Au clusters from presynthesized thiolate-protected<sup>[10]</sup> and protein-protected silver clusters.<sup>[11]</sup>

The interaction of metal ions with quantum clusters is an active topic of research. Metal ions induce new chemical and electrochemical reactivities in clusters and modify their absorption and emission features. Muhammed et al.<sup>[12a]</sup> reported the first metal-ion-induced changes in the optical properties of quantum clusters, and their reactivities with metal ions were investigated.<sup>[12b]</sup> Quantum confinement in nanoparticles significantly alters their redox potentials<sup>[13]</sup> and results in unexpected electrochemical reactions that cannot be explained by conventional electrochemistry.<sup>[14]</sup> Although there are some previous investigations on the size-dependent changes in the reduction potential of metal clusters,<sup>[15]</sup> the electrochemical reactivities of metal clusters remain largely unexplored. Metal-ion-induced changes in

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cluster luminescence has been extensively exploited for highly selective and sensitive detection of these species.<sup>[16]</sup> The distinct roles of the inner core and ligand shells of these clusters and photophysical mechanisms behind these interactions have not been investigated in detail. Recently, metallophilic interactions between closed-shell metal ions with quantum dots<sup>[17]</sup> and protein-protected Au clusters<sup>[18]</sup> were shown to result in quenching of their luminescence. Metallophilic interactions, weak bonding interactions between two closed-shell metal ions, are a well-known phenomenon in Au<sup>I</sup> complexes and heterometallic clusters of transition metals.<sup>[19]</sup> However, this is not a well-recognized phenomenon in monolayer-protected noble-metal quantum clusters. Pyykko et al. presented the first theoretical studies on the interactions between a closed-shell Au<sub>n</sub> cluster and closed-shell Au<sup>I</sup> species.<sup>[20]</sup> This work raised the possibility of metallophilic interactions between the inner  $Au_n$  core and Au<sup>I</sup> ions in the protecting thiolate staple motifs of the clusters and is of immediate relevance to monolayer-protected quantum clusters. Explorations of such interactions in these systems may provide valuable insights into their stability, the origin of their intense luminescence, mechanisms of metal-ion sensing, and may also offer strategic methods for tuning their properties.

Here, we present the utility of the galvanic reduction reaction as a simple method for the synthesis of luminescent monodisperse AgAu quantum clusters protected by mercaptosuccinic acid (AgAu@MSA) derived from polydisperse plasmonic Ag nanoparticles (AgNPs). These clusters are synthesized at room temperature and no external reducing agent is required. The use of Ag nanoparticles as the precursor, instead of atomically precise Ag quantum clusters, makes the method more facile and scalable because of the inherent instability and difficult synthesis of the latter. The intense red luminescence of these clusters under UV irradiation and their high stability in aqueous solutions may make this material useful for biological applications. The luminescence of this cluster is selectively quenched by Cu<sup>II</sup> ions. Detailed X-ray photoelectron spectroscopy (XPS) measurements show that CuII ions are reduced by interactions with the clusters. Even though Murray et al. and Wu et al. have shown that negatively charged as well as neutral Au<sub>25</sub> clusters can reduce more reactive metal ions such as Ag<sup>I</sup> and Cu<sup>II</sup> ions,<sup>[14]</sup> no such reports exist on the redox reactivities of alloy clusters. Time-resolved as well as steadystate luminescence measurements show that this reactivity leads to metallophilic interactions between the Au<sup>I</sup> ions of the clusters and Cu<sup>I</sup> ions formed by the reduction of Cu<sup>II</sup> ions by the clusters. Also, we report the solvent- and protecting-ligand-dependent tunability of these interactions, which are reflected in the changes in the luminescence of the cluster. Reports on such tunable metal-ion interactions with the clusters are scarce in the literature. This is the first report of tunable metallophilic interactions between monolayer-protected quantum clusters and a closed-shell metal ion. These metallophilic-interaction-induced changes in cluster luminescence are useful for the selective detection of Cu<sup>II</sup> ions in water below permissible levels.<sup>[16h]</sup>

#### **Results and Discussion**

#### Synthesis and Characterization of Alloy Clusters

AgAu@MSA clusters were synthesized by galvanic reduction of AgNPs by Au<sup>I</sup>-MSA thiolates. Figure 1 shows the time-dependent changes in the UV/Vis features during the reaction. The plasmonic feature at 410 nm of AgNPs disappeared immediately after the addition of Au<sup>I</sup>-MSA solution, and a new feature appeared at around 600 nm, which gradually disappeared and the spectrum became featureless. The solution was stirred for one hour and centrifuged subsequently to remove larger alloy nanoparticles and AgCl. The UV/Vis spectrum of the resuspended precipitate (Figure S1) showed a broad peak at ca. 600 nm, which is between the Au and Ag plasmonic peaks. This may be due to the formation of larger AgAu alloy nanoparticles. We propose that during the galvanic reduction, larger nanoparticles in the polydisperse AgNP sample react with Au<sup>I</sup>-MSA to form larger AgAu dimetallic nanocrystals, and very small particles undergo galvanic reduction to form AgAu dimetallic quantum clusters (a schematic of the reaction is given in the inset of Figure 1). The TEM images shown in the inset of Figure 1 clearly show the decrease in the particle size from AgNPs to AgAu@MSA clusters. As the larger nanoparticles have lower solubility in methanol, these dimetallic nanoparticles precipitate, and the smaller dimetallic clusters remain in solution. The formation of AgCl was confirmed by XRD analyses of the precipitate (Figure S2).



Figure 1. Time-dependent changes in the UV/Vis features during the reaction between AgNPs and Au<sup>I</sup>–MSA thiolates in methanol. (a) UV/Vis spectra of AgNPs in methanol, (b) immediately after the addition of Au<sup>I</sup>–MSA, and (c) after one hour of the reaction.

Insets a and a' in Figure 2 show the photographs of the precursor AgNPs under visible and UV light, respectively. Immediately after the addition of Au<sup>I</sup>–MSA, this solution showed red emission under UV illumination. The time-dependent evolution of the luminescence features is shown in Figure S3, and typical luminescence features of the cluster are shown in Figure 2. The cluster showed a broad emission peak at 675 nm at 365 nm excitation. Insets b and b' of Figure 2 show the photographs of the AgAu@MSA cluster under visible and UV light, respectively.



Figure 2. Excitation and emission spectra of AgAu@MSA clusters in methanol. The insets are the photographs of (a and a') the AgNP solution, (b and b') the AgAu@MSA solution, and (c and c') the PAGE-separated clusters under visible and UV light, respectively. The discontinuity in the excitation peak shows the position at which the secondary of the emission maximum appears.

Polyacrylamide gel electrophoresis (PAGE) was performed to check the purity of the as-synthesized clusters (details in the Supporting Information). The inset photographs (c and c') of Figure 2 show the presence of a single band of the gel after PAGE separation. The band appeared light yellow under visible light and bright red under a UV lamp. This shows that monodisperse dimetallic clusters can be synthesized from polydisperse plasmonic silver nanoparticles by using the galvanic reduction method.

The XPS survey spectrum shown in Figure 3 (a) indicates the presence of all expected elements. The Au  $4f_{7/2}$  peak at 84.1 eV shows that the Au atoms are in the zero oxidation state. The Ag  $3d_{5/2}$  peak at 368.0 eV<sup>[10]</sup> indicates the presence of metallic Ag in the cluster. The S  $2p_{3/2}$  peak at 162.2 eV suggests that sulfur is attached to the metal core in the form of thiolate.<sup>[22]</sup> Energy-dispersive X-ray analysis (EDAX) also confirmed the presence of the constituent elements in the cluster (Figure S4).



Figure 3. (a) XPS survey spectrum of the AgAu@MSA cluster and expanded regions of it showing the (b) Au 4f, (c) Ag 3d, and (d) S 2p features.

Figure 4 shows the matrix-assisted laser desorption ionization mass spectra (MALDI MS) of the clusters after ligand exchange with hexanethiol. Ligand exchange was attempted because MSA-protected clusters do not give intact ions in MALDI MS. This is also the case with glutathioneprotected clusters.<sup>[23]</sup> The inset of Figure 4 shows the luminescence spectral features of the cluster before (in water) and after (in toluene) ligand exchange. The excitation maximum of the cluster shows a blueshift of ca. 20 nm after ligand exchange, and the emission maximum is blueshifted by ca. 5 nm. These shifts could be caused by the difference in the polarities of water and toluene. The excitation and emission spectral shapes are preserved after ligand exchange, which shows that the composition of the cluster core is unchanged. At the threshold laser fluence, a peak at 17 kDa appears, and the peak shifts to the lower mass region owing to fragmentation of the cluster upon increasing laser fluence.



Figure 4. Laser-intensity-dependent MALDI MS spectra of the AgAu clusters, ligand-exchanged with hexanethiol. The numbers by the side of the arrow indicate the laser intensity as given by the instrument. The inset shows the excitation and emission spectra of the cluster before and after ligand exchange.

#### Tunable Interactions of the Alloy Clusters with Cu<sup>II</sup> Ions

Interactions of metal ions with the quantum clusters affect their absorption and emission features. Metal-ion-induced luminescence changes of quantum clusters were utilized extensively for the selective detection of trace quantities of metal ions.<sup>[16,24]</sup> To study the interaction of metal ions with the AgAu@MSA clusters, the luminescence spectra of these clusters were measured in the presence of various metal ions. Aqueous solutions of various metal ions  $(100 \,\mu\text{L}, 100 \,\text{ppm})$  were added to the cluster solution in methanol. The luminescence of the AgAu@MSA clusters was quenched immediately after the addition of a Cu<sup>II</sup> solution. Figure 5 (a) shows that quenching is selective to  $Cu^{II}$ ions, and there is no significant decrease in luminescence intensity upon the addition of other metal ions. The addition of Cu<sup>II</sup> ions resulted in gradual precipitation of the clusters from the solution. These observations may invoke possibility of aggregation-induced luminescence the





Figure 5. Emission spectra of (a) AgAu@MSA clusters in methanol and (b) AgAu@BBS clusters in THF in the presence of various metal ions.

quenching, resulting from the binding of Cu<sup>II</sup> ions to the carboxylate groups of the MSA ligands, which was suggested as a metal-ion-induced quenching mechanism in noble-metal clusters.<sup>[24]</sup> Aggregation-induced quenching occurs by the reabsorption of the emitted radiation from the fluorophore, and for this phenomenon to be feasible, the Stokes shift of the fluorophore should be very small. The large Stokes shift (ca. 310 nm) of the AgAu@MSA cluster also suggests that this phenomenon is not likely. The UV/ Vis absorption spectrum of the cluster was featureless, and no new features were observed after treatment with the Cu<sup>II</sup> salt (Figure S5). Moreover, aggregation induced by the Cu<sup>II</sup> ions should not affect the cluster core and, hence, the binding energies of the core atoms will not be shifted. XPS measurements (Figure 8) show that the Au binding energy is shifted to higher values. This evidence clearly indicates the absence of aggregation-induced quenching.

To determine whether the observed metal-ion selectivity is due to the specific interaction of  $Cu^{II}$  ions with the core or the carboxylate groups of the ligand shell of the AgAu cluster, quenching experiments were performed with clusters that had been ligand-exchanged with *tert*-butylbenzyl mercaptan (BBSH). As the sulfur atom of this ligand is bound to the AgAu core of the cluster and there are no other functional groups such as –COOH, the possibility of ligand– $Cu^{II}$  interactions is eliminated. Interestingly, the luminescence of the ligand-exchanged cluster (AgAu@BBS) was also quenched by  $Cu^{II}$  ions (Figure 5, b), that is, the selectivity towards  $Cu^{II}$  ions was retained even after ligand exchange. These observations clearly suggest that the origin of metal-ion selectivity is the interaction of  $Cu^{II}$  ions with the AgAu core of the cluster.

The addition of oxalic acid (OA) into the Cu<sup>II</sup>-treated AgAu@MSA solution in methanol (solution 1) resulted in complete recovery of the luminescence (Figure 6, a). This shows that the interaction of Cu<sup>II</sup> ions with the clusters is almost completely reversible in methanol. As oxalic acid is a very strong chelator for Cu<sup>II</sup> ions, it forms stable copper oxalate, which results in the recovery of the luminescence. The addition of Cu<sup>II</sup> ions into a mixture of OA and the clusters did not result in any quenching (Figure S6); this shows that OA effectively prevents cluster–Cu<sup>II</sup> interactions. Interestingly, the luminescence of a AgAu@BBS–Cu<sup>II</sup> mixture in tetrahydrofuran (THF; solution 3) was not at all

recovered by the addition of OA (Figure 6, c). To check the role of change in solvent from methanol to THF on the observed irreversibility, quenching experiments were performed with AgAu@MSA clusters in THF. Interestingly,



Figure 6. Emission spectra of (a) AgAu@MSA clusters in methanol and (b) THF and (c) AgAu@BBS clusters in THF showing the changes in luminescence upon the addition of Cu<sup>II</sup> ions and OA. In each panel, traces a–c corresponds to the pure cluster, the cluster with Cu<sup>II</sup> ions, and the cluster with Cu<sup>II</sup> ions and OA, respectively.



we observed a partial recovery (Figure 6, b) of the luminescence upon the addition of OA into a AgAu@MSA–Cu<sup>II</sup> mixture in THF (solution 2). This indicates that in addition to the solvent, the nature of the protecting ligand is also important in the determination of the reversibility of the interaction (see below for possible effects of solvent and protecting ligands). These observations show that the cluster–Cu<sup>II</sup> interactions that lead to luminescence quenching are tunable with respect to the solvent and the nature of the protecting ligand. However, we observed a redshift of the emission maximum (ca. 5 nm for solution 1 and ca. 10 nm for solutions 2 and 3) upon the addition of Cu<sup>II</sup> ions, and the shift was retained even after the addition of OA.

XPS measurements of the Cu<sup>II</sup>-treated clusters (Figure 7) show that the Cu  $2p_{3/2}$  peak is shifted to lower binding energies compared to that of metallic Cu (935.5 eV). Cu<sup>II</sup> shows a well defined peak shape with characteristic satellite structure. The presence of Cu 2p features in all the treated samples indicates that Cu is part of the samples. Note that the XPS sample preparation involves washing the sample. However, complete absence of the satellite structure in these samples suggests reduction of the Cu<sup>II</sup> ions. For solution 1, the Cu  $2p_{3/2}$  peak is at 932.5 eV, whereas for solutions 2 and 3, these peaks are shifted to 932.3 and 932.2 eV, respectively. As the difference in the Cu<sup>I</sup> and Cu<sup>0</sup> binding energies is only ca. 0.1-0.2 eV,<sup>[25]</sup> it is difficult to assign the exact Cu oxidation states in the mixtures. However, the reduction of Cu<sup>II</sup> to Cu<sup>I</sup>/Cu<sup>0</sup> is evident from these measurements. The characteristic ligand-to-metal charge-transfer satellite of Cu<sup>II</sup> at 946 eV is absent in the treated samples, indicating the complete absence of the Cu<sup>II</sup> state in the samples.



Figure 7. Cu 2p regions in the X-ray photoelectron spectra of pure and Cu<sup>II</sup>-treated AgAu clusters. Traces a–d correspond to CuSO<sub>4</sub> and solutions 1, 2, and 3, respectively.

Figure 8 shows the Au 4f binding energies for the pure and Cu<sup>II</sup>-treated clusters. The Au  $4f_{7/2}$  peak for the pure AgAu@MSA cluster is at 84.1 eV. For solutions 1 and 2, this peak is shifted to 84.6 eV. This peak shifts further to 85.0 eV for solution 3. The binding energies for Ag were almost unchanged for the pure as well as Cu<sup>II</sup>-treated clusters (Figure S7). This clearly indicates that the Cu<sup>II</sup> ions interact preferentially with the Au atoms of the alloy cluster. Elemental analysis (Figure S4) shows that the alloy cluster is mostly composed of Au. Galvanic reduction by Au<sup>I</sup> thiolates is initiated on the surface of the AgNPs, and most of the Ag atoms will be released as Ag<sup>I</sup> ions and a few Ag atoms will be entrapped by Au atoms. Hence, these Ag atoms are more likely to be in the inner core of the cluster, which make them inaccessible to the Cu<sup>II</sup> ions. This could be the reason for the preferential interaction of the Cu<sup>II</sup> ions with the Au atoms of the cluster.



Figure 8. Au 4f region in the X-ray photoelectron spectra of pure and Cu<sup>II</sup>-treated AgAu clusters. Traces a–e correspond to pure AgAu@MSA and solutions 1–4, respectively.

Notably, the observed trend in reversibility of the luminescence parallels the changes in the Au  $4f_{7/2}$  binding energies. The Au  $4f_{7/2}$  peak for solutions 1 and 2 (for which the luminescence was completely and partially reversible, respectively) is at 84.6 eV, which is between those for the pure clusters (84.1 eV) and AgAu@BBS (85.0 eV). The shift in the Au  $4f_{7/2}$  peak (from 84.1 to 85.0 eV) is maximum for solution 3, for which irreversible quenching of luminescence was observed.

We suggest that the clusters can undergo two kinds of interactions with Cu<sup>II</sup> ions. XPS measurements clearly indicate that the Cu<sup>II</sup> ions are reduced as a result of their interactions with the cluster. The decrease in Cu binding energy (to that of Cu<sup>I</sup>/Cu<sup>0</sup>) and increase in that of Au in the clusters may be an indication of a redox reaction between the cluster core and Cu<sup>II</sup> ions (reaction 1). The redshift of the emission maximum of the clusters after treatment with Cu<sup>II</sup> ions could be an indication of the oxidation of the clusters. The reduction of the Cu<sup>II</sup> ions by a noble metal such as Au may seem contradictory when considering the conventional electrochemical potentials. It is to be noted that the standard electrochemical potentials in the literature are those of bulk electrodes. In the case of nanoparticles, especially at the quantum cluster regime, electrochemical potentials will be very much determined by quantum confinement effects. A few earlier investigations<sup>[15c,15d]</sup> on small metal clusters have shown that their reduction potentials were lower than the bulk values. Hence, at the cluster regime, these types of redox processes are feasible. Another possibility for the reduction of the Cu<sup>II</sup> ions is the replacement of some of the



 $Au^{\mathrm{I}}$  ions of the thiolate staple motifs by  $Cu^{\mathrm{II}}$  ions, which may lead to the formation of a mixed thiolate shell containing both Au<sup>I</sup> and Cu<sup>I</sup> ions (reaction 2). This leads to the reduction of Cu<sup>II</sup> to Cu<sup>I</sup> as in the case of copper thiolates.<sup>[26]</sup> We could not confirm this interaction by an analysis of the S 2p binding energies of the samples as there were no significant changes in the S 2p binding energies of solutions 1 and 2, compared to that of pure clusters. Notably, the S 2p binding energies of copper and gold thiolates are almost the same (ca. 162 eV).<sup>[22,26]</sup> However, for solution 3, the S  $2p_{3/2}$  binding energy was 162.7 eV (data not shown), which is higher than that of the pure clusters (162.2 eV). XPS of the OA-treated solution 1 (solution 4; sample for which luminescence was completely recovered) indicates that the Au 4f binding energies remain at higher values (84.6 eV). This shows that the clusters undergo an irreversible oxidation as a result of their interactions with the Cu<sup>II</sup> ions (reaction 1). Reaction 2 is expected to result in complete reversibility of the Au 4f binding energies to those of the pure clusters after treatment with OA, as all of the Cu<sup>1</sup> ions are completely removed by OA. Once the Cu<sup>I</sup> ions have been removed by OA, the Au<sup>I</sup> ions can bind with the ligands (released from Cu<sup>I</sup>), and this will regenerate the pure clusters and should lead to the reversal of the Au 4f binding energies to that of the pure clusters. However, this reversal of binding energies was not observed. These observations indicate that reaction 1, that is, the galvanic reduction, is the most likely interaction between the clusters and Cu<sup>II</sup> ions

To understand the mechanism of the observed fluorescence quenching of AgAu@MSA clusters in presence of Cu<sup>II</sup> ions, we plotted (Figure 9) the fractional fluorescence according to the Stern–Volmer Equation (1).

$$\frac{F_0}{F} = 1 + K_{\rm D}[\mathbf{Q}] \tag{1}$$

 $F_0$  and F are the fluorescence intensity in the absence and presence of quencher, respectively, [Q] is the concentration of quencher and  $K_D$  is the Stern–Volmer quenching con-



Figure 9. Stern–Volmer plot for the luminescence quenching of AgAu@MSA clusters in methanol upon the addition of  $Cu^{II}$  ions. The inset shows the change of  $\tau_0/\tau_{av}$  with  $Cu^{II}$  ion concentration.

stant. From Figure 9, we have found a linear response of fluorescence as a function of  $Cu^{II}$  ion concentration. This result indicates that the nature of quenching is dynamic.

Picosecond time-resolved luminescence measurement is a useful technique to aid understanding of the photophysical processes behind the observed quenching of the AgAu@MSA clusters. A gradual and significant decrease in the average lifetime (Figure S8 and Table S1) with increasing quencher concentration indicates the occurrence of dynamic quenching. The plot of  $\tau_0/\tau_{av}$  against Cu<sup>II</sup> concentration (inset of Figure 9) was linear, as expected for a dynamic quenching process. The decay profiles for pure clusters and solution 1 are shown in Figure 10. The decay transients are fitted tri-exponentially, and the fitting parameters are tabulated in Table 1. Similar decay behaviors were also observed for solutions 2 and 3 (Tables S2 and S3, Figure S9 and S10, respectively). The decay profiles indicate the dynamic nature of quenching for all cases.



Figure 10. Picosecond time-resolved fluorescence transients of AgAu@MSA clusters in methanol. (a) The quenching of luminescence lifetime upon the addition of  $Cu^{II}$  ions and its recovery upon the addition of OA and (b) upon the addition of  $Cu^{II}$  ions and benzoquinone.

Table 1. Picosecond time-resolved luminescence transients of AgAu@MSA before and after the treatment with Cu<sup>II</sup> and recovery by OA in methanol. AgAu@MSA was also treated with BQ.

System	$\tau_1 \text{ [ns] (\%)}$	$\tau_2 [ns] (\%)$	$\tau_3$ [ns] (%)	$\tau_{\rm av}  [{\rm ns}]$
Pure AgAu@MSA cluster in methanol	0.16 (38)	1.12 (20)	50 (42)	21.3
Cluster with Cu <sup>II</sup>	0.10 (65)	1.24 (22)	12 (13)	1.93
Cluster with OA Cluster with BQ	0.09 (52) 0.07 (71)	1.64 (20) 1.58 (9)	50 (28) 55 (20)	14.4 11.2

Dynamic quenching can occur through various processes such as Förster resonance energy transfer (FRET) and photoinduced electron transfer (PET). However, the absence of



any permanent oscillating dipole (owing to the acceptor) rules out the possibility of a FRET process. The absence of an electron transfer processes is further confirmed by control experiments with benzoquinone (BQ), a well-known electron acceptor. In presence of BQ, an ultrafast time component (70 ps) evolves with very sharp decay with a contribution of 71% (Figure 10, b). No such drastic changes in the ultrafast components are observed for the Cu<sup>II</sup>-treated samples, which rules out the possibility of an ultrafast PET process from the cluster to the Cu<sup>II</sup> ions. This is supported by measurements at different Cu<sup>II</sup> ion concentrations, for which the gradual reduction of the longer time component was observed with minor alteration of the shorter time constant (Figure S8 and Table S1).

Similar lifetime decay patterns were reported for Au<sub>25</sub>@BSA clusters upon the interaction with Hg<sup>II</sup> ions.<sup>[18a]</sup> This was attributed to metallophilic bond-induced quenching of the delayed fluorescence. The observations of the reduction of the Cu<sup>II</sup> ions along with the similarity in the decay patterns of the AgAu clusters strongly suggest the possibility of metallophilic interactions. We suggest the following mechanism for the observed selective and tunable quenching interactions. The Cu<sup>II</sup> ions are first reduced to Cu<sup>I</sup> by a redox reaction with the AgAu core of the cluster (reaction 1). The 3d<sup>10</sup> orbital of the Cu<sup>I</sup> ions and the 5d<sup>10</sup> orbital of either the Au<sup>I</sup> ions of the thiolate staple motifs or the Au atoms of the partially oxidized cluster take part in metallophilic interactions. As the staple motifs have a significant role in the electronic structure of monolayer-protected clusters,<sup>[27]</sup> these interactions can lead to changes in the electronic structure and to quenching. Metallophilic interactions between Au<sup>I</sup> and Cu<sup>I</sup> ions are recognized both theoretically and experimentally in diverse systems and have been utilized in applications such as vapochromic sensors.<sup>[28]</sup> We suggest that galvanic reduction-induced metallophilic interactions are the key factor that determines the selectivity towards metal ions. Among the metal ions tested, only the Cu<sup>II</sup> and Hg<sup>II</sup> systems possess positive reduction potentials and, hence, these ions can be reduced most easily by the quantum clusters. The reduction potentials of the other metal ions are negative and, hence, they cannot be reduced by these clusters. In addition to the electrochemical potentials, the requirement of a closed-shell electronic configuration for metallophilic interactions immediately reveals the reason behind the observed metal-ion selectivity. Among Cu<sup>II</sup> and Hg<sup>II</sup>, only Cu<sup>II</sup> can attain a closed-shell configuration (of Cu<sup>I</sup>) from core-induced reduction, and this might be the reason behind the observed selectivity towards Cu<sup>II</sup>. Even though Hg<sup>II</sup> can be reduced to Hg<sup>I</sup> by the cluster, this reduction will lead to the formation of an ion without a closed-shell configuration. Notably, the addition of HgII ions to the clusters does not lead to any immediate luminescence quenching. However, quenching owing to the addition of HgII ions was observable only after about 5 h (data not shown). Earlier studies on the reaction of quantum clusters with Hg<sup>II</sup> ions show that the Hg<sup>II</sup> ions were reduced mostly to Hg<sup>I</sup> and not to Hg<sup>0</sup>.<sup>[12b]</sup> This could

be due to the high affinity of mercury towards the thiolate ligands of the cluster.

We suggest that solvents as well as protecting ligands play a crucial role in the tunability of the luminescence quenching of these AgAu clusters. Solvents can induce structural changes in the cluster core as well as the thiolate staple motifs. Recent studies on Au-Cu clusters protected by alkynyl ligands<sup>[29a]</sup> show that solvents can induce structural reorganization of clusters by affecting the strength of metallophilic bonding, and this leads to changes in the luminescence features. Studies on Au<sup>I</sup> thiolates have shown that solvents can induce Au<sup>I</sup>-Au<sup>I</sup> metallophilic interactions caused by aggregation and lead to visible photoluminescence.<sup>[29b]</sup> These solvent effects will modulate the strength of AuI-CuI boding in these clusters and lead to tunable quenching interactions. As mentioned earlier, changes in the bonding of thiolate staple motifs alter the electronic structure of clusters, which in turn affects the luminescence features of the cluster.[27]

Metallophilic bonding can originate from different types of interactions between the closed-shell species such as van der Waals, ionic, and charge-transfer forces. Theoretical studies on Au<sup>I</sup>–Cu<sup>I</sup> metallophilic bonds<sup>[28a]</sup> show that this bond is mainly due to ionic forces between the two species. Hence, changes in the solvent polarity are expected to significantly affect the nature of this bond. As the polarity of the solvent decreases, the ionic-interaction-based metallophilic bond becomes less feasible and the bond becomes more covalent in nature and, hence, stronger. Therefore, the Au<sup>I</sup>–Cu<sup>I</sup> bond will be weaker (i.e., this bond will be only metallophilic in nature) in a polar solvent such as methanol than in THF. The efficient chelation of Cu<sup>I</sup> species happens in methanol and in turn results in complete recovery of the luminescence. The complete recovery of luminescence was further confirmed by experiments with the clusters in acetone as another polar solvent (Figure S11). We also observe the recovery in the time-resolved measurements (see Figure 10, a). In THF, the metallophilic contributions to the Au<sup>I</sup>-Cu<sup>I</sup> bond will be less, and the covalent nature of the bond increases. This makes the Au<sup>I</sup>–Cu<sup>I</sup> bond stronger and, hence, chelation with OA will be less facile. This could be the reason for the partial recovery of luminescence for the AgAu@MSA clusters in THF. Note that the dielectric constants of methanol, acetone, and THF are 33, 21, and 7.5, respectively. However, we could not demonstrate the irreversible quenching for the MSA-protected sample in a solvent less polar than THF because of the insolubility of the clusters and Cu<sup>II</sup> salts in such solvents. In the case of the ligand-exchanged clusters, the AuI-CuI bond experiences a much more nonpolar environment (owing to the BBSH or hexanethiol ligand shell) compared to the case with the AgAu@MSA clusters in THF. Therefore, the Au<sup>I</sup>-Cu<sup>I</sup> bond will be strongest for the ligand-exchanged cluster in THF. In this case, the chelation of the Cu<sup>I</sup> ions with OA will be least facile. This could be the reason behind the irreversible quenching of the ligand-exchanged cluster. The selectivity towards Cu<sup>II</sup> ions and irreversibility of the lumi-



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nescence quenching are further confirmed with clusters that are ligand-exchanged with hexanethiol.

To check the practical utility of these interactions for  $Cu^{II}$  detection, the luminescence intensities of aqueous solutions of AgAu@MSA with different  $Cu^{II}$  concentrations (in parts per million) were measured (Figure 11). A good linear response of the materials towards  $Cu^{II}$  ions was observed. From this plot, the detection limit is 0.5 ppm, which is well below the permissible limit of  $Cu^{II}$  ions in water (1.3 ppm). As the reduction potentials of the clusters depend on their composition, we expect that these interactions can be utilized for ultra-low-level detection with clusters of varied composition, which can be easily synthesized by our method.



Figure 11. Variation of the luminescence intensity of the AgAu@MSA clusters in water with concentration of  $Cu^{II}$  ions, immediately after the addition.

#### Conclusions

Luminescent AgAu dimetallic quantum clusters were synthesized by a simple method that utilizes the galvanic reduction of polydisperse plasmonic nanoparticles. The clusters were characterized by various spectroscopic and microscopic tools. The luminescence of the clusters is selectively quenched by Cu<sup>II</sup> ions, and the quenching is highly tunable depending on the solvent and the ligand used. Detailed XPS measurements indicate that the clusters undergo a redox reaction with Cu<sup>II</sup> ions. Steady-state as well as timeresolved luminescence measurements prove that the tunability is due to the galvanic reduction-induced tunable metallophilic interactions between the Au<sup>I</sup> ions of the cluster and Cu<sup>I</sup> ions formed by the reduction of Cu<sup>II</sup> ions by the cluster. This is the first report of tunable metallophilic interactions between monolayer-protected quantum clusters and a closed-shell metal ion. We hope that our results draw more attention to the chemistry of quantum clusters with metal ions in general and the metallophilic interactions of clusters in particular.

#### **Experimental Section**

**Materials:** Chloroauric acid (HAuCl<sub>4</sub>·3H<sub>2</sub>O), mercaptosuccinic acid (MSA), and 4-*tert*-butylbenzyl mercaptan (BBSH) were pur-

chased from Sigma–Aldrich. Silver nitrate and tetrahydrofuran (THF) were purchased from RANKEM. Hexanethiol was purchased from Fluka. Oxalic acid, CuSO<sub>4</sub>·5H<sub>2</sub>O, CdCl<sub>2</sub>, ZnSO<sub>4</sub>, HgCl<sub>2</sub>, NiCl<sub>2</sub>, and Co(OAc)<sub>2</sub> were purchased from Merck. All chemicals were used without any further purification.

AgAu@MSA Clusters: MSA-protected AgNPs were synthesized by following a reported procedure.<sup>[21]</sup> A stock solution was prepared by dissolving purified AgNPs (80 mg) in distilled water (10 mL). Au<sup>I</sup> MSA thiolate was prepared in distilled water by dissolving MSA powder (8.5 mg) in aqueous HAuCl<sub>4</sub> solution (5 mL, 5 mM). All reactions were performed at room temperature. For the synthesis of the AgAu@MSA clusters, the stock AgNP solution (0.5 mL) was diluted with methanol (to 10 mL). Au<sup>I</sup>–MSA solution (2 mL) was added into the methanolic AgNP solution with stirring. The reaction was monitored by time-dependent UV/Vis absorption and photoluminescence measurements. Larger AgAu alloy nanoparticles and AgCl were removed by ultracentrifugation, and AgAu@MSA clusters were obtained as a clear solution in methanol. This solution was concentrated by rotary evaporation and then freeze-dried to afford a pasty material; excess thiolates prevented the production of clean powders from the system. Ethyl acetate was added to this pasty material to precipitate the pure AgAu@MSA, which was then collected and dried in nitrogen to afford dry powder samples.

**Ligand Exchange of AgAu@MSA Clusters:** The ligand (BBSH or hexanethiol) was dissolved in methanol (70  $\mu$ L in 2 mL). This solution was added to AgAu@MSA cluster solution (2 mL) in distilled water, and the mixture was stirred at room temperature for 2 min. Toluene (4 mL) was then added, and the mixture was stirred further for 3 min at room temperature. The aqueous layer became colorless, and the toluene layer became light yellow indicating the completion of the ligand exchange. The toluene layer was separated to afford a clear solution of ligand-exchanged AgAu cluster.

Instrumentation: UV/Vis absorption spectra were recorded with a Perkin-Elmer Lambda 25 instrument in the spectral range 200-1100 nm. Transmission electron microscopy (TEM) of the samples was performed by using a JEOL 3010 instrument with an ultrahigh resolution (UHR) polepiece. TEM specimens were prepared by drop-casting one or two drops of the aqueous solution to carboncoated copper grids and allowed to dry at room temperature overnight. All measurements were performed at 200 kV to minimize the damage of the sample by the high-energy electron beam. X-ray photoelectron spectroscopy (XPS) measurements were performed with an Omicron ESCA Probe spectrometer with polychromatic Mg- $K_{\alpha}$  X-rays (hv = 1253.6 eV). The X-ray power applied was 300 W. The pass energy was 50 eV for survey scans and 20 eV for specific regions. Sample solutions were spotted on a molybdenum sample plate and dried in vacuo. The base pressure of the instrument was  $5.0 \times 10^{-10}$  mbar. The binding energy was calibrated with respect to the adventitious C 1s feature at 285.0 eV. Deconvolution of the spectra was performed by using the CASAXPS software. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) studies were conducted with a Voyager-DE PRO Biospectrometry Workstation from Applied Biosystems. A pulsed nitrogen laser of 337 nm was used for the MALDI MS studies. The samples were mixed with a *trans*-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) matrix in 1:1 ratio, spotted on the target plate, and allowed to dry under ambient conditions. Mass spectra were collected in the negative-ion mode and were averaged for 200 shots. Scanning electron microscopy (SEM) and energy-dispersive X-ray (EDAX) analysis were performed with an FEI QUANTA-200 SEM. For measurements, samples were drop-



casted on an indium tin oxide coated conducting glass and dried in ambient conditions. Picosecond-resolved fluorescence decay transients were measured and fitted by using a commercially available spectrophotometer (Life Spec-ps, Edinburgh Instruments, UK) with an 80 ps instrument response function (IRF).

**Supporting Information** (see footnote on the first page of this article): Procedure for the PAGE experiment, UV/Vis spectra of the product and byproducts, XRD pattern of the AgCl formed as a byproduct, time-dependent evolution of the photoluminescence of the clusters during the reactions, EDAX spectrum and elemental composition of the cluster, UV/Vis spectra of the cluster in methanol with and without Cu<sup>II</sup> ions, emission spectra of AgAu@MSA in methanol containing oxalic acid and its stability of fluorescence upon the addition of Cu<sup>II</sup> ions, Ag 3d regions in the X-ray photoelectron spectra of pure and Cu<sup>II</sup>-treated AgAu@MSA clusters, lifetime measurements of alloy clusters containing different Cu<sup>II</sup> concentrations, lifetime decay profiles and fitting parameters for solutions 2 and 3, luminescence data showing the complete reversibility of the quenching in acetone.

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## SUPPORTING INFORMATION

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**<u>Title</u>**: Luminescent AgAu Alloy Clusters Derived from Ag Nanoparticles – Manifestations of Tunable Au<sup>I</sup>–Cu<sup>I</sup> Metallophilic Interactions

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### **Supporting Information 1**

#### Procedure for polyacrylamide gel electrophoresis (PAGE):

A gel electrophoresis unit with 1 mm thick spacer (Bio-rad, Mini-protein Tetra cell) was used to process the PAGE. The total contents of the acrylamide monomers were 30% (bis(acrylamide:acrylamide) = 7:93) and 3% (bis(acrylamide:acrylamide) = 6:94) for the separation and condensation gels, respectively. The eluting buffer consisted of 192 mM glycine and 25 mM tris(hydroxymethylamine). The cluster was dissolved in 5% (v/v) glycerol-water solution (1.0 mL). The sample solution (1.0 mL) was loaded onto a 1 mm gel and eluted for 4 h at a constant voltage of 100 V to achieve separation.

#### **Supporting Information 2**

UV-vis spectra of the AgAu@MSA cluster and AgAuNPs formed during galvanic displacement reaction



**Figure S1.** UV-vis spectra of the product (AgAu@MSA) and the byproduct (AgAuNPs) after the reaction between AgNPs and Au<sup>I</sup>-MSA thiolate.

## **Supporting Infromation 3**



XRD pattern of AgCl formed during galvanic displacement reaction

**Figure S2.** XRD pattern of the precipitate obtained after the reaction between AgNPs and the Au<sup>1</sup>-MSA thiolate, in methanol. The peaks are due to the AgCl crystals formed during the galvanic displacement reaction. The peak at around 37 may be due to the AgAuNPs formed.

## **Supporting Information 4**

Time-dependent evolution of luminescence during galvanic displacement reaction



**Figure S3**. Time-dependent evolution in the photoluminescence showing the formation of AgAu@MSA clusters during the galvanic exchange reaction.

**Supporting Information 5** 

EDAX spectrum and elemental composition of the AgAu@MSA cluster



Figure S4. EDAX spectrum and elemental composition of the AgAu@MSA cluster.

## **Supporting Information 6**

UV-vis spectra of the clusters in methanol with and without Cu<sup>II</sup>



Figure S5. UV-vis absorption spectra of AgAu@MSA clusters in methanol with and without Cu<sup>II</sup>.

### **Supporting Information 7**

Photoluminescence data showing the ability of OA to prevent metal ion quenching



**Figure S6.** Emission spectra of the AgAu@MSA cluster solution in methanol containing oxalic acid and its stability of fluorescence upon the addition of Cu<sup>II</sup>.

**Supporting Information 8** 

Ag 3d regions in the XPS spectra of pure and Cu<sup>II</sup>-treated clusters



Figure S7. Ag 3d regions in the X-ray photoelectron spectra of pure and Cu<sup>II</sup>-treated AgAu@MSA clusters.

## **Supporting Information 9**

Lifetime measurements of alloy clusters containing different concentrations of Cu<sup>II</sup>



#### Figure

**S8.** Picosecond time-resolved fluorescence transients of AgAu@MSA clusters containing different concentration of Cu<sup>II</sup> in methanol.

**Table S1.** Picosecond time-resolved luminescence transients of AgAu@MSA clusters containing different concentration of Cu<sup>II</sup> in methanol. The luminescence of these clusters ( $\lambda_{max} = 675$  nm) is measured using a 375 nm excitation laser.

Concentration of Cu <sup>II</sup>	τ <sub>1</sub> ns (%)	$\tau_2$ ns (%)	τ3 ns (%)	$\tau_{av}$ (ns)
added to				
AgAu@MSA cluster				
in methanol				
0	0.16 (38)	1.12 (20)	50.00(42)	21.28
37.5 μM	0.14 (42)	1.77 (21)	46.77 (37)	17.88
75 µM	0.12 (46)	1.75 (26)	30.45(28)	8.95
112.5µM	0.11(54)	1.60(26)	24.08(20)	5.36
250 μΜ	0.10 (65)	1.24 (22)	12.00 (13)	1.93

**Supporting Information 10** 

Lifetime decay profiles and fitting parameters of solutions 2 and 3



**Figure S9.** Picosecond time-resolved fluorescence transients of AgAu@MSA clusters in THF showing the quenching of the luminescence lifetime upon the addition of  $Cu^{II}$  and its partial recovery upon the addition of OA.



**Figure S10.** Picosecond time-resolved fluorescence transients of AgAu@BBS clusters in THF showing the quenching of the luminescence lifetime upon the addition of  $Cu^{II}$  and its irreversibility upon the addition of OA.

**Table S2.** Picosecond time-resolved luminescence transients of pure and Cu<sup>II</sup>-treated AgAu@MSA cluster in THF. The luminescence of these clusters ( $\lambda_{max} = 680$  nm) is measured using a 375 nm excitation laser.

Cluster system	τ <sub>1</sub> ns (%)	$\tau_2$ ns (%)	τ <sub>3</sub> ns (%)	$\tau_{av}$ (ns)
Pure AgAu@MSA	0.24(23)	1.24 (35)	48.37 (42)	20.74
cluster in THF				
Cluster with Cu <sup>II</sup>	0.18 (27)	1.44 (70)	15.56 (3)	1.39
Cluster with OA	0.21 (25)	1.45 (68)	32.28(7)	3.38

**Table S3.** Picosecond time-resolved luminescence transients of pure and Cu<sup>II</sup>-treated AgAu@BBS cluster in THF. The luminescence of these clusters ( $\lambda_{max} = 670$  nm) is measured using a 409 nm excitation laser.

Cluster system	τ1ns (%)	τ <sub>2</sub> ns (%)	τ <sub>3</sub> ns (%)	$\tau_{av}$ (ns)
AgAu@BBSH cluster	0.15 (43)	2.5 (19)	41.12 (38)	21.28
in THF				

Cluster with Cu <sup>II</sup>	0.082 (71)	1.81 (17)	18.51 (12)	2.60
Cluster with OA	0.106 (69)	1.84 (19)	15.09 (12)	2.17

## **Supporting Information 11**

Luminescence data showing the complete reversibility of the quenching in acetone as solvent



**Figure S11.** Luminescence data showing the quenching of the luminescence of AgAu@MSA clusters in acetone upon the addition of Cu<sup>II</sup> and its complete recovery upon the addition of OA.



# Development of ultralow energy (1–10 eV) ion scattering spectrometry coupled with reflection absorption infrared spectroscopy and temperature programmed desorption for the investigation of molecular solids

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Extremely surface specific information, limited to the first atomic layer of molecular surfaces, is essential to understand the chemistry and physics in upper atmospheric and interstellar environments. Ultra low energy ion scattering in the 1–10 eV window with mass selected ions can reveal extremely surface specific information which when coupled with reflection absorption infrared (RAIR) and temperature programmed desorption (TPD) spectroscopies, diverse chemical and physical properties of molecular species at surfaces could be derived. These experiments have to be performed at cryogenic temperatures and at ultra high vacuum conditions without the possibility of collisions of neutrals and background deposition in view of the poor ion intensities and consequent need for longer exposure times. Here we combine a highly optimized low energy ion optical system designed for such studies coupled with RAIR and TPD and its initial characterization. Despite the ultralow collision energies and long ion path lengths employed, the ion intensities at 1 eV have been significant to collect a scattered ion spectrum of 1000 counts/s for mass selected CH<sub>2</sub><sup>+</sup>. © 2014 AIP Publishing LLC. [http://dx.doi.org/10.1063/1.4848895]

#### I. INTRODUCTION

Low energy ion scattering (LEIS), where mass selected ions in the 10-100 eV energy range collide on a well-defined surface and the product ions are mass analyzed, is an extremely surface sensitive,<sup>1</sup> molecule specific,<sup>2</sup> and structure specific<sup>3</sup> tool attracting significant attention these days.<sup>4–6</sup> Chemical reactivity of polyatomic ions at low energies and the capability to confine them in a spatially specific fashion with energy<sup>7-10</sup> and angle resolution<sup>11,12</sup> have made it possible to explore novel phenomena using this technique. Precise control of ions has made it possible to soft-land ions at surfaces,<sup>5</sup> which has implications to chemistry, biology, and devices. As the interaction time scale is of the order of a few femtoseconds,<sup>13</sup> a surface being sampled may be considered as frozen in the time scale of ion collision.<sup>14</sup> This allows temperature dependent dynamics<sup>15,16</sup> to be probed efficiently. The capacity to modify surfaces at atomic resolution provides new capability to study model systems.

The ultralow energy analog of LEIS is a new variant wherein translational energy of the incoming ion is as low as 1 eV,<sup>17,18</sup> such ions are structure sensitive<sup>19</sup> besides their extreme surface specificity, allowing phenomena such as phase transitions to be studied precisely.<sup>17,20</sup> Coupled with novel surfaces, prepared by a combination of methods such as background deposition, thermal evaporation, sputtering, photochemistry, etc. can create completely new avenues hitherto unexplored.

These studies using LEIS are complementary to diverse analytical methods,<sup>5,21–33</sup> which are normally used to understand molecular surfaces. All of these experimental capabilities must be built around cryogenic conditions<sup>28,34</sup> as well as in environments which can maintain ultralow energy ions. While efforts to create, mass select and transfer ultralow energy ions to well-defined molecular surfaces face challenges in effective ion transmission, there are also hardware restrictions to implement spectroscopies around single crystal surfaces for simultaneous experimentation.

The surface chemistry of various molecular solids at different temperature and low pressures is interesting<sup>34–36</sup> which motivated us to design advanced instrumentation for detailed investigations. In the following, we describe ultralow energy ion scattering spectrometry coupled with reflection absorption infrared spectroscopy (RAIRS) and temperature programmed desorption (TPD), performed in the 10–1000 K window. The experimental capabilities in terms of diversity of measurements and wealth of data are described which present new possibilities to explore fundamental problems of molecular solids.

#### **II. EXPERIMENTAL**

Figure 1 shows an outline of the instrument. A detailed description of mass spectrometer components is given in Fig. 2(b) and will be discussed later. The entire vacuum system is composed of three main chambers [ionization (items 2 and 3), octupole (item 6), and scattering] and a sample manipulator on which a closed cycle He-cryostat is mounted. The interior surface of the chamber was polished by buffing to re-

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FIG. 1. Schematic of the instrument. Various parts of the instrument are: 1. ionization chamber B-A gauge, 2. ionization chamber, 3. quadrupole mass filter (Q1), 4. octupole chamber cold cathode gauge, 5. ion bender, 6. octupole chamber, 7. low energy alkali ion gun (Cs<sup>+</sup>/Li<sup>+</sup>), 8. quadrupole mass analyzer (Q3), 9. scattering chamber B-A gauge, 10. RAIRS setup with KBr view port and external MCT detector (D), 11. sample manipulator fitted with closed cycle He-cryostat, 12. scattering chamber leak valves, 13. TPD probe fitted with ionizer and quadrupole mass analyzer and detector, 14. scattering chamber turbomolecular pump (260 l/s), 16. Backing turbo molecular pump (260 l/s), 17. Pirani gauge, 18. ionization chamber turbomolecular pump (80 l/s), 19. diaphragm pump (9.6 m<sup>3</sup>/h), 20. diaphragm pump (3.8 m<sup>3</sup>/h), 21. gas manifold arrangement with shutoff valve, gas line, pirani gauge, and diaphragm pump, and 22. Ru(0001) single crystal. Standard vacuum symbols are used (wherever necessary) for clarity.

duce outgassing. The ionization chamber is fitted with a 67 l/s turbomolecular pump (TMP, HiPace 80, Pfeiffer Vacuum) marked as item 18. This TMP is backed by a Pfeiffer Vacuum dry pump (MVP 70–3, pumping capacity 3.8 m<sup>3</sup>/h) (item 20). In the octupole chamber, the vacuum was created by one HiPace 300 TMP (capacity 260 l/s, Pfeiffer Vacuum) (item 15). The scattering chamber is evacuated by HiPace 700 TMP (capacity 685 l/s, Pfeiffer Vacuum) (item 14). The octupole chamber and the scattering chamber TMPs are backed by another HiPace 300 (Pfeiffer Vacuum) TMP (item 16) which is further backed by a diaphragm pump (MVP 160-3 from Pfeiffer vacuum, pumping capacity 9.6 m<sup>3</sup>/h) (item 19). All the TMPs are connected to the vacuum chambers through vibration dampers of appropriate dimensions to overcome any vibrational interference arising from the TMPs. The pressure readings in ionization and scattering chambers are measured using Bayard-Alpert type (B-A gauge, model no. PBR 260) (items 1 and 9, respectively) ionization gauges and the octupole chamber pressure is measured by a cold cathode gauge (IKR 270) with a limit of  $5 \times 10^{-11}$  mbar (item 4), all these sensors are controlled by a "MaxiGauge" vacuum gauge controller (Pfeiffer, Model TPG 256 A). An ultimate pressure below 5  $\times$  10<sup>-10</sup> mbar (limit of the sensor) was achieved in both ionization and scattering chambers after bake-out. The octupole chamber pressure is recorded to be  $1 \times 10^{-10}$  mbar.

During the experiment, the sample vapor, i.e., water or other vapors and gases are introduced into the scattering chamber through leak valve (Pfeiffer Vacuum) (item 12). The sample lines of the gas manifold (item 21) are pumped by a small diaphragm pump (MVP 015-4 from Pfeiffer Vacuum), and the samples are separated from the sample line by shut off valves (from Swagelok). The ionization chamber and octupole chamber are separated by a differential pumping baffle and a gate valve, the ions are transferred from the ionization region to the scattering region via the quadrupole mass filter (Q1) (item 3), followed by an ion bender (or ion deflector, item 5). Ions are guided form the octupole chamber to the scattering chamber, through an octupole ion guide. The alignment of ion optical components is achieved using standard laser transit procedures. Argon gas was introduced into the ionization chamber through a leak valve during the experiment when the pressure in it was raised to  $2 \times 10^{-7}$  mbar. The pressure measured in the scattering chamber during experimental condition, i.e., when the Ar source was open, was  $1 \times 10^{-9}$  mbar, which is an indication of effective differential pumping. This was achieved by a gate valve between Q1 and ionization chamber which also incorporates a tube lens. There was an additional vacuum restriction between ionization chamber and the Q1 which further reduces gas flow from the ion source to Q1. The pressure was monitored using a Maxi-Gauge multichannel monitoring system as mentioned before.

A high precision UHV specimen translator (McAllister Corporation) with xyz axis movement and  $\theta$  rotation facility (item 11) was used as the substrate holder. The substrate holder is made of oxygen free high conductivity (OFHC) copper, and the rest of the spectrometer is made with nonmagnetic stainless steel. The mounting copper is electrically isolated from the supporting structure. The heating element is also made of copper. A 1.5 cm diameter ruthenium (Ru) single crystal, Ru(0001) single crystal with 1 mm thickness (point 22) was used as the substrate for deposition during the experiment. This single crystal was mounted on a copper holder which was connected with a closed cycle helium cryostat (from ColdEdge Technologies) through an interface. A heater was also connected to the interface which was electrically isolated from the rest of holder by sapphire balls. Three temperature sensors were connected around the substrate to measure accurately the temperature in the whole range of 10-1000 K. A silicon diode sensor was connected to the top of the interface which measured the cold end of the interface, a Pt-sensor and a K-type thermocouple were attached to the mounting copper (Cu) near to Ru(0001), which was used as the substrate for the growth of molecular solids. The Ru substrate was fixed on the Cu plate by a thin steel clip. The temperature gradient across the sample plate was close to zero. Sample cooling was achieved by a closed cycle He-cryostat and the minimum temperature attained was 7 K whereas the maximum temperature recorded was 1000 K. The sample plate was mounted on the rod connected to the He cryostat. The rod was covered with a polished stainless steel radiation shield. Temperature up to 10 K can be achieved within 2 h, and the variable heating rates like 0.1-50 K/min was controlled by a LakeShore temperature controller (Model 336).



FIG. 2. (a) SimIon simulation of the ion trajectory of 1 eV Ar<sup>+</sup> (m/z 40), colliding on the target. (b) A schematic of the instrument with a description of the components. Appropriate labels are shown on the calculated trajectory so as to relate (a) and (b).

Electron impact ionization (EI) source from Extrel Core Mass Spectrometers (Extrel CMS) was used to generate positive or negative ions. The ionization chamber was fitted with a chamber heater inside the chamber to get the cleaner environment. The generated ions were extracted from the source and transferred to a quadrupole mass filter (Q1) through a set of einzel lenses. The desired mass-to-charge ratio was allowed to pass through Q1. It is possible to get the projectile ions with varying collision energy from 1 to 100 eV by varying the potential of the ion source block and tuning the rest of the ion optics to get a beam current of 1–2 nA for the mass selected ion. The ions were allowed to pass through an ion deflector, an octupole ion guide (q2), and through a set of einzel lenses. The ions collide with the surface at an angle of  $45^{\circ}$  with reference to the surface normal during the ion scattering experiment. The secondary ions generated by the ion collision were collected by a set of einzel lenses and analyzed by a quadrupole mass analyzer (Q3).

Figure 2(a) shows the results of SimIon simulation of a scattering experiment where 1 eV Ar<sup>+</sup> (m/z 40) was allowed to collide on a target. The target was grounded. The overall energy spread of the source ions in the SimIon simulation was 0.0238 eV. This energy is a summation of potential differences at the positions where the ions were created and addition of the allowed kinetic energy distribution which is 0.01 eV. The remaining amount was due to the potential differences at the different positions where ions were born. The computations indicate satisfactory results of ultralow

energy ion scattering onto the target with good ion transmission. In this simulation, the repeller was set at 2 V, ion region was set at 1 V, the ion energy at the target was found to be 1.45 eV. Overall transmission loss was found to be 52% at 1 eV and 56% for 3 eV. Ion spatial spread increased from 1 mm at the source to  $\sim$ 3 mm at the surface. The spread can be reduced, however, by tighter focusing, especially at a slightly higher ion energy of 3 eV where the spread is found to be  $\sim 2$  mm. The optimized instrumental parameters derived from the simulations were used to arrive at the instrumental parameters. Figure 2(b) shows a schematic of the instrument with detailed description of the components. The instrument consists of three chambers (as mentioned above) where there are seven sections, namely, ionizer, Q1, ion bender, q2, scattering chamber, Q3, and TPD probe. Ionizer is composed of an ion volume, a tungsten filament, a repeller, and one electromagnetic lens (L1). From ionizer to Q1, there is gate valve, which also acts as a lens. This was especially chosen to reduce the length of ion trajectory while having a reduced gas load in the scattering chamber from the ion source. After the gate valve lens, ions are mass selected using the Q1 quadrupole assembly. Ions from the source are passed through Q1 entrance lens (Q1 ENT) and Q1 pre/post filter before entering the Q1 mass analyzer. Mass analyzed ions are then channeled through another set of lenses, Q1 pre/post and Q1 exit lens. After these lenses, ions are filtered through ion bender where neutrals, if any, are rejected. As ions come out from the ion bender, those enter the octupole ion guide (q2). The q2 is having q2 entrance lens (q2 ENT), octupole, and q2 exit lens (q2 EXIT). After that there is a set of einzel lenses which focus ions onto the target. During ion scattering experiment, when the target is placed  $45^{\circ}$  with respect to the surface normal, scattered ions are guided to the analyzer quadrupole Q3 by another stack of einzel lenses. These ions are passed through Q3 which consists of Q3 entrance lens (Q3 ENT), quadrupole, and Q3 exit lens (Q3 EXIT). Finally the ions are detected using a conversion dynode (CD) and a channel electron multiplier. During the TPD measurement, the target is rotated to 225° from its ion scattering position. In the TPD probe, there is an ionizer similar to the one described previously. Mass analyzer is similar to Q3. In order to reduce sampling of other regions of the sample holder during TPD, the mass spectrometer has a skimmer, placed in the front of the axial ionizer.

Molecular surfaces were prepared by depositing the corresponding vapors and gases which were in turn delivered very close to the substrate through 1/8 in. stainless steel tubes. The exposure was controlled by a leak valve. The gas-line helps to maintain uniform sample growth on the substrate. Delivery of molecules near the substrate ensured that the vapors were not deposited in unwanted areas. The deposition flux of the vapors was adjusted to ~0.1 ML/s. The thickness of the overlayers was estimated assuming that  $1.33 \times 10^{-6}$  mbar s = 1 ML. 1 ML ice layers have been estimated to be ~1.1 × 10<sup>15</sup> water molecules/cm<sup>2</sup>.<sup>37</sup> The partial pressure of the vapor inside the scattering chamber during deposition time was 1 × 10<sup>-7</sup> mbar. The films were prepared on Ru substrate to make Ru@A (the symbolism implies the creation of layer A over Ru). The spectra presented here

were averaged for 75 scans and the data acquisition time was approximately 0.5 s per scan.

In addition to low energy ion scattering mass spectrometry and TPD probe, the chamber is fitted with RAIRS set-up. The RAIRS experiments were performed using a VERTEX 70 FT-IR spectrometer of Bruker. The IR beam was taken out from the spectrometer to an off-axis paraboloidal gold-coated mirror (focal length 250 mm) which focused the beam at  $80^{\circ}$  $\pm$  7° incident angle onto the Ru single crystal mounted on the cryostat. The reflected beam from the surface was collected by another gold-coated ellipsoidal mirror mounted on an adjustable base plate and ultimately focused onto the detector (see Fig. 1, item 10). The entire IR beam path was purged with dry nitrogen gas. A liquid N2 cooled broadband mercury-cadmium-telluride (MCT) detector (specific detectivity  $D^* \sim 5 \times 10^9$  cm Hz<sup>1/2</sup>/W) was used for the range of 12000-420 cm<sup>-1</sup>. The negative absorption observed around 2080 cm<sup>-1</sup> appeared to be due to background deposition of CO on the clean surface. The total IR path length from the spectrometer exit to the surface is 32.8 cm and the detector is 24.7 cm away from the sample.

#### **III. RESULTS**

In order to measure the distribution of ion kinetic energy (K.E.) of the input beam, stopping potential measurements<sup>18</sup> were performed at Q1. In this measurement, Q1 was kept in the RF (radio frequency) only mode and it transmits all ions formed in the source and Q3 was set to transmit the desired mass. Thereafter, a range of DC voltages are applied across the quadrupoles in order to stop the desired ions. When the ions are stopped at Q1, for example, intensity of the ions detected falls to zero. Figure 3 shows the results of stopping potential measurements of 1, 2, and 3 eV  $Ar^+$  ions. It is evident from the figure that for 1 eV ion, the energy spread is 49% which reduces substantially (5%) in the case of 3 eV ions. With further increase in the input ion kinetic energy up to 8 eV, the spread decreases to 2% (data not shown). It is important to note that this kind of ion energy spread is the best that has been achieved so far in such instrumentation.<sup>6</sup> Increased spread at extremely low energy (1 eV) has been noted before.<sup>18,20</sup> Stopping potential measurement in q2 using Ar<sup>+</sup> (Q1 was set to select the desired ion and Q3 was kept in RF-only mode) showed the same energy spread like Q1 (Fig. 3(a)). After the collision on the Ru target, the stopping potential experiment performed in Q3 indicates a further increase in spread which is 0.52 eV (Fig. 3(b)). This shows the excellent performance of the instrument especially at ultra-low energy range.

After the initial characterization measurements, ion scattering experiment was performed with  $C_6D_6^+$  (*m*/*z* 84) on Ru(0001) at 10 K. The kinetic energy of the ions was 1–6 eV. The results of the experiments are shown in Fig. 4(a). The small shoulder peak next to  $C_6D_6^+$  is attributed to  $C_6D_5^+$ . Figure 4(b) shows the result of chemical sputtering conducted on 100 ML D<sub>2</sub>O grown on Ru(0001) at 10 K. The projectile was 50 eV Ar<sup>+</sup>. Signal at *m*/*z* 22 indicates D<sub>3</sub>O<sup>+</sup>, result of chemical sputtering of D<sub>2</sub>O, the peak *m*/*z* 42 is due to D<sub>5</sub>O<sub>2</sub><sup>+</sup>.


FIG. 3. Plot of  $Ar^+$  stopping potential data at quadrupole 1 (Q1). Data corresponding to octupole 2 (q2) and quadrupole 3 (Q3) are in (a) and (b). The experimental scheme is shown in inset.



FIG. 4. (a) Results of 1–6 eV  $C_6D_6^+$  scattering on Ru(0001) at 10 K. (b) Chemical sputtering spectrum due to 50 eV Ar<sup>+</sup> on amorphous ice at 100 K. Bottom inset shows a schematic of the ultralow energy ion scattering experiment. The ejection of  $D_3O^+$  is due to the proton-transfer reaction upon collision of Ar<sup>+</sup> ions (2D<sub>2</sub>O  $\rightarrow$  D<sub>3</sub>O<sup>+</sup> + OD<sup>-</sup>).

The ions  $D_3O^+$  and  $D_5O_2^+$  are the characteristic features of  $D_2O$  ice. Note that no other ions such as hydrocarbons or  $H_3O^+$  were seen which clearly demonstrate the cleanliness of vacuum, quality of the molecular film, and well-defined ion trajectory. In the absence of all these, the ion scattering spectrum could have shown several other features characteristic of impurities. These spectra indicate the successful performance of the instrument at the entire energy region for which the instrument was designed for.

Having established the instrument performance in the low to high energy window, we carried out TPD measurements. In this process, Argon was chosen as the atomic solid of choice. First the target was cooled to 10 K, which is well below the desorption temperature of Ar. After that, Ar gas was deposited through a leak valve in the scattering chamber for a certain period of time to develop the desired number of monolayers. Thereafter, the substrate was resistively heated at a constant rate (here 5 K/min). The resultant mass spectra were collected, where the surface was at the TPD position. Figure 5, inset (ii, bottom) shows one of the measured TPD spectra for 5 ML thickness of Ar. The ion intensity monitored in this case is m/z 40 due to Ar<sup>+</sup>. Next, a thickness dependent TPD experiment was performed where 0.5, 1, 2, 3, 5 ML of Ar was deposited and heated at the same rate as mentioned previously. From the resultant spectra, areas under the curve are plotted and presented in Fig. 5. Near linearity of the plot indicates successful performance of the TPD experiment at very low coverage and at very low temperature. Inset (ii) shows the TPD spectrum of 5 ML Ar. Curve fitting was done to find the multilayer and monolayer contributions in the TPD spectrum.



FIG. 5. Increase in intensity with respect to number of monolayers of Ar; 0.5, 1, 2, 3, 5 ML during the TPD experiment. Intensity refers to that of the m/z 40 peak. Inset (i) shows a schematic representation of the TPD experiment (MS stands for mass spectrometer). Inset (ii) shows the TPD spectrum of 5 ML Ar.

The residual Ar intensity beyond 30 K (maxima 39.4 K) is attributed to Ar desorption from regions other than the Ru crystal. This area is excluded in the total area measurement in the linear fit. The desorption spectrum is characterized by two peaks, the low temperature peak (marked as 1, maximum at 22 K) due to the multilayer and the high temperature peak (marked as 2, maximum at 25 K) due to the monolayer. Intensities of these two are used in the thickness evaluation.

Subsequently, reflection absorption infrared spectroscopy (RAIRS) was performed on 50 ML CCl<sub>4</sub> layer deposited on Ru at 100 K. Figure 6 shows the observed spectra. It is an average of 512 acquisitions with 4 cm<sup>-1</sup> resolution. The spectrum shows the standard C–Cl stretching vibrations ( $\nu_3$ ) at 794 cm<sup>-1</sup> and  $\nu_1$  (symmetric stretching) +  $\nu_4$  (degenerate deformation) modes of CCl<sub>4</sub> appear at



FIG. 6. RAIR spectrum of 100 ML CCl<sub>4</sub> deposited on Ru(0001) at 100 K.

767 cm<sup>-1</sup>, respectively.<sup>38,39</sup> This spectrum indicates excellent performance of the RAIRS set-up.

### **IV. CONCLUSION**

The instrument developed shows excellent performance during ion scattering experiments in the ultralow energy range. Its capability to achieve low temperatures ( $\sim 10$  K) will help to study molecular and atomic solids of almost any gas (except hydrogen and helium). Simultaneous measurement of ion scattering, RAIRS, and TPD is expected to unravel various unexplored areas of molecular materials. A combination of all these data together can reveal the structure, reactivity, kinetics, and thermodynamics of surface processes. In conjunction with additional facilities such as low energy alkali ion sputtering, thermal evaporation along with surface activation by UV exposure, catalysis at molecular solids (photo and chemical) can be explored.

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# Supramolecular Functionalization and Concomitant Enhancement in Properties of Au<sub>25</sub> Clusters

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**ABSTRACT** We present a versatile approach for tuning the surface functionality of an atomically precise 25 atom gold cluster using specific host—guest interactions between  $\beta$ -cyclodextrin (CD) and the ligand anchored on the cluster. The supramolecular interaction between the Au<sub>25</sub> cluster protected by 4-(*t*-butyl)benzyl mercaptan, labeled Au<sub>25</sub>SBB<sub>18</sub>, and CD yielding Au<sub>25</sub>SBB<sub>18</sub> $\cap$ CD<sub>n</sub> (n = 1, 2, 3, and 4) has been probed experimentally using various spectroscopic techniques and was further analyzed by density functional theory calculations and molecular modeling. The viability of our method in modifying the properties of differently functionalized Au<sub>25</sub> clusters is demonstrated. Besides modifying their optoelectronic properties, the CD moieties present on the cluster surface provide enhanced stability and optical responses



which are crucial in view of the potential applications of these systems. Here, the CD molecules act as an umbrella which protects the fragile cluster core from the direct interaction with many destabilizing agents such as metal ions, ligands, and so on. Apart from the inherent biocompatibility of the CD-protected Au clusters, additional capabilities acquired by the supramolecular functionalization make such modified clusters preferred materials for applications, including those in biology.

**KEYWORDS:** supramolecular chemistry · quantum clusters · Au<sub>25</sub> · cyclodextrin · inclusion complex

istinct properties of nanomaterials arise from diverse attributes, the most important being size, shape, chemical functionalization, and interparticle organization.<sup>1-3</sup> Interfacing individual nanoparticles with functional supramolecular systems is a fascinating prospect which provides them with new capabilities. In this paper, we introduce a method for such surface modifications in atomically precise gold clusters, which are emerging materials due to their unique optical and catalytic properties.<sup>4–8</sup> They are called by various names such as quantum clusters (QCs), nanomolecules, molecular clusters, superatoms, etc. Unique molecule-like absorption (especially that of  $Au_{25}SR_{18}$  (SR denotes the surface thiolate ligand)) and photoluminescence properties of QCs are the result of confinement of electronic wavefunctions<sup>4,5,9–11</sup> which can be manipulated by the modification of the ligand environment.<sup>12-15</sup> The physicochemical interactions occurring at the surface

of QCs may change the efficiency of radiative recombination, which may ultimately result in the enhancement or guenching of their optical properties. QCs, as tunable nanoscale light sources, have found numerous applications in biology, bioanalytics, and optoelectronics.<sup>16–19</sup> Many of these applications require engineering of their surfaces with functional ligands. Different approaches have been developed to achieve this, such as ligand exchange<sup>7</sup> and subsequent isolation of clusters with precise composition,<sup>20</sup> click chemistry, and so on.<sup>21–25</sup> As a complementary and even more versatile concept, especially for solutionstate applications, we introduce the possibility of supramolecular chemistry with QCs, which enables their precise surface functionalization with molecules that can take part in additional events. These surface modifications are important to enable the use of diverse properties of such molecular nanosystems.<sup>26</sup>

Being the most popular supramolecular host molecule,  $\beta$ -cyclodextrin ( $\beta$ -CD), cyclic

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Figure 1. UV-vis absorption spectra (A) and MALDI (L) mass spectra in the positive ion mode (B) of  $Au_{25}SBB_{18}$ . Inset shows a photograph of the cluster (diluted) in THF. Inset of (A) also shows a visualization of the DFT-optimized structure of  $Au_{25}SBB_{18}$ . Gold atoms are shown in gold, sulfur atoms in green, carbon atoms in dark gray, and hydrogen atoms in white. The bridging sulfurs lie along Cartesian axis directions. Here,  $Au_{25}SBB_{18}$  and its Cartesian axes have been oriented so that the environment around the ligands can be seen clearly. In this particular orientation, the x-y plane is in the plane of the paper as shown by the red and green arrows, and the z-axis is going into the plane of the paper as indicated by the blue arrow. Inset of (B) compares the theoretically calculated and experimentally observed molecular ion peak of the cluster. A fragment of ionization ( $Au_{25}SBB_{16}S_2$ )<sup>+</sup> is marked by \*. The peak at m/z 8151 is due to  $Au_{25}SBB_{18}$ .

oligosaccharide comprising seven  $\alpha$ -D-glucopyranose units linked by  $\alpha(1-4)$  glycosidic bonds, has moleculeaccepting cavities which are specific to hydrophobic guest molecules of suitable size and geometry.<sup>26</sup> In addition to other applications, this feature has been exploited for the design and construction of molecular sensors in which the inclusion of the guest molecule triggers a signal which can be detected.<sup>27,28</sup> Binding of  $\beta$ -CD molecules to various guest-functionalized materials has been utilized for various applications in water purification and biology.<sup>29–31</sup> Owing to the high vulnerability of the 4-(t-butyl)benzyl group to form stable host/quest inclusion complexes with  $\beta$ -CD molecules, we synthesized a new 25 atom gold QC protected by 4-(t-butyl)benzyl mercaptan (BBSH) and explored its precise surface functionalization with  $\beta$ -CD molecules. The partial inclusion complex formed due to the host-quest interactions between  $\beta$ -CD and SBB ligand anchored on the Au<sub>25</sub> cluster may be represented as  $X \cap Y$ , where X and Y are substrate and receptor molecules, respectively, as suggested by Lehn<sup>32</sup> and colleagues.<sup>33</sup> Strong inclusion interactions between the inner cavity of  $\beta$ -CD and ligand molecules on the QC have been probed by various spectroscopic techniques and density functional theory (DFT) calculations. Detailed studies on the stability of the functionalized cluster in the presence of metal ions and ligands have also been performed. As the binding of the substrate to its receptor involves a molecular recognition process, presence of a more competitive guest molecule can effectively tune the host-guest reactivity and alter the supramolecular environment around the cluster, suggesting potential applications in sensing. Beyond enhanced stability and sensing

properties, creating such precise CD-functionalized clusters can lead to numerous applications in biology and therapeutics since such materials can be envisaged to develop drug delivery vehicles that can be tracked simultaneously.

### **RESULTS AND DISCUSSION**

There is a strong motivation for making guantum clusters protected with ligands as we wish to use their molecular recognition properties to build supramolecular structures. Here, we synthesized a 25 atom QC of gold with remarkable stability using BBSH as the ligand by following a facile one-pot strategy. BBSH was chosen as the ligand due to its strong tendency to form an inclusion complex with  $\beta$ -CD (binding constant and other data are presented later in the text). The bulkiness of this ligand, while reported to provide higher oxidation resistance to larger Ag clusters (Ag<sub>140</sub> and  $Ag_{\sim 280}$ ), was also viewed as a challenge for the synthesis of clusters with smaller cores.<sup>34,35</sup> The formation of a well-characterized Au<sub>25</sub> cluster with BBSH ligand throws light onto the possibility of such smaller core sizes with Ag, too. In view of various reports on the Au<sub>25</sub> core, protected with other ligands such as GSH and PET,<sup>5,11,36</sup> we present only the relevant characteristics of Au<sub>25</sub>SBB<sub>18</sub> in the main text. Most of the other spectroscopic and microscopic data are presented in Supporting Information (SI).

The cluster has well-defined optical and mass spectral features. The optical absorption spectrum (Figure 1A) of the dark brownish solution revealed discrete molecule-like features which are characteristic, and often described, as the fingerprint of Au<sub>25</sub> QCs.<sup>5,7,37</sup> A key parameter which decides the formation of Au<sub>25</sub>SBB<sub>18</sub>

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is the molar ratio of Au and BBSH, which significantly affects the yield of Au<sub>25</sub>. While the formation of Au<sub>25</sub>SBB<sub>18</sub> required a ratio of 1:6 at an optimized condition, lower thiol ratios resulted in larger clusters which were noticeable from the changes in the optical absorption spectra (see Figure S1 in SI).

The molecular composition of the cluster was confirmed by MALDI (L) MS, where L denotes analysis in the linear mode (Figure 1B). An intact molecular ion peak was observed at m/z 8151, which also indicated the purity of the prepared cluster. The experimental spectrum and the theoretical prediction matched perfectly as shown in the inset of Figure 1B. Molecular ion peak was observed in both the positive and the negative ion modes (Figure S2). An additional fragment corresponding to the C–S bond cleavage (marked with an asterisk in Figure 1) was observed. Due to the bulky nature of the ligand, a mass loss of m/z 294 corresponding to two BB groups  $(-CH_2-C_6H_4-C(CH_3)_3)$  from the parent cluster was identified apart from peaks due to the loss of Au<sub>4</sub>SBB<sub>4</sub> from the parent ion (see Figure S2), a common phenomenon observed in such Au<sub>25</sub> clusters.<sup>11,38</sup> A precise control of the threshold laser intensity was crucial to observe the molecular ion peak without fragmentation (Figure S3). A DFT-optimized model of [Au<sub>25</sub>SBB<sub>18</sub>]<sup>-</sup> is shown in the inset of Figure 1A. While the core and staple motifs are preserved from the Au<sub>25</sub>PET<sub>18</sub> case, there are differences in the directions of the SBB ligands when compared to their PET counterparts, and these are attributed to differences in the rotation angles of ligands about their S-C bonds. We note that, in general, the SBB ligands point away from the core, enabling their inclusion into CD. This scenario may be contrasted with that of Au<sub>25</sub>PET<sub>18</sub>, where the ligands do not point outward from the core,<sup>37</sup> and hence it would be difficult for a CD to form an inclusion complex with it (see later). ESI MS of the cluster (Figure S4) in the negative mode yielded fragments in the low mass region corresponding to  $(AuSBB_2)^-$ ,  $(Au_2SBB_3)^-$ ,  $(Au_3SBB_4)^-$ , and  $(Au_4SBB_5)^-$  due to fragmentation of staples from the cluster surface. The average size of the cluster was <2 nm as confirmed by TEM (Figure S5), and it did not show any electron-beam-induced aggregation, a common phenomenon observed in other Ag and Au clusters. This may be due to the better stability provided by the bulky ligand shell around the cluster. Elemental analysis of the cluster (Figure S6) showed a Au/S ratio of 1:0.73, in agreement with Au<sub>25</sub>SBB<sub>18</sub>. With the confirmation that the cluster formed is Au<sub>25</sub>SBB<sub>18</sub>, we move to the construction of the supramolecular adducts.

The 4-(t-butyl)benzyl group of the SBB ligand on Au<sub>25</sub> is an interesting entity as it acts as a recognition site for stable host/guest inclusion complexes with  $\beta$ -CD molecules. Pure and modified CDs have been widely documented to form stable host/guest inclusion complexes with hydrophobic molecules of appropriate size so as to be included in its cavity.<sup>39,40</sup> Such

complexes are stable, and the products can be isolated. Several inorganic complexes bearing 4-(t-butyl)phenyl groups have been reported to form stable host/guest complexes with  $\beta$ -CD.<sup>41-45</sup> Au<sub>25</sub>SBB<sub>18</sub> $\cap$ CD<sub>n</sub> (n = 1-4) were made as described in the Experimental Methods section. Initially, the Au<sub>25</sub>SBB<sub>18</sub> cluster in THF was mixed with different mole ratios of CD in water and subjected to sonication. Though CD host-guest interactions are known to be most powerful in water, yield of the CD-functionalized cluster analogues (as observed in ESI MS) was poor when the experiment was conducted under conditions of excess water. The tendency of Au<sub>25</sub>SBB<sub>18</sub> to aggregate in highly polar medium may prevent the efficient interaction between CD and the guest molecules from forming inclusion complexes in excess water. The CD molecules themselves form tubular assemblies specifically in THF medium,<sup>46</sup> and this formation is facilitated by the presence of small amounts of water.46,47 This was confirmed from SEM observations (Figure S7). Intermolecular H bonding between the hydroxyl groups on the outer rim of CD molecules, mediated by water, holds them together to form the assembly. Such channel structures of CDs are capable of forming inclusion complexes.<sup>46,47</sup> During the formation of such superstructures, the SBB group present on the cluster may also get entrapped inside the CD cavity. Addition of excess water results in the collapse of

Cluster-entrapped supramolecular adducts of CD  $(Au_{25}SBB_{18}\cap CD_n, where n = 1-4)$  can be extracted into the organic layer. Due to the presence of more hydrophobic SBB groups on the cluster surface (18 - n, 100)where n < 4), the adducts are hydrophobic in nature and allow this preferential extraction into the organic layer. In agreement with this, LDI MS of the aqueous layer showed a broad peak at higher mass range albeit with very low intensity (Figure S8). We noticed that the intensities of MALDI and ESI MS spectra of the CD-incorporated Au<sub>25</sub> cluster in the crude product (before adding excess water) were weak, whereas a significant enhancement in the adduct intensities was observed in both MALDI and ESI MS after addition of excess water. The purified organic layer, devoid of free CD molecules, was used for subsequent characterization as described in the Experimental Methods section. The hydrophobic interactions between the SBB ligandprotected Au<sub>25</sub> cluster and  $\beta$ -CD were studied by a combination of absorption, fluorescence, MALDI MS, ESI MS, and NMR spectroscopies.

CD assemblies releasing the  $Au_{25}SBB_{18}\cap CD_n$  adducts.

MALDI MS of the cleaned organic layer was done with linear (MALDI (L)) and reflectron modes (denoted as MALDI (R)) as well as in TOF TOF mode (MALDI TOF TOF). MALDI (L) MS (Figure S9) measurements of the cluster-CD adduct resulted in a broadened mass spectrum. Factors such as ion kinetic energy distribution of the ejected ions as well as their spatial and temporal distributions are strongly influenced by the

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Figure 2. (A) Effect of MALDI TOF TOF mass spectra of Au<sub>25</sub>SBB<sub>18</sub> (black trace) with increasing SBB/CD ratio (green to brown trace) in solution. Schematic representations of the cluster with different amounts of CD inclusions are also shown. At 1:0.05, some parent Au<sub>25</sub>SBB<sub>18</sub> is also seen, shown with #. UV–vis absorption spectra (B) and luminescence spectra ( $\lambda_{ex}$  992 nm) (C) of the Au<sub>25</sub>SBB<sub>18</sub> cluster with increasing amounts of CD inclusion.

molecular weight, nature of ions, and the matrix,<sup>48,49</sup> which are important in the present case in determining the spectral width. Though the peaks were broad, the peak maximum of samples made with increasing CD concentration in solution shifted toward higher mass numbers, suggesting the complexation of  $\beta$ -CD on the cluster surface. The difference in energy distribution of the ions arising from the desorption ionization event, the possibility of large internal energy distributions of the ejected ions as well as metastable fragmentation due to the presence of flexible supramolecular interactions may be the reason for the significant spread for the ions.<sup>50-53</sup> This spread is evidenced from the fact that the broad distributions in MALDI (L) MS transform to narrow lines over a broad background in the TOF TOF mode with the MALDI (R) MS giving an intermediate distribution. Figure S9 compares the MALDI (L) and MALDI (R) mass spectra.

To confirm this, MALDI TOF TOF mass spectra of mixtures of various SBB/CD ratios were collected wherein better peak resolutions were obtained, as shown in Figure 2A, indicating that improved resolution requires TOF TOF measurements and longer path lengths. The mole ratio of SBB ligand to  $\beta$ -CD in solution for each case is indicated on the figure. Schematic representations of the cluster with various

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amounts of CD inclusions are also shown in Figure 2. Peak corresponding to the parent Au<sub>25</sub>SBB<sub>18</sub> is marked with #. Spectra corresponding to intermediate SBB/CD ratios are shown in Figure S10. Well-defined peaks corresponding to Au<sub>25</sub>SBB<sub>18</sub> $\cap$ CD<sub>n</sub> (where n = 1-4) were observed under different conditions. The trend observed in CD adduct intensities with an increase in SBB/CD is the same as seen in MALDI MS (Figure S9), confirming that the energy spread of the ions in MALDI MS was the reason for the poor resolution.

As in the case of ligand exchange reactions of clusters,<sup>20,54–56</sup> at each ratio of reactants, one can observe multiple peaks due to the existence of various species in solution. However, formation of certain cluster–adduct combinations is indeed higher than the others depending on the incoming  $\beta$ -CD concentration. While a statistical distribution of species always exists in solution and precise control of the formation of exclusively one adduct is difficult, it is indeed possible to create one particular adduct with a higher proportion than the rest by careful control of the precursor ratios.<sup>57</sup> The relative intensities of individual peaks shown in Figure 2A for each ratio suggest such an effect.

It may be noted that optimum laser fluence (lowest fluence needed to observe ion signals) was used for all

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Figure 3. ESI mass spectra in the negative mode for  $[Au_{25}SBB_{18}]^-$  and its CD-functionalized analogues collected using a Q TOF (Synapt G2 HDMS, Waters) mass spectrometer (details are in the Instrumentation section). SBB/CD ratios used for the synthesis were 1:1.2, 1:1, and 1:0 for traces a to c, respectively. Part of trace b is expanded to show the features clearly.

the measurements. The dependence of laser fluence on both the MALDI and MALDI TOF TOF mass spectra for Au<sub>25</sub>SBB<sub>18</sub> $\cap$ CD<sub>4</sub> is shown in Figure S11. It is also important to mention that the MALDI event can cause fragmentation of the adducts and part of the distribution of the lower mass ions may also be due to this. Ion/ molecule reactions in the plasma can lead to gas-phase products at higher masses, not originally present in solution. All of these aspects are inherent complications in the spectrum, and therefore, it is important to study the product distribution using other methods.

We conducted extensive ESI MS measurements to understand the existence of various species in solution. Spectrum in the negative mode confirmed the mass assignment mentioned earlier. Figure 3 shows distinct peaks corresponding to various  $Au_{25}SBB_{18}\cap CD_n$ (where n = 2-4) clusters. Unlike MALDI, matrix interactions and laser-induced fragmentation of the products can be avoided in this case. Although at lower ratios the parent Au<sub>25</sub> peak was dominant compared to the adducts (for n = 2 and 3) and multiple species existed in solution, at a SBB/CD ratio of 1:1.2, greater abundance for Au<sub>25</sub>SBB<sub>18</sub>∩CD<sub>4</sub> species was seen. A possible reason could be the geometric stability of the Au<sub>25</sub>SBB<sub>18</sub>∩CD<sub>4</sub> cluster adducts in comparison to that of others. From our simulations, the higher stability of these species compared to  $Au_{25}SBB_{18}\cap CD_n$ (where n < 4) was attributed to the binding of four CDs in tetrahedral locations (explained later) which would minimize inter-CD interactions and thus lower the total energy of the structure. Second, the tight

packing of the four CDs on the cluster surface sterically hinders further CD molecules from interacting with its surface, which also enhances its stability. Data presented in Figure 3 suggest the existence of one dominant supramolecular adduct, Au<sub>25</sub>SBB<sub>18</sub>∩CD<sub>4</sub> in solution at a SBB/CD ratio of 1:1.2. Further increase in CD concentration in solution did not result in another species, indicating that addition of more CD molecules onto the cluster surface with retention of the Au<sub>25</sub> core is unlikely. Ligand-induced core etching was seen at larger concentrations of CD as we have reported previously.58 The bulky nature of the BBS group may sterically hinder an incoming CD group adjacent to it. Careful control over CD concentration was essential to not cause additional effects. Such control is necessary to achieve specific products in the case of clusters, as seen in the case of ligand exchange and core alloying.<sup>20,54–57</sup> UV-vis absorption spectra (Figure 2B) of these samples showed a nominal decrease in intensity of the characteristic absorption features of the cluster, especially the absorption band found at 685 nm. The  $\sim$ 20 nm shift observed in the UV-vis spectra strongly indicates the modification of the molecule.

Au<sub>25</sub> QCs are known for their luminescence emission in the near-infrared (NIR) region. In order to study the influence of CD encapsulation on the optical property of Au<sub>25</sub> QC, we analyzed the NIR luminescence of Au<sub>25</sub> before and after CD functionalization. The bare Au<sub>25</sub>SBB<sub>18</sub> cluster showed a luminescence maximum at 1030 nm at room temperature (see Figure S12). Though various excitation wavelengths showed slight changes in the emission maxima, emission at 1030 nm was the most dominant and intense among others. Upon  $\beta$ -CD inclusion, the cluster samples showed a pronounced enhancement in their luminescence intensity (Figure 2C). Enhancement of optical properties in such surface-modified clusters is in accordance with previous reports.59,60 Upon silica coating of Au25, both absorption and emission intensities are enhanced.<sup>59</sup> In Au<sub>23</sub>SG<sub>18</sub>, upon phase transfer, due to additional protection of the cluster by the phase transfer agent, the nonradiative decay rate is reduced, enhancing emission.<sup>60</sup> In the present case, this enhanced luminescence remained almost the same even after 2 weeks in ambient conditions, suggesting the enhanced stability of the cluster as a result of complexation with  $\beta$ -CD molecules.

Computational studies were conducted in order to ascertain whether the attachment of cyclodextrin molecules to  $Au_{25}SBB_{18}$  is feasible and, if so, their locations and the maximum number of such attachments.  $Au_{25}$  consists of a 13 atom icosohedral Au core surrounded by six  $-S_{nb}-Au-S_b-Au-S_{nb}-$  staples,<sup>37,61</sup> where  $S_b$  denote the six bridging sulfurs and  $S_{nb}$  the 12 nonbridging sulfurs. The bridging sulfurs join exterior gold atoms to each other in the staple, while the

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Figure 4. (A) View of DFT-optimized structure of  $Au_{25}SBB_{18}$  where the bridging ligands are shown in blue and the nonbridging ligands in magenta. The ligands identified for the attachment of the four CDs are marked by arrows and by the pairs of Cartesian directions marked next to the arrow. The sulfur and gold atoms are colored green and gold, as in Figure 1A inset, and the Cartesian *x*, *y*, and *z* axes are shown by the red, blue, and green arrows. In the front (B) and back (C) views of  $Au_{25}SBB_{18}\cap CD_4$ , the hydrogen atoms are not shown on the SBB ligands for clarity. The four CD molecules are shown in cyan in the stick molecular representation and are approximately arranged in a tetrahedral shape. The binding energies in kcal/mol for the isolated BBSH $\cap$ CD complexes with the *t*-butyl group of BBSH molecule entering (D) the narrow rim and (E) the wider rim of the CD are shown in one color, cyan, while oxygen, carbon, and hydrogen atoms of CD are shown in red, black, and white, respectively.

nonbridging sulfurs connect the core Au atoms to an exterior Au atom. Ligands may be classified as bridging (shown in blue color in Figure 4A) and nonbridging (shown in magenta color in Figure 4A), depending on the type of sulfur they are connected to. While the bridging ligands lie 0.5–0.9 Å farther away from the core, as seen in Figure 4A, and are more easily accessible to CDs in solution, they are fewer than the nonbridging ligands. Due to the six two-fold axes of the icosahedral core,<sup>37,61</sup> we rotated the structure so that the SBB bridging ligands lay along the six Cartesian axes d, where d stands for  $\pm x$ ,  $\pm y$ , or  $\pm z$ . The nonbridging ligands may be associated with a Cartesian plane quadrant or diagonal denoted by the pair  $(d_1, d_2)$ , where the order of  $d_1$  and  $d_2$  is unimportant and they are perpendicular. This notation may be used to identify ligands uniquely; the bridging ligands are specified by Cartesian directions, while the nonbridging ligands are specified by a pair of perpendicular directions. If one examines the model of Au<sub>25</sub>SBB<sub>18</sub>,

one can see that the bridging ligands appear to be more crowded, as shown in the inset of Figure 1A and Figure 4A, while there is greater space around the nonbridging ligands. We confirmed this by studying the ligand orientations of the 3D model of  $Au_{25}SBB_{18}$ (a structure file is provided in XYZ format along with SI). Hence, it would be possible for a CD to make a closer approach and include a greater portion of a nonbridging rather than a bridging ligand. A closer CD position is in better agreement with the NMR data due to the proximity between the aromatic SBB protons and the  $H^3$  and  $H^5$  CD protons.

A model of Au<sub>25</sub>SBB<sub>18</sub> $\cap$ CD<sub>4</sub> is shown in Figure 4B,C, showing the four CDs in an approximately tetrahedral arrangement attached to nonbridging ligands with their narrow end facing the cluster core. A tetrahedral arrangement would be expected to minimize inter-CD interactions. We also note here that the exclusive use of bridging ligands for CD attachment would impose a perpendicular arrangement rather than tetrahedral.

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Figure 5. (A) Schematic showing the inclusion complex between SBB ligand on the QC and  $\beta$ -CD (a–c represent CD, Au<sub>25</sub>SBB<sub>18</sub>, and Au<sub>25</sub>SBB<sub>18</sub>∩CD<sub>4</sub>, respectively). Different types of protons and their interactions are also marked. (B) Twodimensional ROESY spectrum showing interaction between the inner cavity protons of CD and SBB ligand, which appear as cross-peaks in the spectrum (marked by circles).

The nonbridging ligands used were denoted by (-z, -x), (x, -y), (y, z), and (z, -x). We remark here that the structure shown in Figure 4B is one possible local minimum and further simulations would be needed to determine the lowest energy structures completely. Structural isomerism is possible as the choice of ligands for CD attachment is non-unique. Full details of the procedure to construct this model may be found in SI 13.

Interestingly, though there are groups of free ligands which are spread over a large space to fit a fifth CD, albeit with tight packing, the specific orientation of free ligands prevents further attachment of another CD. This region of space can be seen in more detail in the back view of the structure shown in Figure 4C (further views are shown in Figure S13). A ligand which appears to have sufficient space around it can be seen at the center of Figure 4C and is marked with a red star. However, it is still too close to the CD at its lower right to enable another CD to be attached to it. This steric hindrance is in striking agreement with the experimental mass spectral results showing four attached CDs as the maximum observed.

Being an efficient tool in CD complexation studies, NMR spectroscopy (especially 2D NMR) can provide information on the details of interaction of  $\beta$ -CD and the SBB ligands of the cluster such as mode of penetration (through narrow rim or through the wide rim of CD) of the guest molecule, extent of guest inclusion in the CD cavity, orientation of the guest molecule inside the cavity, etc. Various protons corresponding to the ligands and that of the CD are marked in the schematic shown in Figure 5. Inner protons in  $\beta$ -CD are represented as H<sup>3</sup> and H<sup>5</sup>, while the outer protons are marked as H<sup>1</sup>, H<sup>2</sup>, H<sup>4</sup>, and H<sup>6</sup>. In the case of the SBB ligand, aromatic protons are named as H<sup>c</sup> and H<sup>d</sup>, while t-butyl protons and the CH<sub>2</sub> protons are represented as H<sup>e</sup> and H<sup>b</sup>, respectively (see Figure 5A). The <sup>1</sup>H NMR spectrum of the  $\beta$ -CD-encapsulated cluster shows induced chemical shifts for certain protons of  $\beta$ -CD and BBS thiol, which are shown in Figure S14.  $\beta$ -CD protons were shifted further upfield than parent  $\beta$ -CD protons, whereas BBS protons were shifted downfield postencapsulation. The upfield shift of  $\beta$ -CD cavity protons is attributed to the magnetic anisotropy affects in the  $\beta$ -CD cavity,<sup>62,63</sup> arising due to the inclusion of

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a  $\pi$ -electron-rich group (here the aromatic ring of BBS). The formation of the supramolecular complex between  $\beta$ -CD and Au<sub>25</sub>SBB<sub>18</sub> cluster was verified by 2D ROESY spectroscopy (Figure 5). This is critical in the study of the interaction between host CDs and guest molecules since protons of both the species are closely located in space after complex formation. The ROESY spectrum of the supramolecular cluster complex shows clear NOE correlations between BBS protons and the protons of  $\beta$ -CD. Aromatic protons of the BBS group (H<sup>c</sup> and H<sup>d</sup>) show cross-peaks with the inner protons (mainly H<sup>3</sup> and H<sup>5</sup>) of  $\beta$ -CD. This confirms the formation of an inclusion complex. Moreover, strong cross-peaks were also observed between the protons of the *t*-butyl group (H<sup>e</sup>) with inner cavity protons of  $\beta$ -CD, namely, H<sup>3</sup> and H<sup>5</sup>, indicating that they are spatially close to each other. Presence of native as well as complexed *t*-butyl protons was observed in the <sup>1</sup>H NMR spectra after complexation (pink and green traces in Figure S14), indicating that all the BBS ligands are not complexed with CD. CH<sub>2</sub> group protons of BBS (H<sup>b</sup>) did not show any noticeable cross-peaks with inner H<sup>3</sup> and H<sup>5</sup> protons of CD in the ROESY spectrum. This may be because the penetration of BBS ligand on Au<sub>25</sub> into the  $\beta$ -CD cavity is not deep enough for the group to interact with inner cavity protons of the latter. The 2D COSY experiments also provide information on the coupling of protons between the two moieties. The cross-peaks corresponding to coupling between H<sup>e</sup>, H<sup>c</sup>, and H<sup>d</sup> protons of the BBS ligand with that of H<sup>3</sup> and H<sup>5</sup> of CD are marked in Figure S15.

The feasibility of encapsulation of BBSH inside the  $\beta$ -CD cavity was further confirmed by the detailed analysis of the inclusion complex prepared by the reaction between  $\beta$ -CD and free BBSH thiol. LDI MS of BBSH \CD showed the presence of a single sharp peak at m/z 1316, which matched well with the theoretical prediction (Figure S16). ESI MS of BBSH∩CD and pure  $\beta$ -CD are compared in Figure S17. Tandem mass spectrometry data with fragmentation products of peaks at m/z 1316 and 1338 corresponding to the loss of BBSH (180 Da) from parent BBSH∩CD are also shown in Figure S17. Binding constant for BBSH∩CD was measured using fluorescence spectral titration (Figure S18). <sup>1</sup>H NMR and 2D COSY spectrum of BBSH∩CD clearly suggests the complexation between  $\beta$ -CD and BBSH thiol (Figure S19).

In view of getting more insight into the structure of the most stable inclusion complex, DFT calculations were performed on isolated BBSH $\cap$ CD supramolecular adducts. These calculations predict the existence of two different possibilities of encapsulation of BBSH ligand in the  $\beta$ -CD cavity in solution, that is, either through the wide rim (Figure 4E) or through its narrow rim (Figure 4D). The inclusion complex resulting from the entry of BBSH through the narrow end was more stable by 1.99 kcal/mol than that through the wide rim. However, from the <sup>1</sup>H NMR data (see Figure S19), we could not precisely determine the chemical shift for the inner and outer protons of CD after complexation since they appeared as broad and diffused peaks. This could be due to the existence of different types of SBB ligands on the cluster surface, namely, the included ones and the unincluded ones on a given cluster (see Figure S14). The former being in two forms (narrow and wider rim entry). Thus, though complexation of CD on  $Au_{25}$  was confirmed, the direction of the inclusion was not clearly assignable using NMR data. Such difficulties have been reported previously.<sup>64</sup>

In order to study the specificity of  $\beta$ -CD in forming a supramolecular complex with BBSH-protected QC, we extended our study to a different QC system of the same core size (Au<sub>25</sub>) but having PET as the protecting ligand (see Experimental Methods section for details). Au<sub>25</sub>PET<sub>18</sub> was chosen as it is a well-studied and characterized system.<sup>37,61,65,66</sup> Unlike the SBB ligand which readily forms the inclusion complex,  $\mathrm{Au}_{25}\mathrm{PET}_{18}$ did not show such an effect (black trace in Figure 6) upon treatment with similar concentrations of  $\beta$ -CD. The spectrum of a Au<sub>25</sub>PET<sub>18</sub> + CD mixture shows only a peak due to free Au<sub>25</sub>PET<sub>18</sub> at m/z 7391. This matches with the theoretical prediction that formation of an inclusion complex on PET-protected Au<sub>25</sub> may not be facile due to specific orientation of the ligands as noted earlier. This specificity in complexation of the  $\beta$ -CD cavity for certain ligands was exploited subsequently. A complementary protocol for the incorporation of  $\beta$ -CD on such clusters would be the replacement of "ligand 1" (PET) with "ligand 2" (BBSH∩CD) on Au<sub>25</sub>PET<sub>18</sub>. This was achieved by following a simple ligand exchange route. For this, initially the BBSH∩CD complex was prepared (treated as ligand 2) which was subsequently allowed to react with the Au<sub>25</sub>PET<sub>18</sub> cluster. This resulted in replacement of three PET ligands by BBSH∩CD (existing as Na adducts, denoted as SBB∩CD-Na as CD-Na interaction is strong) on the QC, which was evident from the MALDI MS data (Figure 6). The well-defined peak in the positive ion mode found at m/z 10990 corresponds to the ligandexchanged product, Au<sub>25</sub>PET<sub>15</sub>(SBB∩CD-Na)<sub>3</sub>. Loss of the  $CH_2 - CH_2 - C_6H_5$  group from the ligand, PET, due to C−S cleavage leading to Au<sub>25</sub>PET<sub>14</sub>S<sub>1</sub>(SBB∩CD-Na)<sub>3</sub> at m/z 10884 was also observed. Also, peaks due to the loss of BB∩CD-Na and CH<sub>2</sub>-CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub> fragments from the cluster leading to Au<sub>25</sub>PET<sub>14</sub>S<sub>2</sub>(SBB∩CD-Na)<sub>2</sub> and Au<sub>25</sub>PET<sub>13</sub>S<sub>3</sub>(SBB∩CD-Na)<sub>2</sub> were also identified (marked with red and green stars (\*), respectively, in Figure 6). The mass spectrum was in complete agreement with the expected values (see inset of Figure 6). Peaks marked "a" and "b" in the spectrum correspond to the loss of AuL (L = PET) from  $Au_{25}PET_{15}(SBB\cap CD-$ Na)<sub>3</sub> and Au<sub>25</sub>PET<sub>14</sub>S<sub>2</sub>(SBB∩CD-Na)<sub>2</sub>, respectively. Replacement of PET with a CD-containing ligand did not affect optical absorption spectra of the clusters

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Figure 6. Positive ion MALDI mass spectra showing the effect of addition of  $\beta$ -CD (black trace) and increasing amounts of BBSH $\cap$ CD (green to cyan trace) to Au<sub>25</sub>PET<sub>18</sub>. Inset shows the experimental and theoretical match between the predicted values. Ligand-exchanged products are marked in the spectra, and a schematic of the same is also shown. Fragments from the parent cluster are marked with a star.

significantly (Figure S20) and showed an enhancement of luminescence intensity of the cluster. Note that it is important to exercise careful control over the ratio of PET/SBB∩CD during ligand exchange reactions as evident from the traces, green to cyan in Figure 6. The amount of incoming ligand, SBB∩CD, was deliberately kept low to enable minimal exchange. However, three ligand substitution seems to be the most favored among others.

Surface engineering of QCs by supramolecular chemistry brought many added advantages to the QCs. The instability of QCs, particularly in the presence of certain metal ions, is a major issue in terms of utilizing such materials for commercial applications. Metal-ioninduced guenching of cluster luminescence is a commonly observed phenomenon in most QCs.<sup>36,67-69</sup> While being an efficient metal ion sensor, its application capabilities toward sensing other analytes of interest are limited due to this aspect, especially in complex environments containing multitudes of cations. Interaction with metal ions can also result in irreversible damage to the cluster and also can cause its decomposition.<sup>70,71</sup> Incorporation of CDs on cluster systems has advantages such as increased stability due to lack of accessibility to the core by incoming metal ions and ligands. The stability of Au<sub>25</sub>SBB<sub>18</sub>∩CD<sub>4</sub> over the parent cluster was monitored by their reactivity toward metal ion (Cu<sup>2+</sup>) and other ligands. Cu<sup>2+</sup> ions react readily with noble metal QCs.<sup>69,72</sup> Here, Au<sub>25</sub>SBB<sub>18</sub> and its CD-functionalized analogue,  $Au_{25}SBB_{18}\cap CD_4$ , were treated with varying amounts of  $Cu^{2+}$  ions (see SI 21 for details),

and its effect on cluster luminescence was studied. Though luminescence intensities of both Au<sub>25</sub>SBB<sub>18</sub> and its CD-protected analogue were quenched with the addition of  $Cu^{2+}$  ions, the extent of quenching observed in Au<sub>25</sub>SBB<sub>18</sub>∩CD<sub>4</sub> was less than that in bare Au<sub>25</sub>SBB<sub>18</sub> upon treatment with identical concentrations of Cu<sup>2+</sup> ions (Figure S21). This may be due to the reduced accessibility of the metal ions to the Au<sub>25</sub> core owing to the bulky nature of the CD species on the cluster surface. Exposure of the CD-protected cluster  $(Au_{25}SBB_{18}\cap CD_4)$  to lower amounts of  $Cu^{2+}$  ions (0.05 mL, 250 mM) showed only 30% guenching in its luminescence, whereas Au<sub>25</sub>SBB<sub>18</sub> showed 70% guenching. However, with higher amounts of  $Cu^{2+}$  ions, the difference in % quenching observed in both cases showed an exponential decrease. This could be due to the effective penetration of the metal ions, owing to their small size, through the protective CD shell around the cluster core. Direct interaction of CD with metal ions, though possible, is unlikely in this case as such interaction requires a highly alkaline medium (pH >12).<sup>73</sup>

The stability of  $\beta$ -CD-functionalized QCs toward ligand exchange reactions was studied by treating such species with excess ligand of another thiol (thiol-2). This was thought to be another important way to see the difference in core accessibility. Au<sub>25</sub>PET<sub>18</sub> was chosen for this study as it gave better mass spectrum compared to Au<sub>25</sub>SBB<sub>18</sub> systems, post-complexation. Both bare Au<sub>25</sub>PET<sub>18</sub> and Au<sub>25</sub>PET<sub>15</sub>-(SBB∩CD-Na)<sub>3</sub> were treated with excess amounts of thiol-2, in this case free BBSH, and the mass spectrum



was recorded (see Figure S22). In the case of Au<sub>25</sub>PET<sub>18</sub>, the PET ligands were easily replaced by BBS thiols to give ligand-exchanged products with varying amounts of mixed ligand-protected clusters. But for Au<sub>25</sub>PET<sub>15</sub>(SBB∩CD-Na)<sub>3</sub>, the mass spectra showed no shift toward the higher mass region, indicating that ligand exchange was not facile in such systems compared to the bare species. Also, though the CD-functionalized entities were unaffected by BBSH, free Au<sub>25</sub>PET<sub>18</sub>, which was also present in the solution, showed complete ligand exchange to form Au<sub>25</sub>SBB<sub>18</sub> *in situ* (marked on the graph).

Yet another interesting aspect of CDs is their capability in sensing molecules. Competitive guests can replace the existing guests from the CD cavity, and therefore, this can be used in sensing such molecules. An example is provided by 1-adamantanethiol (AdT). Inclusion complexes of CD with adamantyl groups are well-known,<sup>31,74,75</sup> and such products are stable. Many such exchanges of CD guests with adamantyl groups have been reported previously. Both Au<sub>25</sub>SBB<sub>18</sub> and  $Au_{25}SBB_{18}\cap CD_4$  were treated with the same amount of AdT (Figure S23). Quenching of luminescence was observed in both cases, but in Au<sub>25</sub>SBB<sub>18</sub>, although an initial decrease in luminescence intensity was noted, probably due to dilution effect/slight ligand exchange, further exposure to AdT did not seem to have an effect on the cluster luminescence. Addition of similar amounts of AdT on Au<sub>25</sub>SBB<sub>18</sub>∩CD<sub>4</sub> resulted in substantial reduction of its luminescence intensity (red data points in Figure S23). This effect may be attributed to the fact that, as AdT is a better "guest" for CD than BBS, effective removal of CD from the BBS ligand on the Au<sub>25</sub> leads to the drastic quenching of luminescence (note that formation of Au<sub>25</sub>SBB<sub>18</sub> $\cap$ CD<sub>4</sub> resulted in enhanced luminescence). UV optical absorption spectra collected from the samples also gave supporting evidence. While no drastic change was observed upon addition of AdT to bare Au<sub>25</sub>SBB<sub>18</sub> clusters, AdT addition to Au<sub>25</sub>SBB<sub>18</sub> $\cap$ CD<sub>4</sub> indicated gradual evolution of spectral features corresponding to the formation of free Au<sub>25</sub>SBB<sub>18</sub> in the solution (green trace in Figure S23D). This reappearance of the Au<sub>25</sub> cluster features in UV spectra could be due to complex formation between the competitive guest AdT and CD, AdT $\cap$ CD, thereby the BBS ligand becomes free on Au<sub>25</sub> QCs.

### CONCLUSION

In summary, we demonstrated surface functionalization of the Au<sub>25</sub> clusters based on specific hostguest interactions between  $\beta$ -CD and (t-butyl)benzyl groups of Au<sub>25</sub>SBB<sub>18</sub>, which imparts new properties to the clusters. A detailed spectroscopic evaluation of the interactions between the QC and  $\beta$ -CD was conducted. More detailed understanding of the formation of an inclusion complex on the QC surface and a possible structure of Au<sub>25</sub>SBB<sub>18</sub>∩CD<sub>4</sub> were provided by DFT calculations and molecular modeling. The observed experimental results were in accordance with the theoretical predictions. The viability of this method in modifying the surface characteristics of differently functionalized QCs has also been demonstrated. Unusual stability and optical properties of CD-functionalized QCs over bare clusters were observed. Our study opens up new possibilities of supramolecular surface-engineered QCs which could overcome some of the limitations of native QCs for potential applications.

### **EXPERIMENTAL METHODS**

**Materials.** Tetrachloroauric(III) acid (HAuCl<sub>4</sub>·3H<sub>2</sub>O) and methanol were purchased from SRL Chemical Co. Ltd., India. 4-(t-Butyl)benzyl mercaptan (CH<sub>3</sub>)<sub>3</sub>C-C<sub>6</sub>H<sub>4</sub>-CH<sub>2</sub>SH (BBSH), 2-phenylethanethiol C<sub>6</sub>H<sub>5</sub>-CH<sub>2</sub>-CH<sub>2</sub>SH (PET), 1-adamantanethiol (AdT), and sodium borohydride (NaBH<sub>4</sub>) were purchased from Sigma Aldrich.  $\beta$ -CD was purchased from Wako Chemicals, Japan. Tetrahydrofuran was purchased from Rankem, India. All chemicals were of analytical grade and were used without further purification. Glassware was cleaned thoroughly with aqua regia (HCI/HNO<sub>3</sub>, 3:1 vol%), rinsed with distilled water, and dried in an oven prior to use. Triply distilled water was used throughout the experiments.

Synthesis of Au<sub>25</sub>SBB<sub>18</sub>. Au<sub>25</sub>SBB<sub>18</sub> was synthesized using a modified procedure of Jin *et al.* used to prepared Au<sub>25</sub>PET<sub>18</sub>.<sup>76</sup> In a typical synthesis, 10 mL of HAuCl<sub>4</sub>·3H<sub>2</sub>O (14.5 mM in THF) was added to 15 mL of BBSH thiol (89.2 mM in THF) while stirring it at 400 rpm at room temperature (29 °C) in a round-bottom flask. The solution becomes colorless after 15 min, indicating the formation of the Au(I) thiolates. An aqueous solution of 2.5 mL of NaBH<sub>4</sub> (0.4 M) was added rapidly to the reaction mixture under vigorous stirring (1100 rpm), and the solution turned from colorless to black, indicating the formation of clusters. The reaction was allowed to proceed with constant stirring for 3 h under ambient conditions and then for 3 h at 45 °C. The crude

solution thus obtained had a dark brownish color and showed characteristic UV absorption features of Au<sub>25</sub> clusters even without any purification. The solution was left overnight to yield monodisperse species. Solvent was removed under vacuum, and the cluster was first washed with water and later precipitated with methanol. The precipitate (Au<sub>25</sub>SBB<sub>18</sub>) was collected after washing repeatedly with methanol and was dried. For the Au<sub>25</sub>PET<sub>18</sub> cluster, the same protocol was followed with the addition of 15 mL of PET (114 mM in THF) instead of BBSH, maintaining other parameters the same.

Synthesis and Reactivity of Au<sub>25</sub>SBB<sub>18</sub> CD Systems. Approximately 3 mg of purified Au<sub>25</sub>SBB<sub>18</sub> was dissolved in 3 mL of THF, and 0.1 mL of  $\beta$ -CD solution (in water) of appropriate concentration was added, such that specific SBB/ $\beta$ -CD ratio was maintained in the solution (1:0.5, 1:0.8, 1:1, and 1:1.2 for Au<sub>25</sub>SBB<sub>18</sub>∩CD<sub>n</sub>, where n = 1 - 4, respectively). The mixture was carefully sonicated for about 10 min at room temperature. The reaction was allowed to proceed under constant stirring (400 rpm) for 30 min at room temperature with intermittent sonication for 1 min at every 10 min intervals. After the reaction, the CD-encapsulated clusters were recovered by the addition of excess water, which resulted in the separation of two layers. The deep brown upper layer (organic) was collected and washed with water to remove unbound CD which dissolves in it. Note that free BBSH in the cluster solution that forms an inclusion complex with CD (denoted as BBSH∩CD) will also be removed in this process as it becomes hydrophilic due to CD encapsulation.



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Due to the presence of a greater number of hydrophobic BBS groups (in comparison to the BBSH $\cap$ CD moieties), CD-functionalized Au<sub>25</sub> QCs (denoted as Au<sub>25</sub>SBB<sub>18</sub> $\cap$ CD) remained in the organic layer and were used for further studies. SBB/ $\beta$ -CD mole ratio of 1:1.2, corresponding to Au<sub>25</sub>SBB<sub>18</sub> $\cap$ CD<sub>4</sub>, was used for detailed experiments unless otherwise mentioned. For sensing experiments with AdT, 1 mg/mL of both the naked and CD-functionalized Au<sub>25</sub>SBB<sub>18</sub> was treated with 0.025 and 0.2 mL of 30 mM AdT in THF.

Instrumentation. Mass spectral studies were carried out using a Voyager DE PRO biospectrometry workstation (Applied Biosystems) matrix-assisted laser desorption ionization (MALDI) time-of-flight (TOF) mass spectrometer both in the linear and reflectron modes (denoted as MALDI (L) and MALDI (R) MS, respectively) as well as using a MALDI TOF TOF (UltrafleXtreme, Bruker Daltonics) mass spectrometer. In the case of MALDI TOF MS, a pulsed nitrogen laser of 337 nm was employed (maximum firing rate, 20 Hz; maximum pulse energy, 300  $\mu$ J) for the measurements. The MALDI TOF TOF mass spectrometer utilizes a 1 kHz smartbeam-II laser, FlashDetector system, and a minimum 4 GHz digitizer. Mass spectra were collected in positive and negative ion modes and were averaged for 500-700 shots. DCTB (trans-2-[3-(4-t-butylphenyl)-2methyl-2-propenylidene]malononitrile) was used as the matrix for all MALDI MS measurements. All spectra were measured at threshold laser intensity to keep fragmentation to a minimum unless otherwise mentioned. Concentration of the analyte and the mass spectral conditions (laser intensity and spectrometer tune files) were optimized to get good quality spectra. UV-vis absorption spectra were collected using a Perkin-Elmer Lambda 25 spectrophotometer. The experiments were carried out at room temperature, and the absorption spectra were recorded from 200 to 1100 nm. Luminescence measurements were done on a Jobin Yvon NanoLog instrument. The band pass for excitation and emission was set at 5 nm. Electrospray ionization (ESI) mass spectrometric measurements were done in the negative mode using LTQ XL, with a mass range of m/z150-4000 and using a Synapt G2 HDMS, quadrupole time-offlight (Q TOF), ion mobility, orthogonal acceleration mass spectrometer with electrospray (ESI) ionization having a mass range up to 32 kDa. The Synapt instrument used for ESI measurements combined exact-mass guadrupole and highresolution time-of-flight mass spectrometer with Triwave technology, enabling measurements in TOF mode. The purified samples were dispersed in THF and used for both mass spectrometric measurements. The samples were electrosprayed at a flow rate 5  $\mu$ L/min and at a capillary temperature of 150 °C. The spectra were averaged for 80-100 scans. Scanning electron microscopic (SEM) and energy-dispersive analysis of X-ray (EDAX) images were obtained using a FEI QUANTA-200 SEM. For the SEM and EDAX measurements, samples were spotted on a carbon substrate and dried in ambient temperature. Transmission electron microscopy (TEM) was conducted using a JEOL 3011, 300 kV instrument with an ultra-high-resolution (UHR) polepiece. The samples were prepared by dropping the dispersion on amorphous carbon films supported on a copper grid and dried in laboratory conditions. <sup>1</sup>H NMR and 2D rotating frame nuclear Overhauser effect (ROESY) spectra were recorded on a 500 MHz Bruker Avance III spectrometer operating at 500.15 MHz equipped with a 5 mm smart probe. A 1:1 solvent mixture of 99.9% DMSO-d<sub>6</sub> (Aldrich) and 99.9% CDCl<sub>3</sub> (SRL) was used to prepare samples and sealed immediately from the laboratory atmosphere. CDCl<sub>3</sub> solvent signal served as the reference for the field-frequency lock, and tetramethylsilane was used as the internal reference. All experiments were performed at 25 °C. Standard Bruker pulse programs (Topspin 3.0) were employed throughout. The 1D spectra were acquired with 32K data points. The data for phase-sensitive ROESY experiments were acquired with a spectral width of 4464 Hz in both the dimensions. For each spectrum, 4 transients of 2048 complex points were accumulated for 256 t<sub>1</sub> increments and a relaxation delay of 1.975 s was used. A continuous-wave (CW) spin-lock mixing time of 200 ms was employed. Prior to Fourier transformation, zero filling to 1K\*1K complex points was performed and apodized with a weighted function (QSINE) in both

dimensions. All the data were processed on a HP workstation, using Topspin 3.0 software.

Theoretical Calculations. Many properties of QCs have been calculated using DFT efficiently by using smaller CH<sub>3</sub> ligands.<sup>77–79</sup> However, here the formation of an inclusion complex requires keeping all the SBB ligands intact, and this increases the CPU resources needed for the calculations significantly. For the computational modeling of Au<sub>25</sub>SBB<sub>18</sub>, we used density functional theory (DFT) as implemented in the real-space code-package GPAW.<sup>80–82</sup> Structure optimization was performed using full ligands as used in the experiments, Perdew-Burke-Ernzerhof (PBE) functional,<sup>83</sup> 0.2 Å grid spacing, and 0.05 eV/Å criterion for the residual forces for optimization. The GPAW setups for Au include scalar relativistic corrections. The structures of Au<sub>25</sub>SBB<sub>18</sub> and Au<sub>25</sub>SBB<sub>18</sub>∩CD<sub>4</sub> were built up with the help of Ecce builder<sup>84</sup> and Avogadro<sup>85</sup> software packages, and visualizations were created with visual molecular dynamics (VMD)<sup>86</sup> software. We generated the initial structure for the optimization of Au<sub>25</sub>SBB<sub>18</sub> using a model of Au<sub>25</sub>PET<sub>18</sub> taken from one of its known crystal structures<sup>61</sup> and then replacing the PET ligands with SBB ligands. A preoptimization of only the ligand positions, keeping the core and staples fixed, was then carried out using a UFF force field<sup>87</sup> as implemented in Avogadro. The model of Au<sub>25</sub>SBB<sub>18</sub>∩CD<sub>4</sub> was constructed by sequentially attaching four  $\beta$ -CDs to the DFToptimized structure of Au<sub>25</sub>SBB<sub>18</sub> using Avogadro. The ligand and  $\beta$ -CD positions of Au<sub>25</sub>SBB<sub>18</sub> $\cap$ CD<sub>4</sub> were optimized by the UFF force field<sup>87</sup> keeping the Au and S atoms fixed. The calculations on BBSH  $\cap$  CD were carried out with Gaussian 09  $^{88}$ using the B3LYP and hybrid meta-GGA functionals in order to describe the noncovalent forces more accurately, and the basis set was selected according to the size of the system. Further details of all the calculations can be found in the Supporting Information 13.

Conflict of Interest: The authors declare no competing financial interest.

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Supporting Information Available: Additional data on characterization of Au<sub>25</sub>SBB<sub>18</sub>, Au<sub>25</sub>SBB<sub>18</sub>∩CD<sub>4</sub>, and BBSH∩CD adducts along with details of theoretical calculations and optimized structures are provided. XYZ files containing structural coordinates of BBSH∩CD (wide and narrow end entry), Au<sub>25</sub>SBB<sub>18</sub>, and Au<sub>25</sub>SBB<sub>18</sub>∩CD<sub>4</sub> are also given. Data demonstrating the enhanced stability of the Au<sub>25</sub>SBB<sub>18</sub>∩CD<sub>4</sub> supramolecular adduct and its sensing properties are also provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Supporting information (SI) for the paper:

# Supramolecular Functionalization and Concomitant Enhancement in Properties of Au<sub>25</sub> Clusters

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**Figure S1.** Effect of UV-vis optical absorption spectra for various Au:BBSH ratios used for cluster synthesis. An optimum Au:S ratio of 1:6 was employed for typical synthesis of  $Au_{25}SBB_{18}$  (see Figure 1 in paper). While lower thiol ratios (A) showed significant changes in the absorption profile indicating that clusters of higher core sizes are getting formed, even a ten fold increase in thiol (B) compared to the optimised synthesis did not seem to yield still smaller clusters.



**Figure S2.** Full range MALDI (L) mass spectra of  $Au_{25}SBB_{18}$  cluster in both positive and negative ion modes. Fragmentation due to the C-S cleavage of SBB ligand on the cluster surface can be observed apart from the molecular ion peak (these features are expanded in the inset). Loss of  $[Au_4L_4]$  fragment from the parent cluster is a typical phenomenon in  $Au_{25}$  clusters. Here, we observed similar fragments corresponding to  $[Au_4SBB_4BB_{2n}]$  loss, where n = 1, 2, 3 in the negative ion mode from parent  $Au_{25}SBB_{18}$ . The additional BB losses observed in the negative mode (red trace in inset) could be due to the facile C-S cleavage as in the case of the molecular ion peak at 8151 Da. DCTB was used as the matrix and threshold laser intensities were employed for all the measurements.



**Figure S3.** MALDI (L) mass spectra of the purified  $Au_{25}SBB_{18}$  cluster at different laser intensities in the positive mode. Control over the laser intensity is vital to observe the molecular ion peak of the cluster without fragmentation. Laser intensity (shown at the right extreme) is as given by the instrument and has not been calibrated to a standard unit.



**Figure S4.** ESI MS of  $Au_{25}SBB_{18}$  in negative ion mode showing fragments from the cluster in the low mass region.



**Figure S5.** TEM images of  $Au_{25}SBB_{18}$ . Two magnifications are shown. Unlike in typical thiolated clusters, these samples are resistant to electron beam induced aggregation.



# **Supporting information 6**

**Figure S6.** SEM and EDAX characterization of  $Au_{25}SBB_{18}$  cluster. Carbon and aluminium are from the substrate used for the measurement. Contrast of carbon is low due to the use of carbon tape as the substrate. The scale is same for all images.



**Figure S7.** SEM images of (A) native  $\beta$ -CD powder, (B) drop cast  $\beta$ -CD solution in water and (C) drop cast  $\beta$ -CD solution in THF:water (30:1) mixture, after sonication. The formation of needle-like superstructures by self assembly occurred only in the case of reaction in THF:water (30:1) solvent mixture. Presence of minimal amount of water molecules can enhance the possibility of intermolecular hydrogen bonding between the hydroxyl group present on the outer rim of CD molecules. Control experiments in water (B), did not result in formation of superstructures. Thus the dispersion of  $\beta$ -CD molecules by sonication in THF and their subsequent self assembly by re-formation of the strong hydrogen bonding between the CDs with the aid of THF results in these superstructures.



**Figure S8.** LDI mass spectrum of the aqueous layer, post synthesis of the CD-functionalised  $Au_{25}SBB_{18}$  clusters. Addition of excess water to the microtubular arrangement of CD and cluster leads to the formation of  $Au_{25}SBB_{18}\cap CD_n$  (where n=1-4). Though we found better mass spectral intensities for the adducts from the organic layer (see Figure 2 in main text), probably due to the existence of more number of hydrophobic SBB groups on the cluster surface (18-n, where n<4), analysis of the aqueous layer showed a broad peak at higher mass range too albeit with reduced intensity. Inset shows an expanded view. Peak maximum corresponding to  $Au_{25}SBB_{18}\cap CD_4$  is marked with a line.



**Figure S9.** Positive mode MALDI (L) and MALDI (R) mass spectra of  $Au_{25}SBB_{18}$  with increasing SBB:CD ratios in solution. The peak maxima shift with increasing BBS:CD ratio. This gradual increase is marked. Peak corresponding to parent  $Au_{25}SBB_{18}$  is marked using a \*. These peak positions are the same in both the data sets, but in the reflectron mode the peaks are better resolved as the resolution is improved. These peaks resolve even better in the MALDI TOF TOF mode (see S10).



**Figure S10.** MALDI TOF TOF mass spectra of  $Au_{25}SBB_{18}$  with increasing SBB:CD ratios (green to brown) in solution. The peaks are better resolved than in S9.



**Figure S11.** MALDI (L) mass spectra (A) and MALDI TOF TOF mass spectra (B) of  $Au_{25}SBB_{18}\cap CD_4$  at different laser intensities in the positive mode. Note that though the background of the spectra increases with more laser fluence, the peak maxima and relative individual peak intensities remain the same except for red trace in (B) wherein peak due to  $Au_{25}SBB_{13}S_5$  (marked with a \*) gain intensity at higher laser fluence due to cleavage of C-S bond and loss of CDs. There are threshold laser powers above which fragmentations occur.



Figure S12. NIR luminescence observed from the bare Au<sub>25</sub>SBB<sub>18</sub> cluster at (A) various excitation wavelengths and (B) comparison with the spectra ( $\lambda_{ex}$  992 nm) of various starting materials.

### Structural optimization of Au<sub>25</sub>SBB<sub>18</sub>

The cluster was rotated so that the *x*-axis lay along the axis of the cluster passing through its center and the bridging sulfur atoms which were spaced the furthest distance apart.

Cluster boundary conditions were used and the size of the simulation box was chosen to be 34 Å, leaving about 9 Å of buffer space around the molecule. A negative charge was added to the molecule.

### Au<sub>25</sub>SBB<sub>18</sub>∩CD<sub>4</sub>

### Ligand structure of Au<sub>25</sub>SBB<sub>18</sub> and CD attachment

The precise arrangement around any given ligand will affect whether that ligand may be a likely one for CD complexation. It was observed that bridging ligands were generally surrounded by ligands which were quite close to it, while the ligands neighboring a non-bridging ligand were spread further apart. The number of nearest-neighbor ligands to a CD centered on a chosen ligand was four.

The model of  $Au_{25}SBB_{18}\cap CD_4$  was constructed by making attachments of CDs to the DFT optimized structure of  $Au_{25}SBB_{18}$  using molecular builder software. The narrow side of the CD was attached first as this would reduce steric hindrance and this configuration had a lower binding energy as an isolated complex. The choice of ligands also affects the depth of penetration of the CD onto the ligand, which is lesser in the case of the bridging ligands due to greater steric hindrance from the neighbouring ligands. For non-bridging ligands both the aromatic BBS protons and *t*-butyl group protons would be close to the inner CD protons, which also agrees with the NMR data. For bridging ligands the inner H<sup>3</sup> and H<sup>5</sup> protons of the CD would be closer to the *t*-butyl groups.

The non-bridging ligand denoted by (y, -z), in the notation described in the main paper, was easily accessible due to the widely separated positions of the surrounding ligands and hence was chosen for making the first attachment of the CD. The attachment was made in a stepwise fashion starting by including the *t*-butyl group and then by bringing the narrow end of the CD further over the ligand and then reoptimizing using a UFF force field until its position was in agreement with the NMR data. We also rejected position changes which increased the total energy. During the optimization, the core and staple atoms, *i.e.* the Au and S atoms, were kept fixed in their positions from DFT, while the other atoms were allowed to move. This process was repeated three more times by making CD attachments to the (*-z, -x*), (*x,-y*) and (*z,-x*) non-bridging ligands which were easily accessible. The energy of the final structure in the UFF force field was 60,323 kcal/mol.

From our calculations on BBSH∩CD, it is energetically favourable for the included ligand to be at an angle with respect to the CD. Tilting the CD to the angles found in the optimized geometries of BBSH∩CD was found difficult due to the presence of the neighboring ligands. The relative angle of the CD and included ligand varies due to the differing orientations of the included ligand and its neighbors. We remark here that further force-field calculations and molecular dynamics simulations would be necessary to determine more precise attachment

geometries as several different configurations which differ in depth and angle of attachment are consistent with the NMR data.



**Figure S13.** Different views of the Au<sub>25</sub>SBB<sub>18</sub> $\cap$ CD<sub>4</sub> model. Hydrogen atoms are not shown on the SBB ligands for clarity. Sulfur and gold atoms are shown in green and gold, respectively, while the carbon atoms of the bridging and non-bridging ligands are shown in blue and magenta, respectively. The four attached CDs are shown in cyan in the stick molecular representation. The cartesian *x*, *y*, and *z* axes are shown by the red, green and blue arrows, respectively.

### DFT calculations on BBSH∩CD

In this section we give full details of the DFT calculations performed on the BBSH∩CD inclusion complexes and discuss some of the theoretical results presented in the paper in

more detail. All calculations were performed with the Gaussian 09 code.<sup>1</sup> The experimental structure of  $\beta$ -cyclodextrin (C<sub>70</sub>H<sub>42</sub>O<sub>35</sub>) was obtained from the Hic-Up Database and was based on the Protein Data Bank file pdb1z0n.ent.<sup>2</sup> As the downloaded structure was without hydrogen atoms these were added to this structure and the hydrogen positions were optimized at B3LYP/6-31G\* keeping all the other atoms fixed in the same positions as experiment. The geometry of BBSH molecule (C<sub>11</sub>H<sub>15</sub>SH ) was obtained from the web database ChemSpider.<sup>3</sup> A geometry optimization at the B3LYP/6-311+G\*\* level was carried out. The optimization resulted in small changes in the geometry, as the plane of the benzene ring rotated to be perpendicular to the plane containing the C<sub>1</sub>-C<sub>2</sub> bond (carbons are numbered starting from the sulfur end).

The above geometries of CD and BBSH were then used for creating the initial configurations of two BBSH $\cap$ CD adducts. The BBSH molecule was inserted into the CD cavity with the *t*-butyl group going in first. The alignment of the BBSH molecule was such that its C<sub>1</sub>-C<sub>2</sub> axis was along the axis of the CD passing through the CD centre and perpendicular to the planes of its openings. Two such initial configurations were constructed by insertion into the wide and narrow ends of the CD. The geometry optimizations were carried out using the meta-GGA hybrid functional m052-X, which describes more accurately the non-covalent interactions found in the adducts, in conjunction with 6-31G\* and 6-31+G\*\* basis sets. During the optimization, the CD atoms were kept fixed and only the BBSH atoms were allowed to move. This was done not only to speed up the computations but also because  $\beta$ -CD adopts what is known as the anhydrous configuration after a full DFT geometry optimization,<sup>4</sup> which is different from its structure in a solvent.

The optimized geometries of the adducts are shown in Figure 4D (narrow end entry) and 4E (wide end entry), indicating the stability of these adducts due to non-covalent interactions. We did not find a significant change in the geometries with increase in the size of the basis set, and we have presented results using  $6-31G^*$  in Figures 4D and 4E. The BBSH molecule adopted a slanted configuration with its C<sub>1</sub>-C<sub>2</sub> axis parallel to the side of the CD in both the narrow and wide entry cases. Binding energies of the narrow and wide entry configurations were performed using the Boys counterpoise correction method<sup>5</sup> with the m052-X/6-31+G\*\* level of theory. The binding energy is about 2 kcal/mol less for the narrow case. We might attribute this to stronger  $\pi$ -bonding between the BBSH aromatic ring and the inner CD protons in the narrow case because of the shorter inter-proton distance caused by the narrowing of the profile of the CD.

A careful note of the relative positions of BBSH and CD protons was made in order that agreement with NMR experimental data might be evaluated. Referring to Figure 4D and 4E we see the following. In the narrow case, the H<sup>b</sup> group protons are located around the level of the O-H<sup>1</sup> protons, the lower aromatic H<sup>c</sup> protons (closest to the sulfur end) are around the level of the H<sup>2</sup> protons of CD, the upper aromatic H<sup>d</sup> protons are situated around the level of the H<sup>3</sup> CD protons, while the *t*-butyl group H<sup>e</sup> protons are situated between the level of the H<sup>5</sup> and H<sup>6</sup> CD protons. In the wide case, the H<sup>b</sup> protons are slightly below the H<sup>6</sup> protons and not inside the CD, the lower aromatic H<sup>c</sup> protons are at the H<sup>5</sup> proton level, the upper aromatic H<sup>d</sup> and H<sup>7</sup> protons.

NMR data suggests an interaction between both the aromatic and *t*-butyl group protons of BBSH with the H<sup>3</sup> and H<sup>5</sup> inner CD protons, which is also in good general agreement with both the structures. However it is not possible to identify the specific NMR fingerprints of

each of the structures from the experimental data which suggests the possibility of NMR calculations at DFT level. The arrangement of the included ligand and the CD were found to be different for inclusion complexes formed with ligands attached to the cluster rather than isolated ligands. Firstly, the presence of a gold core and -Au-S-Au-S-Au- staples attached to the sulfur of the SBB ligand decreases the penetration depth of the CD. Secondly, the steric hindrance caused by the presence of about four or five ligands around the CD decreases both the CD penetration depth and the angle between the CD and the ligand.



**Figure S14.** <sup>1</sup>H NMR of  $\beta$ -CD, Au<sub>25</sub>SBB<sub>18</sub> and Au<sub>25</sub>SBB<sub>18</sub> $\cap$ CD<sub>x</sub> in 1:1 solvent mixture of DMSO-d6 and CDCl<sub>3</sub> at 25 °C. Here signals due to unreacted H<sup>e</sup> protons of BBS can also be observed (green and pink trace) which suggests the existence of free and complexed BBS on the cluster.







Figure S16. LDI MS of BBSH∩CD in the positive ion mode.



**Figure S17a.** ESI MS of  $\beta$ -CD and BBSH $\cap$ CD inclusion complex in the positive ion mode. Expanded views are given in the inset.


**Figure S17b.** Tandem mass ESI spectra (positive ion mode) for the peak at m/z 1316 (A) and 1338 (B) with increasing collision energy. Fragment ions are also marked. In the MS<sup>2</sup> spectrum of m/z 1338, the peaks formed at m/z 1158, 1316 and 1136 correspond to the loss of BBSH (180 Da) and Na (23 Da) from the parent ions.

The binding constant of a simple host-guest adduct, BBSH $\cap$ CD was measured in the same medium used for complexation of clusters using fluorescence spectral titrations.<sup>6, 7</sup> From the modified Benesi-Hildebrand equation, the linear plot of the reciprocal of the change in fluorescence intensity ( $\Delta$ F) and the reciprocal of the molar concentration of cyclodextrin ([CD]<sub>0</sub>) indicated a 1:1 stoichiometric complex with a binding constant of ~1776 M<sup>-1</sup>. However, for Au<sub>25</sub>SBB<sub>18</sub> and CD, such measurements using normal complexation titration, NMR, etc. were not attempted as multiple stoichiometries, Au<sub>25</sub>SBB<sub>18</sub> $\cap$ CD<sub>n</sub> (where n=1 to 4), can exist in solution thereby making calculation of binding constants difficult.



**Figure S18**. (A) Emission spectra of BBSH solution ( $6.9*10^{-5}$  M) in THF/water mixture in the presence and absence of  $\beta$ -CD. From bottom to top: [ $\beta$ -CD] = 0, 0.5 × 10<sup>-3</sup>, 1 × 10<sup>-3</sup>, 2 × 10<sup>-3</sup>, 3 × 10<sup>-3</sup> and 4 × 10<sup>-3</sup> M. (B) Plot of reciprocal of the change in fluorescence intensity ( $\Delta$ F) and the reciprocal of the molar concentration of cyclodextrin ([CD]<sub>0</sub>)



**Figure 19a**. Comparison of <sup>1</sup>H NMR of CD (blue trace) and BBSH $\cap$ CD (green trace) inclusion complex in 1:1 mixture of DMSO-d6 and CDCl<sub>3</sub> at 25 °C.



**Figure 19b.** 2D COSY spectrum of BBSH∩CD in 1:1 mixture of DMSO-d6 and CDCl<sub>3</sub> at 25 °C.



**Figure S20.** Effect of UV-vis absorption spectra after ligand exchange reaction of  $Au_{25}PET_{18}$  with SBB $\cap$ CD (as incoming ligand). The PET:SBB $\cap$ CD ratios are shown.



**Figure S21.** Quenching of (A) bare  $Au_{25}SBB_{18}$  and (B)  $Au_{25}SBB_{18}\cap CD_4$  upon treatment with an aqueous solution of 250 mM  $Cu^{2+}$  solution (note that clusters were taken in THF solvent so as to allow better miscibility). The spectra were measured after 5 minutes of addition.



**Figure S22.** MALDI (L) mass spectra of bare  $Au_{25}PET_{18}$  (A) and BBSH $\cap$ CD incorporated  $Au_{25}PET_{18}$  QCs (denoted as 'Cluster 2' in the figure) (B) with excess BBSH thiol. In the case of  $Au_{25}PET_{18}$  with excess BBSH (A), peaks corresponding to various ligand exchanged species,  $Au_{25}PET_{18}$ -SBB<sub>x</sub> (where x=0 to 17) separated by m/z 42 due to the exchange of PET (MW 137.2) for BBS (MW 179.3), are seen under various conditions (labelled in figure). Spectrum corresponding to bare  $Au_{25}SBB_{18}$  is also shown for comparison (blue trace in A). For (B), various amounts of BBSH was added to 'Cluster 2' which is a mixture of  $Au_{25}PET_{18}$  and BBSH $\cap$ CD incorporated  $Au_{25}PET_{18}$  QCs. While  $Au_{25}PET_{18}$  ligand exchanges completely with BBSH to give a peak at m/z 8152 corresponding to  $Au_{25}SBB_{18}$  (marked on the graph), peaks due to  $Au_{25}PET_{15}(SBB\cap CD-Na)_3$  and  $Au_{25}PET_{13}S_3(SBB\cap CD-Na)_2$  do not show any shift and their relative intensities are unaffected indicating the absence of ligand exchange.



**Figure S23.** Effect of 1-adamantanethiol (AdT) on both Au<sub>25</sub>SBB<sub>18</sub> and Au<sub>25</sub>SBB<sub>18</sub> $\cap$ CD<sub>x</sub> was studied. Schematic of the possible events upon addition of AdT are depicted in (A). Luminescence from the QCs upon AdT addition is compared in (B). UV-vis absorption spectra of Au<sub>25</sub>SBB<sub>18</sub> (C) and Au<sub>25</sub>SBB<sub>18</sub> $\cap$ CD<sub>x</sub> (D), with addition of AdT are also shown. Re-appearence of Au<sub>25</sub> absorption features with 0.1 mL of AdT (green trace, marked with an arrow) in Au<sub>25</sub>SBB<sub>18</sub> $\cap$ CD is observed in the expanded region of (D).

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# Sequential Electrochemical Unzipping of Single-Walled Carbon Nanotubes to Graphene Ribbons Revealed by *in Situ* Raman Spectroscopy and Imaging

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**ABSTRACT** We report an *in situ* Raman spectroscopic and microscopic investigation of the electrochemical unzipping of single-walled carbon nanotubes (SWNTs). Observations of the radial breathing modes (RBMs) using Raman spectral mapping reveal that metallic SWNTs are opened up rapidly followed by gradual unzipping of semiconducting SWNTs. Consideration of the resonant Raman scattering theory suggests that two metallic SWNTs with chiralities (10, 4) and (12, 0) get unzipped first at a lower electrode potential (0.36 V) followed by the gradual unzipping of another two metallic tubes, (9, 3) and (10, 1), at a relatively higher potential (1.16 V). The semiconducting SWNTs with chiralities (11, 7) and (12, 5), however, get open up gradually at  $\pm$ 1.66 V. A rapid decrease followed by a subsequent gradual decrease in the



metallicity of the SWNT ensemble as revealed from a remarkable variation of the peak width of the G band complies well with the variations of RBM. Cyclic voltammetry also gives direct evidence for unzipping in terms of improved capacitance after oxidation followed by more important removal of oxygen functionalities during the reduction step, as reflected in subtle changes of the morphology confirming the formation of graphene nanoribbons. The density functional-based tight binding calculations show additional dependence of chirality and diameter of nanotubes on the epoxide binding energies, which is in agreement with the Raman spectroscopic results and suggests a possible mechanism of unzipping determined by combined effects of the structural characteristics of SWNTs and applied field.

**KEYWORDS:** graphene · single-walled carbon nanotubes · electrochemistry · Raman spectral mapping · density functional-based tight binding calculations

arbon has been exciting to scientists for centuries and still continues to fascinate the scientific community in the form of nanometer-sized allotropes such as bucky balls<sup>1</sup> and nanotubes<sup>2</sup> and, more recently, in the form of the ideal atomic layer, graphene.<sup>3</sup> Numerous chemical variants of these have also been explored. Both single-walled carbon nanotubes (SWNTs) and graphene possess unique properties with diverse applications in electronics<sup>4–6</sup> and quantum computing<sup>7</sup> and, above all, possess the ability to unravel many fundamental questions related to ballisticthermal and -electronic transport.<sup>8–13</sup> SWNTs require high purity and accurate characterization

in terms of chiralities and length and diameter distribution for them to be used in most of the specific applications. A similar scenario exists in the case of graphene as well, being vulnerable to drastic changes in the band structure with increasing number of layers,<sup>14</sup> changes in the edge states,<sup>15</sup> etc.

It has been understood both theoretically and experimentally that graphene ribbons can have a band gap that could be tuned by varying its width<sup>16,17</sup> and geometry.<sup>18</sup> Nanoribbons are considered important because of the emerging local magnetism with very specific edge states.<sup>19</sup> There are also attempts to use these nanoibbons in electronics by visualizing them as active channel

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materials in field effect transistors.<sup>20,21</sup> Hence, it is desirable to have a precise method without any overoxidation to convert specific SWNTs to graphene nanoribbons and thereby create graphenic materials of desired properties. This concept was also aided by the ability to separate SWNTs according to their metallicity<sup>22</sup> and diameter,<sup>23</sup> which eventually helps in getting graphene ribbons of specific width and edge structure. In this context, recently, Dhanraj et al. devised an electrochemical route to convert multiwalled nanotubes into multilayered graphene nanoribbons (GNRs).<sup>24</sup> In brief, nanoribbons of a few layers of graphene have been prepared from carbon nanotubes (CNT) by a two-step electrochemical approach consisting of oxidation of CNTs at controlled potential, followed by reduction to form GNRs having smooth edges and fewer defects, as evidenced by multiple characterization techniques, including Raman spectroscopy, atomic force microscopy, and transmission electron microscopy (TEM). However, neither the role of electric field nor the mechanism of opening and the sequence of events between CNT breaking (oxidative cleavage of the C-C bond) and GNR formation has been probed. Answers to questions such as, is the unzipping fundamentally different for metallic and semiconducting CNTs, where does the curvature break, and what is the reason for selecting a mixture of semiconducting and metallic CNTs, have not been explored, although both single and multiwalled CNTs have been shown to generate GNRs with controlled widths and fewer defects. An in situ spectroscopic investigation of various stages of the above sequential processes can possibly reveal the mechanism of unzipping of nanotubes and selective breaking, if any. This will also be important to understand the mechanism of unzipping of SWNTs to GNRs by other methods such as laser cutting and chemical unzipping.25,26

#### **RESULTS AND DISCUSSION**

We report an in situ Raman spectroscopic and microscopic investigation (see Methods and Materials for a detailed description) of the electrochemical unzipping of SWNTs to form graphene ribbons. It was desirable to have a different electrochemical setup that enables this process to be observable in real time. An electrochemical cell was constructed by making a discontinuity on a conducting indium tin oxide (ITO)coated glass plate to have both electrodes (working and counter) laterally mounted on the same surface in order to suit Raman measurements. The constraint due to the microscopic setup (limited working distance of the objective used) did not allow us to have a cell thicker than 0.24 mm. SWNT dispersion (Methods and Materials) in N,N-dimethyl formamide (DMF) was deposited on the working electrode, which was kept under the microscope. A particular portion of the



Figure 1. Schematic of the experimental setup used for the *in situ* Raman spectroscopic investigation of the unzipping of SWNTs with orthogonal laser illumination and spectral collection in the backscattering geometry, inside the electrochemical cell. Various parts of the electrochemical cell and essential parts of the Raman spectrometer are labeled. The connections to the electrodes from the dc source are given using a 0.1 mm thick Pt wire.

nanotube sample, say a bundle which contains many SWNTs, was selected and continually imaged using Raman spectral features keeping the same region (20  $\mu$ m  $\times$  20  $\mu$ m) by varying the potentiostatic conditions. A schematic of the experimental setup used for the study is given in Figure 1 (details are given in the Materials and Methods).

An average micro-Raman spectrum from the dropcasted SWNT on the working electrode shows all the expected features such as the radial breathing modes (RBMs) appearing in the spectral window of 180-280 cm<sup>-1</sup>, a not so prominent D band (1345 cm<sup>-1</sup>), a G band (1593 cm<sup>-1</sup>), and a 2D band (2660 cm<sup>-1</sup>). A high-resolution RBM spectrum collected for the same sample using a grating of 1800 grooves/mm shows three distinct features at 196 (designated here on as RBM I), 240 (RBM II), and 276 (RBM III)  $cm^{-1}$ . RBMs II and III indicate the presence of a number of metallic nanotubes (mSWNT), and RBM I is due to a couple of semiconducting tubes (sSWNT) with different chiralities. By considering the resonance (532 nm laser, 2.33 eV) condition of our measurement, the bundling of SWNTs, the peak positions, and the peak width of  $\omega_{\text{RBM}}$ , a more reasonable assignment of the chiralities can be suggested as follows: 196 cm<sup>-1</sup>  $[(11, 7) \text{ or } (12, 5), \text{ interband transition } E_{33} = 2.37 \text{ or}$ 2.35 eV, diameter d = 1.25 or 1.2 nm, semiconducting], 240 cm<sup>-1</sup> [(10, 4) or (12, 0),  $E_{11}$  = 2.24 or 2.23 eV, d = 0.98 or 0.94 nm, metallic], 276 cm<sup>-1</sup> [(9, 3) or (10, 1),  $E_{11} = 2.43$  or 2.41 eV, d = 0.85 or 0.83 nm, metallic].<sup>27–29</sup> While the data from one set of SWNT bundles is presented here, data from other bundles are presented in the Supporting Information. Each data set has also been checked for reproducibility.

Spatially resolved Raman spectra (see Materials and Methods) were collected in the spectral window of  $0-3900 \text{ cm}^{-1}$  for various electrochemical conditions

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Figure 2. Raman spectra of gradual unzipping of SWNTs. Inset displays the averaged RBM spectra from the sample for various conditions. The black trace is that of the parent material. The red trace (immediately after the application of 0.36 V) shows near-complete disappearance of the second RBM. The third RBM disappears with various conditions, as one can see from the decrease in intensity of the peak around 276 cm<sup>-1</sup>. Various conditions are labeled by different color. A considerable decrease in the width of the G band is observed, suggesting the reduction in metallicity along with an increase in the D band, which implies increased defects formed during unzipping. The variations in the 2D band at 2660 cm<sup>-1</sup> band have been discussed elsewhere in the text. Featureless regions of the spectra are used to place the insets.

(labeled in Figure 2). Figure 2 shows the evolution of the average spectral features of the SWNT sample upon various cycles of electrochemical processes. A decrease in peak width of the G band was observed as time evolves and with increased potentials, which is indicative of reduction in the metallicity of the SWNT bundle. There was also an increase in the D band intensity, suggesting increased defects (see Figures 4 and 5 and the subsequent text for detailed discussion). The spectral position of the 2D band remains unchanged with a slight decrease in the peak width, suggesting the single-layer nature of the formed graphene ribbon with uncoupled ribbons. The inset of Figure 2 gives the evolution of the three RBMs of the average spectrum collected from the SWNT bundle, say RBM I (196 cm<sup>-1</sup>), RBM II (240 cm<sup>-1</sup>), and RBM III  $(276 \text{ cm}^{-1})$ , which are labeled in the graph as I, II, and III, respectively. It is evident that immediately after the application of 0.36 V (red trace) to the working electrode, the intensity of feature II, corresponding to SWNTs with chiralities (10,4) and (12, 0), gradually disappears along with a considerable decrease in the intensity of feature III.

This remarkable change in RBM II suggests rapid unzipping at a relatively lower anodic potential. The subsequent steps show a gradual decrease in the intensity of RBM III, although the intensity of feature I was almost constant. However, after 7 h of application of 1.66 V to the working electrode, the intensity of RBM III (mSWNT) almost disappears (wine red colored trace), while the intensity of RBM I (another type of sSWNT) disappears only after the application of -1.66 V. The electrochemical potentials have been calibrated by carrying out separate experiments under identical conditions of the two-electrode in situ electrochemical cells in a three-electrode setup using a mercury/ mercurous sulfate reference electrode. The hump still existing at the position of RBM II at the higher potentials is due to the fact that the spectra given in Figure 2 are averages of all the spectra collected throughout the region of the SWNT bundle. Upon examination of smaller areas, we see that there is a complete disappearance of this band immediately after the application of 0.36 V (Figure S1). We believe that there are inhomogeneities in the potential across a large area, and unzipping proceeds only slowly in such regions, explaining this overall spectral behavior.

Images corresponding to different phonon modes in SWNTs were filtered from the spectral map, and they reveal similar morphology, confirming the presence of high-quality SWNTs. A comparison of the images obtained from specific vibrational features for various electrochemical oxidizing conditions further confirms the sequential unzipping of different kinds of SWNTs to form graphene ribbons. Figure 3 compares the morphological features filtered using RBM I (178-206 cm<sup>-1</sup>), RBM II (228–256 cm<sup>-1</sup>), and RBM III  $(264-288 \text{ cm}^{-1})$  for potentials of 0 V (open circuit with no external bias), 0. 36 V (immediately after the application), and 1.16 and 1.66 V, applied to the working electrode for 7 h each. Three columns contain images filtered using RBM features of three pairs of SWNTs. The first column (RBM I) is the image due to sSWNTs (11, 7) or (12, 5), and the second (RBM II) and third (RBM III) columns correspond to mSWNTs (10, 4) or (12, 0) and (9, 3) or (10, 1), respectively. The spectral window for each set of RBMs is given at the top of each column. Each row corresponds to various potentiostatic conditions (as labeled at the left of each column) showing a different extent of oxidation of various types of nanotubes. The corresponding images after intermittent reducing potentials (by the application of -0.36, -1.16, and -1.66 V for 7 h) are shown in Figure S2 (Supporting Information).

The first row (Figure 3a-c) shows the presence of the three RBMs prior to the application of potential (*i.e.*, open circuit with no external bias denoted by 0 V) to the electrodes of the cell. The second row shows the presence of RBM I (d) and RBM III (f) in the imaged structure with a disappearance of the image filtered using RBM II (e) immediately after the application of 0.36 V to the working electrode. This is indicative of the rapid unzipping of two of the mSWNTs, (10, 4) and (12, 0). Figure 3 h and i show the absence of RBMs II

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Figure 3. Evolution of the RBM region during electrochemical unzipping. Three columns contain images filtered using the RBMs of three different types of SWNTs. RBM I is due to sSWNTs (filtering windows is 178-206 cm<sup>-1</sup>), whereas RBMs II and III are from mSWNTs (228-256 and 264-288 cm<sup>-1</sup>, respectively). Each row corresponds to various stages of unzipping (corresponding to different oxidizing potentials as labeled at the left of each column) for the disappearance of various types of nanotubes. Raman images a-c show the presence of the three types of SWNTs prior to the application of potential (open circuit, i.e., zero applied bias) to the electrodes of the cell. The second row shows the presence of RBM I (d) and RBM III (f) in the imaged structure with a disappearance of the image due to RBM II (e) immediately after the application of 0.36 V to the working electrode; images h and i show the absence of RBM II and III (mSWNTs), respectively, after the application of 1.16 V for a period of 7 h to the working electrode. Image j shows clear reduction in the intensity of RBM I throughout the bundle, along with the absence of RBM II (k) and III (l) after the application of 1.66 V to the working electrode for a period of 7 h. The color scaling varies slightly in each of the figures as the absolute intensities may not be the same in all the images. While the scale bar is 4  $\mu$ m for images in the first two rows (a–f), it is 3  $\mu$ m for the last two rows (g-l).

and III, suggesting the unzipping of another two types of mSWNTs, (9, 3) and (10, 1), after the application of 1.16 V for a period of 7 h. Figure S1 (Supporting Information) shows the disappearance of RBM I, suggesting the opening of sSWNTs (11, 7) and (12, 5) with the disappearance of the 196  $cm^{-1}$ peak after the application of -1.66 V for a period of 7 h.

Although specific morphological features due to the three RBMs disappear sequentially with the application of the electric field, other morphological features filtered using D, G, and 2D remain more or less invariant. This is especially significant for unraveling

the sequence of events associated with defect generation and lose of curvature. Figure 4 shows the Raman images filtered from 1320 to 1380 cm<sup>-1</sup> (D band), 1565–1615 cm<sup>-1</sup> (G), and 2620–2700 cm<sup>-1</sup> (2D) before (a, b, and c) and after (d, e, and f) electrochemical processing. It is self-evident that the image filtered from G and 2D remains intact, whereas the features due to the D band are enhanced during the process, indicating additional defects formed upon unzipping (shown in Figure 4d). These preserved features arising from the planar sp<sup>2</sup>-hybridized hexagonal carbon lattice along with the disappearance of RBMs suggest the unzipping of SWNTs to form GNRs. The images filtered using D, G, and 2D bands for the intermediate steps (immediately after the application of 0.36, 0.36, -0.36, 1.16, -1.16, and 1.66 V applied continuously for 7 h) are shown in Figures S3, S4, and S5. Additional measurements have been conducted on different bundles to confirm this phenomenon (Figures S6, S7, and S8). Formation of graphene ribbons was confirmed by TEM (Figures S9 and 10).

The relative intensity of RBM III with respect to that of RBM I (blue scatter) plotted in Figure 5a clearly shows a reduction at various steps (electrochemical conditions), numbered from 1 to 7 (same order as in Figure 2). We have excluded the eighth step (i.e., -1.66 V applied to the working electrode) as in most cases the RBMs I and III are not present or are negligible to take a ratio. A considerable increase in the intensity of the D  $(1345 \text{ cm}^{-1})$  band is observed with each step. The  $I_{\rm D}/I_{\rm G}$  ratio has increased (Figure 2) from 0.039 (for open circuit) to 0.246 (after the application of -1.66 V for 7 h), suggesting the unzipping of SWNTs along with the addition of some undesirable defects. We have tried to analyze the G band and a broad shoulder present at its lower wavenumber region by deconvoluting the region from 1520 to  $1610 \text{ cm}^{-1}$  with two adjacent Lorentzian peaks (see a representative fit in Figure S11). The peak in the spectral range of 1520 to 1580 cm<sup>-1</sup> (labeled as G\* here on) accounts for the metallicity of the bundle, whose position and width vary with the electrochemical conditions. The peak width of the G (1596 cm<sup>-1</sup>) band decreased from 23  $\text{cm}^{-1}$  for the pristine SWNT to 18  $\text{cm}^{-1}$  for the seventh step with a small increase to 19 cm<sup>-1</sup> for the last step, i.e., application of -1.66 V for 7 h. This variation in the peak width of the G band is also displayed in Figure 5a (wine color scatter), against various steps. The data plotted are the average of the information from four sets of in situ Raman spectroscopic data. The standard deviation is given as the error bar. The G\* band shows a large decrease in its area and width along with a shift of the center maximum of the Lorentzian peak (details are given in Table S1 and Figure S12). This along with the variation in the RBM intensity ratio (blue scatter) explicitly confirms reduction

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Figure 4. Transformation of SWNTs to graphene ribbons. The first row (a, b, and c) presents the images of the SWNT prior to the electrochemical unzipping, filtered using D (1320–1380 cm<sup>-1</sup>), G (1565–1615 cm<sup>-1</sup>), and 2D (2620–2700 cm<sup>-1</sup>) bands, respectively (scale bar is 4  $\mu$ m). The second row (e, f, and g) shows the images of the unzipped SWNTs filtered using D, G, and 2D bands. The presence of the G and 2D bands suggests that the sp<sup>2</sup>-hybridized carbon structure is intact with an increase in the defect density (scale bar is 3  $\mu$ m).



Figure 5. (a) Variation in the intensity ratios of the third RBM to that of the first RBM (blue) and variation of the peak width of the G band (wine color) for various steps (electrochemical conditions labeled in Figure 2 in the same order). Each point is the mean of the ratios from all four sets of *in situ* Raman data considered in the article. Their standard deviation is given as the error bar. (b) Cyclic voltammograms of pristine SWNT mixture, SWNT oxide, and graphene nanoribbons in the potential window from -0.7 to 0.7 V vs MMS in 0.5 M  $H_2SO_4$  (same as used for the *in situ* Raman measurements) using a glassy carbon electrode at 100 mV/s scan rate. Arrows in the figure indicate the potential where SWNTs are selectively oxidized or reduced.

in metallicity.<sup>30</sup> We have also fitted the 2D band with a Lorentzian to measure the variation accurately. It is seen that there is a decrease in the intensity of the 2D band as the transformation progresses. We have also found from the spectral deconvolution data that there is a decrease in the width of the 2D band (Figure S13), which is an indication of the decoupling of the layers: the separation of individual tubes from one another upon unzipping in this particular experiment. As the sample under study was a bundle

(not isolated SWNTs), it is intuitive that the integrity of the bundle might be affected by the unzipping process, as evident from Figure S10. However, it is difficult to know the details of the modification happening to the bundle using the available observations. The variation of the RBM at negative electrode potentials can happen only after the prior application of a positive potential. This suggests that the oxidation followed by reduction enhances unzipping.

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The applied electric field initiates the breaking of sp<sup>2</sup> carbon bonds, perhaps at the middle (longitudinal) region of the side wall of the nanotubes, where a few topological defects can act as the epicenter (Stone-Wales defects). The above argument is supported by molecular dynamic simulations on MWNTs<sup>31</sup> and our TEM measurements (Figure S10 D). This defect generation continues in the longitudinal direction due to the field gradient, as evidenced by the subtle changes in the voltammogram (Figure 5b) similar to the changes seen in in situ Raman features. Broken SWNTs along a straight line are stretched farther away by the tension in the curved surface, which could result in the transformation into graphene oxide layers.<sup>32</sup> Cyclic voltammograms for the oxidation of SWNTs within the potential window from -0.7 to 0.7 V (vs mercury/ mercurous sulfate (MMS) reference electrode) show surface-confined peaks at the beginning with a capacitance value of 50 F/g. However, after 7 h of oxidation at a potential of 0.7 V, there is a large increase in the capacitance (83 F/g), partly due to the change in surface area originating from the morphological changes and the remaining contribution due to the creation of oxygen-containing moieties.

More significantly, the increase in nonfaradaic current with time suggests subtle morphological changes, including that of the area. By keeping the potential at 0.7 V for 7 h, the oxidation of SWNTs generates an enormous number of oxygen functionalities (mainly for semiconducting types). At the end, interestingly the open-circuit potential also increases by 55 mV, clearly revealing the formation of many of these groups, which usually happens because of the creation of functional groups due to oxidation. Oxidative unzipping, which increases the surface area per SWNT, also enhances the capacitance, accounting for the increase in area as well as the formation of functional groups (especially, oxygen-containing functional groups). After selective reduction of SWNT oxide at -0.7 V for 7 h, there is a gradual decrease in the capacitance ascribed to the removal of oxygen functionalities (from X-ray photoelectron spectra in ref 18) from unzipped tubes implying faster kinetics compared to that during the oxidation step.33 Peaks at -0.57 and -0.38 V correspond to oxygen reduction and hydroxide formation.

In addition to the electric field effects as addressed above, structural characteristics of SWNTs such as chirality and diameter could also lead to specified preferences for the unzipping process, as evidenced by the *in situ* Raman spectroscopic measurements. In order to clarify their influence and to understand further the fundamental role of oxygen in unzipping, we have performed calculations using the spin-polarized density-functional tight-binding (DFTB) method. On the basis of geometrical optimization of three sets of SWNTs with different chiralites and diameters,



Figure 6. Optimized configurations of (4, 4), (5, 2), and (7, 0) SWNTs with *n*O (n = 1, 2) by self-consistent charge formalism (SCC-DFTB-D). These chiralities were chosen so that the *n*O addition is visually identifiable along with clarity in the direction of oxygen addition. A number of various chiralities were used for the DFTB-D study of the dependence of the *n*O binding energy on the oxygen attachment direction and diameter of the SWNT.

relative energy changes in forming epoxy groups on the outer walls were studied. We classify carbon nanotubes by their diameter into three sets; the first set includes chiralities (4, 4), (5, 2), and (7, 0), the second set (5, 5), (6, 3), and (9, 0), and the last set (6, 6), (7, 4), and (11, 0). All three sets of SWNTs have lengths between 2 and 3 nm. Both ends of the SWNTs are terminated chemically by hydrogen atoms.

By forming epoxy groups on graphitic carbon structures, the underlying sp<sup>2</sup> carbon–carbon bonds will be elongated; thus SWNTs can be unzipped, or cut, by oxidation into graphene nanoribbons with specific width depending on the structures of SWNTs.<sup>34</sup> It is shown that, by preferential aligning, the total energy of epoxidized SWNTs could be lowered.<sup>35,36</sup> As illustrated in Figure S14, there are several possible pathways for cutting by binding oxygen atoms, as described by their relative orientation to the axis of SWNTs. In the first set of SWNTs for example, there are two cutting directions for armchair (4, 4) nanotubes, with different angles to the axis,  $0^{\circ}$  and  $30^{\circ}$ , respectively. For chiral (5, 2) SWNTs, angles are 16.1°, 52.9°, and 76.1°, and for zigzag (7,0) SWNTs, the angles are  $30^{\circ}$  and  $60^{\circ}$  for zigzag nanotube (7,0). The other two sets have similar cutting mechanisms with multiple pathways. All the structures have been optimized, and the most stable structures in the first set of SWNTs and their oxidized derivatives are shown in Figure 6. For the armchair and chiral SWNTs, the ground states of the products are closed-shell singlet, while for zigzag ones, quintet states with four unpaired electrons on the nanotube ends are preferred energetically. The energies of epoxidized structures with one and two oxygen atoms are listed in Table S2.

According to the Bell-Evans-Polanyi (BEP) principle, the difference in activation energy between two reactions of the same family is proportional to the

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Figure 7. Dependance of oxygen binding on the diameter of SWNT. Variation of the binding energy ( $E_b$ ) of the SWNTs + O (n = 1, blue data) (a) and (n = 2, maroon) (b) with the diameter of SWNTs.

difference of their enthalpy of reactions. Thus the binding energies calculated here offer explicit evidence to assess the reaction barrier of SWNT oxidation.<sup>37</sup> This is confirmed by direct comparison between calculated binding energies and reaction barriers using the climbing nudged energy band (CNEB) method using the first-principles method (details given in the Supporting Information as text and Figure S15). From the results we can clearly see that, for SWNTs with the same chirality, it turns out that SWNTs with smaller diameters have higher oxygen-binding energies; that is, one oxygen cutting through epoxidation is energetically more favorable for SWNTs with smaller diameters than those with larger diameters. As an example,  $E_{\rm b}$  for (7, 0) is 5.1436 eV, while for (9, 0) and (11, 0), it is 4.3011 and 4.1384 eV, respectively. For all structures under investigation, we find the direction of epoxidation prefers to be aligned to the nanotube axis. There is also a distinct dependence of  $E_{\rm b}$  on the angle of chirality  $\theta$  (for a nanotube with chiral index  $(n, m), \theta = \tan^{-1}[\sqrt{3m}/(m+2n)], e.g., \theta = 0^{\circ} \text{ and } 30^{\circ} \text{ for}$ zigzag and armchair CNTs, respectively) of an SWNT, as shown in Figure S16. As the chiral angle of graphene lattice increases, E<sub>b</sub> decreases and the binding of oxygen atoms through epoxy groups is less preferred.

In order to correlate the DFT calculations with the *in situ* Raman spectroscopic observations, we have performed similar calculations for SWNTs with the chiralities, the same as in the Raman studies (Table S3 and Figure S17). The calculations matched well with our experimental results for certain SWNTs, as the binding energies of 2O addition for (12, 0), (12, 5), and (11, 7) with relatively large diameters are 9.65, 9.86, and 9.90 eV, whereas for (9, 3), (10, 1), and (10, 4) the binding energies are 10.47, 10.14, and 10.20 eV, respectively (Figure 7). The data indicate that, for the first three SWNTs, oxygen addition is more difficult when compared to the later ones. The energetics of oxygen addition is a direct measure of the ease of unzipping; that is, (9, 3) and (10, 1) can be be unzipped with lesser energy (low electrode potentials), whereas (12, 5) and (11, 7) need relatively higher energy to get unzipped. These structural characteristics, with additional effects of the applied field clarified above (not included in the calculations), and potentially the interaction between substrate and SWNTs<sup>38</sup> determine the preference of oxidation and cutting processes of SWNTs.

#### CONCLUSIONS

Here we report an in situ Raman spectroscopic and microscopic investigation of the electrochemical unzipping of SWNTs. From careful observation of the RBMs and by using inputs from resonant Raman scattering theory, we understand that two types of metallic SWNTs with chiralities (10, 4) and (12, 0) are opened up rapidly at 0.36 V followed by a gradual opening of another two metallic SWNTs with chiralities (9, 3) and (10, 1) at 1.16 V. This is again followed by the slow unzipping of another two kinds of semiconducting nanotubes with chiralities (11, 7) and (12, 5) at a relatively high potential (-1.66 V). It has been observed that smaller size SWNTs are unzipped at relatively low electrode potentials. A gradual decrease in the metallicity of the SWNT ensemble was confirmed from the careful observation of the width of the G band. An increase in the D (defect) band with retention of the 2D band suggests unzipping of nanotubes forming graphene ribbons. A CV study confirms selective oxidation of SWNTs at an applied potential of 0.7 V for 7 h. Oxidative unzipping is evidenced by the improvement in capacitance. In the next reduction step, SWNT-oxide becomes graphene, which is clear from the subtle changes in the voltammograms with a decrease in the capacitance. On the basis of DFTB calculations, we show that there is a dependence of the diameter and chirality of an SWNT on the binding energies of single and double oxygen atoms as in-line epoxy groups. This trend is similar to the in situ Raman spectroscopic

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agnanc www.acsnano.org observations, suggesting that the mechanism of unzipping is likely to be the formation of epoxides on SWNTs and their successive transformation to graphene ribbons.

Preparation of the SWNT Sample. The sample of SWNTs from Carbon Nanotechnologies Inc., which is a mixture of semiconducting and metallic nanotubes, was purified according to the following protocol. The mixture was heated for 12 h at 250 °C in a furnace. It was further treated with 15 mL of concentrated HCl, thereby removing the metal catalysts as their chlorides. The acid-treated sample was then filtered using a membrane (0.2  $\mu$ m pore size) filter to obtain bucky paper, which was neutralized with a 1 M solution of NaHCO<sub>3</sub>, until the filtrate showed a pH greater than 7.0. The unreacted acid was removed followed by washing with copious amounts of water. The residue collected was dried at 70 °C for 6 h and preserved under vacuum until further use. The dispersion of the purified SWNT was prepared by taking 1 mg of the sample in 10 mL of dimethyl formamide (purchased from Qualigens) and sonicating it for 2 h with control over temperature.

Electrochemical Cell for in Situ Raman Measurements. A 50  $\mu$ L sample of the SWNT dispersion in DMF was drop casted on the working electrode of the electrochemical cell. The cell was designed in such a way that both the electrodes (working and counter) are on the conducting side of the indium-doped tin oxide coating (conductivity of 40  $\Omega$  cm<sup>-1</sup>, purchased from Nikon Sheet Glass Ltd.). We made a discontinuity on the conducting side of the glass slide by removing the conducting layer by scratching to create the electrodes (inset of Figure 1). The cell was covered with a coverglass, through which the process of unzipping can be observed using Raman spectroscopy. Three sides of the cell were sealed with Teflon tape to make it a cavity with one side open in order to allow the insertion of the electrolyte. The electrolyte (0.5 M H<sub>2</sub>SO<sub>4</sub>) was injected into the cell with the help of a syringe, which was then distributed into the cell by means of capillary action as a cell column thickness of around 0.15 mm was used. A coverglasscorrected ( $\infty$ /0.17) 100 $\times$  oil immersion objective (NA 1.3) whose working distance is 0.23 mm was used for spectral measurements. The potential was applied using a dc source in the range -5.0 to +5.0 V.

Confocal Raman Spectroscopy and Imaging. Confocal Raman measurements were done with a WiTec GmbH, CRM  $\alpha$ S300 instrument having a 532 nm Nd:YAG laser as the excitation source. The effective scan range of the spectrometer was 0–3800 cm<sup>-</sup> with a 600 grooves/mm grating, and the dispersed light intensity was measured by a Peltier-cooled charge coupled device (CCD). Raman imaging was done using the same grating, with an integration time of 100 ms. The piezoelectric scanner with maximum scanning area of 100  $\mu$ m  $\times$  100  $\mu$ m enabled the movement of the electrochemical cell (which is at the focal plane of the objective) for scanning. Each image contains 200 pixels in 200 lines (40 000 pixels) with each pixel having a Raman spectrum of a particular spatial position. Single-spot spectra were also acquired with larger integration times. For improved resolution and to ascertain peak positions, 1800 grooves/mm grating was used while acquiring single-spot spectra. The intensities of the desired portion of the spectra, collected over all of the pixels, were compared by Scan CTRL Spectroscopy Plus Version 1.32 software, to construct colorcoded images. Also, the image corresponding to various features of graphene, namely, RBM I, RBM II, RBM III, D, G, and 2D, was filtered from the image using WiTec Project 3.2

**Transmission Electron Microscopy.** TEM imaging was performed using a JEOL 3010 instrument. The accelerating potential used for imaging the graphene ribbons was 200 kV. The sample was drop cast on a copper grid and was dried in ambient conditions prior to the TEM analysis.

**Density Functional Theory Calculations.** All calculations here are carried out using the DFTB+ program.<sup>39,40</sup> DFTB is an approximate density functional theory method based on the

tight-binding approach that utilizes an optimized minimal linear combination of atomic orbital (LCAO) Slater-type all-valence basis set and a two-center approximation for Hamiltonian matrix elements. The Coulombic interaction between partial atomic charges was determined using the self-consistent charge (SCC) formalism. Slater–Kirkwood-type dispersion was employed for van der Waals and  $\pi$ – $\pi$  stacking interactions. This approach has been shown to give a reasonably good prediction of carbon nanostructures and their functional derivatives.<sup>41,42</sup>

*Conflict of Interest:* The authors declare no competing financial interest.

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Supporting Information Available: Raman images filtered from RBM I, RBM II, RBM III, D band, G band, and 2D band for all eight electrochemical conditions (steps) are given along with the average Raman spectra obtained from automated cluster analysis (which showed similarity to the manually averaged spectra) by the WiTec Project software. TEM images are also included to show the unzipped SWNTs. Supporting evidence for the Bell–Evans–Polanyi principle by additional first-principles calculations is provided, which compares binding energies of oxygen atoms and the reaction barriers. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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# Supplementary Information

# Sequential Electrochemical Unzipping of Single Walled Carbon Nanotubes to Graphene Ribbons Revealed by *in-situ* Raman Spectroscopy and Imaging

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## Complete disappearance of RBM from specific sample regions.



**Figure S1.** Images showing the bundle at two electrochemical conditions (given at the top of the images) and the RBM spectra corresponding to these conditions from specific sample areas (marked green) show the complete disappearance of RBM II.

Note: It might appear that there is considerable intensity of RBM II for some electrochemical conditions other than open circuit (Figure 2). This is basically due to an averaging effect. Generally, we take spectra from regions where there is an increase in the D band intensity in

order to study the spectral evolution, as presented in Figure 2. It should be noted that the unzipping process need not happen throughout the sample region under study due to the possible inhomogeneity in the local potential. In order to clarify the statement that there is disappearance of RBM II at 0.36 V, we have selected certain specific regions of the sample (marked by green color in the above images) for two electrochemical conditions and averaged spectra from those regions are presented above. It is evident that there is clear disappearance of RBMII.

Variations of the spectral images at the intermittent electrode potentials.



**Figure S2.** Evolution of RBM for various intermediate reduction steps corresponding to the oxidizing steps given in Figure 2 of the main article. The types of RBMs are being labeled at the top of each column. Each row gives the images of three RBMs at the conditions given at the left of the respective row.



after 7 h of 1.16 V after 7 h of -1.16 V after 7 h of 1.66 V

**Figure S3.** Evolution of the D band for various intermediate potentiostatic conditions such as just after 0.36 V (a), after 7 h of 0.36 V (b), after 7 h of -0.36 V (c), after 7 h of 1.16 V (d), after 7 h of -1.16 V (e), and after 7 h of 1.66 V (f).

# immediately after the application of 0.36 V



after 7 h of 1.16 V

after 7 h of -1.16 V

after 7 h of 1.66 V

Figure S4. Evolution of the G band for various intermediate potentiostatic conditions such as just after 0.36 V (a), after 7 h of 0.36 V (b), after 7 h of - 0.36 V (c), after 7 h of 1.16 V (d), after 7 h of -1.16 V (e) and after 7 h of 1.66 V (f).

# immediately after the application of 0.36 V

after 7 h of 0.36 V after 7 h of -0.36 V



after 7 h of 1.16 V

after 7 h of -1.16 V

after 7 h of 1.66 V

**Figure S5.** Evolution of the 2D band for various intermediate potentiostatic conditions such as just after 0.36 V (a), after 7 h of 0.36 V (b), after 7 h of - 0.36 V (c), after 7 h of 1.16 V (d), after 7 h of -1.16 V (e) and after 7 h of 1.66 V (f.)



Reproducibility of Raman spectroscopic observations.

**Figure S6.** Variation of the different spectral features of a particular SWNT bundle under various electrochemical conditions as labeled in the graph. Inset is showing the variation of the RBM regions showing a response similar to what is described in Figure 2. This set of data also shows a similar kind of response for the variation of the peak structure and width in the G band region. There is also a reduction in the intensity of the 2D band along with a slight decrease in

its width.  $I_{RBM III}$  /  $I_{RBM I}$  vary from 1.86 to 0.54 along with an increase in D band intensity. Featureless regions of the spectra are used to place the insets.



**Figure S7.** Variation of the different spectral features of another SWNT bundle under various electrochemical conditions as labeled in the graph. Inset is showing the variation of the RBM regions showing a response similar to what is described in Figure 2. A similar kind of response for the variation of the peak structure and width in the G band region was observed in this case

too.  $I_{RBM III}$  /  $I_{RBM I}$  vary from 1.26 to 0.25 along with an increase in the D band intensity. Featureless regions of the spectra are used to place the insets.



**Figure S8.** Variation of the different spectral features of another SWNT bundle under various electrochemical conditions as labeled in the graph. Inset is showing the variation of the RBM regions showing a response similar to what is described in Figure 2. This set of data also a similar kind of response for the variation of the peak structure and width in the G band region.  $I_{RBM III} / I_{RBM I}$  vary from 2.5 to 0.19 along with an increase in D band intensity. This along with

Figure S6 and S7 shows the reproducibility of the experimental results presented in the manuscript. Featureless regions of the spectra are used to place the insets.



Transmission electron microscopy to verify the formation of GNRs from SWNTs.

**Figure S9.** (A) TEM image showing the pristine SWNT bundle (scale bar is 20 nm) and (B) High resolution image of another bundle showing the well defined edges of the SWNTs (scale bar - 10 nm). The guide to eye lines in B with colors magenta, red and dark yellow are placed parallel to the walls of SWNTs with diameters 1.2, 1.0 and 1.6 nm, respectively. The SWNT image of the sample shows the integrity of the bundle.



**Figure S10.** TEM images (A-D) showing unzipped SWNTs as graphene nanoribbons. The scale bar is 20 nm for all figures except for C in which the scale bar is 10 nm. Figure A shows criss-crossed graphene ribbons whereas B shows a twisted ribbon. Both A and B in effect show the separation of the ribbons from the bundle upon unzipping. Figure C shows a single graphene ribbon whose average width is around 6 nm. A closer look at image D reveals that an SWNT (1.4

nm, wine red colored arrows) opens up into a graphene ribbon (5 nm, blue colored arrows). The width is 2.5 nm where the red arrows are present and it is 4 nm where the dark yellow arrows are pointed at. The bottom part of the image shows a narrow ribbon of width 3.5 nm (magenta arrows). Considering the diameters of the SWNTs used, this image represent most likely the partial unzipping of an SWNT and it appears that the unzipping originates at the middle portion (lengthwise) of the side wall rather than at the ends of the nanotubes.





**Figure S11. Deconvolution of the Raman spectrum.** Raman spectra fitted with 4 Lorentzian peaks, one at D band centered around 1345 cm<sup>-1</sup>, another 2 peaks at the G band region, out of which the peak at lower Raman shift is denoted G\* and the one around 1598 cm<sup>-1</sup> is the G band itself with the last one being the 2D band. As it was obvious that there will be an increase in the D band intensity, we focused our attention majorly on to the last 3 peaks namely, G\*, G and 2D.

Three parameters of each of the component peaks, such as peak position, width and area are evaluated for all 4 sets (Figure 2, S17, S18 and S19) of unzipping data out of which only that of the Raman data in Figure 2 are given below as a table (Table S1). Statistical variations of some of these parameters are given in Figure 5a, S12 and S13.

Various	Electrochemical	Peak	Area	Peak width	$R^2$ value	Reduced
steps	conditions	position cm <sup>-1</sup>		$(cm^{-1})$		$\chi^2 (10^{-4})$
		1551 (G*)	31.8	89.8		
1	0 V – open	1596 (G)	25.5	18.7	0.965	2.54
	circuit	2659 (2D)	39.1	52.8		
		1568 (G*)	28.5	50.6		
2	Immediately	1596 (G)	25.4	18.7	0.970	2.06
	after 0.36 V	2660 (2D)	29.8	53.5		
		1564 (G*)	26.7	67.1		
3	7 h of 0.36 V	1597 (G)	26.1	18.4	0.962	2.43
		2661 (2D)	28.2	49.7		
		1565 (G*)	25.5	68.4		
4	7 h of -0.36 V	1598 (G)	25.4	18.1	0.968	2.11
		2661 (2D)	33.7	50.3		
		1570 (G*)	24.5	63.0		
5	7 h of 1.16 V	1598 (G)	24.7	17.5	0.959	2.44
		2662 (2D)	26.2	49.6		
		1569 (G*)	23.0	61.2		
6	7 h of -1.16 V	1598 (G)	25.3	17.6	0.959	2.39
		2662 (2D)	25.4	49.8		
		1576 (G*)	19.4	49.3		
7	7h of 1.66 V	1597 (G)	23.4	15.9	0.955	2.44
		2663 (2D)	20.7	48.6		
		1578 (G*)	29.9	56.3		
8	7 h of -1.16 V	1598 (G)	30.2	19.1	0.922	3.82
		2662 (2D)	16.2	46.4		

Table S1. Deconvolution of the Raman spectra with 3 Lorentzian components.

The values of the coefficient of determination ( $R^2$ ) and the reduced  $\chi^2$  are given in the table to assess the quality of the fit. The  $R^2$  values give a better estimate of the quality of the fit as the reduced  $\chi^2$  values might get scaled with a scaling of the intensity values (all spectra are

normalized using the G band and translated vertically for clarity). The peak widths given in the table are full width at half maximum (FWHM). The possibility of giving a similar deconvolution data for the RBM region will be improper as the intensity of RBM peaks are generally very less and also because of the lack of proper peak shape owing to the low intensity. Also there can be a confusion arising due to the averaging effect as discussed in Figure S1 and the following text.



**Figure S12.** (a) Variation of the average of G\* (labeled in Figure S11) band peak width for the successive variations in the electrochemical conditions and (b) Variation of the average position of the G\* band. These data were obtained by considering all 4 sets of unzipping data (Figure 2, S6, S7 and S8) and their standard deviation from the mean value is presented as the error bar.



**Figure S13.** Variation of the width of the Lorentian peak fitted to the 2D band centered at 2660 cm<sup>-1</sup>. This data was obtained by considering all 4 sets of unzipping data (Figure 2, S6, S7 and S8) and their standard deviation from the mean value is presented as the error bar.

Details of the DFTB calculations.



Figure S14. Schematic showing various cutting (oxygen attachment) directions of CNTs with different chiralities. The 1-oxygen and 2-oxygen binding energies were calculated for similar

directions of the SWNTs having chiral indices of interest and presented in the following discussion.

## Relation between the oxygen binding energies and reaction barrier of oxidation

In physical chemistry, there is a general rule as the Bell–Evans–Polanyi (BEP) principle,<sup>1-4</sup> which observes that the difference in activation energy between two reactions of the same family is proportional to the difference of their enthalpy of reaction. It indicated that for a series of SWCNTs, the oxidation process happened on the tubes are coincident of the BEP principle. The reaction energy barrier is proportional to the binding energy calculated in this work.



**Figure S15.** The transition state and barrier (in eV) of the sencond Oxygen adding process in SWCNT (4,4).

To certify the validity of this principle in our system, we performed density functional theory (DFT) based first-principles transition-state calculations for a (4,4) nanotube with oxygen atoms added in different directions. The generalized gradient approximation (GGA) with the Perdew-Burke-Ernzerhof (PBE) functional was used in the calculations.<sup>5</sup> The plane wave basis set with

an energy cutoff of 400 eV was used and the criterion of convergence is set as the force on atom below 0.03 eV/Å. The transition states were searched and their barriers were calculated by the climbing nudged energy band (cNEB) method as implemented in the Vienna Ab initio Software Package (VASP).<sup>6,7</sup> It is found that if the oxygen adding direction (defined by the angle  $\theta$  along the tube axis) of (4,4) nanotube is 0°, the transition state does not exist, indicating a spontaneous reaction. However, if  $\theta = 60^{\circ}$ , there is an energy barrier of 0.498 eV (see Figure S15). It turns out that SWNTs with smaller diameters have higher oxygen binding energies and binding oxygen atoms through epoxy groups is preferred. The transition states calculations are consistent with the BEP principle, allowing us to make the abovementioned conclusion with DFTB calculations for the oxygen binding energies.

CNTs(+O)	Singlet (eV)	Quintet (eV)	Binding energy (eV)
(4,4)	-6921.31		
(4,4)-0°-O	-7011.18		5.90
(4,4)-0°-2O	-7101.49		12.23
(4,4)-60°-O	-7009.55		4.27
(4,4)-60°-20	-7097.03		7.77
(5,2)	-7455.52		
(5,2)-16.1°-O	-7545.43		5.92
(5,2)-16.1°-20	-7635.81		12.32
(5,2)-52.9°-O	-7543.83		4.33
(5,2)-52.9°-20	-7632.66		9.18
(5,2)-76.1°-O	-7543.79		4.28
(5,2)-76.1°-20	-7631.52		8.04
(7,0)		-6710.62	
(7,0)-30°-O		-6799.74	5.14
(7,0)-30°-2O		-6889.64	11.07
(7,0)-90°-O		-6798.84	4.25
((7,0)-90°-20		-6886.51	7.93
(5,5)	-8667.97		

Table S2. The binding energies and chiralities of the three sets of SWNTs

	1		
(5,5)-0°-O	-8757.33		5.38
(5,5)-0°-2O	-8847.40		11.47
(5,5)-60°-O	-8756.01		4.07
(5,5)-60°-2O	-8843.42		7.50
(6,3)	-8078.05		
(6,3)-19.11°-O	-8167.47		5.44
(6,3)-19.11°-20	-8257.51		11.50
(6,3)-79.11°-O	-8166.00		3.98
(6,3)-79.11°-20	-8253.46		7.45
(6,3)-139.11°-O	-8166.15		4.12
(6,3)-139.11°-20	-8253.91		7.90
(9,0)		-8646.05	
(9,0)-30°-O		-8734.33	4.30
(9,0)-30°-2O		-8824.29	10.29
(9,0)-90°-O		-8733.98	3.95
(9,0)-90°-2O		-8821.39	7.39
	10412 22		
	-10412.23		5.04
(6,6)-0°-0	-10501.24		5.04
(6,6)-0°-20	-10591.20		11.01
(6,6)-60°-0	-10499.99		3.79
$(6,6)-60^{\circ}-20$	-105/7.52		/.36
(7,4)	-11895.93		<b>7.11</b>
(7,4)-21.05°-0	-11985.02		5.11
(7,4)-21.05°-20	-120/4.85		10.96
(7,4)-81.05°-0	-11983.78		3.88
(7,4)-81.05°-20	-120/1.16		1.27
(7,4)-141.05°-0	-11983.91		3.99
(7,4)-141.05°-20	-120/1.52	10570.00	/.64
		-105/8.29	
(11,0)-30°-0		-10666.41	4.14
(11,0)-30°-20		-10/56.09	9.84
(11,0)-90°-0		-10666.11	3.84
(11,0)-90-20		-10753.44	7.19
0	-83.98		


**Figure S16**. The relationship between the binding energy ( $E_b$ ) of the CNTs + nO (n = 1, 2) and and the nO addition angle with respect to the tube axis of SWNTs in the first set of calculations. Details are given in Table S2.

Table S3.	Details of th	e DFT	calculations	for the	SWNTs	with	same	chiralities	as in	the	Raman
spectrosco	opic observati	ons.									

CNTs	Diameter (nm)	Singlet (eV)	Triplet (eV)	Quintet (eV)	Binding energy (hartree) (eV)	Binding energy (eV)
(9,3)-13.90°-O	0.791		-434.38		-0.16	4.34
(9,3)-46.10°-O			-434.37		-0.15	3.99
(9,3)-73.90°-O			-434.36		-0.14	3.83
(9,3)-13.90°-20			-437.69		-0.38	10.47
(9,3)-46.10°-20			-437.59		-0.28	7.73
(9,3)-73.90°-20			-437.57		-0.26	7.17
(10,1)-4.72°-O	0.829			-416.22	-0.16	4.23
(10,1)-55.28°-O				-416.21	-0.15	4.10
(10,1)-64.72°-O				-416.21	-0.14	3.85

(10,1)-4.72°-20			-419.52	-0.37	10.14
(10,1)-55.28°-20			-419.50	-0.35	9.64
(10,1)-64.72°-2O			-419.42	-0.27	7.22
(10,4)-16.10°-O	0.893	-505.36		-0.15	4.21
(10,4)-43.90°-O		-505.35		-0.14	3.91
(10,4)-76.10°-O		-505.35		-0.14	3.77
(10,4)-16.10°-20		-508.67		-0.37	10.20
(10,4)-43.90°-20		-508.57		-0.28	7.55
(10,4)-76.10°-20		-508.56		-0.27	7.08
(12,5)-16.63°-O	1.068	-611.78		-0.15	4.06
(12,5)-43.37°-O		-611.77		-0.14	3.84
(12,5)-76.63°-O		-611.77		-0.14	3.71
(12,5)-16.63°-20		-615.08		-0.36	9.86
(12,5)-43.37°-20		-614.99		-0.27	7.42
(12,5)-76.63°-20		-614.98		-0.26	6.99
(11,7)-22.69°-O	1.074	-692.37		-0.15	4.06
(11,7)-37.31°-O		-692.36		-0.14	3.81
(11,7)-82.69°-O		-692.360		-0.14	3.72
(11,7)-22.69°-20		-695.67		-0.36	9.90
(11,7)-37.31°-20		-695.58		-0.27	7.26
(11,7)-82.69°-2O		-695.57		-0.26	7.03

Two sets of results came out of the DFTB calculations as given in the above tables. (i) The nO binding energy increases with decrease in SWNT diamater and (ii) as the angle between the cutting direction (nO addition) and the tube axis is smaller, the cutting is easier to happen, evidenced by an increase in binding energy.



**Figure S17**. The relationship between the binding energy (Eb) of the CNTs + nO (n = 1, 2) and the angle of epoxy addition with respect to the axis of SWNTs used in the Raman experiment.

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# Mixed-Monolayer-Protected Au<sub>25</sub> Clusters with Bulky Calix[4]arene Functionalities

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**Supporting Information** 

**ABSTRACT:** Although various complex, bulky ligands have been used to functionalize plasmonic gold nanoparticles, introducing them to small, atomically precise gold clusters is not trivial. Here, we demonstrate a simple one-pot procedure to synthesize fluorescent magic number  $Au_{25}$  clusters carrying controlled amounts of bulky calix[4]arene functionalities. These clusters are obtained from a synthesis feed containing binary mixtures of tetrathiolated calix[4]arene and 1-butanethiol. By systematic variation of the molar ratio of ligands, clusters carrying one to eight calixarene moieties were obtained. Structural characterization reveals unexpected binding of the calix[4]arenes to the  $Au_{25}$  cluster surface with two or four thiolates per moiety.



**SECTION:** Physical Processes in Nanomaterials and Nanostructures

etal clusters in the size range below 2 nm are interesting materials because of their size-dependent properties, which differ from the properties of larger nanoparticles, bulk metal, or single atoms.<sup>1-3</sup> Within this size range, cluster sizes can be controlled with atomic precision, thus allowing precise investigations of structure-function relationships. These metal cores are typically passivated by an organic ligand shell, and therefore, these species are often referred to as monolayerprotected clusters (MPCs). Gold clusters passivated by thiolates,  $Au_n(SR)_m$  are one of the most studied MPCs because of their high stability. Due to the stability, these clusters can be prepared by wet chemistry methods in ambient conditions and processed like ordinary chemicals. As a result of developments in synthetic procedures, separation methods, and analysis techniques, several stable "magic number" MPCs, such as  $Au_{25}(SR)_{18}$ ,  $Au_{38}(SR)_{24}$ ,  $Au_{102}(SR)_{44}$ , and  $Au_{144}(SR)_{60}$ , have been isolated, and reasons for their stability have been recognized.1,4,5

Various bulky or structurally complex thiols (e.g., drug analogues, cyclodextrin derivatives, DNA oligonucleotides, polymers, dendrimers) have been used to produce plasmonic gold MPCs with applications including sensing,<sup>6</sup> diagnostics,<sup>7</sup> and drug delivery.<sup>8</sup> However, most studies related to magic number MPCs are focused on using simple alkane- or arenethiols,<sup>9–11</sup> though more complex, bulky ligands could bring valuable functionalities also to smaller clusters. This

shortcoming stems from major difficulties in reaching atomic precision by direct synthesis with structurally complex thiols. Atomically precise mixed-monolayer-protected clusters (MMPCs), on the other hand, can be realized by using, for example, ligand exchange reactions to existing magic number MPCs.<sup>12–14</sup> However, ligand exchange of bulky ligands is often severely hampered by kinetic effects (i.e., steric hindrance), thus restricting the extent of exchange or even making ligands completely unusable.<sup>15</sup> To overcome this hindrance, we investigate here a novel approach for synthesis of magic number MMPCs prepared directly by using thiol mixtures. This procedure enables a straightforward incorporation of functional, bulky ligands, whereas secondary small ligands facilitate size-focusing to discrete magic numbers and provide additional chemical stability by filling the possible defect sites on MMPCs.

Without doubt, capping clusters with specific functional groups is crucial regarding possible applications. Calixarenes are an interesting group of compounds that possess specific host–guest interactions with organic as well as metal cations.<sup>16</sup> Because of these interactions, calixarenes find applications, for example, in ion-sensitive electrodes and sensors,<sup>17</sup> stationary

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phases in chromatography,<sup>18</sup> and catalysis.<sup>19</sup> After the first report of calixarene-protected gold nanoparticles by Arduini et al.,<sup>20</sup> there has been an increased interest toward calixarene-modified gold nanostructures.<sup>21–25</sup> Recently, atomically precise MPCs (Au<sub>11</sub>) with five phosphine-bound calixarene moieties were realized by de Silva et al.,<sup>26</sup> and their structure was theoretically verified by Chen et al.<sup>27</sup> Important to note is that the number of calixarene ligands was fixed, and their bulkiness led to incomplete capping, leaving the gold core partly exposed.

Here, we aim at a one-pot synthesis for atomically precise MPCs with a tunable number of bulky ligands, where a second smaller ligand would allow a complete ligand monolayer to cover the gold core. A mixture of thiol-modified calix[4] arene (briefly, Calix-4SH) and 1-butanethiol (BuSH) is used to prepare atomically precise  $Au_{25}(Calix-4S)_x(BuS)_y$  clusters passivated by a mixed monolayer of these thiols (Chart 1).

#### Chart 1. Structures of Thiol Ligands



Calix-4SH in cone conformation was synthesized using a reported protocol;<sup>28</sup> details are given in the Supporting Information. Cone conformation, locked by four 4-mercaptobutanolate chains, was chosen to facilitate multidentate binding to the cluster surface. By systematically varying the Calix-4SH concentration in the synthesis feed, we are able to control the amount of calixarene functionalities on the clusters. To the best of our knowledge, there are no previous reports of creating atomically precise cluster cores carrying thiolated calixarenes. In addition, no studies of fluorescent gold clusters prepared using a mixed ligand feed could be found in the literature.

Au<sub>25</sub> clusters passivated with mixtures of Calix-4SH and BuSH were synthesized by modifying the method by Qian et al.<sup>10</sup> Details of the synthetic procedure can be found in the Supporting Information. Briefly, a mixture of Calix-4SH and BuSH was added to a solution containing HAuCl<sub>4</sub> and tetraoctylammonium bromide (TOAB) in tetrahydrofuran (THF). After the formation of colorless Au-thiolates, NaBH<sub>4</sub> was added in aqueous solution to obtain a polydisperse cluster mixture. This polydisperse cluster mixture was size-focused by allowing it to react until Au<sub>25</sub> cluster absorption features became prominent. The clusters were purified by repeated centrifugal washing with methanol followed by size-exclusion chromatography (SEC) to ensure removal of free Calix-4SH from the final cluster product. SEC served well to separate free Calix-4SH as well as a minor portion of larger cluster species from the final product (Figure 1, inset). The Au<sub>25</sub> cluster yields were typically 12-20%.

Absorption spectroscopy was initially used to get information about the core size of the clusters. Prominent absorption features were observed from all cluster samples at 680, 442, 400, and 320 nm, which are well-known features of the  $Au_{25}(SR)_{18}$  cluster core (Figure 1). In addition, details in the absorption spectrum revealed the charge state of the cluster. It has been shown that a broad absorption at 800 nm is an



**Figure 1.** UV-vis absorption spectra of  $Au_{25}$  clusters with varying Calix-4SH concentration (spectra are vertically shifted for clarity). The inset shows spectra before and after SEC.

indication of a negatively charged, reduced gold core as well as a ratio of 1.2 between ~400 and ~440 nm absorptions.<sup>29,30</sup> All Calix-4S-functionalized cluster samples showed these features, thereby indicating a majority of negatively charged cluster cores as observed also in previous reports from similar syntheses.<sup>10,29,30</sup> The counterion of these negatively charged clusters was TOA<sup>+</sup>, which was detected in the nuclear magnetic resonance (NMR) spectra of all Au<sub>25</sub>(Calix-4S)<sub>x</sub>(BuS)<sub>y</sub> clusters.

To further analyze the structure of the clusters, the ligand compositions were probed by electrospray ionization mass spectrometry (ESI-MS) and NMR analyses. The negative polarization ESI-MS spectra were, surprisingly, dominated by the pure  $Au_{25}(BuS)_{18}$  cluster. In positive polarization measurements, however, more abundant  $Au_{25}(Calix-4S)_r(BuS)_r$  clusters could be observed, with x ranging from 0 to 8 (neutral clusters as Cs<sup>+</sup> adducts and anionic clusters as 2Cs<sup>+</sup> adducts). Apart from Au<sub>25</sub>, no other cluster sizes were observed in ESI-MS.  $Au_{25}$  clusters are easily oxidized by atmospheric oxygen, as reported by Jin's group.<sup>29</sup> We also observed the same phenomenon, and the rate of oxidation was more pronounced with dried samples compared to clusters stored in THF solution. In vacuum-dried samples, signs of oxidation could be noticed in hours, and the samples were fully oxidized in a few days. Comparison of negative and positive polarization mass spectra shows that oxidation is more pronounced in the case of  $Au_{25}(Calix-4S)_x(BuS)_y$  clusters than for  $Au_{25}(BuS)_{18}$ . However, when stored free of oxygen, in the dark and at 4 °C, clusters exhibited no change in their spectral features even after 2 months of storage.

As expected, the use of a thiol mixture led to varying ligand compositions in the final products (see Table 1 for results from a sample with 0.36% Calix-4SH in the synthesis feed; data from other samples are presented in the Supporting Information). However, it can be easily observed that the amount of Calix-4S units attached to clusters clearly correlates with the amount of Calix-4SH inserted into the synthesis feed (Figure 2). Samples with 0-2, 1-3, 2-5, and 5-8 calixarene units were obtained by varying the Calix-4SH feed in the 0.36-7.0% range of the amount of BuSH. Even though this kind of synthesis with a binary mixture of thiols will rarely end up in a single ligand

Table 1. Cluster Compositions in Sample with 0.36% Calix-4SH in the Synthesis Feed

	Calix		
ion composition $(+nCs^+)$	tetradentate	bidentate	BuS
$Au_{25}(BuS)_{18}$	0	0	18
Au <sub>25</sub> (Calix-4S)(BuS) <sub>14</sub>	1	0	14
Au <sub>25</sub> (Calix-4S)(BuS) <sub>16</sub>	0	1	16
$Au_{25}(Calix-4S)_2(BuS)_{10}$	2	0	10
$Au_{25}(Calix-4S)_2(BuS)_{12}$	1	1	12
$Au_{25}(Calix-4S)_2(BuS)_{14}$	0	2	14



**Figure 2.** Variation of the amount of Calix-4S units on clusters when increasing the Calix-4SH content in the synthesis feed. For better visibility of the trend, the normal distribution for each sample has been overlaid on the histogram.

composition, we were still able to maintain good control on the final ligand compositions.

Au<sub>25</sub> clusters are known to be passivated by 18 thiolate ligands, arranged as 6 RS-(Au-SR)<sub>2</sub> units on top of a 13-atom icosahedral gold core.<sup>31-33</sup> All ions observed in our ESI-MS analysis could be interpreted to have 18 thiolates attached to the gold surface, suggesting that the well-known  $Au_{25}(SR)_{18}$ structure is retained regardless of the ligand composition. The clusters are also eight-electron "superatoms" as negatively charged species after the synthesis. The cluster compositions observed in ESI-MS measurements revealed interesting details on the binding of Calix-4S units. Interestingly, the vast majority of clusters (>99%) had an even number of BuS ligands attached. This suggests that Calix-4SH ligands are attached to the cluster surface predominantly with two or four thiolates because binding with one or three thiolates would also result in species containing an odd numbers of BuS. Binding only with two or four thiolates is also evident in clusters containing only one calixarene unit; Au<sub>25</sub>(Calix-4S)(BuS)<sub>15</sub> and Au<sub>25</sub>(Calix-4S)(BuS)<sub>17</sub> were not observed in ESI-MS analysis. Binding with two or four thiolates also supports our recent study of Calix-4S protected nanoparticles, in which a binding with an average of three thiolates was observed (unpublished results).

Calix-4SH binding to gold with two thiolates leaves the two other thiolates dangling. Chemical structure of the dangling thiols was investigated with ESI-MS and NMR studies. In NMR spectra, all resonances were broadened due to the existence of different ligand compositions and also various isomers in each composition due to different Calix-4S binding sites on Au<sub>25</sub>. The signals from thiol-neighboring methylene protons at 2.6 ppm were shifted downfield from free calixarene signals,



**Figure 3.** <sup>1</sup>H NMR spectra of Calix-4SH (bottom) and calixarenemodified  $Au_{25}$  clusters before (middle) and after (top) the Iodine Death reaction (7.0% Calix-4SH in the feed). Signals arising from the different parts of Calix-4SH are marked in color, and the signals from THF, THF stabilizing agent butylated hydroxytoluene, and TOA<sup>+</sup> are marked with *#*, *\**, and *§*, respectively.

suggesting no free thiol groups being present in the system (Figure 3). Additionally, the mass accuracies in ESI-MS experiments suggest no thiol groups being present in the clusters. Absence of S–H bonds was further confirmed by infrared absorption spectroscopy (see the Supporting Information). Therefore, it can be proposed that two dangling thiolates of bidentately bound Calix-4SH form a disulfide bridge with each other. Also, the peak positions in <sup>1</sup>H NMR studies support the formation of disulfides, as seen from the downfield shifts of CH<sub>2</sub> protons next to sulfur atoms.

The intensities of peaks in ESI-MS are influenced by the ionization efficiencies and transmission efficiencies of the ions through the ESI-MS interface. Considering the chemical and physical similarities of calixarene-decorated clusters, their ionization and transmission efficiencies can be approximated to be independent of ligand composition, thus allowing semiquantitative analysis of abundances of clusters with different ligand compositions (Figure 2). In addition to ESI-MS, the average ligand composition was probed with <sup>1</sup>H NMR analysis. The cluster cores were decomposed with the Iodine Death reaction, which quantitatively liberates the alkanethiolate monolayer as disulfide compounds and thus allows determination of ligand concentrations through integration of NMR signals.<sup>24,34</sup> The average ligand compositions measured by these independent methods are well in line with each other (Table 2).

It is worth noticing that the amount of calixarene finally attached to clusters is very high compared to the amount used in the synthesis. In all samples, the ratio n(Calix-4S)/n(BuS) on the cluster surface was 20–25 times higher than the n(Calix-4SH)/n(BuSH) ratio in the synthesis feed. This highly efficient binding is likely caused by the multidentate nature of Calix-4SH as it does not detach easily during the size-focusing process in which larger clusters are etched into Au<sub>25</sub> cores. Hence, polythiolated molecules are highly useful in cluster syntheses with thiol mixtures. Incorporation of thiols into complex molecules is often a tedious task, and cluster synthesis typically consumes large amounts of thiol ligands (usually Au/S  $\leq 1/5$ ). By using a mixture of thiols, the consumption of Calix-4SH is minimized because Calix-4SH efficiently binds to the cluster surface and a large excess of commercially available BuSH acts

	n(Calix-4S)/n(B)	buS) on clusters, %	
Calix-4SH in the feed, %	NMR	ESI-MS	average composition <sup>a</sup>
0.36	8	5	$Au_{25}(Calix-4S)_1(BuS)_{16}$
0.72	17	11	$Au_{25}(Calix-4S)_2(BuS)_{14}$
2.0	51	52	$Au_{25}(Calix-4S)_4(BuS)_8$
7.0	152	155	$Au_{25}(Calix-4S)_6(BuS)_4$
<sup><i>a</i></sup> Rounded to integers.			

as a secondary passivating ligand and, even more importantly, as an etchant that allows one to reach atomic precision of the gold core. It is also worth mentioning that ligand exchange of Calix-4SH to  $Au_{25}(BuS)_{18}$  clusters could not be accomplished, most probably due to the bulkiness of the incoming Calix-4SH ligand. Therefore, the use of thiol mixtures is the only suitable option for preparing clusters with Calix-4SH ligand.

The <sup>1</sup>H NMR spectra of clusters showed an interesting resonance pattern in the aromatic region consisting of a center signal and doublet side signals (Figure 3). The side signals were present in all samples, and their positions were independent of the amount of Calix-4S moieties on the cluster surface. Signals in the aromatic region were further investigated by 2D NMR measurements (see the Supporting Information). In COSY and TOCSY spectra, couplings were observed only between the doublets of the side signals, indicating that all three resonances (~7.1, 6.6, and 6.1 ppm) form separate spin systems. In the ROESY spectrum, however, an additional coupling was observed between the side signals at 7.1 and 6.1 ppm. This coupling suggests that these protons belong to a specific type of cluster-bound Calix-4S, which is further supported by the symmetric shape and magnitude of the side signals. The center signal at 6.6 ppm shows no coupling in ROESY, indicating that it originates from a cluster-bound Calix-4S with a chemical shift similar to the aromatic signal from free Calix-4SH.

Considering the 2/4 thiolate binding mechanism indicated by ESI-MS analysis, it is reasonable to suggest that the center signal originates from Calix-4S binding with four thiolates and the side signals from species binding with two thiolates. The side signals arise from a conformational distortion of the symmetric calixarene cup to a pinched cone conformation. "Locking" to a pinched cone conformation has been observed in tetrakis(n-alkoxy)calix[4] arenes at low temperatures, while the interconversion between cone and pinched cone conformations is rapid at room temperature.<sup>35</sup> Functionalization of upper and lower rims has also been used to lock calix[4]arenes to pinched cone conformation through covalent and hydrogen-bonding interactions.<sup>35–38</sup> On the contrary, conformational locking to a pinched cone by a steric impediment, as seen now in Au<sub>25</sub> cluster surfaces, has not been previously reported. It is worth noticing that the pinched cone conformation is not observed on larger nanoparticle surfaces protected by dithiolated<sup>24</sup> or tetrathiolated calix[4]arenes (unpublished results). Therefore, the origin of the preferential pinched cone formation can be attributed to the high surface curvature of the subnanometer-sized Au25 core, which sets specific requirements on the S-Au binding positions.

The effect of ligand composition on cluster fluorescence was also investigated. All clusters showed fluorescence in the near-infrared region (800 nm), and their quantum yields varied slightly (0.1-0.3%) with the amount of Calix-4S (see the Supporting Information). However, fluorescence from Au<sub>25</sub> clusters also strongly depends on the oxidation state of the

cluster core, as reported by Wu and Jin.<sup>39</sup> As the clusters easily oxidize, it is difficult to separate the effect of the ligand composition from slight variations in the oxidation state of clusters. It is worth noting that the shape of the excitation spectra is similar to the absorption spectra of clusters, a feature not present in existing literature of Au<sub>25</sub> clusters.<sup>12,39</sup>

In conclusion, atomically precise  $Au_{25}$  clusters passivated with Calix-4SH and BuSH were prepared in a robust one-pot synthesis by using a mixture of thiols in the synthesis feed. This type of synthesis could provide a general strategy for the incorporation of other bulky ligands to cluster surfaces. By varying the molar ratio of the ligands, clusters carrying up to eight calixarene moieties could be obtained. Calix-4SH was found to preferentially bind to a high curvature  $Au_{25}$  surface by two or four thiolates, and in the case of two thiolate binding, the calixarene cavity undergoes a distortion to a pinched cone conformation due to steric impediment. These clusters show promising potential for building cluster assemblies via host– guest interactions, which is a matter of further study.

#### ASSOCIATED CONTENT

#### Supporting Information

Details of the synthesis procedures and characterization techniques as well as further UV–vis, IR, NMR, and ESI-MS data. This material is available free of charge via the Internet at http://pubs.acs.org.

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#### Notes

The authors declare no competing financial interest.

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### SUPPORTING INFORMATION

# Mixed-Monolayer-Protected Au<sub>25</sub> Clusters with Bulky Calix[4]arene Functionalities

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#### Experimental methods and characterization techniques

#### Chemicals

Tetrachloroauric(III) acid (HAuCl<sub>4</sub> · 3 H<sub>2</sub>O,  $\geq$  99,9 %), tetraoctylammonium bromide (TOAB, 98 %), 1-butanethiol (BuSH, 99 %), sodium borohydride (NaBH<sub>4</sub>, 99 %), chloroform-d (CDCl<sub>3</sub>, >99,96 %), tetrahydrofuran (THF,  $\geq$  99,0 %), methanol (HPLC grade) were purchased from Sigma-Aldrich. Bio-Beads® S-X1 were purchased from Bio-Rad. All the chemicals were used as received without further purification. The water used in experiments was Milli-Q grade with a resistivity of 18.2 M $\Omega$ ·cm. The thiol-modified calix[4]arene (25,26,27,28-tetrakis(4-mercapto-n-butoxy)calix[4]arene, shortly Calix-4SH) was synthesized as described in the following section.

#### Synthesis of thiol-modified calix[4]arene (25,26,27,28-tetrakis(4-mercapto-n-butoxy)calix[4]arene)

Calix-4SH was synthesized as reported<sup>1</sup>. Dry dimethylformamide (190 ml) was placed into a flask and purged with nitrogen. Calix[4]arene-25,26,27,28-tetrol (9.43 mmol), dibromobutane (188.5 mmol) and NaH (56.6 mmol) were added into the flask. (Caution: NaH reacts violently with water!) The mixture was stirred for 20 min, after which the flask was heated to 80 °C. The reaction mixture was stirred under N<sub>2</sub> for five days.

The reaction was quenched with careful water addition and the mixture was extracted twice with chloroform. The organic phase was washed with distilled water twice and dried with anhydrous sodium sulfate. Organic phase was evaporated under high vacuum (3 mbar, 140 °C) in order to remove volatile organic substances and the most of the DBB. The residue was purified using column chromatography (chloroform:hexane 75:25, Rf 80%.). The yield was 49%. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) 6.3 (12H, s), 4.1 (4H, d), 3.6 (8H, t), 3.2 (8H, t), 2.9 (4H, d), 1.7 (16H, m).

A part of the product (3.77 mmol) of previous synthesis phase was placed in a nitrogen purged flask containing 125 ml of dry DMF. Thiourea (76.1 mmol) was added and the mixture was stirred for 20 min, after which the flask was heated to 80 °C. The mixture was stirred under nitrogen for 12 hours and then, was quenched by pouring the mixture into NaOH solution (3.8 %, 580 ml). The reaction mixture was stirred for one hour and finally the pH was adjusted to 4–5 using HCl. Product was filtered, washed with water, dried in vacuum and further purified using column chromatography (chloroform, Rf 75%.) Yield: 70%. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) 6.6 (12H, s), 4.4 (4H, d), 3.9 (8H, t), 3.2 (4H, d), 2.6 (8H, q), 2.0 (8H, m) 1.7 (8H, m), 1.4 (4H, t).

#### Synthesis of calixarene-modified Au<sub>25</sub> clusters

The calixarene functionalized clusters were synthesized by slightly modifying a method proposed by Qian *et al.*<sup>2</sup> Tetrachloroauric(III) acid (40 mg) was dissolved in 7.5 ml THF and 65 mg of TOAB was added while stirring the solution. The color of the reaction mixture changed from yellow to orange when continuing the stirring for 15 min. In a separate vial, a mixture of 55  $\mu$ l BuSH and a varying amount of Calix-4SH was prepared and dissolved into 500  $\mu$ l THF. The molar amount of Calix4SH in the thiol mixtures was varied between 0-7.0 % of the amount of BuSH. The thiol mixture was rapidly added to the reaction mixture under vigorous stirring (1200 RPM). The stirring was continued for 2 h during which the solution turned colorless. After that, 39 mg NaBH<sub>4</sub> dissolved in 2.5 ml ice-cold water was rapidly added to the reaction solution under vigorous stirring and the stirring was continued until distinct Au<sub>25</sub> cluster core absorption features were observed (Figure S1). The size-focusing process lasted typically 18-27 hours, the time increasing with the amount of Calix-4SH in the reaction mixture. When preparing clusters without Calix-4SH (Au<sub>25</sub>(BuS)<sub>18</sub>), the size-focusing period was reduced to five hours. After the size-focusing period, the solvent was removed from the reaction mixture by rotary vacuum evaporation and majority of excess thiols and TOABr was removed by centrifugal washing with methanol (4 times, 3000 RCF). Subsequently, the product was dissolved to THF and the white insoluble matter consisting most likely of Au(I)-thiolates was removed by centrifugal-tion. The clusters in THF were further purified by size-exclusion chromatography. In the case of Au<sub>25</sub>(BuS)<sub>18</sub>, methanol:water mixture (3:1 v/v) was used in centrifugal washing.

#### Size-exclusion chromatography

Bio-Beads® S-X1 (200-400 Mesh) was used as the stationary phase for size exclusion chromatography (SEC) as suggested in a recent work<sup>3</sup>. Briefly, nine grams of beads were swollen overnight in 90 ml THF and loaded in a column equipped with a glass frit. The beads were washed with several bed volumes of THF until a constant bed height (40 cm) was reached. The cluster samples were dissolved into 200  $\mu$ l THF and eluted at 0.5–1 ml/min. The product was collected in 1 ml fractions which were analyzed by absorption spectroscopy. After combining fractions with Au<sub>25</sub> clusters, the solvent was evaporated and product washed twice with methanol. The solid powder was dissolved in THF and oxygen was removed by bubbling nitrogen through the solution. The product was stored at 4 °C.

#### UV-visible absorption spectroscopy

Absorption spectra were recorded in UV-visible range with PerkinElmer Lambda 950 UV/Vis/NIR absorption spectrophotometer. Spectra were recorded in high quality quartz cells with 10 mm path length.

#### Fourier transform infrared spectroscopy

Transmission spectra were recorded with Thermo Nicolet Avatar 380 FT-IR spectrometer using a Thermo Scientific Smart Orbit attenuated total reflection (ATR) accessory with a type II diamond tungsten carbide crystal. The spectra were acquired by averaging 64 scans with 4 cm<sup>-1</sup> resolution. Cluster samples were drop-casted directly onto the ATR crystal from tetrahydrofuran and evaporated to dryness with nitrogen flow. Background spectrum (air) was acquired before measurements and a blank spectrum recorded with no sample was subtracted from the raw data to obtain final spectra of clusters.

#### Fluorescence spectroscopy

Fluorescence spectra of cluster solutions were recorded using a QuantaMaster 40 spectrofluorometer from Photon Technology International. A double excitation monochromator was used in the measurements to decrease the stray light level and the slits in excitation and emission monochromators were set to 5 nm. Fluorescence spectra were recorded using standard 90° measurement geometry and no filters in excitation or emission channel. The fluorescence spectra were corrected by subtracting a blank solvent background and by using instrument's excitation and emission corrections provided by the manufacturer.

#### Quantum yield determination

The quantum yields ( $\Phi_f$ ) of the clusters were measured by comparing the integrated emission intensities of cluster samples in THF to a known reference fluorophore 4-(Dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran (DCM) in ethanol ( $\Phi_f = 0.435$ ).<sup>4</sup> The absorbances ( $A_x$ ) at the excitation wavelength ( $\lambda_{ex} = 440$  nm) were adjusted to approximately 0.1 when determining quantum yields. Quantum yields were calculated according to equations 1 and 2, where the integrated emission intensities  $F^x$ , absorption factors  $f_x$  and refractive indices of sample (S) and reference fluorophore (R) are taken into account.

$$\Phi_f^S = \frac{f_R(\lambda_{ex})}{f_S(\lambda_{ex})} \frac{\int_{\lambda_{em}} F^S(\lambda_{em})}{\int_{\lambda_{em}} R^R(\lambda_{em})} \frac{n_S^2}{n_R^2} \Phi_f^R \tag{1}$$

$$f_x(\lambda_{ex}) = 1 - 10^{-A_x(\lambda_{ex})} \tag{2}$$

#### Electrospray ionization mass spectrometry

The mass spectrometric experiments were performed with a QSTAR Elite ESI-Q-TOF mass spectrometer equipped with an API 200 TurboIonSpray ESI source from AB Sciex (former MDS Sciex) in Concord, Ontario (Canada). The samples for positive polarization experiments were prepared by diluting THF stock solutions with THF/MeOH 7:1 ( $\nu/\nu$ ) where CsOAc was added to enhance the ionization. The final concentration of clusters in each sample solution was ~ 30 µM. The sample solutions for negative polarization experiments were prepared without CsOAc addition by diluting THF stock solutions with THF to give final sample concentration of ~ 40 µM. The samples were injected into the ESI source with a flow rate of 5 µl/min. The parameters were optimized to get maximum abundance of the ions under study. Room-temperature nitrogen was used as nebulization (10 psi ESI- and 35 psi ESI+) and as curtain gas (18 psi). The ion-source voltages of 5.5 kV for capillary, 50 V for the oriface plate (declustering potential), 10 V as potential difference between skimmer and pre-quadrupole, and between 250 V for the potential difference between the focusing ring and pre-quadrupole were used on positive polarization experiments (in negative polarization experiments the corresponding voltages were -4.5 kV, -20 V, -10 V and -250 V). Accumulation delay of 2 s, ion release delay of 6 ms and ion release width of 5 ms were used. Each spectrum was an average of spectra collected within 2 to 5 min, each of these containing 20 individual scans that were averaged before being sent from the instrument to data system. The measurement and data handling was accomplished with Analyst® QS 2.0

Software. Mass spectra were externally calibrated by using tetramethylated C2-resorcarene dendrimer (C2G3, compound 8)<sup>5</sup>. The monoisotopic resolution was not always obtained, but the charge states of the ions were determined by characteristic Cs<sup>+</sup> mass differences and by comparison of the peak shape to shape of theoretic isotopic distributions. The compositions of the ions were finally verified by comparing experimental *m*/*z* values with the theoretical ones. The n(Calix-4S)/n(BuS) values were calculated based on the intensities of the peaks of different compositions in ESI-MS spectra.

#### Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) analyses were performed with Bruker Avance III spectrometer operating at 400 MHz. Typically, 1-2 mg of clusters dissolved in 600  $\mu$ l CDCl<sub>3</sub> was used in analyses. For the analysis of mixed monolayer compositions, Iodine Death reaction was utilized. After measuring <sup>1</sup>H-NMR spectra of clusters, a 20  $\mu$ l drop of saturated iodine solution in CDCl<sub>3</sub> was added to the NMR tube and the tube was shaken for few minutes. After few hours, a deposit was detected at the bottom of the NMR-tube. <sup>1</sup>H-NMR spectrum was recorded again and broadened signals were replaced with sharp signals from free ligands. The Calix:BuS ratios were calculated from spectra after Iodine Death reaction by integrating the Calix-4SH aromatic signal (6.6 ppm, 12H) and BuSH methylene signal next to the sulfur atom (2.7 ppm, 2H).



Figure S1. UV-vis absorption spectra of the reaction mixture during the size-focusing process of Au<sub>25</sub> clusters with 2.0 % Calix-4SH (Inset: purified clusters after size-focusing of 23 hours).



Figure S2. IR-spectra of Calix-4SH and calixarene-functionalized  $Au_{25}$  clusters (left) as well as BuSH and  $Au_{25}(BuS)_{18}$  (right). The band assigned to S-H stretching mode at 2560 cm<sup>-1</sup> disappears as ligands bind to clusters.



Figure S4. TOCSY spectrum of Au<sub>25</sub> clusters prepared by using 7.0 % Calix-4SH in the synthesis feed.



Figure S5. ROESY spectrum of  $Au_{25}$  clusters prepared by using 7.0 % Calix-4SH in the synthesis feed. The coupling of the aromatic protons is highlighted with circles.



Figure S6. Fluorescence excitation and emission spectra of  $Au_{25}(Calix-4S)_x(BuS)_y$  clusters. The second order excitation peak (400 nm) and THF raman peak (360 nm) have been omitted for clarity. It should be noted that the NIR emission can be limited by the detector at > 820 nm.

Table S1	. Fluorescence	Quantum	Yields of	Cluster	Products
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Calix-4SH in feed (%)	Quantum yield (%)
0	0.20
0.36	0.12
0.72	0.18
2.0	0.28
7.0	0.24



Figure S7. ESI(-)-TOF mass spectrum measured from sample obtained from 0.0 % Calix-4SH in the synthesis feed.



Figure S8. ESI(-)-TOF mass spectrum measured from sample obtained from 0.36 % Calix-4SH in the synthesis feed.



Figure S9.

	Calix-4S		BuS bound		Calix	-4S	- BuS bound		
Cluster composition <sup>a</sup>	Tetradentate	Bidentate	to Au	Cluster composition <sup>a</sup>	Tetradentate	Bidentate	to Au		
0 %	o Calix-4SH in	feed		2.0 % Calix-4SH in feed					
Au <sub>25</sub> (BuS) <sub>18</sub>	0	0	18	Au <sub>25</sub> (Calix-4S) <sub>2</sub> (BuS) <sub>12</sub>	1	1	12		
				Au <sub>25</sub> (Calix-4S) <sub>2</sub> (BuS) <sub>13</sub> <sup>d</sup>	1	0	13		
				Au <sub>25</sub> (Calix-4S) <sub>2</sub> (BuS) <sub>14</sub>	0	2	14		
				Au <sub>25</sub> (Calix-4S) <sub>2</sub> (BuS) <sub>16</sub>	0	2	14 <sup>b</sup>		
				Au <sub>25</sub> (Calix-4S) <sub>3</sub> (BuS) <sub>6</sub>	2	1	8		
				Au <sub>25</sub> (Calix-4S) <sub>3</sub> (BuS) <sub>10</sub>	1	2	10		
0.26	0/ C-1: 4011	f 1		Au <sub>25</sub> (Calix-4S) <sub>3</sub> (BuS) <sub>12</sub>	0	3	12		
0.36	% Calix-4SH	in feed		Au25(Calix-4S)3(BuS)14	0	3	12 <sup>b</sup>		
Au <sub>25</sub> (BuS) <sub>18</sub>	0	0	18	Au <sub>25</sub> (Calix-4S) <sub>4</sub> (BuS) <sub>4</sub>	3	1	4		
Au <sub>25</sub> (Calix-4S)(BuS) <sub>14</sub>	1	0	14	Au <sub>25</sub> (Calix-4S) <sub>4</sub> (BuS) <sub>6</sub>	2	2	6		
Au25(Calix-4S)(BuS)16	0	1	16	Au <sub>25</sub> (Calix-4S) <sub>4</sub> (BuS) <sub>8</sub>	1	3	8		
Au <sub>25</sub> (Calix-4S) <sub>2</sub> (BuS) <sub>10</sub>	2	0	10	Au <sub>25</sub> (Calix-4S) <sub>4</sub> (BuS) <sub>10</sub>	0	4	10		
Au <sub>25</sub> (Calix-4S) <sub>2</sub> (BuS) <sub>12</sub>	1	1	12	Au <sub>25</sub> (Calix-4S) <sub>4</sub> (BuS) <sub>12</sub>	0	4	10 <sup>b</sup>		
Au <sub>25</sub> (Calix-4S) <sub>2</sub> (BuS) <sub>14</sub>	0	2	14	Au25(Calix-4S)5(BuS)4	2	3	4		
				Au25(Calix-4S)5(BuS)6	1	4	6		
				Au25(Calix-4S)5(BuS)8	0	5	8		
				Au <sub>25</sub> (Calix-4S) <sub>5</sub> (BuS) <sub>10</sub>	0	5	8 <sup>b</sup>		
0.72	% Calix-4SH	in feed		7.0	% Calix-4SH i	n feed			
Au <sub>25</sub> (BuS) <sub>18</sub>	0	0	18	Au <sub>25</sub> (Calix-4S) <sub>5</sub> (BuS) <sub>2</sub>	3	2	2		
Au <sub>25</sub> (Calix-4S)(BuS) <sub>14</sub>	1	0	14	Au <sub>25</sub> (Calix-4S) <sub>5</sub> (BuS) <sub>4</sub>	2	3	4		
Au <sub>25</sub> (Calix-4S)(BuS) <sub>16</sub>	0	1	16	Au <sub>25</sub> (Calix-4S) <sub>5</sub> (BuS) <sub>6</sub>	1	4	6		
Au25(Calix-4S)(BuS)18	0	1	16 <sup>b</sup>	Au25(Calix-4S)5(BuS)8	0	5	8		
Au25(Calix-4S)2(BuS)10	2	0	10	Au <sub>25</sub> (Calix-4S) <sub>5</sub> (BuS) <sub>10</sub>	0	5	8 <sup>b</sup>		
Au <sub>25</sub> (Calix-4S) <sub>2</sub> (BuS) <sub>12</sub>	1	1	12	Au <sub>25</sub> (Calix-4S) <sub>6</sub> (BuS) <sub>2</sub>	2	4	2		
Au <sub>25</sub> (Calix-4S) <sub>2</sub> (BuS) <sub>14</sub>	0	2	14	Au <sub>25</sub> (Calix-4S) <sub>6</sub> (BuS) <sub>4</sub>	1	5	4		
Au <sub>25</sub> (Calix-4S) <sub>2</sub> (BuS) <sub>16</sub>	0	2	14 <sup>b</sup>	Au <sub>25</sub> (Calix-4S) <sub>6</sub> (BuS) <sub>5</sub> <sup>d</sup>	1	4	5		
Au <sub>25</sub> (Calix-4S) <sub>3</sub> (BuS) <sub>6</sub>	2	1	8	Au <sub>25</sub> (Calix-4S) <sub>6</sub> (BuS) <sub>6</sub>	0	6	6		
Au <sub>25</sub> (Calix-4S) <sub>3</sub> (BuS) <sub>10</sub>	1	2	10	Au25(Calix-4S)6(BuS)8	0	6	6 <sup>b</sup>		
Au <sub>25</sub> (Calix-4S) <sub>3</sub> (BuS) <sub>12</sub>	0	3	12	Au <sub>25</sub> (Calix-4S) <sub>7</sub> (BuS) <sub>2</sub>	1	6	2		
Au <sub>25</sub> (Calix-4S) <sub>3</sub> (BuS) <sub>14</sub>	0	3	12 <sup>b</sup>	Au25(Calix-4S)7(BuS)4	0	7	4		
				Au25(Calix-4S)7(BuS)6	0	7	4 <sup>b</sup>		
				Au25(Calix-4S)8(BuS)4	0	8	2 <sup>b</sup>		
				Au <sub>25</sub> (Calix-4S) <sub>8</sub> (BuS) <sub>6</sub>	0	8	2 <sup>c</sup>		
a. Charge and adduct ions b. Two BuS are presumed c. Four BuS are presumed	(Cs <sup>+</sup> ) are omitted to bind to Calix- to bind to Calix- ed to bind magnet	l 4S with disulfi 4S with disulfi	ide bridges ide bridges						

# Table S2. $Au_{25}(Calix-4S)_x(BuS)_y$ Clusters Observed in the ESI(+)-MS Spectra of Samples With Varying Calix-4SH Concentration in the Synthesis Feed and Interpretation of Ligands' Binding Modes

d. One Calix-4S is presumed to bind monodentately



Figure S10. ESI(+)-TOF mass spectrum measured from sample obtained from 0.0 % Calix-4SH in the synthesis feed.

Table S3. The Ions Observed in the ESI(+)-MS Spectrum Measured from 0.0 % Calix-4SH Feed: Theoretical and Experimental *m/z* values (Most Abundant) and Absolute Mass Accuracies

Ion	Ion Charge State	Core Charge	Composition	m/z (theor.)	m/z (exp.)	Mass Accuracy
$[Au_{25}(BuS)_{18}]^+$	1+	1+	$C_{72}H_{162}S_{18}Au_{25}\\$	6528.9274	6528.8747	0.05
$\left[Au_{25}(BuS)_{18}+Cs\right]^+$	1+	0	$C_{72}H_{162}S_{18}Au_{25}Cs$	6661.8328	6661.8106	0.02
$[Au_{25}(BuS)_{18} + Cs_2]^+ \\$	1+	1-	$C_{72}H_{162}S_{18}Au_{25}Cs_2$	6794.7382	6794.6839	0.05
$[Au_{25}(BuS)_{18} + TOA]^+$	1+	0	$C_{104}H_{230}S_{18}Au_{25}N$	6995.4643	6995.4264	0.04



Figure S11. ESI(+)-TOF mass spectrum measured from sample obtained from 0.36 % Calix-4SH in the synthesis feed.

Ion	Ion Charge State	Core Charge	Composition	m/z (theor.)	<i>m/z</i> (exp.)	Mass Accuracy
[Au <sub>25</sub> (BuS) <sub>18</sub> ]	1+	1+	$C_{72}H_{162}S_{18}Au_{25}\\$	6528.9274	6528.8747	0.05
$[\mathrm{Au}_{25}(\mathrm{Bu}\mathrm{S})_{18} + \mathrm{Cs}]$	1+	0	$C_{72}H_{162}S_{18}Au_{25}Cs$	6661.8328	6661.8106	0.02
$[Au_{25}(BuS)_{18} + Cs_2] \\$	1+	1-	$C_{72}H_{162}S_{18}Au_{25}Cs_2$	6794.7382	6794.7096	0.03
[Au <sub>25</sub> (calix)(BuS) <sub>14</sub> +Cs]	1+	0	$C_{100}H_{178}O_4S_{18}Au_{25}Cs$	7077.9392	7077.8774	0.06
[Au <sub>25</sub> (calix)(BuS) <sub>14</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{100}H_{178}O_4S_{18}Au_{25}Cs_2$	7210.8447	7210.8037	0.04
[Au <sub>25</sub> (calix)(BuS) <sub>16</sub> +Cs]	1+	0	$C_{108}H_{196}O_4S_{20}Au_{25}Cs$	7256.0243	7255.9776	0.05
[Au <sub>25</sub> (calix)(BuS) <sub>16</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{108}H_{196}O_4S_{20}Au_{25}Cs_2$	7388.9297	7388.9334	0.00
[Au <sub>25</sub> (calix)(BuS) <sub>14</sub> +Cs <sub>2</sub> ]	2+	0	$C_{100}H_{178}O_4S_{18}Au_{25}Cs_2$	3605.4220	3605.4316	-0.01
[Au <sub>25</sub> (calix)(BuS) <sub>16</sub> +Cs]	2+	1+	$C_{108}H_{196}O_4S_{20}Au_{25}Cs$	3628.0119	3628.0231	-0.01
[Au <sub>25</sub> (calix)(BuS) <sub>14</sub> +Cs <sub>2</sub> ]	2+	0	$C_{100}H_{178}O_4S_{18}Au_{25}Cs_3$	3671.8748	3671.8745	0.00
[Au <sub>25</sub> (calix)(BuS) <sub>16</sub> +Cs <sub>2</sub> ]	2+	0	$C_{108}H_{196}O_4S_{20}Au_{25}Cs_2$	3694.4646	3694.4735	-0.01
[Au <sub>25</sub> (calix)(BuS) <sub>16</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{108}H_{196}O_4S_{20}Au_{25}Cs_3$	3760.9173	3760.9473	-0.03
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>10</sub> +Cs <sub>2</sub> ]	2+	0	$C_{128}H_{194}O_8S_{18}Au_{25}Cs2$	3813.4750	3813.5258	-0.05
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>12</sub> +Cs <sub>2</sub> ]	2+	0	$C_{136}H_{212}O_{20}S_{20}Au_{25}Cs_2$	3902.5175	3902.5188	0.00
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>12</sub> +Cs <sub>2</sub> ]	2+	0	$C_{136}H_{212}O_{20}S_{20}Au_{25}Cs_2$	3968.9703	3969.0439	-0.07
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>14</sub> +Cs <sub>2</sub> ]	2+	0	$C_{144}H_{230}O_8S_{22}Au_{25}Cs_2$	3992.0603	3992.0604	0.00
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>14</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{144}H_{230}O_8S_{22}Au_{25}Cs_3$	4058.5130	4058.4983	0.01

Table S4. The Ions Observed in the ESI(+)-MS Spectrum Measured from 0.36 % Calix-4SH Feed: Theoretical and Experimental *m/z* Values (Most Abundant) and Absolute Mass Accuracies



Figure S12. ESI(+)-TOF mass spectrum measured from sample obtained from 0.72 % Calix-4SH in the synthesis feed.

Ion	Ion Charge State	Core Charge	Composition	m/z (theor.)	<i>m/z</i> (exp.)	Mass Accuracy
[Au <sub>25</sub> (BuS) <sub>18</sub> ]	1+	1+	$C_{72}H_{162}S_{18}Au_{25}$	6528.9274	6528.8747	0.05
$[\mathrm{Au}_{25}(\mathrm{Bu}\mathrm{S})_{18} + \mathrm{Cs}]$	1+	0	$C_{72}H_{162}S_{18}Au_{25}Cs$	6661.8328	6661.8106	0.02
$[Au_{25}(BuS)_{18} + Cs_2] \\$	1+	1-	$C_{72}H_{162}S_{18}Au_{25}Cs_2$	6794.7382	6794.5836	0.15
[Au <sub>25</sub> (calix)(BuS) <sub>14</sub> ]	1+	1+	$C_{100}H_{178}O_4S_{18}Au_{25}$	6945.0337	6944.9274	0.11
[Au <sub>25</sub> (calix)(BuS) <sub>14</sub> +Cs]	1+	0	$C_{100}H_{178}O_4S_{18}Au_{25}Cs$	7077.9392	7077.8774	0.06
[Au <sub>25</sub> (calix)(BuS) <sub>16</sub> ]	1+	1+	$C_{108}H_{196}O_4S_{20}Au_{25}$	7123.1188	7123.1122	0.01
[Au <sub>25</sub> (calix)(BuS) <sub>14</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{100}H_{178}O_4S_{18}Au_{25}Cs_2$	7210.8447	7210.8037	0.04
[Au <sub>25</sub> (calix)(BuS) <sub>16</sub> +Cs]	1+	0	$C_{108}H_{196}O_4S_{20}Au_{25}Cs$	7256.0243	7255.9776	0.05
[Au <sub>25</sub> (calix)(BuS) <sub>16</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{108}H_{196}O_4S_{20}Au_{25}Cs_2$	7388.9297	7388.9334	0.00
[Au <sub>25</sub> (calix)(BuS) <sub>18</sub> +Cs]	1+	0	$C_{116}H_{214}O_4S_{22}Au_{25}Cs$	7434.1095	7434.0400	0.07
$[\mathrm{Au}_{25}(\mathrm{calix})_2(\mathrm{BuS})_{10} + \mathrm{Cs}]$	1+	0	$C_{128}H_{194}O_8S_{18}Au_{25}Cs$	7494.0453	7493.9500	0.10
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>10</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{128}H_{194}O_8S_{18}Au_{25}Cs_2$	7626.9506	7626.9038	0.05
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>12</sub> +Cs]	1+	0	$C_{136}H_{212}O_8S_{20}Au_{25}Cs$	7672.1302	7672.2019	-0.07
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>12</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{136}H_{212}O_8S_{20}Au_{25}Cs_2$	7805.0356	7805.0594	-0.02
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>14</sub> +Cs]	1+	0	$C_{144}H_{230}O_8S_{22}Au_{25}Cs$	7851.2157	7851.2176	0.00
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>14</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{144}H_{230}O_8S_{22}Au_{25}Cs_2$	7984.1211	7984.0894	0.03
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>16</sub> +Cs]	1+	0	$C_{152}H_{248}O_8S_{24}Au_{25}Cs$	8029.3006	8029.2488	0.05

Table S5. The Ions Observed in the ESI(+)-MS Spectrum Measured from 0.72 % Calix-4SH Feed: Theoretical and Experimental *m/z* Values (Most Abundant) and Absolute Mass Accuracies

[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>8</sub> + Cs]	1+	0	$C_{164}H_{228}O_{12}S_{20}Au_{25}Cs$	8089.2064	8089.1204	0.09
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>10</sub> +Cs]	1+	0	$C_{172}H_{246}O_{12}S_{22}Au_{25}Cs$	8267.3214	8267.4367	-0.12
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>10</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{172}H_{246}O_{12}S_{22}Au_{25}Cs_2$	8400.2268	8400.1320	0.09
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>12</sub> + Cs]	1+	0	$C_{180}H_{264}O_{12}S_{24}Au_{25}Cs$	8445.4063	8444.9200	0.49
[Au <sub>25</sub> (calix)(BuS) <sub>14</sub> +Cs]	2+	1+	$C_{100}H_{178}O_4S_{18}Au_{25}Cs$	3538.9693	3538.9736	0.00
[Au <sub>25</sub> (calix)(BuS) <sub>14</sub> +Cs <sub>2</sub> ]	2+	0	$C_{100}H_{178}O_4S_{18}Au_{25}Cs_2$	3605.4220	3605.4016	0.02
[Au <sub>25</sub> (calix)(BuS) <sub>16</sub> +Cs]	2+	1+	$C_{108}H_{196}O_4S_{20}Au_{25}Cs$	3628.0119	3628.0231	-0.01
[Au <sub>25</sub> (calix)(BuS) <sub>14</sub> +Cs <sub>2</sub> ]	2+	0	$C_{100}H_{178}O_4S_{18}Au_{25}Cs_3$	3671.8748	3671.8745	0.00
[Au <sub>25</sub> (calix)(BuS) <sub>16</sub> +Cs <sub>2</sub> ]	2+	0	$C_{108}H_{196}O_4S_{20}Au_{25}Cs_2$	3694.4646	3694.4735	-0.01
[Au <sub>25</sub> (calix)(BuS) <sub>16</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{108}H_{196}O_4S_{20}Au_{25}Cs_3$	3760.9173	3760.8314	0.09
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>10</sub> +Cs <sub>2</sub> ]	2+	0	$C_{128}H_{194}O_8S_{18}Au_{25}Cs2$	3813.4750	3813.5258	-0.05
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>12</sub> +Cs <sub>2</sub> ]	2+	0	$C_{136}H_{212}O_{20}S_{20}Au_{25}Cs_2$	3902.5175	3902.9911	-0.47
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>12</sub> +Cs <sub>2</sub> ]	2+	0	$C_{136}H_{212}O_{20}S_{20}Au_{25}Cs_2$	3968.9703	3969.0439	-0.07
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>14</sub> +Cs <sub>2</sub> ]	2+	0	$C_{144}H_{230}O_8S_{22}Au_{25}Cs_2$	3992.0603	3992.0604	0.00
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>14</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{144}H_{230}O_8S_{22}Au_{25}Cs_3\\$	4058.5130	4058.4983	0.01
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>16</sub> +Cs <sub>2</sub> ]	2+	0	$C_{152}H_{248}O_8S_{24}Au_{25}Cs_2$	4081.1028	4081.1685	-0.07
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>8</sub> +Cs <sub>2</sub> ]	2+	0	$C_{164}H_{228}O_8S_{24}Au_{25}Cs_2$	4111.0707	4110.5646	0.51
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>18</sub> +Cs <sub>2</sub> ]	2+	0	$C_{160}H_{266}O_8S_{24}Au_{25}Cs_2$	4170.6449	4170.6498	0.00
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>8</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{164}H_{228}O_8S_{24}Au_{25}Cs_3\\$	4177.5234	4177.6498	-0.13
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>10</sub> +Cs <sub>2</sub> ]	2+	0	$C_{172}H_{246}O_{12}S_{22}Au_{25}Cs_2 \\$	4200.1131	4200.1209	-0.01
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>12</sub> +Cs <sub>2</sub> ]	2+	0	$C_{180}H_{264}O_{12}S_{24}Au_{25}Cs_2$	4289.1557	4289.2803	-0.12
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>12</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{180}H_{264}O_{12}S_{24}Au_{25}Cs_{3}\\$	4355.6083	4355.1426	0.47
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>14</sub> +Cs <sub>2</sub> ]	2+	0	$C_{180}H_{264}O_{12}S_{24}Au_{25}Cs_2$	4378.6980	4378.2505	0.45



Figure S13. ESI(+)-TOF mass spectrum measured from sample obtained from 2.0 % Calix-4SH in the synthesis feed.

Ion	Ion Charge State	Core Charge	Composition	m/z (theor.)	<i>m/z</i> (exp.)	Mass Accuracy
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>12</sub> +Cs]	1+	0	$C_{136}H_{212}O_8S_{20}Au_{25}Cs$	7672.1302	7672.8548	-0.72
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>13</sub> +Cs]	1+	0	$C_{140}H_{221}O_8S_{21}Au_{25}Cs\\$	7762.1731	7762.5171	-0.34
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>12</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{136}H_{212}O_8S_{20}Au_{25}Cs_2$	7805.0356	7805.8736	-0.84
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>14</sub> +Cs]	1+	0	$C_{144}H_{230}O_8S_{22}Au_{25}Cs$	7851.2157	7851.5278	-0.31
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>14</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{144}H_{230}O_8S_{22}Au_{25}Cs_2$	7984.1211	7983.2086	0.91
[Au <sub>25</sub> (calix) <sub>2</sub> (BuS) <sub>16</sub> +Cs]	1+	0	$C_{152}H_{248}O_8S_{24}Au_{25}Cs$	8029.3006	8030.0553	-0.75
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>8</sub> + Cs]	1+	0	$C_{164}H_{228}O_{12}S_{20}Au_{25}Cs$	8089.2364	8089.6137	-0.38
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>10</sub> +Cs]	1+	0	$C_{172}H_{246}O_{12}S_{22}Au_{25}Cs$	8267.3214	8267.7189	-0.40
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>10</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{172}H_{246}O_{12}S_{22}Au_{25}Cs_2$	8400.2268	8400.5783	-0.35
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>12</sub> + Cs]	1+	0	$C_{180}H_{265}O_{12}S_{24}Au_{25}Cs$	8445.4063	8445.8527	-0.45
[Au <sub>25</sub> (calix) <sub>4</sub> (BuS) <sub>4</sub> +Cs]	1+	0	$C_{192}H_{244}O_{16}S_{20}Au_{25}Cs$	8505.3420	8504.6046	0.74
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>12</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{180}H_{265}O_{12}S_{24}Au_{25}Cs_2$	8578.3119	8579.0796	-0.77
[Au <sub>25</sub> (calix) <sub>4</sub> (BuS) <sub>6</sub> +Cs]	1+	0	$C_{200}H_{262}O_{16}S_{22}Au_{25}Cs$	8684.4269	8683.9105	0.52
[Au <sub>25</sub> (calix) <sub>4</sub> (BuS) <sub>6</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{200}H_{262}O_{16}S_{22}Au_{25}Cs_2$	8817.3325	8816.8649	0.47
[Au <sub>25</sub> (calix) <sub>4</sub> (BuS) <sub>8</sub> +Cs]	1+	0	$C_{208}H_{280}O_{16}S_{24}Au_{25}Cs$	8862.5119	8861.8226	0.69
[Au <sub>25</sub> (calix) <sub>4</sub> (BuS) <sub>8</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{208}H_{280}O_{16}S_{24}Au_{25}Cs_2$	8995.4175	8995.5890	-0.17
[Au <sub>25</sub> (calix) <sub>4</sub> (BuS) <sub>10</sub> +Cs]	1+	0	$C_{216}H_{298}O_{16}S_{26}Au_{25}Cs$	9040.5970	9040.9995	-0.40

Table S6. The Ions Observed in the ESI(+)-MS Spectrum Measured from 2.0 % Calix-4SH Feed: Theoretical and Experimental *m/z* Values (Most Abundant) and Absolute Mass Accuracies

[Au <sub>25</sub> (calix) <sub>4</sub> (BuS) <sub>10</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{216}H_{298}O_{16}S_{26}Au_{25}Cs_2$	9173.5022	9173.934	-0.43
[Au <sub>25</sub> (calix) <sub>4</sub> (BuS) <sub>12</sub> +Cs]	1+	0	$C_{224}H_{316}O_{16}S_{28}Au_{25}Cs$	9218.2856	9217.9771	0.31
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>4</sub> +Cs]	1+	0	$C_{236}H_{296}O_{20}S_{24}Au_{25}Cs$	9278.6178	9278.1398	0.48
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>4</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{236}H_{296}O_{20}S_{24}Au_{25}Cs_2$	9411.5233	9411.6998	-0.18
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>6</sub> +Cs]	1+	0	$C_{244}H_{314}O_{20}S_{26}Au_{25}Cs$	9456.2011	9456.1256	0.08
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>6</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{244}H_{314}O_{20}S_{26}Au_{25}Cs_2$	9589.6083	9588.9201	0.69
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>8</sub> +Cs]	1+	0	$C_{252}H_{332}O_{20}S_{28}Au_{25}Cs$	9635.7877	9635.0603	0.73
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>8</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{252}H_{332}O_{20}S_{28}Au_{25}Cs_2$	9768.6933	9767.9801	0.71
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>10</sub> +Cs]	1+	0	$C_{260}H_{350}O_{20}S_{30}Au_{25}Cs$	9813.8727	9813.1319	0.74
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>8</sub> +Cs <sub>2</sub> ]	2+	0	$C_{164}H_{228}O_8S_{24}Au_{25}Cs_2$	4111.0707	4110.6945	0.38
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>10</sub> +Cs <sub>2</sub> ]	2+	0	$C_{172}H_{246}O_{12}S_{22}Au_{25}Cs_2$	4200.1131	4200.2444	-0.13
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>10</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{172}H_{246}O_{12}S_{22}Au_{25}Cs_{3}\\$	4266.5658	4266.5276	0.04
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>12</sub> +Cs <sub>2</sub> ]	2+	0	$C_{180}H_{264}O_{12}S_{24}Au_{25}Cs_2$	4289.1557	4289.1499	0.01
[Au <sub>25</sub> (calix) <sub>3</sub> (BuS) <sub>12</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{180}H_{264}O_{12}S_{24}Au_{25}Cs_{3}\\$	4355.6083	4355.0056	0.60
[Au <sub>25</sub> (calix) <sub>4</sub> (BuS) <sub>6</sub> +Cs <sub>2</sub> ]	2+	0	$C_{200}H_{262}O_{16}S_{22}Au_{25}Cs_2$	4408.6659	4408.0513	0.61
[Au <sub>25</sub> (calix) <sub>4</sub> (BuS) <sub>6</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{200}H_{262}O_{16}S_{22}Au_{25}Cs_{3}\\$	4475.1187	4475.0630	0.06
[Au <sub>25</sub> (calix) <sub>4</sub> (BuS) <sub>8</sub> +Cs <sub>2</sub> ]	2+	0	$C_{208}H_{280}O_{16}S_{24}Au_{25}Cs_2$	4497.7085	4497.2168	0.49
[Au <sub>25</sub> (calix) <sub>4</sub> (BuS) <sub>8</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{208}H_{280}O_{16}S_{24}Au_{25}Cs_{3}\\$	4564.1611	4564.1341	0.03
[Au <sub>25</sub> (calix) <sub>4</sub> (BuS) <sub>10</sub> +Cs <sub>2</sub> ]	2+	0	$C_{216}H_{298}O_{16}S_{26}Au_{25}Cs_2$	4586.7509	4586.7630	-0.01
[Au <sub>25</sub> (calix) <sub>4</sub> (BuS) <sub>10</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{216}H_{298}O_{16}S_{26}Au_{25}Cs_{3}\\$	4653.2037	4652.6646	0.54
[Au <sub>25</sub> (calix) <sub>4</sub> (BuS) <sub>12</sub> +Cs <sub>2</sub> ]	2+	0	$C_{224}H_{316}O_{16}S_{28}Au_{25}Cs_2$	4675.7934	4675.3825	0.41
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>4</sub> +Cs <sub>2</sub> ]	2+	0	$C_{236}H_{296}O_{20}S_{24}Au_{25}Cs_2$	4705.7613	4705.6725	0.09
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>4</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{236}H_{296}O_{20}S_{24}Au_{25}Cs_{3}\\$	4772.2140	4772.5505	-0.34
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>6</sub> +Cs <sub>2</sub> ]	2+	0	$C_{244}H_{314}O_{20}S_{26}Au_{25}Cs_2\\$	4794.8039	4795.1661	-0.36
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>6</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{244}H_{314}O_{20}S_{26}Au_{25}Cs_{3}\\$	4861.2565	4861.0934	0.16
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>8</sub> +Cs <sub>2</sub> ]	2+	0	$C_{252}H_{332}O_{20}S_{28}Au_{25}Cs_2$	4884.3464	4883.9176	0.43
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>8</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{252}H_{332}O_{20}S_{28}Au_{25}Cs_{3}\\$	4950.7990	4950.5830	0.22
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>10</sub> +Cs <sub>2</sub> ]	2+	0	$C_{260}H_{350}O_{20}S_{30}Au_{25}Cs_2$	4973.3887	4973.3496	0.04
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>10</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{260}H_{350}O_{20}S_{30}Au_{25}Cs_{3}$	5039.8415	5039.6813	0.16



Figure S14. ESI(+)-TOF mass spectrum measured from sample obtained from 7.0 % Calix-4SH in the synthesis feed.

Ion	Ion Charge State	Core Charge	Composition	m/z (theor.)	m/z (exp.)	Mass Accuracy
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>2</sub> +Cs]	1+	0	$C_{228}H_{278}O_{20}S_{22}Au_{25}Cs$	9100.5330	9099.5010	1.03
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>2</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{228}H_{278}O_{20}S_{22}Au_{25}Cs_2$	9233.4382	9232.2867	1.15
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>4</sub> +Cs]	1+	0	$C_{236}H_{296}O_{20}S_{24}Au_{25}Cs$	9278.6178	9278.8680	-0.25
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>4</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{236}H_{296}O_{20}S_{24}Au_{25}Cs_2$	9411.5233	9411.3165	0.21
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>6</sub> +Cs]	1+	0	$C_{244}H_{314}O_{20}S_{26}Au_{25}Cs\\$	9456.2011	9456.1256	0.08
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>6</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{244}H_{314}O_{20}S_{26}Au_{25}Cs_2$	9589.6083	9589.4738	0.13
[Au <sub>25</sub> (calix) <sub>6</sub> (BuS) <sub>2</sub> +Cs]	1+	0	$C_{272}H_{330}O_{24}S_{26}Au_{25}Cs$	9873.8088	9873.6423	0.17
[Au <sub>25</sub> (calix) <sub>6</sub> (BuS) <sub>2</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{272}H_{330}O_{24}S_{26}Au_{25}Cs_2$	10006.7141	10006.501	0.21
[Au <sub>25</sub> (calix) <sub>6</sub> (BuS) <sub>4</sub> +Cs]	1+	0	$C_{280}H_{348}O_{24}S_{28}Au_{25}Cs_1$	10051.8934	10052.273	-0.38
[Au <sub>25</sub> (calix) <sub>6</sub> (BuS) <sub>4</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{280}H_{348}O_{24}S_{28}Au_{25}Cs_2$	10184.7989	10184.838	-0.04
[Au <sub>25</sub> (calix) <sub>6</sub> (BuS) <sub>6</sub> +Cs]	1+	0	$C_{288}H_{366}O_{24}S_{30}Au_{25}Cs$	10229.9785	10231.819	-1.84
[Au <sub>25</sub> (calix) <sub>6</sub> (BuS) <sub>6</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{288}H_{366}O_{24}S_{30}Au_{25}Cs_2$	10362.8838	10361.671	1.21
[Au <sub>25</sub> (calix) <sub>7</sub> (BuS) <sub>2</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{316}H_{382}O_{28}S_{30}Au_{25}Cs_2 \\$	10779.9899	10780.946	-0.96
[Au <sub>25</sub> (calix) <sub>7</sub> (BuS) <sub>4</sub> +Cs]	1+	0	$C_{324}H_{400}O_{28}S_{32}Au_{25}Cs$	10825.1692	10824.861	0.31
[Au <sub>25</sub> (calix) <sub>7</sub> (BuS) <sub>4</sub> +Cs <sub>2</sub> ]	1+	1-	$C_{324}H_{400}O_{28}S_{32}Au_{25}Cs_2$	10958.0747	10957.698	0.38
[Au <sub>25</sub> (calix)7(BuS)6+Cs2]	1+	1-	$C_{332}H_{418}O_{28}S_{34}Au_{25}Cs_2$	11136.1596	11135.688	0.47
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>2</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{236}H_{296}O_{20}S_{24}Au_{25}Cs_{3}\\$	4683.1716	4683.0519	0.12

Table S7. The Ions Observed in the ESI(+)-MS Spectrum Measured from 7.0 % Calix-4SH Feed: Theoretical and Experimental m/z Values (Most Abundant) and Absolute Mass Accuracies

[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>4</sub> +Cs <sub>2</sub> ]	2+	0	$C_{236}H_{296}O_{20}S_{24}Au_{25}Cs_2$	4705.7613	4705.1973	0.56
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>4</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{236}H_{296}O_{20}S_{24}Au_{25}Cs_{3}\\$	4772.2140	4772.7306	-0.52
[Au <sub>25</sub> (calix) <sub>5</sub> (BuS) <sub>6</sub> +Cs <sub>2</sub> ]	2+	0	$C_{244}H_{314}O_{20}S_{26}Au_{25}Cs_2$	4794.8039	4795.0868	-0.28
[Au25(calix)5(BuS)6+Cs3]	2+	1-	$C_{244}H_{314}O_{20}S_{26}Au_{25}Cs_3\\$	4861.2565	4860.8874	0.37
[Au25(calix)5(BuS)8+Cs2]	2+	0	$C_{252}H_{332}O_{20}S_{28}Au_{25}Cs_2$	4884.3464	4884.3736	-0.03
[Au25(calix)6(BuS)2+Cs2]	2+	0	$C_{272}H_{330}O_{24}S_{26}Au_{25}Cs_2$	5003.3568	5003.3221	0.03
[Au25(calix)6(BuS)2+Cs3]	2+	1-	$C_{272}H_{330}O_{24}S_{26}Au_{25}Cs_3$	5069.8095	5069.647	0.16
[Au25(calix)6(BuS)4+Cs2]	2+	0	$C_{280}H_{348}O_{24}S_{28}Au_{25}Cs_2$	5092.3992	5092.4946	-0.10
[Au <sub>25</sub> (calix) <sub>6</sub> (BuS) <sub>4</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{280}H_{348}O_{24}S_{28}Au_{25}Cs_3\\$	5158.8519	5158.2616	0.59
[Au25(calix)6(BuS)6+Cs2]	2+	0	$C_{288}H_{366}O_{24}S_{30}Au_{25}Cs_2$	5181.4417	5180.8219	0.62
[Au <sub>25</sub> (calix) <sub>6</sub> (BuS) <sub>5</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{284}H_{357}O_{24}S_{29}Au_{25}Cs_3$	5203.3732	5204.8419	-1.47
[Au25(calix)6(BuS)6+Cs3]	2+	1-	$C_{288}H_{366}O_{24}S_{30}Au_{25}Cs_{3}\\$	5247.8944	5248.3874	-0.49
[Au25(calix)6(BuS)6+Cs4]	2+	2-	$C_{288}H_{366}O_{24}S_{30}Au_{25}Cs_4\\$	5314.3471	5314.4613	-0.11
[Au <sub>25</sub> (calix) <sub>6</sub> (BuS) <sub>8</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{296}H_{384}O_{24}S_{32}Au_{25}Cs_{3}\\$	5337.4368	5337.9127	-0.48
[Au <sub>25</sub> (calix)7(BuS)4+Cs2]	2+	0	$C_{324}H_{400}O_{28}S_{32}Au_{25}Cs_2\\$	5479.0371	5478.4458	0.59
[Au <sub>25</sub> (calix)7(BuS)4+Cs3]	2+	0	$C_{324}H_{400}O_{28}S_{32}Au_{25}Cs_{3}\\$	5545.4898	5545.1033	0.39
[Au <sub>25</sub> (calix)7(BuS)6+Cs2]	2+	0	$C_{332}H_{418}O_{28}S_{34}Au_{25}Cs_2 \\$	5568.0795	5568.2112	-0.13
[Au <sub>25</sub> (calix)7(BuS)6+Cs3]	2+	1-	$C_{332}H_{418}O_{28}S_{34}Au_{25}Cs_{3}\\$	5634.5323	5634.4177	0.11
[Au <sub>25</sub> (calix) <sub>8</sub> (BuS) <sub>4</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{368}H_{452}O_{32}S_{36}Au_{25}Cs_{3}\\$	5932.1276	5932.2377	-0.11
[Au <sub>25</sub> (calix) <sub>8</sub> (BuS) <sub>6</sub> +Cs <sub>3</sub> ]	2+	1-	$C_{376}H_{470}O_{32}S_{38}Au_{25}Cs_{3}$	6021.1702	6021.0844	0.09

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#### P Mass Spectrometry Very Important Paper

### Molecular Ionization from Carbon Nanotube Paper\*\*

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Dedicated to Professor C. N. R. Rao on the occasion of his 80th birthday.

**Abstract:** Ambient ionization is achieved by spraying from a carbon nanotube (CNT)-impregnated paper surface under the influence of small voltages ( $\geq 3$  V). Organic molecules give simple high-quality mass spectra without fragmentation in the positive or negative ion modes. Conventional field ionization is ruled out, and it appears that field emission of microdroplets occurs. Microscopic examination of the CNT paper confirms that the nanoscale features at the paper surface are responsible for the high electric fields. Raman spectra imply substantial current flows in the nanotubes. The performance of this analytical method was demonstrated for a range of volatile and nonvolatile compounds and a variety of matrices.

 ${f R}$  ecent progress in mass spectrometry has depended heavily on advances in the methods of ion formation. The creation of stable molecular ions of complex molecules with minimum internal energy has been a primary goal of such experiments. The most widely used methods to achieve this are electrospray ionization (ESI) and matrix-assisted laser desorption/ ionization (MALDI). More recently developed ambient ionization methods,<sup>[1]</sup> such as desorption electrospray ionization (DESI),<sup>[2]</sup> allow samples to be examined in their native state with minimal or no sample pre-treatment. These advantages and the resulting speed of analysis have led to the introduction of about fifty different variants of ambient ionization. Direct analysis in real time (DART),<sup>[3]</sup> extractive electrospray ionization (EESI),<sup>[4]</sup> desorption atmospheric pressure chemical ionization (DAPCI),<sup>[5]</sup> desorption atmospheric pressure photoionization (DAPPI),<sup>[6]</sup> laser ablation electrospray ionization (LAESI)<sup>[4b]</sup> and paper spray ionization,<sup>[7]</sup> are some of the methods that have been introduced

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over the past decade. Herein, we show that ionization can be achieved from a substrate that is coated with carbon nanotubes (CNTs) at a potential of just a few volts. It is suggested that the high electric fields that are produced at the small CNT protrusions are responsible for low-voltage ionization, which appears to occur by field emission of charged microdroplets.<sup>[8]</sup> With this "nanotube spray" method, various analytes, which are applied to the tip of the coated substrate, are detectable in small amounts. Neutral molecules typically appear as either their protonated or deprotonated forms, whereas salts yield both positive and negative ions. The fact that a high voltage (HV) is not needed sets this method apart from other ambient spray ionization methods, except for easy ambient spray ionization (EASI).<sup>[9]</sup>

Experiments were done with triangles of CNT-coated filter paper, which were wetted with MeOH/water and connected to a 3 V battery (Figure 1 A, B; see also the Experimental Section). Mass spectra that were recorded for triphenylphosphine (TPP) using the CNT paper and a 3 V battery source (Figure 1 C) exhibited a peak at m/z 263, which is due to protonated triphenylphosphine,  $[M+H]^+$ . Spectra could be collected for two to three seconds using 2 µL of the sample solution. When the voltage on the paper was reduced



**Figure 1.** A) Schematic representation of the ionization process from CNT paper. B) Photograph of the ionization source showing paper triangle and battery along with a grounding electrical connection. C) Mass spectra of triphenylphosphine (*M*) at 3 kV, 3 V, and 1 V from wet CNT paper. D) Field emission scanning electron microscopy (FE-SEM) image of CNT-coated paper. E) Isotope distribution of the protonated molecule at 3 V. F)  $MS^2$  spectrum of the parent ion at *m/z* 263.

to 1 V, the spectrum disappeared completely. The full-range mass spectrum of TPP on CNT-coated paper (Supporting Information, Figure S1) is similar to the conventional ESI mass spectrum recorded at 3 kV (Figure S2). Although the intensity of the molecular ion at 3 V is as much as  $10^4$  times lower than that in the ESI spectrum, the conditions are less harsh; in particular, the oxidation product of triphenylphosphine at m/z 279 as well as the oxidation product of a trace homologous impurity (product at m/z 293) are not observed, nor are their fragmentation products at m/z 203 and m/z 219. Moreover, the mass spectrum shows a well-defined isotopic pattern (Figure 1E) of the molecular ion, and its structure was confirmed by a tandem mass spectrum, which showed the expected benzene loss and further loss of H<sub>2</sub> (Figure 1F).

An increase in the applied potential increases the ion intensity, until it is saturated at 4 kV; at this point, the signal was almost of the same magnitude as the ESI signal. However, no additional features were observed. The two paper spray spectra (at 3 kV and 3 V, both from CNT-coated paper) that are shown in Figure 1C are identical in terms of the ions observed but the signal/noise ratio is higher at 3 V. A minimum applied voltage of 3 V is essential for detectable ion signals. Control experiments confirmed the fact that CNTs were essential for the ionization process at 3 V. Filter paper without the CNT coating, but cut similarly and using the same solvent did not produce detectable ions with a range of analytes, even at up to 500 V (Figure S3A). A closer examination of the edge of the coated paper revealed protruding nanotubes (Figure 1D). From these results and the experiments described below, we suggest that field emission of microscale solution droplets containing analyte occurs at these nanoscale protrusions, and this is responsible for the observed ionization event.

Additional experiments were conducted to explore the mechanism of ionization. Clearly, the absence of fragment ions in the mass spectrum may be attributed to the occurrence of soft ionization events. The occurrence of ionization at 3 V strongly implies a process that is associated with a very high electric field. The field must be due to the small conductive CNT structures (Figure 1D) that protrude from the surface of the filter paper and act as electrodes.<sup>[10]</sup> The voltage (from the battery) that is applied at the CNT electrode induces an electric field between the paper tip and the mass spectrometer inlet. The field intensity is high at the paper tip, where ionization occurs.

To differentiate the contributions of the protruding CNT structures from the macroscopic paper point to ionization, in another experiment, a rectangle of CNT-coated paper was held in front of the mass spectrometer inlet (with one of the long sides facing the MS inlet), and ionization of TPP was attempted. All other parameters except the shape of the paper were held constant. The mass spectra showed ionization of TPP at 3 V from this paper rectangle (Figure S3D). This confirms that in this case, the pointed paper tip is not involved in the ionization, but that the protruding CNTs are responsible for this process. Furthermore, the complete absence of molecular ion peaks when using a similarly cut filter paper without the CNT coating revealed the role of the nanoscale features in providing a field strength that is high enough to

cause field emission. Figure S3 E and S3 F show the change in intensity of the molecular ion peak with voltage for CNT-coated and normal paper triangles, respectively. The ion signals for both of these papers saturate at high voltages, but with CNTs, the onset of ion ejection occurs much earlier. Therefore, it is reasonable to conclude that at lower voltages CNTs play a role in ionization, and that with the increase in voltage a Taylor cone forms at the paper tip so that the macroscopic electric field is responsible for ionization.

In conventional field ionization,<sup>[11]</sup> vapor-phase molecules that are placed in a strong electric field lose an electron to form positively charged radical cations. Many of the analytes used are simple volatile organic molecules, which are expected to give  $M^+$  radical cations when ionized by this mechanism, for example, m/z 262 in the case of triphenylphospine, not the observed m/z 263. To test whether field ionization of vapor-phase compounds might contribute, triethylamine (vapor pressure:  $\rho = 57$  torr at 20 °C) was dissolved in acetone ( $\rho = 184.5$  torr at 20 °C) and introduced into the field (the gap between the CNTs and the MS inlet) as a vapor, and ionization was attempted at low voltages. It was found that the use of analyte vapors did not lead to a detectable amount of ionization. We thus conclude that field emission occurs from a solvated analyte or droplet, as in all cases, only the  $[M+H]^+$  ion and not the radical cation  $M^+$ . was detected.

To further investigate the proposed mechanism, which involves field emission of charged droplets, the experiment with TPP and three other analytes was repeated in the presence and absence of a protic acid.<sup>[9]</sup> Addition of acid will generate the salt and should inhibit simple field ionization (to give  $M^+$ ), but it should increase the field emission from droplets (to give  $[M+H]^+$ ). Therefore, analytes that contain basic functional groups (phosphines and amines) were selected, and they were analyzed before and after the addition of dilute acid (0.01N HCl). The relative intensities of the peaks that correspond to the protonated molecules are enhanced after the addition of dilute acid to the analytes with basic functional groups (Figure 2). This enhancement supports the hypothesis that ionization of a solvated species has occurred.

Various preformed ions (derived from the salts tetramethylammonium chloride, tetramethylammonium bromide, tetramethylammonium nitrate, and tetrabutylammonium iodide) were studied under the same conditions. In accordance with the proposed mechanism, both positive and negative ions were observed in the CNT-derived mass spectra (Figure S4 and S5). No fragmentation was observed, and the extreme softness of the process, even compared to other soft ionization methods, is indicated by the presence of hydrated halide anions. These studies showed that preformed ions can also be ejected from the surface in droplets, and that conventional field ionization is not responsible for ion formation.

To further investigate the applicability of the CNT ionization technique, this method was employed for the qualitative analysis of various analytes, including pesticides, antibiotics, and amino acids. All of these compounds gave characteristic mass spectra; therefore, it has been shown that



**Figure 2.** A–D) Intensity enhancement upon the addition of dilute HCl for various analytes (*M*) at 3 V, namely triphenylphosphine (A), tributylphosphine (B), diphenylamine (C), and triethylamine (D). The upper and lower traces in each plot are on the same scale and show spectra that were recorded before and after HCl addition, respectively. The y axes correspond to the intensity.

this low-voltage ionization method is useful for diverse analytical needs. Direct analysis of various contaminants on fruit is possible with this method. Three common insecticides (carbofuran, methyl parathion, and parathion) that are used for the protection of fruit were applied on the surface of an orange at a concentration of 50 ppm. Then, CNT-coated paper was rubbed on the surface and held in front of the MS inlet for analysis. The molecular ion peaks of different pesticides that were obtained using the battery-powered spray MS method are shown in Figure 3. In reality, the amount of sample extracted from the fruit surfaces during rubbing may be several orders of magnitude lower than the applied quantity, and therefore, the limit of detection is much lower than the applied sample concentration. The molecular ion peaks of each of these pesticides are shown in Figure S6.

The same method was used to analyze medicines. CNTcoated paper was rubbed on the surfaces of three commercially available tablets, namely Crocin, Combiflam, and Xyzal (trade names), and held in front of the MS inlet with the 3 V battery set-up. Both Crocin and Combiflam contain paracetamol (acetaminophen) as the major ingredient (Figure S7). Direct analysis of these tablets using the CNT-coated paper gave a peak corresponding to protonated paracetamol. The other tablet, Xyzal, is a non-sedative antihistamine and contains levocetirizine dihydrochloride as the active ingredient. Analysis of this tablet (Figure S7B) under the same conditions gave a peak in the mass spectrum that corresponds to protonated levocetirizine. The identity of the analytes was confirmed by MS<sup>2</sup> studies (Figure S7, insets).

Direct analysis of amino acids is also possible by spraying from CNT-coated paper. Several amino acids (30 ppm) were dropped onto the tip of the CNT-coated paper with a micro-



Figure 3. A-C) Detection of pesticides that were examined individually from the surface of an orange; carbofuran (A), methyl parathion (B), and parathion (C). The y axes correspond to the intensity.

pipette (injected volume:  $3 \mu L$ , which corresponds to a total loading of 10 ng). Intense peaks that correspond to the protonated amino acids were observed by mass spectrometry (Figure S8). The zwitterionic nature of amino acids may lead to easy extraction of ions from the nanotube tips in the electric field.

To probe the effect of the ionization event on the paper electrode itself, Raman spectra of CNT-coated paper were recorded before and after a series of experiments (ionization of TPP at 3 V over a period of 20 min with continuous sampling (50 times) using 3 µL of solution each time; Figure 4 A).<sup>[12]</sup> The data revealed a large red shift<sup>[13]</sup> of the D and G bands, which implies the acquisition of electrons by the CNT during ionization in the positive ion mode.<sup>[14]</sup> It appears that as the ionization occurs, a charge builds up, as would be expected for field-assisted ionization.[11] However, there appears to be electron transfer from the developing microdroplet to the CNT, which is effectively a polarization of the electrons in the long thin CNT fiber (see Figure 1D) that is driven by the high field, the mobility of electrons in the CNTs, and their large electron affinity.<sup>[15]</sup> As the positively charged droplet breaks away, the residual charge appears to lead to a reduction of the CNTs, which is reflected by the red-shifted D and G bands in the Raman spectrum. The hypothesis that electron transfer from the charged solvent microdroplet to CNT occurs was supported by a blank experiment, where only solvent and potential were applied to CNT-coated paper for the same period of time; afterwards, the CNT paper was studied by Raman spectroscopy. The recorded spectrum revealed red-shifted D and G bands.



**Figure 4.** Raman spectra of CNT-coated paper before and after ionization for A) neutral molecules (30 ppm TTP in MeOH/H<sub>2</sub>O) and preformed ions (tetramethylammonium bromide) in the B) positive and C) negative ion modes. The y axes correspond to the intensity.

Raman spectra of the nanotube sample were also recorded before and after ionization of the salt tetramethylammonium bromide in both the positive and negative ion modes. When preformed ions were employed, a red shift of the D and G bands for the CNT-coated paper was only observed for the measurement in the positive ion mode (Figure 4B). As before, this may be due to the high electric field that is needed to cause the ejection of solvated ions in microdroplets. Such a reduction did not occur during the negative ion mode measurement, as Raman spectra showed unshifted D and G bands (Figure 4C), presumably because the CNTs are already electron-rich under these conditions, and the field replenishes the lost charge. These measurements also confirm the reusability of the nanotube-coated electrodes.

The results presented herein suggest a versatile strategy for the direct analysis of diverse chemical species. This method can be modified to suit various analytical requirements. Nanoscale surfaces have previously been used for ionization in matrix-free laser-based techniques.<sup>[17]</sup> Replacement of the high-voltage power supply with a 3 V battery simplifies mass spectrometry through ion formation from a nanoscale antenna. This nanotube ionization method has been applied to analyze a variety of samples from different sources, including fruit surfaces, tablets, and a range of organic molecules, including amino acids, antibiotics, and pesticides, at relatively low concentrations.<sup>[18]</sup>

#### **Experimental Section**

Most of the experiments were carried out using multi-walled carbon nanotubes (MWNTs), referred to as CNTs. They were dispersed in water (2 mg in 25 mL water) assisted by sodium dodecyl sulfate (6 mg) as a surfactant using a probe sonicator.<sup>[16]</sup> This stable CNT suspension was drop-cast onto Whatman 42 (particle retention: 2.5  $\mu$ m) filter paper (3  $\mu$ L of the CNT suspension coating 5 mm<sup>2</sup>). The uniformity of this dispersion and its consequent stability are essential aspects of the success of the experiment. The paper was

dried in air and cut into triangles with dimensions of  $2 \times 5 \text{ mm}$ (base × height). As mentioned in the text, a triangular shape is not essential. The CNT-coated paper triangle was connected to a 3 V battery and held close (2 mm) to the mass spectrometer inlet. Then it was loaded with sample (typically as 30 ppm solutions). The volume of solvent used was 2 µL, and repeated measurements using the same paper used the same aliquot of pure solvent. All routine measurements were made at 3 V. Mass spectra were recorded in the positive ion mode for all analytes, except for preformed ions. For preformed ions that are derived from salts, both positive and negative ion mode spectra were recorded at  $\pm 3$  V. Spectra were recorded under the following experimental conditions: solvent: methanol/water (1:1); source volt-

age:  $\pm 3$  V; capillary temperature: 150 °C; capillary voltage:  $\pm 15$  V; and tube lens voltage:  $\pm 55$  V. Single-walled carbon nanotubes (SWNTs) were also used for measurements, but no detectable enhancement of the signal was seen. The following parameters were used for all ESI experiments: Source voltage: -5 kV; sheath gas (nitrogen) flow rate: 8 (manufacturer's unit); solvent flow rate:  $2 \mu L \min^{-1}$ ; all other parameters were the same as for the paper spray experiments. All ESI mass spectra correspond to an average of 100 scans.

SWCNTs and MWCNTs were purchased from Nanocyl s.a, USA; SDS from RFCL Ltd., Gujarat, India; triphenylphosphine from Spectrochem Pvt. Ltd., Mumbai, India; tributylphosphine from Wako Pure Chemical Industries Ltd.; diphenylamine and triethylamine from Merck Ltd., Mumbai, India. The pesticides carbofuran, methyl parathion, and parathion were purchased from Sigma Aldrich, India. All of the tablets used (Crocin, Combiflam, and Xyzal, all trade names) were purchased from a local pharmacy. Amino acids used in the experiments were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. All analytes (other than pesticides and tablets) were used at concentrations of 30 ppm. HPLC-grade methanol (Sigma Aldrich) and MeOH/water (1:1) were used as the solvents.

The probe sonicator (750 W, model number VCX 750) was obtained from M/s Sonics, USA. All mass spectra were recorded using an ion trap LTQ XL (Thermo Scientific, San Jose, California).  $MS^2$  analysis using collision-induced dissociation was performed to confirm the identity of the ions. Raman measurements were made using a Witec GmbH Confocal Raman microspectrometer, Germany with 532 nm and 633 nm laser excitations. A field emission scanning electron microscope by FEI was used to image the CNT-coated paper samples.

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- [18] Note added in proof (March 7, 2014): We can now collect mass spectra even at an applied potential of 1 V.



# Supporting Information © Wiley-VCH 2014

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## **Molecular Ionization from Carbon Nanotube Paper\*\***

Rahul Narayanan, Depanjan Sarkar, R. Graham Cooks, and Thalappil Pradeep\*

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## Supporting information

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#### **Supporting information 1:**



Figure S1. Full range mass spectrum of triphenylphosphine at 3 V.

### **Supporting information 2:**



**Figure S2.** ESI mass spectrum (MeOH:H<sub>2</sub>O, 1:1) of triphenylphosphine at 3 kV. The spectrum shows an enhanced oxidation peak at m/z 279 and its  $C_6H_6$  fragment at m/z 221, in comparison to the CNT-coated paper (Figure 1C). MS/MS spectrum is shown in the inset.

#### **Supporting information 3:**



**Figure S3.** A) Mass spectrum of TPP below 500 V using normal paper as seen on the spectrometer (no signal is seen), B) spectrum of TPP at 3 V using CNT-coated paper (same as that in Figure 1C, given for comparison), C) spectrum at 500 V from a normal paper, D) spectrum using rectangular CNT-coated paper and the inset shows the schematic of the paper (with mass spectrometer facing it), E) variation of intensity of the m/z 263 peak with voltage for CNT-coated paper and F) the same for normal paper.

#### **Supporting information 4:**



**Figure S4.** Analysis of preformed ions (positive and negative ion modes) at 3 V; A) tetramethylammonium chloride and B) tetramethylammonium bromide.

#### **Supporting information 5:**



**Figure S5.** Analysis of preformed ions (positive and negative ion modes) at 3 V; A) tetramethylammonium nitrate and B) tetrabutylammonium iodide.

### **Supporting information 6:**



**Figure S6.** Analysis of a pesticide mixture at 3 V from the surface of an orange. Isotopic distribution of the peaks is not clearly visible due to low intensity.

**Supporting information 7:** 



**Figure S7.** Analysis of tablets from CNT-coated paper at 3 V with their mass spectral and MS<sup>2</sup> data. A) Crocine (paracetamol), B) xyzal (levocetirizine dihydrochloride) and C) combiflam (paracetamol).



#### **Supporting information 8:**

**Figure S8.** Detection of various amino acids (90 ng) loaded on CNT-coated paper and spectra recorded at 3 V: A) phenylalanine, B) methionine, C) glutamic acid, D) glutamine, E) isoleucine, F) valine, G) proline and H) serine.





# Noble Metal Clusters: Applications in Energy, Environment, and Biology

Ammu Mathew and Thalappil Pradeep\*

Sub-nanometer-sized metal clusters, having dimensions between metal atoms and nanoparticles, have attracted tremendous attention in the recent past due to their unique physical and chemical properties. As properties of such materials depend strongly on size, development of synthetic routes that allows precise tuning of the cluster cores with high monodispersity and purity is an area of intense research. Such materials are also interesting owing to their wide variety of applications. Novel sensing strategies based on these materials are emerging. Owing to their extremely small size, low toxicity, and biocompatibility, they are widely studied for biomedical applications. Primary focus of this review is to provide an account of the recent advances in their applications in areas such as environment, energy, and biology. With further experimental and theoretical advances aimed at understanding their novel properties and solving challenges in their synthesis, an almost unlimited field of applications can be foreseen.

#### 1. Introduction

The unique physiochemical properties of soluble/dispersible noble metal and semiconducting nanomaterials have contributed to several areas of research pertaining to energy, environment, and medicine in the past few decades.<sup>[1-6]</sup> For noble metals, the excitement has been largely due to plasmonic (metallic) nanoparticles (NPs) of diverse shapes.<sup>[3,7,8]</sup> In the recent past, a new class of nanoscale materials made of a few to tens of atoms, having size <2 nm, often called nanoclusters or quantum clusters (QCs)<sup>[9]</sup> are receiving large attention due to their unique physical and chemical properties. They are believed to have greater implications to the aforementioned areas. Consequently, precise control of the clusters by developing easy synthetic strategies became an active area of research. These materials fall in the NP-to-atom/molecule transition region and exhibit molecule-like properties owing to the gradual emergence of discrete electronic states.<sup>[10]</sup> Analogous to the size-dependent bandgap and quantum confinement

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effects in semiconductor quantum dots, QCs with sizes comparable to the Fermi wavelength of electrons show interesting properties such as size-dependent fluorescence, making them distinctly different from their NP counterparts. Unlike semiconductor quantum dots, QCs are less toxic and are smaller in dimension, making them superior candidates than the former in terms of biological applications. This review is an attempt to look at the emerging applications of this fascinating branch of materials science.

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Naming of these materials is still a point of debate and a fully acceptable terminology has not appeared so far. As a result, authors use a number of names, which include nanomolecules, nanoclusters, NPs, faradaurates, monolayer-protected clusters (MPCs), artificial atoms,

super atoms, QCs, etc. MPC has been a more acceptable name in the literature, although this has been used before in the context of plasmonic NPs, since 1995.<sup>[11]</sup> As clusters being discussed these days are atomically precise, naming them as MPCs seems inappropriate and often makes one confuse them with particles of the past. The name "clusters" is suggestive of gas-phase analogues of these materials, which are unprotected and unstable in the condensed phase. The name "nanomolecule" appears to imply that other prefixes such as pico, femto, etc. are possible for molecules, which does not make chemical sense, although we note that macromolecules do exist. "Faradaurate" is not appropriate as the suffix "ate" appears to imply a complex ion. Superatom and artificial atom would have been acceptable if the system was only an aggregate of atoms, while the materials are indeed molecules and possess properties of ligands quite distinctly. It is in fact these properties that are being used extensively in many of the applications. Moreover, with the recent addition of the crystal structures of non-superatomic systems such as  $[Au_{23}SR_{16}]^{-}$ , where SR is SC<sub>6</sub>H<sub>11</sub>, the term "superatoms" fails to describe this class of materials appropriately. It is in this context that a more suitable name, QCs, is used, which suggests distinct optical and electronic properties of the system and also resembles the name, quantum dots which indeed they are, although the former are composed of metals. Molecules are indeed quantized and therefore, the prefix "quantum" is, at least, partly redundant. We do note that search for a more appropriate name is continuing.

## Particle Systems Characterization www.particle-journal.com

Although noble metals generally refer to eight elements (ruthenium, rhodium, palladium, silver, osmium, iridium, platinum, and gold), QCs of gold,<sup>[10,12–15]</sup> silver,<sup>[16,17]</sup> as well as their alloys<sup>[18–20]</sup> are the most studied, although several clusters of copper,<sup>[21–23]</sup> platinum,<sup>[24–26]</sup> and palladium<sup>[27,28]</sup> are also reported. Over the past two decades, metallic QCs of various core sizes and ligands with unique properties have been utilized in diverse applications of energy, environment, and biomedicine, as depicted in **Figure 1**.

Syntheses and isolation of such clusters can be achieved by different means. The current synthetic capabilities allow the large-scale synthesis of several stable and isolable clusters.<sup>[9,29]</sup> Discrete molecule-like electronic transitions, size-dependent physicochemical properties, especially photoluminescence as well as other properties such as magnetism and electrochemical properties have generated wide interest. Catalytic activities of both supported and unsupported Au clusters<sup>[30-33]</sup> have been explored intensely. Additionally, the possibility of tuning optical and electrical properties of QCs by independently modifying their surface chemistry has given another handle to control their properties. A range of organic, biological, and polymeric molecules such as thiols, aminoacids, proteins, dendrimers, and DNA and their derivatives have been exploited for tuning the surface chemistry of QCs. Although several noble metal clusters have been studied for catalytic applications, they are largely unprotected and distinct molecular formulae cannot be assigned to many.[34-37]

Several excellent reviews exist on diverse synthetic strategies,<sup>[9,17,29,38,39]</sup> unique structure,<sup>[10,40,41]</sup> properties,<sup>[13,15,16,32,38,41–45]</sup> and bio-applications<sup>[17,46–49]</sup> of Au and AgQCs. Practical applications of these materials in environment, energy, and biology become possible due to their distinct chemistry (both of the ligand and the core). The diverse optical and catalytic properties of these systems, being used in the application areas mentioned, are mostly manifested in the solution state. Even in the case of supported clusters, the role of ligands is important. Solubility and compatibility with the solvent system are a crucial requirement, which is enabled by the ligand chemistry. Use of the core electronic structure in the biological context is enabled by surface conjugation. From the above, it is evident that practical utility of these materials is largely due to their unique chemistry. In this review, we highlight the molecular properties of QCs enabling applications. Structure of the review is as follows. Brief descriptions of the common synthetic techniques utilized are presented in Section 2, which is followed by a discussion of their unique properties in Section 3. Applications of QCs are discussed in Section 4. Finally, a summary and future prospects of this area are provided in Section 5. In the discussion, clusters are often described using their core size such as Au35, Au38, etc. without reference to their ligand structure such as Au25L18, Au38L24, etc. (where "L" denotes the ligand) as the cluster composition is well defined even with the metal core itself in such cases. In other words, most of the clusters except Ag<sub>32</sub> (which is reported to have 19 and 21 ligands) have a fixed number of ligands. Clusters whose atomic compositions are ill defined are also discussed. They are labeled as M@L, where there is no atomic precision in the composition, while distinct cluster properties such as visible luminescence exist.



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Ammu Mathew holds an M.Sc. in chemistry from Stella Maris College, Chennai, and is currently pursuing her Ph.D. under the guidance of Prof. T. Pradeep in the Department of Chemistry, Indian Institute of Technology Madras, Chennai. Her work is concerned with the chemistry of noble metal quantum clusters, applications of

mesostructures in ultra-trace detection of analytes, and creation of hybrid structures of the two.



T. Pradeep, after his Ph.D. from the Indian Institute of Science and postdoctoral training at University of California, Berkeley, and Purdue University, West Lafayette, joined IIT Madras in 1993, where he is a full professor since 2004. Prof. Pradeep's research interests are in molecular and nanoscale materials, and he

develops instrumentation for those studies. He is involved in the development of affordable technologies for drinking water purification, and some of his technologies have been commercialized.

#### 2. Common Synthetic Approaches

Creation of clusters with varying core sizes and ligands is fascinating as it opens up immense opportunities to study the emergence of novel physical and chemical properties and provides an insight into their structure–property relationships. While for NPs, changes in the physiochemical properties are manifested by change in size (diameter) or shape of the particles, modification of a single metal atom in the core can alter properties in the cluster size regime. Several protocols have been developed and they have been documented in several excellent reviews.<sup>[9,17,29,38,39]</sup>

After the pioneering work of Brust and co-workers in 1994,<sup>[50]</sup> the two-phase system of synthesis of thiol stabilized  $\approx$ 3 nm AuNPs, comprising an organic layer (toluene) and an aqueous layer gained much interest. A phase-transfer agent was used to transfer the metal ion precursor to the thiol containing organic phase. Efficient routes for the synthesis of monolayer protected sub-nanometer-sized clusters of various ligands using both biphasic and monophasic solvent mixtures were realized later using modified versions of the initial Brust–Schiffrin method.<sup>[51–54]</sup> Following this, several protocols were developed to make atomically precise, monodisperse clusters. Based on the precursors used, synthetic protocols

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**Figure 1.** Applications of QCs are spanned over three major domains: energy, environment, and biology. Images in the figure reproduced with permission.<sup>[94,170,264,269,273,290]</sup> Copyright 2010, 2011, and 2012, American Chemical Society; Reproduced with permission.<sup>[135,173,206]</sup> Copyright 2010, 2011, and 2012, Wiley-VCH; Reproduced with permission.<sup>[210]</sup> Copyright 2013, Royal Society of Chemistry; Reproduced with permission.<sup>[291]</sup> Copyright 2010, Springer.

can be broadly classified into two major categories, bottom up and top down methods, as shown in Figure 2. In the bottom up synthesis of QCs, metal ions are used as precursors. Here, the choice of ligands, reducing agent, metal precursor, and experimental conditions plays significant roles in determining the core size, purity, and yield of the clusters. Apart from traditional wet chemical routes (solution-phase synthesis), new protocols such as solid-state synthesis, interfacial synthesis, gel-mediated synthesis, etc. offer alternate ways of making clusters with diverse properties. In a typical solid-state synthesis, the reactants, namely, the metal ion precursor, reducing agent and ligand are ground well in their native form in air and the cluster is extracted into a suitable solvent. Ag9,<sup>[55]</sup> Ag32,<sup>[56]</sup> Ag44.<sup>[57]</sup> and Ag152<sup>[58]</sup> were synthesized utilizing the above protocol. Cluster synthesis using various templates such as polymers,<sup>[59,60]</sup> proteins,<sup>[61-63]</sup> dendrimers,<sup>[64,65]</sup> gels,<sup>[66,67]</sup> DNA,<sup>[68,69]</sup> etc. are widely employed for the synthesis of fluorescent QCs of Au, Ag, Cu, etc. Such templates can serve as suitable environments for cluster synthesis owing to their size, distinct conformation, and multiple binding sites, leading to distinct core sizes. After the synthesis of Au clusters protected by bovine serum albumin (BSA) by Xie et al.,<sup>[70]</sup> protein-protected clusters have emerged as an area of research due to their immense potential in biological studies. They are summarized well in several reviews.<sup>[17,39,47,71]</sup> Photoreduction method,<sup>[60,67]</sup> sonochemical method,<sup>[72]</sup> microemulsion method,<sup>[73]</sup> radiolytic method,<sup>[74,75]</sup> electrochemical method,<sup>[76]</sup> microwave-assisted synthesis,<sup>[77]</sup> etc. are other routes for making clusters. Among the various top down routes used for the synthesis of clusters, ligand-mediated etching from larger NPs<sup>[78]</sup> or clusters<sup>[79]</sup> is a useful method. Ligand-exchange<sup>[80,81]</sup> or place-exchange<sup>[82]</sup> reactions from preformed clusters is another popular protocol. The above synthetic routes, upon careful control of precursor metal ion and ligand concentration yields fairly monodisperse clusters of definite nuclearity. However, it is important to note that an additional purification step is often employed, post-synthesis, in order to discard other impurities in solution such as excess ligands, metal complexes, other clusters, etc. Isolation and separation of clusters are carried out via various techniques such as polyacrylamide gel electrophoresis (PAGE),<sup>[55,83,84]</sup> solvent selective precipitation,[85,86] size-exclusion chromatography,<sup>[87]</sup> etc. Recently, high-resolution separation and isolation of mixed ligand clusters containing a distribution of chemical compositions to yield individual clusters have been realized using high-performance liquid chromatography (HPLC) for clusters of various metal cores such as PdAu<sub>24</sub>, Au<sub>25</sub>, and Au<sub>38</sub> as well as various regioisomers of Au<sub>38</sub>.<sup>[81,88]</sup> The difference in polarity between the clusters induced by various ligand functionalization was used for the separation of the products. Enantiomeric separation of Au<sub>38</sub> cluster protected by achiral thiolates was achieved by chiral HPLC.<sup>[89]</sup>

Studies of gas-phase clusters of Au and Ag were initiated way back in 1980s.<sup>[90]</sup> Chemical sputtering of metal targets of gold and silver by pulsed laser or inert gas ions produced metal atoms, which later coalesced and nucleated to form "naked" (without ligand protection) clusters. While the reports of QCs stabilized by various ligands are numerous, synthesizing their corresponding gas-phase analogues poses a major challenge to

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Figure 2. Various routes for the synthesis of atomically precise, sub-nanometer-sized, noble metal quantum clusters.

researchers. Dissipation of the excess internal energy of such clusters in the form kinetic energy leading to gas-phase collisions and subsequent nucleation is a major limiting factor for the stability and isolation of such clusters. Recently, creation of clusters of magic numbered unprotected metal cores such as Au\_{18}^+, Au\_{25}^+,  $\tilde{\text{Au}}_{38}^+,$  and Au\_{102}^+ with unusual stability in the gas phase was demonstrated in laser desorption ionization experiments using protein templates.<sup>[91]</sup> It was proposed that the cluster nucleation occurred in the vicinity of the protein, in the gas phase, leading to the formation of magic numbered Au clusters. Later, naked alloy clusters of the type Au<sub>24</sub>Pd<sup>+</sup> were also detected in the gas phase using a similar approach.<sup>[92]</sup> A recent perspective article by Rao and Pradeep<sup>[29]</sup> summarizes advances in the synthesis of atomically precise QCs of silver, gold, and their alloys with special emphasis on silver QCs. Table 1 highlights several synthetic routes discovered so far along with a few examples.

#### 3. Unique Characteristics

#### 3.1. Structure

Understanding the crystal structure of QCs is important in exploiting their unique properties for various applications in diverse fields. Information on the nature of Au–S interaction, atomicity of the core, orientation of the ligands around the metal core in the form of staples, etc. is derived by solving the crystal structure of the material. Moreover, observation of crystals from such clusters serves as an irrefutable confirmation of their existence in solution. X-ray crystallography and computational studies have led to significant progress in deriving the total structure of atomically precise nanoclusters. Many crystal structures of gold clusters such as  $Au_{102}SR_{44}$ ,<sup>[93]</sup>  $Au_{25}SR_{18}$ ,<sup>[94,95]</sup>  $Au_{38}SR_{24}$ ,<sup>[96]</sup>  $Au_{36}SR_{24}$ ,<sup>[97]</sup>  $[Au_{24}(PPh_3)_{10}(SR)_5Cl_2]^+$ ,<sup>[98]</sup>  $Au_{28}SR_{20}$ ,<sup>[99]</sup> and  $[Au_{23}SR_{16}]^{-[100]}$  (where SR = thiolate) were reported in the past few years mainly due to their better stability, resistance to oxidation, and optimized synthetic protocols, facilitating the growth of large single crystals. However, crystal structures of silver clusters such as  $Ag_{14}$ ,<sup>[101]</sup>  $Ag_{16}$ ,<sup>[102]</sup>  $Ag_{32}$ ,<sup>[102]</sup> and  $Ag_{44}SR_{30}$ ,<sup>[103,104]</sup> as well as bimetallic alloy clusters such as  $Au_{13}Cu_x$  (x = 2, 4, 8) clusters,<sup>[105]</sup> and  $Au_{12}Ag_{32}SR_{30}$ ,<sup>[103]</sup>

Gold clusters such as  $Au_{102}(p-MBA)_{44}$ ,  $Au_{25}(SCH_2CH_2Ph)_{18}$ , and  $Au_{38}(SCH_2CH_2Ph)_{24}$  are composed of non-FCC (face-centered cubic) kernels such as decahedral  $Au_{79}$ , icosahedral  $Au_{13}$ , and face-sharing bi-icosahedral  $Au_{23}$ , respectively. Surface of each  $Au_n(SR)_m$  clusters contain unique "staple"-like motifs such as dimeric staples (-SR-Au-SR-Au-SR-) and monomeric staples (-SR-Au-SR-).  $Ag_{44}SR_{30}$  cluster has a double-shell core made of concentric shells of an inner  $Ag_{12}$  icosahedron cage within an  $Ag_{20}$  dodecahedron cage. These 32-atom cages were further protected by six  $Ag_2(SR)_5$  units in which Ag(I) ion binds to three SR ligands in a  $Ag(SR)_3$  planar configuration, which is unlike the case of Au clusters, in which Au(I) ions were coordinated linearly to two thiolate ligands.

The very first crystal structure, among thiolated noble metal QCs was of  $Au_{102}(p-MBA)_{44}$  in 2007 by Kornberg and co-workers.<sup>[93]</sup> The structure is composed of a central Marks decahedron core made of 49 gold atoms, two 20-atom caps

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Table 1. List of various common synthetic approaches for making noble metal QCs along with some examples. Schematic of the synthetic procedures is shown in Figure 2.

Method				Some examples of QCs synthesized	Refs.
Bottom up approach	Brust–Schiffrin method and its modifications			Au <sub>25</sub> SR <sub>18</sub> , <sup>[94,152,307]</sup> Au <sub>44</sub> SR <sub>28</sub> , <sup>[51]</sup> Pt@DT, <sup>[308]</sup> Cu@MPP <sup>23</sup>	[23, 51,152,307–309]
	Template-based synthesis	based Polymers sis		Ag@LA-PEG, <sup>[310]</sup> Ag@(PG-b-PAA), <sup>[59]</sup>	[59,60,310]
		Proteins		Au <sub>25</sub> @BSA, <sup>[70]</sup> Ag <sub>15</sub> @BSA, <sup>[62]</sup> Au@NLf, <sup>[63]</sup> Cu@BSA, <sup>[21]</sup> AuAg@BSA, <sup>[20]</sup>	[61–63]
		Dendrimers		Ag@PMAA, <sup>[72]</sup> ,311 Ag@PAMAM, <sup>[65]</sup>	[64,65,72,311]
		Gels		Ag <sub>25</sub> @SG, <sup>[66]</sup> Au <sub>15</sub> @CD, <sup>[170]</sup>	[66,67,170]
		DNA		Ag@DNA, <sup>[69]</sup> ,312-315	[68,69,312–315]
		Thiol- or amine- functionalized smal molecules	I	Ag <sub>7</sub> DMSA <sub>4</sub> , <sup>[52]</sup> Ag <sub>8</sub> (H <sub>2</sub> MSA) <sub>8</sub> and Ag <sub>7</sub> (H <sub>2</sub> MSA) <sub>7</sub> , <sup>[169]</sup> Ag <sub>-4,5</sub> @DHLA, <sup>[193]</sup> Au <sub>25</sub> SG <sub>18</sub> , <sup>[79]</sup> ,83	[52,169,193]
	Photoreduction			Ag@PAMAM, <sup>[65]</sup>	[60,65,67]
	Sonochemical synthesis	i		Ag@PMAA <sup>[69]</sup> ,72	[72]
	Solid-state synthesis			$Ag_{9}$ , <sup>[55]</sup> $Ag_{32}$ , <sup>[56]</sup> $Ag_{44}$ , <sup>[57]</sup> and $Ag_{152}$ , <sup>[58]</sup>	[55–58]
	Microemulsion technique			Ag <sub>n</sub> QCs (n < 10) <sup>[73]</sup>	[73]
	Microwave-assisted synthesis			Ag@L-GSH <sup>[77]</sup>	[77]
	Electrochemical synthesis			Cu@TBAN <sup>[76]</sup>	[76]
	Radiolytic approach			Ag <sub>3</sub> <sup>2+</sup> , <sup>[43]</sup> Ag <sub>4</sub> <sup>2+[44]</sup>	[74,75]
Top down approach	From NPs	Etching	Using excess ligand	Au <sub>25</sub> SG <sub>18</sub> , <sup>[79]</sup>	[79]
			High temperature	Au@DT, <sup>[316]</sup> Ag <sub>38</sub> MSA <sub>24</sub> , <sup>[78]</sup> Ag <sub>75</sub> SG <sub>40</sub> <sup>[317]</sup>	[78,316]
			Other	$Ag_8(H_2MSA)_8$ and $Ag_7(H_2MSA)_7$	[169]
	From other QCs	Etching		Au <sub>25</sub> SR <sub>18</sub> , <sup>[79]</sup> Au <sub>38</sub> SR <sub>24</sub> , <sup>[318]</sup> Au <sub>75</sub> <sup>[319]</sup>	[79,318,319]
		Ligand exchange		Au <sub>25</sub> (DDT <sub>18-n</sub> SBB <sub>n</sub> ), <sup>[81]</sup> Pd <sub>1</sub> Au <sub>24</sub> (DDT <sub>18-n</sub> SBB <sub>n</sub> ) <sup>[81]</sup>	[80-82]
		Alloying		$\begin{array}{l} Ag_{7}Au_{6}(H_{2}MSA)_{10},^{[320]}\left(Cu_{n}Au_{25-n}\right)SR_{18}\ (n=1-5),\ ^{[163]},321\ (Pd_{1}Au_{24})SR_{18},^{[164]}\ (Au_{24}Pt)SR_{18},^{[322]} \end{array}$	[163,164,320–322]

\*Abbreviations used: Dodecanethiol-stabilized platinum clusters (Pt@DT), 2-mercapto-5-n-propylpyrimidine (MPP), polyethylene glycol appended with lipoic acid groups (LA-PEG), polyglycerol-block-poly(acrylic acid) (PG-*b*-PAA), meso-2,3-dimercaptosuccinic acid (DMSA), mercaptosuccinic acid (H<sub>2</sub>MSA), dihydrolipoic acid (DHLA), bovine serum albumin (BSA), glutathione (GSH), poly(amidoamine) (PAMAM), poly(methacrylic acid) (PMAA), single-stranded DNA (ssDNA), tetrabutylammonium nitrate (TBAN), L-glutathione (L-GSH), mercaptosuccinic acid (MSA/(H<sub>2</sub>MSA), dodecanethiol (DDT).

with fivefold rotational (C5) symmetry on opposite poles, and a 13-atom equatorial band. The sulfur atom of the ligands (p-MBAs) binds to two gold atoms in a bridge conformation and thus forms a rigid surface layer on the metal core. The crystal structure also revealed the chiral nature of the cluster due to the number and specific geometry of the atoms in the equatorial band. Chirality in atomically precise clusters was later studied in greater detail using several clusters (described below). Later Murray and co-workers<sup>[94]</sup> and Jin and co-workers<sup>[95]</sup> reported the crystal structure of  $Au_{25}(SCH_2CH_2Ph)_{18}$  clusters. It consists of three types of gold atoms:

- 1. a central gold atom with coordination number 12;
- 2. 12 gold atoms with coordination number 6 forming the vertices of an icosahedron around the central atom (where five bond to gold atoms and one to a sulfur atom) and
- 3. 12 gold atoms stellated on 12 of the 20 faces of the  ${\rm Au}_{13}$  icosahedron (with six orthogonal semi-rings of

 $Au_2(SCH_2CH_2Ph)_3$  around the  $Au_{13}$  core, wherein each sulfur atom on the central  $Au_{13}$  core is connected to two gold atoms).

In 2010, the total structure of  $Au_{38}(SC_2H_4Ph)_{24}$ , derived by Jin and co-workers,<sup>[96]</sup> showed a face-fused  $Au_{23}$  bi-icosahedral core with three monomeric  $Au(SR)_2$  staples capped at the waist of the  $Au_{23}$  rod and six dimeric  $Au_2(SR)_3$  staples. Due to the staggered arrangement of the dimeric  $Au_2(SR)_3$  staples, three on the top icosahedron and the other three on the bottom icosahedron,  $Au_{38}SR_{24}$  cluster framework has a  $C_3$  rotational axis. Following these, several reports on total structure of clusters of various atomicities emerged. Though a vast number of clusters of various metal cores, ligands, and functionalities are known to date, only a few of them have been successfully crystallized. **Table 2** lists the noble metal cluster crystals reported till date in the order of their publication. Details of conditions used for crystallization are also provided. **Figure 3** shows their crystal structures.

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<b>Table 2.</b> List of crystal structures of noble metal quantum clusters reported till date in the order of their publication. The crystal structures are shown in Figure 3.						
No.	Year	Cluster*	Core	Shell/staples	Solvents and crystallization conditions	Refs.
1.	2007	Au102(p-MBA)44	Au39	2[RS—(AuSR) <sub>2</sub> ] and 19(RS—Au—SR)	Water (solvent) $300 \times 10^{-3}$ M NaCl, 100	[93]

1.	2007	Au <sub>102</sub> (p-MBA) <sub>44</sub>	Au <sub>39</sub>	2[RS—(AuSR) <sub>2</sub> ] and 19(RS—Au—SR)	Water (solvent) $300 \times 10^{-3}$ M NaCl, 100 $\times 10^{-3}$ M sodium acetate, pH 2.5, 46% methanol	[93]
2.	2010	[N(C <sub>8</sub> H <sub>17</sub> ) <sub>4</sub> ][Au <sub>25</sub> (PET) <sub>18</sub> ]	Au <sub>13</sub>	6(RS—Au—SR—Au—SR)	DCM (solvent)1:1 mixture of PET and thiophenol, excess methanol	[94]
3.	2010	Au <sub>25</sub> (PET) <sub>18</sub>	Au <sub>13</sub>	6(RS—Au—SR—Au—SR)	20 mg mL <sup>-1</sup> cluster in ethanol:toluene (4:1)	[95]
4.	2010	Au <sub>38</sub> (PET) <sub>24</sub>	Au <sub>23</sub>	3(RS—Au—SR) and 6(RS—Au—SR —Au—SR)	Toluene/ethanol (solvent), ambient conditions	[96]
5.	2011	$[Au_{25}(PPh_3)_{10}(PET)_5Cl_2]^{2+}$	2(Au <sub>13</sub> )	5 bridging (—SR—), 10 terminally coordinated (PPh <sub>3</sub> ) and 2Cl	DCM/ethanol (1:1)	[323]
6.	2012	Au <sub>36</sub> (SPh-tBu) <sub>24</sub>	Au <sub>28</sub>	4(SR—Au—SR—Au—SR) and 12 bridging (—SR—)	Toluene (solvent) ≈5 mg cluster in 1.5 mL DCM and 1 mL ethanol	[97]
7.	2012	$Ag_{14}(SC_6H_3F_2)_{12}(PPh_3)_8$	$(Ag_6)^{4+}$	8[Ag <sup>+</sup> (SC <sub>6</sub> H <sub>3</sub> F <sub>2</sub> <sup>-</sup> ) <sub>2</sub> PPh <sub>3</sub> ] tetrahedra sharing one (SC <sub>6</sub> H <sub>3</sub> F <sub>2</sub> <sup>-</sup> ) between them	DCM/hexane solvent mixture at 4 °C	[101]
8.	2012	$[Au_{24}(PPh_3)_{10}(PET)_5 \times_2]^+$	2 (Au <sub>12</sub> )	5 bridging (—SR—), 10 terminally coordinated (PPh₃) and 2X	Vapor diffusion of hexane into a toluene solution of clusters	[98]
9.	2013	$Ag_{16}(DPPE)_4(SPhF_2)_{14}$	$(Ag_8)^{6+}$	$[Ag_8(DPPE)_4(SPhF_2)_{14}]^{6-}$	Clusters in DCM/hexane solvent mixture at 4 °C in presence of 5.0 mg PPh₄Br	[102]
10.	2013	[PPh <sub>4</sub> ] <sub>2</sub> [Ag <sub>32</sub> (DPPE) <sub>5</sub> (SPhCF <sub>3</sub> ) <sub>24</sub> ]	$[Ag_{22}]^{12+}$	$1[Ag_6(DPPE)_3 (SPhCF_3)_{12}]^{6-},$ $2[Ag_2(DPPE) (SPhCF_3)_4]^{2-}$ and $4(SPhCF_3)^{-}$		
11.	2013	$[Au_{13}Cu_2(PPh_3)_6(SPy)_6]^+$	Au <sub>13</sub>	6(PPh <sub>3</sub> ) and 6(SPy) holds two face- capping Cu atoms	DCM/hexane solvent mixture at 4 $^{\circ}\text{C}$	[105]
12.	2013	[Au <sub>13</sub> Cu <sub>4</sub> (PPh <sub>2</sub> Py) <sub>4</sub> (SPh- tBu) <sub>8</sub> ] <sup>+</sup> [ClO <sub>4</sub> ] <sup>-</sup>	Au <sub>13</sub>	4(PPh <sub>2</sub> Py) and 8(SPh-tBu) holding four surface-capping Cu atoms		
13.	2013	[Au <sub>13</sub> Cu <sub>8</sub> (PPh <sub>2</sub> Py) <sub>12</sub> ] <sup>+</sup>	Au <sub>13</sub>	2[Cu(PPh <sub>2</sub> Py) <sub>3</sub> ] and 6[Cu(PPh <sub>2</sub> Py)]		
14.	2013	Au <sub>28</sub> (SPh-tBu) <sub>20</sub>	Au <sub>20</sub>	4(—SR—Au—SR—Au—SR—) and eight bridging (—SR—)	1.5 mL DCM and 1 mL ethanol solvent mixture	[99]
15.	2013	Na <sub>4</sub> Ag <sub>44</sub> (p-MBA) <sub>30</sub>	Ag <sub>12</sub> @Ag <sub>20</sub> two shell core	$6[Ag_2(SR)_5]$	Water/methanol (solvent); cluster washed with 1% acetic acid in DMF; crystallized from DMF	[104]
16.	2013	$[PPh_4]_4[Ag_{44}(SPhF_2)_{30}]$	Ag <sub>12</sub> @Ag <sub>20</sub> two shell core	6[Ag <sub>2</sub> (SR) <sub>5</sub> ]	Layering hexane into the DCM solutions of clusters at 4°C	[103]
17.	2013	$[PPh_4]_4[Ag_{44}(SPhCF_3)_{30}]$				
18.	2013	[PPh <sub>4</sub> ] <sub>4</sub> [Ag <sub>44</sub> (SPhF) <sub>30</sub> ]				
19.	2013	[PPh <sub>4</sub> ] <sub>4</sub> [Au <sub>12</sub> Ag <sub>32</sub> (SPhF) <sub>30</sub> ]	Au <sub>12</sub> @Ag <sub>20</sub> two shell core			
20.	2013	$[PPh_4]_4[Au_{12}Ag_{32}(SPhF_2)_{30}]$				
21.	2013	$[PPh_4]_4[Au_{12}Ag_{32}(SPhCF_3)_{30}]$				
22.	2013	[Au <sub>23</sub> (c-C <sub>6</sub> ) <sub>16</sub> ] <sup>-</sup>	Au <sub>15</sub>	2[Au <sub>3</sub> (SR) <sub>4</sub> ], 2[Au(SR) <sub>2</sub> ] and 4 bridging (—SR—)	≈4 mg cluster in 1 mL DCM followed by vapor diffusion of pentane into the cluster solution for 1–2 d	[100]
23.	2014	Au <sub>18.3</sub> Ag <sub>6.7</sub> (PET) <sub>18</sub>	Au <sub>6.3</sub> Ag <sub>6.7</sub>	6(RS—Au—SR—Au—SR)	Incoming Au/Ag molar ratio was 1:0.25. Toluene or DCM was used as solvent and EtOH or MeOH as the nonsolvent	[324]
24.	2014	$Au_{30}S(S\text{-}t\text{-}Bu)_{18}$	Au <sub>20</sub>	2(—SR—Au—SR—Au—SR—Au— SR—) units and 4(—SR—Au—SR—) units and 2 bridging (—SR—)	Vapor–vapor diffusion of ethanol into a toluene solution of cluster	[325]

\*Abbreviations used are p-mercaptobenzoic acid (p-MBA), phenylethane thiol (PET), dichloromethane (DCM), 4-tert-butylbenzenethiol (SPh-tBu), pyridine-2-thiol (SPy), triphenylphosphine (PPh<sub>3</sub>), diphenylphosphinopyridine (PPh<sub>2</sub>Py), 3,4-difluorothiophenol (SPhF<sub>2</sub>), 4-fluorothiophenol (SPhF), 4-(trifluoromethyl)thiophenol (SPhCF<sub>3</sub>), dimethylformamide (DMF), 1-cyclohexanethiol (c-C<sub>6</sub>), 1,2-bis(diphenylphosphino)ethane (DPPE), 2-Methyl-2-propanethiol (S-t-Bu).



**Figure 3.** Crystal structures of the various noble metal clusters crystallized till date. Note that the clusters are numbered as shown in Table 2. Panel 1 reproduced with permission.<sup>[94-96,98-100,323-326]</sup> Copyright 2007, American Association for the Advancement of Science. Panels 2–5, 8, 11–14, 22–24 reproduced with permission.<sup>[94-96,98-100,323-326]</sup> Copyright 2010, 2011, 2012, 2013, and 2014, American Chemical Society. Panel 6 reproduced with permission.<sup>[97]</sup> Copyright 2012, Wiley-VCH. Panels 7, 9, 10 reproduced with permission.<sup>[101,102]</sup> Copyright 2012 and 2013, Royal Society of Chemistry. Panels 15–21 reproduced with permission.<sup>[103,104]</sup> Copyright 2013, Macmillan Publishers Ltd.

#### 3.2. Absorption Spectroscopy

Appearance of distinct molecule-like absorption features is often treated as the fingerprint of QCs.<sup>[106–110]</sup> In this size regime, plasmon band, typically observed in metal NPs, is absent and unique step-like features in the absorption spectra due to molecular highest occupied orbitals and the lowest unoccupied orbitals (HOMO–LUMO) transitions emerge. This happens as a result of the conversion of the electronic band structure to discrete energy levels.<sup>[107,110,111]</sup> Density functional theory (DFT) calculations help in providing an in-depth understanding of the

optoelectronic properties of the clusters.<sup>[95,112–114]</sup> Tsukuda and co-workers<sup>[83]</sup> observed distinct optical absorption and emission profiles for glutathione (GSH)-protected AuQCs [Au<sub>10</sub>SG<sub>10</sub>, Au<sub>15</sub>SG<sub>13</sub>, Au<sub>18</sub>SG<sub>14</sub>, Au<sub>22</sub>SG<sub>16</sub>, Au<sub>22</sub>SG<sub>17</sub>, Au<sub>25</sub>SG<sub>18</sub>, Au<sub>29</sub>SG<sub>20</sub>, Au<sub>33</sub>SG<sub>22</sub>, and Au<sub>39</sub>SG<sub>24</sub>], which confirm their quantized electronic states. For example, Au<sub>25</sub>SR<sub>18</sub> (where SR = phenylethane thiol) show multiple absorption bands. Three distinct bands at 670 nm (intraband sp  $\leftarrow$  sp, LUMO  $\leftarrow$  HOMO transition), 450 nm (mixed intraband (sp  $\leftarrow$  sp), and interband (sp  $\leftarrow$  d) transitions), and 400 nm (interband (sp  $\leftarrow$  d) transition) are found and they assert the role of quantum size effects in the

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QCs. The labels of the energy levels (sp, d, etc.) are due to the Au 6s and Au 6p or Au 5d levels, which make the bands. Interband and intraband transitions refer to those between states derived from the same (6s6p to 6s6p) or different (5d to 6s6p) principal quantum numbers. The observed spectrum is in good agreement with time-dependent DFT calculations of the observed crystal structure.<sup>[95]</sup> The band at ≈670 nm is associated with the electronic transitions within the Au13 core of the cluster, clearly demonstrating the significance of the optical absorption features in determining the cluster structure. A superatom picture has been proposed by Grönbeck and co-workers<sup>[115]</sup> to account for the stability of [Au<sub>25</sub>SR<sub>18</sub>]<sup>-</sup>. According to them, each SR ligand localizes one 6s electron of gold. Though the nominal 8 electron shell closure in the case of [Au<sub>25</sub>SR<sub>18</sub>]<sup>-</sup> appears to conform to the superatom model, stability of [Au25SR18]<sup>0</sup> and [Au25SR18]<sup>+</sup> is not explained by this. In the case of Au<sub>38</sub>SR<sub>24</sub> QCs, the prominent absorption band at 700 nm is due to the overlap of the absorption features at 675 and 770 nm.<sup>[86,116]</sup> The face-fused bi-icosahedral Au<sub>23</sub> core of the Au<sub>38</sub> cluster<sup>[96]</sup> is attributed to such an effect. The absorbance band edge at 1.33 eV matched with the calculated bandgap energy for the Au<sub>38</sub> cluster. The optical absorption spectrum of [Ag44SR30]4- cluster[117] in solutions exhibited multiple bands and as a result they were initially described as IBANs (intensely and broadly absorbing NPs).<sup>[118]</sup> Eight distinct absorption bands were observed in their optical spectra ranging from 380 to 850 nm with extinction cross-sections as high as  $2.59 \times 10^5$  L mol<sup>-1</sup> cm<sup>-1</sup>. Change of ligand from thiolate<sup>[117,118]</sup> to selenolate<sup>[57]</sup> seemed to have little or no effect in their absorption features. Recently, crystal structures of various [(M12Ag32)  $SR_{30}$ ]<sup>4-</sup> clusters where M stands for Au<sup>[103]</sup> and Ag<sup>[103,104]</sup> were reported using various ligands. While the absorption spectrum showed no apparent change in peak positions for clusters with different thiols for M = Ag, a distinct shift was observed in the case of alloy clusters were M = Au, clearly indicating the significance of the core metal atoms in the overall optical properties of the clusters.<sup>[103]</sup>

Recently, Häkkinen and co-workers<sup>[119]</sup> studied the changes in the localized surface plasmon resonance (LSPR) of Au clusters upon growth of their core size from 1.5 to 2 nm, using ab initio calculations and atomistic models. Using time-dependent density functional perturbation theory (TD-DFPT), the threshold size for the occurrence of LSPR in gold clusters was predicted to fall between metal cores of diameter between 1.5 and 2 nm. In 1.5 nm clusters, the protecting layer confined the plasmon-like resonance within the core and for clusters above 2 nm, the LSPR was enhanced. The origin of plasmonic features in QCs or the threshold at which plasmonic behavior emerges has been probed by various groups.<sup>[109,111,120]</sup> In 1997, Whetten and co-workers<sup>[109]</sup> studied the changes in optical absorption spectra for thiol-protected nanocrystal gold molecules with core diameters ranging from 1.4 to 3.2 nm. From their studies, a systematic broadening of the surface plasmon (SP) band was observed with decreasing core size of the crystallites, however, the SP band becomes unidentifiable and onset of step-like structure emerges in the absorption spectrum for crystallites of sizes less than 2.0 nm. Murray and co-workers<sup>[111]</sup> interpreted this loss of the SP band with decrease in core size as the onset of quantum size effects in AuQCs. Emergence of a distinct



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plasmonic feature at 495 nm in the optical absorption spectrum was observed from a 76.3 kDa AuQC.  $^{\left[120\right]}$ 

#### 3.3. Photoluminescence

Photoluminescence, yet another interesting property exhibited by these clusters. It originates as a result of the breakdown of the continuous energy bands of NPs into discrete energy levels and subsequent electronic transitions between the HOMOs-LUMOs. The resulting strong emission from these materials can be used for a wide array of applications. It has been proposed that the high-energy visible fluorescence can be attributed to the radiative interband recombination between sp and d bands. Whereas the low-energy near-infrared (NIR) emission originates from the radiative intraband transitions within the sp-band across the HOMO-LUMO gap. The fluorescence property of QCs largely depends on their chemical environments such as the cluster core size, protecting ligands, solvents, etc.<sup>[9,43,121,122]</sup> Synthesis of different luminescent QCs with emission ranging from blue to red and NIR was realized by tuning the core size as well as the protecting ligand shell of the clusters.

While luminescence from metal films was noticed much earlier,<sup>[123,124]</sup> NIR photoluminescence from 1.1 and 1.7 nm Au nanocrystals at room temperature was reported by Whetten and co-workers<sup>[125]</sup> in 2000. The observed emission had a quantum yield of  $(4.4 \pm 1.5) \times 10^{-5}$ , five orders of magnitude greater than bulk gold, and was attributed to the intraband sp to splike transitions. Following the discovery of its crystal structure, Au25SR18 clusters had been studied intensely and visible and NIR emissions of these clusters (originally thought as Au<sub>28</sub>)<sup>[126]</sup> were correlated to their structure.<sup>[121,127,128]</sup> Though the luminescence observed was independent of the protecting ligands and was mainly ascribed as a core property,<sup>[128]</sup> use of ligands with electron-rich atoms and groups can indeed enhance the luminescence.<sup>[121]</sup> Link et al.<sup>[126]</sup> in 2002 reported the presence of two luminescence maxima (at 1.5 and 1.15 eV) for Au<sub>28</sub> (later identified as Au<sub>25</sub>) clusters with a total quantum yield of approximately  $(3.5 \pm 1.0) \times 10^{-3}$ . The steady-state luminescence studies of Au<sub>25</sub> QCs show two distinct features, a strong feature in the NIR region and a weaker luminescence in the visible region. Luminescence in the NIR region has a higher quantum yield ( $\approx 1 \times 10^{-3}$ ) compared to the latter ( $\approx 10^{-6} - 10^{-7}$ ). NIR luminescence of Au<sub>25</sub> QCs was used as a probe to follow the excited state dynamics of the core Au states by Ramakrishna and co-workers.<sup>[128]</sup> Unlike larger clusters, ultrafast growth and decay kinetics were observed in the luminescence decay traces of Au25SR18 QCs. While the time constants of emergence of luminescence were independent of the passivating ligands, the luminescence decay was influenced by the same. This indicates that though luminescence arises from the Au<sub>25</sub> core states and is independent of the protecting ligands, its decay happens via relaxation of the core Au states to S-Au-S-Au-S semi-ring states. A finite luminescence lifetime of 200 fs up to a few picoseconds (ps) was observed for  $Au_{25}$  QCs using femtosecond time-resolved measurements. An extremely fast (<200 fs) internal conversion process was observed within the Au<sub>13</sub> core, while the core to semi-ring relaxation required



1.2 ps.<sup>[129]</sup> Photoluminescence from various QCs passivated using diverse ligands has been studied in great detail.<sup>[17,39,43]</sup> Visible luminescence is mostly observed in case of water-soluble clusters with hydrophilic ligands. Among them clusters entrapped in dendrimers, proteins, DNA, polymers, biothiols, etc. are most studied as they are relatively more stable than their thiolate counterparts owing to better protection of the cluster core against destabilizing agents. Size-dependent emission, ranging from UV to IR, was observed from dendrimerprotected Au clusters of different sizes such as Au<sub>5</sub>, Au<sub>8</sub>, Au<sub>13</sub>, Au23, and Au31.[130] However, change in visible emission might not always be an indication of change in core size of the cluster. In Ag<sub>15</sub>@BSA clusters,<sup>[62]</sup> the emission observed from the cluster solution undergoes a sequential color change, from green to yellow to orange and finally to red, upon addition of NaBH<sub>4</sub>. Further examination using matrix-assisted laser desorption ioniszation (MALDI) MS and photoluminescence (PL) studies revealed the existence of both green emitting Ag-BSA conjugate and red emitting Ag<sub>15</sub>@BSA clusters in solution and the observed change in visible luminescence under UV lamp was attributed to increasing formation of red emitting Ag<sub>15</sub>@ BSA in solution compared to the former. It is important to mention that the luminescence from such clusters is now identified as fluorescence. Tang and co-workers<sup>[131]</sup> studied the fluorescence from red-emitting BSA-protected Au<sub>25</sub> QCs in detail and observed the presence of dual fluorescence bands at 710 (band I) and 640 nm (band II) via temperature-dependent measurements. From their studies, band I originates exclusively from the icosahedral Au13 core while the [-S-Au-S-Au-S-] staples are responsible for band II. Time-resolved photoluminescence and transient absorption measurements<sup>[132]</sup> revealed that the fluorescence seen in these clusters consisted of both fast (nanoseconds) and slow (microseconds) components due to prompt fluorescence and thermally activated delayed fluorescence. The unusually efficient intersystem crossing found in these systems was attributed as a consequence of the small energy gap (30 meV) between the triplet and the singlet states.

Solvatochromism from clusters, demonstrated by Ras and co-workers<sup>[122]</sup> in silver clusters protected by poly(methacrylic acid) (PMAA), wherein emission from the clusters showed a shift upon change in polarity of the dispersing medium (water-methanol mixtures) is yet another interesting phenomenon. This was followed by several reports of Ag clusters protected by DNA,<sup>[30]</sup> polystyrene-block-PMAA block copolymer,<sup>[133]</sup> and polyethyleneimine<sup>[134]</sup> exhibiting such solvent polarity-dependent emission tunability.

The luminescence property of these clusters is widely utilized in most applications involving QCs (discussed later). Owing to their tunable emission, high quantum yields, large stokes shift, resistance to photodegradation, etc. such fluorescent Au and AgQCs are used in diverse applications.<sup>[17,39,48]</sup>

#### 3.4. Metal-Enhanced Luminescence

Another interesting phenomenon exhibited by these clusters is metal-enhanced luminescence (MEL). MEL from Au@BSA QCs in presence of AgNPs was first demonstrated by Pradeep and co-workers<sup>[61]</sup> A fluoropohore in close proximity to a metal NP typically experiences quenching of its luminescence whereas when it is separated from the NP by a distance, it experiences an enhancement. This effect is called MEL. Approximately, ninefold enhancement in luminescence of Au@BSA QCs was observed in the presence of AgNPs. Though an exact mechanism is not known yet, here the protein shell on the QC was acting as the spacer between the luminescent cluster and the NPs. Absence of luminescence enhancement in the case of Au and Au/AgNPs could be attributed to the poor matching between the excitation wavelength of the cluster and the SP oscillations of the NPs. MEL was also observed in the case of Ag<sub>15</sub>@BSA clusters when coated on bimetallic Au/Ag mesoflowers (MFs).<sup>[135]</sup> As mentioned previously, this phenomenon is still in its infancy with regard to QCs and needs to be understood in greater detail.

#### 3.5. Chirality

Chirality is a common structural property exhibited by many natural molecules. Stable structures of bare gold QCs show interesting chiroptical properties as a function of cluster size and the way they respond to circularly polarized light. This was first reported by Whetten and co-workers,<sup>[136]</sup> when they found that 38-atom Au clusters are very sensitive for the polarization of light as evidenced by CD in the near-IR, visible, and near-UV regions.

Kornberg and co-workers<sup>[93]</sup> established the existence of chirality in Au<sub>102</sub>(p-MBA)<sub>44</sub> cluster from their crystal structure studies. The electron density map of Au<sub>102</sub>(p-MBA)<sub>44</sub> is shown in **Figure 4**A. The chirality in Au<sub>102</sub>(p-MBA)<sub>44</sub> structure is apparent from a view of the cluster down the Marks decahedral (MD) axis (Figure 4B). Both the number and geometry of the equatorial atoms in the cluster core are imparting chirality to the cluster core. Sulfur atoms bonded to Au atoms in two different shells and to a phenyl ring are also found to be the chiral centers in the cluster. One enantiomer has R configuration with 22 sulfur centers while S configuration had 18. Apart from this, two sulfur centers with no readily assigned chirality were also found, as they are bonded to two gold atoms in the same shell.

In 2010, Aikens and co-workers<sup>[137]</sup> reported the structural, electronic, and optical properties of thiolate-protected Au<sub>38</sub>(SR)<sub>24</sub> cluster by density-functional theory calculations (R=CH<sub>3</sub> and  $R=C_6H_{13}$ ) and powder X-ray crystallography ( $R=C_{12}H_{25}$ ). This study provided a new mechanism for the strong chiral activity of thiolate-protected gold clusters with achiral metal cores and ligands. They verified the existence of a stable cluster unit that consists of a bi-icosahedral core and a chiral arrangement of the protecting gold-thiolate Au<sub>v</sub>(SR)<sub>v</sub> units. The low-energy structure of this cluster has been assigned to Au23@(Au(SR)2)3(Au  $_2(SR)_3)_6$ , which is in excellent agreement with the theoretically predicted structure (for  $R=C_6H_{13}$ ). This isomer was found to have an intrinsically chiral structure due to special arrangement of the protective  $SR(AuSR)_x$  units on the surface of its  $Au_{23}$ core. The computed absorption and circular dichroism (CD) spectra of the lowest energy structure of Au<sub>38</sub>(SCH<sub>3</sub>)<sub>24</sub> was in agreement with the previously measured low-energy CD signal of GSH-protected Au<sub>38</sub>(SG)<sub>24</sub>. Recently, Bürgi and co-workers<sup>[89]</sup> successfully separated the enantiomers of Au<sub>38</sub> cluster, covered



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**Figure 4.** A) Electron density map depicting the X-ray crystal structure of the  $Au_{102}$  (p-MBA)<sub>44</sub> clusters. Gold, sulfur, carbon, and oxygen atoms are shown as yellow, cyan, gray, and red spheres, respectively). B) The two enantiomeric  $Au_{102}$  (p-MBA)<sub>44</sub> clusters, as viewed down from the cluster axis indicating chirality. Gold and sulfur atoms are depicted as yellow and cyan spheres in the image. Panels A, B reproduced with permission.<sup>[93]</sup> Copyright 2007, American Association for the Advancement of Science. C-a) Top and b) side-view of the crystal structure of the left-handed enantiomer of  $Au_{38}SR_{24}$  (where  $SR = SCH_2CH_2Ph$ ). Gold adatoms are shown in yellow, Au core atoms in green and sulfur atoms in orange;  $CH_2CH_2Ph$  units in the thiol were removed for clarity. c) Schematic representation of a top view of the  $Au_{38}SR_{24}$  cluster along the C3 axis highlighting the handedness of the QC. The top three core atoms binding to the long staples are represented by the inner triangle while the outer triangle denotes the core Au atoms binding to the "end" of the staple. The long staples are represented by arrows and the two triangles are not in the same plane. d) Top and e) side view of the cluster in space-filling mode. Reproduced with permission.<sup>[89]</sup> Copyright 2012, Macmillan Publishers Ltd. D) CD spectra of  $Au_{25}$  nanoclusters capped by chiral thiol ligands (SCH<sub>2</sub>C\*H(CH<sub>3</sub>)Ph). Inset of figure shows a schematic of the cluster. Reproduced with permission.<sup>[140]</sup> Copyright 2011, American Chemical Society.

with achiral thiolates (2-phenylethylthiolate, denoted as PET) by HPLC. Crystal structure of the left-handed enantiomer of  $Au_{38}(SCH_2CH_2Ph)_{24}$  is shown in Figure 4C. This cluster has a prolate shape with face-fused biicosahedral  $Au_{23}$  core, which is protected by three short  $Au(SR)_2$  and six long  $Au_2(SR)_3$  staples (Figure 4C). The staples are arranged in a chiral fashion, wherein the long staples have a staggered configuration of two triblade fans (composed of three staples), which can rotate either clockwise or anti-clockwise, depending on the enantiomer. The short staples at the equator of the cluster follow the handedness of the long staples, but are slightly tilted with respect to the threefold axis.

Among  $Au_{25}(SR)_{18}$  QCs, GSH-protected  $Au_{25}$  clusters  $[Au_{25}(SG)_{18}]$  showed distinct CD signals whereas phenylethylthiolate-protected  $Au_{25}$  i.e.  $[Au_{25}(SCH_2CH_2Ph)_{18}]$  did not.<sup>[138,139]</sup> This was due to the intrinsic chiral nature of the former ligand. Whereas,  $Au_{25}(PET^*)_{18}$  clusters synthesized using chirally modified phenylethylthiols  $[(HSCH_2C^*H(CH_3)Ph, abbreviated$ as PET\*] at the second position, showed mirror-imaged CD spectra.<sup>[140]</sup> The CD spectra of  $Au_{25}(PET^*)_{18}$  showed intense bands at 310 and 425 nm with positive sign for the peaks of the *R*-Au<sub>25</sub> isomer and negative for the *S*-Au<sub>25</sub> isomer (Figure 4D). The observed CD signals are not from the R- or S-pet\* ligands, as the ligand itself shows neither absorption nor CD signals in this wavelength region. On the basis of the atomic structure and electronic properties of well-defined  $Au_{25}$  nanoclusters, it was found that the chirality of  $Au_{25}(SR^*)_{18}$  is not caused by the metal core but by the surface ligands and surface gold atoms of the cluster  $^{\rm [41,140]}$ 

#### 3.6. Two-Photon Absorption

The noble metal QCs behave differently from NPs in terms of their emission lifetimes as well as two-photon cross-sections. In view of their biological applications, the nonlinear optical properties such as two/multi-photon-excited emissions are beneficial for low power medical imaging as two-photon excitation (TPE) in the NIR region increases the penetration depth, spatial resolution, and minimizes autofluorescence. Two photon absorption (TPA) properties of AuQCs have been extensively investigated by Goodson and co-workers<sup>[141]</sup> two-photon excited emission was first observed for Au25 QCs under 1290 nm excitation, showing an emission peak at 830 nm (Figure 5A). The pumppower dependence of fluorescence with a slope of  $\approx 2$  indicates that it is a two-photon excited emission (Figure 5B). The TPA cross-section of Au25 in hexane was measured to be 42 700 GM (Göppert-Mayer unit, 10-50 cm<sup>4</sup>s), which is superior to the TPA cross-sections of many organic chromophores. Apart from the NIR luminescence for Au<sub>25</sub> clusters, the additional luminescence observed in the visible region with maximum around 510 nm was later found to be two-photon allowed.<sup>[141]</sup>

In 2008, Dickson and co-workers developed three watersoluble Ag clusters, emitting at 660, 680, and 710 nm, using





Figure 5. A) Two-photon excited fluorescence from Au25. B) Pump power dependence of Au25 for the two-photon excited emission. Reproduced with permission.<sup>[141]</sup> Copyright 2008, American Chemical Society.

single-stranded (ss) DNA, which exhibited large two-photon cross-sections of ≈50 000 GM providing bright, photostable emission with versatile tunability of excitation and emission wavelengths.<sup>[142]</sup> Recently, two-photon excited fluorescence from a DNA-templated Ag QC, showing an emission at 630 nm when excited at 800 nm, with a two-photon absorption crosssection of ≈3000 GM was reported.<sup>[143]</sup>

#### 3.7. Magnetism

In 2004, ferromagnetic behavior was observed in 1.4 nm<sup>[144]</sup> and 1.9 nm<sup>[145]</sup> thiol-capped gold NPs. This generated much interest as bulk Au is diamagnetic. The hysteresis loops were measured at 5 and 300 K for thiol-capped AuNPs of  $\approx$ 1.4 nm diameter.<sup>[144]</sup> From the curve, the ferromagnetic behavior exhibited by the NPs is evident and high coercive field values, ranging between 250 and 860 Oe at 300 and 5 K, respectively, were observed. Magnetic moment of the gold atoms was estimated to be 0.036 µB. However, diamagnetic behavior was observed in the case of similar sized Au particles protected by a surfactant. Thus the localized magnetic moment arises due to the 5 d localized holes generated through Au-S bonds, which in turn is a result of the high spin-orbit coupling (1.5 eV) of Au and the reduction in symmetry associated with Au-Au and Au-S bonds. Miyake and co-workers<sup>[146]</sup> discussed the observance of Au ferromagnetism and studied the diameter-dependent magnetization behavior of Au particles and predicted a maximum magnetic moment per Au atom in the particles of 3 nm diameter. The ferromagnetic polarization mechanism of metallic Au is different from that of transition metals and existence of a spin-correlation effect at the nanoscale was proposed by the authors.

Paramagnetism has been reported in several noble metal QCs.<sup>[41,147,148]</sup> Jin and co-workers<sup>[148]</sup> reported the paramagnetic behavior exhibited by charge neutral Au<sub>25</sub> nanoclusters, [Au<sub>25</sub>SR<sub>18</sub>]<sup>0</sup>. The magnetic properties of these monodisperse Au<sub>25</sub> QCs were evaluated with electron paramagnetic resonance (EPR) spectroscopy using microcrystal powders of these QCs, which showed an S = 1/2 signal with g = 2.56, 2.36, and 1.82. EPR quantification indicates that [Au<sub>25</sub>(SR)<sub>18</sub>]<sup>0</sup> has one unpaired spin per particle. Magnetism is strongly dependent on the charge state of the cluster.  $(Au_{25}SR_{18})^0$  is

magnetic and  $(Au_{25}SR_{18})^{-1}$  is nonmagnetic and this enabled the reversible switching of magnetism in such clusters (Figure 6B). The reason for the observed magnetic property has been attributed to the presence of one unpaired spin per particle, delocalized in the Au<sub>13</sub> core. This is contrary to the previous belief that the magnetism in gold arose from the particle surface via charge transfer in the Au-S bonds. Magnetization measurements of the clusters using superconducting quantum interference device (SQUID) magnetometer also revealed their paramagnetic nature between 5 and 300 K with no hysteresis at 5 K.

Magnetic moment measurements using X-ray magnetic circular dichroism (XMCD) of various GSH-protected AuQCs (Au<sub>n</sub>SR<sub>m</sub>) by Tsukuda and co-workers<sup>[147]</sup> showed an increase in magnetic moment with increase in cluster size. However, the magnetic moment observed per Au-S bond remained constant. Thus, the inherent spin polarization observed in the gold QCs was identified as a consequence of the localized hole created by Au-S bonding at the Au-S interface rather than due to a quantum size effect. XMCD data on Au<sub>18</sub>SG<sub>14</sub> cluster revealed its paramagnetic nature with a magnetic moment of 0.0093  $\mu B$ per Au-S obtained from SQUID measurements. The absence of hysteretic behavior in the magnetization curves of Au<sub>18</sub>SG<sub>14</sub> at 2, 5, 200, and 290 K, as shown in Figure 6C, suggests the paramagnetic nature of the cluster. Induced magnetism in clusters via chemical oxidation was demonstrated recently in the case of Au102MBA44 QCs.[149]

#### 3.8. Stability

Stability of QCs is important for their application in diverse fields. Thiolate-protected gold clusters (Au<sub>n</sub>SR<sub>m</sub>) of certain compositions show higher stability compared to others. Stability of QCs is governed by various electronic and geometric factors and is often explained in terms of magic numbers,<sup>[84]</sup> superatom model,<sup>[150]</sup> closing of electronic shells,<sup>[151-153]</sup> etc. Tsukuda and co-workers<sup>[84]</sup> isolated various GSH-protected magic numbered AuQCs such as Au<sub>18</sub>SG<sub>11</sub>, Au<sub>21</sub>SG<sub>12</sub>, Au<sub>25±1</sub>SG<sub>14±1</sub>, Au<sub>28</sub>SG<sub>16</sub>, Au<sub>32</sub>SG<sub>18</sub> and Au<sub>39</sub>SG<sub>23</sub> using PAGE and distinct optical properties were observed for each composition. Role of electronic factors in deciding the stability of clusters is also evident from



**Figure 6.** A) Hysteresis loops for thiolate-protected Au particles at 5 K and 300 K. Reproduced with permission.<sup>[144]</sup> Copyright 2004, American Physical Society. B) Schematic showing the magnetic reversibility of neutral and anionic Au<sub>25</sub>QCs. Reproduced with permission.<sup>[147]</sup> Copyright 2009, American Chemical Society. C) Magnetization curves of Au<sub>18</sub>SG<sub>14</sub> clusters at 2, 5, 200, and 290 K. Reproduced with permission.<sup>[147]</sup> Copyright 2006, American Chemical Society.

the isolation of charged Au clusters such as Au<sub>147</sub>,<sup>[151]</sup> Au<sub>44</sub>,<sup>[51]</sup> and Au<sub>25</sub>.<sup>[152,153]</sup> The unusual stability of Au<sub>25</sub>SR<sub>18</sub> QCs has been a subject of intense research, both experimentally and theoretically.<sup>[79,115,154,155]</sup> Passivating ligands play an important role on the electronic and thermodynamic stability of the QCs.[153,156-<sup>159]</sup> Among the water-soluble AuQCs, captopril-protected Au<sub>25</sub> exhibited improved thermal stability compared to their GSHprotected analogues.<sup>[160]</sup> Another interesting report by Negishi and co-workers<sup>[161]</sup> describes the increased stability of Au<sub>25</sub> QCs made with selenolate ligands compared to thiolate ligands against degradation in solution. In general, the thermal stability of AuQCs is higher than that of AgQCs.<sup>[155,162]</sup> Decomposition of GSH-protected Ag<sub>25</sub> clusters above 50 °C to yield Ag<sub>2</sub>S NPs  $(3 \pm 1 \text{ nm})$ ,<sup>[150]</sup> crystallizing in monoclinic acanthite polymorph, via S-C bond cleavage of the cluster monolayer, illustrates this phenomenon.<sup>[162]</sup>

Core alloying of Au<sub>25</sub> QCs with foreign atoms, such as Pd and Cu, affects their stability differently. While copper doping distorted the geometric structure and stability of Au<sub>25</sub> QCs,<sup>[163]</sup> mono-Pd-doped Au<sub>25</sub> (Pd<sub>1</sub>Au<sub>24</sub>SR<sub>18</sub>) enhanced their stability against degradation.<sup>[164]</sup> Recently, enhanced stability of Au<sub>25</sub> QCs in solution was demonstrated by supramolecular functionalization of  $\beta$ -cyclodextrin (CD) on 4-(*t*-butyl)benzyl mercaptan (BBSH) protected Au<sub>25</sub> QC, via host–guest interactions.<sup>[157]</sup> CD molecules act as an umbrella protecting the fragile cluster core from the direct interaction with destabilizing agents such as metal ions, ligands, etc.

#### 4. Applications

Unique properties of clusters combined with their robust nature enable them to be developed into useful materials. Energy, environment, and biology are the three main domains in which clusters have shown significant promise. Applications in each of these domains are discussed in the following sections.

Owing to their extremely small size and high reactivity, sensors made of such materials can markedly improve the sensitivity and specificity of analyte detection. For an efficient sensing strategy, the sensor material should be structurally robust and stable under ambient conditions. Combining the unique properties of QCs with other nanoscale materials can lead to creation of hybrid materials with enhanced properties of both. In spite of their highly sensitive nature, clusters are highly amenable for loading on various substrates without loss of properties enabling their use in diverse avenues. Several reports of clusters loaded on various substrates such as chitosan films,<sup>[165]</sup> silica particles,<sup>[166-168]</sup> alumina,<sup>[169]</sup> cyclodextrin gels,<sup>[170]</sup> graphene sheets,<sup>[171,172]</sup> bimetallic Au/Ag MFs,<sup>[135]</sup> crystals of polypeptide hormone (insulin),<sup>[173]</sup> boron nitride sheets,<sup>[174]</sup> SnO<sub>2</sub> nanowire networks,<sup>[175]</sup> etc. exist. Figure 7 shows a few examples of clusters embedded/coated on various substrates. Such hybrid materials can serve as functional multimodal materials for diverse applications. For example, a strategy for sub-zeptomole level visual detection of TNT, an explosive molecule, was developed using a hybrid material combining materials of two different size regimes, namely, Au MFs and fluorescent AgQCs.<sup>[135]</sup> Apart from advantages of the unique morphological features of Au MFs and the optical properties of the QCs, anchoring QCs on Au MFs can lead to surface-enhancement of their luminescence and thus ultrasensitive detection (discussed later).

#### 4.1. Applications in Environment

Recent research shows great promise of QCs in providing solutions to a number of environmentally relevant issues, which include pollution control and access to clean water. In view of





**Figure 7.** QCs embedded/supported on various substrates. Photographs under A,D, and F) visible light and under B,E, and G) UV light of clusters loaded on A,B) chitosan film, D,E) alumina, and F,G) cyclodextrin gels. TEM images of C) Au clusters on SiO<sub>2</sub> nanoparticles, H) AuQCs on graphene sheets, I) AuQCs on boron nitride sheets, and J) AgQCs on SnO<sub>2</sub> nanowires. K) Fluorescence microscopic image of AuQCs in insulin crystals. Panels A–C, F-G, and J reproduced with permission.<sup>[165,166,170,175]</sup> Copyright 2012 and 2011, American Chemical Society. Panels D-E, H, and K reproduced with permission.<sup>[169,171,173]</sup> Copyright 2011, Wiley-VCH. Panel I reproduced with permission.<sup>[174]</sup> Copyright 2013, Royal Society of Chemistry.

this, some noteworthy applications of QCs are described in the subsequent sections.

#### 4.1.1. Chemical Sensing

For chemical sensing applications, optical absorption and luminescence are two most employed optical properties utilized in sensor development. Advantages such as selectivity, sensitivity, and miniaturizability in addition to properties such as strong luminescence, good colloidal stability, and ease of functionalization make QCs better optical sensors than NPs and other molecules. In the case of QCs, both the ligand and the metal core are important in sensing molecules and their well-defined structures and compositions allow detailed exploration of the underlying mechanisms. Furthermore, owing to their small size, QCs can be anchored on NPs and thus used to create hybrid materials, having properties of both constituents, which can serve as novel platforms for ultrasensitive detection of various analytes of societal interest.  $^{[135]}$ 

*Heavy Metal Ion Sensing*: Heavy metal contamination in drinking water is a major problem faced by the global community.<sup>[176,177]</sup> Though heavy metals such as Mn, Cu, Fe, Zn, etc. are nutritionally essential in trace quantities to the body, they have significant toxicological and carcinogenic effects beyond a certain safe limit. Apart from their non-biodegradable nature, their inherent tendency to form complexes with biomolecules leading to rupture of hydrogen bonds, changes in the conformation and structure of proteins, inhibition of enzymes, etc. pose a threat to the society. Heavy metal ions are known to have adverse effects to humans, especially to the central nervous system, kidneys, liver, skin, bones, teeth, etc.<sup>[50,178,179]</sup> Limits of permissible contamination of such species in drinking water have been recommended by WHO (World Health Organization) and EPA (Environmental Protection Agency).<sup>[180]</sup>

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www.particle-journal.com www.MaterialsViews.cor (C1) (D) Au₁ (F)  $(E_1)$ Pb Zn Cu<sup>2+</sup> 100 ppb 50 ppb 1 ppm 5 ppm 50 ppm Ni (G) 0.8 Mg Intensity (%) Hg 0.6 0.6 0| / (|-0|) Co (E,) Cd 0.2 Ca Control 0.0 Al<sup>3+</sup>Ba<sup>2+</sup>Ca<sup>2+</sup>Cd<sup>2+</sup>Ce<sup>3+</sup>Cr<sup>3+</sup>Fe<sup>3+</sup>Li<sup>+</sup>Mr<sup>2+</sup>Pb<sup>2+</sup>Sr<sup>2+</sup> Hydrodynamic diameter (nm) 1000 Metal ions

**Figure 8.** A) Schematic illustration of fluorescence quenching of Au clusters in presence of  $Hg^{2+}$  ions due to formation of metallophilic  $Hg^{2+}$ –Au<sup>+</sup> bonds. B) Photographs showing the selective quenching of Au cluster solution with  $Hg^{2+}$  ions in presence various metal ions (at  $50 \times 10^{-6}$  M), under UV light. Reproduced with permission.<sup>[138]</sup> Copyright 2010, Royal Society of Chemistry. Fluorescence images ( $C_1-C_3$ ) showing the gradation of color with addition of  $Hg^{2+}$  ions of different concentrations into  $Au@SiO_2$ -FITC@Ag<sub>15</sub> MFs. Scale bars in the optical images of the MFs shown as inset correspond to 3 µm. Reproduced with permission.<sup>[135]</sup> Copyright 2012, Wiley-VCH. D) Illustration of  $Cu^{2+}$  ion sensing approach using an  $Au_{15}$  cluster embedded chitosan film and corresponding photographs showing the luminescence quenching of the film before and after exposing to varying concentrations of  $Cu^{2+}$  solutions. Reproduced with permission.<sup>[165]</sup> Copyright 2012, American Chemical Society. Fluorescence images of cells incubated with red emitting Au@BSAclusters before ( $E_1$ ) and after ( $E_2$ ) treatment with  $Cu^{2+}$  ions. Inset shows corresponding higher magnification images. Reproduced with permission.<sup>[204]</sup> Copyright 2011, Royal Society of Chemistry. F) Dynamic light scattering spectra of Cu@BSA clusters in presence of different metal ions validating the induction of protein–protein interaction and spherical aggregation of the cluster in presence of Pb<sup>2+</sup> ions. Reproduced with permission.<sup>[211]</sup> Copyright 2011, American Chemical Society. G) Plot showing the effect of fluorescence quenching  $[(I_0-I)/I_0]$  of the Ag clusters at 575 nm with different metal ions ( $20 \times 10^{-3}$  M) and its high selectivity towards  $Cr^{3+}$  ions. Reproduced with permission.<sup>[200]</sup> Copyright 2011, Royal Society of Chemistry.

Tolerance limits for Pb<sup>2+</sup>, Cd<sup>2+</sup> and Hg<sup>2+</sup> in drinking water are 0.015, 0.002, and 0.005 mg L<sup>-1</sup>, respectively. Although various conventional techniques such as atomic absorption spectroscopy, inductively coupled plasma optical emission/mass spectrometry, UV–vis absorption spectroscopy, etc<sup>[181–185]</sup> provides sensitive and selective analysis of metal ions in solution; tedious sample preparation, pre-concentration steps, sophisticated instrumentation and need of skilled professionals are disadvantages aspects of such methods.

QCs of gold and silver with bright luminescence in the visible window are utilized for sensing toxic metal ions.<sup>[135,186–188]</sup> Among the heavy metal ion contaminants, mercuric ions are the most studied ones using QCs of Au, due to the strong metallophilic interaction between the d<sup>10</sup> centers of Au<sup>+</sup> (5d<sup>10</sup>) and Hg<sup>2+</sup> (5d<sup>10</sup>) ions. Relativistic effects and huge dispersion forces between these closed shell atoms are responsible for this effect. Selective detection of Hg<sup>2+</sup> ions has been demonstrated by many groups using luminescent clusters of gold<sup>[188–191]</sup> and silver.<sup>[66,192,193]</sup> Compared to the absorption bands, fluorescence originating from QCs are much more sensitive to changes in their immediate environment. Thus most studies on metal ion sensitivity are carried out based on changes in fluorescence as it is more sensitive to the changes in particle size. Proteinprotected clusters of Au and Ag are promising candidates for metal ion sensing experiments due to their high quantum yields (6%-10%). In most cases, intrinsic luminescence from the clusters is quenched upon interaction with the Hg2+ ions as a result of formation of Hg<sup>2+</sup>-Au<sup>+</sup> or Hg<sup>2+</sup>-Ag<sup>+</sup> bonds with the cluster core as shown in the schematic in Figure 8A. Selective interaction of mercury ions with BSA-protected Au clusters was reported by Ying et al.<sup>[188]</sup> Red luminescence from the clusters was quenched instantaneously upon addition of Hg<sup>2+</sup> ions in comparison to other ions (Figure 8B) and the intensity of quenching was found to be linearly dependent on the concentration of mercury ions with a detection limit of  $0.5 \times 10^{-9}$  M. This method was also extended to a paper test strip system using nitrocellulose membrane as a support for the clusters facilitating rapid visual detection. Several other reports on selective interaction of mercury ions with BSA-protected clusters of Au and Ag exist.<sup>[188,190,194]</sup> Mercury ions were detected visually at sub-zeptomole level from solution among other metal ions using 15 atom silver clusters<sup>[62]</sup> anchored on silica-coated gold mesoflowers (denoted as Au@SiO2@Ag15 MFs).[135] Incorporation of an additional dye, fluorescein isothiocyanate (FITC) on the hybrid sensor enabled visual color change of the MFs from red to green in presence of the analyte. Figures  $8(C_1-C_3)$ show the distinct change in fluorescence of the MFs from red to green upon exposure to increasing Hg<sup>2+</sup> concentration. The





intermediate yellow (Figure 8C<sub>2</sub>) color could be due to the additive effect of the unquenched red luminescence of the cluster on the MF surface and the underlying green luminescence of FITC-incorporated silica shell.

Interaction of mercury ions with the passivating ligands on the cluster surface and subsequently quenching the cluster luminescence is yet another strategy for sensing. However, multivalent ions such as Pb2+ and Cd2+ possess similar binding affinity with carboxylic anions of the ligand. Quenching of the green luminescence of 11-mercaptoundecanoic acid (MUA)protected Au clusters upon addition of Hg<sup>2+</sup> ions reported by Chang and co-workers<sup>[195]</sup> was attributed to a similar iontemplated chelation process but the clusters were unable to differentiate Hg<sup>2+</sup> ions from Pb<sup>2+</sup> and Cd<sup>2+</sup> ions. Thus an additional chelating ligand 2,6-pyridinedicarboxylic acid (PDCA), known to form highly stable complexes with Hg<sup>2+</sup> compared to other ions, was introduced into the solution. The adsorbed PDCA ligands on the cluster surface effectively masked other ions and improved the selectivity of the cluster towards Hg<sup>2+</sup> ions via a cooperative effect. A luminescent, freestanding film for the detection of Cu<sup>2+</sup> ions, in aqueous solution was developed utilizing the sensitivity of Au<sub>15</sub> cluster towards Cu<sup>2+</sup> ions.<sup>[165]</sup> The cluster-embedded chitosan film, similar to the pH paper, showed a visual sensitivity to Cu<sup>2+</sup> ions up to 1 ppm (Figure 8D). Several other reports also exist on the detection of Cu ions using Ag<sup>[196]</sup> NPs, core-shell polymer NPs<sup>[197,198]</sup> and Au<sup>[199]</sup> clusters. Zhu and co-workers<sup>[200]</sup> demonstrated a fluorescent silver cluster probe for the detection of Cr<sup>3+</sup> ion with high sensitivity and selectivity, based on the fluorescence quenching of the cluster. The detection limit was found to be 28 × 10<sup>-9</sup> м.

As yet another strategy, aggregation-induced fluorescence quenching mechanism of clusters was utilized to detect ions such as  $Hg^{2+}$ ,  $Fe^{3+}$ , [201]  $Pb^{2+}$ , [202]  $Cu^{2+[61,63,203]}$  etc. Mercurv ions can form chelation complexes with free carboxylic acid groups of the ligands such as dihydrolipoic acid (DHLA),<sup>[193]</sup> GSH, etc. resulting in interparticle aggregation and loss of cluster luminescence. Compared to the chelation tendencies of other ions, the relatively high binding affinity of mercury ions with simple carboxylic acids in water may be the reason for the greater quenching observed in presence of Hg<sup>2+</sup>. Similar strategy was utilized for the detection of copper ions in live cells (Figure 8E) using BSA-protected Au clusters under various pH conditions.<sup>[204]</sup> The red emission observed from the cells incubated with Au–BSA (Figure 8E<sub>1</sub>) was completely quenched upon exposure of  $Cu^{2+}$  ions (Figure 8E<sub>2</sub>). Copper ions are known to chelate with GSH in 1:2 (Cu<sup>2+</sup>:GSH) ratio. This principle was utilized to demonstrate a "turn-on" luminescence sensor for GSH using Au@BSA clusters.<sup>[61]</sup> Addition of GSH to a mixture containing QCs and Cu2+ ions led to the formation of a complex between Cu<sup>2+</sup> ions and GSH resulting in the deaggregation of clusters and thereby luminescence recovery. Bovine serum albumin-protected copper clusters (Cu@BSA) were demonstrated as a potential sensor for Pb2+ ions in water up to ppm concentrations.<sup>[21]</sup> The quenching was associated to the complexation between BSA and Pb2+ ions via the carboxylate groups leading to cluster aggregation. DLS measurements of the system showed an increase in the hydrodynamic diameter (Figure 8F) of the cluster solution post-treatment with Pb<sup>2+</sup> ions

due to the protein–protein interaction and subsequent spherical aggregation of the cluster.

Another plausible mechanism for the luminescence quenching of clusters in presence of metal ions was the transfer of electron density from the clusters to the adsorbed cations leading to partial oxidation and subsequent amalgam formation<sup>[205]</sup> of the cluster. A simple static quenching mechanism was proposed by Wang and co-workers<sup>[192]</sup> using Ag@oligonucleotide clusters based on the resulting unchanged fluorescence lifetime and the linear Stern-Volmer plot observed upon interaction with mercury ions with the cluster. Here, formation of a nonfluorescent complex as a result of the interaction was associated with the quenching of cluster luminescence. Use of Ag<sub>25</sub> clusters for the detection of Hg<sup>2+</sup> ions at low concentrations (LOD – 1 ppb) has been demonstrated.<sup>[66]</sup> Both colorimetric and fluorescence detection were demonstrated and the changes in cluster features were utilized for the quantitative analysis of the ions. Detection of Cr<sup>3+</sup> ions from solutions with high sensitivity was demonstrated by fluorescent Ag nanoclusters<sup>[200]</sup> in presence of other metal ions such as Al<sup>3+</sup>, Ba<sup>2+</sup>, Ca<sup>2+</sup>, Cd<sup>2+</sup>, Ce<sup>3+</sup>, Fe<sup>3+</sup>, Li<sup>+</sup>, Mn<sup>2+</sup>, Pb<sup>2+</sup>, Sr<sup>2+</sup>, Y<sup>3+</sup>, and Zn<sup>2+</sup> (Figure 8G). Table 3 lists some of the cluster-based heavy metal ion sensors and their properties.

Anion Sensing: QC-based sensors for the detection of anions such as halides, sulfides, cyanides, nitrites, etc are also known. These anions have significant role in environmental pollution. A gold-cluster-based fluorescent sensor for the detection of cyanide ions from aqueous solutions was demonstrated by Lu et al.<sup>[206]</sup> The authors proposed a cyanide etching-induced fluorescence quenching of the gold clusters (**Figure 9**A) based on the unique Elsner reaction (given below) between cyanide and gold atoms.

#### $4Au + 8CN^{-} + 2H_2O + O_2 \rightarrow 4Au(CN)_2^{-} + 4OH^{-}$

The quenching effect was strongly dependent on the pH of the solution and maximized at pH 12, because under low pH conditions CN- ions can capture available protons in solution to form hydrocyanic acid (HCN). The detection limit of 200  $\times$  $10^{-9}$ M achieved was much lower than the allowed limit of CN<sup>-</sup> in drinking water ( $2.7 \times 10^{-6}$  M) stipulated by WHO. Moreover, the technique was demonstrated to be effective in detecting CN<sup>-</sup> from real water samples such as local groundwater, tap water, pond water, and lake water spiked with cyanide. A fluorescent and colorimetric platform (Figure 9B) for the sensitive and selective detection of halide ions (e.g., Cl<sup>-</sup>, Br<sup>-</sup>, and I<sup>-</sup>) was developed by Luo and co-workers<sup>[207]</sup> using hyperbranched polyethyleneimine-functionalized Ag clusters based on halideinduced oxidative etching and aggregation of Ag nanoclusters. Reaction between halide ions and silver atoms and the difference in solubility constants (Ksp) of the silver compounds serve as the indicators for detection. Though oxidative etching is proposed as the dominant mechanism at lower halide concentrations, an increase in halide ions chemisorbed on the surface of the clusters could neutralize the surface charge of Ag@PEI clusters thereby giving rise to an increase in van der Waals forces among the particles leading to their aggregation at higher halide concentrations. LODs for Cl<sup>-</sup>, Br<sup>-</sup>, and I<sup>-</sup> ions are  $200 \times 10^{-9}$ ,  $65 \times 10^{-9}$ , and  $40 \times 10^{-9}$ M, respectively. Besides,

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 Table 3. List of various clusters exhibiting metal ion sensitivity and associated properties.

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Detection technique	Analyte	Cluster/hybrid system used	Selectivity among other ions	LOD	Sample matrix	Ref.
Fluorescence quenching	Hg <sup>2+</sup>	Ag@oligonucleotide	Co <sup>2+</sup> , Ni <sup>2+</sup> , Pb <sup>2+</sup> , Zn <sup>2+</sup> , Cu <sup>2+</sup> , Fe <sup>2+</sup> , Fe <sup>3+</sup> , Mn <sup>2+</sup> , and Cd <sup>2+</sup>	5 × 10 <sup>-9</sup> м (1 ppb)	Aqueous media	[192]
		Au <sub>25</sub> @BSA	$\begin{array}{l} Ag^{*},Cu^{2+},Zn^{2+},Mg^{2+},K^{+},Na^{+},Ni^{2+},Mn^{2+},Fe^{3+},\\ Cd^{2+},Pt^{4+},Pd^{2+},Co^{2+},Pb^{2+},Ca^{2+},Cl^{-},NO_{3}^{-},\\ SO_{4}^{-2},andPO_{4}^{-3-} \end{array}$	0.5 × 10 <sup>-9</sup> м (0.1 ppb)	Aqueous media	[188]
		Ag@DHLA	K <sup>1+</sup> , Cs <sup>1+</sup> , Sr <sup>2+</sup> , Ba <sup>2+</sup> , Mg <sup>2+</sup> ,Mn <sup>2+</sup> , Fe <sup>2+</sup> ,Co <sup>2+</sup> , Pb <sup>2+</sup> ,Cu <sup>2+</sup> , Zn <sup>2+</sup> , Sn <sup>2+</sup> , and Pd <sup>2+</sup>	1 × 10 <sup>-9</sup> м	Aqueous media	[193]
		Au@MUA	$Li^{1+},Na^{1+},K^{1+},Mg^{2+},Ca^{2+},Sr^{2+},Ba^{2+},Cu^{2+},Co^{2+},\\ Ni^{2+},Zn^{2+},Pb^{2+},Hg^{2+},Cd^{2+},Fe^{2+},Al^{3+},Cr^{3+},and\\ Au^{3+}$	5×10 <sup>-9</sup> м (1 ppb)	Aqueous media	[189]
		Au@BSA	Na <sup>1+</sup> , K <sup>1+</sup> , Mg <sup>2+</sup> , Ca <sup>2+</sup> , Ba <sup>2+</sup> , Pb <sup>2+</sup> , Mn <sup>2+</sup> , Fe <sup>3+</sup> , Ni <sup>3+</sup> , Zn <sup>2+</sup> , Cd <sup>2+</sup> , Al <sup>3+</sup> , Cr <sup>3+</sup> , Cu <sup>2+</sup> , Br <sup>-</sup> , I <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , SO <sub>3</sub> <sup>-</sup> , Ac <sup>-</sup>	80 nm	Aqueous media	[327]
		Au@SiO2@Ag15 MFs	Pb <sup>2+</sup> , Ni <sup>2+</sup> , Cd <sup>2+</sup> , and Cu <sup>2+</sup>	0.1 zeptomoles	Aqueous media	[135]
		Ag <sub>25</sub> @GSH	Cr <sup>3+</sup> , Mn <sup>2+</sup> , Fe <sup>3+</sup> , Co <sup>2+</sup> , Ni <sup>2+</sup> , Cu <sup>2+</sup> , Zn <sup>2+</sup> , Pd <sup>2+</sup> , Cd <sup>2+</sup> , Pt <sup>2+</sup> , Au <sup>3+</sup>	1 ppb	Aqueous media	[66]
	Hg <sup>2+</sup> and CH <sub>3</sub> Hg <sup>+</sup>	Au@Lys VI	Li <sup>+</sup> , Na <sup>+</sup> , K <sup>+</sup> , Mg <sup>2+</sup> , Ca <sup>2+</sup> , Sr <sup>2+</sup> , Ba <sup>2+</sup> , Fe <sup>2+</sup> , Fe <sup>3+</sup> , Co <sup>2+</sup> , Ni <sup>2+</sup> , Cu <sup>2+</sup> , Zn <sup>2+</sup> , Mn <sup>2+</sup> , Ag <sup>+</sup> , Cd <sup>2+</sup> , Pb <sup>2+</sup> , Cr <sup>3+</sup> , F <sup>-</sup> , Cl <sup>-</sup> , Br <sup>-</sup> , l <sup>-</sup> , ClO <sub>4</sub> <sup>-</sup> , BrO <sub>3</sub> <sup>-</sup> , IO <sub>3</sub> <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , CO <sub>3</sub> <sup>2-</sup> , HCO <sub>3</sub> <sup>-</sup> , SO <sub>3</sub> <sup>-</sup> , SO <sub>4</sub> <sup>2-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , PO <sub>4</sub> <sup>3-</sup> , HPO <sub>4</sub> <sup>2-</sup> , H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> , AcO <sup>-</sup> , citrate <sup>3-</sup> , BO <sub>3</sub> <sup>3-</sup>	$3\times10^{-12}$ $_{M}$ (Hg $^{2+})$ and $4\times10^{-9}$ $_{M}$ (CH $_{3}Hg^{+})$	Aqueous media and sea water	[191]
	Cr <sup>3+</sup>	Ag@PMAA-Na	$Al^{3+},Ba^{2+},Ca^{2+},Cd^{2+},Ce^{3+},Fe^{3+},Li^+,Mn^{2+},Pb^{2+},Sr^{2+},Y^{3+},andZn^{2+}$	28 × 10 <sup>-9</sup> м	Aqueous media	[200]
	As <sup>3+</sup>	Au@Cys	Ba <sup>2+</sup> , Co <sup>2+</sup> , Mn <sup>2+</sup> , Mg <sup>2+</sup> , Fe <sup>2+</sup> , Pb <sup>2+</sup> , Zn <sup>2+</sup> , Ni <sup>2+</sup> ,Ca <sup>2+</sup> , Sr <sup>2+</sup> , Sb <sup>3+</sup> , Bi <sup>3+</sup> , Al <sup>3+</sup> , Cr <sup>3+</sup> , and Au <sup>3+</sup>	53.7 × 10 <sup>-9</sup> м	Aqueous media	[328]
	Pb <sup>2+</sup>	Cu@BSA (Cu5 and Cu13 core)	Hg <sup>2+</sup> , Ca <sup>2+</sup> , Co <sup>2+</sup> , Zn <sup>2+</sup> , Ni <sup>2+</sup> , Cd <sup>2+</sup> , Mg <sup>2+</sup> , Na <sup>+</sup> , and K <sup>+</sup>	>20 ppm	Aqueous media	[21]
	Cu <sup>2+</sup>	Ag@PMAA	$ \begin{array}{l} K^{+},Na^{+},Ag^{+},Ca^{2+},Ba^{2+},Zn^{2+},Mg^{2+},Pb^{2+},Co^{2+},\\ Ni^{2+},AI^{3+}andFe^{3+},F^{-},CI^{-},Br^{-},CIO_4^{-},BrO_3^{-},\\ IO_3^{-},Ac^{-},C_2O_4^{-},NO_2^{-},B_4O_7^{2-},SO_3^{2-},SO_4^{2-},\\ PO_4^{3-}andcitrate^{-} \end{array} $	8 × 10 <sup>-9</sup> м	Reversible aqueous media	[329]
		Au@GSH	Hg <sup>2+</sup> , Pb <sup>2+</sup> , Cd <sup>2+</sup> , Fe <sup>2+</sup> , Co <sup>2+</sup> , Ni <sup>2+</sup> , Zn <sup>2+</sup> , Mn <sup>2+</sup> , Ca <sup>2+</sup> , Mg <sup>2+</sup> , Ba <sup>2+</sup> , Al <sup>3+</sup> , Fe <sup>3+</sup>	3.6×10 <sup>-9</sup> м	Aqueous media	[203]
		Au <sub>15</sub> @αCD	Fe <sup>3+</sup> , Zn <sup>2+</sup> , Ag <sup>1+</sup> , Cd <sup>2+</sup> , Hg <sup>2+</sup>	${<}1{\times}10^{-6}$ м Not tested	Aqueous media	[170]
		Au@NLf	$Ag^{1+},Ca^{2+},Ni^{2+},Co^{3+},Fe^{3+},andZn^{2+}$	10 ppm	Aqueous media	[63]
		Au@BSA	Ni <sup>2+</sup> , Co <sup>2+</sup> , Fe <sup>2+</sup> , Fe <sup>3+</sup> , Cd <sup>2+</sup> , Pb <sup>2+</sup>	50 × 10-6 м	Live cells	[204]
Fluorescence enhancement		Cu/Ag@DNA	Na+, K+, Mg^+, Ca^+, Zn^+, Pb^+, Cd^+, Ni^+, Cr^+, and Fe^{3+}	2.7 × 10 <sup>-9</sup> м	Aqueous media	[198]
	Ag+	Au <sub>16</sub> @BSA	Al <sup>3+</sup> , Ca <sup>2+</sup> , Cd <sup>2+</sup> , K <sup>+</sup> , Mg <sup>2+</sup> , Mn <sup>2+</sup>	$1 \times 10^{-6}$ м	Aqueous media	[18]
	Al <sup>3+</sup>	AgAu@MSA	Fe <sup>3+</sup> , Fe <sup>2+</sup> , Hg <sup>2+</sup> , Cu <sup>2+</sup> , Pb <sup>2+</sup> , Mg <sup>2+</sup> , Zn <sup>2+</sup> , Ni <sup>2+</sup> , Mn <sup>2+</sup> , Cr <sup>3+</sup> , Cd <sup>2+</sup> , Co <sup>2+</sup> and Ba <sup>2+</sup>	$0.8  imes 10^{-6}$ m	Aqueous media	[330]

\*Abbreviations used are, limit of detection (LOD), bovine serum albumin (BSA), dihydrolipoic acid (DHLA), mercaptoundecanoic acid (MUA), Ag<sub>15</sub> cluster- functionalized silica-coated Au mesoflower (Au@SiO<sub>2</sub>@Ag<sub>15</sub> MFs), glutathione (GSH), lysozyme type VI (Lys VI), sodium salt of polymethacrylic acid (PMAA-Na), α-cyclodextrin (α-CD), native lactoferrin (NLf), polyethyleneimine (PEI), DNA-templated bimetallic Au/Ag nanoclusters [(Au/Ag)@DNA)] and mercaptosuccinic acid (MSA).

selective detection of Br<sup>-</sup> and I<sup>-</sup> ions coexisting with Cl<sup>-</sup> ions was demonstrated under conditions of higher ionic strength. This sensor has been successfully applied for the detection of Cl<sup>-</sup> in real water samples. Sulfide ions have been detected with a LOD of  $0.83 \times 10^{-9}$  M from solutions utilizing the fluorescence quenching of DNA-templated gold/silver nanoclusters (Au/Ag@DNA QCs) in presence of sulfide ions.<sup>[208]</sup> Changes to the conformation of the DNA template from packed hairpin to

random coil structures as a result of interaction between sulfide ions and gold/silver atoms were responsible for this effect. Addition of sodium peroxydisulfate to the mixture reduced the interference from I<sup>-</sup> ions in the detection protocol. Yang and coworkers<sup>[209]</sup> constructed a molecular Boolean NAND logic gate for the detection of nitrite ion using Au@BSA clusters. Cluster luminescence, acting as the optical transducer signal, was quenched only in presence of both nitrous acid and hydrogen



**Figure 9.** A) Schematic illustration of the detection of cyanide ions from aqueous solution using Au clusters. Inset shows photographs of the cluster solution 1 and 1') before and 2 and 2') after addition of  $5 \times 10^{-3}$  M cyanide ions under 1 and 2) visible and 1' and 2') UV light, respectively. Reproduced with permission.<sup>[206]</sup> Copyright 2010, Wiley-VCH. B) Photographs showing the colorimetric and fluorescence detection of halide ions using hyperbranched polyethyleneimine-capped Ag clusters upon addition of different concentrations of halide ions. Reproduced with permission.<sup>[208]</sup> Copyright 2012, American Chemical Society. C) Schematic representation of the binary Boolean NAND logic gate based on Au@BSA clusters with H<sub>2</sub>O<sub>2</sub> and nitrite ions as inputs. Symbolic expressions for the logic gate are also shown in each case. Reproduced with permission.<sup>[209]</sup> Copyright 2013, Royal Society of Chemistry.

peroxide. This property was used as the readout to build the NAND logic gate and was used to monitor presence of nitrite ions in real samples.

TNT Sensing: Ultra trace sensors for analytes of societal interest such as 2,4,6-trinitrotoluene (TNT) is an ongoing quest due to their importance in national security and welfare of humanity. TNT is a man-made compound, which is used as an explosive material for military applications. TNT from the explosion and production sites is generally released into the environment through wastewater effluents where it can persist for many years. This nitroaromatic compound is toxic to many organisms including animals, plants, and microorganisms. A novel strategy for visual detection of TNT at sub-zeptomole level<sup>[135]</sup> was achieved using a hybrid material made by combining two systems and their variants with specific properties; one in the mesoscale regime (Au MFs)<sup>[7]</sup> having unique structural features observable under an optical microscope and another in the sub-nanometer regime (Ag<sub>15</sub>@ BSA)<sup>[62]</sup> having sensitivity to the analyte. Figure 10A shows a schematic of the sensing strategy. Change in the luminescence color in presence of an analyte being a more desirable indicator, a TNT-insensitive fluorophore, FITC was precoated on the MFs resulting in a bright green emission from the Au@SiO2-FITC MFs (Figure 10B(1 and 1')). After further functionalization with AgQCs, the Au@(SiO<sub>2</sub>-FITC)@Ag<sub>15</sub> MFs showed a red emission (Figure 10B(2 and 2')) wherein the FITC emission was suppressed. Upon exposure to 10 ppb TNT, a green emission from the underlying FITC was observed (Figure 10B(4 and 4')), as the red luminescence from the cluster had been completely

quenched. Even at 100 ppt, an observable color change was evident (Figure 10B(3 and 3')). The observation of green luminescence is in agreement with the solution-phase data, wherein the disappearance of cluster emission and the emergence of FITC emission are observed upon TNT exposure (Figure 10C). Owing to their highly anisotropic nature, MFs can act as highly sensitive probes for surface-enhanced Raman spectroscopy (SERS). Upon exposure to TNT, luminescence from the QCs on the bimetallic Ag-coated Au MFs (Au/Ag MFs) is lost and the Raman features from TNT (at 1209, 1361, 1535, 1619, and  $2960 \text{ cm}^{-1}$ ) are detectable on the particle, as shown in Figure 10D. This method stands unique compared to other nanomaterialsbased detection approaches as it involves the combination of multiple detection techniques such as SERS and luminescence to detect TNT at ultratrace levels and avoid false alarms. This approach can be used in terms of a single-particle, single-molecule detection technique, which is probably the ultimate in ultra-trace sensitivity with selectivity.

*Halocarbon Sensing*: Among the various contaminants present in the environment, halocarbons such as chlorofluorocarbons (CFC),  $C_2Cl_4$ ,  $C_2ClF_3$ ,  $CCl_4$ , etc. in water and air pose a threat to humanity owing to their potential for ozone depletion and global warming. In spite of regulations on their use, many of them find applications as industrial solvents, lubricants, plasticizers, and refrigerants due to lack of suitable replacements or financial constraints. Complete degradation of chlorocarbons such as  $CCl_4$ ,  $CHCl_3$ , and  $C_6H_5CH_2Cl$  at room temperature using mercaptosuccinic acid (MSA) protected Ag clusters,  $Ag_9MSA_7$ , was demonstrated by Pradeep and co-workers<sup>[210]</sup>

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**Figure 10.** A) Schematic of the TNT sensor. B) Optical (1, 2) and fluorescence (1', 2') images of green emitting  $Au@SiO_2$ -FITC MFs before (1, 1') and the red emitting  $Au@(SiO_2$ -FITC)@Ag<sub>15</sub> MFs (2, 2') after cluster functionalization. Optical (3, 4) and fluorescence (3', 4') images of the later upon exposure to 100 ppt (3, 3') and 10 ppb (4, 4') TNT, respectively. C) Effect of emission spectra of the bare cluster upon mixing with FITC dye and subsequent exposure to TNT of varying concentrations. The photographs of solution containing a mixture of FITC dye and Ag<sub>15</sub> cluster before and after TNT addition, taken under visible (1 and 2) and UV light (1' and 2'), respectively, are shown in the inset. D) Raman spectra showing the gradual evolution of TNT features from the  $Au/Ag@Ag_{15}$  MFs with addition of increasing concentration of TNT. Optical and fluorescence images of  $Au/Ag@Ag_{15}$  MFs are given in the inset. Gradual appearance of Raman feature at 2960 cm<sup>-1</sup> is shown in the inset. Reproduced with permission.<sup>[135]</sup> Copyright 2012, Wiley-VCH.

From their studies, Ag clusters showed increased efficiency towards such halocarbon degradation compared to their NP analogues. Importance of isopropyl alcohol (IPA) for the reaction was highlighted as it can improve the solubility of halocarbons in water. The authors propose that  $Cl^-$  ions formed due to the cleavage of  $Cl_3C$ —Cl can replace the thiolates on the surface of the cluster leading to loss of its stability and formation of AgCl.

#### 4.1.2. Catalysis

The field of gold catalysis has seen many advances over the past years. Extensive studies are carried out to fabricate novel catalysts with enhanced selectivity, activity, and stability. The excellent catalytic activity exhibited by gold in the NP regime is distinctly different from bulk. Gold was considered to be one of the least catalytically active metals until 1987 when gold NPs deposited on semiconducting transition-metal oxides showed surprising activity in carbon monoxide oxidation even at a temperature of  $-77 \, ^\circ C.^{[211]}$  The remarkable catalytic ability demonstrated by the metal was ascribed to its size. The greater number of low coordination surface atoms present on particles of smaller size is considered to facilitate the chemisorption of reactant molecules on the surface of the metal leading to their high catalytic activity. Aptly pointed out by Bond and Thompson in their review, "The long neglect of gold as a catalyst

is chiefly due to the failure to appreciate the necessity of creating particles that are sufficiently small and, for oxidations, of selecting a helpful support."<sup>[212]</sup> Subsequently, catalysts made of nanogold received enormous attention.<sup>[211,213–219]</sup> Studies of the onset of catalytic activity in gold clusters of various sizes, prepared on single crystalline titania surfaces showed that the activity of such QCs originates only when their diameters are less than 3.5 nm.<sup>[35]</sup> This threshold was associated to the metalto-nonmetal transition observed upon the decrease of cluster size below  $\approx$ 300 atoms per cluster.

Atomically precise clusters of gold, such as Au<sub>25</sub>, Au<sub>38</sub>, Au102, Au144, etc. have stimulated much interest in this area due to their unique structure-property relationships and known crystal structures. Recently, gold clusters stabilized by thiolate, phosphine, halides, and polymers as ligands have also been employed as catalysts for various reactions.<sup>[32,44,45]</sup> Apart from advantages due to monodisperse nature and large number of highly active surface sites for catalysis, correlation of their complete structure and catalytic properties stimulated much interest as it provided insights into their size-dependent catalytic properties. Catalytic activities of NPs are strongly dependent on their particle size. The broad size distribution of the NPs employed in such reactions hinders precise measurements of size-dependent catalytic performance, which is crucial for improving catalytic performance. Atomically precise QCs on the other hand, having solved X-ray crystal structures are advantageous in this context. For example, among



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the three AuQCs (Au<sub>25</sub> (diameter 1.0 nm), Au<sub>38</sub> (diameter 1.3 nm), and Au<sub>144</sub> (diameter 1.6 nm)) utilized for the selective oxidation of styrene, catalytic performance was found in the order, Au<sub>25</sub> > Au<sub>38</sub> > Au<sub>144</sub> indicating a strong size-dependence in catalytic performance.<sup>[220]</sup> Several reports exist on the catalytic activities of clusters for reactions such as oxidation of styrene,<sup>[219–222]</sup> carbon monoxide,<sup>[223–226]</sup> alcohol,<sup>[34,227–229]</sup> and cyclohexane,<sup>[230]</sup> hydrogenation of aldehydes and ketones,<sup>[33,231]</sup> reduction of nitrophenol,<sup>[232]</sup> and electrochemical reduction of O<sub>2</sub>.<sup>[23,233–236]</sup>

Both supported and unsupported clusters have shown enormous potential for catalytic activities. Clusters immobilized on various supports such as, graphene, SiO2, [222,225,237] titania,<sup>[238,239]</sup> iron oxide,<sup>[31]</sup> ceria,<sup>[240]</sup> etc. are important in the fabrication of heterogeneous catalysts. Gas-phase clusters deposited on various substrates are also excellent catalysts for various reactions. Carbon monoxide oxidation by Au<sub>8</sub> clusters deposited on MgO surfaces showed catalytic activity even at a temperature of 150 K.<sup>[241]</sup> Hutchings and co-workers<sup>[31]</sup> demonstrated similar CO oxidation activity using Au-10 clusters immobilized on iron-oxide supports. Similarly, sub-1 nm gold particles on ceria substrates were found to be highly active in methanol-steam-reforming and water-gas-shift reactions, compared to particles larger than 3 nm in size.<sup>[242,243]</sup> Ambient pressure X-ray photoelectron spectroscopy was used to study such reactions using various clusters of Au, Pt, Pd, and Cu clusters embedded in mesoporous ceria.<sup>[240]</sup> The oxidation of CO using mass-selected Pd13 clusters on thin MgO films was modeled using microkinetic simulation of the reaction by Heiz and coworkers.<sup>[244]</sup> The model allowed predictions of mole fractions, turn-over frequency, reaction probability, and sticking coefficients of the clusters in addition to providing an understanding of the substrate effects during catalysis. Effect of substrate on the cluster-ripening mechanisms was also studied by depositing monodisperse Pd clusters on three different model catalysts namely, (1) bare Rh(1 1 1), (2) superstructures of Moirépatterned graphene grown on Rh(1 1 1) and Ru(0 0 0 1), and (3) hexagonal boron-nitride film that was grown on Rh(1 1 1).<sup>[245]</sup> Mechanism of CO oxidation catalyzed by AuQCs on a thin defect-free MgO film supported on a Mo(100) surface was predicted by Landman and co-workers<sup>[246]</sup> to occur via a Langmuir-Hinshelwood or an Eley-Rideal mechanism on 2D gold cluster islands. The excess electronic charge at the gold cluster/ magnesia interface as a result of the penetration of metal states through the MgO thin film was considered to be the impetus for the observed catalytic activity. The dependence of the thickness of the substrate on the catalytic reaction was also studied using mass-selected Au<sub>20</sub> clusters via first-principles DFT calculations.<sup>[247]</sup>

Zeng et al.<sup>[248]</sup> conducted ab initio studies of the catalytic properties of 12 different clusters in the size range of  $Au_{16}$ - $Au_{35}$  towards CO oxidation reaction and proposed a quantitative assessment of the site-size–activity relationship. From their studies, anionic clusters showed stronger adsorbtion of CO and  $O_2$  in comparison to their neutral counterparts. **Figure 11**A shows the computed reaction pathways of various anionic Au clusters towards CO oxidation. While both activation energy and size of the clusters were important parameters in deciding the strength of CO adsorption, the effective indicator to access

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**Figure 11.** A) Computed pathways for the reaction,  $CO + O_2 \rightarrow CO_2 + O$ , catalyzed by various anionic gold clusters from Au<sub>16</sub> to Au<sub>35</sub>. The species adsorbed on the cluster is denoted by a star (\*). Reproduced with permission.<sup>[248]</sup> Copyright 2011, American Chemical Society. B) Plot showing the extremely high turnover number (TON) and turnover frequency (TOF) for different amounts of AuCl in the ester-assisted hydration reaction (shown in the inset) of in situ-formed alkynes using small gold clusters (Au<sub>3</sub> to Au<sub>10</sub>) as catalysts at room temperature. Reproduced with permission.<sup>[37]</sup> Copyright 2012, American Association for the Advancement of Science.

catalytic activities of the clusters was dependent on the CO and O<sub>2</sub> adsorption energies on them. Role of anionic clusters of gold  $Au_n^-$  (n = 1-7) on O<sub>2</sub> activation was probed by Wang et al.<sup>[249]</sup> using photoelectron spectroscopy.

Supported Au<sub>55</sub> clusters were used as efficient catalysts for the selective oxidation of styrene by dioxygen.<sup>[219]</sup> The catalytic activity was associated with the altered electronic structure of the Au<sub>55</sub> clusters. Further, the authors demonstrated that a sharp size threshold existed for the catalytic activity and particles with size (diameter) above 2 nm were completely inactive. Yet another promising report in this context was the huge turnover number of  $10^7$  atoms reported for the ester-assisted hydration reaction of alkynes using small gold clusters (Au<sub>3</sub> to Au<sub>10</sub>) as catalysts at room temperature at parts per billion concentrations.<sup>[37]</sup> Turnover number (TON) of a catalyst denotes its catalytic activity, it is the number of substrate molecules that can be converted to products by one catalyst site/species before its www.particle-journal.com

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**Figure 12.** A) Density functional theory model of the stable  $Au_{25}$ – $CO_2$  couple denoting the interaction of oxygen atom (shown in red) with three sulfur atoms (blue) of the  $Au_{25}$  shell during the reduction of  $CO_2$ . B) Potential-dependent rate of formation of  $H_2$  and CO for carbon black (CB) supported  $Au_{25}$  (denoted as  $Au_{25}/CB$  in figure) during electrolysis. Panels A,B reproduced with permission.<sup>[250]</sup> Copyright 2012, American Chemical Society. C) Cyclic voltammograms of Cu clusters deposited on glassy carbon electrode (0.1 M KOH) saturated with N<sub>2</sub> (black curve) or O<sub>2</sub> (red curve) under a potential scan rate of 0.1 V s<sup>-1</sup>. Reproduced with permission.<sup>[23]</sup> Copyright 2011, American Chemical Society. D) Schematic illustration of the proposed mechanism of  $Au_{25}SR_{18}$  catalyzed, chemoselective hydrogenation of  $\alpha$ , $\beta$ -unsaturated ketone to unsaturated alcohol. Au atoms of the core are shown in magenta and that of the shell are shown in cyan. Thiolate ligands are not shown for clarity. Reproduced with permission.<sup>[33]</sup> Copyright 2010, Wiley-VCH.

deactivation. The time component depicting the speed at which the catalyst operates is given by the turnover frequency (TOF), which is the number of substrate molecules converted per unit time. Plot showing the extremely high TON and TOF for different amounts of AuCl in the ester-assisted hydration reaction using small gold clusters (Au<sub>3</sub> to Au<sub>10</sub>) as catalysts are shown in Figure 11B.

Reduction of CO<sub>2</sub>, an important greenhouse gas, was studied both experimentally and theoretically using negatively charged atomically precise Au<sub>25</sub> clusters by Jin and co-workers.<sup>[250]</sup> They investigated the effect of catalytic properties upon interaction between  $Au_{25}(C_2H_4Ph)_{18}$  and  $CO_2$ .<sup>[250]</sup> A spontaneous and reversible electronic interaction similar to that seen during Au<sub>25</sub> oxidation was observed spectroscopically and electrochemically. Atomic-scale determination of the favorable binding sites and adsorption structures were realized with the help of DFT calculations. DFT model of the Au25-CO2 couple (as shown in Figure 12A) shows the interaction of oxygen atom (shown in red) with three sulfur atoms (blue) of the Au<sub>25</sub> shell during the reaction. Reversible perturbation of the electronic structure and CO2 induced charge redistribution within Au25 cluster as a result of molecular physisorption on the cluster was associated to the change in its properties. The potential-dependent formation rate of the reaction products of CO and H<sub>2</sub>, shown in Figure 12B, provided an insight into the mechanism of reduction and a two electron, two proton pathway through adsorbed CO2 -- intermediate was proposed.

In another report, sub-nanometer-sized Cu<sub>n</sub> clusters (where  $n \leq 8$  showed high electrocatalytic activity towards oxygen reduction).<sup>[23]</sup> Cyclic voltammograms of the Cu clusters deposited on glassy carbon electrode in 0.1 M KOH saturated independently with N<sub>2</sub> (black trace) and O<sub>2</sub> (red trace) are shown in Figure 12C. The onset potential of O<sub>2</sub> reduction of -0.07 V was comparable to commercial Pt catalysts. The catalytic capability of Au<sub>25</sub>SR<sub>18</sub> clusters towards selective hydrogenation capability of the C=O bond in  $\alpha$ , $\beta$ -unsaturated ketones and aldehydes at low temperature (0 °C) with 100% chemoselectivity showed the efficiency of the clusters in catalytic reactions.<sup>[33]</sup> The unique structure of Au<sub>25</sub> cluster with an electron-rich Au<sub>13</sub> core and low-coordinated (N = 3) surface gold atoms are responsible for the observed high catalytic activity. Figure 12D shows the proposed mechanism.

In short, QCs have immense potential for developing novel catalysts for highly specific and selective reactions. A summary of the catalytic properties of thiolate-protected Au clusters can be found in elsewhere.<sup>[32,44,45,251]</sup> Experimental results and theoretical calculations can offer excellent inputs into understanding the high catalytic activities evidenced in this class of materials. Moreover, correlation of the crystal structures of the QCs with their catalytic properties will lead to an in-depth understanding of catalytic mechanisms, active centers involved, etc. and thus allow the design and development of new "nano" catalysts for specific reactions with improved selectivity. **Table 4** illustrates more examples of clusters acting as catalysts in various reactions.



Table 4. Various clusters exhibiting catalytic activity.



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Cluster	Size (diameter)	Support	Type of reaction	Conditions	Ref.
Au	1–6 nm	TiO <sub>2</sub>	Carbon monoxide oxidation	Ultrahigh vacuum	[35]
Au <sub>8</sub>	_	MgO		Soft landing conditions, 150 K	[241]
Pd <sub>13</sub>		MgO		Soft landing conditions, 478 K	[244]
Au <sub>~10</sub>	0.5 nm	FeOOH		25 °C, air, pretreatment of cata- lyst at 120 °C	[31]
$Au_{25}(SCH_2CH_2Ph)_{18}$	-	$\rm TiO_2, CeO_2, and Fe_2O_3$		60–80 °C, water vapor, O <sub>2</sub> pretreatment at 150 °C	[223]
$Au_{38}(SCH_2CH_2Ph)_{24}$	-	CeO <sub>2</sub>		60–80 °C, water vapor, O <sub>2</sub> pretreatment at 175 °C	[224]
$Au_{144}(SC_2H_4Ph)_{60}$	-	CuO-mSiO <sub>2</sub>		calcination at 300 °C in air prior to reaction	[225]
Au <sub>25</sub> SR <sub>18</sub>	_	НАР	Styrene oxidation	Toluene, TBHP	[221]
$Au_{25}SR_{18}, Au_{38}SR_{24}, and Au_{144}SR_{60}$	-	-		Toluene, $O_2$ atmosphere, 100 °C	[220]
$Pt_1Au_{24}SC_2H_4Ph_{18}$	-	TiO <sub>2</sub>		Acetonitrile, N <sub>2</sub> , 70 °C PhI(OAc) <sub>2</sub>	[331]
$Au_{55}(PPh_3)_{12}Cl_6$	≈1.4 nm	SiO <sub>2</sub>		Toluene, 100 °C, O <sub>2</sub> atmosphere	[222]
Au <sub>11</sub> @TPP	$0.8\pm0.3~\text{nm}$	mSiO <sub>2</sub>	Alcohol oxidation	Water, 80 °C, H <sub>2</sub> O <sub>2</sub>	[237]
Au@PVP	≈l nm	-		Water, air, 27 °C	[34,227,228]
Au@ poly(EOEOVE)	<4 nm	-			[332]
Pd <sub>1</sub> Au <sub>24</sub> (SC <sub>12</sub> H <sub>25</sub> ) <sub>18</sub>	_	CNT		Water, 30 °C, 1 atm	[229]
AuAg@PVP	1.3–2.2 nm	_		Water, air, 25 °C	[333]
Au <sub>25</sub> (SCH <sub>2</sub> CH <sub>2</sub> Ph) <sub>18</sub>	_	CeO <sub>2</sub>	Homocoupling of aryl iodides	DMF, 130 °C, K <sub>3</sub> PO <sub>4</sub>	[334]
Au <sub>25</sub> SR <sub>18</sub>	-	TiO <sub>2</sub>	Photocatalytic degradation of methyl orange	water, h <i>v</i> , air	[335]
$Au_nSG_{m_i}$ (n, m) = (10, 10), (18, 14), (25, 18), and (39, 24)	<2 nm	НАР	Cyclohexane oxidation	150 °С, ТВНР	[230]
Cu@(PAMAM-OH)	_	_	Hydrogenation of carbonyl and olefin groups	Water, 25–10 °C	[336]
Au <sub>25</sub> (SCH <sub>2</sub> CH <sub>2</sub> Ph) <sub>18</sub>	_	TiO <sub>2</sub> , Fe <sub>2</sub> O <sub>3</sub> , and CeO <sub>2</sub>	Oxidation of sulfide to sulfoxide	DCM, 40 °C, N $_2$ atmosphere	[337]
Au <sub>25</sub> SR <sub>18</sub> , Au <sub>38</sub> SR <sub>24</sub> , and Au <sub>144</sub> SR <sub>60</sub>	-	-	Hydrogenation of aldehydes and ketones	Toluene:acetonitrile (1:1), H <sub>2</sub> atmosphere, 60 °C	[220]
Au <sub>25</sub> SR <sub>18</sub>	-	-		Toluene:ethanol, 0 °C, H <sub>2</sub> atmosphere	[33]
$Au_{25}(SCH_2CH_2Ph)_{18}$	-	-		Ethanol:toluene (10:2), RT, H <sub>2</sub> atmosphere	[231]
Ag <sub>7,8</sub> (H <sub>2</sub> MSA) <sub>7,8</sub>	-	SiO <sub>2</sub> , TiO <sub>2</sub> , Fe <sub>2</sub> O <sub>3</sub> , and Al <sub>2</sub> O <sub>3</sub>	Reduction of nitrophenol	Water, 35 °C, air	[338]

\*Abbreviations: tert-butyl hydroperoxide (TBHP), polyvinylpyrrolidone (PVP), 2-(2-ethoxy)ethoxyethyl vinyl ether (EOEOVE), poly(amidoamine) dendrimer with hydroxyl surface groups (PAMAM–OH), carbon nanotubes (CNT), hydroxyapatite (HAP), triphenylphosphine (TPP), iodobenzene diacetate (PhI(OAc)<sub>2</sub>), room temperature (RT), mesoporous silica (mSiO<sub>2</sub>), mercaptosuccinic acid (H<sub>2</sub>MSA).

#### 4.1.3. Surface-Enhanced Raman Scattering Substrates

Noble metal NPs of diverse shapes and sizes have been employed as SERS-active substrates<sup>[7,252,253]</sup> for enhancing the Raman signals of many different molecules.<sup>[254–256]</sup> The most commonly used substrates include colloidal silver and gold particles between 10 and 50 nm in size and their films. Recently, Pradeep and co-workers<sup>[257]</sup> described the use of thiolate-protected Ag<sub>152</sub> cluster as an efficient SERS substrate using several dyes and biomolecules. An enhancement factor of  $1.58 \times 10^9$  was obtained in the case of crystal violet molecules using the QC substrate. The unusually high SERS enhancement observed was attributed to the formation of Ag<sub>152</sub> crystallites in solution, which could act as hot spots for Raman enhancement. Absence of visible luminescence from the cluster and its plasmonic nature may also be the reasons for its better SERS

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activity compared to other smaller QCs and larger NPs. Use of atomically precise QCs as efficient SERS-active substrates thus opens up hitherto unprecedented possibilities of such QCs to a number of analytes in various areas.

#### 4.2. Applications in Energy

Recent research focuses on developing new materials for clean and affordable energy along with means to reduce energy consumption and lessen toxicity on the environment. Noble metal nanoclusters have shown their capability for H<sub>2</sub> production and solar cells applications. Research in organic and inorganic photovoltaic materials has seen considerable growth worldwide due to the increasing need to make materials with lower cost and higher power conversion efficiencies. Though hybrid systems consisting of multiple components can be an efficient solution, increased complexity of the device structure can hinder commercial applications. Studies on the efficiency of QCs in solar cell applications were pursued owing to the improved efficiency achieved using metallic NPs-incorporated polymer solar cells.<sup>[258-261]</sup> Zhu and co-workers<sup>[262]</sup> demonstrated the use of Au nanocluster decorated multi-layer graphene as transparent anode in polymeric solar cells. Apart from the better power conversion efficiencies and enhanced fill factor of these electrodes compared to other modified multi-layer graphene devices, they showed improved interfacial contact, which substantially decreased the series resistance of the nanocluster-embedded polymer solar cells.

Recently, thiolate-protected AuQCs were used to develop high-efficiency solar cells by incorporating them in mesoscopic TiO<sub>2</sub> films.<sup>[263]</sup> A stable photocurrent of 3.96 mA cm<sup>-2</sup> with a relatively high power conversion efficiency of 2.3% was demonstrated under AM 1.5 illumination. The higher photovoltage observed was attributed to the effective electron injection as a result of the greater HOMO–LUMO gap of QCs and their stronger interaction with TiO<sub>2</sub>. The overall absorption features and cell performance of the QC-sensitized solar cells, with an open-circuit voltage of 832 mV and fill factor of 0.7, were comparable to that of their quantum dot analogues emphasizing their potential as viable candidates for the next generation of solar cells.

Hydrogen's potential role as a way to store renewable energy has been hampered due to challenges in production and storage. Despite the potential to reduce environmental pollution, use of this fuel is severely hampered due to challenges in its production and storage. Generation of hydrogen through methanolysis of ammonia-borane (AB) using poly(*N*-vinyl-2-pyrrolidone) (PVP)-stabilized palladium(0) nanoclusters at room temperature was demonstrated by Özkar and co-workers<sup>[27]</sup> The clusters showed high stability and turnover number of 23 000 in 27 h at room temperature. Kinetic studies on the catalytic methanolysis of AB triggered by PVP-stabilized Pd clusters showed a first-order reaction with activation energy of  $35 \pm 2 \text{ kJ mol}^{-1}$ . Castellano and co-workers<sup>[264]</sup> demonstrated the use of Pt clusters in hydrogen production. The clusters supported on a titania surface was used to photocatalyze the reduction of protons to hydrogen. The net catalytic ability for the heterogeneous hydrogen production, was however, dependent on various



factors including the surface coverage of the metal precursor, Pt(dcbpy)Cl<sub>2</sub> (dcbpy = 4,40-dicarboxylic acid-2,20-bipyridine).

Another interesting report was the impressive electrocatalytic performance of a hybrid system comprising Au clusters on reduced graphene oxide (rGO) towards oxygen reduction reaction (ORR).<sup>[240]</sup> The Au/rRO hybrid system showed superior methanol tolerance, enhanced electrocatalytic stability, and comparable onset potential to commercially available Pt/C catalyst. This work is promising in view of developing low cost and high- performance alternatives for Pt catalysts in fuel cells.

High oxygen reduction activity of Pt clusters embedded on genomic DNA/graphene oxide nanocomposites was demonstrated by Kim and co-workers<sup>[26]</sup> The strong interaction between the Pt clusters and the DNA/graphene oxide nanocomposite can cause modulation in the electronic structure of the cluster leading to its high performance in electrocatalysis of ORR. Such hybrid materials can be utilized for applications in high-performance fuel cells and batteries.

Use of hydrogen as a means of storing energy produced from other sources can help in solving our ever-increasing energy demand owing to its abundance and non-contaminating nature. Use of noble metal clusters in this area is still in its infancy. Combining such catalytically active materials with atomic tunability in various aspects of this science can possibly yield new and better candidates for clean energy applications.

#### 4.3. Applications in Biology

Medicinal benefits of noble metals, especially gold, date back to several thousands of years.<sup>[265,266]</sup> Optical properties of metal NPs (Au/Ag) such as absorption, scattering, and their surfaceenhancing characteristics offered promising results in medical diagnosis and treatment. But their huge size limits their applications in biological matter to a large extent. The lower QYs of plasmonic NPs indeed hinder their applications in terms of bioimaging and labeling applications. The ultrasmall size (<2 nm), enhanced photoluminescence and better QYs exhibited by QCs in comparison to metal NPs prove them to be better candidates in biological applications. Moreover, the smaller sizes of the QCs lead to lower cytotoxicity and thus efficient renal clearance unlike NPs. In vivo applications of noble metal NPs are still severely hampered mainly by their slow renal clearance and high nonspecific accumulation in the organs of the reticuloendothelial system (RES), such as liver and spleen. Owing to their small size, these materials can be used to target-specific areas inside cells hitherto inaccessible to larger sized NPs. While good photostability, lower toxicity, and smaller size make QCs advantageous in bioapplications over semiconductor quantum dots; large Stokes shift, ease of functionalization, and better stability against photobleaching are the added advantages of QCs in comparison with traditional fluorescent dyes. In addition, tendency of aggregation and multivalency is concerns for quantum dots that hinder their use in both in vitro and in vivo applications. The QYs of protein-protected QCs are much higher than that of thiolate-capped clusters. For example, red emitting Au@BSA clusters have a QY of 6%,[70,267] Au@Lyz clusters<sup>[268]</sup> exhibit a QY of 5.6% and Ag<sub>15</sub>@BSA clusters<sup>[62]</sup> show a QY as high as 10.7%. Though QYs of QCs are much



lower than fluorescent dyes, capabilities of QCs such as luminescence tunability (based on core size), surface modification without compromising luminescence, better sensitivity (luminescence quenching/enhancement, etc.) to external events in the cells, etc. make them ideal candidates for bioimaging, therapy, and drug delivery applications. QCs functionalized with drug molecules can serve multiple purposes; 1) they can be used as efficient drug carriers due to their small size, which allows easy cell penetration, 2) luminescence from the QC can be used to simultaneously track the payload and thus ensure their delivery in targeted areas, and 3) release of drugs in cell organelles and subsequent changes can be monitored using changes in luminescence of the QC. Thus, fluorescent metal QCs having better biocompatibility, high quantum yields, excellent photostability, and NIR luminescence are better candidates for multiple applications in molecular biotechnology and biomedical engineering. Towards this aim, synthesis of various clusters of Au and Ag protected by biocompatible ligands such as proteins, DNA, biothiols, etc. has been well explored.<sup>[47]</sup>

#### 4.3.1. Biomolecular Sensing

Sensing biomolecules and reactive oxygen species (ROS) is important in biomedical diagnosis as it can provide vital information on the stages of many diseases including cancer. Florescent noble metal clusters have also been utilized for detection of biomolecules such as biothiols (cysteine, GSH etc.), small molecules, aminoacids, proteins, DNA, nucleic acids, etc. In addition, they have also used for sensing ROS such as  $H_2O_2$ .

Fluorescent gold clusters protected by the enzyme horseradish peroxidase (HRP) were used to detect the presence of hydrogen peroxide in solutions.<sup>[269]</sup> Addition of  $H_2O_2$ quenched the cluster luminescence quantitatively, as shown in **Figure 13**A, indicating that HRP enzyme remains active even after cluster formation and possesses its intrinsic catalytic capability. A change in the structure/conformation of HRP in presence of  $H_2O_2$  that can further change the microenvironment of the clusters was attributed as the reason for the loss of fluorescence. A linear quenching was observed over the range of  $100 \times 10^{-9}$  M to  $100 \times 10^{-6}$  M with a detection limit of  $30 \times 10^{-9}$  M. ROS plays a very important role in cellular metabolism. Reactivity of Au@HRP clusters towards other ROS such as  $O_2^-$ , *t*-butyl hydroperoxide (TBPH), OCl<sup>-</sup>, and 'OH also showed a quenching of cluster luminescence. The quenching mechanism was attributed to the oxidation of the Au-S bond between Au core and the HRP scaffolds by the catalytic effect of HRP enzyme resulting in fewer HRP molecules protecting the cluster and therefore leading to aggregation of clusters. Fluorescent probes for other important molecules such as glutaral-dehyde,<sup>[270]</sup> methotrexate (antimetabolite drug),<sup>[271]</sup> etc have also been reported.

Biothiols such as cysteine (Cys) and GSH commonly seen in biological systems have numerous functions in cellular metabolism and redox reactions.<sup>[272]</sup> Deficiency of Cys can lead to various medical ailments such as edema, depigmentation of hair, liver damage, etc. Thus, a facile analysis of Cys level in our body is important for early diagnosis and treatment. Xie and co-workers<sup>[273]</sup> demonstrated a simple method of detection of Cys using GSH-protected Ag clusters combining the thiol-silver chemistry and steric hinderence of the ligand shell protecting the cluster surface. Ag@GSH clusters showed a superior selectivity for Cys compared to the other 19 non-thiol containing-natural aminoacids (Figure 13B) due to the specific thiol-Ag interaction. Further differentiation based on size of thiol molecules compared to the GSH ligand on the cluster provided additional sensitivity for Cys and a detection limit of <3  $\times \, 10^{-9}$  м was achieved. A dual optical signal change for detection of Cys, both fluorometrically and colorimetrically, avoids false positives. The change is attributed to the decomposition of the cluster to smaller species. Ag cluster-based sensors for biothiols<sup>[274–276]</sup> involving luminescence quenching of the cluster as a result of thiol-adsorption-accelerated oxidation are also



**Figure 13.** A) Change in emission spectra of Au@HRP clusters with increasing addition of  $H_2O_2$ . Inset of (A) shows the schematic of the formation of Au@HRP clusters and its quenching with  $H_2O_2$ . Reproduced with permission.<sup>[269]</sup> Copyright 2011, American Chemical Society. B) Photographs of the Ag@GSH cluster solution in presence of different aminoacids, under visible (top panel) and UV (bottom panel) light. Relative fluorescence intensities of the corresponding solutions are shown. Schematic of the sensing mechanism is also shown. Reproduced with permission.<sup>[273]</sup> Copyright 2012, American Chemical Society.

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Table 5. Various sensors used for biopolymer detection along with their properties.

Analyte	Detection technique (Fluorescence)	Cluster/material used	Analyte/Antigen	Antibody/additional conditions used	LOD	Sample matrix	Ref.
Proteins	Turn off	Au@PAMAM	hIgG	Goat-derived polyclonal anti-human IgG	mM to nM levels	Aqueous solution	[277]
	Turn on	Au@MUA and Au NPs	PDGF AA	Thiol-derivatized aptamer (Apt) molecules	0.5 × 10 <sup>-9</sup> м	Cell media, urine etc	[278]
	Turn on		lpha-Thrombin	Apt <sub>thrombin</sub>	0.15 × 10 <sup>-9</sup> м		
	Turn off	Au@S-Man	Con A	lpha-mannopyranosyl residues	75 × 10 <sup>-12</sup> м	Aqueous solution	[279]
		Ag@DNA	DNA, ATP	Formation of G-Quadruplex/ Hemin complex			
	Turn on	Au@Man	Tg	Con A	$48\times 10^{-12}~{\rm m}$	Serum	[282]
	Turn on	Au@S-Man	Tg	Con A, BSA (50×10 <sup>-6</sup> м)	$90  imes 10^{-12}$ M	Solution	[283]
	Turn on	Au@Man + Au NPs@anti-Tg	Tg	Con A	65 (±16) × 10 <sup>-9</sup> м	Complex serum samples	[282]
	Naked eye	Au@SG	GST-tagged proteins	-	750 × 10 <sup>-9</sup> м	Complex cell lysate samples	[281]
	Turn on	Au@PA	hIgG	-	10 × 10 <sup>-9</sup> м	Solution	[280]
	Turn on		Protein G	-	85 × 10 <sup>-9</sup> м	Plasma samples	
	Turn off	Ag@DNA-Apt	Thrombin	Aptamers APT15 and APT29	1 × 10 <sup>-9</sup> м	Solution	[285]
Oligonucle- otides	Turn off	Ag@DNA	miRNA	-	0.5 × 10 <sup>-6</sup> м	Whole plant endog- enous RNA	[339]
	Turn on	Ag@DNA	HBB-SCA	-	-	-	[314]
	Turn on	Ag@DNA	Human <i>Braf</i> oncogene	guanine-rich DNA sequences	10×10 <sup>-9</sup> м	Various DNA targets	[340]
	Turn on	Ag <sub>-10</sub> @DNA	DNA	-	<1 × 10 <sup>-6</sup> м	Blood and serum samples	[341]

\*Abbreviations used are, poly(amido) amine (PAMAM), platelet-derived growth factor AA (PDGF AA), 11-mercaptoundecanoic acid (MUA), 27-nt DNA aptamer (Aptthrombin), concanavalin A (Con A), mannose-conjugated Au NCs (Au@Man), adenosine triphosphate (ATP), glutathione S-transferase (GST), human immunoglobulin G (hIgG), protein A (PA), thyroglobulin (Tg), anti-Tg antibody conjugated Au NPs (Au NPs@anti-Tg), single-stranded DNA binding protein (SSB), DNA aptamer-templated AgNC (Ag@DNA-Apt), microRNA (miRNA), Homo sapiens hemoglobin beta chain (HBB) gene responsible for sickle-cell anemia mutation (HBB-SCA).

known. A "turn-on" assay for biothiols utilizing specific nature of the DNA template protecting the Ag clusters was reported by Qu and co-workers recently.<sup>[276]</sup> Apart from achieving a detection limit of  $6.2 \times 10^{-9}$  M, this study opens up the possibility of fabricating specific DNA templates for the selective detection of specific analytes by virtue of the template-dependent fluorescence properties of the cluster.

Detection of biopolymers such as proteins and DNA is yet another interesting application of these nanoclusters. Sensors employed for such applications typically use antibody or aptamer-functionalized clusters to selectively conjugate with the analyte. Leblanc and co-workers<sup>[277]</sup> developed an immunoassay for the detection of nanomolar concentrations of human IgG in aqueous solutions using PAMAM dendrimer encapsulated luminescent gold clusters. The immunofluorescent assay was based on the electrostatic conjugation of an antibody (polyclonal, goat-derived anti-human IgG antibody) to the human IgG antigen. Thus appropriate selection of the analyte specific antibody is pivotal in designing the sensor. Competitive fluorescence quenching demonstrated by Chang and co-workers<sup>[278]</sup> for analyzing proteins is yet another sensing strategy. A fluorescence "turn-on" strategy was proposed for platelet-derived growth factors (PDGFs) and their receptors using bioconjugated 13 nm Au NPs and luminescent Au clusters. Clusters have also been used for the sensitive detection of many other proteins such as concanavalin A,<sup>[279]</sup> immunoglobulin G,<sup>[280]</sup> GSH S-transferase-fusion proteins,<sup>[281]</sup> thyroglobulin,<sup>[282,283]</sup> single-stranded DNA binding protein,<sup>[284]</sup> thrombin,<sup>[285]</sup> etc. **Table 5** summarizes the literature on detection of various proteins and oligonucleotides using QCs.

Dual-detection strategies such as colorimetry and fluorescence were used to quantitatively determine the presence of Human IgG using an optical immunosensor employing fluorescent Au cluster as the signal transducing agent.<sup>[286]</sup> A schematic of the portable biosensor consisting of biomolecules immobilized on an ITO chip using poly(dopamine) film is shown in **Figure 14**A. A sensitivity of 5 pg mL<sup>-1</sup> was achieved via this technique suggesting the potential of the sensor for clinical analysis. Fluorescent protein sensors based on the silver cluster aptamers, having strong binding affinities to specific proteins, were used for the detection of thrombin.<sup>[285]</sup> Figure 14B shows the effect of fluorescence emission spectra of the Ag cluster in presence of specific (thrombin) and nonspecific proteins (streptavidin, PDGF, and BSA).

Adenosine triphosphate (ATP), often described as the "molecular unit of currency" for intracellular energy transfer



**Figure 14.** A) Schematic design of the optical immunosensor used for detection of biomolecules employing Au cluster as labels. Reproduced with permission.<sup>[286]</sup> Copyright 2011, American Chemical Society. B) Change in the fluorescence emission spectra of Ag clusters in presence of specific (thrombin) and nonspecific proteins (BSA, PDGF, and streptavidin). Inset shows the fluorescence (top panel) and gel shift analysis (bottom panel) of 1) aptamer–Ag clustersand its interaction with 2) thrombin protein, 3) streptavidin, 4) PDGF, and 5) BSA. Reproduced with permission.<sup>[285]</sup> Copyright 2011, Royal Society of Chemistry. C) Schematic illustration of the photoinduced electron transfer between Ag@DNA clusters and the 1) G-Quadruplex/ Hemin Complex and that of analysis of 2) target DNA, and 3) ATP using the conjugates. Reproduced with permission.<sup>[258]</sup> Copyright 2013, American Chemical Society.

is an important constituent of the various metabolic processes taking place inside cells. A simple route using DNA-protected Ag clusters as fluorescent molecular beacons for selective detection of ATP was reported by Ye and co-workers<sup>[287]</sup> Guanine-rich DNA sequence on Ag nanoclusters was also used as the signal transducer to monitor the activity of the enzyme, adenosine deaminase in solutions.

Recently, selective detection of DNA and ATP molecules was demonstrated by Zhang et al.<sup>[258]</sup> based on the photoinduced electron transfer processes between luminescent DNA-protected Ag clusters and G-quadruplex/hemin complexes. Figure 14C shows the schematic of the sensing strategy employed. A parallel G-quadruplex blocked by a duplex is released upon specific combination of the target (DNA/ATP) allowing it to form a stable G-quadruplex/hemin complex. This triggers the electron transfer from the Ag@DNA clusters to the hemin Fe(III) center resulting in a decrease in the fluorescence intensity of the Ag@DNA QCs, thereby facilitating detection. Electrocatalytic activity of oligonucleotide-encapsulated Ag clusters utilized for the detection of microRNA (miRNA) was described by Zhang and co-workers<sup>[288]</sup> The efficient catalytic property of the cluster towards H<sub>2</sub>O<sub>2</sub> reduction was used to design the electrochemical miRNA biosensor probe.

Recently, luminescent DNA-stabilized Ag nanoclusters were described to act as fluorescent labels for various biocatalytic transformations<sup>[289]</sup> such as oxidation of glucose, tyrosine, dopamine, or tyramine. Fluorescence quenching of Ag clusters by  $H_2O_2$  and quinones enabled the detection of  $H_2O_2$ -generating oxidases and tyrosinase during biocatalytic processes. Moreover, use of Ag clusters as optical labels for bienzyme biocatalytic cascades was demonstrated using two such systems namely, 1) alkaline phosphatase/tyrosinase coupled hydrolysis and oxidation of o-phospho-l-tyrosine and 2) acetylcholine esterase/choline oxidase hydrolysis of acetylcholine and subsequent oxidation of choline. Ultrasensitve detection of alkaline phosphatase and o-phospho-l-tyrosine was also reported using this protocol.

#### 4.3.2. Detection of Single-Nucleotide Polymorphism

Precise identification of genetic variations, such as single-nucleotide polymorphisms (SNPs), is critical as a slight alteration of a single base can cause diseases. In view of this, a new inexpensive method based on fluorescent silver QCs is reported for quick identification of base switches in a gene. It has been reported that certain nonemissive DNA-templated silver QCs can light up into distinct colors through interactions with different enhancer DNA sequences.<sup>[69]</sup> On the basis of this finding, Werner and co-workers<sup>[290]</sup> have designed a QC-based molecular probe, which fluoresce upon binding-specific DNA targets.







**Figure 15.** A) Schematic shows three relative positions (-3, +2, and +7) between the enhancer sequence (red fill) and the NC-nucleation sequence (blue fill). A cartoon of a Ag NC is shown for positions -3, +2, and +7, which results in a red light-up color for positions -3 and +7 and a yellow/orange color for position +2. B) DNA-templated Ag NC consist of a NC probe and a guanine (G)-rich probe lights up into different colors upon binding SNP targets. Probes remain dark in the absence of targets. Upon binding the wild-type target, the Ag NCs probe lights up into one color (orange) and upon binding the mutant type of target, the Ag NCs lights up into another color (red). The difference between wild-type and mutant-type targets is a single-nucleotide substitution. C) 2D Fluorescence contour plots of the 11 hybridized samples and a control sample having only the NC-bearing strand, with the corresponding position number shown on the upper left corner of each plot. Reproduced with permission.<sup>[290]</sup> Copyright 2012, American Chemical Society.

Different relative positions between the enhancer and the NC nucleation sequences were produced by hybridizing a common NC-bearing strand with 11 different guanine-rich (G-rich) strands (Figure 15A). The fluorescence emission of the AgQCs substantially changed (a shift of 60–70 nm in the emission maximum) depending upon the alignment between the AgQCs and the DNA enhancer sequence. A schematic representation of this phenomenon is given in Figure 15B. This new property has been exploited for the sensitive detection and identification of a number of disease-related SNPs. The hybridized samples generated multiple spectral peaks when excited in the visible to NIR region (450–800 nm), which was probed by the 2D fluorescence contour plots (Figure 15C). This method has been validated in

three synthetic DNA targets with SNP and in two clinical samples taken from patients with ovarian cancer. In these samples, SNP can be easily identified by the naked eye under UV excitation, making this method reliable and cost effective.

In another study, Wang and co-workers<sup>[22]</sup> have successfully demonstrated the capability of an intelligent CuQC probe in distinguishing match and mismatch sequences with 15-mer probe DNA in solution. The high sensitivity of Cu nanoclusters to base types located in the major groove of DNA leading to associated modifications in their fluorescent property made these QCs fluorimetric indicators of the DNA hybridization event. This method allows the detection of not only a single mismatch but also its mismatch type in a specific DNA sequence.





**Figure 16.** A) Epifluorescence microscopic images of amyloid fibrils stained with Ag clusters in combination with thioflavin T after an exposure time of 1) 0.02 s and 2) 1.6 s, respectively. The scale bars correspond to 10 µm. Reproduced with permission.<sup>[292]</sup> Copyright 2005, Elsevier. B) Time-gated luminescence microscopic images of NIH3T3 cells stained with silver nitrate showing the fast cluster emission at short times. Intensity scales for the images are also shown. Reproduced with permission.<sup>[293]</sup> Copyright 2007, Wiley-VCH. C) Endogeneous labeling of biotin within human hepatoma cells (HepG2) using Au clusters is demonstrated by the 1–3) fluorescence and 1′–3′ phase-contrast images from cells exposed to 1 and 1′ unconjugated Au clusters, 2 and 2′) streptavidin-conjugated Au clusters, and 3 and 3′) streptavidin-conjugated FITC. Scale bars in the images correspond to indicate 50 µm. Reproduced with permission.<sup>[293]</sup> Copyright 2009, American Chemical Society. D) Fluorescence image showing the internalization of Au@insulin clusters (red) inside C2C12 myoblast cells after exposure to the clusters for 2 h. Green and blue colors are from the dyes used to stain the cell boundary and cell nucleus, respectively, for ease of visual identification. Reproduced with permission.<sup>[173]</sup> Copyright 2011, Wiley-VCH.

#### 4.3.3. Bioimaging and Biolabeling

The unique photophysical properties of noble metal QCs give rise to a myriad of applications in imaging techniques ranging from cellular staining to imaging specific proteins inside live cells. Size, biocompatibility, fluorescence quantum yield, and stability against photobleaching are essential components for any fluorescence based optical probe.

While limited photostability and small Stokes shift are problems crippling the use of conventional organic dyes as fluorescent reporters; bigger size and toxicity of semiconductor quantum dots hinder their use as biomarkers. Luminescent properties of fluorescent metal clusters make them excellent biological labels especially in the NIR region. Interference from the short-wavelength (400–600 nm) emission in biological media and scattering light can be avoided by use of such NIR probes wherein biological tissues are optically transparent. Smaller size, low cytotoxicity, high biocompatibility, high quantum yields, better emission rates, large Stokes shift, excellent photostability, and NIR luminescence make them better candidates than organic dyes and quantum dots for biomedical applications.<sup>[291]</sup>

Baskakov and co-workers<sup>[292]</sup> in 2005 demonstrated the use of Ag clusters in bioimaging for the first time in combination with a fluorophore, thioflavin T. The modified clusters were

used to stain amyloid fibrils. The clusters showed bright green fluorescence in aqueous solution without any detectable photobleaching and could easily be detected using a fluorescence microscope (Figure 16A). Later Dickson and co-workers used intracellularly synthesized Ag clusters using argyrophilic proteins and imaged living cells using cluster emission.<sup>[293]</sup> The short fluorescence lifetimes [220 ps (33%) and 1760 ps (67%)] of the clusters allowed them to be monitored efficiently using time-gated luminescence microscopy (Figure 16B). Conjugation with biologically active molecules that can retain activity post-treatment is yet another way to target specific locations. Owing to their ease of functionalization, luminescence tunability and specific binding capabilities, QCs functionalized with aptamers are commonly employed for cellular labeling, nuclei staining and tumor detection. Dickson and co-workers<sup>[294]</sup> utilized DNA-encapsulated Ag clusters conjugated with proteins such as avidin for surface labeling in live cells by exploiting the specific avidin-biotin interactions. Ag clusters functionalized with avidin stained the cell surface and was internalized subsequently. Later Chang and co-workers<sup>[295]</sup> demonstrated that dihydrolipoic acid (DHLA)-protected water-soluble Au clusters can be conjugated effectively to biomolecules such as polyethylene glycol (PEG), BSA, avidin, and streptavidin via EDC coupling and used them for biological imaging applications. Endogeneous labeling of biotin inside human hepatoma cells

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(HepG2) was demonstrated effectively by streptavidin-conjugated Au@DHLA clusters.<sup>[295]</sup> While unconjugated Au@DHLA clusters showed weak emission [Figure 16C (1 and 1')] from the cells, streptavidin-conjugated clusters stained the biotin containing cells with high intensity [Figure 16C (2 and 2')]. Streptavidin conjugated with FITC [Figure 16C (3 and 3')] was used as the positive control as it is known to specifically bind biotin. Similar approach was used by Pradeep and co-workers to image human hepatoma cells (HepG2) using streptavidin-functionalized red emitting Au<sub>23</sub> clusters<sup>[296]</sup> and oral carcinoma KB cells using folic-acid-conjugated Au@BSA clusters.<sup>[61]</sup> In both cases, the inherent luminescence of the internalized QCs was used in imaging the cells.

While use of various biopolymers for synthesis and subsequent imaging of clusters has been demonstrated by exploiting its luminescence, use of a biologically active protein such as insulin, for synthesis of clusters and retaining its biological activity was advantageous as it adds another modality towards diagnosis and imaging of such clusters. Chou and coworkers<sup>[173]</sup> synthesized fluorescent Au clusters using insulin and demonstrated its activity in blood glucose regulation. Figure 16D shows the internalization of the Au@insulin clusters in C2C12 mouse myoblasts cells. The red fluorescence in the image is from the clusters while the green and the blue are from the cell nucleus and cell boundary stained using dyes, 4',6-diamidino-2-phenylindole and Alexa Fluor 488 phalloidin, respectively, for easy visual identification.

#### 4.3.4. Cancer Therapy

In two early reports, the over expression of folic acid receptors in certain cancerous cells such as oral carcinoma KB cells was used to internalize and image folic-acid-functionalized Au clusters via receptor-mediated endocytosis.[61,267] Here, the higher internalization of folic acid (FA)-conjugated red luminescent BSA-protected Au25 QCs in FR +ve oral squamous cell carcinoma (KB) and breast adenocarcinoma (MCF-7) cell lines (compared to negative control cell lines) confirmed the receptor-targeted imaging and cancer detection capability of the clusters. A similar study conducted on mouse fibroblast L929 cells without folate receptors served as the negative control, wherein significant luminescence was not observed.<sup>[61]</sup> These studies demonstrated detection of cancerous cells for the first time using Au clusters. Later, potential of various Au and Ag clusters for simultaneous cancer cell targeting and imaging was demonstrated.<sup>[297,298]</sup> Efficacy of such materials for therapy was limited due to the lack of effective nuclear drug delivery vehicles to assist the transport of the drug from cytoplasm to the nucleus. Passage of the drug into the nucleus can significantly enhance its therapeutic capability. Irudayaraj et al.<sup>[299]</sup> reported nuclear localization and targeting capability of fluorescent BSAprotected Au clusters conjugated with Herceptin. Herceptin is a humanized monoclonal antibody capable of targeting ErbB2 receptors, which are overexpressed in breast cancer cells and tumour tissues. Combined use of fluorescent correlation spectroscopy (FCS) and fluorescence lifetime imaging microscopy (FLIM) was used to track the dynamics of the fluorescent cluster probes inside the nucleus of live cells with single particle sen-



sitivity. Figure 17A shows the FLIM of SK-BR3 cells stained by Lamin A antibody labeled with Alexa350 and incubated with AuQCs. The presence of Au cluster inside the nucleus of the cell was clearly identified based on the difference in fluorescence lifetime of the Au clusters (1.5 ns) and Lamin A antibody labeled with Alexa350 (1.9 ns) as shown in the left panel of Figure 17A. Comparison of the FLIM from the cells for Herceptin conjugated and unconjugated clusters emphasized the advantage of the former for nuclear delivery. Moreover, the capability of escaping the endolysosomal pathway enhanced their potential to be efficient nuclear drug delivery vehicles. Figure 17B shows the confocal fluorescence images of endolysosomes of SK-BR3 cells incubated with Herceptin-conjugated Au clusters stained by lysosensor marker blue. Lysosensor, used to stain the endosomes and lysosomes of live SK-BR3 cells, was used to monitor the uptake of clusters after incubation. Majority of the clusters did not accumulate within the endosome (right panel of Figure 17B), signifying their endosomal escaping ability. Nuclear localization enhanced the anticancer therapeutic efficacy of Herceptin by the induction of DNA damage as shown in Figure 17C,D. Another report by Wang et al.<sup>[171]</sup> shows the transport of anticancer molecular drugs such as doxorubicin across HepG2 hepatocarcinoma cell membranes using Au cluster/reduced graphene oxide (GNC-RGO) nanocomposites. Furthermore, they used Raman spectroscopy to study the interaction of GNC-RGO nanocomposites and the proteins and DNA in cancer cells to gain mechanistic insights into their inhibitory action on cancer cells.

In a recent report, biosynthesis of fluorescent QCs by cancerous cells has been monitored by florescence imaging.<sup>[298]</sup> Clusters were formed inside human hepatocarcinoma (HepG2) and leukemia cell lines (K562) upon incubation with micromolar concentrations of chloroauric acid solutions, while noncancerous human embryo liver cells (L02) showed no such effect. Fluorescent biolabels formed as a result of subcutaneous injections of gold precursors near xenograft tumors in mouse models having hepatocellular carcinoma or chronic myeloid leukemia labels showed noninvasive tumor diagnostic capability (Figure 17E,F).

#### 4.3.5. Other Diagnostic Tools (MRI and CT Imaging)

Magnetic resonance imaging (MRI) is a powerful and widely used imaging technique owing to its fast scan rate, excellent spatial resolution, and deep tissue penetration. However, requirement of large doses of gadolinium-based agents for adequate image contrast is a cause of concern. Use of Au@ BSA clusters as bimodal MRI/optical nanoprobes was demonstrated by covalently grafting gadolinium complex of dieth-ylenetriaminepentacetic acid (DTPA), denoted as Gd-DTPA, onto the Au clusters (**Figure 18**A).<sup>[300]</sup> T1-weighted MR images of the Gd-DTPA-conjugated cluster for various Gd<sup>3+</sup> concentrations are shown in Figure 18B. These nanoprobes show a higher relaxivity of 23.7 mM<sup>-1</sup> s<sup>-1</sup> per Gd<sup>3+</sup> relative to clinical Gd-DTPA (4.3 mM<sup>-1</sup> s<sup>-1</sup>), while their fluorescence emission intensity is preserved (Figure 18C).

In another report, a similar strategy was used by Kong and coworkers.<sup>[301]</sup> for multimodal imaging using Gd<sup>3+</sup>-functionalized



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**Figure 17.** A) FLIM of SK-BR3 cells stained by Lamin A antibody labeled with Alexa350 showing the nuclear uptake of Herceptin-conjugated Au cluster (left) and unconjugated Au clusters (right) incubated for 4 h. Scale bar for FLIM is 0 to 2 ns. B) Confocal fluorescence images of endolysosomes of SK-BR3 cells incubated with Herceptin-conjugated Au clusters stained by lysosensor marker blue showing the emission from the lysosensor (Left panel) and from that of the Au cluster (right panel). C) Fluorescence images showing the induction of DNA damage and apoptosis by 1) unconjugated Au clusters, 2) Herceptin-conjugated Au clusters, and 3) Herceptin by staining the nucleus with Hoechst 33258. D) Bar diagram showing the quantitative evaluation of DNA damage of the cells as a percentage of the total number of cells for different treatments. Panels A-D reproduced with permission.<sup>[299]</sup> Copyright 2011, American Chemical Society. Representative xenograft tumor mouse models of hepatocellular carcinoma observed in visible light (E) and by in vivo fluorescence imaging (F1) 24 h after a subcutaneous injection of HAuCl<sub>4</sub> solution near the tumor. Inset shows an enlarged view of the xenograft tumor. Xenograft tumor mouse models of chronic myeloid leukemia observed by in vivo fluorescence imaging (F2) 24 h after a subcutaneous injection of 10 mmol L<sup>-1</sup> HAuCl<sub>4</sub> solution near the tumor. (F3) Control mouse observed by in vivo fluorescence imaging 48 h after a subcutaneous injection of 10 mmol L<sup>-1</sup> HAuCl<sub>4</sub> solution in the right side of their abdomen. Panels E,F reproduced with permission.<sup>[298]</sup> Copyright 2013, Nature Publishing Group.

gold clusters (Gd-AuQCs) protected by cyclodecapeptide (CP) for dual model (fluorescence/magnetic resonance) imaging. The Gd-Au QC probes emit bright red fluorescence under UV light, while exhibiting a high longitudinal relaxivity of  $41.5 \pm 2.5 \text{ mM}^{-1} \text{ s}^{-1}$  and low relaxivity ratio (r2/r1, where r2 and r1 are the relaxivities determined from the influence on the relaxation times *T*2 and *T*1) of 1.2 at 0.55 T. Figure 18D shows the unmodified intense red luminescence from the Gd-AuQCs under UV light along with remarkable *T*1 signal enhancement. The MR image of Gd-AuQCs was much brighter than Gd-CP and Gd-AuNPs under similar Gd<sup>3+</sup> concentrations. Comparison of the *T*1-weighted MRI of Gd-AuQCs and Gd-DTPA showed better results for the former (Figure 18E) as also observed from their r1 relaxivity curves (Figure 18F).

Strong X-ray computed tomography(CT) signal elevation from Au clusters stabilized by insulin (Au@insulin)<sup>[173]</sup> brought to light yet another imaging modality using these clusters. Clusters showed a dose-dependent enhancement in contrast (Figure 18G<sub>1</sub>) when tested for CT imaging with C2C12 myoblast cells and the uptake was clearly distinguishable in the presence and absence of Au@insulin clusters (Figure  $18G_2$ ).

#### 4.3.6. The Issue of Toxicity

In spite of their applications in diverse avenues of biology, a primary concern for use of such materials in clinical trials is involving their toxicity to living organisms especially, humans. Efficient renal clearance is an important parameter as ideally any nanomaterial-based contrast agent or fluorescent label should be effectively cleared out of the body and show very little accumulation in organs. In vivo applications of noble metal NPs is still severely hampered mainly by their slow renal clearance and high nonspecific accumulation in the organs of the RES, such as liver and spleen, after systematic administration. NPs with smaller diameters (<10 nm) generally considered to be stealthy to the RES organs, are still often found in the liver (Figure 19A). In this context, sub-nanometer-sized clusters are advantageous compared to NPs for biomedical applications due





**Figure 18.** A) Schematic representation of functionalization of Au@BSA clusters with Gd-DTPA. B) T1-weighted MR image of Gd-DTPA-conjugated Au@BSA cluster for various Gd<sup>3+</sup> concentrations in water from a 1.5 T clinical MRI system. C) Photographs of water (tube 1) and Gd-DTPA-conjugated Au@BSA cluster (tube 2) under white light (left) and under an excitation wavelength of 475 nm (right). Panels A-C reproduced with permission.<sup>[300]</sup> Copyright 2013, Royal Society of Chemistry. D) Photographs of the AuQCs under visible (left panel), UV light (middle panel), and their T1-weighted MR images (right panel) of the 1) Gd-CP, 2) AuQCs, 3) Gd-AuQCs, and Gd-AuNP samples. E) Comparison of the T1-weighted MRI of Gd-AuQCs (top) and Gd-DTPA for various Gd concentrations and r1 F) their relaxivity curves at 0.55 T. Panels D-F reproduced with permission.<sup>[301]</sup> Copyright 2013, Royal Society of Chemistry. G) CT imaging of Au@insulin QCs in sequential dosage (1) and differentiated C2C12 myoblasts with (right) and without (left) the clusters. Reproduced with permission.<sup>[173]</sup> Copyright 2011, Wiley-VCH.

to their extremely small size. Both the nature of the ligand and the particle size are crucial for efficient renal clearance.

Glutathione-protected luminescent Au clusters (Au@SG) showed efficient renal clearance through urine within 24 h after intravenous (IV) injection.<sup>[302]</sup> Real-time accumulation of the luminescent Au@SG clusters in the bladder of a live mouse was visualized by X-ray computed tomographic (CT) images as shown in Figure 19B. More than 50% of the cluster was excreted out of the body through urine within 24 h (Figure 19C) and up to 65% was observed in the urine, 72 h post-injection.

The importance of choice of the surface capping ligands during in vivo applications of clusters was revealed in experiments conducted with various ligand-protected clusters. Cliffel and co-workers<sup>[303]</sup> demonstrated that the histological damage to the renal tubules caused by the use of tiopronin-passivated Au clusters during in vivo applications can be eliminated by the incorporation of PEG on the clusters. The amount of PEGylation, chain length of the PEG chains, etc. had an effect on the clearance rate and thus the circulation lifetime of the particles in the body.<sup>[304]</sup> An optimum of 1% PEGylation using shortchain ligands and alcohol-terminated PEG was identified to achieve short circulation lifetimes in addition to zero toxicity, no immune response, and high water solubility.

However, it is important to mention that conflicting data exist in the literature about the cytotoxicity of small gold particles. A size-dependent toxicity study using gold NPs of sizes



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**Figure 19.** A) Schematic showing the efficiency of renal clearance of clusters in comparison with nanoparticles due to difference in particle size. B) X-ray computed tomography (CT) images of a live mouse 1) before and 2) after 30 min post-injection of Au@SG clusters. C) Fluorescence images of the urine after 1) 2 h and 2) 24 h post-injection of Au@SG clusters along with control urine (3) sample under UV light with a 630/75 bandpass filter. Panels B,C reproduced with permission.<sup>[302]</sup> Copyright 2011, Wiley-VCH.

ranging from 0.8 to 15 nm using various cell lines indicated that AuNPs of 15 nm size are nontoxic while the 1.4 and 1.2 nm AuNPs resulted in rapid cell death by necrosis and apoptosis, respectively, within 12 h of incubation.<sup>[305]</sup> Au<sub>55</sub> clusters have also been shown to interact with DNA and cause significant toxicity towards various human cell lines compared to NPs,<sup>[306]</sup> but this enhanced reactivity shown by such clusters was envisioned as a possible mode for cancer treatment.

Thus, while water-soluble QCs have undoubtedly proven to have immense potential in biological applications, caution should be exercised in the choice of suitable ligand and their functionalization and core size in order to avoid additional effects.

#### 5. Conclusions and Future Prospects

Novel properties arising as a result of size quantization in clusters, such as luminescence, a phenomenon not prominent in NPs, make such materials promising candidates for applications in diverse fields. Such sub-nanometer-sized materials can be easily conjugated with molecules, thus making them ideal candidates in biological applications such as multimodal imaging, sensors for biomolecules, nuclear targeting, drug delivery, oncotherapy, etc. Issues such as selectivity and stability of these clusters in complex media can be addressed by combining them with other molecular species or NPs, to obtain hybrid materials with multimodal properties. Novel sensing strategies based on these materials can be envisaged. Extremely

small size and tunable luminescence properties of QCs may be useful for the development of hybrid, multimodal gold QCsbased nano-formulations for future therapeutics against various diseases, including cancer. Apart from this, the possibilities of conjugating QCs with various aptamers could possibly bring new capabilities in gene therapy. The recent progress in the computational capabilities and the current knowledge in the crystal structures of QCs provide sufficient background for designing new catalysts with predictable reactivity and selectivity. New capabilities in assembling nanoclusters precisely on various substrates may lead to the development of QC-based optical devices. Synthetic strategies in making hybrid QCs can open up new possibilities in more efficient and bio-friendly solar cells. The unique capabilities and biocompatibility make these materials promising candidates for the development of "next"-generation "quantum medicine" for disease diagnosis and treatment. As the properties of many of the QCs are studied only to a limited extent, many promising avenues in terms of their medical and materials science applications are yet to be explored. With further experimental and theoretical advances towards understanding these materials and by solving challenges in their synthesis, an almost unlimited field of applications can be foreseen. At present, it is necessary to a) have an appropriate terminology for describing such materials, b) crystallize as many of them as possible, c) correlate structures with associated properties, d) come up with a general rule or rules for the formation of these materials, and e) expand their science (chemistry, physics, biology, and applications). In this, inputs of computational studies will be highly beneficial. With



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the expansion of computational power and developments in methodologies, accurate predictions of spectra become possible and this is demonstrated in a number of recent publications.<sup>[103,104]</sup> It is clear that expansion of science at the interface of molecules and NPs will be a result of strong overlap of experiments with theory.

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testimonies may not be verifiable accurately by the principles of modern medicine. The unique properties offered by noble metal QCs are currently being studied and exploited in a range of potential applications in biology.

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## Manifestation of the Difference in Reactivity of Silver Clusters in Contrast to Its Ions and Nanoparticles: The Growth of Metal Tipped Te Nanowires

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Supporting Information

**ABSTRACT:** Reactivity of two different nanosystems of silver, namely nanoparticles and atomically precise clusters, toward 1D tellurium nanowires (NWs) was probed and compared with the reaction of silver ions. While the reaction of nanoparticles and ions led to silver telluride nanowires, a different reactivity was exhibited by clusters which resulted in silver islands at different positions on the Te NWs. These hybrid Ag nodule-decorated Te NWs are sensitive to temperature, and they transform to dumbbell-shaped silver-tipped Te NWs upon solution phase annealing. Differences in chemical reactivity of nanoparticles of two different size regimes with nanowires are demonstrated. Synthetic methods of this kind will be useful in creating complex nanostructures which are difficult to be made in the solution phase.



#### INTRODUCTION

Nanosystems have become enormously diverse in the past several years in terms of their chemical variety and structural attributes. However, the most common and widely researched nanosystems belong to zero- and one-dimensional categories.<sup>1</sup> While there have been many zero-dimensional particles belonging to metals, semiconductors and dielectrics, noble metal nanoparticles are the most widely researched systems. The most emerging category of zero dimensional noble metal nanosystems is their atomically precise analogues, called by a number of names such as quantum clusters (QCs),<sup>2</sup> nanoclusters,<sup>3</sup> superatoms,<sup>4</sup> or nanomolecules.<sup>5</sup> While gold nanoclusters have been extensively studied,<sup>6,7</sup> reports on their silver counterparts are limited.<sup>8–10</sup> Most of such studies are on synthesis,<sup>11–15</sup> characterization,<sup>16–19</sup> and properties,<sup>20,21</sup> while their chemistry is beginning to evolve.<sup>22-27</sup> They are typically labeled in terms of the number of atoms in the core such as Au<sub>25</sub>, although the cluster is composed of ligands as well, protecting the core.

In the one-dimensional nanosystems, large chemical varieties have evolved in the past decade. Among these, tellurium, due to its inherent structural anisotropy, is prone to one-dimensional growth, and Te nanowires (NWs) of precise aspect ratio can be synthesized chemically.<sup>28,29</sup> Chemistry of these wires results in binary 1D systems,<sup>30–33</sup> and in special cases biphasic NWs<sup>34–39</sup> can also be synthesized. Properties of such systems with unusual morphological features have been rarely explored, although when done have led to intriguing applications.<sup>38,40</sup>

In this article, we introduce the chemistry of atomically precise clusters with 1D nanosystems, taking Ag QCs and Te NWs as reactants. As an example of Ag QCs, we have taken  $Ag_{32}SG_{19}$  (SG refers to glutathione thiolate), a cluster reported by our group<sup>41</sup> as well as others.<sup>42</sup> The unprecedented and

unique chemistry of these reactants lead to new nanosystems which have not been observed when the clusters were replaced with metal ions or nanoparticles (NPs). While the precise reasons for this unique chemistry are unknown, the observed structures, their reproducibility, and synthetic control this reaction offers, suggest new possibilities of nanochemistry. Extension of the reactivity to chemically similar systems and exploration of processes *in situ* would provide an insight into the chemistry between nanosystems.

### EXPERIMENTAL SECTION

**Chemicals.** All the chemicals were commercially available and were used without further purification. Silver nitrate (AgNO<sub>3</sub>, 99%) and glutathione (GSH, 97%) were purchased from SRL Chemical Co. Ltd., India. Sodium dodecyl sulfate (SDS,  $C_{12}H_{25}O_4SNa$ , 99%) was obtained from Acros. Tellurium dioxide (TeO<sub>2</sub>, 99.9%) powder was purchased from Alfa Aesar. Hydrazine monohydrate (N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O, 99–100%) was purchased from SD Fine Chemicals, India. Sodium borohydride (NaBH<sub>4</sub>, 99.99%, Aldrich), ethanol (HPLC grade, 99.9%, Aldrich), and methanol (HPLC grade) were used as received.

**Synthesis of Ag<sub>32</sub>SG**<sub>19</sub>. Synthesis followed a method reported previously.<sup>41</sup> About 23 mg of AgNO<sub>3</sub>(s) was added to 200 mg of GSH(s) at room temperature, and the mixture was ground well in a mortar to make Ag(I)SG. About 25 mg of NaBH<sub>4</sub>(s) was added, and grinding was continued further for 10 more minutes. After that, 10 mL of distilled water was added slowly (in one mL step) which resulted in the formation of a reddish brown solution. Clusters were then precipitated immediately by the addition of excess ethanol. The resulting precipitate was collected and washed repeatedly with ethanol through centrifugal precipitation. Finally, precipitate was dried and

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**Figure 1.** (A) UV-visible absorption spectra of as prepared (crude) [trace a] and gel separated [trace b]  $Ag_{32}$  cluster. Photographs of the solution of crude cluster under visible and UV light are shown in inset (a) and (b), respectively. The red emission is clearly visible only at the top as the UV excitation coming from the top attenuates with depth. Excitation and emission spectra of the crude cluster are shown in inset (c). (B) The UV-visible extinction spectrum of Te NWs reacted with Ag cluster (trace c) is shown along with the spectra of Te NWs (trace a) and citrate capped Ag NPs (trace b). Emergence of a new peak in the spectrum around 400 nm indicates the formation of Ag NPs. (C) The TEM image of parent Te NWs, diameter distribution of the NWs is shown in the inset a. A high resolution TEM image of a Te NW is shown in inset b. (D) The TEM image of NWs decorated with nodules obtained through Ag cluster reaction with Te NW. Diameter distribution of the nodule-free regions of the NWs is shown in the inset. Scale bar in the TEM images (C) and (D) is 100 nm and is 2 nm for the inset of (C).

collected as a reddish brown powder (~26 mg). This was termed as crude cluster (CC) in this paper.

**Synthesis of Te NWs.** Te NWs were prepared by the chemical method; originally reported by Chang et al.<sup>28</sup> In a typical procedure, 24 mg of TeO<sub>2</sub> powder was slowly added to a beaker containing 10 mL of hydrazine monohydrate. The reaction was allowed to continue at room temperature under constant magnetic stirring. The powder dissolved completely, and the color of the solution changed from colorless to blue indicating formation of Te NWs. After 1 h, the solution was diluted 10-fold with 10 mM SDS, in order to control the length of the NWs. The as-prepared solution was purified by centrifugation at 8000 rpm for 10 min. The residue was redispersed

in deionized water. Centrifugation-redispersion cycle was repeated twice to remove any unreacted species and excess surfactant.

**Synthesis of Ag NPs.** Citrate capped Ag NPs were synthesized according to the Turkevich method.<sup>43</sup> 2 mL of 1 wt % trisodium citrate solution was added to a boiling 50 mL silver nitrate (1 mM) solution, and heating was continued further for a few minutes. The solution turned light yellow in color, indicating the formation of NPs. The suspension was cooled in an ice bath to allow the growth of NPs.

Glutathione capped Ag NPs were synthesized following the Creighton method of Ag colloid synthesis. Twelve mg of NaBH<sub>4</sub> was added to 100 mL of 0.1 mM solution of AgNO<sub>3</sub> under vigorous magnetic stirring at room temperature. To this yellow colored solution 2 mL of 1 mM glutathione (GSH) solution was added dropwise, and



**Figure 2.** (A) A large area TEM image of nodule decorated NWs. The EDS spectrum taken from the same area is shown in the inset which shows the presence of both Ag and Te. Scale bar in the image is 200 nm. (B) XRD patterns of decorated Te NWs. All peaks are indexed to Ag (indexed in green) and Te (indexed in red). Standard peaks of Ag (JCPDS: 04-0783) and Te (JCPDS: 36-1452) are shown as stick patterns in the inset which match with the observed XRD pattern. (C) The HRTEM image of the decorated NWs showing crystalline nature of both the NW and the nodule. While the NW shows the Te phase, nodules are made up of Ag. A low magnification image of the corresponding NW is shown in the inset. Scale bar in the HRTEM is 5 nm and is 100 nm for the inset image. Characteristic planes of Te (001) and Ag (111) are marked.

stirring was continued for another 2 h in the dark to obtain stable colloidal Ag NPs.

Both citrate and glutathione capped Ag NPs were cleaned by centrifugal precipitation followed by redispersion in distilled water prior to reaction with Te NWs to ensure the removal of excess capping agent.

**Reaction between Ag<sub>32</sub> QC and Te NWs.** Five mL of cleaned Te NW dispersion was mixed with different volumes of  $Ag_{32}$  QC solution (1 mg mL<sup>-1</sup>) under constant magnetic stirring at room temperature, and the reaction was monitored with UV–visible spectroscopy at regular intervals until the completion of the reaction. Then the resulting dispersion was centrifuged at 7500 rpm for 10 min to precipitate the NWs. These NWs were then redispersed in distilled water and used for TEM analysis.

For solution phase heating experiments, an Eyela organic synthesizer was used to ensure uniform heating. The dispersion containing NWs after reaction with Ag QCs was maintained at various temperatures for different durations. Then the dispersion was centrifuged, redispersed, and spotted for TEM analysis. Structure of NWs was assessed from these images, and structural changes upon heating were identified.

#### ■ INSTRUMENTATION

UV–visible absorption spectra were recorded using a PerkinElmer Lambda 25 spectrophotometer in the range 200–1100 nm. High-resolution transmission electron microscopy (HRTEM) was performed with a JEOL 3010, 300 kV instrument equipped with a UHR polepiece. Energy dispersive X-ray analysis (EDS) was carried out with an Oxford EDAX housed in the TEM. Samples were prepared by dropping the dispersion on carbon coated copper grids and drying in ambient condition. X-ray diffraction (XRD) data were collected with a Bruker AXS, D8 Discover diffractometer using Cu K<sub>a</sub> ( $\lambda = 1.54$  Å) radiation. Samples were scanned in the 2 $\theta$  range from 10 to 90°. All the peaks were assigned and compared with the database published by the Joint Committee on Powder Diffraction Standards (JCPDS).

#### RESULTS AND DISCUSSION

**Formation of Ag NPs Decorated Te NWs.** Quantum clusters, being molecular in nature, exhibit molecule-like absorption features. Position and shape of these absorption bands depend on the size of the metal core.<sup>8</sup> For this reason, one cluster of a given atomicity can be distinguished from

another by looking at their UV-visible absorption spectrum. A solid state method, developed originally for Ag<sub>9</sub> clusters,<sup>44</sup> was utilized for the synthesis of glutathione protected Ag<sub>32</sub> clusters,<sup>41</sup> which was used in the studies reported here. The main reason to select Ag<sub>32</sub> as the model Ag cluster for this study lies in its solubility in water. Although MSA (mercaptosuccinic acid) and MBA (mercaptobenzoic acid) protected Ag clusters are also water-soluble, they were not chosen to avoid possible structural deformation to the Te NWs induced by the ligands.<sup>45</sup> Another possible choice was Ag<sub>75</sub> protected with SG,<sup>46</sup> but it is synthesized by formic acid reduction and when added to Te NWs dispersion, pH of the solution changes drastically and NWs tend to aggregate. As there are no other water-soluble clusters known, a comparative study of clusters has to wait some time. The effect of GSH on Te NWs was also investigated. Crystallinity of Te NWs was unaffected by GSH upon exposure for 24 h at room temperature. No other changes were seen in TEM.

As the characterization of Ag<sub>32</sub> has been discussed earlier, we discuss only the essential aspects. The synthesis resulted in a brown colored, water-soluble solid, namely crude cluster (CC). The UV-visible spectrum (trace a, Figure 1A) of this brown solution (inset a, Figure 1A) shows a peak at 480 nm, accompanied by two shoulders at 350 and 550 nm. The characteristic feature of plasmonic Ag NPs was absent. The solution is red luminescent as can be seen from the photograph under UV light irradiation (inset b, Figure 1A) and also from the photoluminescence spectrum shown in inset c, Figure 1A. Further analysis with polyacrylamide gel electrophoresis (PAGE) proved that the crude cluster is a mixture of five different Ag@GSH clusters, with Ag32SG19 being the principal component. The UV-visible spectrum of gel separated Ag<sub>32</sub>SG<sub>19</sub> (trace b) is shown in Figure 1A for comparison. As the crude cluster is by and large composed of Ag<sub>32</sub>, for all the studies carried out in this paper, we used this sample.

Te NWs show two distinct interband transitions in their UV–visible extinction spectrum. Peak I originates from electronic transitions from p-bonding valence band (VB2) to p-antibonding conduction band (CB1) and appears in the range of 250-350 nm, whereas Peak II is due to the transition from p-lone pair valence band (VB3) to the p-antibonding conduction band (CB1) and appears around 600-850 nm.<sup>28</sup> In



**Figure 3.** (A) Time dependent UV-visible extinction spectra of a solution containing Ag NPs and Te NWs. Traces a, b, c, and d represent the spectrum after 0, 6, 12, and 24 h, respectively. The spectrum of citrate capped Ag NPs is shown in the inset. (B) The TEM image of the NWs formed after 24 h. Diameter distribution of the NWs is shown in the inset. (C) The TEM image and the EDS spectrum of the NWs. EDS intensity map for Ag (D) and Te (E) for the same area are shown. Scale bar is 200 nm in (B) and 100 nm in (C).

our synthesis, the positions of these peaks were 280 and 680 nm, respectively (trace a, Figure 1B). The spectrum of Te NWs after reaction with Ag<sub>32</sub> QCs is shown in trace b, and it shows a small hump around 400 nm where the surface plasmon resonance (SPR) peak of Ag NPs appears (trace c). The two peaks characteristic to Te NWs remain, albeit a slight shift in their positions and intensities was observed. A similar trend was observed for the growth of Au NP on Te NWs,<sup>47</sup> though the Au plasmon peak was found to appear only at a very high concentration of Au NPs. The presence of the 400 nm band in the spectrum possibly results from higher surface electron density in Ag than Au, which gives rise to a better plasmonic property in Ag.<sup>48</sup> A representative TEM image of the parent Te NWs is shown in Figure 1C. The HRTEM image of the Te NW (inset b) shows the (001) directed anisotropic growth in it. Diameter distribution of these NWs (inset a) was obtained by measuring diameters of NWs from several TEM images. Parent Te NWs had an average diameter of 32 nm. Reaction of Te NWs with Ag<sub>32</sub> QC leads to the formation of nodular growth at different locations of the NW. A TEM image of such NWs is shown in Figure 1D. Diameter distribution of regions without such growth is shown in the inset of the image, and average diameter of those regions is the same as that of the

parent NWs. From these analyses, we presumed the resulting products to be Ag-Te hybrid NWs.

Structural characterization of the NWs having nodular growth was performed using spectroscopy, diffraction, and microscopy. A large area TEM image of the NWs with nodular growth on them is shown in Figure 2A. The EDS spectrum taken from the same area is shown in the inset of the figure and the presence of both Ag and Te can be seen. The XRD pattern of these NWs is presented in Figure 2B. The combined JCPDS pattern for hexagonal Te (JCPDS: 36-1452) and cubic Ag phase (JCPDS: 04-0783) is shown in the inset, and it resembles the observed pattern. Final structural conformation of the NW structures came from the analyses of the HRTEM image presented in Figure 2C. The nodular growth was identified to be due to Ag, while Te NW retained its original (001) oriented hexagonal structure. A low magnification TEM image of the same NW is shown in the inset.

Difference in Reactivity of Silver Cluster Compared to  $Ag^+$  and Ag Nanoparticle. Reactivity of  $Ag_{32}$  QCs with Te NWs in solution is in stark contrast with that of  $Ag^+$ . With the addition of  $Ag^+$ , blue colored Te NWs dispersion convert to brown colored  $Ag_2$ Te NWs dispersion almost instantaneously.<sup>30</sup> This transformation is reflected in the UV–visible



Figure 4. Increase in the extent of nodule coverage of the NWs with increasing amount of  $Ag_{32}$  QCs added: (A) 200  $\mu$ L, (B) 1 mL, and (C) 2 mL. Scale bar in the images is 100 nm.

spectra of the NWs (Supporting Information, SI, Figure S1A), where peaks due to Te NWs (trace a) diminish with the emergence of a broad band around 480 nm (trace b). Reactivity of Te NWs with another Ag(0) nanosystem, namely Ag NPs (citrate capped), was also investigated. The TEM image of the citrate capped Ag NPs used for this study is shown in Figure S1B. They were found to form the same end product of  $Ag_2Te$ NWs. UV-visible spectra of a mixture of Te NWs and Ag NPs after 0, 6, 12, and 24 h (traces a, b, c and d, respectively) are shown in Figure 3A. Peaks due to both Ag NPs and Te NWs diminished over time, and the spectrum of the solution after 24 h (trace d) appeared exactly similar to that of Ag<sub>2</sub>Te NWs. The TEM image taken from the solution after 24 h of reaction showed the presence of only NWs. These NWs were 33% larger in diameter (inset, Figure 3B) and 15% longer (Figure S2) than the parent Te NWs, and this matches with the reported volume change for Te to Ag<sub>2</sub>Te conversion.<sup>32</sup> The EDS spectrum of the formed NWs (inset, Figure 3C) showed the presence of Ag and Te in a 2:1 atomic ratio, and equal distribution of these elements throughout the NWs (Figure 3D and E, respectively) confirmed NWs composition to be Ag<sub>2</sub>Te. XRD and HRTEM (Figure S2) of the NWs further established their identity.

This similar but slow reactivity of Ag NPs compared to  $Ag^+$  can be explained by the slow  $Ag^+$  leaching property of Ag NPs. Ag NPs are known to release  $Ag^+$  in water,<sup>49</sup> which react with the Te NWs present in the solution. Consumption of the released  $Ag^+$  by reaction enhances the leaching, and all the Ag NPs present slowly get consumed. Te NWs transform into  $Ag_2$ Te NWs in due course, but  $Ag_{32}$  QCs, being highly capped with strong thiolate ligands, do not release  $Ag^+$  into the solution and silver telluride does not form.

We probed the reactivity of GSH capped Ag NPs also with Te NWs to understand the difference in reactivity between particles of two different size regimes but with the same capping agent. However, GSH capped Ag NPs did not attach themselves to the Te NWs to form a hybrid Ag–Te nanostructure, nor did they form  $Ag_2Te$  NWs by reacting with Te NWs at room temperature, even after 24 h. Te NWs and GSH capped Ag NPs remained as separated entities in solution (Figure S3).

Probable Mechanism of Ag Nodule Decorated Te NW Formation. With decrease in size of a metal particle, the population of atoms with reduced coordination number increases, resulting in the increase in surface free energy of

the particle. This induces a tendency toward aggregation in small clusters. These small clusters, when put on a surface, tend to diffuse across the surface and during this movement, as one cluster encounters another; they coalesce to form bigger particles.<sup>50–52</sup> In the case of monolayer protected Ag clusters, although the core silver atoms are protected by a monolayer of thiolates, this protection is incomplete, and certain core atoms are still accessible as evident from the formation of alloy clusters.<sup>26</sup> Due to this incomplete protection from thiolate, these clusters have an inherent tendency to coalesce to form nanoparticles. This process of coalescence does not happen in solution as SG groups carry charge, and so the clusters repel each other; but when put on surfaces, charge neutralization can happen, and the clusters can come together through diffusion and form bigger NPs. In our experiment, Ag<sub>32</sub> clusters coalesce on the surface of Te NWs when put together in solution, and they form Ag islands on the NW surface.

This phenomenon, being diffusion controlled, leads to the formation of polydispersed particles on the surfaces. Again, for the same reason, particles do not grow beyond a certain size. To check the validity of the conjecture of NP formation by diffusion-controlled aggregation of Ag<sub>32</sub> QCs on the Te NW surface, different volumes of the QC solution were reacted with the same amount of Te NW dispersion. As 200  $\mu$ L of QC solution was reacted, formation of very few small sized nodules on Te NWs was observed (Figure 4A). When the volume of QC solution was increased to 1 mL, the number of nodules formed per NW increased and so did their size (Figure 4B); but as the amount of cluster was further increased to 2 mL, though the number of nodules and their coverage increased, the size of the formed nodules remained nearly the same (Figure 4C). Size distribution of the nodules formed in 1 and 2 mL cases is shown in Figure S4. UV-visible spectra for all these three NWs are shown in Figure S5A. The NWs obtained by 2 mL of QC addition are almost completely covered with Ag nodules, and with further addition of QC, no change in the UV-visible spectrum was observed.

**Change in Nanowire Morphology upon Heating.** The morphology of these Ag-nodule decorated Te NWs was found to be very sensitive to the temperature of the solution. Figure 5 shows morphology evolution of the NWs as the NWs dispersion was maintained at 60 °C. The shape evolution was checked by collecting the NWs by centrifugal precipitation from the heated solution and subjecting them to TEM analysis at different time intervals. Figure 5A shows the TEM image of



Figure 5. Effect of solution phase heating on the morphology of the Ag nodule-decorated Te NWs. (A) The TEM image of the parent decorated NWs. (B) After 3 h of heating, nodules are bigger in size and occur only at the two ends and very few on the NW sidewalls. (C) After 6 h of heating, bigger nodules are present toward the NW ends and these resemble dumbbells. (D) The TEM image of a single dumbbell-shaped NW. Scale bar in (A)-(C) is 100 nm, and it is 50 nm in (D).

the NWs before heating. On solution phase heating, aggregation and movement of the nodules toward the tips of the NWs were observed. In Figure 5B, a TEM image of the NWs after 3 h of heating is presented. These NWs have a few big nodules present on their side walls with almost all of them having two big nodules present at the tips. After 6 h of heating, nodules present on the NW side walls vanish, and the resulting NWs morphology resembles that of a nano dumbbell in which two big sphere-shaped particles are joined by a NW section (Figure 5C). The TEM image of a single nano dumbbell is given in Figure 5D. At this point, we would also like to stretch that this morphological evolution in the nodule decorated NWs was observed only at a higher solution temperature. Keeping the as-prepared NWs in solution at room temperature did not bring out such morphological changes in them.

Structural identification of these nano dumbbells was performed with EDS and HRTEM. Ag and Te EDS intensity maps of such a NW were combined and that is shown in Figure 6A. Te was found only in the middle section, whereas the ballshaped structures had only Ag in them. This kind of Ag—Te— Ag structure of the nano dumbbells was also confirmed from the HRTEM image of one end of a nano dumbbell (Figure 6B), where the presence of Ag in the end segment and Te in the middle can be observed. The XRD pattern of these NWs is given in Figure S5B, and coexistence of both Ag and Te phases in them can be observed.

Growth of Au NPs is traditionally employed for the identification of high energy and defect sites in nanostructures.<sup>53,54</sup> In Te NWs, due to their unique *c*-axis oriented growth, the (001) planes are present at both ends, whereas the sidewalls are composed of (110) and its centrosymmetric



Figure 6. (A) Overlaid Ag and Te EDS intensity maps showing the distribution of Te and Ag in the dumbbell-shaped NW. While Te is the component of the NWs, the ball-shaped ends are composed of Ag. The corresponding TEM image and the EDS spectrum are given in the insets. Scale bar in the TEM image is 50 nm. (B) The HRTEM image of the dumbbell-shaped NWs showing the presence of Ag at the ends and Te in the middle. Scale bar is 5 nm.

planes. Surface energy of (001) plane is higher than that of (110).<sup>55</sup> Due to this reason, the ends are more reactive than the body which was revealed in the growth of Au NPs on Te NWs.<sup>56</sup> In the present case, the presence of the Ag caps on the tips of Te NWs reduces the overall surface energy of the NWs. The presence of two bigger NPs also decreases the surface energy of Ag system than the presence of several small Ag nodules, and this overall minimization of surface energy in a dumbbell structure leads to the transformation observed at elevated temperature.

This process of Ag-Te-Ag nano dumbbell formation from the Ag nodule-decorated Te NWs was found to be sensitive to the solution temperature. When the temperature was maintained at 80 °C, silver was found to diffuse within Te lattice forming silver incorporated NWs (Figure S6). While the exact nature of the transformation of nodule decorated NWs into dumbbell-shaped NWs is not fully understood, this may be seen as a "nanoscale zone refining" process. The bigger nodules acquire sufficient energy to become mobile on the NW surface<sup>51</sup> at high solution temperature (60 °C), coalesce with each other, and move toward the tips; but, at even higher temperature (80 °C), Ag atoms in the nodules gain enough energy to diffuse into the Te lattice. The nature of solution also plays an important role in this process. As we analyzed the mother liquor with electrospray ionization (ESI) mass spectrometry, the presence of glutathione (as Na salt) and its dimer was found in the solution (Figure S7). These species probably originate during the cluster coalescence where excess ligand is thrown into the solution. We presume that these species play an important role in the stabilization of the Ag nodules. As the mother liquor was discarded through centrifugation and decorated NWs were redispersed in distilled water, propensity of silver diffusion into Te increases, and the incorporation of Ag into Te lattice happens at 60 °C (Figure S8).

We checked the fate of the mixture of GSH capped Ag NPs and Te NWs solution at elevated temperature as ligand binding of SG to the NP surface is expected to weaken at higher temperature and that may lead to reaction with Te NWs; but even at 60 °C, we did not observe any reaction between the two species, while at 80 °C they slowly (over a period of 40 h) react with each other to give  $Ag_2$ Te NWs.

#### SUMMARY AND CONCLUSIONS

In summary, we probed the unique chemistry of a glutathione protected, water-soluble Ag QC upon its interaction with a 1D nanosystem, Te NWs. The QC reactivity was found to be quite different than that of silver ions as well as Ag NPs. Clusters coalesce on the NW surface to form NPs. This leads to the formation of a new material, Ag nodule-decorated Te NWs. A controlled solution phase heating of this material brings about changes in the morphology, and dumbbell-shaped Ag-Te-Ag NWs are formed. Both of these new materials (nodule decorated Te NWs and double dumbbell Ag-Te-Ag NWs) can have potential applications in the fabrication of electronic devices. The study suggests an unusual difference in the reactivity of nanosystems leading to the formation of novel structures. Although the formation of such structures may be reasoned, an accurate molecular understanding requires additional efforts.

#### ASSOCIATED CONTENT

#### Supporting Information

UV-visible spectra of Te and Ag<sub>2</sub>Te NWs, TEM images of Ag NPs, length distribution of Te and Ag<sub>2</sub>Te NWs, HRTEM and XRD of Ag<sub>2</sub>Te NWs, TEM and HRTEM images of a mixture of Te NW and GSH capped Ag NP after 24 h of reaction, diameter distribution of the nodules formed with the addition of 1 and 2 mL of cluster solution, UV-visible spectra of Ag nodule decorated NWs for different cluster concentrations, XRD of dumbbell-shaped Ag–Te–Ag NWs, TEM and EDS of NWs heated at 80 °C, the ESI mass spectrum of the mother liquor, TEM and EDS of NWs heated at 60 °C after removal of mother liquor and redispersion in distilled water. This material is available free of charge via the Internet at http://pubs.acs.org

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#### Notes

The authors declare no competing financial interest.

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## **Supporting Information**

## Manifestation of the difference in reactivity of silver clusters in contrast to

## its ions and nanoparticles: The growth of metal tipped Te nanowires

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## S1. Supporting information 1



**Figure S1**(A) UV-visible extinction spectra of Te (trace a) and  $Ag_2Te$  (trace b) NWs.  $Ag_2Te$  NWs were prepared by reacting Te NWs with aqueous solution of  $AgNO_3$ . (B) Large area TEM image of citrate capped Ag NPs showing the presence of a few anisotropic particles. Most of the NPs were spherical as seen in the inset image. Scale bar is 200 nm for the large area image and 20 nm for the inset image.



## S2. Supporting information 2

**Figure S2** (A) Length distribution of Te NWs. (B) Length distribution of  $Ag_2Te$  NWs. (C) HRTEM image of  $Ag_2Te$  NWs formed by the reaction of Ag NPs with Te NWs. Scale bar is 5 nm. (D) XRD pattern of the  $Ag_2Te$  NWs. Standard pattern of monoclinic  $Ag_2Te$  (JCPDS: 34-0142) is given as sticks which matches with the measured pattern.

### **S3.** Supporting information 3



**Figure S3.** (A) TEM image of the mixture of Te NW and GSH capped Ag NP after 24 h of reaction. Presence of both NPs and NWs are seen. Scale bar is 200 nm. (B) HRTEM image from one of NWs after 24 h of reaction. Te lattice can be observed showing no silver incorporation from GSH capped Ag NPs. (C) HRTEM image of one of the NPs showing Ag lattice. Scale bar is 5 nm for both (B) and (C).

### S4. Supporting information 4



**Figure S3.** (A) Diameter distribution of Ag nodules formed with 1 mL of the cluster. (B) Diameter distribution of the nodules formed with 2 mL of the cluster.

## **S5.** Supporting information 5



**Figure S5.** (A) UV-visible extinction spectra of decorated NWs plotted along with Te NWs. a. Parent Te NWs and the samples with b. 100  $\mu$ L, c. 1 mL and d. 2 mL of cluster addition after 24 h. (B) XRD pattern of Ag-Te-Ag dumbbell shaped NWs. Peaks corresponding to Te and Ag are marked in black and green, respectively.

## **S6.** Supporting information 6



**Figure S6.** (A) TEM image of the NWs and broken pieces produced after heating the Ag nodule-decorated NWs at 80°C. (B) TEM image of a single such NW along with a broken piece. This area was chosen for EDS mapping. EDS intensity maps for Ag (C) and Te (D). Scale bar in the TEM images is 100 nm.

**S7.** Supporting information 7



**Figure S7.** ESI mass spectral analysis of the mother liquor after  $Ag_{32}$  QC reaction with Te NWs. Glutathione and glutathione dimer were observed in this solution.

## **S8.** Supporting information 8



**Figure S8.** (A) TEM image of a NW produced when the Ag nodule-decorated NWs were subjected to heat treatment at 60 °C after the removal of the mother liquor and redispersal of the material in distilled water. EDS spectrum is given in the inset. (B) EDS intensity map for Ag in the NW. (C) EDS intensity map for Te along the NW.

## Nanoscale

## PAPER

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# Emergence of metallicity in silver clusters in the 150 atom regime: a study of differently sized silver clusters<sup>†</sup>

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We report the systematic appearance of a plasmon-like optical absorption feature in silver clusters protected with 2-phenylethanethiol (PET), 4-flurothiophenol (4-FTP) and (4-(t-butyl)benzenethiol (BBS) as a function of cluster size. A wide range of clusters, namely,  $Ag_{44}(4-FTP)_{30}$ ,  $Ag_{55}(PET)_{31}$ ,  $\sim Ag_{75}(PET)_{40}$ ,  $\sim Ag_{114}(PET)_{46}$ ,  $Ag_{152}(PET)_{60}$ ,  $\sim Ag_{202}(BBS)_{70}$ ,  $\sim Ag_{423}(PET)_{105}$ , and  $\sim Ag_{530}(PET)_{100}$  were prepared. The UV/ Vis spectra show multiple features up to  $\sim Ag_{114}$ ; and thereafter, from  $Ag_{152}$  onwards, the plasmonic feature corresponding to a single peak at  $\sim 460$  nm evolves, which points to the emergence of metallicity in clusters composed of  $\sim 150$  metal atoms. A minor blue shift in the plasmonic peak was observed as cluster sizes increased and merged with the spectrum of plasmonic nanoparticles of 4.8 nm diameter protected with PET. Clusters with different ligands, such as 4-FTP and BBS, also show this behavior, which suggests that the 'emergence of metallicity' is independent of the functionality of the thiol ligand.

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## Introduction

The strong collective oscillation of electrons referred to as plasmon resonance,1 which produces characteristic colors in optical absorption, is the most fascinating property of noble metal nanoparticles,<sup>2-5</sup> and is the basis for most of their applications.<sup>6-8</sup> The occurrence of this collective phenomenon is attributed to the existence of metallicity9 in such systems; therefore, it is important in the cluster size regime of metals. Investigation of electronic structures by photoelectron spectroscopy has suggested the emergence of metallicity in the 200-400 atom window in several naked (ligand-free) metal systems such as Hg, Cu and Au.<sup>10-12</sup> For several metals such as Na, K, and Al, the appearance of plasmon has been extensively studied.<sup>13-17</sup> Investigations of single particle conductivity by scanning electron spectroscopy and similar studies18 have also proposed the emergence of metallicity in this size window. On the other hand, large nanoparticles protected by monolayers of organic molecules, possessing metallicity in transport measurements<sup>19</sup> have been brought into the cluster size regime by core etching protocols<sup>20-22</sup> wherein distinct luminescence in the visible region, characteristic of clusters has been observed.<sup>23</sup> Therefore, metallicity occurs beyond the regime of these clusters<sup>24-31</sup> but below the size regime of nanoparticles in the range of silver nanoclusters. Thiolated gold clusters are normally more stable as compared with silver clusters because Ag(0) is easily oxidisable under atmospheric conditions. Several gold clusters, such as Au<sub>25</sub>,<sup>32,33</sup> Au<sub>28</sub>,<sup>34</sup> Au<sub>36</sub>,<sup>35</sup> Au<sub>38</sub>,<sup>36</sup> and Au<sub>102</sub>,<sup>37</sup> have been crystallized recently, and there are many reports of their mass spectrometric assignments.<sup>38,39</sup> Recently, Dass *et al.*<sup>40</sup> and Jin *et al.*<sup>41</sup> demonstrated the plasmonic feature in gold for ~76 kDa particles protected by monolayers. In the case of silver, although clusters analogous to gold have not been prepared, there are reports of Ag<sub>7,8</sub>,<sup>22</sup> Ag<sub>9</sub>,<sup>30</sup> Ag<sub>32</sub>,<sup>27</sup> and Ag<sub>152</sub><sup>42</sup> and a few others such as Ag<sub>14</sub>,<sup>43</sup> Ag<sub>16</sub><sup>44</sup> and Ag<sub>44</sub>,<sup>26,31</sup> the latter clusters have been crystallised.

In this paper, we report the emergence of plasmon-like optical absorption spectra in thiol-protected silver clusters in solution, which points to the appearance of metallicity in clusters composed of ~150 metal atoms. Our proposal of emergence of plasmon resonance is in accordance with the studies reported so far<sup>12</sup> and points to similar results in other metal systems.<sup>6-8</sup>

## **Experimental section**

#### Materials

All the chemicals were commercially available and were used without further purification. Silver nitrate (AgNO<sub>3</sub>, 99% Aldrich), silver trifluroacetate (AgCOOCF<sub>3</sub>, 99%, Aldrich),



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 $<sup>\</sup>dagger$  Electronic supplementary information (ESI) available: Details of laser dependent fragmentation, SEM-EDAX of  $\sim\!\!Ag_{202}$ , TEM of  $\sim\!\!Ag_{530}$  and nanoparticles and corresponding energy-dependent UV/Vis spectra, DLS, and comparative MALDI MS. See DOI: 10.1039/c4nr00679h

sodium borohydride (NaBH<sub>4</sub>, 99.9%, Aldrich), tetraoctylammonium bromide (TOAB, 99%), 2-phenylethanethiol (PET, 98%, Aldrich); 4-(*t*-butyl)benzenethiol (BBS, 98%, Aldrich), 4flurothio phenol (4-FTP, 98%, Aldrich) ethanol (Changshu Yangyuan Chemical, China, AR grade), acetonitrile, tetrahydrofuran, methanol and toluene (all obtained from Ranken, AR grade) were used in this work.

#### Synthesis of PET-protected clusters

The clusters protected with PET were prepared using the solid state method,<sup>22</sup> and a solution-phase synthetic route was followed for BBS-protected clusters. The clusters were synthesized by carefully controlling the conditions and were purified using solvent extraction protocols. These can be clearly understood in the light of Table 1 given below, which lists the synthetic details. Initially, at room temperature (35-40 °C in Chennai, relative humidity, 31%) X mg of AgNO<sub>3</sub> and Y µL of PETH were ground thoroughly in a clean agate mortar using a pestle. In some cases, tetraoctylammonium bromide (TOAB) was also used along with the ligand (see Table 1). The color of the mixture changed to pale orange, showing the formation of silver thiolate. To this mixture, Z mg of solid NaBH<sub>4</sub> was added and the contents were mixed well. 5 mL of ethanol was added for washing the mixture. The mixture was maintained for 15-30 s until the color changed from pale orange to deep grey, which happened within 1 min. The contents were then taken in a centrifuge tube and centrifuged at 1600 rpm. The centrifugate was removed (except in the case of Ag<sub>55</sub>, where ethanol was used as the extracting solvent) and the residue was dissolved in Q mL solvent (S). The clusters obtained from the solvent extract were dark brown in color. The synthesis of ~Ag<sub>530</sub> required more time; initially, ~Ag<sub>75</sub> clusters were formed but after one day they transformed to  $\sim Ag_{530}$ , which is stable. TOAB facilitated this transformation.45

#### Synthesis of the BBS-protected cluster

20 mg of AgNO<sub>3</sub> was dissolved in 3 mL of methanol, and 3 mL of toluene was also added to this solution. Then BBS (1 : 6 mole ratios with respect to silver) was added under stirring, and an Ag-thiolate complex was formed. During this process, the color of the solution changed from deep yellow to light yellow. After 30 min, 2 mL fresh ice-cold solution of NaBH<sub>4</sub> (1 : 10 mole ratio

**Table 1** Amounts of starting materials used for synthesizing various clusters<sup>a</sup>

with respect to silver) was added. The yellow color changed to dark brown. Stirring was continued for 30 min. Two separate layers were observed; the top layer contained the cluster, which was removed and further characterized.

#### Synthesis of Ag<sub>44</sub> cluster

The Ag<sub>44</sub> cluster was synthesised using a reported procedure.<sup>28</sup>

#### Instrumentation

UV/Vis spectra were measured with a Perkin Elmer Lambda 25 instrument in the range of 200-1100 nm. The spectra were corrected by the Jacobian factor (see below). High resolution transmission electron microscopy of clusters was carried out with a JEOL 3010 instrument with a UHR polepiece. The samples were drop-cast on carbon-coated copper grids and allowed to dry under ambient conditions. Matrix-assisted desorption ionization mass spectrometry (MALDI MS) studies were conducted using a Voyager-DE PRO Bio-spectrometry Workstation (Applied Bio-systems). A pulsed nitrogen laser of 337 nm was used for the MALDI MS studies. Mass spectra were collected in the positive ion mode and were averaged over 200 shots. For sample preparation, the as-synthesized clusters were mixed with DCTB (trans-2-[3-(4-t-butylphenyl)-2-methyl-2-propenylidene]malononitrile) matrix (12.5 mg mL<sup>-1</sup> in toluene) in 1:1 and 2:1 volume ratios, followed by an immediate spotting on the MALDI plate. Scanning electron microscopy (SEM) and energy dispersive X-ray (EDAX) measurements were performed with a FEI QUANTA-200 SEM. For measurements, samples were drop-cast on an indium tin oxide (ITO)-coated glass as well as on carbon tape and subsequently dried under vacuum. DLS was performed with a Horiba instrument. X-ray photoelectron spectroscopy (XPS) measurements were conducted using an Omicron ESCA probe spectrometer with polychromatic MgKa Xrays ( $h\nu = 1253.6$  eV). The samples were spotted as drop-cast films on a sample stub. A constant analyzer energy of 20 eV was used for the measurements.

## **Results and discussion**

The core size of each cluster sample was characterized by matrix assisted laser desorption ionization mass spectrometry (MALDI

Take 1 Amounts of starting matchais used for synthesizing various clasters									
Clusters	AgNO <sub>3</sub> (X/mg)	TOAB (in mg)	PETH $(Y/\mu L)$	NaBH <sub>4</sub> (Z/mg)	Solvent (Q/mL)	Yield (%)	Stability <sup><math>\Phi</math></sup> (day)		
Ag <sub>55</sub>	23	5	100	25	Ethanol (5)	64%	3		
$\sim Ag_{75}$	20	—	Glutathione* (12.5 mg)	Formic acid* (200 μL)	Water*	—	8		
$\sim Ag_{114}$	23	—	100	25	$CH_3CN(5)$	72%	4		
Ag <sub>152</sub>	23	—	100	25	Toluene (5)	82%	$10^{\$}$		
$\sim Ag_{423}$	23	—	100	25	50 : 50 mixture of toluene–methanol (5)	64%	5		
$\sim Ag_{530}$	47	34	200	30	Toluene (20)	76%	$14^{\#}$		
Ag NP	47	_	100	30	Toluene (5)	84%	30		

 $a^{a}$  \*, # and \$ refer to the corresponding references given below and  $\Phi$  refers to stability in the ambient laboratory conditions. \* ref. 46, \$ ref. 42, # ref. 45.

MS) with DCTB as the matrix (Fig. 1), which is effective for organic soluble clusters.<sup>42,46-48</sup> The spectra show the existence of eight distinctly different clusters with characteristic peaks at 10.3, 13.7, 18.6, 24.5, 33.4, 60 and 70.9k. Assuming the ions to be monocationic, as suggested by their peak width, the clusters responsible for these peaks were assigned to  $Ag_{55}(PET)_{31}$ ,  $\sim Ag_{75}(PET)_{40}$ ,  $^{46} \sim Ag_{114}(PET)_{46}$ ,  $Ag_{152}(PET)_{60}$ ,  $^{42} \sim Ag_{202}(BBS)_{70}$ ,  $\sim Ag_{423}(PET)_{105}$ , and  $\sim Ag_{530}(PET)_{100}$ ,<sup>45</sup> respectively, (where peaks were ill defined in a few cases, the composition is indicated with a ' $\sim$ ' symbol, as practiced by other researchers<sup>49</sup>). Compositions suggested here have also been supported by elemental analysis and XPS (see below). In most of the cases (labeled as 'a', 'b', 'c', 'd' in Fig. 1), the spectra correspond to sharp and single features. This is especially noticeable in 'a' and 'd' corresponding to Ag<sub>55</sub> and Ag<sub>152</sub>, respectively. For 'b', there is a weak feature at the high mass side and for 'c', there is a weak shoulder at the high mass side. For these reasons, we suggest only approximate compositions for these cases. Detailed characterization of each cluster is beyond the scope of this work, and some of the clusters (Ag<sub>44</sub>, ~Ag<sub>75</sub>, Ag<sub>152</sub> and ~Ag<sub>530</sub>) have been described previously.<sup>26,28,31,42,45,46</sup> It is important to note that the spectra were collected at the threshold laser powers (fluences) where observable fragmentation is not observed. Above a characteristic laser power, fragmentation was observed, normally resulting from the loss of the monolayer, AgSR, Ag(SR)<sub>2</sub> or R.42,46 Such systematic losses and fragmentation arising from

cleavages in  $Ag_n$ -S-R are well-known on UV laser irradiation, and such processes have been useful in understanding the core sizes.<sup>50</sup> For example, in the case of  $Ag_{152}$ PET<sub>60</sub>, we could see a gradual fragmentation with an increase in laser power (Fig. S1, ESI†) and above a certain laser power, no further fragmentation was observed. The spectra are also composed of a few other clusters of higher masses (traces 'e', 'f' and 'g'), which exhibit multiple features. Specific clusters have not been obtained in such cases to date.

However, even in those cases the spectra are dominated by one major feature with a few other clusters existing with reduced intensity. Another interesting feature was the full width at half maximum (FWHM) of each spectrum. The FWHM varied from 1.5 to 5k. In a, c, d and f, the peak width is very small (below 3k), which is not observed in any silver clusters reported to date.

It is important to note that all the peaks are broad in comparison with molecules of similar mass such as smaller proteins. However, we must note that the peak is sharper than protein-protected silver, gold and silver–gold alloy clusters, which are similar to those considered here.<sup>51–53</sup> In Fig. S2A, ESI,† we have plotted the MALDI MS spectra of the Ag<sub>152</sub> cluster (of the most narrow width), a small protein, a lysozyme in its native state and that of a lysozyme–gold cluster (all in linear positive ion mode). We see that while the native protein spectrum is sharper ( $\Delta m = 0.3$  Da), the mass feature of the protein cluster is



Fig. 1 MALDI MS spectra (collected in positive mode) of silver clusters prepared in solution. All the clusters were purified by solvent extraction before spotting for MALDI MS studies. Threshold laser fluence was used throughout the experiment to avoid fragmentation. The spectra show a series of clusters with peak maxima ranging from 10.2 to 70.9k. Almost all the spectra (except 'f' and 'g', which have some other features with reduced intensity) show sharp single features, confirming the formation of one dominant cluster in each case. The FWHM varied from 1.5 to 5 kDa. The peaks (from bottom to top) were assigned as:  $Ag_{55}(PET)_{31}$  [a],  $\sim Ag_{75}(PET)_{40}$  [b],  $\sim Ag_{114}(PET)_{46}$  [c],  $Ag_{152}(PET)_{60}$  [d],  $\sim Ag_{202}(BBS)_{70}$  [e],  $\sim Ag_{423}(PET)_{105}$  [f], and  $\sim Ag_{530}(PET)_{100}$  [g]. Spectra have been shifted vertically for clarity.

#### Paper

broader ( $\Delta m = 2.5$  Da) and the width is comparable to that of the silver cluster. This broadening of the protein–gold cluster is due to the changes in the native structure of the protein upon cluster formation. It may also be noted that the protein-protected clusters shown are those of Au, which has only one isotope. In the case of silver, there is a natural isotope width, and its spread is large in the case of a multi-atom cluster (see below). The data suggest that the inherent width in silver clusters is comparable to that of molecular systems of similar masses. For comparison, the MALDI MS of Ag<sub>152</sub> cluster was plotted along with the well-characterised Ag<sub>44</sub> cluster (Fig. S3, ESI†). The data suggest that the spectra of silver clusters are broad in nature even if they are atomically precise.

Electrospray ionization (ESI) is a preferred method for retrieving data on several thiolated clusters. However, for silver clusters, ESI MS is still not ideal because intact ionization is difficult. More ESI MS data are available for gold clusters; however, it is important to note that gold has only one isotope, whereas silver has two isotopes at m/z 107 and m/z 109 with 50: 50 intensity. This adds additional width to the mass feature of silver clusters. In addition, silver-sulfur binding is weaker, which makes the intact cluster less stable, and the ligand losses occur upon ionization. Both these factors contribute to the increased width of the mass spectrum of silver clusters (in comparison with that of gold). In the case of  $Au_{25}PET_{18}$ , the spectrum (Fig. S2B, ESI<sup>+</sup>) is measurable with improved resolution in the reflectron mode. Because typical reflectron measurements are limited to m/z 10 000, the present silver clusters at  $m/z > 10\ 000$  could not yield better peak shapes in such measurements.

The SEM/EDAX data further support the composition of the clusters. Fig. 2 shows the corresponding EDAX spectra of Ag<sub>55</sub>

(a),  $\sim Ag_{75}$  (b),  $\sim Ag_{114}$  (c),  $Ag_{152}$  (d),  $\sim Ag_{423}$  (e) and  $\sim Ag_{530}$  (f). Here, clusters were drop-cast on indium tin oxide (ITO)-coated glass plates. The spectrum of  $\sim Ag_{202}$  was collected from a dropcast sample (on a carbon tape) and is shown in Fig. S4, ESI.† The expected elements are seen in each case, and the Ag : S ratios were 1:0.60, 1:0.58, 1:0.39, 1:0.43, 1:0.40, 1:0.34 and 1:0.37 for Ag<sub>55</sub>,  $\sim Ag_{75}$ ,  $\sim Ag_{114}$ , Ag<sub>152</sub>,  $\sim Ag_{202}$ ,  $\sim Ag_{423}$  and  $\sim Ag_{530}$ , respectively, whereas the expected ratios are 1:0.56, 1:0.53, 1:0.36, 1:0.39, 1:0.34, 1:0.25 and 1:0.18. For the last two cases, there is an excess amount of thiol in the sample, which is probably required for cluster stabilisation.<sup>42</sup> As  $\sim Ag_{423}$ and  $\sim Ag_{530}$  samples are not pure, as seen from their mass spectra, their atomic ratios deviate significantly from the expected ratios.

These clusters with precise core sizes are observable in TEM, where well-defined cores ranging from 1.12 nm to 4.0 nm are observed (Fig. 3 and S5, ESI<sup>†</sup>). The particle size increases with the nuclearity of the cluster. The size distribution shown in the insets of each of these images (Fig. 3) confirmed that the synthetic protocol yielded the desired results. A narrow size distribution suggests the monodispersity of the as-synthesised clusters. The average core diameters were 1.12, 1.32, 1.75, 2.0, 2.61 and 3.20 nm for Ag55, ~Ag75, ~Ag114, Ag152, ~Ag202 and ~Ag<sub>423</sub>, respectively. Polydispersity is seen in image 'f', which in agreement with the mass spectrum (Fig. 1f). The cluster  $\sim Ag_{530}$ is about 3.61 nm in diameter (Fig. S5, ESI<sup>+</sup>), which also exhibits polydispersity. To further support monodispersity, dynamic light scattering measurements were carried out for the PETprotected clusters (Fig. S6, ESI<sup>+</sup>), which also shows a narrow size distribution for smaller clusters up to  $Ag_{152}$ . For  $\sim Ag_{423}$ , the peak becomes broader, as expected from MALDI MS data, which suggests the existence of other species in minor proportions.



Fig. 2 SEM/EDAX of Ag<sub>55</sub> (a), ~Ag<sub>75</sub> (b), ~Ag<sub>114</sub> (c), Ag<sub>152</sub> (d), ~Ag<sub>423</sub> (e) and ~Ag<sub>530</sub> (f). The expected elements are seen. The spectra were collected from a drop-cast sample on an ITO plate. Peaks corresponding to Si, Sn, Ca and O are from the substrate.



Fig. 3 HRTEM images of clusters:  $Ag_{55}$  [a],  $\sim Ag_{75}$  [b],  $\sim Ag_{114}$  [c],  $Ag_{152}$  [d],  $\sim Ag_{202}$  [e] and  $\sim Ag_{423}$  [f]. The images show a gradual increase in cluster size from left to right ('a' to 'c' and 'd' to 'f'). Insets in all the images show the size distribution of clusters, which varies from 1.12 to 3.20 nm. Some bigger nanoparticles were also seen, which could have resulted from aggregation upon electron beam irradiation.

Because such clusters are prone to electron beam-induced damage<sup>21</sup> and subsequent core size evolution with increasing electron beam irradiation, a few aggregates and cores of larger sizes are observed in the images. For comparison, TEM images of the Ag@PET nanoparticle are shown in the Fig. S7, ESI.† It has a wide range of sizes from 3 to 8 nm with an average diameter of 4.8 nm.

The optical absorption spectra of clusters of smaller sizes show multiple features corresponding to the distinct transitions between the discrete energy states (Fig. 4 and S8, ESI<sup>†</sup>). In the still smaller regime of Ag<sub>7</sub>,<sup>22</sup> Ag<sub>8</sub> and Ag<sub>9</sub>,<sup>30</sup> Ag<sub>14</sub>,<sup>43</sup> Ag<sub>16</sub>,<sup>44</sup> Ag<sub>32</sub>,<sup>27</sup> Ag<sub>44</sub>,<sup>28</sup> *etc.* many more features are observed. In the case of Ag<sub>7</sub>, two humps corresponding to 550 and 600 nm were observed.<sup>22</sup>

Similarly for Ag<sub>9</sub>, four features at 450, 489, 629 and 886 nm were observed.<sup>30</sup> Multiple features were also observed from the cluster reported by Kitaev.<sup>24</sup> In the magic-numbered glutathione-protected silver clusters,<sup>29</sup> in the bands 2, 6, 9 and 13 separated by polyacrylamide gel electrophoresis (PAGE), distinct optical features are seen. Multiple step-like features were seen in the absence of plasmon in their optical spectra. Recently, Bigoni's group identified Ag<sub>32</sub> by mass spectrometry<sup>27</sup> where we also see the same step-like features along with the absence of plasmon-like absorption, which appear at about 390–430 nm region for water-soluble clusters. The case is similar for the Ag<sub>44</sub> cluster, as reported by Dass *et al.*<sup>28</sup> Surprisingly, compared with other silver clusters, this system shows five pronounced and three weak bands in its optical spectrum.

From the data presented in Fig. 4, we see that as the sizes increase these low energy features disappear and a large oscillator strength accumulates around 450 nm. Smaller clusters show molecule-like properties with discrete energy levels, which are reflected in their optical spectra. The Ag<sub>44</sub> cluster shows five

intense bands along with three weak bands similar to that reported by Harkness et al.28 For Ag55, two distinct features at 450 (2.75) and 550 (2.25) nm (eV) are seen along with a hump at 495 (2.50) nm (eV). In  $\sim$ Ag<sub>75</sub>, the features appear at 475 (2.61) and 630 (1.96) nm (eV) along with a shoulder at 430 (2.88) nm (eV). ~Ag114 shows two features at 464 (2.67) and 540 (2.29) nm (eV). Ag<sub>152</sub>, ~Ag<sub>202</sub>, ~Ag<sub>423</sub>, ~Ag<sub>530</sub> and AgNP show a single peak at 462 (2.68), 460 (2.69), 458 (2.70), 457 (2.71) and 454 (2.73) nm (eV), respectively. The features merge at around  $Ag_{152}$ . All the smaller clusters also show significant absorption in the 3-4 eV (413-310 nm) window, possibly arising from the Ag-thiolate shell protecting the metal core. Note that the Ag-SR thiolate in toluene solutions typically show absorption in the ultraviolet region. At Ag<sub>152</sub> and beyond, only one feature is seen. Beyond this, the peak position shifts only marginally upon increase in cluster size from ~Ag<sub>202</sub> to ~Ag<sub>530</sub>. Because larger clusters, such as  $\sim Ag_{202}$  and beyond, are not pure, their spectra may also have contributions from impure particles. However, from Ag<sub>152</sub> itself, which is atomically precise, the spectra resemble that of Ag nanoparticles protected with PET, whose peak maximum occurs at 462 nm (Fig. S8, ESI<sup>†</sup>). For water-soluble nanoparticles, SPR occurs in the 390-440 nm window, depending on size. Part of the reason for a red-shifted peak in PET-protected particles is the organic shell, and the other reason is the medium surrounding the particles. Surface plasmon resonance is greatly influenced by the dielectric constant of the solvent. In this case, the solvents are organic, which have much lower dielectric constants (toluene = 2.4 at 20  $^{\circ}$ C) than water (80.4 at 20  $^{\circ}$ C). This could be the reason for the plasmon shift of organic soluble nanoparticles, as suggested by the Mie theory.<sup>1,2</sup> The spectra as a function of energy are plotted in Fig. S8, ESI.<sup>†</sup> For more clarity, the peak position is plotted against the reciprocal of diameter (Fig. 5a). The average diameter was taken from the



**Fig. 4** UV/Vis spectra of clusters with wavelength on the *x*-axis. Reading from bottom up:  $Ag_{44}$  [a],  $Ag_{55}$  [b],  $\sim Ag_{75}$  [c],  $\sim Ag_{114}$  [d],  $Ag_{152}$  [e],  $\sim Ag_{202}$  [f],  $\sim Ag_{423}$  [g],  $\sim Ag_{530}$  [h] and AgNPs [i]. The spectra have been shifted vertically for clarity. The spectra show multiple features up to 'd' (namely, eight bands for  $Ag_{44}$ , two energy bands for  $Ag_{55}$  and  $\sim Ag_{114}$ , and three for  $\sim Ag_{75}$ ). But from 'h' to 'i', only a single plasmon-like feature was observed with a small blue shift (*i.e.*, at higher energy).

TEM analysis. It shows an almost linear behavior, in agreement with the spherical shell model.<sup>54–56</sup> Optical absorption spectra of smaller clusters for both gold and silver exhibit multiple features. As the size decreases below 55, as in the case of Ag<sub>44</sub>, Ag<sub>32</sub>, Ag<sub>9</sub>, for example,<sup>27,28,30,31</sup> many more features appear in the spectra, especially in the red region. Many of these transitions have large contributions from the thiolate shell as shown by time-dependent density functional theory (TDDFT) calculations.<sup>57,58</sup> A comparison of the calculated optical spectrum of various clusters suggests a systematic trend for the core-derived transitions.<sup>57,58</sup> In the limit of large cluster sizes, these transitions converge to the plasmon resonance. From a simplistic argument, one can see that as the proportion of ligand-derived states decrease the dipole transition of the core is dominant in the optical absorption spectra. Comparing the spectra of the clusters listed here, it is seen that large optical density, similar to plasmon resonance, appears in the vicinity of Ag<sub>152</sub>. This cluster is expected to have 92 free electrons, whereas metallicity



Fig. 5 A plot of absorption peak positions with respect to the reciprocal of cluster diameter (a). The average core diameter was obtained from the TEM size distribution. Expanded XPS of the Ag3d region of  $Ag_{55}$ ,  $Ag_{152}$ , AgNPs and Ag(i)-thiolate (b).

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is suggested to occur at around 60 atoms in the case of naked gas phase clusters.<sup>12</sup> However, the thiolated cluster systems are not identical to the bulk fcc solid. For some well-characterized clusters of the type Ag<sub>44</sub>, the cage is known to be a Keplerate solid.<sup>26,31</sup> This hollow cage structure is distinctly different from the molecular analogues of the bulk metal, which can result in a variation of the optical absorption spectrum.

Ultraviolet photoelectron spectroscopic studies of these clusters were attempted but nothing significant was observed from that study. X-ray photoelectron spectroscopy gives some good information regarding the valence state of silver. XPS was also used in evaluating the elemental composition of the samples, although this data are not presented here. For consistency in analysis, we collected XPS of four consecutive systems:  $Ag_{55}$ ,  $Ag_{152}$ , AgNPs and Ag(1)-thiolate (Fig. 5b). The  $Ag_{55}$  cluster shows the  $3d_{5/2}$  peak at 368.0 eV, which is close to the Ag-thiolate peak (368.1 eV). This could be because a higher number of Ag(1)s is present as compared with Ag(0). On the other hand, the  $Ag_{152}$  cluster shows a peak at 367.7 eV, which is closer to that of the AgNPs, further suggesting a change in the properties of materials in the  $Ag_{152}$  regime.

There have been several photoelectron spectroscopic investigations regarding the emergence of metallicity in metal clusters.<sup>10,12,59</sup> These are generally discussed in the light of initial and final state effects.<sup>60</sup> Electronic relaxation of the final state and electronic interaction with the support contributing to the additional screening are important factors for determining the final state effect. Intrinsic electronic structure stabilizing the cluster contributes to the initial state effect.60 Whereas systematic energy shifts of the 4f binding energy is observed in supported gold clusters, no such effect is observed in supported silver clusters.<sup>60</sup> The change in ionization energy as well as the increased width of the photoelectron spectra is proportional to 1/R, as suggested by spherical shell model.<sup>54-56</sup> Electronic and geometric effects contribute to changes in the behavior. In the case of supported Ag clusters, no binding energy shift was observed, which may be attributed to the large separation of the 4d and 5s orbitals in comparison with those of gold because of the lack of relativistic contraction for silver.<sup>60</sup> As a consequence, no large redistribution occurs between the s and d orbitals. While this is the case of un-passivated clusters, in the data presented in Fig. 5b we see a small shift in the increased binding energy in clusters and thiolate in comparison with that of the nanoparticles. This is attributable to the large proportion of Ag<sup>+</sup>. However, for Ag<sub>152</sub>, the spectrum is almost identical to that of AgNPs. Thus, while photoemission using the core level is important to distinguish the clusters from bulk for gold, no large difference are seen for silver.<sup>60</sup> The data are consistent for both the monolayer-protected and naked clusters, as shown here.

The present results suggest the emergence of plasmon-like optical absorption around  $Ag_{152}$ . In silver clusters, metallicity is seen at about 60 atoms in photoelectron spectroscopic studies, as mentioned in a previous study.<sup>12</sup> Unlike the case of naked clusters used for photoelectron spectroscopy, the clusters used here are monolayer protected and have distinct cores of smaller dimensions. A cluster with a different ligand (BBS) also shows

the plasmonic feature, which confirms that the emergence of metallicity is the inherent property of the metal. The origin of metallicity is suggested to be around  $Ag_{152}$ , which has a core of 92 atoms and a shell composed of  $Ag_{60}SR_{60}$ .<sup>42</sup>

## Conclusions

In summary, we report the occurrence of a plasmon-like optical absorption feature in silver clusters prepared in solution. A range of clusters with varying nuclearities were synthesised and analysed through MALDI MS. Well-defined TEM with a gradual increase in size were observed. Different ligands were used to understand the silver-thiolate binding chemistry and its influence on the appearance of the plasmon. Based on the emergence of collective electron resonance in the 150 atom regime, we suggest that this is the window where metallicity originates for silver in monolayer-protected clusters.

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# Supplementary Information Emergence of Metallicity in Silver Clusters in the 150 Atom Regime: A Study of Differently Sized Silver Clusters

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## Supporting information

## Method of Jacobian correction

To amplify the less-intense absorption features at the red end of the spectrum, the data have been corrected with the Jacobian factor. For this, the experimentally obtained absorbance values as a function of wavelength  $[I(\omega)]$ , were converted to energy-dependent numbers [I(E)], using the expression,

$$I(E) = \frac{I(\omega)}{\partial E/\partial \omega} \propto I(\omega)^* \omega^2$$

Where  $\partial E / \partial \omega$  represents the Jacobian factor.

S1. Supporting information 1 Laser dependency of Ag<sub>152</sub> cluster



Fig.S1. Laser intensity dependent mass spectrum of  $Ag_{152}$  cluster. With increase (by 1000 units) in laser intensity from 1832 to 2432 (numbers refer to instrument settings and not absolute value of laser power), the peak shifts to lower values of m/z. Further increase in laser intensity does not change the peak position.

## S2. Supporting information 2

**Comparative mass spectra clusters** 



Fig.S2. A: Comparison of the mass spectra of PET protected silver cluster  $[Ag_{152}(PET)_{60}]$ , native lysozyme (Lyz) and lysozyme-gold cluster (Au@Lyz).  $M_L^+$  refers to the molecular ion on Lysozyme. The peak shift in Au@Lyz peak position is due to the Au cluster nucleated within the protein.  $2M_L^+$ ,  $3M_L^+$ ,... are dimer, trimer, etc. of the protein and the corresponding clusters. ML also shows a dication ( $M_L^{2+}$ ) feature. B: MALDI MS data of  $Au_{25}PET_{18}$ 

S3. Supporting information 3 Comparative MALDI MS of Ag<sub>152</sub> and Ag<sub>44</sub> clusters



Fig. S3. MALDI MS spectra of  $Ag_{44}$  and  $Ag_{152}$  clusters using DCTB as a matrix.

## S4. Supporting information 4 SEM/EDAX of ~Ag<sub>202</sub> cluster



Fig.S4. SEM/EDAX of  $\sim Ag_{202}$  cluster. The spectrum was collected from the solid sample spotted on a carbon tape. Absence of sodium shows the purity of the cluster.

**S5.** Supporting information 5

TEM image of ~Ag<sub>530</sub> cluster



Fig.S5. TEM image of  $\sim Ag_{530}$  cluster showing nearly homogeneous particles. Inset shows the size distribution of clusters ranging from 3 to 4 nm with an average diameter of 3.61 nm. The image also shows particles of a few different sizes, in agreement with the mass spectrum (Fig.1, main text).
S6. Supporting information 6 DLS of PET protected clusters



Fig.S6. DLS spectra of different sized PET protected silver clusters.

S7. Supporting information 7 MALDI MS and TEM of Ag@PET NPs



Fig.S7. The MALDI MS of Ag@PET nanoparticles which does not exhibit any distinct feature. Inset shows the corresponding TEM images which shows various sizes. Inset of inset shows the size distribution with an average diameter of 4.8 nm. In a typical synthesis, Ag nanoparticles are polydisperse.

## **S8.** Supporting information 8

Absorption spectra of clusters in terms of energy



Fig.S8. Absorption spectra of clusters plotted in terms of energy, after normalization. The spectra correspond to (from bottom to up)  $Ag_{44}$  [a],  $Ag_{55}$  [b],  $\sim Ag_{75}$  [c],  $\sim Ag_{114}$  [d],  $Ag_{152}$  [e],  $\sim Ag_{202}$  [f],  $\sim Ag_{423}$  [g],  $\sim Ag_{530}$  [h] and AgNPs [i].

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## Blue Emitting Undecaplatinum Cluster†

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A blue luminescent 11-atom platinum cluster showing steplike optical features and absence of plasmon absorption was synthesized. The cluster was purified using high performance liquid chromatography (HPLC). Electrospray ionization <sup>10</sup> (ESI) and matrix assisted laser desorption ionization (MALDI) mass spectrometry (MS) suggest a composition,  $Pt_{11}$ (BBS)<sub>8</sub> which was confirmed by a range of other experimental tools. The cluster is highly stable and compatible with many organic solvents.

- <sup>15</sup> Noble metal quantum clusters (QCs), also referred to as monolayer protected clusters, artificial atoms, and nanomolecules belong to a fascinating area of current research owing to their unique optical and photochemical properties.<sup>1-6</sup> Smaller size (less than 2 nm), high quantum confinement and absence of surface <sup>20</sup> plasmon resonance make these materials molecule-like. Due to the enhanced optical and photochemical properties, they have been used in many applications such as bio-labeling,<sup>7, 8</sup> drug delivery,<sup>9</sup> cancer cell imaging<sup>10</sup> and many others.<sup>11, 12</sup> Among the noble metals, gold has been explored significantly. Crystal <sup>25</sup> structures of clusters such as Au<sub>23</sub>,<sup>13</sup> Au<sub>25</sub>,<sup>6, 14</sup> Au<sub>36</sub>,<sup>15</sup> Au<sub>38</sub>,<sup>16, 17</sup>
- and Au<sub>102</sub><sup>18</sup> have been reported. Unlike in the case of gold, very few reports namely, Ag<sub>7,8</sub>,<sup>19</sup> Ag<sub>9</sub>,<sup>20</sup> Ag<sub>32</sub>,<sup>21</sup> Ag<sub>44</sub><sup>22, 23</sup> and Ag<sub>152</sub><sup>24</sup> exist for silver clusters with detailed characterization. Crystal structures of a few of the silver analogues of QCs are available <sup>30</sup> recently.<sup>25-28</sup> There are very few reports on luminescent Pt
- <sup>30</sup> recently.<sup>25-28</sup> There are very few reports on luminescent Pt clusters<sup>29-33</sup> which are either polymer (pentaerythritol tetrakis 3-mercaptopropionate-polyvinylacetate, PTMP-PVAc)-protected,<sup>30</sup> dendrimer-protected,<sup>29</sup> or glutathione (GSH)-protected.<sup>31</sup> However, improved characterization, especially detailed mass
- <sup>35</sup> spectrometric studies are needed to know each of these systems in much more detail, particularly their precise compositions as many atomically precise clusters have still not been crystallized. <sup>18-22,34</sup> Besides synthesizing new clusters with versatile methods, accurate and reproducible separation would enable improved <sup>40</sup> characterization of these materials. As Pt has high catalytic activity,<sup>35</sup> discovery of such clusters can enhance the field tremendously.

In this work, we report the synthesis of a blue emitting monolayer protected atomically precise Pt<sub>11</sub> cluster. Detailed <sup>45</sup> characterization of the chromatographically-isolated cluster was made with electrospray ionization mass spectrometry (ESI MS) and matrix assisted laser desorption ionization mass spectrometry (MALDI MS) in order to identify its precise composition.

The synthesis of Pt cluster follows a solid state route<sup>20</sup> which <sup>50</sup> was originally developed for Ag<sub>9</sub> clusters protected with mercaptosuccinic acid (MSA). In the method, H<sub>2</sub>PtCl<sub>6</sub>(s) was ground with 4-(tert-butyl)benzyl mercaptan (BBSH) (l) at 1:10 molar ratio in a mortar and pestle. Then 40 mg of NaBH<sub>4</sub>(s) was added and grinding was continued for 2 minutes to complete the <sup>55</sup> reaction. Immediate extraction of excess thiol in the reaction mixture with 5 mL of ethanol and subsequent dissolution of the residue in toluene makes the crude cluster (details are in supporting information 1). This was further analyzed with high performance liquid chromatography (HPLC) and the Pt<sub>11</sub> cluster <sup>60</sup> was isolated as a deep yellow solution and was used for further characterization.



Fig. 1. UV/Vis spectrum of the as-synthesized Pt cluster plotted in terms of energy. Jacobian-corrected intensities are plotted. Spectrum shows two humps near 3.13 and 3.98 eV. Inset 'a' shows the luminescence spectral data. Three emission spectra, so collected at the maxima shown by the excitation spectrum are shown. The excitation spectrum was collected for emission at 450 nm, which is the maximum exhibited in all the emission spectra. Insets 'b' and 'c' are the photographs under visible and UV light, respectively.

The UV/Vis of the crude cluster shows (Fig. 1A) two broad bands at 3.13 eV (395 nm) and 3.98 eV (310 nm) with a threshold of absorption at 1.06 eV. The latter one may correspond to the interband (sp-d) transition from metal to thiolate and the former <sup>90</sup> one may be due to intraband (sp-sp) transition.<sup>31</sup> The step-like feature confirms the formation of molecule-like species. It is important to note that unlike in the case of gold and silver nanoparticles, platinum does not exhibit any surface plasmon absorption in the visible region.<sup>29</sup> None of the known clusters of <sup>95</sup> platinum show distinct features.<sup>29-33</sup>

**ARTICLE TYPE** 

The cluster shows blue luminescence (Fig. 1c) under UV light with a quantum yield of 3 x  $10^{-3}$  (details of the QY calculation is given in supporting information) which is quite low compared to the dendrimer encapsulated blue emitting <sup>5</sup> cluster, proposed to be Pt<sub>5</sub>(MAA)<sub>8</sub> (MAA = mercaptoacetic acid), reported by Tanaka *et al.*<sup>29</sup> Three excitation features were observed for our cluster at 358, 275 and 399 nm (Fig. 1a). All of them give raise to the same emission feature at 450 nm.

The as-synthesized crude cluster was run through a HPLC 10 system with normal phase column (Fig. S2A, ESI<sup>+</sup>). Toluene was used as the mobile phase with 1 mL flow rate in isocratic condition and a UV/Vis detector was set for this experiment. Three peaks were observed (Fig. S2A, ESI<sup>†</sup>); among them, only the first one (marked as 1), eluted at 6.3 min retention time, 15 shows the UV/Vis pattern nearly identical to the spectrum of the crude cluster (Fig. S2B, ESI<sup>+</sup>). The blue luminescence and emission features (data not shown) were also identical. The other two peaks (marked 2 and 3) did not show any UV/Vis features (Fig. S3, ESI<sup>†</sup>). Individual components were collected and used 20 for further characterization. The last two components did not show any MALDI MS feature (Fig. S3, ESI<sup>+</sup>), which are likely to be decomposed thiolates. SEM/EDAX (Fig. S4, ESI<sup>+</sup>) was used to know the elemental ratio in these samples. Both of them have the same atomic ratio of Pt and S (1:4) and they form needle-like <sup>25</sup> microcrystals which suggest the possibility of PtL<sub>4</sub> type thiolates. As we are interested in the cluster with molecular features we progressed further only with this species.



Fig. 2. MALDI MS data of the purified cluster. Molecular peak at m/z 3600 was observed at threshold laser power. Inset shows the ESI mass spectrum of the purified cluster. CsOAc was used as ionization enhancer. The red sticks show the calculated spectra <sup>35</sup> for the corresponding composition.

The purified cluster eluted at 6.3 min. in HPLC was subjected to MALDI MS studies. Here, trans-2-[3-(4-tert-Butylphenyl)-2methyl-2-propenylidene]malononitrile (DCTB) was used as the <sup>40</sup> matrix as it is known to give a better resolved mass spectrum.<sup>24, <sup>34, 36</sup>, <sup>37</sup> The spectrum shows (Fig. 2) a prominent peak centered at m/z 3600 ± 50. Compared to the Au<sub>25</sub>(PET)<sub>18</sub> cluster, the FWHM of Pt cluster is broad. This is mainly because of its isotopes (<sup>192</sup>Pt, <sup>194</sup>Pt, <sup>195</sup>Pt, <sup>196</sup>Pt, <sup>198</sup>Pt) whereas gold has only one (<sup>197</sup>Au). So, in <sup>45</sup> comparison to gold the FWHM of Pt cluster will be significantly large. However, it is obvious that the observed peak width is not</sup>

only due to the isotope width alone, there are other factors such as fragmentation. Laser intensity dependence of the spectrum was studied (Fig. S5A, ESI<sup>†</sup>). From our previous study,<sup>24</sup> we know 50 that increase in laser power gives fragmentation which may be used to understand the structure of the monolayer protected cluster. Increase in laser power (from 1840 to 2640, in instrument units) results in an additional broad hump around m/z 7200±100 and a sharp peak at ~ m/z 2150 (Fig. S5A, ESI<sup>+</sup>). Further 55 increase in laser power does not show any change in the spectrum. The broad shoulder at m/z 7200 may be due to the dimer generated at higher laser power and the sharp peak at m/z 2150 may correspond to the metallic core or the fragmented species. The cluster species may also contain weakly bound 60 ligands which may have desorbed during desorption-ionization. As mass spectra of platinum clusters are not available in the literature, we compare the MALDI MS data with that of the Ag<sub>44</sub> system (Fig. S5B, ESI<sup>+</sup>) which is also broad. The data presented suggest the number of ligands present in the cluster may be 8 ((3600-2150)/179 = 8.10) and the core may contain 11 (2150/196)

= 10.96) Pt atoms.

ESI MS analysis was carried out to know the composition precisely. For this experiment, an external ionizing agent, caesium acetate (CsOAc) was used, as the ligand is completely 70 non-polar. Toluene-methanol mixture (1:1) was used as a solvent and the spectrum was collected in the positive mode. The ESI MS shows a very clean spectrum with a peak at m/z 3730 along with some fragmented peaks in the lower mass region (Fig. 2 and Fig. S6, ESI<sup>†</sup>). Fragmentation does occur in elctrospray conditions 75 and which has been observed for many clusters.<sup>23-24</sup> This is mainly because of the high stability of the specific fragments under the ionizing conditions. The fragments form even in softer conditions. Spectra under various conditions are plotted in Fig. S7, ESI<sup>†</sup>. The peak at m/z 3730 was assigned to  ${}_{80}$  [Pt<sub>11</sub>(BBS)<sub>8</sub>Cs(H<sub>2</sub>O)]<sup>+</sup>. The attachment of Cs and H<sub>2</sub>O to a cluster may be surprising, but similar attachments have been observed in clusters.<sup>35, 37, 38</sup> Attachment of  $Cs^+$  is common to neutral species in secondary ion mass spectrometry (SIMS).<sup>39</sup> The experimental spectrum matches exactly with the calculated spectrum 85 (compared in inset of Fig. 2). It is important to note that corresponding gold analogue with a composition of Au<sub>11</sub>(PPh<sub>3</sub>)<sub>8</sub>Cl<sub>3</sub> is reported, although these two may have complexly different structures.<sup>40, 41</sup> No chlorine incorporation was seen in EDAX or in other measurements. In the lower mass 90 region, the intense peak at m/z 1791 is due to a stable fragment of composition, Pt<sub>3</sub>(BBS)<sub>6</sub>Cs (Fig. S6, ESI<sup>†</sup>). The isotope distribution is matching with the corresponding calculated spectrum. Next to the stable fragment, a less intense peak at m/z 1836 is assigned to Pt<sub>3</sub>(BBS)<sub>7</sub>, again supported by the calculated 95 spectrum. We did not observe any multiply charged species in the spectrum. MS/MS was also tried but nothing significant came out from this analysis.

TEM image shows the presence of clusters and they appear as tiny dots with an average diameter of 0.7 nm (Fig. S8, ESI<sup>†</sup>). The narrow size distribution also suggests the monodispersity of the clusters. Another important aspect is that the clusters are sensitive to the electron beam. With longer time irradiation, they aggregate themselves to form bigger particles. Dynamic light scattering (DLS) confirms the monodispersity of the product. A very narrow size distribution curve was seen with an average particle size of 1.5 nm (Fig. S9, ESI<sup>†</sup>). We note that the hydrodynamic diameter of the cluster includes the ligand shell as well. The SEM/EDAX (Fig. S10, ESI<sup>†</sup>) also supports the composition where the Pt:S ratio was 1:0.737 (expected ratio is 1: 0.727). The IR spectra of Pt<sub>11</sub> cluster and BBSH thiol are given in Fig. S11, ESI<sup>†</sup>. Absence of S-H stretching at 2587 cm<sup>-1</sup> suggests the binding mode of thiol as thiolate (RS<sup>-</sup>). Thermogravimteric analysis (Fig. 3A) showed 39.91% mass loss (calculated, 40.02%) which further supports the composition. X-ray photoelectron spectroscopy (XPS) was s carried out to confirm the valence state of the elements. Survey spectrum (Fig. S12, ESI<sup>+</sup>) shows the presence of expected

- elements such as C 1s, Pt 3d, Pt 4f, Pt 4p, Pt 4s and S 2p. Pt:S ratio was found as 1: 0.729. The expanded spectrum in the Pt 4f region shows (Fig. 3B) two peaks at 71.9 eV and 75.3 eV  $_{10}$  (corresponding to  $4f_{7/2}$  and  $4f_{5/2}$ , respectively), corresponding to
- <sup>10</sup> (corresponding to 41<sub>7/2</sub> and 41<sub>5/2</sub>, respectively), corresponding to the zero valent state of Pt.<sup>31</sup> C and S show peaks at the expected positions (Fig. S13, ESI<sup>†</sup>). S 2p peak at 162.4 eV confirms the binding of thiol in the thiolate form. Powder X-ray diffraction (PXRD) analysis (Fig. S14, ESI<sup>†</sup>) was carried for the cluster in the 20 range of 10,00°. The nettern shows bread differentian peaks.
- ts the 2 $\theta$  range of 10-90°. The pattern shows broad diffraction peaks centered around 39°, 45° and 66°. Nanoclusters having moleculelike properties are small to contain a periodic lattice in them and so they do not show sharp peaks as seen for noble metal nanoparticles.



Fig. 3. TG analysis of  $Pt_{11}(BBS)_8$  cluster (A). Extended XPS (B) <sup>35</sup> and <sup>195</sup>Pt NMR (C) of  $Pt_{11}$  cluster. For NMR measurement  $K_2PtCl_6$  was taken as the standard.

<sup>195</sup>Pt NMR studies were conducted on the HPLC purified cluster to understand structural details about the cluster (experimental <sup>40</sup> details are presented in S1). Fig. S15, ESI<sup>†</sup> shows the spectrum of K<sub>2</sub>PtCl<sub>6</sub> standard, which is used for this purpose in similar studies.<sup>42</sup> Note that <sup>195</sup>Pt is having only a natural abundance of

- 33.7 % with a wide range of chemical shifts which cover about 15000 ppm, ranging from +11840 ppm ( $[PtF_6]^{2-}$ ) to -3000 ppm <sup>45</sup> (Pt(0) complexes). This makes it very difficult in studying such NMR unless one knows about the possible chemical shift for the
- peaks. It is difficult to extract information also because no such report about such clusters exists in the literature.  $K_2PtCl_6$  was taken as the standard in our measurements which shows a line at
- <sup>50</sup> 0.0008 ppm. Negative shift was seen (Fig. 3C) in the spectrum of the cluster as expected for a Pt(0) cluster.<sup>43</sup> Two peaks appearing at -2509 and -2534.5 ppm, respectively (Fig. 3C) suggest the possibility of two distinct environments in the cluster. For the Au<sub>11</sub> system, two kinds of distinct environments exist.<sup>44</sup> The <sup>55</sup> intensity ratio of the peaks is 6:5 (taking the area of the peaks),

which is distinctly different from 8:3, expected if 8 ligands are directly connected to the Pt atoms. The observed intensity ratio suggests that there are bridging ligands as in the case of gold clusters.

The purified cluster is stable for 10 days in ambient conditions (Fig. S16A, ESI<sup>†</sup>). Gradually it degrades to form thiolates. The cluster is compatible with many solvents (Fig. S16B, ESI<sup>†</sup>). Efforts are being made to crystallize the cluster.

In summary, undecaplatinum cluster was synthesized <sup>65</sup> successfully through a solid state route. The crude cluster was purified using HPLC. It shows a blue luminescence with a quantum yield of 3 x 10<sup>-3</sup>, comparable to that of gold clusters. Precise composition of Pt<sub>11</sub>(BBS)<sub>8</sub> was determined from MALDI MS and ESI MS analyses. Several other studies support <sup>70</sup> the results. Atomically precise and soluble platinum clusters of this kind may be useful for homogeneous organic catalysis. Use of other ligands and adsorption on proper supports may enhance their stability.

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#### Notes and references

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<sup>†</sup> Electronic Supplementary Information (ESI) available: [Details of experimental procedures, instrumentation, chromatogram of the crude cluster; SEM/EDAX, DLS, PXRD, TEM, FT-IR, and <sup>90</sup> XPS of the isolated Pt<sub>11</sub> cluster; UV/Vis, MALDI MS and

- <sup>90</sup> XPS of the isolated  $Pt_{11}$  cluster, UV/V18, MALDI MS and SEM/EDAX of isolated 2 and 3; and <sup>195</sup>Pt NMR of K<sub>2</sub>PtCl<sub>6</sub> standard.] See DOI: 10.1039/b000000x/.
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Supporting information for the paper

# **Blue Emitting Undecaplatinum Cluster**

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#### Materials and methods:

#### 1. Chemicals

Chloroplatinic acid (H<sub>2</sub>PtCl<sub>6</sub>, 99% Aldrich), sodium borohydride (NaBH<sub>4</sub>, 99.9%, Aldrich); 4-(tert-butyl) benzyl mercaptan (BBSH, 98%, Aldrich); ethanol (Changshu Yangyuan Chemical, China, AR grade) and toluene (Ranken) were used in this synthesis. All the chemicals were commercially available and were used without further purification.

#### 2. Synthesis of Pt<sub>11</sub>(BBS)<sub>8</sub>

The synthesis of Pt cluster protected by BBSH (4-(tert-butyl) benzyl mercaptan) involves the following steps. Initially, 20 mg of H<sub>2</sub>PtCl<sub>6</sub>.6H<sub>2</sub>O and 60  $\mu$ L of 4-(tert-butyl) benzyl mercaptan (BBSH) were ground and mixed well at room temperature in a clean mortar using a pestle. The color changes to orange yellow showing the formation of platinum thiolate. To this mixture, 40 mg of solid NaBH<sub>4</sub> was added and the contents were ground well. The orange yellow color changes to deep brown suggesting the reduction of Pt and formation of Pt clusters. Then 5 mL of ethanol was added to remove the extra thiol and it was centrifuged for 4 minutes at 4000 rpm. Centrifugate was discarded and the precipitate was re-extracted with 7 mL of toluene to get the crude cluster which is deep yellow in color.

#### 3. Instrumentation:

UV-Vis spectra were measured with a Perkin Elmer Lambda 25 spectrometer in the range of 200-1100 nm. Luminescence measurements were carried out on a Jobin Vyon NanoLog instrument. The band passes for excitation and emission were set as 2 nm. HPLC measurement was done using a Shimadzu HPLC system equipped with a normal phase column (Shimadzu) and a UV/Vis detector. Matrix-assisted desorption ionization mass spectrometry (MALDI MS)

studies were conducted using a Voyager-DE PRO Biospectrometry Workstation from Applied Biosystems. DCTB (trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile) was used as matrix (at 1:100 ratio of sample to matrix). A pulsed nitrogen laser of 337 nm was used for the MALDI MS studies. Mass spectra were collected in positive ion mode and were averaged for 200 shots. ESI MS measurements was done one LTQ XL mass spectrometer from Thermo Scientific, San Jose, CA. Methanol-toluene mixture was used for this experiment. CsOAc was used as the external ionizing agent. The spectra were collected in positive mode. High resolution transmission electron microscopy of clusters was carried out with a JEOL 3010 instrument. The samples were drop casted on carbon-coated copper grids and allowed to dry under ambient conditions. Scanning electron microscopic (SEM) and energy dispersive X-ray (EDAX) analyses were performed with a FEI QUANTA-200 SEM. For measurements, samples were drop casted on an indium tin oxide (ITO) coated glass and dried in vacuum. FT-IR spectra were measured with a Perkin Elmer Spectrum One instrument. X-ray photoelectron spectroscopy (XPS) measurements were conducted using an Omicron ESCA Probe spectrometer with polychromatic MgKa X-rays (hu=1253.6 eV). The samples were spotted as drop-cast films on a sample stub. Constant analyzer energy of 20 eV was used for the measurements. DLS was done with a Horiba instrument. Powder XRD patterns of the samples were recorded using PANalytical X'pertPro diffractometer. A thin film of cluster was made on a glass slide which was used for this experiment. <sup>195</sup>Pt NMR measurements were performed using a Bruker (700 MHz) instrument. K<sub>2</sub>PtCl<sub>6</sub> was taken as standard. Whole Pt chemical shift (-3000 to +11800 ppm) range was covered for the Pt<sub>11</sub> cluster to see the peaks. Overnight runs were necessary to observe adequate signals.

#### 4. QY calculation

It has been calculated by taking R<sub>6</sub>G as a reference<sup>1</sup> using the following equation

$$\Phi = \Phi_r \frac{A_r}{I_r} \frac{I}{A}$$

where  $\phi$  is the quantum yield, I is the measured integrated emission intensity, A is the absorbance and the subscript "r" refers to the reference.

Chromatogram and UV/Vis of the crude Pt cluster



**Fig. S2.** Chromatograph of as-synthesized Pt cluster taken with a normal phase column with UV/Vis detector. Well separated cluster (peak 1) was isolated at 6.3 min retention time along with two other features (marked 2 and 3). Isolated (1) shows the same UV/Vis as the as–synthesized cluster (data shown in the inset). Other peaks do not show any characteristic UV/vis features.

MALDI MS and UV/Vis spectra of isolated 2 and 3



**Fig. S3.** MALDI MS (A) and UV/Vis (B) spectra of isolated 2 and 3, respectively. The data suggest that there are decomposed products.

SEM/EDAX of isolated 2 and 3



**Fig. S4.** SEM/EDAX of the isolated 2 (A) and 3 (B), spotted on an ITO plate. Ca, Sn, Si are from the plate. Inset show the corresponding SEM images.

Laser intensity-dependent MALDI MS and comparison with  $Ag_{44}(4-FTP)_{30}$  cluster



Fig. S5. A: Laser power dependency on the  $Pt_{11}(BBS)_8$  cluster. The numbers on the right of the figure are laser intensities shown by the instrument. B: Comparative MALDI mass spectra of  $Pt_{11}(BBS)_8$  and  $Ag_{44}(4$ -FTP)<sub>30</sub> cluster.

Full range ESI MS



**Fig. S6.** Full range ESI mass spectrum of the purified cluster. Inset shows the expanded view of a fragment. The mass spectrum (black) shows nearly an exact match with the calculated spectrum (sticks).

ESI MS of  $Pt_{11}(BBS)_8$  cluster under different ionizing conditions



**Fig. S7.** ESI MS spectra of  $Pt_{11}(BBS)_8$  cluster under different ionizing conditions. In this case, the instrument parameters have been varied to get a maximum intense molecular peak.

#### **Supporting Information 8**

TEM image and size distribution of the  $Pt_{11}(BBS)_8$  cluster



**Fig. S7.** A: HRTEM image of the  $Pt_{11}$  cluster shows tiny particles. Inset shows an expanded view of the selected area. B: Size distribution fitted with an appropriate polynomial. The average diameter was found to be 0.66 nm. Electron beam-induced damage causes an increased particle size distribution.



DLS of the  $Pt_{11}(BBS)_8$  cluster

Fig. S9. DLS correlation (A) and size distribution curve (B) of the cluster.

SEM/EDAX of the  $Pt_{11}(BBS)_8$  cluster



**Fig. S10.** A: SEM/EDAX spectrum of purified cluster showing all the expected elements. Pt:S ratio also supports the composition. Insets are the SEM image (a) and elemental maps of Pt L (b), Pt M (c) and S K (d).

## **Supporting Information 11**

FT IR spectra of the thiol and cluster



**Fig. S11.** The IR spectra of pure BBSH (a) and  $Pt_{11}(BBS)_8$  cluster (b). The S-H stretching frequency at 2580 cm<sup>-1</sup> is absent in cluster which confirms the binding of thiol to the metal.

## **Supporting Information 12**

XPS survey spectrum of the  $Pt_{11}(BBS)_8$  cluster



Fig. S12. XPS survey spectrum of the  $Pt_{11}(BBS)_8$  cluster. All the peaks are marked.

## **Supporting Information 13**

Extended XPS spectra in the C 1s and S 2p regions



**Fig. S13.** Extended XPS spectra for C 1s (A), and S 2p (B) of the purified sample. Each spectrum was fitted with components.





**Fig. S14.** X-ray diffraction patterns of as-synthesized  $Pt_{11}(BBS)_8$  cluster. Positions of metallic platinum are shown for comparison.

**Supporting Information 15** 

<sup>195</sup>Pt NMR of K<sub>2</sub>PtCl<sub>6</sub> standard



Fig. S15. <sup>195</sup>Pt NMR of K<sub>2</sub>PtCl<sub>6</sub> standard (in D<sub>2</sub>O).

Time dependent UV/Vis and solvent compatibility



Fig. S16. Time dependent UV/Vis spectra of  $Pt_{11}$  cluster (A) and UV/Vis spectra of the cluster extracted in different solvents (B).

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# **Coalescence of Atomically Precise Clusters on Graphenic Surfaces**

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**Supporting Information** 

**ABSTRACT:** The interaction of ultrasmall metal clusters with surfaces of graphene is important for developing promising future applications of graphenic materials. In the experiment, chemically synthesized reduced graphene oxide (RGO) in water was mixed with  $Au_{25}SR_{18}$  (where SR,  $SCH_2CH_2Ph$ , is a ligand protecting the cluster core) in tetrahydrofuran, and a completely new cluster, larger in mass, was formed at the liquid—liquid interface. Matrix assisted laser desorption ionization mass spectrometry of the product attached to RGO show that the peak due to  $Au_{25}SR_{18}$  disappears gradually upon reaction and a single sharp peak referred to here as "135  $\pm$  1 kDa cluster" appears. The composition of the new cluster



is very close to the well-known magic cluster,  $Au_{144}SR_{60}$  while the peak maximum is at  $Au_{135}SR_{57}$ . The formation of  $35 \pm 1$  kDa cluster from the parent  $Au_{25}$  is proposed to be governed by the trapping of smaller clusters in a deep potential well generated at the graphene surface. We theoretically model the active role of the surface in stabilizing the large clusters. Our studies indicate a general mechanism of stabilization of clusters of precise size via the competition between the interfacial fluctuations and the energy scales of interaction of the clusters with the surface. The chemical transformation occurs at deformable surfaces at reduced particle densities which is in good agreement with the theoretical model. Transformations of this kind are important in controlled tuning of particles at graphenic surfaces.

#### **1. INTRODUCTION**

Atomically precise ultrasmall clusters have different structural, electronic, magnetic, and catalytic properties compared to their bulk counterparts.<sup>1–5</sup> This makes studies of such clusters a growing area of nanoscience.<sup>6–8</sup> The interactions of ultrasmall metal clusters with graphenic surfaces are both pedagogically and technologically important for their ability to tailor the electronic and magnetic properties of graphene,<sup>9,10</sup> as required for many potential applications.<sup>10–13</sup> Very recently, graphene–metal and graphene–metal nanoparticle composites have become hot topics of research because of their biological, magnetic, electrochemical, optical, and environmental implications.<sup>14–16</sup>

Diffusion,<sup>17,18</sup> migration,<sup>19</sup> bonding,<sup>20</sup> and growth of transition metals on graphene<sup>21</sup> have been studied in detail. The mobility of naked clusters of palladium on graphene surfaces has also been studied.<sup>22</sup> The clusters of noble metals, protected with monolayers, referred to as quantum clusters (QCs), nanomolecules, or super atoms are stable materials with unusual optical, structural and catalytic properties<sup>1,23–25</sup> and such properties are modified by the monolayers.<sup>26</sup> Among the thoroughly studied noble metal clusters are  $Au_{23}L_{16}^{,27}$   $Au_{25}L_{18}^{,28,29}$   $Au_{36}L_{24}^{,30}$   $Au_{38}L_{24}^{,31}$   $Au_{102}L_{44}^{,32}$  and  $Ag_{44}L_{30}^{,2}$  for which crystal structures are known (where L is the ligand protecting the cluster core). Interaction of naked noble metal clusters with supports, for example, magnesia has been well

studied and such supported clusters show unusual properties.<sup>33–35</sup> Molecular dynamics simulations predict that the cluster, Au<sub>140</sub> undergoes Lévy-type power-law flight-length and sticking-time distributions on the basal plane of graphite.<sup>18</sup> However, the interaction of protected clusters or QCs with graphite as well as graphene is yet to be explored. The protecting monolayers are likely to affect the interaction between the graphite substrate and noble metal clusters. In particular, it is of immense interest how the stability and hence, the functionalities of these clusters be tuned over the graphite surface.

In the present work, we study the growth of noble metal clusters at graphenic interfaces. We use the large and atomically thin surface of graphene for the coalescence of  $Au_{25}(SCH_2CH_2Ph)_{18}$  to  $35 \pm 1$  kDa cluster (tentatively assigned as  $Au_{135}(SCH_2CH_2Ph)_{57}$ ), where  $SCH_2CH_2Ph$  is a ligand protecting the cluster core of finite number of gold atoms. We will refer to these molecules as  $Au_{25}$  and  $Au_{135}$  in the subsequent discussion. We observe that the conversion takes place on the graphene surface via a sluggish dynamics, and it depends on both graphene and  $Au_{25}$  concentrations.

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#### 2. EXPERIMENTAL METHODS

**Synthesis of Graphene.** Details about the synthesis of graphene have been presented in the experimental section (Supporting Information S1). Brifely, graphene oxide (GO) was prepared by the modified Hummer's process.<sup>36</sup> Then GO was reduced to reduced graphene oxide (RGO) by hydrazine.

**Synthesis of Au**<sub>25</sub>. We have prepared the parent material Au<sub>25</sub>, by our in-house-developed method. The concept of slow reduction was used to prepare this material. To HAuCl<sub>4</sub> in a methanol-tetrahydrofuran (THF) mixture (100 mg, 25 mM), 3 equiv of phenylethanethiol (SCH<sub>2</sub>CH<sub>2</sub>Ph) was added under stirring at room temperature. After 30 min, 10 equiv of ice cold solution of NaBH<sub>3</sub>CN in 2 mL of cold water was added and the solution was kept stirring at room temperature. Evolution of Au<sub>25</sub> was investigated by time dependent MALDI MS study using DCTB (*trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile) as the matrix. This matrix is important to get clusters without fragmentation as reported previously.<sup>37</sup>

**Reaction of Graphene with the Au<sub>25</sub> Cluster.** About 100  $\mu$ L of as-synthesized Au<sub>25</sub> in tetrahydrofuran (THF) was added to a 3 mL suspension of chemically synthesized graphene<sup>36</sup> (0.01 wt %) in water, which was thoroughly cleaned and contained no impurities. Rapid disappearance of the color of the mixture was noticed and almost simultaneously a black matter suspended at the top. The aqueous phase became colorless. Time dependent MALDI MS study of this black suspended matter was performed. Initial MALDI MS measurement of this matter shows the presence of different cluster masses. After a long time (120 min), for a required amount of cluster and graphene (0.01 wt %), we got a single peak in MALDI MS. In Scheme 1, we show a cartoon representation of this reaction.

Scheme 1. Schematic Representation of the Reaction of  $Au_{25}(SCH_2CH_2Ph)_{18}$  with Aqueous Suspension of Graphene<sup>*a*</sup>



<sup>*a*</sup>A magnetic pellet used for stirring is shown at the bottom of the glass bottle, used for synthesis.

**UV-vis Measurement.** PerkinElmer Lambda 25 UV-vis spectrometer was used for the measurements. Spectra were typically measured in the range of 190–1100 nm.

**MALDI MS Measurement.** The mass spectrometric studies were conducted using a Voyager DE PRO Bio spectrometry Workstation (Applied Biosystems) MALDI TOF MS instrument. A pulsed nitrogen laser of 337 nm was used for desorption ionization and TOF was operated in the delayed extraction mode. Typical delay times employed were of the order of 75–150 ns. The mass spectra were collected in the positive ion mode and were averaged for 200 shots. Most of the measurements were done in the linear mode. To study the evolution of Au<sub>25</sub> by MALDI MS, 1.5  $\mu$ L of the reaction mixture was taken out during its synthesis and mixed with 2.5  $\mu$ L of DCTB matrix, prepared in toluene. The mixture was spotted on the target plate. The plate was left to dry in air and inserted into the spectrometer. It was assumed that after spotting that there was no progress in the reaction.

In the case of the black suspended matter (during Au<sub>25</sub>– graphene reaction), each time, a small amount of the product (graphene and the cluster) was taken out using a 10  $\mu$ L pipet tip. Then the material was mixed with freshly prepared 2.5  $\mu$ L of toluene solution of DCTB. The sample was dried in air and inserted into the spectrometer.

**TEM Measurement.** TEM images were collected using a JEOL 3010 microscope. A diluted solution of  $Au_{25}$  was spotted on carbon coated copper grid and was dried in laboratory ambience. Images were collected at 200 keV. In the case of the black suspended matter, a small amount of it was taken out from the bottle and spotted on the grid. It was dried in laboratory ambience.

#### 3. RESULTS AND DISCUSSION

As  $Au_{25}$  has been characterized in great detail previously<sup>38</sup> by various studies, we present only essential details here. Typically, the samples are characterized by their well-defined optical absorption features.<sup>1</sup> The positions of peak maxima, peak shape, and intensities are specific to  $Au_{25}(SCH_2CH_2Ph)_{18}$  in THF (Figure 1). As the spectrum of  $Au_{25}(SCH_2CH_2Ph)_{18}$  has



**Figure 1.** UV-vis spectrum of the as-synthesized sample. The spectrum matches with the reported spectrum of  $Au_{25}(SCH_2CH_2Ph)_{18}$ .<sup>1</sup> Inset: MALDI MS spectrum of the sample in the positive ion mode. The peak at m/z 7391 confims the formation of  $Au_{25}(SCH_2CH_2Ph)_{18}$ . The fragmented product,  $Au_{21}(SCH_2CH_2Ph)_{14}^+$  is shown with an asterisk (\*).

been discussed thoroughly, we merely note that the spectrum is comparable to the published data.<sup>1,37,38</sup> The purity of the sample is proven by its unique MALDI MS spectrum (inset of Figure 1), showing a narrow ( $\Delta m = 15$ ) peak at 7391 due to Au<sub>25</sub>(SCH<sub>2</sub>CH<sub>2</sub>Ph)<sub>18</sub><sup>+</sup> along with its characteristic fragmentation product at m/z 6055 due to Au<sub>21</sub>(SCH<sub>2</sub>CH<sub>2</sub>Ph)<sub>14</sub><sup>+</sup>, as mentioned previously.<sup>37</sup> The data were collected at threshold laser intensity to reduce fragmentation. Formation of Au<sub>25</sub> was completed after 36 h of stirring (Figure S2). Chemically synthesized graphene samples were characterized in the solution phase by optical absorption spectroscopy and in the drop-cast form by transmission electron microscopy (data to be presented later). MALDI MS of the black suspended material formed by the reaction between  $Au_{25}$  and graphene is shown in Figure 2. This



**Figure 2.** (a–d) Time dependent positive ion MALDI MS study of the conversion of Au<sub>25</sub>. At zero time (a) a peak corresponding to pure Au<sub>25</sub> (main peak at m/z 7391 and a fragment at m/z 6055) is seen. The fragment is shown with an asterisk (\*). The main peak is expanded on the top. After 15 min, mixtures of clusters, including Au<sub>135</sub> were formed (b). With increasing time, the peak of Au<sub>135</sub> increases and peaks corresponding to lower m/z decrease in intensity (c). (d) Spectrum shows that Au<sub>135</sub> has been formed after 120 min for a mixture of 0.01 wt % graphene (3 mL) and 27  $\mu$ M Au<sub>25</sub> (100  $\mu$ L). Dication (2+) of Au<sub>135</sub> is seen in MALDI MS, marked on trace (d). Insets: (i) The presence of two phases of the reaction mixture, cluster (Au<sub>25</sub>) and graphene; and (ii) the final product of Au<sub>135</sub> on the graphene surface.

transformation shows a gradual evolution of a peak at m/z 34.4 kDa assigned to Au<sub>135</sub>(SCH<sub>2</sub>CH<sub>2</sub>Ph)<sub>57</sub><sup>+</sup> along with the simultaneous disappearance of the peak due to Au<sub>25</sub>(SCH<sub>2</sub>CH<sub>2</sub>Ph)<sub>18</sub><sup>+</sup>. The assignment of

Au<sub>135</sub>(SCH<sub>2</sub>CH<sub>2</sub>Ph)<sub>57</sub><sup>+</sup> may have a slight uncertainty as other Au/SCH<sub>2</sub>CH<sub>2</sub>Ph combinations are possible (see below). We have seen a dication, assigned to  $[Au_{135}(SCH_2CH_2Ph)_{57}]^{2+}$  at m/z 17.2 kDa, which further supports the assignment. Occurrence of dication is a standard feature seen in MALDI MS of such large clusters.<sup>39</sup> However, as mass measurements at this mass range have some inaccuracy, it is safe to describe these clusters as "35 ± 1 kDa clusters".

One may wonder whether the new mass peak at 34.4 kDa is an adduct of Au<sub>25</sub>(SCH<sub>2</sub>CH<sub>2</sub>Ph)<sub>18</sub> with graphene as such ultrathin sheets are expected to wrap around a cluster. This possibility was investigated. Graphene sheets prepared are of different sizes (to be seen below from the TEM data) and obviously the adduct peaks derived from them should be composed of multiple maxima, reflecting the polydispersity of graphene in a given preparation. Also at higher laser powers, such peaks are expected to fragment, giving carbon or carbon cluster losses which were not observed. Multiple preparations of graphenes produced the same peak. These made us conclude that graphene is not part of the mass peak. Larger width of the peak in comparison to the narrow peak of Au<sub>25</sub> was a concern. To find out whether this is due to the inherent reduction in resolution at higher masses in a liner time-of-flight measurement, the spectra of similar samples at higher masses were compared. MALDI MS spectra of gold clusters of lysozyme (Lyz)<sup>40</sup> and parent Lyz are shown in Figure S3. These comparisons suggest that the observed peak is due to single cluster. The broadness of the feature is due to reduced instrumental resolution at high masses as revealed from the protein mass spectrum (Figure S3).

It is important to point out that MALDI MS was measured at threshold laser powers which result in spectra at no or reduced fragmentation.<sup>37</sup> This complete conversion occurs at a finite time interval (more details are below) after which no  $Au_{25}$  was detected in the solution, at the interface. In between these two times, several features were observed in the lower mass region (m/z 8000–18000) which are attributed to intermediate products. From our time dependent study, we have shown that  $Au_{33}$ ,  $Au_{38}$ ,  $Au_{55}$ , and some other clusters (the ligand shell is neglected in this description) were formed at the lower mass



**Figure 3.** (A) MALDI MS spectra with increasing graphene concentration for a constant  $Au_{25}$  concentration. (a<sub>1</sub>) Data corresponding to  $Au_{25}$  alone. The peak marked \* is due to the fragment,  $Au_{21}L_{14}^+$ . (a<sub>2</sub>) Data at lower concentration (0.005 wt %) of graphene where complete conversion of  $Au_{25}$  to  $Au_{135}$  has not taken place. It shows some peaks at lower mass. (a<sub>3</sub>) At higher concentration (0.01 wt %) of graphene, complete conversion to  $Au_{135}$  has happened. Both the spectra were collected after 180 min of mixing the reactants. Dication of  $Au_{135}$  is marked on the above figure. (B) Time dependent conversion of  $Au_{25}$  for a fixed graphene concentration at different  $Au_{25}$  concentrations. I<sub>P</sub> and I<sub>R</sub> represent the intensity of  $Au_{135}$  and  $Au_{25}$  in the MALDI MS spectra. Traces  $b_{1y}$   $b_{2y}$  and  $b_3$  represent final concentrations of  $Au_{25}$  in solutions (3.17, 1.68, and 0.87  $\mu$ M, respectively). Statistics refer to data from multiple measurements. (C) A plot of slope vs surface concentration derived from panel B.



**Figure 4.** (A) TEM image of chemically synthesized graphene alone. The nanometer thin folding (marked) indicates that the sheets imaged contain two-three layers of chemically synthesized graphene. (B) Image of graphene surface containing clusters ( $Au_{135}$ ). Some clusters are marked with circles. Outside the graphene surface, there was no cluster. It proves that the conversion happened only on graphene surfaces. Number of folding has decreased significantly in panel B.

region during the conversion process (Figure S4). This confirms that the transformation is nonstoichiometric. The product ion,  $Au_{135}(SCH_2CH_2Ph)_{57}^+$  has much larger width than  $Au_{25}(SCH_2CH_2Ph)_{18}^+$  due to distribution in ligand composition as well as reduced resolution at the higher mass range. Due to this reason, the exact composition of the product may have some variation. A stable cluster known in this mass range is  $Au_{144}(SCH_2CH_2Ph)_{60}$ . Actual mass of  $Au_{144}(SCH_2CH_2Ph)_{60}$  is 36.5 kDa as measured by electrospray ionization mass spectrometry (ESI MS). But MALDI MS for the product shows a peak around ~34 kDa.<sup>41</sup> It is likely that  $Au_{144}(SCH_2CH_2Ph)_{60}$  with some fragmentation is observed here. Although the exact composition of the product is uncertain, we note that only one product was seen.

The transformation is sensitive to both concentration of the cluster and graphene. For a given cluster concentration, increasing graphene content converts all the clusters to  $Au_{135}$ . As shown in Figure 3A, at a lower graphene concentration (0.005 wt %, trace  $a_2$ ),  $Au_{25}$  is observed even after 48 h of reaction. At higher concentration (0.01 wt %, trace  $a_3$ ) of graphene, conversion to  $Au_{135}$  takes place where the  $Au_{25}$  peak is not any more visible. Further, upon increasing cluster concentration, for a fixed graphene concentration (0.01 wt %), we see reduced conversion (Figure 3B). While at lower cluster concentration all the clusters convert to  $Au_{135}$ , at increasing cluster concentration, more clusters remain without conversion. This reduced efficiency of conversion continued even upon a longer reaction time.

While the transformed clusters are less susceptible for electron beam-induced aggregation, the parent  $Au_{25}$  is extremely sensitive to electron beam-induced aggregation and undergoes rapid coalescence (Figure S5). Smaller clusters such as  $Au_{25}$  grow in size with exposure to electron beam.<sup>42</sup> The molecular nature of  $Au_{135}$  is evident in the mass spectrum which exhibits a well-defined peak assigned to a specific composition. From the MALDI MS study, we have seen that  $Au_{135}$  is resistant toward fragmentation upon increasing laser intensity used to perform desorption-ionization (Figure S6). Generally, clusters show severe laser intensity-dependent fragmentation in which more of lower mass ions are observed at increasing laser fluence. The increased stability observed is due to the fact that the clusters are attached to the graphene surface which efficiently removes the excitation energy.

The slope of the curves in Figure 3B gives the rate of conversion to Au135 at different times. The rate is rapid at initial times, slows down gradually, and finally becomes zero at very large times. The low time values of the rate of conversion,  $k_i$ have been plotted as a function of the Au<sub>25</sub> surface concentration, c, on the graphene surface (Figure 3C). The best fitted line shows that  $k = \lambda/c_i$ , the dynamical information being incorporated in  $\lambda$  which has the dimension  $[L^2T]^{-1}$ . Such dynamical quantity is dimensionally consistent with mean squared displacement (MSD)  $\langle r^2 \rangle \propto t^{-1}$  over the surface as a function of time, t. Such MSD can be found if the particle motion is in a trapping potential of strength  $V_0$  in the presence of strong damping  $\Gamma$ . The mean squared fluctuations from the trapping center would decay as  $[1 + (V_0/\Gamma) t]^{-1}$ . Clearly the formation of Au135 clusters from the parent Au25 clusters is governed by the trapping of the smaller clusters in a deep potential well generated by the graphene surface.

Figure 4 compares the TEM images of chemically stable graphene and gold cluster-nucleated graphene. The size of  $Au_{135}$  is in the 2 nm range which agrees with the observed particle sizes, which are the expected sizes of clusters of this range.<sup>39</sup> Number of folds of the sheets has reduced after the reaction (Figure 4B). As the clusters are strongly adherent to the graphene surface, our efforts to separate  $Au_{135}$  in solution for additional examination was unsuccessful.

The energy gain due to reduction of the surface curvature may be the main drive to trap the smaller clusters, leading to their coalescence, as suggested by the concentration dependence of the conversion rate. In the following we present a general mechanism of stabilization of clusters of precise size via the competition between the interfacial fluctuations and the energy scales of their interaction with the surface. Let us assume that the small clusters are driven by the curvature. The smaller clusters thus experience a chemical potential proportional to the local curvature on the surface. Let  $h(\vec{r})$  be the height of the surface and  $\rho(\vec{r})$  be the density of the smaller clusters on the surface at a point  $\vec{r}$ . The chemical potential experienced by the smaller clusters is then  $\mu(\vec{r}) = \lambda \nabla^2 h(\vec{r}), \lambda$ being the coupling parameter. We use the density functional free energy for a strongly interacting classical system,<sup>43</sup> consisting of the smaller clusters and the surface. The Gibbs free energy of the system consists of several components: The entropy of the smaller clusters given by  $\int d\vec{r}\rho(\vec{r}) \ln(\vec{r})$ ; the entropy of height fluctuations of the surface  $\int d\vec{r}h(\vec{r}) \ln h(\vec{r})$ ;

the contributions due to correlated changes in density of the smaller clusters and in the surface height, given by -(1/2) $d\vec{q}c(\vec{q})|\delta\rho(\vec{q})|^2$  and  $-(1/2)\int d\vec{q}\alpha(\vec{q})|\delta h(\vec{q})|^2$ , respectively. The wave vector modes  $\delta \rho(\vec{q})$  and  $\delta h(\vec{q})$  indicate heterogeneity with respect to the mean density and average surface height, while  $c(\vec{q})$  and  $\alpha(\vec{q})$  denote their correlations, respectively. Finally we add the contributions due to the chemical potential coupling between the smaller clusters and the surface, resulting in  $\int \mu(\vec{r}) \delta \rho(\vec{r}) d\vec{r}$ . The net free energy F of the system is given by the sum of these contributions. The equilibrium heterogeneity of the structure spontaneously supported by the system is given by the simultaneous conditions  $\partial F/\partial \rho(\vec{q}) =$  $\partial F/\partial h(\vec{q}) = 0$ . We consider the standard Ornstein–Zernike form for the correlation functions:<sup>43</sup>  $c(q) = c_0/(q^2 + \xi_0^2), \xi_0$ being the particle correlation length and  $c_0$  related to the inverse of the bulk compressibility; and similarly  $\alpha(\vec{q}) = \alpha_0/q^2 + \alpha_0/q^2$  $\xi_s^2$ ;  $\xi_0$  being the correlation length of the surface height fluctuations and  $\alpha_0$  given by the inverse of surface compressibility. For large  $\lambda$ ,  $L \approx q^{-1} \propto \lambda \xi_0 / c_0^{1/2}$ , for a given concentration of graphene. At very low particle densities,  $c \approx 1$ , the correlation length typically extends to a few particle diameters so that L is about a few nanometers so far as  $\lambda$  is a finite number. L compares well to the size of Au<sub>135</sub> clusters. The theory of interacting fluids shows<sup>43</sup> that  $c_0 \approx -\infty$  for a high density incompressible fluid and  $c_0$  decreases with decreasing particle density. Thus, lower density of the particles facilitates the formation of bigger clusters which is qualitatively supported by the experimental observations. On the other hand,  $\lambda$  can be taken to increase with increasing graphene concentration so that the stabilization of large clusters would be favored, as found in the experiments.

Our phenomenological model hints at the most dominant factors underlying the experimental observations. This also indicates what aspects should be looked into in ab initio calculations. We must add here that the dynamics of the system can be captured only within Carr–Parinello type ab initio calculations which are too expensive to carry out, unless the specific aspects of the phenomenon are known from simplistic calculations. We know that the system under discussion is not a metal particle alone; it has a molecular shell. Nevertheless, it is a particle on a deformable surface. The dynamics of such a complex system in its simplest form is considered here.

#### 4. CONCLUSION

In summary, we established the coalescence of clusters of finite size to form a larger cluster at graphenic interfaces. In particular, the role of the interface has been ascertained by both experiments and model theoretical calculations in stabilizing the large clusters. Our methodology with diverse clusters and high surface area substrates will allow the creation of atomically precise clusters directly on such supports. Extension of this study to fullerene and carbon nanotubes may be useful to produce new clusters. Such materials are expected to have novel applications via controlled tuning of the structural and electronic properties of both the deposited clusters and the support surfaces.

#### ASSOCIATED CONTENT

#### **S** Supporting Information

Figure S1–S6 are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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#### Notes

The authors declare no competing financial interests.

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## Supporting information for the paper:

# **Coalescence of Atomically Precise Clusters on Graphenic Surfaces**

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#### S1. Supporting information

#### **Experimental Section**

**Synthesis of graphene:** Graphene has been prepared by the modified Hummer's process followed by reduction of GO to RGO.<sup>1</sup> Initially, 1 g of graphite powder (purchased from R. K. Scientific) was taken in a round bottle flask. To that, 12 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was added and the mixture was kept at 90 °C for an hour. 2 g of  $K_2S_2O_8$  and 2 g of  $P_2O_5$  were added under stirring condition. The mixture was kept at the same temperature for 6 h for pre-oxidation of graphite. Then the mixture was cooled to room temperature and filtered. The filtrate was discarded and the pre-oxidized GO was kept for drying in hot air overnight. About 24 mL of concentrated  $H_2SO_4$  was added to this pre-oxidized GO and kept at ice cold condition. Then 3 g of KMnO<sub>4</sub> was added slowly with constant stirring and allowed to stand for 6 h. Then 400 mL of distilled water was added slowly under stirring condition and kept at room temperature for an hour. To stop the reaction, 5 mL of 20% H<sub>2</sub>O<sub>2</sub> was added to it and kept undisturbed overnight. The bright yellow precipitate confirmed the formation of GO. The solution was decanted and the precipitate was collected. It was washed with 1% HCl for three times. This solution was centrifuged to collect GO and then dried in vacuum for 24 h. About 1 g of dried GO was weighed and re-dispersed in 500 mL of water such that the concentration of the GO solution was 0.5 wt %. Unwanted ions were removed from the solution by dialysis. This GO solution was used as the stock solution for the preparation of graphene. About 100 mL of this GO (0.1 wt %) solution was taken in a round bottom flask. To that 700 µL of ammonia (25%) and 65 µL of hydrazine (35%) was added and the solution was kept at 80 °C for two hours. The solution was cooled to room temperature. Then it was centrifuged and washed with distilled water for two times. The ppt was dispersed in requisite amount of water so that final concentration is 0.01 wt %.

## **S2.** Supporting information

Evolution of Au<sub>25</sub>(PET)<sub>18</sub> - MALDI MS study



*Figure S2.* Time dependent MALDI MS data during the synthesis of  $Au_{25}PET_{18}$ . It shows that the formation of  $Au_{25}$  takes 36 hours. (Where PET, SCH<sub>2</sub>CH<sub>2</sub>Ph, is a ligand protecting the cluster core)
#### **S3.** Supporting information

Comparison of the MALDI MS spectra of PET protected gold cluster [Au<sub>135</sub>(PET)<sub>57</sub>] with native lysozyme (Lyz) and lysozyme-gold cluster (Au@Lyz)



*Figure S3.* Comparison of the spectra of PET protected gold cluster  $[Au_{135}(PET)_{57}]$  with native lysozyme (Lyz) and lysozyme-gold cluster (Au@Lyz). The peak that is shifted from the native protein in Au@Lyz is due to the Au cluster nucleated within the protein.  $2M_L$ ,  $3M_L$ , are dimer, trimer, etc. of the protein and the corresponding cluster features.  $M_L$  also shows a dication  $(M_L^{2+})$  feature. Lysozyme and Au@Lysozyme data have been published by Ananya *et. al.*<sup>2</sup>

#### **S4.** Supporting information



MALDI MS spectrum of the intermediate product and its laser flux-dependent study

*Figure S4.* (A) MALDI MS spectrum of the intermediate product shows that  $Au_{33}$ ,  $Au_{38}$ ,  $Au_{55}$  have been formed during the conversion process of  $Au_{25}$  to  $Au_{135}$ . (B) Laser flux-dependent study of the intermediate product.

#### **S5.** Supporting information



TEM, effect of electron beam irradiation on Au<sub>25</sub> and graphene-supported Au<sub>135</sub>

*Figure S5.* (A) and (A1) TEM images of  $Au_{25}$  clusters before and after electron beam irradiation. Size of  $Au_{25}$  grows fast upon electronbeam irradiation. A1 was collected after 10 minutes of irradiation. (B) and (B1) Same data for supported  $Au_{135}$  clusters. Some clusters are marked with circles. Size of the graphene-supported  $Au_{135}$  clusters do not change upon electron beam irradiation.

#### **S6.** Supporting information



Laser flux-dependent study of Au<sub>25</sub> and graphene-supported Au<sub>135</sub>

*Figure 6.* (A) Laser flux-dependent study of  $Au_{25}$  showing that as the laser intensity increases, intensity of the parent peak ( $Au_{25}$ ) decreases and fragmented peak ( $Au_{21}$ ) increases. At very high laser power, the parent peak has almost vanished. (B) Laser flux study-dependent of graphene-supported  $Au_{135}$  shows that with the increase of laser intensity, peak broadening occurs with a peak at lower mass, due to fragmentation. Inset: Change of dication with the increase of laser intensity.

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### Synthesis of Atomically Precise Silver Clusters by Using the Miscibility Principle

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Keywords: Cluster compounds / Phase diagrams / Silver / S ligands / Solvent effects

A new strategy to synthesize a diverse array of organic-soluble, atomically precise silver clusters has been developed. The technique is based on the miscibility principle of solvents and uses no phase-transfer agents; various clusters of masses 8.0, 13.4, 22.8, 29.2, and 34.4 kDa were synthesized by changing the reactant composition. We have also synthesized the well-known  $Au_{25}(SR)_{18}$  cluster by the same method. Among the silver clusters formed, we have studied the new

#### Introduction

Among the emerging categories of nanosystems are the atomically precise clusters of noble metals protected with monolayers, which are referred to as quantum clusters (QCs), nanomolecules, clusters, or super atoms. The interesting structural, optical, and electronic properties of QCs<sup>[1]</sup> as well as their potential applications in catalysis,<sup>[2]</sup> biomedicine,<sup>[3]</sup> and nanoelectronics<sup>[4]</sup> have made these systems important for chemistry and materials science as a whole. The systematic size evolution of these clusters to bulk materials allows the investigation of the emergence of size-dependent properties.

Although the two-phase method for the synthesis of noble metal nanoparticles has been highly effective,<sup>[5]</sup> the materials synthesized by this route contain the phase-transfer agent (PTR, discussed in the Supporting Information), which is a persistent impurity in detailed analysis owing to the interdigitation of monolayers.<sup>[6]</sup> Although methods that avoid PTRs exist, they have not produced a large variety of materials, especially for silver, and the materials are most often polydisperse.<sup>[7]</sup> The solubility of the nanosystems formed in the organic phase and the requirement of reducing agents in the aqueous phase lead to reduction at the interface, and the products formed phase-separate naturally. However, a careful control of the composition of a threecomponent system can also lead to phase separation, which

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13.4 kDa species, which has unique steplike features in its UV/Vis spectrum, in detail by mass spectrometry and other analytical techniques. The compound has been assigned as  $Ag_{68}(SR)_{34}$ , which is reported for the first time. By time-dependent studies, we have shown that the synthetic route follows the bottom-up approach. The material forms micro-crystals. We hope that the proposed synthetic strategy will extend the area of atomically precise clusters.

can be conveniently used for reduction, cluster growth, and isolation of products without the use of a PTR. A threecomponent system that exhibits two well-defined phases can be converted to one phase by the precise control of the phase compositions. This control can result in the transfer of solutes from one phase to the other. In that process, a chemical reaction may occur, and the products can be separated without the use of additional chemicals. For atomically precise clusters of noble metals, the most rapidly expanding family of noble metal nanosystems, water is an essential ingredient of the reaction as most of the reduction is performed by NaBH<sub>4</sub> or its variants.<sup>[5b,7b,8]</sup> The transfer of metal ions into a suitable organic solvent or mixture and reduction by the nascent hydrogen liberated in a homogeneous phase can eventually lead to clusters, which phaseseparate at the newly formed phase boundary. This phase transfer can be controlled with suitable pairs of solvents and, thus, the use of a PTR can be avoided completely. Here, we introduce this versatile strategy for the synthesis of an array of atomically precise clusters. Although several clusters are known, mostly of gold, new synthetic methodologies are required to realize many of the unknown clusters, especially those of silver.

#### **Results and Discussion**

The phase diagram of the three-component system used for the experiments is shown in Figure 1 (A). Multiple solvent compositions were used for cluster synthesis, as indicated in the phase diagram. As shown in the photographs (Figure 1, B), the syntheses yielded different clusters in each of these solvent compositions with the same quantities of reagents (more details of the procedure are provided in the Supporting Information). The MALDI MS and UV/Vis

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spectra of the products formed (Figure S2) confirm the formation of different cluster cores. At each of the solvent compositions, different clusters can also be formed by varying the reactant ratios. In this communication, we introduce this method by describing one of the new clusters, namely,  $Ag_{68}SBB_{34}$  (SBB = the thiolate form of BBSH, 4-*tert*-butylbenzyl mercaptan), in some detail. This corresponds to the solvent composition marked "d" in the phase diagram (Figure 1).



Figure 1. (A) Phase diagram of the three-component (water, methanol, and toluene) system in which the synthesis was performed. The four different regions in the phase diagram at which the reactions were performed are indicated. (B) Photographs of the reaction products. Different clusters were formed under each of these conditions.

The miscibility principle may be illustrated with a specific example close to region "d" in Figure 1 (A). Initially, methanol (3 mL) was used (Figure S3A), and toluene (3 mL) was added; this mixture formed a single phase as they are completely miscible (Figure S3B). Next, water (2 mL) was added to this homogeneous phase. As water is completely miscible with methanol and partly miscible with toluene, water/methanol formed a phase and separated from the toluene phase (Figure S3C). In Figure S3D, toluene is replaced by chloroform (3 mL). As the chloroformrich phase is denser than water/methanol, the former moved to the bottom when water was added. To extend this phase separation to cluster synthesis, we performed the reduction of Ag<sup>I</sup>SBB thiolates in a three-component (water/toluene/ methanol) system. Initially, AgNO<sub>3</sub> (20 mg, 0.12 mmol) was dissolved in methanol (3 mL). To this solution, toluene (3 mL) containing BBSH (132 µL, 0.72 mmol) was added; this resulted in a color change from colorless to turbid yellow-white owing to the formation of Ag<sup>I</sup>SBB thiolates (Fig-

ure 2,  $a_1$ ). At this point, toluene and methanol are miscible, and the mixture appears as a uniform phase. The solution changes to brown upon the addition of ice cold water (2 mL) containing NaBH<sub>4</sub> (45 mg, 1.20 mmol) with constant stirring; the color change indicates the reduction of Ag<sup>1</sup>SBB (Figure 2, a<sub>2</sub>). Upon standing for 10 min, the final result is the separation of two distinct phases. The bottom phase is a water/methanol mixture and is colorless, and the top dark brown portion contains the cluster in a toluenerich phase (Figure 2,  $a_3$ ). When we used chloroform instead of toluene, the bottom phase contained the cluster (Figure 2,  $a_4$ ). The phase boundaries can be seen clearly owing to the extraction of the clusters into these phases. Nearly pure clusters were collected from the top layer in Figure 2  $(a_3)$ , whereas impurities such as thiolates and metal ions were collected in the bottom phase (Figure S4a). The only possible impurity along with cluster is excess thiol (Figure S4b). The clusters were precipitated by the addition of excess methanol, which also removes the thiol present in the sample. The precipitate was washed four to five times with methanol, and the cluster powder was obtained by rotary evaporation. The synthetic method is highly robust and can be reproduced easily. It does not strongly depend upon conditions such as temperature or the purity of the materials, unlike typical cluster syntheses. We performed the reaction during the prevailing summer (ca. 40 °C) and winter (ca. 25 °C) conditions in Chennai and also used normal and dry solvents. We confirmed the identity of the products by UV/Vis spectroscopy and matrix-assisted laser desorption



Figure 2. The UV/Vis spectrum of the as-synthesized cluster. The spectrum has steplike features, which are characteristic of small clusters. The arrows indicate the peak positions. (a) Photograph of the synthetic process: (a<sub>1</sub>) Ag and thiol in methanol/toluene mixture (single phase), (a<sub>2</sub>) after the addition of NaBH<sub>4</sub>, (a<sub>3</sub>) 10 min after stopping the reaction (two well-separated phases with the cluster at the top), and (a<sub>4</sub>) separated phases with chloroform instead of toluene, the cluster is in the bottom phase. A magnetic pellet is at the bottom of each of the test tubes. (b) Single peak in the HPLC chromatogram of the crude product, which confirms the formation of a single cluster. (c) TEM image of the purified cluster. The approximate core size of the cluster is 1.2 nm. Some clusters are marked with circles.

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ionization mass spectrometry (MALDI MS). These data are discussed below. The purity is estimated to be ca. 95% on the basis of the MALDI MS data. Similar results were obtained by replacing toluene with CHCl<sub>3</sub>. All of the results presented here are for the clusters obtained from the water/ toluene/methanol system. In Figure S5, we have shown the importance of the solvent mixture. Larger quantities of the cluster were prepared by scaling up the synthesis (the details are in Supporting Information). The precipitate was washed with methanol and then dissolved in toluene for the various measurements.

The UV/Vis spectrum of the crude cluster in toluene is shown in Figure 2. Two well-defined steps are seen, as is observed for atomically precise clusters.<sup>[9]</sup> The purity of the sample is the most important issue in this type of synthesis. We have performed high-performance liquid chromatography (HPLC) of the sample with tetrahydrofuran/MeOH (THF/MeOH, 70:30) as the mobile phase (Figure 2, b) to check the purity of the sample. Only one peak was seen in the HPLC chromatogram, and the chromatographed sample has the same UV/Vis spectrum as that of the crude product (Figure 2). In this experiment, a C18 column  $(250 \times 4.6 \text{ mm})$  was used with a flow rate of 1 mL/min under isocratic conditions (the details are in the Supporting Information). These results confirm the formation of a single cluster,<sup>[10]</sup> which was identified as Ag<sub>68</sub>SBB<sub>34</sub> from studies presented below. The optical spectrum shows moleculelike transitions at 555 and 665 nm, unlike those of plasmonic nanoparticles. These peaks are redshifted in comparison with those of the analogous Au<sub>68</sub>SR<sub>34</sub>.<sup>[11]</sup> The TEM image of the cluster is shown in Figure 2 (c). The cluster core size is nearly 1.2 nm. In Figure S7, we have shown that the cluster is stable towards electron beam irradiation, unlike other atomically precise clusters such as  $Au_{18}$  and Au<sub>25</sub>.<sup>[8,12]</sup>

Detailed mass analyses were conducted by MALDI MS and laser desorption ionization (LDI) MS to determine the composition of the cluster. For several of the monolayerprotected gold and silver clusters, the exact compositions were determined by MALDI MS.<sup>[10a,10c,11,13]</sup> The LDI MS and MALDI MS analyses show that the cluster is highly pure as no peaks other than those of interest were found. The MALDI MS of the sample was recorded with trans-2-[3-(4-tertbutylphenyl)-2-methyl-2-propenylidene]malononitrile (popularly known as DCTB) as the matrix. The cluster gave a single peak at m/z = 13.4 kDa in the MALDI MS spectrum (Figure 3). No other peaks were observed in a wide range from m/z = 15 to 100 kDa. It is important to point out that the spectrum was measured at the threshold laser power, which results in spectra with no or reduced fragmentation.<sup>[11]</sup>

We assigned the peak to a cluster of composition  $Ag_{68}SBB_{34}$ . To confirm this assignment, we have performed LDI MS studies of the sample. In MALDI MS measurements, we can obtain the true mass of the cluster. However, during the LDI MS measurements, the laser can cleave the C–S bonds upon ionization. Instead of  $Ag_mSBB_n$ , we can obtain the mass spectrum corresponding to the  $Ag_mS_n$  core,



Figure 3. MALDI MS spectrum of the cluster, which shows a single peak at m/z = 13.4 kDa. (a) Representation of MALDI and LDI MS. The MALDI MS shows peaks of  $Ag_mL_n$  (L is Ligand), whereas LDI MS shows peaks of the  $Ag_mS_n$  core (LDI breaks the C–S bonds). (b) Expanded MALDI MS (red trace) and LDI MS (gray trace) spectra of the cluster. The red trace was fitted with a Gaussian function, which is shown in blue. The mass difference between the center of the Gaussian curve fitted for  $Ag_{68}(SBB)_{34}$  cluster and the peak position from where  $Ag_{25}$  loss started is 5 kDa. Inset:  $Ag_{25}$  loss in the MALDI MS spectrum, which begins at ca. 8.4 kDa. This overlaps with the mass of the  $Ag_{68}S_{34}$  core. (c) The formation of the  $Ag_mS_n$  core is confirmed by the successive loss of  $Ag_{25}$  molecules (m/z = 248) in the LDI MS spectrum, shown as a series.

in addition to systematic fragmentations. The C-S bond cleavage can also happen during MALDI but it occurs at increased intensity only with higher laser powers. A representation of the observed MALDI and LDI processes is shown in Figure 3 (a). The MALDI MS and LDI MS spectra are compared in Figure S8 and show a mass difference of ca. 5 kDa. This mass loss supports the presence of 34 ligands on the cluster  $[147 \times 34 = 4998; 147$  is the mass of the ligand after C-S cleavage)]. The expanded Gaussianfitted MALDI MS and LDI MS spectra for the cluster are shown in Figure 3 (b). The mass difference between the center of the Ag<sub>68</sub>(SBB)<sub>34</sub> peak and the position from which Ag<sub>2</sub>S loss started is 5 kDa. The highest mass at which Ag<sub>2</sub>S loss is clearly seen in the MALDI MS spectrum is ca. 8.4 kDa, which corresponds to the  $Ag_{68}S_{34}$  core. The formation of the  $Ag_mS_n$  core is further confirmed from the series of silver sulfide peaks spaced at m/z = 248, which are due to the fragmentation of the parent  $Ag_mS_n$  core (Figure 3, c).

The thermogravimetric (TG) analysis of  $Ag_{68}(SBB)_{34}$  under a nitrogen atmosphere displayed a sharp mass loss of 44.8% in the 110–500 °C window (Figure S9). The observed

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mass loss matches the organic weight fraction (45.0%) expected for Ag<sub>68</sub>(SBB)<sub>34</sub>. The SEM images with elemental mapping and the energy-dispersive X-ray spectroscopy (EDAX) spectrum are shown in Figure S10. In SEM/ EDAX, the Ag/S ratio seen was uniform throughout the sample and almost matched the calculated value for Ag<sub>68</sub>(SBB)<sub>34</sub> (1:0.51). The absence of a sodium peak (remnant of the reducing agent) indicated that the sample is highly pure. The SEM image of the material after crystallization is shown in Figure S11. Although microcrystals were seen, they were not large enough for single-crystal studies. The ligation of BBSH in the form of thiolate (SBB) attached to the Ag core was confirmed by the absence of an S-H stretching peak at 2562 cm<sup>-1</sup> in the Fourier transform infrared (FTIR) spectrum of the cluster (Figure S12). The existence of clusters was confirmed by the X-ray diffraction (XRD) pattern of the sample, which showed a broad peak centered at  $2\theta \approx 38^\circ$ . The spectrum was compared with the characteristic face-centered cubic (fcc) diffractions exhibited by 15 nm diameter metallic Ag@H<sub>2</sub>MSA (H<sub>2</sub>MSA: mercaptosuccinic acid) nanoparticles (Figure S13).<sup>[14]</sup> Several silver clusters show only broad features.<sup>[7b,10d,15]</sup> A broad peak at  $2\theta \approx 35^\circ$  was also observed for gold clusters.<sup>[10e,16]</sup> The nature of the metal and monolayer binding were confirmed by X-ray photoelectron spectroscopy (XPS). The XPS survey spectrum of the as-synthesized cluster is shown in Figure S14A. Only the expected elements are present; therefore, this also shows that the sample is very pure. The Ag atoms are almost in the zero oxidation state (Figure S14B). The S 2p<sub>3/2</sub> binding energy (BE) of 163.2 eV is characteristic of the thiolate ligand, which supports the IR data (Figure S14C). The BE is calibrated with respect to that of C 1s at 284.5 eV.

For any synthesis, it is always important to know the synthetic route. To gain some understanding of the synthesis process, we have performed time-dependent mass and UV/Vis analyses. Such UV/Vis spectra showed that the cluster formation is complete within 120 min (insets of Figure 4). A broad peak at 550 nm appeared initially at 15 min. A shoulder peak at 665 nm started to appear at 60 min and is more prominent at 120 min. The absence of further changes at 180 and 240 min (Figure S15) confirmed the conclusion of the reaction. The time-dependent MALDI MS spectrum after 15 min of reaction shows a single peak corresponding to 10.3 kDa for the cluster (Figure 4). At 60 min, the presence of a larger cluster at m/z = 11.5 kDa is seen. Finally, at 120 min, the sample shows a dominant peak at 13.4 kDa. As discussed earlier, in the LDI process, the C-S bond cleavage occurs, which will give information about the  $Ag_m S_n$  core. We also performed LDI for the 10.3, 11.5, and 13.4 kDa clusters to understand their core masses. As the reaction time increases, the core mass and mass loss increase, which implies that the number of silver atoms and ligands increase with time. In the LDI experiment, the formation of  $Ag_m S_n$  was confirmed by the observation of a series of silver sulfide peaks owing to the fragmentation of the parent  $Ag_m S_n$  core. This data supports that the cluster evolution follows a bottom-up approach. The yield of the

Ag<sub>68</sub> synthesis depends on the solvent (toluene or chloroform) used. For toluene, the yield was ca. 55%, whereas the yield was ca. 60-65% for chloroform.



Figure 4. Time-dependent mass spectra of the cluster during synthesis. The black traces represent the MALDI MS spectra, and the red traces represent the LDI MS spectra. The corresponding UV/ Vis spectra are shown in the insets.

By using the same process, we have also synthesized several silver clusters of masses 8.0, 22.8, 29.2, and 34.4 kDa. The UV/Vis spectra and MALDI MS spectra of these assynthesized clusters are shown in Figure S16. We have assigned those peaks as  $Ag_{=38}L_{=24}$ ,  $Ag_{=130}L_{=52}$ ,  $Ag_{=170}L_{=64}$ , and  $Ag_{=202}L_{=70}$  (L is *p-tert*-butylbenzenethiol). The larger spectral width and poor resolution at larger mass numbers made the assignments imprecise. However, for gold clusters, especially  $Au_{25}SBB_{18}$  synthesized by this method, the spectrum was well-defined (Figure S17). It may be noted that silver cluster peaks are inherently broader as it has two stable isotopes (107 and 109), whereas gold has only one natural isotope (197).

#### Conclusions

We have developed a new strategy for the synthesis of a diverse array of clusters without a PTR, which is a persistent impurity for the previously reported two-phase methods. This process will help to create a series of monodisperse organic-soluble metal clusters. In particular, we have characterized a new cluster,  $Ag_{68}SBB_{34}$ , by using different analytical tools. The product is crystalline. We expect that the proposed synthetic strategy and the cluster synthesized will help to extend the area of atomically precise clusters.

#### **Experimental Section**

Only the synthesis and characterization of  $Ag_{68}$  are discussed here. More details are provided in the Supporting Information. Silver nitrate (20 mg) was dissolved in methanol (3 mL). To that solution, toluene (3 mL) was added, and the solution was constantly stirred. Date: 28-07-14 11:53:50

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BBSH (ca. 132  $\mu$ L) was then added. The solution changed from yellow to turbid yellow-white owing to the formation of AgISBB thiolates. After 30 min of stirring, ice cold water (2 mL) containing sodium borohydride (45 mg) was added to the solution with stirring. The solution changed to dark brown. Upon standing for 10 min, two distinct phases separated. The top phase contained the cluster in toluene. The toluene solution was used for the measurement of the time-dependent UV/Vis and MALDI MS spectra. The time-dependent study showed that the reaction was complete within 120 min. After 120 min, the toluene phase containing the cluster was separated and dried by rotary evaporation. Then, the dried material was washed four to five times with methanol to remove excess thiol and other impurities. HPLC of this cleaned sample was performed with THF/MeOH (70:30) as the mobile phase. This confirmed the presence of a single cluster. From the MALDI and LDI MS study, we assigned the material as Ag<sub>68</sub>SBB<sub>38</sub>. The composition was further confirmed by thermogravimetric (TG) and EDAX analyses.

**Chemicals:** Silver nitrate (AgNO<sub>3</sub>, AR grade) was purchased from RANKEM, India. Methanol (AR grade), toluene (AR grade), and chloroform (AR grade) were purchased from R. K. Scientific, India. 4-*tert*-Butylbenzenethiol (97%), 4-*tert*-butylbenzyl mercaptan, *trans*-2-[3-(4-tertbutylphenyl)-2-methyl-2-propenylidene]malono-nitrile (DCTB, 98%) matrix, and sodium borohydride (NaBH<sub>4</sub>, 95%) were purchased from Sigma–Aldrich.

Instrumentation: Details are provided in the Supporting Information.

**Supporting Information** (see footnote on the first page of this article): MALDI MS, LDI MS, UV/Vis, and EDAX spectra; TEM images; SEM images; TGA curve; and XRD pattern.

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#### **Silver Clusters**

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/KAP1

Synthesis of Atomically Precise Silver Clusters by Using the Miscibility Principle

Keywords: Cluster compounds / Phase diagrams / Silver / S ligands / Solvent effects



Silver and gold clusters have been synthesized by using the miscibility principle of solvents. Three solvents, that is, water, methanol, and toluene (or chloroform) have been used to synthesize different silver clusters by this method without phasetransfer agents. Separate regions of the phase diagram (a, b, c, and d) produce distinctly different clusters from the same reactant compositions. Eur. J. Inorg. Chem. 2014 · © WILEY-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, 2014 · ISSN 1099–0682

#### **SUPPORTING INFORMATION**

<u>**DOI**</u>: 10.1002/ejic.201402587 <u>**Title**</u>: Synthesis of Atomically Precise Silver Clusters by Using the Miscibility Principle <u>**Author(s)**</u>: Atanu Ghosh, Thalappil Pradeep\*

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## S1. Supporting information

**Phase transfer agent:** Phase transfer agent (PTR) is a catalyst which transfers reactant from one phase to another phase where a process occurs. Tetraoctylammoniumbromide (TOABR), one such PTR, has been widely used in gold nanoparticle synthesis which transfers gold ions from the aqueous to the organic phase, to carry out reactions in the organic medium. To transfer 30 mL 30 mM HAuCl<sub>4</sub> from water to toluene phase, 80 mL 50 mM TOABR is needed (M. Brust, M. Walker, D. Bethell, D. J. Schiffrin, and R. Whyman, *J. Chem. Soc., Chem. Commun.*801, 1994). It has been well-established that PTR is a persistent impurity in monolayer protected nanoparticles and it affects the properties of the monolayers (Mukhopadhyay, R.; Mitra, S.; Johnson, M.; Rajeev Kumar, V. R.; Pradeep, T. *Phys. Rev. B* 2007, 75, 075414; Kumar, V. R. R.; Mukhopadhyay, R.; Pradeep, T. *J. Chem. Sci. (Bangalore, India)* 2008, *120*, 537).

**Experimental section:** Compositions used in Figure 1: First we dissolved 20 mg of silver nitrtate in a fixed quanity of methanol (see below for the volumes used for various points in the figure). To that, a fixed quanity of toluene and 132  $\mu$ L BBSH (4-tert-Butylbenzyl mercaptan) were added under stirring. The color of the solution changes colorless to yellowish white. After 30 min, we added a definite volume of ice cold water containing 45 mg of NaBH<sub>4</sub> under stirring. We continued stirring for 90-120 min. This ended up with two phases, toluene rich and water rich phases. Then small amount of the sample from the toluene rich phase was taken out and MALDI MS was taken using DCTB (trans-2-[3-(4-tertbutylphenyl)-2-methyl-2-propenylidene] malononitrile) as the matrix. UV-vis spectrum of the sample was measured using toluene as the solvent. In case of points a, b, c and d, we have done the reaction at the same condition and reactant compositions but varied the solvent compositions. Below we have shown the solvent percentages (mole fraction) used at respective points in Figure 1.

Point	Methanol	Water	Toluene
а	20%	65%	15%
	1.82 mL	2.63 mL	3.55 mL
b	65%	15%	20%
	4.19 mL	0.45 mL	3.36 mL
С	15%	20%	65%
	0.63 mL	0.37 mL	6.97 mL
d	35%	52%	13%
	3.00 mL	2.00 mL	3.00 mL

## Analytical methods:

**UV-vis measurement:** Perkin Elmer Lambda 25 UV-vis spectrometer was used for the measurements. Spectra were measured typically in the range of 190-1100 nm.

**TEM measurement:** TEM images were collected using a JEOL 3010 microscope. A diluted solution of Ag<sub>68</sub> was spotted on a carbon coated copper grid and was dried in laboratory ambience. Images were collected at 200 keV to reduce beam-induced damage.

**Fourier-transform infrared (FT-IR):** FT-IR spectrum of Ag<sub>68</sub> was measured with a Perkin Elmer Spectrum One instrument. For sample preparation, KBr crystals were used as the matrix.

**SEM and EDAX analyses:** Scanning electron microscopic (SEM) and energy dispersive X-ray (EDAX) analyses were done in a FEI QUANTA- 200 SEM. For measurements, samples were drop-casted on an indium tin oxide coated conducting glass and dried in vacuum. It was died in air and used for analysis.

**HPLC:** HPLC experiments were conducted on a Shimadzu instrument consisting of a CBM-20A controller, DGU-20AR on-line degasser, LC-20AD pump, SIL-20A auto-sampler, CTO-20A column oven, and SPD-M20A photodiode array (PDA) detector. A stainless steel column (250x4.6 mm inner diameter) packed with 5-µm C18 bonded silica with 300-Å pore size (Theromo Scientific) was used as the reverse-phase column. The column temperature was maintained at 25 °C. The absorbance chromatogram was monitored by a PDA detector at 320 nm. The absorption spectra of the eluted peaks were collected over 190–800 nm by the PDA detector.

**MALDI and LDI MS:** The mass spectrometric studies were conducted using a Voyager DE PRO Bio spectrometry Workstation (Applied Biosystems) MALDI TOF MS instrument. A pulsed nitrogen laser of 337 nm was used for desorption ionization and TOF was operated in the delayed extraction mode. Typical delay times employed were of the order of 75–150 ns. The mass spectra were collected in the positive mode and were averaged for 200 shots. Most of the measurements were done in the reflectron mode. To study the time dependent MALDI MS of Ag<sub>68</sub>, each time 1.5  $\mu$ L of the sample was taken from the reaction bottle and mixed with 2.5  $\mu$ L of DCTB matrix which was prepared in toluene. Then the mixture was spotted on the target plate. The plate was left to dry in air and inserted into the spectrometer. It was assumed that after spotting, there was no progress of the reaction. It is important to point out that MALDI MS was measured at threshold laser powers which result in spectra at no or reduced fragmentation (A. Dass, *J. Am. Chem. Soc.* 2008, *130*, 5940). In case of LDI MS, only 2.5  $\mu$ L of the sample was spotted on the plate and dried in air. Then the plate was inserted into the spectrometer.

## S2. Supporting information

MALDI MS and UV-vis spectra of the samples prepared at different solvent compositions



Figure S2. MALDI MS of the samples prepared at different regions of the phase diagram, keeping the same reactant composition. It confirms the formation of different cluster cores. Inset: UV-vis spectra of the samples, in toluene solvent.

## S3. Supporting information Blank experiments with solvents



**Figure S3.** Photographs represent miscibility experiment of solvents. (A) Only 3 ml methanol, (B) mixture of 3 ml methanol and 3 ml toluene, appearing as a single phase and (C) when 2 ml water is added to (B), water-methanol form a phase which is separated from toluene rich phase. (D) If toluene is replaced with chloroform, chloroform rich phase will come down and the methanol rich phase goes up. Phase boundaries are marked.

### S4. Supporting information EDAX spectra of two separated phases

Upon centrifugation, insouble thiolates precipitate in methanol/water part. By this way we have discarded the excess thiolate. Below Figure 1a and 1b are the EDAX spectra of methanol/water and toluene phases, respectively. To prepare the samples for EDAX, first we have separated the two phases. Then we had drop cast the samples on two different conducting glass plates and dried under vacuum. Figures 1a and 1b (below) are the EDAX spectra of methanol/water and toluene phases, respectively. Figure 1a shows that silver and sodium ions are present in methanol/water and sulphur is absent. This means that thiol/thiolate is absent in this phase. Figure 1b shows that higher amount of sulphur i.e thiol is present in toluene phase and sodium ions are almost absent in it.



Figure S4. EDAX spectra of (a) methanol/water and (b) toluene phases, respectively.

## S5. Supporting information Reaction in methanol

Silver nitrate is soluble in methanol but insoluble in toluene. We have performed the same reaction only in methanol. The following figures show the importance of mixed solvents in comparison to methanol alone.



**Figure S5.** (A-C) Photographs for the synthesis done in methanol alone. (A) Ag(I)SBB thiolate in methanol alone. B) After addition of 45 mg 2 mL aq NaBH<sub>4</sub> to Ag(I)SBB thiolate. C) The precipitate in B was dispersed in toluene. D) Synthesis done in methanol-toluene mixture.

## S6. Supporting information Large scale synthesis

In a larger scale synthesis, 160 mg of silver nitrate was dissolved in 24 ml of methanol. To that 24 ml of toluene was added. 800  $\mu$ L of BBSH thiol was added to it under stirring condition. The color of the solution changes to light yellow and then yellowish white. It confirms the formation of Ag(I)SBB thiolate. The solution contained single phase at this stage. After 30 minutes, 16 ml of ice cold solution containing 360 mg of NaBH<sub>4</sub> was added to it under stirring condition. The color changes from light brown to dark brown. Time dependent UV-vis spectra were taken. The reaction took 120 minutes to complete. After 10 minutes of waiting, cluster containing toluene rich phase was separated from the methanol rich phase. It was rotavapored and the product was washed with methanol 3-4 times to get the dark brown powder of Ag<sub>68</sub>SBB<sub>34</sub>.



**Figure S6.** A photograph of the large scale synthesis. Top brown colored toluene rich layer contains the cluster. Bottom methanol rich layer contains the reduction byproducts and other unreacted materials.

## S7. Supporting information TEM, effect of electron beam irradiation



**Figure S7.** (A) TEM image of Ag<sub>68</sub>SBB<sub>14</sub>. (B-D) The cluster upon the irradation with the electron beam. TEM images were taken from the same area after 5, 10 and 15 minutes of irradiation in B, C and D, respectively. Size of the cluster increases due to electron beam irradation. Insets of figure (A) and (D) represent the histrograms of size distribution.

## S8. Supporting information MALDI and LDI MS



Figure 8. i) Schematic illustration of the effect of direct laser irradiation on ligand on the cluster. ii) Comparison of MALDI and LDI MS.

## S9. Supporting information TGA



**Figure S9.** Thermogravimetric analysis under nitrogen atmosphere shows a weight loss of 44.8% which supports the expected value (45%) due to the organic fraction present in the cluster.

# S10. Supporting information SEM/EDAX



**Figure S10.** (A) EDAX spectrum of Ag<sub>68</sub>SBB<sub>34</sub> and (B) SEM image of Ag<sub>68</sub>SBB<sub>34</sub> aggregate from which the EDAX spectrum was taken. Ag:S atomic ratio measured is 1:0.51, as expected (actual is 1:0.50).

## S11. Supporting information SEM image of the crystallites



Figure S11. SEM image of the sample after crystallization.

## S12. Supporting information

## FT-IR



**Figure S12**. Expanded FT-IR of BBSH and Ag<sub>68</sub>SBB<sub>34</sub>; the disappearance of the peak at 2562 cm<sup>-1</sup> (in the spectrum of Ag<sub>68</sub>SBB<sub>34</sub>) indicated that the S-H bond was absent in it. This proved that sulphur is connected to the metal ion in the thiolate form. Inset: An expanded cluster spectrum.

# S13. Supporting information XRD



**Figure S13.** Comparison of the X-ray diffraction patterns (XRD) of the Ag<sub>68</sub>SBB<sub>34</sub> cluster (red) and Ag@H<sub>2</sub>MSA (15 nm) nanoparticle (green).The diffraction peaks are marked.

## S14. Supporting information

XPS



**Figure S14.** (A) XPS survey spectrum of the as synthesized Ag<sub>68</sub>(SBB)<sub>34</sub>. (B), (C) and (D) represent the XPS spectra for Ag 3d, S 2p and C 1s, respectively with multiple component fitting. It shows that Ag is almost in the zero oxidation state.

## S15. Supporting information

Time dependent UV-vis spectra



Figure 15. Time dependent UV-vis spectra for the cluster during synthesis.

## S16. Supporting information Different clusters prepared



**Figure S16.** (A-D) Mass spectra for the four different clusters prepared by this method. Insets show the UV-vis spectra for the corresponding cluster. Here L is 4-tert-butylbenzenethiol. The conditions are listed below.

20 mg AgNO₃	1: 4 thiol (88 μL)	1: 6 thiol (132 μL)
1:5 NaBH <sub>4</sub> (22.5 mg)	8.0 kDa	-
1:7 NaBH <sub>4</sub> (37 mg)	22.8 kDa	-
1:10 NaBH <sub>4</sub> (45 mg)	29.2 kDa	34.4 kDa

## S17. Supporting information



**Figure S17.** UV-vis spectrum of Au<sub>25</sub>(SBB)<sub>18</sub> prepared by the reported method. The spectrum matches with that of a standard sample. Inset: MALDI MS spectrum of Au<sub>25</sub>(SBB)<sub>18</sub> in the negative mode. Fragmented product is marked with an asterisk (\*). It is due to Au<sub>21</sub>(SBB)<sub>14</sub>.

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## Spatiotemporal mapping of three dimensional rotational dynamics of single ultrasmall gold nanorods

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Spatiotemporal mapping of the position and orientation of nano-machinery inside complex and dynamic cellular environments is essential for the detailed understanding of many bio-physical processes. For the genuine observation of such biomolecular dynamics with high signal to noise ratio and reduced disturbance from the labeling probes, reduction in the size of nano-bio labels and simplification of techniques for their observation are important. Here we achieve this using polarized dark field scattering micro-spectroscopy (PDFSMS), in its simplest form so that it is deployable in several experiments. We not only locate tiny gold nanorods (GNRs) of size 30 (length)  $\times$  10 nm (diameter) inside HEK293 cells but also demonstrate mapping of their *in-situ* polarization patterns using a novel method. Real time observations of rotating GNR with DFSMS and PDFSMS are used to resolve in-plane and out-of-plane rotational modes of GNR. We have shown that PDFSMS itself can provide complete information about the state of GNR. A step ahead, we demonstrate the application of PDFSMS to track three dimensional rotational dynamics of transferrin-conjugated GNRs inside live HEK293 cells. These first-time observations of the three dimensional intracellular rotational dynamics of tiny GNRs using PDFSMS present a new landmark in single particle scattering spectroscopy.

evelopment of techniques to study nano-bio interactions at a single particle level is of great importance to understand the complexity of biological functions<sup>1-3</sup>. In these studies, it is important to monitor spatial as well as temporal behavior of systems of interest. Although there has been extensive use of gold nanoparticles (GNPs) in biomedical applications<sup>4</sup> and studies at nano-bio interfaces<sup>5</sup>, when it comes to monitoring orientational and rotational dynamics of molecules, gold nanorods (GNRs) are better nano-bio labels<sup>6</sup> due to their anisotropic thermal<sup>7</sup> and optical properties<sup>8,9</sup>. Different experimental methods have used these properties of GNRs to image and track single molecule orientation *in vitro* and *in vivo*<sup>6,10</sup>. Different techniques involve defocused or dual wavelength dark field imaging, two photon luminescence imaging or dark field imaging through bifringent crystal etc<sup>11-14</sup>. Differential interference contrast (DIC) imaging technique has been developed for single particle orientation and rotational tracking (SPORT) of GNR-labeled motor proteins and intracellular cargos in live cells<sup>10,15-22</sup>.

While there have been a few observations of single GNRs by optical techniques, the dimensions of the particles were above 60–75 nm (L)  $\times$  20–40 nm (D) for *in vivo* observations<sup>15–19</sup>. However, full potential of such single particle studies is realized when size of the probe is smaller than the system under observation. Smaller probes have lesser influence on cellular processes and allow observation of genuine dynamics. For example, observations of the rotational dynamics involved in functions of FoF1-ATP synthase<sup>6</sup> and F1-ATPase<sup>23</sup> were done using probes such as 77 (L)  $\times$  39 (D) nm GNRs and 1,000–2,600 (L)  $\times$  10 (D) nm actin filaments, respectively while size of these proteins is in the range of  $\sim$ 10 nm<sup>24,25</sup>. Even smaller probes would enable better understanding of the dynamics. Rotational dynamics of plasmonic particles below 40 nm have never been observed in cellular systems, although orientations have been discerned from the samples deposited on a substrate<sup>11,12,26</sup>. In the present work, we present polarized dark field scattering micro-spectroscopy (PDFSMS) of small GNRs of 30 (L)  $\times$  10 (D) nm (labeled GNR<sub>30X10</sub> later) in complex cellular environments. Use of ultrasmall GNRs and VNIR light source instead of a laser source reduces the possibility of heating and minimizes the effect of probe itself on the system under observation<sup>13,14,27-29</sup>. Unlike previous studies, we have not used any confocal set-up or complex arrangement for polarized illumination or collection<sup>11-14,27-29</sup>. With a regular dark field set-up equipped with linear

polarizer (analyzer), we not only determine orientation of GNRs but also map their *in-situ* polarization patterns (referred as polar maps). We also discuss the effect of cellular environment on the changing polarizability and scattering spectrum of GNR. Real time observations of rotating GNRs with DFSMS and PDFSMS have also shown that in-plane and out-of-plane rotational modes of GNR can be resolved using simple dark field microscopy. The observations on three dimensional movements of GNRs of the kind reported here has not been done so far. We have provided novel methods to map *in-situ* polar patterns of GNRs and resolve their rotational modes. Hence PDFSMS alone can provide complete information about the state of GNR such as three dimensional motion, orientation, rotational modes and whether it is an aggregate or single GNR. To the best of our knowledge, these are first time observations of three dimensional ultrasmall nanoparticle dynamics with simpler instrumentation.

#### Results

Set-up and principle of mapping the *in-situ* polar maps of GNRs. The key to achieve successful mapping of polarization patterns is to utilize the refraction of light rays when they pass through an analyzer with minute angular tilt. As shown in Fig. 1a, a regular dark field setup was used for these measurements. Magnified view of the set-up in Fig. 1b explains the principle of *in-situ* polar mapping. Due to the intrinsic polarizability of GNR, scattered light has a major component polarized along the longitudinal axis of GNR. When this light passes through an analyzer, a minute angle in the mounting of the analyzer gives rise to refraction which causes the image to be shifted by a few pixels in the direction of the analyzer axis. This results in the mapping of a filled circle for scattering sources with no polarizability, as in the case of spherical GNP (Fig. 2b and supporting information (SI) Video S1). But for GNRs, circular mapping is modulated by variations in the scattering intensity (Fig. 2a and Video S2). Size distribution for the same is shown in (SI Fig. S1). Fig. 2b shows dark field images (b1, b2) of a single spherical GNP and an anisotropic GNP captured at different

angles of the analyzer. As all GNPs are not perfectly spherical in a typical synthesis, such collection of particles can always be seen in a sample. Unlike  $GNR_{30X10}$ , in this case, the aspect ratio is less with larger diameter as suggested by the scattering spectrum (Fig. S2). On rotation of the analyzer, paths tracked by both these particles shown in Fig. 2b (b3) follow a circle. Corresponding polar maps, calculated by SI Equation 1, are shown in Fig. 2b (b4). Comparison of polar plots calculated by SI Equation 2 and polar maps prove that the particle orientation is reproduced sincerely (Fig. S2). Here polar plots provide angular distribution of scattering intensity integrated over particle spot (in image) area. But polar maps provide visualization of how these changes occur in space. Polar mapping is sensitive to even smaller deviations in the shape of the nanostructure from an isotropic sphere. Hence it works also for GNPs which are not exactly spherical (Fig. S3, S4). For such GNPs, value of polarization anisotropy (P, calculated by SI Equation 3) was  $\sim$ 0.4 whereas for GNRs it was  $\sim$ 1.0. A transmission electron microscopic (TEM) image of GNR<sub>30x10</sub> used in this study is shown in Fig. 1c, along with a high resolution image of one particle.

Figure 2c shows the lamp spectrum with maximum in the region where LSPR (longitudinal surface plasmon resonance) of  $GNR_{30X10}$  occurs (check Fig. S5 for DDA simulated spectrum). This helps in reducing the noisy background from the cellular components which scatter mostly in the blue-green region (Fig. S6). This facilitates thorough hyperspectral analysis of the changes in the scattering spectra of GNR which are discussed in greater detail in the next section.

Spectroscopic identification of single GNPs, GNRs and GNR aggregates *in vitro* and *in vivo*. It is well known that SPR (surface plasmon resonance) of nanostructures is very sensitive to the changes in the surrounding environment<sup>8</sup>. Hence before proceeding to *in vivo* mapping measurements, we have established a method to identify distinct scattering features of small GNRs in different environments. GNP<sub>40</sub> are included in this comparison because GNR<sub>30X10</sub> aggregates and GNP<sub>40</sub> look almost similar in their dark



Figure 1 | *In-situ* polarization pattern mapping was achieved by displacement of the image caused by a tilted analyzer. (a) Schematic illustration of the experimental set-up used for *in-situ* polar mapping of GNRs. (b) Principle of polar mapping is explained in this ray diagram. Briefly, this is possible due to minute angular tilt ( $\delta\phi$ ) in the mounting of the analyzer which causes refraction of the polarized light scattered by GNR. Figures are not to scale. (c) A transmission electron microscopic (TEM) image of the GNR<sub>30x10</sub> is shown along with a high resolution image of the tip of one GNR (inset).



**Figure 2** | **Imaging, spectroscopy and polar mapping of single GNRs.** (a) Dark field images of a single  $GNR_{30X10}$  captured at different angles of the analyzer. Corresponding variations in the scattering intensity are shown in the form of a polar plot. (b) Dark field images of a single spherical GNP and a single anisotropic GNP at different angles of analyzer are shown in b<sub>1</sub> and b<sub>2</sub>. Only difference between the images is the analyzer orientation, shown by the double headed arrow, ' $\uparrow$ ' for 0 degree (b<sub>1</sub>) and ' $\leftrightarrow$ ' for 90 degrees (b<sub>2</sub>). Path tracked by both these particles are shown in the image b<sub>3</sub> and corresponding polar maps determined by SI Equation 1 are shown in the image b<sub>4</sub>. (c) Scattering spectra of a single GNR and an aggregate of GNRs formed upon interaction with bovine serum albumin (BSA) are shown along with the spectrum of halogen lamp used for illuminating the sample.

field images but can be distinguished only on the basis of their spectral features as explained later. Figure 3 shows the consolidated data obtained after statistical analysis of the single particle SPR peak positions of GNP40, GNR30X10 and GNR30X10 aggregates in vitro and in vivo. Examples of spectra corresponding to each SPR peak distribution are given in Fig. S7 and Gaussian curve fits for their peak positions are given in Fig. S8. Figures 3a & 3b show optical dark field images of GNP40 and GNR30X10. Although the volume of  $GNR_{30X10}$  is much smaller than that of  $GNP_{40}$  (~1/15 of  $GNP_{40}$ ), GNP<sub>40</sub> has peak distribution from 500-600 nm but GNR<sub>30X10</sub> has a distribution of SPR peaks from 600-800 nm (Fig. 3e). Gold nanorods exhibit this property due to aspect ratio dependence of their SPR. It is expected that cetyltrimethylammonium bromide (CTAB)-stabilized particles (although cleaned) aggregate on cell membrane either due to receptor clustering<sup>30</sup> or due to interaction with proteins in cell culture medium<sup>31</sup>. Hence to monitor changes in the scattering signal of GNR<sub>30X10</sub> after aggregation or clustering, we have done analysis of GNR<sub>30X10</sub> samples incubated with BSA (GNR@BSA) and compared their SPR peak distribution with the scattering signals obtained from GNR<sub>30X10</sub> treated HEK293 cells. TEM images and UV-Vis absorption spectra of GNR@BSA samples are shown in Fig. S9 and S10, respectively. Figure 3e shows the SPR distribution of GNR@BSA. The various SPR peaks are labeled as shown in Fig. 3e. We see that those scattering spectra of GNR@BSA have only one SPR peak (labeled, GNR@BSA:S1P1); their peak distribution is centered at  $\sim$ 740 nm with small fraction of peaks at  $\sim$ 530 nm (see their relative area). However, many scattering spectra of GNR@BSA have two SPR peaks; first one centered at ~530 nm (labeled, GNR@BSA:S2P1) and the other one centered at ~730 nm (labeled, GNR@BSA:S2P2). To confirm the presence of two SPRs, we have simulated the scattering spectrum of an aggregate of six GNR<sub>30X10</sub> as shown in Fig. 3f & 3g. We see that although GNR<sub>30X10</sub> exhibits only one prominent SPR, an aggregate exhibits two strong SPRs. The possible reason behind this is the plasmonic coupling between multiple GNR<sub>30X10</sub> which enhances the TSPR (transverse surface plasmon resonance) of GNR<sub>30X10</sub> and closely packed GNRs in an aggregate tend to behave as a single particle equivalent to a GNR of reduced aspect ratio. This would give equally prominent TSPR and LSPR. As a result of this plasmonic coupling, a red shift in LSPR of GNR@BSA can also be observed. GNP40:S1P1 is red shifted as compared to GNR@ BSA:S2P1 (Fig. 3e); possibly due to smaller diameter of GNR<sub>30X10</sub>. Ensemble UV-Vis absorption spectra also show that SPR for GNP<sub>40</sub> occurs at 532 nm, whereas TSPR of GNR<sub>30X10</sub> occurs at 514 nm (Fig. S11). It suggests the possibility that major component of GNR@ BSA:S1P1 arises due to chains or chain-shaped aggregates of GNR<sub>30X10</sub>. It is known from previous studies that zwitterionic molecules such as small peptides present in cellular environment may give rise to chain-shaped aggregates of GNRs<sup>32</sup>. Observation of two prominent peaks was also supported by the results obtained by analyzing hyperspectral image of HEK293 cells treated with GNRs (GNR@ HEK293). It was observed that the distribution of GNR@ HEK293:S2P1 and GNR@HEK293:S2P2 matches with the distribution of GNR@BSA:S2P1 and GNR@BSA:S2P2, except that there is a small increase in the baseline for GNR@HEK293 scattering spectra in the region around 600 nm (Fig. S6). Increase in the baseline can be due the background scattering from surrounding cellular components. It should be noted that, although appearance of two prominent SPR peaks in experimental results matches the simulated data, this observation cannot be generalized. This is because GNR aggregation is hard to control in complex biological environments and many different geometries or combinations can exist inside cells. Hence on the basis of these observations, we can not only locate aggregates of small GNRs in complex cellular environment but also differentiate between an intact GNR and an aggregate based on the number and position of SPR peaks. It should be emphasized that although GNP<sub>40</sub> and GNR@BSA look almost similar in optical dark field images, they can be distinguished clearly using their spectra. In the next section we compare the polar mapping and effect of various environments on the polarizability of GNR<sub>30X10</sub>.

Effect of various environments on the polarizability of GNR<sub>30X10</sub>. To check the applicability of polar mapping in different environments, multiple observations were made. Figure 4a and 4b show the polar plots and corresponding maps for a single GNP<sub>40</sub> and a single GNR<sub>30X10</sub>, respectively. For GNP<sub>40</sub> with small polarization anisotropy, the mapped pattern is just a filled circle but for GNR<sub>30X10</sub>, it exhibits a filled 'figure 8' pattern. In Fig. 4b, 4c and 4d we can see how the scattering spectra, polar plots and polar maps of GNR<sub>30X10</sub> change sequentially from the free state, during interaction with HEK293 cell membrane and after entering the cell. Scattering spectrum for GNR<sub>30X10</sub> on HEK293 cell surface (Fig. 4c) suggests that the GNR is at an initial stage of clustering or aggregation. It should be noted that, although due to diffraction limit of optical microscopy, an aggregate appears like a single particle; it can be very well distinguished by its spectroscopic features. It can be seen that polar plot and map exhibited by GNR<sub>30X10</sub> aggregate inside the cell is similar to an aggregate of GNR<sub>30X10</sub> treated with BSA (Fig. 4e & 4e). Figure S12 provides an example of such a mapping





Figure 3 | Spectroscopic identification of single GNPs, GNRs and GNR aggregates *in vitro* and *in vivo*. Dark field images of various samples at  $100 \times$  magnification. (a) GNPs of 40 nm diameter (GNP<sub>40</sub>). (b) GNR<sub>30X10</sub> (c) GNR<sub>30X10</sub> aggregates obtained by treating it with BSA. (d) GNR<sub>30X10</sub> aggregates in HEK293 cells. (e) Consolidated data obtained from the Gaussian curve fitting of the statistical distributions of single particle SPR peaks of the samples (a-d). In labels, 'S' stands for the total number of SPR peaks in the scattering spectrum and 'P' stands for a serial number of SPR peak when it is counted from lower wavelength to higher wavelength. Area of each of the Gaussian peak represents the number of pixels in the hyperspectral image whose scattering maximum falls within its base width. (f) Surface images of the model dipole arrays generated for DDA simulations of samples (a-d). Please note the relative size of these nanostructures. (g) Corresponding DDA simulated scattering cross sections of nanostructures on unpolarized white light excitation. Color coding is given such that simulated spectra can be compared with the experimental results obtained for the samples (a-c).

done over the whole HEK293 cell, treated with  $\text{GNR}_{30X10}$ . Please note the changes in *P* for all these samples, it justifies that unless the nanostructure under observation is a perfect sphere, despite the small value of *P*, maps are efficient in determining *in-situ* orientation. Hence with the criterion discussed above, PDFSMS alone can provide complete information about the state of small GNRs in cellular environments.

Real time rotational dynamics of GNR<sub>30X10</sub> using PDFSMS. For in vitro observations of rotational dynamics, we have monitored freely moving GNRs in a viscous solution of PEG (GNR@PEG). Rotational dynamics of GNRs can occur at the time scale of microseconds to seconds. In this study, due to limitations of the temporal resolution of the camera, we could perform time lapse measurements only at the temporal resolution of 100 ms. This allows us to observe longer time scale rotational dynamics of GNRs. Figures 5a and 5b show the timelapse images of a freely moving single GNR<sub>30X10</sub> in such a solution captured with and without analyzer, respectively. In our previous observations with GNR@BSA, we have observed that SPR peak position of GNR changes after interaction with BSA; but forGNR@ PEG, SPR position remains constant (Fig. S13). This suggests the existence of single isolated GNRs instead of aggregates. For GNRs with deionized water as surrounding medium (GNR@DI), TSPR in single GNR scattering spectrum is nearly zero. But in the case of GNR@PEG, possible adsorption of PEG on the GNR surface gives rise to increased SPR width and non-zero TSPR component (Fig. S13). Due to this, when orientation of GNR@PEG is perpendicular

discrimination between orientation change and Z axis movement (see below). Change in color - mainly variations in the red channel scattering intensity of GNR<sub>30X10</sub> - can be used to determine its orientation. Whereas width of the 2D Gaussian fitted to GNR spot  $(W_G)$  in the RGB (red-green-blue) image can be used to determine its movement along the Z axis. From the temporal changes in  $W_G$ (Fig. 5a (blue curve) and 5b (green curve)), we see that  $W_G$ decreases to less than ~60% of the  $W_G$  value for in focus GNR, only if its movement occurs along the Z axis. Generally on defocusing, scattering based image of GNR becomes diffuse, ring shaped and reduced signal to noise ratio leads to reduction in  $W_{G}$ . Such images are shown for GNR@DI in Video S3. As per these observations, our system can resolve 1 micron movement along Z axis. Since color is important for the determination of in focus GNR<sub>30X10</sub> orientation, images were color saturated instead of improving brightness and contrast required for the observation of defocused GNR<sub>30X10</sub> patterns. For orientation measurements, intensity variations that occur due to Z axis movement of the scatterer can be eliminated by subtracting the green channel intensity from the red channel intensity. Details of this baseline correction are explained in SI Discussion 1 with an example of a rotating GNR<sub>30X10</sub> in HEK293 cell, in comparison with a vesicle, the latter can well be considered as an isotropically scattering particle. Figure 5b shows that changes in color and scattering intensity of GNR can also be observed up to some extent even

to the analyzer axis, its scattering intensity is not zero. For particles

like this, non-zero TSPR gives an additional advantage of



Figure 4 | Effect of various environments on the polarizability of GNR<sub>30X10</sub>. Each column in this figure shows (top to bottom) a polar plots, polar maps and single particle scattering spectrum for, (a) GNP<sub>40</sub>, (b) GNR<sub>30X10</sub>, (c) GNR<sub>30X10</sub> on the HEK293 cell surface, (d) GNR<sub>30X10</sub> inside HEK293 cell, and (e) GNR<sub>30X10</sub> treated with BSA. Polar plots indicate the nature of GNP or GNR being examined. Inset images in each of the polar map images show dark field images of the particles when analyzer was (left) parallel ( $\leftrightarrow$ ) and (right) perpendicular ( $\ddagger$ ) to the longitudinal axis defined by the orientation of the analyzer where maximum scattering occurs.

without analyzer in the optical path. The reason behind this is explained in the next section, with the help of DDA simulations.

Observations of a rotating GNR with and without analyzer. Figures 5(c,d & e) show the results of DDA simulations of GNR<sub>30X10</sub> performing in/out of plane rotation. Upon unpolarized white light excitation, scattering cross section of GNR performing out-of-plane rotation will vary, as shown in Fig. 5c. Hence orientation of GNR performing such rotation can be sensed even without polarized collection as we have said before. But for a GNR performing in-plane rotation, polarized illumination is necessary to determine its orientation (Fig. 5d & 5e); conversely, upon unpolarized illumination, this rotational mode can be observed by polarized collection. This also suggests the possibility that rotational modes of GNR illuminated with unpolarized light can be resolved by simultaneous observation with and without analyzer. In the absence of a simultaneous DFSMS and PSFSMS imaging setup, separate measurements were performed on GNRs rotating inside a viscous PEG solution using DFSMS and PSFSMS separately. As shown in Fig. 5a, when observed with PDFSMS, changes in the color of GNR can be seen for both in-plane and out-of-plane rotations. But when GNR was seen with DFSMS, changes in the color of GNR can be seen only for out-of-plane rotation. Here in this study we have focused only on the observations that can be done with a simple setup of PDFSMS. Next section discusses the feasibility of such observations of real time rotational dynamics of GNR<sub>30X10</sub> inside HEK293 cell and development of a methodology for the same.

**3D** Three dimensional spatiotemporal mapping of GNR@Tf inside HEK293 cell. In case of GNR@PEG, when GNR moves along the Z axis, we observed a decrease in  $W_{G}$ . However, for GNR@Tf@HEK293 (transferrin-conjugated GNR inside HEK293

cell), when GNR@Tf goes drastically out of focus, an increase in  $W_G$  was observed. This is attributed to the noise in the environment inside cell; because of which when GNR goes completely out of focus, noisy surrounding results in a fit with the 2D Gaussian of larger width. Also, in such observations, GNR movement occurs in three dimensions in space; because of which, camera gain and focus settings need to be changed and optimized time to time. All these changes are mentioned in detail at each place wherever it has been made. Figure 6a shows the scattering spectrum of GNR@Tf when it was attached on the cell membrane. It still shows a sharp SPR at  $\sim$ 600 nm, suggesting the existence of single GNR<sub>30X10</sub>. Figure 6c shows the position of GNR inside the cell; it shows that GNR under observation has tracked its path in a constricted environment between the nucleus and the cell membrane. Figure S14 shows an atlas of all the images of GNR corresponding to the movement of GNR inside cell. Observed events can be explained in detail as follows. During the first one minute, apart from translational motion, no considerable change in the orientation and  $W_G$  of GNR was observed (red color track in Fig. 6f). This initial data collected at slightly lower gain of camera is shown in Fig. S15. After that, the gain was optimized for proper observation of GNR and kept constant throughout the measurements (indicated by green arrow in Fig. 6f). Then fusion between GNR and another vesicle was observed. Video S4 shows this binding event; where it can be seen that when GNR moves or gets attracted towards the vesicle, its scattering intensity increases. This is reflected in the increase in  $W_G$  with corresponding increase in the scattering intensity (Fig. 6d & 6e). Observation of this fusion event also explains the possible reason behind red shift in the scattering spectrum of GNR (Fig. 6b). Then after minor focus adjustment, GNR was monitored for  $\sim 1$  min; during which it kept moving towards the interior of the cell. Then it started fluctuating



Figure 5 | Real time rotational dynamics of GNR<sub>30X10</sub> using PDFSMS. (a) Time-lapse (100 ms) images of a single GNR<sub>30X10</sub> in viscous solution of PEG captured through the analyzer. To fit in smaller space, images are stacked such that time (seconds) for each image can be calculated by adding  $t_x$  and  $t_y$  time coordinates and can be correlated with the graph shown below. Corresponding variations in the scattering intensity (magenta curve) and 2D Gaussian width (blue curve) of the particle spot are shown in the graph below. Arrow indicates a time point when GNR moves along Z axis, goes completely out of focus and reverts back to the focal plane. (b) Images of a single GNR<sub>30X10</sub> from the same sample captured without the analyzer are shown with corresponding variations in the scattering intensity (red curve) and spot width (green curve). Arrow has same meaning as in (a). (R-G) indicates that variations are shown for the difference between red and green channel scattering intensity. Next figures show DDA simulated scattering cross sections of a single GNR<sub>30X10</sub> performing different rotational modes. Arrows with letter *P* show allowed polarizations while illumination of GNR in Z direction. Descriptions of rotational modes are: (c) out-of-plane rotation mode with unpolarized illumination, (d) in-plane rotation mode with unpolarized illumination.

along the Z direction and went slightly out of focus which was reflected in the decrease of  $W_G$  but still it was possible to monitor it (hence no focus adjustment was necessary at this point). But after  $\sim$ 20 seconds, when it reached in the crowded and constricted interior of the cell, its movement along the Z direction increased and it went quite out of focus. Then the focus was adjusted to an optimized position to monitor the GNR. In this highly interactive environment, it resulted in enormous fluctuations in the value of  $W_G$ and orientational changes for the next 2 min; which we have tentatively interpreted as a process in which TfR dissociates from GNR. However, after this process, when GNR started coming out of the interior, its fluctuations in the Z direction decreased considerably. Then it kept moving towards cell membrane with steady values of  $W_{G}$ , intervened by large variations at some places. These large variations can be interpreted as due to collisions with other vesicles and attempts of GNR to come outside the cell. Here due to large scattering from vesicles, a minor fluctuation in the path tracking occurs. By correlating temporal variations in the scattering intensity (R-G), and  $W_G$  (shown by blue line in Fig. 6d & 6e), it can be inferred that GNR movements along Z direction occur by out-of-plane rotations. Studies have shown that for Tf or GNR@Tf an average time required for penetration of the cell membrane is  $\sim 7 \min^{10,33}$ . This implies that movement of GNR inside cell starts 7 min after it begins interacting with cell surface proteins<sup>10,33</sup>. Our observations over a time span of  $\sim 10$  min starts with the movement of GNR@Tf from cell membrane towards

interior of the cell and then back towards the cell membrane at the end of the observation (Video S5). This is  $\sim$ half of the transit time (21 min) that is reported for transferrin receptors<sup>33</sup>, which suggests that our observations start much after the entry of GNR inside the cell and may involve dissociation of GNR from TfR so that GNR follows a different path to return towards the cell membrane.

#### Discussion

To conclude, we have used PDFSMS in its simplest form to develop a methodology for three dimensional spatiotemporal orientational mapping of tiny GNR<sub>30X10</sub>. We have shown that minute angular tilt in the analyzer can be used as an advantage to map in-situ polarization patterns (called as polar maps) and hence orientation of GNRs. Using statistical analysis of single particle SPR peaks and DDA simulations, we have given a criterion to distinguish GNR aggregates from GNPs and single GNRs. It is shown that aggregation of GNR<sub>30X10</sub> induced by proteins in cellular environment gives rise to two prominent SPR peaks. This criterion was used to compare the spectroscopic features of samples such as GNP and GNR on glass substrate, GNR on cell membrane and an aggregate of GNR on glass substrate and inside the cell; the data were correlated with the polar plots and maps to understand the effect of environment on the polarizability of these particles. We have also demonstrated PDFSMS for real time observations of the rotational dynamics of GNR<sub>30X10</sub> in cellular environment. From the observations of freely moving GNR in viscous solution of PEG, it was inferred that movement of GNR along Z


**Figure 6 3D Three dimensional spatiotemporal mapping of GNR@Tf inside HEK293 cell**. (a) Scattering spectrum of a single GNR@Tf attached on HEK293 cell membrane. (b) Scattering spectrum of the same GNR@Tf after entering inside the cell. Insets show corresponding hyperspectral images. (c) Image of a HEK293 cell showing the position of the monitored GNR@Tf inside the cell. The GNR is marked with a square. Inset shows an enlarged view of the GNR. Light scattered in the Z direction was collected through analyzer, whose orientation is shown by yellow double arrow. (d) Temporal variations in the scattering intensity (R-G) of GNR@Tf. Time scale corresponds to the axis of the graph shown below. Pink vertical bars show the region where microscope focus was adjusted on the particle after it went out of the focal plane. (e) Temporal variations in the 2D Gaussian width of GNR spot. Labeled events are discussed in the manuscript text. (f) Path of GNR@Tf inside the HEK293 cell. Green arrow shows the time point from where temporal data of the GNR is shown. Color of the trace corresponds to the time scale of graphs (d,e). Please note that the background image is just to give a rough idea of the position of GNR inside the cell.

axis can be tracked by decrease in  $W_G$ , the width obtained by 2D Gaussian fitting of GNR spot in dark field image. In contrast, in a noisier cellular environment,  $W_G$  increases whenever GNR movement occurs along the Z axis. Observation made on tracking of GNR@Tf inside HEK293 cells suggest that motion along Z axis occurs with out-of-plane rotations. To summarize, we have shown that simply DFSMS with an analyzer can be potentially used for understanding the overall state of an anisotropic nanostructure in complex environments.

As a future direction, our data suggests the possibilities that Intracellular path of Tf and GNR@Tf might be different and this may also involve dissociation of Tf itself from GNR. We plan to investigate behavior of GNRs using simultaneous polarized and unpolarized DFSMS to resolve the rotational modes of GNR. Combination of this technique with real time signal controlled microscope stage will be useful for further improvements in the three dimensional rotation and orientation tracking of GNR-labeled intracellular components.



#### **Methods**

Polarized dark field scattering micro-spectroscopy. HSI measurements were performed using Cytoviva hyperspectral imaging system equipped with Cytoviva<sup>TM</sup> high resolution dark field condenser (oil immersion). UPLFLN 100XOI2 oil immersion objective (Olympus) with NA of 1.3–0.6 and working distance of 0.2 mm was used for magnification. Analyzer was kept in the optical path after the objective. A L1090 - Halogen Lamp (400–1,000 nm) with Aluminum Reflector (International Light Technologies Inc., USA) was used for illumination of the sample. Optical images were collected using Dagexcel-M Color Camera – Cooled (CCD, 2,048 × 2,048). Spectra were measured using the Specim V10E spectrometer (400–1,000 nm) at a spectral resolution of  $\pm$ 1.3 nm. To tackle with the lower scattering cross section of ultrasmall nanoparticles, measurements were performed at full power illumination of source (150 W) and enhanced camera gain. For live cell experiments CV-30 CytoViva<sup>TM</sup> Environment Chamber provided by Warner Instruments was used. DMEM and GNR solutions were pumped using NE-300 Just Infusion<sup>TM</sup> Syringe Pump.

**Cell culture and maintenance.** HEK293 cells were cultured in *Dulbecco's Modified Eagle Medium* (DMEM) containing 10% foetal bovine serum (FBS) and antibiotics (100 units of penicillin and 10  $\mu$ g streptomycin/mL). For steady state observations with fixed cells, following protocol was used. HEK293 cells were cultured on poly-Llysine-coated 0.145 mm thick Nexterion® clean room cleaned glass coverslips (SCHOTT) in six well plates. Once cells reached ~80% confluency, they were washed twice with 1X phosphate buffered saline (PBS) and 2 mL DMEM containing 100  $\mu$ L GNR was added. These cells were incubated for 10 min inside the incubator at 37°C. Then cells were washed thrice with 1XPBS and incubated for 8 min with 4% paraformaldehyde solution in 1XPBS. Then cells were washed thrice with PBS and incubated with ethanol gradient (25%, 50%, 75%, 100%) 10 min each. Then cells were dried and mounted with DPX mountant on 1 mm thick ultrasonically cleaned glass (SCHOTT) for HSI measurements.

For live cell experiments, HEK293 cells were cultured on poly-L-lysine-coated 40 mm round glass slides (Warner instruments) in 60 mm petri dishes. After reaching 80% confluency, cells were washed once with 1XPBS and fresh DMEM. Then the slide was mounted on CV-30 set-up immediately and after initial pumping, flow of DMEM was maintained at 0.05 mL/hr. A three way valve was used to switch between DMEM and (DMEM + GNR) solutions.

**Image analysis**. Image processing, particle tracking and RGB analysis was done using NIH Image<sup>34</sup> which included particle tracker plugins developed by MOSAIC group (ETH, Zurich)<sup>35</sup> and Fabrice Cordeli (Institut Curie, Orsay, France). Details of image processing algorithms are given in SI Method 1. MATLAB scripts were developed for polar mapping, tracking the integrated RGB intensity of moving particle images and to extract particle images separately from large area images. For 2D Gaussian fitting of particle images, a script developed by Gero Nootz (MATLAB CENTRAL File ID: #37087) was used. Spectral image analysis was done using ENVI and homebuilt software LSPRPEAK1.001 (Fig. S16).

**DDA simulations.** DDA simulations were done using DDSCAT7.3 package developed by Draine and Flatau<sup>36–38</sup>. Refractive index data provided by Johnson and Christy et al. was used for gold<sup>39</sup>. Particle or aggregate models were generated by DDSCAT7.3 code<sup>36–38</sup> and images were generated by Paraview software<sup>40</sup>. In order to simulate spectra with unpolarized excitation, scattering cross sections were averaged for different polarizations of incident electromagnetic wave varying from 0–360° at an interval of 10°. To match with the experimental data, simulated spectra were corrected using lamp spectrum.

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#### **Author contributions**

K.C. and T.P. designed the experiments. K.C. performed experiments. K.C. and T.P. analyzed the data and wrote the manuscript.

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**Supporting information** 

# Spatiotemporal mapping of three dimensional rotational dynamics of single ultrasmall gold nanorods

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#### Materials and protocols in detail

#### Chemicals

Tetrachloroauric acid trihydrate (HAuCl<sub>4</sub>·3H<sub>2</sub>O) (99.9%), cetyltrimethylammonium bromide (99.9%) (CTAB), sodium borohydride (99%, Fluka), (3-mercaptopropyl)trimethoxysilane (95%), trisodium citrate (>99%), glutaraldehyde (70% in H<sub>2</sub>O), DPX mountant, poly (ethylene glycol) (average Mn 950-1,050), poly-L-lysine solution (0.1 % in H<sub>2</sub>O) and trypsin-EDTA (0.25% in H<sub>2</sub>O) were purchased from Sigma Chemicals. L(+) ascorbic acid (99.7%), silver nitrate (99.9%), sodium hydroxide (98%), sodium carbonate (99.8%) and ferric chloride anhydrous (98.5%) were purchased from RANKEM, India. Antibiotic (100X, Penicillin-Streptomycin-Glutamine), DMEM (Dulbecco's Modified Eagle Medium with high glucose, GlutaMAX<sup>TM</sup> Supplement and pyruvate) and FBS (Foetal Bovine Serum) were purchased from Invitrogen, USA. Details of other chemicals used are as follows, chloroform (99.5%, Thermo Fisher Scientific India Pvt. Ltd.), cystamine dihydrochloride (97%, TCI Chemicals (India) Pvt. Ltd.), bovine serum albumin (96-98%, pH 7, SRL Pvt. Ltd., India.). Apo-transferrin (Tf human recombinant) was purchased from ProSpec-Tany TechnoGene Ltd., Israel. Plastic ware for cell culture were purchased from Tarson, India. Millipore deionized water (DI) (~18.2 MΩ) was used throughout the experiments.

**Synthesis of GNRs.** GNRs of the dimension 30 (L) nm  $\times$  10 (D) nm were synthesized by a protocol previously reported from our group.<sup>1</sup> Briefly growth solution was prepared by adding 50 mL of 100 mM CTAB, 2.5 mL of 10 mM HAuCl<sub>4</sub>·3H<sub>2</sub>O, 325 µL of 10 mM AgNO<sub>3</sub>, and 350 µL of 100 mM ascorbic acid. Growth solution was incubated for five minutes and then 50 µL NaBH<sub>4</sub> (1.67 mM in ice-cold DI water) was added. After that mixture was kept undisturbed at room temperature for overnight to complete the growth of nanorods. This solution was washed using DI water after centrifugation at 7,500 rpm (20 min) once and then twice at 12,000 rpm (20 min) to remove excess CTAB. Considering that

40% of the gold retained in the form of GNRs after washing (as estimated by us), final molarity of GNR in solution (in terms of elemental gold) was ~0.2  $\mu$ M. Details of these calculations are given in SI Equation 4.

**Synthesis of GNPs.** GNPs of the diameter 40 nm were synthesized by the Turkevich method.<sup>2</sup> Briefly 60  $\mu$ L of 100 mM HAuCl<sub>4</sub> solution was added to 20 mL DI water and heated in a synthesizer at 400 rpm. On boiling, 240  $\mu$ L of the 100 mM solution of trisodium citrate was added. After 20 minutes of continuous boiling, color of the solution changed to wine red. The solution was then cooled at room temperature. To prepare GNRaggregates, after overnight incubation of GNR<sub>30X10</sub> with BSA (GNR@BSA), sample was washed to remove excess BSA (10 min, 8000 rpm) and spotted for PDFSMS.

**Immobilization of GNRs on glass slide.** GNRs were immobilized on a glass slide by the pinpoint immobilization method. In this method, 1 mm thick ultrasonically cleaned glass slide (SCHOTT) was flushed with 5 mL solution of (3-mercaptopropyl)trimethoxysilane (0.2  $\mu$ M in chloroform). Then it was thoroughly flushed with DI water and immediately 10  $\mu$ L solution of as synthesized GNR (0.02  $\mu$ M) was dropped and covered with 0.145 mm thick Nexterion® Clean room cleaned glass coverslip (SCHOTT). After five minutes of incubation, coverslip was removed and glass slide was thoroughly flushed with DI water to remove loosely bound GNRs. Then 2  $\mu$ L of DI water was dropped on GNR immobilized region and covered with coverslip. It was sealed with nail paint on sides to avoid drying of samples. In the overall procedure, care was taken such that only one side of slide was exposed to chemicals.

**Preparation of iron loaded transferrin (holo-transferrin).** For iron loading, ferric chloride solution was neutralized by sodium hydroxide (1 M) to a pH of 7. Then apo-transferrin was incubated for 1 hr with neutralized ferric chloride (molar ratio 1:2) in presence of carbonate ions followed by dialysis against DI water in 3.5 kDa molecular weight cut off Snakeskin<sup>™</sup> Dialysis Tubing (Thermo Scientific Pierce, USA).

**Conjugation of holo-transferrin to GNR surface.** For conjugation of transferrin to GNR surface, amine functionalized GNRs were prepared by modifying previously reported protocol by Wang et al.<sup>3</sup> For this, GNRs were treated with cystamine dihydrochloride (molar ratio 1:2) with sonication for 10 mins. Then these GNRs were activated by incubation with glutaraldehyde (molar ratio 1:2) for 1 hr. Then these GNRs were washed with DI water by centrifugation at 12,000 rpm (20 min) and incubated with holo-transferrin (molar ratio 1:1) for 1 hr. Overall procedure was performed in the dark.

**UV-Vis spectroscopic analysis.** The samples were diluted in DI water and UV/Vis spectra were measured with a Perkin Elmer Lambda 25 instrument in the range 400-1,000 nm.

**Transmission electron microscopic analysis.** High-resolution transmission electron microscopy (HRTEM) of the GNR and GNR@BSA samples were carried out using a JEOL 3010 instrument at 300 kV. TEM specimens were prepared by drop casting one or two drops of aqueous solution on carbon-coated copper grids and drying in ambient.

#### **De-pixelation of the images**

For de-pixelation of the images using ImageJ software, following protocol was used.

- 1. Images were filtered using a Gaussian blur filter with sigma (radius) of 1.0.
- 2. Length and width of image was increased ten-fold in pixel units.

Contrast and brightness of the image was set to an optimum position that image should not saturate but most of the particles are visible.

#### **Supporting Equations**

#### **Equation 1**

For polar mapping, an image set collected at different angles of the analyzer was processed using the formula,

$$I_{p(x, y)} = \max\left(i_{p(x, y)}(\theta)(\theta = 0^\circ - 360^\circ)\right)$$
(1)

where,  $I_{p(x,y)}$  is the intensity value at the pixel coordinate (x,y) in an image to be constructed for polar mapping and  $i_{p(x,y)}$  is the scattering intensity at pixel coordinate (x,y) in the captured image as a function of  $\theta$ . In this case,  $\theta$  takes discrete values between 0° to 360° at the interval of 22.5°.

# **Equation 2**

Polar plot for each particle was determined using the following formula applied to the cropped image squares containing the particle of interest,

$$I_r(\theta) = \left( \left( i_{(m \times n)}(\theta) \right) / \max\left( i_{(m \times n)}(\theta) \right)_{(\theta = 0^\circ - 360^\circ)} \right) \times 100$$
<sup>(2)</sup>

$$i_{(m \times n)}(\theta) = \left( \sum_{x=1}^{m} \sum_{y=1}^{n} I_{p(x, y)}(\theta) \right) / (m \times n)$$

where,  $I_r(\theta)$  is the radial intensity in polar plot at an angle  $\theta$  of the analyzer, expressed as percentage. For an image *i* captured at particular angle  $\theta$ , size of the image is represented by *m* and *n*, the number of pixels in *X* and *Y* directions.  $I_{p(x,y)}(\theta)$  provides the intensity at particular pixel (*x*, *y*) as a function of  $\theta$ .

# **Equation 3**

Polarization anisotropy was calculated using the following formula,

$$P = \frac{I_{\max} - I_{\min}}{I_{\max} + I_{\min}}$$
(3)

 $I_{max}$  and  $I_{min}$  are the maximum and minimum scattering intensities that are observed at mutually perpendicular positions of analyzer while rotation is from 0-360 degrees.

### **Equation 4**

# Molarity of GNRs in solution was calculated using the following formula,

$$M_{NP} = \frac{\left(Molarity \ of \ Au^{3+} \ in \ the \ solution\right) \times (Volume \ of \ one \ gold \ atom)}{(Volume \ of \ one \ GNR)}$$

$$M_{NP} = \frac{12MA_r}{\left(2\pi D^3 + 3\pi D^2 l\right)\rho N_A}$$
(4)

Where,

M = Molarity of Au<sup>3+</sup> stock in  $\mu$ M

 $A_r$  = Atomic weight of Au in g

D = Diameter of GNR in cm

l = Length of GNR excluding caps in cm

 $\rho$  = Density of gold in g/cm<sup>3</sup>

 $N_A$  = Avogadro number



Figure S1. Size distribution of  $GNR_{30X10}$ . Histograms of the length and diameter of gold nanorods as determined from TEM show that nanorods have average length of 30 nm and diameter of 10 nm, suggesting an aspect ratio of ~3. Inset shows TEM image of the sample.



**Figure S2.** Polar plots and scattering spectra of a spherical gold nanoparticle (GNP) and and an anisotropic equivalent. (a) Polar plots of a spherical GNP (green) and an anisotropic GNP (red) for which polar maps and dark field images are shown in (b). (b) Scattering spectra suggest that they are indeed from single GNP and a single rod-shaped anisotropic GNP, respectively.



**Figure S3. Anisotropy in the shape of GNPs.** Graph shows percentage change in the scattering intensity of single GNPs and GNRs. It can be seen that GNPs also exhibit anisotropy depending on deviation from perfectly spherical shape. Adjacent images of GNPs are captured at different analyzer angles.



**Figure S4. Polar mapping of anisotropic GNPs. (a)** Dark field image of GNPs. (b) Corresponding polar maps of GNPs suggest that particles are not perfect spheres. These polar maps are determined using SI Equation 1.



**Figure S5. DDA simulations of GNR**<sub>30X10</sub>. (a) DDA simulated scattering cross section of GNR<sub>30X10</sub> on unpolarized white light excitation (averaged over wavelengths 425-1000 nm and polarization of incident electric field varying from  $0^{\circ}$ -360°). Inset shows magnified view of the TSPR of GNR which is negligible as compared to LSPR of GNR. (b) DDA simulated electric near-field of GNR<sub>30X10</sub> is shown where electromagnetic wave just touches the GNR. Polarization of incident electric field is along the longitudinal axis of nanorod in this simulation.



**Figure S6. Scattering spectra of cellular components.** It can be seen from the scattering spectra shown above that although scattering of vesicles is substantially higher than the background spectra collected from cellular components, their scattering spectra are broad with a maximum in the blue green region.



Figure S7. Examples of spectra of single GNPs, GNRs and GNR aggregates *in vitro and in vivo*. Figure shows dark field images and single particle scattering spectra of various samples collected at 100X magnification. (**a,d**) GNPs of 40 nm diameter (GNP<sub>40</sub>). (**a,e**) GNRs of the dimensions 30 (L)  $\times$  10 (W) nm (GNR<sub>30X10</sub>). (**b,f**) GNR<sub>30X10</sub> aggregates obtained by treating it with BSA (bovine serum

albumin) protein. (**c**,**g**) GNR<sub>30X10</sub> aggregates in HEK293 cells. Next figures are the same as the ones in the manuscript and shown here just for direct comparison. (**h**) Surface images of the model arrays generated for DDA simulations of GNP<sub>40</sub>, GNR<sub>30X10</sub>, GNR<sub>90X30</sub> and a GNR aggregate constructed from six GNR<sub>30X10</sub>. (**i**) Corresponding DDA simulated scattering cross sections of nanostructures on unpolarized white light excitation are shown here.



**Figure S8.** Gaussian curve fits to the SPR peak distributions of single GNPs, GNRs and GNR aggregates *in vitro and in vivo*. Dark field images and statistical distribution of SPR peaks determined from hyperspectral measurements of various samples at 100X magnification. (a,d) GNP40. (a,e) GNR<sub>30X10</sub> (b,f) GNR<sub>30X10</sub> aggregates obtained by treating it with BSA. (c,g) GNR<sub>30X10</sub> aggregates in

HEK293 cells. Next figures are the same as the ones in the manuscript and shown here just for direct comparison. (h) Surface images of the model arrays generated for DDA simulations of  $GNP_{40}$ ,  $GNR_{30X10}$ ,  $GNR_{90x30}$  and a GNR aggregate constructed from six  $GNR_{30X10}$ . (i) Corresponding DDA simulated scattering cross sections of nanostructures on unpolarized white light excitation are shown here for comparison. Color coding for all SPR statistical distributions is provided in (c).



Figure S9. Aggregation of GNRs on treatment with BSA was confirmed by transmission electron microscopy (TEM). TEM images of  $GNR_{30X10}$  treated with BSA (GNR:BSA molar ratio is 1:10). Scale bar is 0.2 µm.



Figure S10. Aggregation of GNRs was confirmed by UV-Vis absorption. Figure shows ensemble phase UV-Vis absorption spectra of  $GNR_{30X10}$  incubated with different ratios of GNR:BSA. For uniformity in the data, 1:10 sample was used for further measurements with HSI. The spectrum of pure GNR is in Figure S11.



Figure S11. Ensemble phase UV-Visible spectra of  $GNP_{40}$  and  $GNR_{30X10}$ . UV-Visible absorption spectra of  $GNP_{40}$  and  $GNR_{30X10}$  solutions in deionized (DI) water. Inset shows dark field images of both these samples (red spots –  $GNR_{30X10}$  and yellowish green spots –  $GNP_{40}$ ).



**Figure S12.** *In-situ* polar mapping of GNR<sub>30X10</sub> in HEK293 cells. (a) Dark field image of HEK293 cell treated with GNR<sub>30X10</sub>. Circles show nanorods for which polar plots and maps are shown in the next image. (b) *In-situ* polar mapping of whole cell gives an idea about polarization pattern and orientation of GNRs.



**Figure S13. Scattering spectra of GNR**<sub>30X10</sub> **in viscous PEG solution.** (a) Hyperspectral image (HSI) of freely moving  $GNR_{30X10}$  in viscous solution of poly (ethylene glycol) (PEG). Since hyperspectral imaging is not as fast as images captured with CCD camera, during the capture of HSI, moving nanorods change their position and give a streaked appearance as shown in the above image. (b) Scattering spectrum of one such GNR@PEG is compared with GNR@DI (DI – deionized water) and Lamp to show that it is still single GNR and not an aggregate.



**Figure S14.** Color changes in GNR@Tf during its journey inside HEK293 cell. (a) Image shows changes in the color of GNR when images were captured through analyzer while its movement inside HEK293 cell. These 5670 images of GNR whose color and spot size changes as per the rotation and movement of GNR inside cell are extracted from real time data and stacked such that each column in the image roughly visualizes behavior of GNR in the time regime corresponding to time scale of graph in (c). Stacking is similar to the way images of GNR are stacked in Fig. 5 of the manuscript. (b) Temporal variations in the scattering intensity (red-green, R-G) of GNR@Tf. Time scale corresponds to the graph in (c). Pink color strips show the region where microscope focus was adjusted on particle whenever it went completely out of the focal plane. (c) Temporal variations in the  $W_G$  of GNR spot.



Figure S15. Behavior of GNR@Tf during initial time of intracellular observation. (a) Images show changes in the color of GNR when images were captured through analyzer before it starts rigorous activity inside cell. These ~630 images of GNR whose color and spot size changes as per the rotation and movement of GNR inside cell are extracted from real time data and stacked such that each column in the image roughly visualizes behavior of GNR in the time regime corresponding to time scale of graph in (c). Stacking is similar to the way images of GNR are stacked in Fig. 5 of the manuscript. (b) Temporal variations in the scattering intensity (R-G) of GNR@Tf. Time scale correspond to the graph shown below. (c) Temporal variations in the  $W_G$  of GNR spot shown at the same scale as in manuscript Fig. 6e.



**Figure S16. Efficiency of LSPRPEAK1.001 in detecting SPR peaks.** Figure shows sample spectra collected from the aggregates of citrate capped silver nanoparticles. Inset of graph 1 shows dark field image of silver nanoparticles. Hyperspectral image of the same was analyzed using LSPRPEAK1.001. Peaks detected by LSPRPEAK1.001 from the four example spectra (1-4) are shown in the table.

#### **Supporting Discussion**

#### **Discussion 1**



**Figure S17. Real time rotational dynamics of GNR**<sub>30X10</sub> **inside HEK293 cell.** (a) Time-lapse images of a GNR<sub>30X10</sub> rotating inside cell. (b) Time dependant variations in the scattering intensity (R-G) of a single rotating GNR<sub>30X10</sub> in HEK293 cell. (c) Optical dark field image of live HEK293 cell with arrows indicating GNR (white arrow) and a vesicle (black arrow) under observation. Inset shows region of interest (ROI) selected for time-lapse imaging, with the particles marked. (d) Time dependant variation in the scattering intensity (R-G) of a vesicle inside HEK293 cell. (e) Time-lapse images of a vesicle. It should be noted that vesicle inside the ROI in the beginning goes slightly outside the ROI during the capture of time-lapse image sequence. See the difference between (a) and (e). (f) Scattering spectrum of a single GNR<sub>30X10</sub> in HEK293 cell. Sharp LSPR peak position at ~626 nm suggests that it is indeed a single GNR and not an aggregate. (g) Temporal variations in the 2D Gaussian width ( $W_G$ ) of a GNR@HEK293 spot in the image. It suggests that there is no considerable decrease or increase in the width observed and hence there was no considerable movement along the Z axis.

**Real time rotational dynamics of GNR<sub>30X10</sub> inside HEK293 cell.** Figure S17c shows an optical dark field image of live HEK293 cell with a single GNR non-specifically entered inside HEK293 cell (GNR@HEK293). Scattering spectrum of this GNR@HEK293 shown in SI Fig. S17f confirms that it is a single GNR<sub>30X10</sub>. Yellow arrow in Fig. S17c indicates the orientation of the analyzer axis which was kept constant throughout the measurement. Due to temporal variations in the scattering intensity, rotating GNR appears to be blinking inside cell. This rotating GNR with large area view of the cells is shown in Video S6 and magnified view of the same with smoothed scattering intensity variations is shown in Video S7. Figure S17b shows variations in the time (t)-dependent red, green and blue scattering intensity of GNR calculated by Equation 5,

$$I_{(C)(m \times n)}(t) = \sum_{x=1}^{m} \sum_{y=1}^{n} i_{(C)(x, y)}(t)$$
(5)

where *C* refers to R, G or B, the isolated red, green or blue pixel images obtained after splitting the color channels of an original RGB image. Indices *m* and *n* indicate the number of pixels in the *X* and *Y* direction.  $I_C$  is the scattering intensity collected by color channel *C* at particular time *t* and  $i_{(C)(x,y)}$  is the scattering intensity at pixel coordinate (x,y) in the captured image as a function of *t*. Since the prominent plasmonic scattering from LSPR of GNR occurs in the red region, most variations were observed in the value of  $I_R$ .  $I_B$  appears to be almost inert to the changes in the orientation of GNR and  $I_G$  exhibits slight variations due to the tail of LSPR peak that lies in this region (Fig. S17b). But for the vesicle (Fig. S17d),  $I_R$ ,  $I_G$  and  $I_B$  follow the same trend in the variations of scattering intensity and hence confirm good S/N ratio provided by GNR<sub>30X10</sub> labels. Although the vesicle inside the region of interest (ROI) goes slightly outside during the capture of time-lapse image sequence, spatial uniformity in its color justifies that scattering intensity variations can still be monitored with the available signal. Since  $I_G$ variable is close to  $I_R$  than  $I_B$  it can be used well to correct the baseline of  $I_R$  which also helps in removing the noise contributed by changes in the surrounding environment. Temporal variations in parameter  $W_G$  (Fig. S17g) suggest that there was no considerable movement along Z axis. On the basis of these preliminary studies, we performed real time observations on the interaction of transferrin conjugated GNR (GNR@Tf) with HEK293 cells (GNR@Tf@HEK293). These observations are discussed in the manuscript.

#### **Supporting Notes**

Note 1

#### **Description of Supporting Videos**

#### Video S1

Three panels in this video shows, optical dark field images of a spherical GNP and an anisotropic GNP captured at different angles of the analyzer, path tracked by these particles due to displacement in the image caused by rotation of analyzer and corresponding polar maps, respectively.

#### Video S2

This video shows blinking of GNRs immobilized on glass substrate when their images were captured at different angles of the analyzer.

#### Video S3

This video shows how scattering pattern of GNRs immobilized on glass substrate changes when their images were captured at different distances from the focal plane (glass substrate), in steps of 1 µm.

#### Video S4

Two panels of this video show original time lapse images and images overlaid by particle tracks to indicate the fusion event between GNR and a vesicle inside the cell.

#### Video S5

Time lapse images of transferrin conjugated GNR moving inside HEK293 cell. A dark blue color dot is overlaid in these images to highlight the position of GNR. Other GNR appearing from the surrounding medium intermittently are indicated.

#### Video S6

A large area view of live HEK293 cells showing a single  $GNR_{30X10}$  (indicated by arrow) blinking due to rotation inside the cell.

#### Video S7

Magnified and de-pixelated view of the ROI (region of interest) showing a blinking-rotating GNR in a HEK293 cell. Corresponding variations in the scattering intensity are shown after smoothing.

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# Using Ambient Ion Beams to Write Nanostructured Patterns for Surface Enhanced Raman Spectroscopy\*\*

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Abstract: Electrolytic spray deposition was used to pattern surfaces with 2D metallic nanostructures. Spots that contain silver nanoparticles (AgNP) were created by landing solvated silver ions at desired locations using electrically floated masks to focus the metal ions to an area as little as 20 µm in diameter. The AgNPs formed are unprotected and their aggregates can be used for surface-enhanced Raman spectroscopy (SERS). The morphology and SERS activity of the NP structures were controlled by the surface coverage of landed silver ions. The NP structures created could be used as substrates onto which SERS samples were deposited or prepared directly on top of predeposited samples of interest. The evenly distributed hot spots in the micron-sized aggregates had an average SERS enhancement factor of 10<sup>8</sup>. The surfaces showed SERS activity when using lasers of different wavelengths (532, 633, and 785 nm) and were stable in air.

Metallic nanoparticles have attractive properties in catalysis, photonics, and chemical sensing.<sup>[1]</sup> Raman spectroscopy is a powerful nondestructive technique,<sup>[2]</sup> the sensitivity of which can be significantly improved through surfaceenhanced or tip-enhanced methods.<sup>[3]</sup> The enhancement arises from the proximity of the analytes to intense localized fields created by nanoscale objects.<sup>[4]</sup> The capability to modify, coat, and pattern surfaces with nanostructures is important for SERS and also for a wider range of nanomaterials applications.<sup>[5]</sup> Conventionally, modified surfaces are constructed by delivering intact nanoparticles to target locations through dropcasting or spin coating.<sup>[6]</sup> However, the difficulty

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in positioning discrete particles with control over orientation, position, and degree of aggregation means that drop casting of nanoparticles has not been widely used in the high-throughput preparation of SERS substrates. Immobilized and shell-isolated nanosystems<sup>[5b, 6c, 7]</sup> address these issues, but the necessary vacuum preparation procedures significantly increase the complexity of such approaches.

Ion/surface collisions including ion soft-landing have been used to fabricate surface structures under vacuum.<sup>[8]</sup> Recently an electrolytic spray ionization method<sup>[9]</sup> has been developed that is capable of generating noble metal ions directly from their solids under ambient conditions as precursors for nanoparticle synthesis. Herein, we report the in situ fabrication of SERS-active spots and micro-scale patterns by landing ionized silver at desired locations where spontaneous cathodic reduction takes place, allowing the creation of nanostructure assemblies.

Silver is a widely used SERS material<sup>[10]</sup> and the plasmon resonance of silver nanostructures is tunable through the visible to mid-infrared regions of the electromagnetic spectrum.<sup>[11]</sup> Electrolytic spray deposition readily creates spots of approximately 3 mm in diameter composed of silver particles (AgNP) at desired locations, both on top of previously deposited analyte as well as prior to analyte deposition



**Figure 1.** a) Two AgNP-containing spots created on a penny coin by electrolytic spray ionization deposition under ambient conditions. The top spot was created before drop casting a crystal violet sample ("NP-below") while the lower spot was created on top of a layer of crystal violet ("NP-on-top"). 10 Monolayers (ML) of silver ions were landed to create both spots, which showed similar morphology (c, d) and enhanced Raman signals (b).

(Figure 1). Both the NP-on-top and the NP-below configurations prepared in this way showed uniformly distributed silver NPs in SEM images (Figure 1 c,d). The particles were polydispersed in size and shape, yet the morphology was uniform across each spot (Figure 2, and Figures S4–S7 in the Supporting Information). On the one hand, the polydispersity conferred surface plasmon resonance activity over a wide energy range, making the spots SERS-active when using lasers of different wavelengths (532, 633, and 785 nm) in the cases of crystal violet and Rhodamine 6G as probe molecules

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**Figure 2.** Effect of Ag coverage on SERS signal intensity from copper foil surface. See Figure S4 for the SEM images of the corresponding surfaces. Crystal violet ( $\approx 10^5$  molecules per  $\mu$ m<sup>2</sup>) was applied over the whole Ag deposition region by dropcasting. Each spectrum was acquired using 8.6 mW 633 nm laser excitation, with an acquisition time of 1 s.

(Figures S1 and S2). A resonance effect is evident using a 532 nm laser, but is avoided by using a 785 nm laser or by using R6G with a 633 nm laser. On the other hand, the uniform NP distribution resulted in numerous evenly distributed hot spots within each nanoparticle assembly (Figure S3). The robustness of this SERS surface is greatly enhanced by these features. Note that both the NP-on-top and the NPbelow surfaces showed similar SERS enhancements, (Figure 1b).

Uniform spatial distribution of the landed silver ions  $(10 \text{ nA})^{[9]}$  is critical for highly active surfaces. With the emitter tip (1–5 µm diameter) placed 5 mm from the cm-sized target, a roughly uniform charge distribution is created in the 2–5 mm diameter droplet plume, as mapped using a CCD atmospheric pressure ion detector (Figure S8).<sup>[12]</sup> The approximate uniformity in the central region of the spray was evident when examining the prepared structures using optical and electron microscopy. Coverage values were calculated from the accurately measured spot sizes and the logged deposition currents. The uniform SERS activity is most readily seen in Raman images (Figure S3a).

Using a copper foil as the support material, a coverage dependent study of the SERS enhancement was performed. As shown in Figure 2, the Raman signal for crystal violet (1  $\mu$ M in MeOH, 2  $\mu$ L dropcast to an approximately 3 mm spot) increased more than 10 times as the silver coverage increased from 1.6 ML to 5.5 ML and continued to increase until the CCD detector began to saturate at 9.9 ML when the average enhancement factor was  $4 \times 10^8$ .

SEM images for these surfaces (Figure S4) show single nanoparticles and a small number of aggregates at low surface coverage (1–3 ML). As the silver coverage was increased to 9.9 ML, the granules/particles grew larger and then aggregated with neighboring particles. This coverage-controlled in situ fabrication method produced uncapped NP structures. An important phenomenon is that features of the individual NPs were maintained during this aggregation process, creating numerous 1–5 nm gaps and crevices across the surface. This might be due to the fact that the particles were anchored to the metal surfaces during their growth. The nanojunctions and nanogaps (Figures S4 and S5d) observed are believed to be ideal for creating SERS hot spots,<sup>[10,13]</sup> although the stability of the surfaces in air is more noteworthy. The SERS peak intensities are summarized in Tables S1 and S2. The enhancement factors (Table S2) were calculated using a previously reported method (see the Supporting Information, Section 2).<sup>[7,14]</sup>

For a circular landing spot of 3 mm diameter, the 10 nA ion current is equivalent to 0.03 ML/minute. At this rate, it took 5 h to prepare a 10 ML spot. A higher landing current density was achieved by positioning the emitter closer to the surface or by increasing the spray voltage from 1.5 kV to around 2.5 kV, which also increased the fluctuation of both landing current and spot size. By placing a mask of nonconductive material (or electrically floated conductive material) on top of the deposition surface, the landing current density was increased reproducibly (see the Supporting Information, Section 5). The local electric field that produced a focusing effect is generated by charge buildup on the mask material during ion deposition.<sup>[15]</sup> The simplest form of this idea was realized by applying a perforated plastic foil, or electrically floated metal mesh, on top of the deposition target, as shown for one particular experiment (Figure 3).



**Figure 3.** Schematic of the ionization, focusing, and deposition experiment. The drawing is not to scale, but the important dimensions (diameters and distances) are labelled. As a result of the charge-induced focusing effect, the spot sizes in the deposited patterns are 10 times smaller than those in the mask.

In typical experiments, this focusing effect increased the landing current density by a factor of around nine, with a 5–25% decrease in total ion current. Arrays of AgNP-containing spots were created in a single deposition process, simply by using masks with an array of apertures (Figure 3, and Figures S9–11).

The operations just described are essentially lithographic approaches similar to stencil vapor deposition in vacuum.<sup>[16]</sup> Under ambient conditions, ion beams and charged droplets manipulated by electric fields, magnetic fields, and pneumatic forces,<sup>[17]</sup> should be useful in lithography applications. Beyond static patterns, a coupled moving stage allowed writing of more detailed subpatterns (Figure 4).

Aside from the other applications inherent in these 2D structures,<sup>[18]</sup> these patterns are easily identifiable under microscopes and the patterned images also help to distinguish the synthesized nanoparticles from adventitious particles inevitably present in ambient experiments.

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**Figure 4.** Optical images of a) AgNP patterns created using a grounded TEM grid as a static mask, and b) AgNP patterns created using a floated metal mesh as a mask while moving the copper target discontinuously underneath it. The movement created three-spot patterns visible at a number of locations. Images (c) and (d) are Raman maps of the selected ( $150 \times 150 \ \mu m^2$ ) regions in (a) and (b), respectively. Raman signals of the dropcast ( $\approx 3 \ mm$  diameter circle) crystal violet sample are only observable in the AgNP regions. The Raman intensity in the 1154 to 1204 cm<sup>-1</sup> range was used for imaging. The highest peaks in the images correspond to a Raman signal (1176 cm<sup>-1</sup> peak) of 41870 counts s<sup>-1</sup>·mw<sup>-1</sup> in (c), and 37690 counts s<sup>-1</sup>·mw<sup>-1</sup> in (d).

The choice of support material was also found to significantly influence the SERS activity of the deposited silver nanostructures, as is the case for other modification methods.<sup>[7,10,19]</sup> Generally, flat-polished slide supports gave much lower SERS enhancement factors under the same conditions ( $\approx 10$  ML Ag coverage, Table S2). Copper, aluminum, and gold foils (not flat at the microscale) gave the highest average SERS enhancement factors (exceeding  $10^7$ and reaching 10<sup>9</sup>), while brass and stainless steel foils gave weak enhancements. Although detailed mechanisms are not clear, the results for a gold foil support demonstrate that displacement plating<sup>[20]</sup> and oxide participation is not critical to the generation of SERS active surfaces by the electrospray deposition method. The flexible foils used here serve as efficient SERS sampling media, allowing dropcasting, wiping, spin coating, and spray deposition of samples. More importantly, samples can be present on the surface and hot spots can be generated in situ by depositing silver ions onto the sample spot (NP-on-top). Enhanced Raman signals were observed for all these methods.

In conclusion, the fabrication of nanostructures through electrolytic spray deposition is a "green", one-pot preparation method at ambient conditions that eliminates vacuum, lasers, and solution procedures associated with conventional nanofabrication. Micrometer-scale patterns can easily be made for SERS imaging and other purposes. Only sub-nanogram amounts of silver are consumed for each SERS substrate, spectra can be recorded in under a second, and the surfaces are stable in air for days. The enhancement factor is not unusual, but the simplicity of the fabrication method and the stability of the surfaces are noteworthy. Operation under atmospheric pressure further increases the ease of nanoscale surface modification. Electrolytic spray ionization deposition may serve well as a complement to sputtering or vapor deposition in other applications, such as the generation of plasmonic superstructures, catalysts, and in lithography.

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### Communications

#### Nanoparticle Spots

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Using Ambient Ion Beams to Write Nanostructured Patterns for Surface Enhanced Raman Spectroscopy



#### Electrolytic spray deposition was

employed for the formation of nanoparticle spots on various substrates in air. These materials are rugged, versatile substrates for surface-enhanced Raman spectroscopy, in which they lead to good enhancements. Lithographic applications of this method of ion deposition were also investigated.



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## Using Ambient Ion Beams to Write Nanostructured Patterns for Surface Enhanced Raman Spectroscopy\*\*

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### **Supporting Information**

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#### 1. Experimental

#### **Materials and Chemicals**

The support surfaces used in this experiment included ITO slides, aluminum coated (~100 nm) microscope glass slides (Deposition Research Lab, St. Charles, MO), gold (120 nm) with titanium (100 nm) adhesion layer coated microscope glass slide (Deposition Research Lab, St. Charles, MO), ITO coated glass slides (1.1 mm thickness, 1" × 3", (Nanocs, New York, NY)), heavy duty aluminum foil of 0.01 mm thickness (Durable Packaging International, Wheeling, IL), soft annealed copper foil of 0.05 mm thickness (McMaster-Carr, Elmhurst, IL). Silver foil, stainless steel foils, and gold foil of 0.01 mm thickness were purchased from Aldrich Chemical Company (Milwaukee, WI). P400 silicon carbide abrasive paper (Buehler, IL) was used to remove oxide layer and roughen surfaces when needed. TEM grids (Electron Microscopy Science) were used as received.

The metal electrodes used for electrolytic spray ionization were assembled as previously described.<sup>[1]</sup> HPLC grade acetonitrile and methanol (Chromasolv, Sigma–Aldrich) were used as received. Crystal violet and Rhodamine 6G (reagent grade, Sigma–Aldrich) were used as received.

#### **Chemical Instrumentation**

A home-built ambient ionization and deposition set-up was used to accurately log the number of ions delivered onto any collection surface.<sup>[1]</sup> Briefly, a wire-in-acetonitrile nanoESI source was subjected to a high voltage of ~1.5 kV. The ionic species generated by the ion source were recorded using an Orbitrap mass spectrometer (LTQ Orbitrap XL, Thermo, CA) before and after deposition. Metal-containing ions were directed to a grounded target surface. The recombination current through ground was monitored and logged once a second. Target surfaces were grounded and positioned 5-10 mm away from the tip of the spray emitter under ambient conditions. Monolayer coverage (ML) was calculated based on the total deposited charge and the measured size of deposition spot using electron or optical microscopes. Perforated masks were used when focused ion beams or specific spot sizes were needed.

The spatial distribution of ion current at the deposition surface was measured using an IonCCD detector system (OI Analytical, College Station, TX, USA).<sup>[2]</sup> The IonCCD<sup>TM</sup> is a pixelated charge detector consisting of an array of 21  $\mu$ m wide TiN pads or pixels 1.5 mm in height, separated by 3  $\mu$ m. When ions come in contact with the floated electrode surface they are neutralized and their charge is stored over a user-determined integration time. Following integration the charge on each pixel is read out serially and the resulting signal is reported in the form of a digital number (dN). The detector array and associated electronics are housed in a stainless steel enclosure with a 1.5 mm wide, 49 mm long slit

exposed to the detector surface. A detailed description of the detector operation is provided by Hadjar et al.<sup>[3]</sup> Unless otherwise noted, the integration time was set to 100 ms with 25 V being applied to the stainless steel housing of the detector.

SEM images and EDAX data were taken on a FEI Philips XL-40 Scanning Electron Microscope with a Schottky field emission gun. High resolution TEM images of the samples were obtained using a JEOL 3010 instrument with a UHR pole piece. Specimens for TEM analysis were prepared by placing a lacey carbon grid on top of the collecting surface.

Several Raman instruments equipped with different lasers were used to evaluate prepared SERS active surfaces. The first instrument was an Alpha-SNOM 300 S confocal Raman microscope (WITec GmbH, Germany) with a 532 nm laser as excitation source. Large area scans (4 mm × 4 mm) used 200 spots per line. Large area optical images were taken using the image stitch option in the software of this Raman instrument. The second instrument was an Alpha 300 confocal Raman microscope (WITec GmbH, Germany) with a 633 nm laser as excitation source. The third instrument was a near Infrared Raman imaging microscope (Olympus BX60) equipped with a 785 nm laser. The fourth instrument was a home built portable Raman microscope equipped with 532 nm laser. Raman signals were collected using objective lenses, laser power intensities and with an integration times denoted individually in each figure.

All the Raman spectra shown here have been background-corrected. The background correction was done using the WITec instrument software, initially the spectrum was fitted with a best fit polynomial and then that was subtracted from the original spectra. Raman images were generated based on the intensity of Raman peaks using the WITec software.

#### 2. Enhancement factor calculations and uniformity evaluation

The enhancement factor (EF) was calculated based on the measured Raman spectra. First the SERS intensities were compared with normal Raman intensities, corrected for the number of molecules under the laser spot. The formula to measure the EF is given as:<sup>[4]</sup>

$$EF = \frac{I_{SERS}/N_{surface}}{I_{normal}/N_{bulk}}....(1)$$

 $I_{SERS}$  and  $I_{normal}$  are the observed SERS intensities arising from the coating of analyte molecule (here, crystal violet (CV) or Rhodamine 6G (R6G)) on the Ag nanoparticle spot and the Raman intensity of analyte molecule in absence of nanoparticle (normal Raman signal).  $N_{bulk}$  and  $N_{surface}$  are the number of analyte molecules excited under the laser spot for the bulk specimen and the number of analyte molecules under the laser spot on the Ag nanoparticles, respectively. In this report,  $I_{SERS}$  and  $I_{normal}$  were taken from the normalized (for power and acquisition time) intensity of Raman shift at 1176 cm<sup>-1</sup> for CV and Raman shift of 1365cm<sup>-1</sup> for R6G.  $N_{surface}$  values are calculated using the formula given below:

$$N_{surface} = 4\pi r^2 \cdot C \cdot A \cdot N \dots (2)$$

where *r*, *C*, *A*, *N* are average particle radius of the Ag nanoparticles in the spot, surface density of the analyte monolayer, area of the laser spot and the average number of particles per square micrometer area, respectively. The average particle radius *r* was taken (from SEM measurement) as 32 nm, surface density of analyte molecule *C* was calculated as  $10^{5}/\mu$ m<sup>2</sup>, the area of the laser spot (50× objective, Numerical Aperture = 0.55) diameter was 3  $\mu$ m (*A* = 7.1 $\mu$ m<sup>2</sup>), and the number of particles per square micrometer *N* from SEM measurement is 255.

 $N_{bulk}$  was calculated using the formula:

$$N_{bulk} = N_A \cdot A \cdot h \cdot \rho / M_{\dots} \tag{3}$$

where A is area of the laser spot, h is penetration depth of the laser,  $\rho$  is density of the solid analyte (0.83 g/cm<sup>3</sup> in case of crystal violet), molecular weight of the analyte (in this work, 408 for crystal violet and 479 for Rhodamine 6G). The laser spot was 3µm diameter; penetration depth of laser h was taken as 20 µm.

Using these parameters and the previously quoted equation (1-3), the highest EF for the AgNP structured copper foil was calculated to be  $2 \times 10^{10}$ , Fig. S1 (a). Table S1 summarizes the enhancement factor of AgNP spots on various support materials.

Molecular electronic resonance Raman (RR) and surface-enhanced Raman effects were observed to increase Raman signal synergistically. Crystal violet has a wide absorption spectrum with an absorption maximum ranging from 420 to 600nm depending on the environmental pH. The resonance Raman contribution to the enhancement factor ( $EF_{RR}$ ) can be as much as  $10^{5-7}$  when the laser wavelength matches the electronic excitation energy of the analytes.<sup>[5]</sup> This might be one reason for the extremely high signal intensity when crystal violet was probed using a 532 nm laser, as shown in Figure S1. This contributing factor complicates interpretation of the SERS enhancement factor.



**Figure S1.** SERS spectra and average enhancement factor of crystal violet ( $10^5$  per  $\mu$ m<sup>2</sup>) on top of a AgNP nanostructure on copper foil substrate under excitation using (a) 532 nm, 20 mW (b) 633 nm, 8.6 mW (c) 785 nm, 52 mW laser sources. This is a copper foil surface modified by only ~7 ML of Ag ion, to avoid CCD saturation when using 532 nm laser.

The 785 nm laser is far away from the resonance of crystal violet. This experimental combination should give enhancement without interference of a resonance contribution. Fig. S1 (c) shows that the

near-IR laser gave an average enhancement factor of  $2 \times 10^5$ . The decrease of EF from  $10^8$ , however, may also be due to the different interaction between created nanostructures with the near IR laser. For this reason, R6G was tested at 633 nm (which is far from R6G's resonance)<sup>[5a]</sup> for a better comparison. The result was intermediate but still a very high enhancement factor of  $\sim 1 \times 10^8$  was seen, as shown in Fig. S2.

Another interesting phenomenon observed in this SERS experiment is that the SERS signal always decreased while recording of the spectrum. If the sample was slightly moved, the signal would rise (sometimes beyond the CCD saturation level) to a high value and then immediately decrease within the 1 second integration time. In the imaging mode, the excitation laser was attenuated to 1 mW to avoid possible saturation. Raman signals were taken over each pixel with a 0.01 second integration time and then the sample stage was moved to next pixel. Much higher signal intensities, as well as enhancement factor were obtained in this short acquisition time imaging mode.



**Figure S2.** SERS spectra of R6G ( $10^5$  per  $\mu$ m<sup>2</sup>) on top of AgNP nanostructure on top of copper foil using excitation from a 1 mW, 633 nm laser. The two spectra were taken with (a) 1 second (b) 0.01 second recording time from the same spot region. The peak height (relative to baseline) of the 1365cm<sup>-1</sup> band is labeled in both the spectra.

The above figure shows that the highest signal intensity found in the 1s integration scans is only 9 times higher than the highest signal intensity in 0.01 s integration scans. Similarly, when the laser power was turned down, the Raman signal decreased less than proportionately. For example, for the same substrate, the highest Raman intensity of 7,310 (Table S1) when the laser power was 8.6 mW only decreased to 4,253 when laser power was turned down to 1 mW. These phenomena could be the results of

thermal desorption of the molecule from the hotspot driven by laser heating, or the result of laser-induced melting of nanoparticles since no capping agent was used to protect the AgNPs. Even though the 0.01 integration time gave much better results, most of the EFs reported in Table S1 are based on 1 second integration time and with the consideration that most Raman spectrometers are built without image scanning functions. Also, for consistency, 8.6 mW laser power was also kept constant for the values in the Tables.

In summary, a resonance contribution may have increased the overall enhancement factor while laser induced damage could have decreased the actual enhancement factor. Future modifications to the surface may give even better performance for SERS applications.

#### SERS Uniformity of the modified surfaces

For high throughput SERS applications, surface uniformity is an important measure that determines the robustness of the experiment. Densely and evenly distributed hotspots would be ideal for rapid Raman analysis. In this experiment, the SERS uniformity of the modified surface within the same AgNP spot was evaluated by repeating measurements on randomly selected regions in that spot. The corresponding Raman signal intensity values from the AgNP spots on top of three different support materials were summarized in Table S1.

Support Material	region 1	region 2	region 3	region 4	region 5	mean	%RSD
Copper Foil	9747	12733	11510	12921	11204	11623	11%
Al foil	2302	1213	2173	1690	1739	1823	24%
Au foil	7310	5324	4880	6160	5259	5787	17%

Table S1. SERS intensity of band 1176 cm<sup>-1</sup> in different regions of spots created on different support materials, 10 ML Ag coverage, 8.6 mW, 633 nm laser excitation

Another way to evaluate SERS uniformity is to take Raman images of the surface. Fig. S3 (a) shows a randomly selected AgNP array composed of small spots. Evaluation of uniformity was done by Raman imaging of the different areas composing the spot. As shown in the below Fig. S3 (b) and (c), these spots are effectively identical for SERS purposes.



**Figure S3.** Raman images (3D plot viewed 45 degree angle) of (a) a randomly selected  $150 \times 150 \ \mu m^2$  region in a ~3 mm AgNP spot (~5 ML on Cu foil); and two spots (b, c) in an array (Fig. S11). Crystal

violet (~10<sup>5</sup> per  $\mu$ m<sup>2</sup>) was applied over the whole region by dropcasting a solution onto the copper foil. The images were generated using the 1176 cm<sup>-1</sup> peak intensity of crystal violet (using 1 mW, 633 nm laser excitation, and acquisition time of 0.01 second). The SERS uniformity within the bigger spot gave a maximum-minimum difference of only ~50% in a randomly selected 150×150  $\mu$ m<sup>2</sup> region. The SERS uniformity of smaller array spots is demonstrated by the similarity (in shape and intensity) of the Raman images of CV from two randomly selected spots in the array pattern. The volcano shape of the Raman imaging might be due to the flux distribution in the depositing ion plume when focused to 20  $\mu$ m.

There was no detectable change in surface activity when storing a substrate in air for three days. Even after 1 month, there was still ~10% activity for the surfaces. For an uncapped NP this is highly satisfactory. We believe that the surface anchoring contributes to the stability. In summary, the net enhancement signal is highly uniform, especially when considering the variation in the surface distribution of the analyte brought about by dropcasting.

#### 3. SEM images of Ag<sup>+</sup> modified surfaces

A series of surfaces was tested as supported materials for in situ preparation of AgNP by metal electrolytic spray ionization deposition. Different SERS performance was found for the different support materials as summarized in Table S2. These modified surfaces show different morphologies as imaged by scanning electron microscope. Even among the "good" substrates, different morphologies can be observed. Interestingly, the SERS surfaces retained >10% activity after exposure to SEM analysis.



**Figure S4.** Morphologies of surface nanostructures created by depositing different amounts of silver ions onto a copper foil using metal electrolytic spray ionization deposition. Coverage turned out to be one determining factor for the SERS performance of spots created by this surface modification method.



**Figure S5.** AgNP structures created by 10 monolayer coverage of  $Ag^+$  deposited onto aluminum foil. Polydispersed morphology was uniform throughout each spot created. (a), (b), (c) and (d) are images from four randomly selected regions located >200 µm from each other in the same deposition spot.



Figure S6. AgNP structures created by 10 monolayer coverage of Ag<sup>+</sup> deposited onto gold foil.



**Figure S7**. AgNP structures created by on top of (a) ITO coated slide and (b) aluminum coated glass slide. The AgNP nanostructures created on these polished flat surfaces do not give high quality SERS data at silver coverage 1-20 ML. Nanostructures created on these surfaces are either aggregates which are too large or individual particles are too small to have appropriately sized nano gaps.

## **Table S2.** SERS intensity of band 1176 cm<sup>-1</sup> of CV on AgNP spots created on different support materials(~10 ML Ag coverage), 8.6 mW, 633 nm laser excitation and 1 second acquisition time.

Support Material	Highest Peak Intensity (1176 cm <sup>-1</sup> )	Enhancement Factor
Copper Foil	12921	4E8
Gold foil	7310	2E8
Aluminum Foil	2302	7E7
Copper Tape	3731	1E8
Brass foil	40	1E6
Stainless Steel Foil	181	5E6
Silver foil	N. A.	N.A.
ITO coated slide	30	8E5
Aluminum coated slide	N.A.	N.A.
Penny coin, AgNP first	590	2E7
Penny coin, Sample first	471	1E7

#### 4. Deposition plume: Spatial flux distribution, coverage, morphology and SERS signal

Once loaded with anhydrous acetonitrile and in contact with the high voltage, the metal electrolytic spray ionization source readily generated silver-containing ions as the dominant ion signal as observed using an atmospheric pressure sampling mass analyzer.<sup>[1]</sup> The diameter of the charged droplet emitter tips was typically 1-5  $\mu$ m. After moving in ambient air along the electric gradient for ~5 mm, the spray plume diameter expanded to 1-5 mm. The metal ion distribution in this expanded plume may result in an uneven distribution of precursor ion concentrations on the collecting surface. When mapped using an Ion CCD (Fig. S7 (a) and (b)), a spray plume of ~3 mm diameter showed the maximum current in the center dropping slowly to less than 30% in the first 1.5 mm from the center. For the next 1 mm, the current dropped a lot more rapidly and accounted for the remaining 70% of the signal. For this reason, we assume a uniform distribution of deposited metal ions across most of the area inside the deposition circle. This "uniform in the center" assumption is largely valid as observed by optical and electron microscopes. Coverage can be controlled by deposition time with an estimation based on deposition current and spot size. The actual coverage of each experiment was calculated afterwards with the accurately measured spot sizes and the deposition currents logged by a computerized system.

$$Coverage = (\frac{N_A}{A \cdot F} \sum Idt) / ML$$

A is the measured spot area, F is the Faraday constant,  $N_A$  is the Avogadro constant, I is logged landing current, dt is the logging interval. ML is the monolayer atom density for silver,  $1.6 \times 10^{15}$ atom/cm<sup>2</sup>.



**Figure S8**. (a) A cross section scan and (b) the reconstructed contour plot of the ion intensity at the deposition surface as measured by the scan of the ionCCD. The elongation along the x-axis of the figure is due to distortion caused by the distance (1.5 mm) between the entrance slit and the detector boards. (c) Spot created by depositing silver ions for 12 hours with an average coverage of 100 ML. (d) On the edge of this spot, where the actual coverage varied due to the current density drop, rainbow-like color transition was observed. This means that the surface plasmon resonance of this modified surface area can be roughly tuned by **just** varying the coverage of depositing silver ions between 0 and 100 ML.

## 5. Ion beam focusing and creation of surface patterns using metal electrolytic spray ionization deposition with masks

Static patterns of nanoparticle containing spots were created by putting masks between the ion emitter and the deposition targets. Grounded conductive masks create a negative pattern by simply blocking ion deposition on the positive regions. Non-conductive and floated conductive masks, however, provides additional focusing effect that gives higher flux and smaller (than the mask holes' dimensions) spots.



**Figure S9.** (a) AgNP spot 4.9 mm<sup>2</sup> created on copper foil by direct deposition without using any focusing or masking. This spot of averaged 8 ML coverage of silver was created by 136min deposition of 13 nA landing current. (b) Using a perforated plastic tape mask (50  $\mu$ m thick, ~500 $\mu$ m diameter) on top of the deposition target, only 17 min of deposition was needed to get twice as much coverage for this 0.23 mm<sup>2</sup> spot even though the total depositing current dropped to 8 nA. Improved color uniformity was also achieved by this spot compared to (a). (c) An array of even smaller spots was created by using an arrayed mask. This dark green color is from ~ 50 ML coverage. 100  $\mu$ m spots (d) separate by < 2 mm is as good as hand-craftsmanship can get.



**Figure S10**. An array of AgNP spots deposited on top of a copper foil using a floated conductive stainless steel mesh as mask. The spots are 560 $\mu$ m away from each other. The uniform 20  $\mu$ m spots are created by mask with 200  $\mu$ m holes shown in the Fig. S11. This type of focusing might be useful for further downsizing the fabrication dimensions. The SERS activity of these spots is seen from the images in Fig. S3.



**Figure S11**. Stainless mesh used to create the pattern shown in Fig. S10. This mesh is a flat 200  $\mu$ m thick plate composed of with ~200  $\mu$ m holes. The floating/insulation from ground is achieved by 50  $\mu$ m separation with the deposition target.

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# Ag<sub>11</sub>(SG)<sub>7</sub>: A New Cluster Identified by Mass Spectrometry and Optical Spectroscopy

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Supporting Information

**ABSTRACT:** We report a one-step and high yield synthesis of a redluminescent silver cluster with the molecular formula,  $Ag_{11}(SG)_7$  (SG: glutathionate) via reduction of silver ions by sodium borohydride in the presence of the tripeptide, glutathione (GSH). The as-prepared cluster shows prominent absorption features at 485 and 625 nm in its UV–vis absorption spectrum. Aging of the as-prepared cluster solution led to the disappearance of the 625 nm peak, followed by broadening of the 485 nm peak to give three maxima at ~487, 437, and 393 nm in its absorption spectrum. These peaks remain unchanged even after polyacrylamide gel electrophoresis (PAGE), where a single band was observed confirming high purity of the cluster formed. Electrospray ionization mass spectrometry (ESI MS) reveal the composition of the cluster to be  $Ag_{11}(SG)_7$  with multiple sodium attachments to the ligand to give -3 and -2 charged species. These compositions match



well with their calculated isotope patterns. Extensive MS/MS was performed to understand the fragmentation. Potential atomic structures are discussed based on density functional theory calculations and comparisons for optical absorption spectra using  $Ag_{11}(SCH_3)_7$  as the model. Photoluminescence of this cluster was selectively quenched in the presence of Hg(II) and Cu(II) separately. Detection limit was found to be below their permissible limits in drinking water set by US EPA.  $Ag_{11}(SR)_7$  cluster is reported for the first time.

#### INTRODUCTION

Exploration of atomically precise monolayer-protected clusters of metals, particularly those of Au,<sup>1-4</sup> Ag,<sup>5,6</sup> Cu,<sup>7</sup> Pt,<sup>8</sup> and Pd,<sup>9</sup> is progressing enormously in the past decade due to interest in their unique optical, chemical, and biological properties. A series of gold and silver clusters with monolayer protection have been synthesized by several means, although synthesis of these clusters with atomic precision and their thorough characterization to obtain exact molecular formulas are challenging. The most common and well-established synthetic route is the reduction of an appropriate metal precursor in the presence of ligands of choice (water- or organic-soluble)<sup>10,11</sup> under suitable experimental conditions. Some of the other routes are core and interfacial etching of nanoparticles,<sup>12</sup> galvanic exchange,<sup>13</sup> microwave irradiation,<sup>14</sup> reduction by CO,<sup>15</sup> solid state route,<sup>16–18</sup> sonochemical,<sup>19</sup> and sunlightmediated<sup>20</sup> methods. Protein- and polymer-coated silver and gold nanoclusters have also been prepared.<sup>21</sup> Cavities within gels were used to control the growth of clusters.<sup>22</sup>

The most difficult task in characterization of clusters is finding their molecular formulas. Many reports are limited to the synthesis and studies of optical properties. The most reliable way to obtain the molecular formula of the clusters is from their crystal structures. Several crystal structures of noble metal nanoclusters exist although a large number of clusters are awaiting crystallization. A good number of thiol-protected gold clusters, namely,  $Au_{25}(SR)_{18}$ ,  $^{23,24}$   $Au_{36}(SR)_{24}$ ,  $^{25}$   $Au_{38}(SR)_{24}$ ,  $^{26}$   $Au_{102}(SR)_{44}$ ,  $^{27}$   $Au_{30}S(SR)_{18}$ ,  $^{28}$  etc., have been crystallized due to their better stability. However, inherently poor stability limited the crystallization of silver clusters to just a few; namely,  $[Ag_{14}(SC_6H_3F_2)_{12}(PPh_3)_8]$ ,  $^{29}$   $[Ag_{16}(DPPE)_4(SC_6H_3F_2)_{14}]$ ,  $^{30}$   $[Ag_{32}(DPPE)_5(SC_6H_4CF_3)_{24}]$ ,  $^{30}$  and  $[Ag_{44}(SR)_{30}]$ .  $^{31,32}$  Apart from the crystal structure, mass spectrometry is another way to understand the molecular formulas of the clusters.  $^{33-38}$  There are many reports of mass spectrometric studies of monolayer-protected gold clusters, but the number is still limited in the case of silver clusters especially when they are water-soluble. There are reports on silver clusters such as  $Ag_7(DMSA)_{41}$ ,  $^{39}Ag_{7,8}(MSA)_{7,8}$ ,  $^{12}Ag_9(MSA)_{7,1}$ ,  $^{17}Ag_9(SG)_{6,4}$ ,  $^{40}Ag_{15}(SG)_{11}$ ,  $^{41,42}Ag_{16}(SG)_{9,4}$ ,  $^{45}Ag_{152}(PET)_{60}$ ,  $^{18}$  etc., whose chemical formulas were obtained using mass spectrometry (DMSA, MSA, SG, and PET refer to dimercaptosuccinic acid, mercaptosuccinic acid, glutathione and phenylethanethiol, all in the thiolate form).

With regard to applications of noble metal clusters, there are reports on their use in energy, environment, biology, catalysis,

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etc.<sup>46</sup> One of the important advantages of water-soluble clusters is their use in sensing toxic metal ions in drinking water. Metal ions such as mercury, cadmium, and lead cause severe health effects in human and aquatic life.<sup>16</sup> It is very important to develop sensors for selective detection of this kind of ions down to their permissible levels. Absorption and photoluminescence features are found to be affected by the interactions of metal ions (and anions) with clusters. Possible reasons for photoluminescence quenching are aggregation of clusters,<sup>47</sup> redox reactions,<sup>16</sup> metallophilic interactions,<sup>13</sup> etching,<sup>48</sup> etc.

In this article, we report a one-step and high yield synthesis of monodispersed red-luminescent silver cluster of composition  $Ag_{11}(SG)_7$ . This core is reported for the first time. This cluster is characterized using mass spectrometric (ESI MS), spectroscopic (UV–vis absorbance, photoluminescence, X-ray photoelectron, and infrared spectroscopy), and microscopic (transmission electron microscopy) tools. Computational modeling suggests a structural motif that is validated by the comparison of calculated and measured optical absorption spectra. These clusters were employed for sensing Hg(II) and Cu(II) in water.

#### EXPERIMENTAL SECTION

**Chemicals.** All the chemicals were procured from various sources and used without further purification. Silver nitrate (AgNO<sub>3</sub>, 99%), glutathione reduced (GSH, 97%, Aldrich), acrylamide (AR grade),  $N,N^{I}$ -methylenebis(acrylamide) (BIS) (AR grade), ammonium persulfate, and  $N,N,N^{I},N^{I}$ -tetramethylethylene diamine (TEMED) were obtained from SRL Chemical Co. Ltd., India. Sodium borohydride (NaBH<sub>4</sub>, 99.99%, Aldrich), ethanol (HPLC grade, 99.9%, Aldrich), and methanol (HPLC grade) were used as received.

Synthesis of Ag<sub>11</sub>(SG)<sub>7</sub>. About 650 mmol of GSH was added to 50 mL of MeOH under ice cold conditions maintained through ice bath and stirred for 10 min. About 130 mmol of AgNO<sub>3</sub>, dissolved in 0.5 mL of Millipore water was mixed with the GSH solution, and the mixture was stirred for 15 min to form silver thiolates. About 7 mL (1.4 mol) of ice cold sodium borohydride was added to the mixture dropwise, and the solution stirred for another 15 min for complete reduction of thiolates to clusters. The as-formed clusters were not completely soluble in methanol, and they started precipitating. Excess MeOH was added for complete precipitation. The sample was centrifuged at 7000 rpm and washed repeatedly with methanol to remove the excess ligand and thiolates. The precipitate was dried by rotavapor evaporation to get a powder. About 55 mg of powder can be achieved in a single synthesis, and hence, the yield is about 85% in terms of Ag content in the reactant and the product.

**Polyacrylamide Gel Electrophoresis (PAGE).** PAGE was performed as per the literature.<sup>17</sup> A Biorad, Mini-protein Tetra cell with 1 mm thick spacer was utilized to process the PAGE. The as-prepared cluster was dissolved in water (10 mg/mL) and kept at 20 °C for 30 min. After that, the solution was centrifuged at 5000 rpm for 3-4 min and dissolved in 5% (v/v) glycerol–water solution (1 mL). The sample solution (1 mL) was loaded onto a 1 mm gel and eluted for 5 h at a constant voltage of 150 V. Only a single band was observed. The gel fraction containing the clusters was cut out, and the cluster was extracted into distilled water. The extracted cluster solution was centrifuged to remove the gel material and used further for UV–vis, PL, and ESI MS study.

**Sensing Metal lons.** Solutions of the cluster (~1 mg/mL water) and metal ions of known concentration were prepared

initially. Equal volumes of cluster and metal ion solutions (typically 3 mL each) were mixed, and PL measurements were taken after 5 min. It should be noted that the concentrations of metal ions mentioned are the final values in these solutions.

**Instrumentation.** UV–vis spectra were recorded with a PerkinElmer Lambda 25 instrument in the range of 200–1100 nm with a band-pass of 1 nm. Photoluminescence measurements were carried out on a Jobin Yvon NanoLog instrument. The band-pass for excitation and emission was set at 3 nm. X-ray photoelectron spectroscopy (XPS) measurements were done using an Omicron ESCA Probe spectrometer with polychromatic Mg K<sub> $\alpha$ </sub> X-rays ( $h\nu = 1253.6$  eV) with a constant analyzer energy of 20 eV. High resolution transmission electron microscopy of clusters was carried out with a JEOL 3010 instrument. The samples were drop-cast on carbon-coated copper grids and allowed to dry under ambient conditions. FTIR spectra were measured with a PerkinElmer Spectrum One instrument. KBr crystals were used as the matrix for preparing samples.

**Mass Spectrometric Analysis.** Thermo scientific LTQ XL ESI MS was used for mass spectrometric analysis. Experiments were carried out in both positive and negative ion modes, but nothing significant was observed in the positive mode other than the ligand. Therefore, further experiments were done in the negative ion mode. MS<sup>2</sup> experiments were performed in the collision induced dissociation (CID) mode with varying collision energy. We have also performed a capillary temperature-dependent study, but no extra peak was observed. As the instrumental settings are important, we list them below:

solvent: 1:1 (v/v)  $H_2O/MeOH$ sample flow rate: 10  $\mu$ L/min vaporizer temperature: 582 °C capillary temperature: 275 °C source voltage: 5.47 kV source current: -6.31  $\mu$ A capillary voltage: -42.97 V tube lens voltage: -82.66 V

We have performed several control experiments to confirm that our cluster is not a fragment from a bigger cluster due to the temperature or potentials used in ESI MS. We have performed a capillary temperature-dependent experiment ranging from 80 to 300 °C, and no change in the mass spectrum was seen. We have also changed source voltage from 1.5 kV to 8 kV and found that only peak intensity changes while changing the voltage. No new peak appeared at higher mass at lower voltages or reduced temperatures.

#### RESULTS AND DISCUSSION

**Spectroscopic Characterization.** Clusters were synthesized following the above-mentioned method. The reaction was done in the presence of excess MeOH at ice cold conditions. MeOH was used for precipitating the clusters and to remove excess ligand and thiolate, which probably helped us to get this stable cluster in solution. In our method, we have done the reaction in MeOH, and hence, once the cluster was formed, it started precipitating due to the presence of excess MeOH in the solution. By this process we could avoid contamination of excess ligand and thiolates, which are soluble in methanol. The precipitate was centrifuged and washed repeatedly to remove excess thiol (if any) and dried by rotavapor to get a powder. The yield was 85% in terms of the silver content.



Figure 1. (A) UV-vis absorption spectra of as-synthesized, washed, and aged silver cluster. The clusters show two peaks at 485 and 625 nm (black and red traces) where the 625 nm peak disappeared with time and the other peak broadened into three smaller humps positioned at 393, 437, and 487 nm (blue trace). Photoluminescence spectra are shown in B, where all the three excitation maxima (385, 440, and 510 nm) resulted in a single emission maximum at 705 nm. Photographs of the as-synthesized cluster under UV and visible light are shown in inset (i).

Optical absorption spectrum of the as-synthesized (crude, without washing) cluster showed two humps positioned at 625 nm (1.98 eV) and 485 nm (2.55 eV), with the latter being more prominent. The peak positions remain the same after washing with MeOH. The peak shape started changing with the disappearance of the 625 nm peak and broadening of the 485 nm peak, which resulted in three relatively smaller humps centered at 487, 437, and 393 nm. This happened at room temperature (25 °C) when the clusters were dissolved in water and kept for 30 min (see Figure 1A). This conversion implies that in the beginning a metastable cluster was formed, which was converted to a stable one in solution. After this, the spectrum remained the same without any change in absorbance.

Three distinct excitation peaks were observed at 385, 440, and 510 nm in the photoluminescence spectrum, all of which resulted in the same emission maximum at 705 nm (Figure 1B). The clusters are red-luminescent under UV irradiation as shown in Figure 1A. As reported before, water-soluble silver clusters do not show high quantum yield unlike gold clusters. We have obtained a quantum yield of 0.8% for these clusters using Rhodamine 6G as the reference.

To check whether the cluster is a single one or a mixture, we have performed PAGE separation, a well developed technique in cluster science. We have observed a single band in PAGE (see the inset of Figure 2) confirming the high purity of the assynthesized cluster. There are reports<sup>49</sup> where PAGE was used for the analysis of mixture of clusters.

We have cut the band, and the cluster was extracted into water for further study. The same UV–vis absorption features were observed confirming that the cluster is monodisperse in as-synthesized form (Figure 2). The band was also fluorescent under UV irradiation and showed similar excitation and emission feature in the photoluminescence spectra as shown in Figure 1B. We have compared our UV–vis absorption data with previously reported glutathione protected silver clusters.<sup>49</sup> Our absorption spectral data are nearly matching with band 2 in this report, which was assigned to be Ag<sub>15</sub>. This implies our cluster is unlikely to be bigger than Ag<sub>15</sub>. Monodisperse clusters



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**Figure 2.** UV–vis absorption spectra of silver clusters remain the same before and after PAGE separation. A single band in PAGE (inset (i)) confirms the high purity of the cluster in the as-synthesized form. The band was cut and the cluster was extracted into water. This cluster exhibits red luminescence under UV irradiation (shown on the right).

were observed in TEM analysis with an average size of 1 nm as shown in Figure S1 (Supporting Information). XPS survey spectrum of clusters confirms the presence of elements, Ag, S, C, O, N, and Na (Figure 3). Silver in the cluster close to the zerovalent state was confirmed from the Ag  $3d_{5/2}$  position in XPS (368.0 eV) (Figure 3i). However, the S  $2p_{3/2}$  peak at 162.5 eV proved the Ag–S bonding in the cluster (Figure 3ii). Thiolate bound to noble metals is seen at this binding energy (BE).<sup>12</sup> Infrared spectroscopy was performed to check the corresponding changes in the ligand after cluster formation. The S–H stretching frequency at 2525 cm<sup>-1</sup> was absent after cluster formation, which confirms successful binding of Ag to sulfur of glutathione (Figure S2, Supporting Information).

**Mass Spectrometric Understanding.** A 1:1 MeOH: $H_2O$ (v/v) mixture was used for further characterization of the



Figure 3. XPS survey spectrum of AgSG cluster showing all the expected elements, Ag, S, C, O, N, and Na. Ag 3d and S 2p regions are expanded in (i) and (ii), respectively, showing Ag in  $Ag^0$  state and sulfur bound to metal.

cluster using ESI MS. A negative ion ESI MS is shown in Figure 4 where two distinct envelopes (labeled \*) were observed in



Figure 4. ESI MS of the clusters in the negative ion mode showing -2 and -3 charged species along with some thiolates. Main peaks are marked with the \* symbol. Experimental spectrum (blue trace) is in good agreement with the calculated mass spectrum (red trace) of the species as shown in the inset.

the mass range of m/z 1100–1200 and 1650–1800 with specific separation between the neighboring peaks due to sodium attachment. Besides, a few thiolates were also seen. The optimized conditions are given in the experimental section. No peak was observed in the higher mass region confirming absence of any bigger cluster core. We have thoroughly examined each and every parameter affecting the mass spectrum. We have performed flow rate, capillary voltage, and capillary temperature dependence in details. At lower flow rate, peaks were not resolved well with proper isotope distribution. At higher flow rate, peaks become broader. About 10  $\mu$ L/min was the optimum flow rate where we achieved a good signal intensity as well as resolution. We have tried to see the effect of capillary temperature over a range of 80 to 300 °C with 10 °C increase in each step. Here, no difference in the peak positions other than signal intensity was noticed. As the cluster is smaller in size, it might be a fragment from a bigger parent cluster, which can happen in the presence of high voltage. We did not find any new peak at lower (1.5 kV) as well as higher (8 kV) source voltages, confirming that this is a new cluster and not a fragment. Note that smaller clusters like Ag<sub>7</sub>, Ag<sub>8</sub>, Ag<sub>9</sub>, etc., were reported and characterized before, using ESI MS.<sup>12,16,17</sup> We also have checked ESI MS of the PAGE separated sample, which resulted in the same mass peaks. Experimental and calculated spectra are in agreement with the assigned species as shown in the inset of Figure 4 for a triply charged species.

Peaks in the m/z 1100–1200 region are separated by m/z 7.3 corresponding to a triply charged species (Figure 5A),



**Figure 5.** Expanded negative mode ESI MS of  $Ag_{11}(SG)_7$  clusters in m/z 1100–1150 (A) and 1650–1800 (B) ranges. Multiple sodium attachments to glutathione ligand is evident from both the double (A) and triply (B) charged species. Good match of the experimental and calculated mass spectra for  $Ag_{11}(SG)_7$  with and without sodium adducts is also seen.

suggesting the presence of a species with Na attachment (m/z [Na-H]/3 = 7.33), whereas the separation is m/z 11 (m/z [Na-H]/2 = 11) for doubly charged species (in the range of m/z 1650–1800, Figure 5B). On the basis of these, the cluster is assigned to have a formula Ag<sub>11</sub>(SG)<sub>7</sub>.

Analogous Au clusters have been reported as well as crystallized with phosphine ligand in 1970.<sup>4,50</sup> The calculated mass spectra of the clusters with specific charge state matches well with the experimentally observed one as shown in the inset of Figure 5A,B where both triply charged and doubly charged envelopes are expanded and plotted along with the calculated spectra. Multiple Na attachments are possible with GSH due to the presence of two –COOH groups. Such attachments were observed in Ag clusters before.<sup>12,16,17</sup> For this specific cluster of interest, which contains 7 ligands, a maximum of 13, 12, and 11 Na attachments are possible for singly, doubly, and triply negative species, respectively. This observation was clear for the –2 charge region where a series of peaks separated by m/z 11 were observed and a maximum of 12 Na attachments is clear confirming the exact number of ligands (Figure SB).

Extensive MS/MS analysis was performed to understand the fragmentation pattern of the ions (Figure 6). When the triply charged species (m/z 1130 was selected with an isotope width of 60, i.e., m/z 1100–1160, assigned to Ag<sub>11</sub>(SG)<sub>7</sub>Na<sub>n</sub><sup>3-</sup>) was allowed to fragment under collision induced dissociation (CID) with varying collision energy (Figure S3, Supporting Information), it readily loses one AgSG and forms Ag<sub>10</sub>(SG)<sub>6</sub><sup>2-</sup>. At higher collision energy, we can also see some smaller thiolate fragments such as Ag<sub>3</sub>SG<sub>2</sub><sup>-</sup>. When this Ag<sub>10</sub>(SG)<sub>6</sub><sup>2-</sup> fragment was further allowed to dissociate under

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**Figure 6.** (A) ESI MS/MS of  $[Ag_{11}(SG)_7Na_n]^{3-}$  showing one AgSG loss, and the corresponding peak is  $[Ag_{10}(SG)_6Na_n]^{2-}$ . Collision energy dependence study of  $Ag_{11}(SG)_7Na_n^{3-}$  is shown in Figure S5, Supporting Information. When  $[Ag_{10}(SG)_6Na_n]^{2-}$  fragment was further dissociated in MS<sup>3</sup>, it fragments to various thiolates like  $Ag_3(SG)_2^-$ ,  $Ag_5(SG)_4^-$ ,  $Ag_8(SG)_6^-$ , etc., as shown in B. Same fragments were also observed with  $Ag_8(SG)_6Na_n^{2-}$  alone under MS<sup>4</sup> to produce  $Ag_5(SG)_4Na_n^-$  and  $Ag_3(SG)_2^-$  as shown in C.

CID (MS<sup>3</sup>), it leads to various thiolates namely,  $[Ag_3(SG)_2]^-$ (m/z 935),  $[Ag_8(SG)_6Na_n]^{2-}$  (m/z 1349 for n = 0),  $[Ag_5(SG)_3Na_n]^ (m/z \ 1457 \ \text{for} \ n = 0), \ [Ag_5(SG)_4Na_n]^- (m/z)^$ z 1763 for n = 0, and  $[Ag_7(SG)_6Na_n]^- (m/z 2591$  for n = 0)(Figure 6B). Further fragmentation of  $[Ag_8(SG)_6Na_n]^{2-}$  led to  $[Ag_3(SG)_2]^-$  and  $[Ag_5(SG)_4Na_n]^-$  fragments (Figure 6C). MS/ MS of doubly charged species resulted in one AgSG loss similar to the triply charged one (Figure S4, Supporting Information). Some species of higher masses were also observed when higher collision energies were used. These species include  $[Ag_{12}(SG)_{10}Na_n]^{2-}$   $(m/z \ 2177 \ for \ n = 0)$  and  $[Ag_{16}(SG)_{15}Na_n]^{3-}$  (m/z 2105 for n = 0) along with other thiolates. This phenomenon can be explained in terms of gas phase association of the fragments in the trap to form larger thiolates. If we look at the fragments carefully, most of them consist of at least one extra metal atom compared to the ligand. Considering a linear chain of thiolates, the Ag atoms in them may be positioned at the tail of the chain, and they may associate with other fragments of free ligands (generated by CID).

This in fact proves the accessibility of reacting molecules to the Ag sites in the cluster. This observation proposes new possibilities for catalysis and gas phase reactions. Single cluster (by trapping specific ion) catalysis may be achieved for reactions such as oxidation of CO and alkene to  $CO_2$  and epoxide, respectively. Note that such types of catalysis are reported for gas phase gold and silver clusters.<sup>51–53</sup> Au<sub>25</sub>(SR)<sub>18</sub> and Au<sub>38</sub>(SR)<sub>24</sub> were used successfully as a catalyst for the conversion of CO to CO<sub>2</sub> where a metal oxide, CeO<sub>2</sub>, was used as the support.<sup>54–56</sup>

**Theoretical Calculation.** Structural search via density functional theory (DFT) by using  $Ag_{11}(SCH_3)_7$  as a model

system indicated a family of low-energy structures that all share an Ag<sub>7</sub> core protected with two Ag(SR)<sub>2</sub> and one Ag<sub>2</sub>(SR)<sub>3</sub> motifs. Figure S5, Supporting Information, shows six structural candidates that are all located within 0.85 eV (Table S1, Supporting Information). Their HOMO–LUMO energy gaps vary within 1.65 to 2.29 eV indicating a remarkable electronic stability. The computed optical spectra show two to three absorption features (Figure S6a–f, Supporting Information). The best agreement to experiment is shown by structure "f" that has absorption maxima 360, 410, and 509 nm as shown in Figure 7. These compare quite well to the experimental peaks



**Figure 7.** Computed optical spectrum of  $Ag_{11}(SCH_3)_7$ , structure f. The individual optical lines are broadened with 0.05 eV gaussians. The structure is shown as an inset.

at 393, 437, and 487 nm (Figure 1). This structural isomer is within 0.2 eV from the lowest-energy structure. Use of a different (simple, noncharged) thiol in the computational models may explain the uncertainty to assign a prominent "best" structural candidate in this case. Use of the actual glutathione as a ligand would increase the computational effort significantly due to requirements to consider ligand-charging and solvent interactions as well. We also searched structures based on the crystallographically known Au<sub>11</sub>(PR<sub>7</sub>)Cl<sub>3</sub> cluster.<sup>44</sup> The resulting cluster is a very high-energy isomer (1.65 eV) and its HOMO–LUMO gap is rather small (0.84 eV) indicating that structural motifs that are known for gold may not be formed for silver.

Clusters for Metal Ion Sensing. The as-synthesized clusters are red luminescent under UV light. This property was utilized for sensing metal ions. A series of transition metal ions, namely, As(III), As(V), Cd(II), Co(III), Cu(II), Fe(III), Hg(II), Pb(II), and Zn(II) were used for this study. Among them, Cu(II) and Hg(II) showed significant quenching in luminescence as shown in Figure 8A. Relative luminescence intensity is plotted in Figure 8B. Corresponding photographs are shown in the inset. Hg(II) contamination in water is one of the examples of heavy metal toxicity. It is known that Hg(II)interacts with the metal cluster core and oxidize the metal, and by this redox process, the luminescence is quenched.<sup>16</sup> We examined the detection limit of Hg(II) using fluorescence intensity. Just after the addition of 1 ppb (final concentration), there is an instant decrease of 15% in the fluorescence intensity. There was a linear decrease with subsequent increase in Hg(II)

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**Figure 8.** (A) Sensitivity of the photoluminescence of  $Ag_{11}(SG)_7$  cluster toward different metal ions (5 ppm, final metal ion concentration). The photoluminescence was totally quenched in the presence of Cu(II) and Hg(II). (B) Bar diagram representing the relative decrease in fluorescence intensity in the presence of different metal ions where Hg(II) is showing maximum quenching. Photographs of the samples are shown in the inset. (C) Hg(II) concentration-dependent quenching of cluster luminescence. (D) Relative luminescence intensity vs Hg(II) concentration is plotted showing a linear decrease in emission intensity in the 10–1000 ppb range. Photographs of the corresponding samples are shown in the inset.

concentration up to 1000 ppb, as shown in Figure 8C,D. The detection limit was found to be 1 ppb based on photoluminescence quenching. This is below the limit (2 ppb) set by United States Environmental Protection Agency (US EPA) for drinking water. However, for a practical application, significant additional work is needed. The cluster was sensitive to copper also down to 0.5 ppm due to metallophilic interactions.

#### SUMMARY AND CONCLUSIONS

In summary, we present a combined experimental and theoretical study of a new silver cluster with molecular formula,  $Ag_{11}(SG)_7$ . One-step, high yield synthesis resulted in a monodisperse red-luminescent cluster. Theoretical modeling on the structure and UV-vis absorption spectrum support our experimental findings and the formula of the cluster. DFT calculations suggest a seven atom core protected with two  $Ag(SR)_2$  and one  $Ag_2(SR)_3$  motifs. Detailed mass spectrometric investigation confirmed the presence of a single cluster. Extensive MS/MS was performed to understand the fragmentation pattern and thiolate formation under collision induced dissociation process. Gas phase association of smaller fragments to create larger analogues opens up new possibilities of catalysis, and studies proved the availability of free Ag sites on the cluster fragments, which could yield specific reactions. Finally, Ag<sub>11</sub> clusters were used in specific sensing of Cu(II) and H(II) ions down to permissible limits in drinking water.

#### ASSOCIATED CONTENT

#### **Supporting Information**

TEM, FTIR, collision energy-dependent ESI MS/MS, computational methods, calculated low energy isomers, and corresponding absorption profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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#### Notes

The authors declare no competing financial interest.

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# Supporting Information Ag<sub>11</sub>(SG)<sub>7</sub>: A new cluster identified by mass spectrometry and optical spectroscopy

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**Figure S1:** TEM image of the as-synthesized AgSG clusters. A few clusters are marked with circles in the inset (an expanded view).



**Figure S2:** FTIR spectra of GSH and AgSG cluster showing absence of S-H stretching (at 2525 cm<sup>-1</sup>) for clusters which confirms successful binding of the ligand with Ag.



**Figure S3:** Collision energy dependent ESI MS/MS of  $Ag_{11}(SG)_7Na_n^{3-}$  showing one AgSG loss to give  $Ag_{10}(SG)_6Na_n^{2-}$ . As we keep on increasing the collision energy selectively,  $Ag_{10}(SG)_6Na_n^{2-}$  species forms. The Na attachment series is expanded in the inset. The separation in m/z corresponds to Na attachment for a -2 charged species. The experimentally observed isotope pattern matches well with the theoretically calculated one as shown for  $Ag_{10}(SG)_6Na_8^{2-}$ .



**Figure S4:** Collision energy dependent ESI MS/MS of  $[Ag_{11}(SG)_7Na_n]^{2-}$  showing AgSG loss to give  $[Ag_{10}(SG)_6Na_n]^{2-}$ . Some higher mass species are also formed. These can be attributed to gas phase association of the fragments in the ion trap.

#### **Computational Methods**

The DFT calculations were performed with the GPAW code (1), which implements projectoraugmented wave method in a real-space grid. The grid spacing is 0.2Å. Ag(4d<sup>10</sup>5s<sup>1</sup>), S(3s<sup>2</sup>3p<sup>4</sup>), C(2s<sup>2</sup>2p<sup>2</sup>) and H(1s<sup>1</sup>) electrons were regarded as the valence. The PAW setups for Ag included scalar-relativistic corrections. Total energies were evaluated at the GGA-PBE (gradient-corrected functional of Predew, Burke, and Ernzerhof) level (2). All the atoms were relaxed during the geometry optimization until the maximum force acting on atoms was below 0.5eV/Å. Optical absorption spectrum was calculated at the GGA-PBE level using linear-response (LR) timedependent DFT (LR-TDDFT) formalism in GPAW (3).

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**Figure S5:** Two views of each of the six low-energy isomers of Ag<sub>11</sub>(SCH<sub>3</sub>)<sub>7</sub>. Ag: grey, S: yellow, C: dark grey, H: white.


Figure S6: Computed optical spectra of cluster isomers **a** to **f** shown in Figure S10. The individual optical transitions have been folded into a smooth curve by using a Gaussian width of 0.05 eV.

**Table S1:** Calculated relative total energies and HOMO-LUMO (HL) gaps of isomers  $\mathbf{a}$  to  $\mathbf{f}$  of $Ag_{11}(SCH_3)_{7.}$ 

Isomer	Rel. energy (eV)	HL gap (eV)
a	0.854	1.79
b	0.020	2.08
c	0.647	1.65
d	0	2.29
e	0.535	1.78
f	0.196	2.03

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# Controlled synthesis and characterization of the elusive thiolated Ag<sub>55</sub> cluster†

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A stable, Ag<sub>55</sub> cluster protected with 4-(*tert*-butyl)benzyl mercaptan (BBSH) was synthesized which exhibits two prominent absorption bands with maxima at 2.25 and 2.81 eV. A molecular ion peak at m/z 11500  $\pm$  20 in matrix assisted laser desorption ionization mass spectrum (MALDI MS), assigned to Ag<sub>55</sub>(BBS)<sub>31</sub> was observed. Electrospray ionization (ESI MS) shows a prominent trication along with higher charged species. An analogous Ag<sub>55</sub>(PET)<sub>31</sub> (PET = 2-phenylethanethiol, in the thiolate form) was also synthesized under optimized conditions which proves the amenability of this cluster and the synthetic methodology to other ligands.

Monolayer protected noble metal quantum clusters (QCs) composed of few atoms are completely different from their corresponding bulk analogues.<sup>1-3</sup> Due to their unique optical and luminescent properties, they have been applied in diverse applications such as sensing,<sup>4-6</sup> catalysis,<sup>7,8</sup> antibacterial,<sup>9</sup> drug delivery,<sup>10</sup> etc. Stable QCs satisfy the criteria of either geometric (*e.g.*  $Au_{13}$ ,<sup>11</sup>  $Au_{55}$ ,<sup>12</sup> *etc.*) or electronic (*e.g.*  $Au_{11}(PH_3)_7(SMe)$ ,<sup>13</sup>  $Au_{25}SR_{18}$ ,<sup>14,15</sup>  $Au_{102}(SR)_{44}$ ,<sup>16</sup> *etc.*) closed shells; the latter may form by ionic cores as well. Among such stable clusters, Au<sub>55</sub>(PR<sub>3</sub>)<sub>12</sub>Cl<sub>6</sub> is an early example.<sup>17</sup> Inertness of the Au<sub>55</sub> nucleus, ability to make bare clusters<sup>18</sup> and crystals,<sup>19</sup> observation of phenomena such as single-electron tunneling<sup>20</sup> and interactions with several bio-systems<sup>21</sup> made this cluster interesting. Recently, new methods have been developed to synthesize thiol protected Au<sub>55</sub> in the organic phase which facilitated further characterization.<sup>22-25</sup> Although several QCs of silver with known chemical composition such as water soluble Ag<sub>7</sub>,<sup>26</sup> Ag<sub>7,8</sub>,<sup>27</sup> and Ag<sub>9</sub>,<sup>28</sup> as well as organic soluble Ag<sub>44</sub>,<sup>29,30</sup> Ag<sub>140</sub>,<sup>31</sup> Ag<sub>152</sub>,<sup>32</sup> and Ag<sub>280</sub>,<sup>33</sup> have been made and crystal structures of Ag<sub>14</sub>,<sup>34</sup> Ag<sub>16</sub>,<sup>35</sup> Ag<sub>32</sub>,<sup>35</sup> and Ag<sub>44</sub>,<sup>36,37</sup>

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have been reported, the growth of the area is not comparable to that of gold analogues.<sup>1,14,16,24,38-40</sup> This is especially noticeable from the absence of reports of clusters of the kind, Ag<sub>13</sub> and Ag<sub>55</sub> for which the gold counterparts have been known since 1981. Recently, we have shown the optical spectrum of the 2-phenylethanethiol protected Ag<sub>55</sub> cluster while exploring the appearance of plasmon excitation in silver clusters.<sup>41</sup>

Here, we report thiolated  $Ag_{55}$  clusters and their most essential characterization. The synthesis involves reaction of the metal salt (AgNO<sub>3</sub>) and the thiol (BBSH) in a 1:4 ratio in a mortar and pestle to form the thiolate in the solid state. Addition of NaBH<sub>4</sub> powder under the laboratory atmosphere and continued grinding makes the clusters which are extracted with toluene, to give a crude sample. The toluene extract was precipitated by MeOH and re-extracted in toluene which results in the purified cluster sample. The cluster is stable for 8 days in solution under ambient conditions. The stability enhances under reduced temperatures. A powder sample is stable for extended periods (additional information is given in ESI 1<sup>†</sup>).

MALDI MS analyses (all experimental details are given in ESI 1<sup>†</sup>) were done on crude (red trace in Fig. 1) and purified (black trace in Fig. 1) clusters using trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) as the matrix which is known to be ideal for organic thiol-protected metal nanoclusters.<sup>41,42</sup> Threshold laser power  $(f_{th})$  was used to detect the molecular ion peak without fragmentation. For both the cases, molecular ion peak at m/z 11 500  $\pm$  20 (the uncertainty is largely due to the isotope distribution and also due to inherent instrumental limitations) was seen and was assigned as Ag<sub>55</sub>(BBS)<sub>31</sub> (expected mass: 11 510). Even though the peak maximum was the same for the purified and crude cluster samples, there was a difference in peak width between the two. The purified cluster has a full width at half maximum (FWHM) of 3.3 kDa whereas it is 5.5 kDa for the crude cluster. This increased width may be due to the presence of extra thiols or thiolates in the crude cluster which is responsible for peak broadening. FWHM of silver clusters are broad in nature compared to gold clusters and we have studied them extensively.<sup>41</sup> Laser intensity-dependent study (Fig. S2, ESI<sup>†</sup>) shows



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<sup>†</sup>Electronic supplementary information (ESI) available: Details of experimental procedures and characterization using ESI MS, XPS, SEM/EDAX, XRD of Ag<sub>55</sub>(BBS)<sub>31</sub> clusters. See DOI: 10.1039/c4dt02476a



**Fig. 1** MS spectra of the as-synthesized crude cluster (red trace) and the purified Ag<sub>55</sub> cluster (black trace). Inset shows the UV/Vis spectra for the same (a: the crude cluster, b: purified cluster) plotted as a function of energy. Jacobian-corrected intensities are plotted (details are given in ESI†). The purified cluster shows two prominent bands at 2.25 and 2.81 eV and a weak band at 1.93 eV (marked) whereas the crude one shows only a broad band at 2.28 eV. Inset of inset shows the ESI† mass spectrum of the purified cluster (in positive mode) which shows a prominent peak at m/z 3870 corresponding to  $[Ag_{55}(BBS)_{31}Cs]^{3+}$ . The corresponding calculated isotope distribution is shown in green.

similar observation as reported in the case of Ag<sub>152</sub>(PET)<sub>60</sub>.<sup>42</sup> With increase in laser fluence,  $f = xf_{\rm th}$ , x > 1.02, a systematic mass loss was observed till x = 1.37. No further loss was seen after that. Like the Ag<sub>152</sub> cluster,<sup>42</sup> a similar sharp rise at the low mass side and steep fall at the high mass side was seen when laser fluence was varied. Significant difference was also observed in the optical spectra (inset of Fig. 1) for both the clusters. The crude cluster shows a broad feature at 2.28 eV (543 nm) whereas the purified cluster shows two prominent bands at 2.25 eV (550 nm) and 2.81 eV (440 nm) along with a week band at 1.93 eV (640 nm). The prominent step-like feature and absence of plasmon (seen in nanoparticle) confirmed the formation of a molecular species.

ESI MS is an ideal tool to determine the precise composition of nanoclusters.<sup>25,27,28,43</sup> As BBSH is completely nonpolar, cesium acetate (CsOAc) was used to ionize the molecule. The cluster solution was taken in a toluene-methanol mixture and the spectra (Fig. S3, ESI<sup> $\dagger$ </sup>) were collected in the *m*/*z* range of 200-4000 (maximum limit of the instrument). A prominent peak at m/z 3870 (inset of Fig. 2) corresponding to [Ag<sub>55</sub>(BBS)<sub>31</sub>Cs]<sup>3+</sup> which matches exactly with the calculated spectrum (green trace) was observed. The two isotopes of silver, namely, 107 and 109, with equal natural abundances and the isotopes of carbon and sulfur together make the overall cluster peak too complex and specific peaks were not resolved for this charge state. However, the spectral envelope was reproduced clearly and was identical to the calculated spectrum. Systematic Cs addition to this peak was also observed. As the peaks are due to 3+ charge, the peak separation was 133/3 = 44.3, which is marked in the figure. This

type of Cs induced ionization and corresponding Cs addition peaks have been reported for the Au<sub>333</sub>(SCH<sub>2</sub>CH<sub>2</sub>Ph)<sub>79</sub> cluster.44 The composition was further supported by the presence of  $[Ag_{55}(BBS)_{31}Cs_4]^{4+}$ ,  $[Ag_{55}(BBS)_{31}Cs_5]^{5+}$  and  $[Ag_{55}(BBS)_{31}Cs_6]^{6+}$  at m/z 3005, 2431 and 2048, respectively (Fig. S3, ESI<sup>†</sup>), although these peaks are not prominent. It is important to recall that well-defined mass spectra of this kind are rare in the case of silver clusters (except for the Ag<sub>44</sub> cluster which shows distinct mass spectral features). We could not see the 4+, 5+ or 6+ ions of [Ag<sub>55</sub>(BBS)<sub>31</sub>] without Cs. This is probably due to the fact that the cluster is inefficient to take these many charges on its own. Some fragments were also seen in the spectrum and few of them have been identified. The highest intense pattern is expanded in the inset of Fig. S3, ESI<sup>†</sup> which shows a precise isotope distribution. It matches exactly with the calculated spectrum of  $[Ag_5(BBS)_6Cs_2]^+$ . Although the unit charge on a Cs<sub>2</sub>-bound species is surprising, it may be noted that Ag<sub>5</sub>(BBS)<sub>6</sub> is likely to be an anion due to an excess BBS ligand and therefore, [Ag<sub>5</sub>(BBS)<sub>6</sub>Cs<sub>2</sub>] is a singly charged cation. These types of fragments are most stable which explains their high intensity. Even under softer ionizing conditions, the fragment intensity has not been reduced.

TG analysis of the  $Ag_{55}(BBS)_{31}$  cluster shows a weight loss of 47.9 ± 0.5% which corresponds to the total organic content of the material (Fig. 2A). The calculated organic content of the cluster is 48.5% which matches with the experimental results further supports the composition. Interestingly, two kinds of losses were seen, one at 200 °C (42.30%) and another at 800 °C (5.58%). The major loss may be due to the carbon–hydrogen (CH) content of the ligand which happens at a lower temperature and the remaining fragment of the ligand, namely sulfur which sits on the metal surface to form a nearly stoichiometric  $Ag_2S$  and the sulfur leaves at higher temperature. This kind of



Fig. 2 TGA spectrum of the  $Ag_{55}$  cluster (green trace) which shows two types of weight losses (A). Red trace is a differential plot of the same. 'B' shows the TEM image of the  $Ag_{55}$  cluster and inset is the corresponding size distribution curve. 'C' and 'D' are the extended XPS spectra for Ag 3d and S 2p regions. All the spectra are properly fitted.

sulfide formation is consistent with the solution phase decomposition of thiolated silver clusters reported previously<sup>45</sup> where facile C–S cleavage occurs, facilitating the formation of  $Ag_2S$  in the acanthite form.

PXRD analysis (Fig. S4, ESI<sup>†</sup>) of the Ag<sub>55</sub>(BBS)<sub>31</sub> cluster powder in the  $2\theta$  range of  $10^{\circ}$  to  $90^{\circ}$  showed broad diffraction peaks at  $2\theta \sim 37^{\circ}$ ,  $44^{\circ}$  and  $64^{\circ}$ . Individual silver clusters do not contain a periodic lattice in them and so they do not show sharp peaks. However, the broad lines are observed.<sup>28</sup> The Ag<sub>55</sub>BBS<sub>31</sub> composition was further verified with SEM/EDAX data (Fig. S5, ESI<sup> $\dagger$ </sup>) where the Ag : S ratio was found as 1 : 0.61 ± 0.05 (calculated value 1:0.57). The absence of sodium in the EDAX spectrum confirms the purity of the cluster. The TEM image (Fig. 2B) shows the presence of the cluster as tiny particles with an average size (diameter) of 1.15 nm. Bigger sized nanoparticles were not seen. XPS analysis was performed to know the chemical states of elements, although there is not much difference (only 0.5 eV) in the binding energies of Ag(0) and Ag(1).<sup>46</sup> A survey spectrum (Fig. S6, ESI<sup>†</sup>) shows the expected elements. Expanded spectra of Ag 3d (Fig. 2C) show the presence of Ag(0) and a small portion of Ag(I) which might be coming from the thiolate shell. The S 2p peak at 162.5 eV (corresponding to  $2p_{3/2}$ ) is because of thiolate (Fig. 2D).

FTIR spectra of BBSH and  $Ag_{55}(BBS)_{31}$  are given in Fig. 3A. The spectrum of BBSH shows characteristic stretching and bending modes of various bonds present in it. The absence of S–H stretching at 2585 cm<sup>-1</sup> in the  $Ag_{55}$  cluster confirms the binding of thiol to the silver core. Interesting difference was found between the cluster and BBSH in the C–H stretching (Fig. 3B) and bending regions (Fig. 3C). Intensities of the 3063 and 3086 cm<sup>-1</sup> peaks in the C–H stretching region (of –CH<sub>2</sub>–) decrease and show a red shift when the thiol binds to the cluster which suggests that this C–H part of the ligand is



**Fig. 3** (A) FT-IR spectra of pure BBSH (blue trace) and  $Ag_{55}(BBS)_{31}$  cluster (red trace). The –SH stretching featured at 2586 cm<sup>-1</sup> in BBSH is marked by a dotted-circle which is absent in the cluster. The structure of BBSH is shown as an inset in which different IR-active parts are numbered. The expanded views of C–H stretching and bending regions are given as (B) and (C), respectively. For clarity, the spectra have been shifted slightly.



Fig. 4 SAXS analysis of the Ag<sub>55</sub> cluster. Experimental and simulated (nonlinear least squares method) SAXS curves are shown in the top (A) and the corresponding particle size distribution obtained from the SAXS profile is shown in the bottom panel (B). UV/Vis spectra of the Ag<sub>55</sub>(BBS)<sub>31</sub> cluster compared with the PET protected Ag<sub>55</sub> cluster (C). Inset is the MALDI mass spectrum of the as-synthesized Ag<sub>55</sub>(PET)<sub>31</sub> cluster. Clustering of the molecular ion is seen.

nearest to the cluster core (marked 1 in inset of Fig. 3A). Similarly, the peak at 2870 cm<sup>-1</sup> also shows a shift (but here it is a blue shift) which might be because of the other C–H bond on the same carbon, closer to the cluster core. In the C–H bending region, two peaks at 1380 cm<sup>-1</sup> and 1363 cm<sup>-1</sup> in free thiol merge with each other giving rise to a single broad peak while forming the cluster, which could be attributed to the C–H bend of the nearest carbon (marked 1 in inset of Fig. 3A). Small angle X-ray scattering (SAXS) suggest the high monodispersity of the cluster (Fig. 4A and B) and the average particle size was found to be 1.796 nm (more details are given in ESI 1†).

Synthesis was optimized to yield a 2-phenylethane thiolate (PET) protected  $Ag_{55}$  cluster, which suggests the universality of the synthetic strategy and proposes the feasibility of stabilizing this cluster core with other ligands. UV/Vis spectra show (Fig. 4C) similar bands as presented for the BBS-protected  $Ag_{55}$  cluster. The peak at m/z 10.2 kDa in MALDI MS (inset of Fig. 4C) using DCTB as a matrix confirms the formation of  $Ag_{55}(PET)_{31}$ .<sup>41</sup> Here we could see the dimer and trimer also at respective m/z values, similar to the case of ~Ag\_{75} cluster.<sup>42</sup>

### Conclusions

In summary, we have successfully synthesized  $Ag_{55}$  clusters protected with BBS and PET ligands through a solid state route. Solvent selective extraction and a MeOH induced precipitation technique yields the cluster. From the ESI MS and MALDI MS data, the compositions,  $Ag_{55}(BBS)_{31}$  and  $Ag_{55}(PET)_{31}$  were confirmed. TGA and SEM/EDAX further supported the composition. Extensive characterization was done to know the cluster system in detail. The PET protected  $Ag_{55}$ cluster was prepared to check the adaptability of the cluster core to other ligands. We believe that  $Ag_{55}(BBS)_{31}$  and other much sought after stable clusters in organic environments will expose new directions in cluster research, in both experiment and theory. Work towards crystallization is underway.

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Supplementary information for paper

# Controlled Synthesis and Characterization of the Elusive Thiolated Ag<sub>55</sub> Cluster

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#### Materials and methods:

### 1. Chemicals

Silver nitrate (AgNO<sub>3</sub>, 99% Aldrich), sodium borohydride (NaBH<sub>4</sub>, 99.9%, Aldrich); 4-(tertbutyl) benzyl mercaptan (BBSH, 98%, Aldrich); Methanol (Changshu Yangyuan Chemical, China, AR grade) and toluene (Ranken) were used in this synthesis. All the chemicals were commercially available and were used without further purification.

#### 2. Synthesis of Ag<sub>55</sub>(BBS)<sub>31</sub>

The synthesis of  $Ag_{55}$  cluster protected by BBSH (4-(tert-butyl) benzyl mercaptan) involves the following processes. Initially, at room temperature 23 mg of AgNO<sub>3</sub> and 97.65 µL of 4-(tert-butyl) benzyl mercaptan (BBSH) was ground and mixed well in a clean mortar using a pestle. The color of the mixture changes to pale orange showing the formation of silver thiolate. To this mixture, 25 mg of solid NaBH<sub>4</sub> was added and the content was ground well. Then 5 mL of toluene was added to extract the cluster and it was centrifuged for 4 minutes at 4000 rpm. Finally, the supernatant was collected, which was black in color. This contains the crude cluster which was further purified using MeOH. Methanol was added drop wise on the extracted cluster resulting in the precipitation of Ag<sub>55</sub> cluster. The supernatant was removed and the precipitate was redispersed in toluene is required to observe the clusters. The solid state method was originally reported for Ag<sub>9</sub> clusters.<sup>1</sup> The cluster is stable for a month. It decomposed to greenish yellow colored thiolates.

#### 3. HPLC separation

HPLC separation was tried with the purified cluster, here we could see a single peak but the cluster was found to be degrading and it lost some of its characteristic features under the HPLC condition. We have tried the separation with solvents such as THF and toluene but the features

were getting lost within the column. This is probably due to the high pressure and the interaction with the stationary phase which leads to degradation of the cluster. We present the best chromatogram (A) and corresponding UV/Vis spectrum (B) using a normal phase column (A8-ST5SIL120-98). Broad hump-like baseline indicates that the broken cluster is sticking inside the column.



**Fig. S1:** (A) The chromatograph obtained using the purified cluster and (B) the optical absorption spectra (Jacobian corrected) due to the peak at 5 min retention time (black trace) and the cluster before passing through HPLC (red trace).

### 4. Synthesis of Ag<sub>55</sub>(SCH<sub>2</sub>CH<sub>2</sub>Ph)<sub>31</sub>

Similar to  $Ag_{152}$  synthesis,<sup>2</sup> the silver and thiol ratio was taken as 1:4. The cluster was washed with heptanes after ethanol wash followed by the extraction in toluene.

#### 5. Instrumentation:

UV-Vis spectra were measured with a Perkin Elmer Lambda 25 instrument in the range of 200-1100 nm. The absorbance of raw spectral data are corrected [I(E)] using the following equation and plotted in terms of energy (1239.8/ Wavelength in nm = Energy (eV)).

$$I(E) = \frac{I(w)}{\partial E/\partial w} \propto I(w)w^2$$

HPLC separation was performed using a Shimadzu HPLC system equipped with a normal phase column (A8-ST5SIL120-98) and a UV/Vis detector. High resolution transmission electron microscopy of clusters was carried out with a JEOL 3010 instrument. The samples were drop casted on carbon-coated copper grids and allowed to dry under ambient conditions. Matrixassisted desorption ionization mass spectrometry (MALDI MS) studies were conducted using a Voyager-DE PRO Biospectrometry Workstation from Applied Biosystems. DCTB was used as the matrix (at 1:100 ratio of sample to matrix). A pulsed nitrogen laser of 337 nm was used for the MALDI MS studies. Mass spectra were collected in positive ion mode and were averaged for 200 shots. Scanning electron microscopic (SEM) and energy dispersive X-ray (EDAX) analyses were performed with a FEI QUANTA-200 SEM. For measurements, samples were drop casted on an indium tin oxide (ITO)-coated glass and dried in vacuum. FT-IR spectra were measured with a Perkin Elmer Spectrum One instrument. TGA was measured using a TA instrument. A temperature range of 30°C to 910°C was used and the analysis was done in nitrogen atmosphere. Powder XRD patterns of the samples were recorded using PANalytical X'pertPro diffractometer. The powder samples of parent silver nanoparticles and clusters were taken on a glass plate and the X-ray diffractogram was collected from 5 to 100 degrees in 2 theta using Cu Ka radiation. ESI MS measurements were done using a LTQ XL mass spectrometer from Thermo Scientific, San Jose, CA. Methanol-toluene mixture of solvent was used for this experiment. Small angle Xray scattering (SAXS) experiments were performed on the sample solutions in transmission mode using Rigaku Smart Lab X-ray diffractometer operating at 9 kW (Cu-  $K_{\alpha}$  radiation;  $\lambda$ =1.54059 Å). The sample solutions were placed in borosilicate capillary tubes (~1.5 mm internal diameter). NANO-Solver programme of Rigaku have been used to solve the SAXS profiles. The raw SAXS profiles obtained were corrected for background absorption and air scattering. Particle size distributions were obtained from simulated fittings of the corrected SAXS profiles using 'core-shell' model. The 'core-shell' model exhibited very good fittings and the results are in agreement with the size of the metal nanoclusters (NCs) from TEM studies. The capping agents have been considered as shells while the metal NCs as core. Densities of the capping agent (shell) and respective metal nanoclusters (core) are used to fit the SAXS profiles. The shell thickness and the size of the particles have been evaluated from the auto-fitted

simulated profiles. The particles (i.e. metal NCs-capping agent) are highly monodisperse in nature.

 Table 1: SAXS parameters

Sample	Avg. size	Vol (%)	Normalised	Shell	Avg metal	R-factor
no.	(nm)		dispersion (o)	diameter	Nanocluster	
					Core size*	
Ag <sub>55</sub>	1.796	99.84	0.032	0.6957	1.1003	1.45
		(rest are				
		bigger				
		particles#)				



**Fig. S2.** Laser fluence-dependent MALDI mass spectra of the purified  $Ag_{55}BBS_{31}$  cluster. Inset is an expanded view. With increase in laser fluence (up to X = 1.37; X is defined as  $f/f_{th}$  where f is the laser fluence.), the peak position shifts to lower mass. Further increase in the laser fluence does not change the peak position.



**Fig. S3.** ESI mass spectrum of the purified  $Ag_{55}$  cluster [in toluene-methanol (1:1) mixture] in the positive ion mode. Insets; is the isotope distribution of a well-defined fragment [ $Ag_5(BBS)_6Cs_2$ ]<sup>+</sup>.



**Fig. S4.** X-ray diffraction pattern of the as-synthesized  $Ag_{55}$  cluster. It shows a broad feature at  $2\theta \sim 37^{\circ}$  and  $44^{\circ}$  as expected.





Fig. S5. EDAX spectrum of the purified Ag<sub>55</sub> cluster.





Fig. S6. XPS survey spectrum of the  $Ag_{55}$  cluster. Peaks are labeled.

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# Nanoscale

### COMMUNICATION



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### Reversible formation of Ag44 from selenolates†

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The cluster Ag<sub>44</sub>SePh<sub>30</sub>, originally prepared from silver selenolate, upon oxidative decomposition by  $H_2O_2$  gives the same cluster back, in an apparently reversible synthesis. Such an unusual phenomenon was not seen for the corresponding thiolate analogues. From several characterization studies such as mass spectrometry, Raman spectroscopy, *etc.*, it has been confirmed that the degraded and as-synthesized selenolates are the same in nature, which leads to the reversible process. The possibility of making clusters from the degraded material makes cluster synthesis economical. This observation makes one to consider cluster synthesis to be a reversible chemical process, at least for selenolates.

Synthesis and characterisation of noble metal quantum clusters is becoming one of the most fascinating topics of materials research due to their unique size-dependent properties.<sup>1–5</sup> Ultra small size and enhanced optical properties make them widely applicable in a variety of applications such as surface enhanced Raman scattering (SERS),<sup>6</sup> catalysis,<sup>7</sup> biolabelling,<sup>8,9</sup> sensing<sup>10,11</sup> and many others.<sup>12–14</sup>

Starting from the Brust protocol,<sup>15</sup> several synthetic routes such as modified Brust synthesis,<sup>16,17</sup> high temperature route,<sup>18</sup> solid state route,<sup>19–22</sup> interfacial synthesis,<sup>23</sup> *etc.* have been developed to create such atomically precise pieces of matter. As of now, most of the reports are on gold clusters and crystal structures of Au<sub>23</sub>,<sup>24</sup> Au<sub>24</sub>,<sup>25</sup> Au<sub>25</sub>,<sup>5,26</sup> Au<sub>28</sub>,<sup>27</sup> Au<sub>30</sub>,<sup>28</sup> Au<sub>36</sub>,<sup>29</sup> Au<sub>38</sub>,<sup>30,31</sup> and Au<sub>102</sub>.<sup>32</sup> Compared to gold, very few reports exist for silver clusters<sup>33,34</sup> with detailed characterization, which include Ag<sub>7,8</sub>,<sup>23</sup> Ag<sub>9</sub>,<sup>20</sup> Ag<sub>32</sub>,<sup>35</sup> Ag<sub>44</sub>,<sup>36</sup> and Ag<sub>152</sub>.<sup>19</sup> Crystal structures of mixed ligand protected Ag<sub>14</sub>,<sup>37</sup> Ag<sub>16</sub>,<sup>38</sup> and  $Ag_{32}^{38}$  clusters have been solved. Recently, the crystal structure of the very first complete thiolate protected  $Ag_{44}$  cluster has been reported by Bigioni<sup>39</sup> and Zheng<sup>40</sup> groups. The  $Ag_{44}(SR)_{30}$ cluster forms a Keplerate solid of concentric icosahedral and dodecahedral atom shells to form a hollow cage which is further protected by six  $Ag_2(SR)_5$  units in an octahedral geometry. A similar structure has been proposed for the selenolate analogue of the  $Ag_{44}$  cluster<sup>41</sup> which shows an identical optical spectrum with a shift, as expected from the difference in ligands. In most of the cases, the clusters have been examined in terms of their stability.<sup>42–44</sup> As silver clusters are easily oxidisable under aerobic conditions, they degrade rapidly to form thiolates or selenolates and studies on intact clusters are limited.

In this work, we report the reversible formation of the Ag<sub>44</sub> cluster from selenolates. The reversibility has been checked for other corresponding clusters also but interestingly, except for selenolated Ag<sub>44</sub>, no other systems (including thiolated Ag<sub>44</sub>, which is chemically most similar) show this property. The clusters examined include, Ag<sub>44</sub>(SPh)<sub>30</sub>, Ag<sub>44</sub>(4-FTP)<sub>30</sub>, and Ag<sub>44</sub>-(3-FTP)<sub>30</sub>, where SPh, 4-FTP and 3-FTP correspond to the thiolate forms of thiophenol, 4-flurothiophenol and 3-flurothiophenol, respectively. A larger silver cluster, Ag<sub>152</sub>(PET)<sub>60</sub>, was also studied. To explore this phenomenon in more detail, we have oxidised the cluster using peroxide to form selenolates and then the reversibility was checked using borohydride reduction. Several other characterisation studies were done to understand the reversibility.

A two phase solution state route as described in our previous report<sup>41</sup> has been used to synthesize the cluster. Initially, silver trifluoroacetate (0.0714 mmol) was dissolved in 7.2 mL acetonitrile and stirred for 5 min. Benzeneselenol (0.0471 mmol) was added to that solution and was left to stir for another 15 min (resulting in solution A). In another conical flask, 28.6 mL acetonitrile solution of NaBH<sub>4</sub> (0.286 mmol) was kept for stirring for 30 min (solution B). Then, solution B was added to solution A and the reaction mixture was left to stir for 3 h at room temperature. A wine red colored cluster was formed after 3 h and it was stored in a refrigerator at ~4 °C.

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<sup>†</sup> Electronic supplementary information (ESI) available: Details of experimental procedures; instrumentation; reversible cycles, UV/Vis spectra of thiophenol, 4-FTP, 3-FTP protected Ag<sub>44</sub>, and Ag<sub>152</sub> cluster; UV/Vis, SEM images and Raman spectra of as-synthesized and degraded thiolates & selenolates; SEM/EDAX of degraded selenolates, UV/Vis of the Ag<sub>44</sub>(SePh)<sub>30</sub> cluster under different selenol concentrations and temperatures. See DOI: 10.1039/c4nr03267e



**Fig. 1** A: Photographs of the  $Ag_{44}(SePh)_{30}$  cluster solution showing time dependent changes during oxidation (i to vi) and reduction (vi to x). Oxidation was accomplished by  $H_2O_2$  and reduction was by NaBH<sub>4</sub>. B: Time dependent UV/Vis spectra during oxidation of the  $Ag_{44}$  cluster to form selenolates. C: Time dependent UV/Vis spectra for the reduction of selenolates to form the  $Ag_{44}$  cluster. Each spectrum has been collected at 1 min interval.

Similar methodologies have been followed for thiol protected clusters also. More details are given in the ESI.†

The Ag<sub>44</sub>(SePh)<sub>30</sub> cluster<sup>41</sup> shows five intense bands at 1.41 (879), 1.82 (681), 2.16 (574), 2.40 (516) and 2.82 (440) eV (nm) along with three broad bands centered around 1.27 (970), 1.95 (635) and 3.14 (395) eV (nm) in its absorption spectrum. The cluster kept under aerobic conditions will lose its optical identity gradually and a yellow precipitate appears. Reversible cluster formation was observed first from the degraded cluster. Upon addition of adequate amount of borohydride and constant stirring, we observed that the degraded selenolate, formed from the cluster (selenolate 1) can be reformed to the Ag44 cluster in 15 minutes. However, degradation under aerobic conditions typically takes 5-7 days and time dependent observation was difficult. So, an external oxidizing agent, hydrogen peroxide, was added to a controlled amount so that we can monitor oxidation in real time. Photographs at different stages of the reaction are given in Fig. 1A. The cluster is wine red in color but upon addition of H<sub>2</sub>O<sub>2</sub>, the color changes to yellowish-brown and finally to yellow which confirms the formation of silver selenolate. The reaction was monitored through absorption spectroscopy where the distinct features of the Ag<sub>44</sub>(SePh)<sub>30</sub> cluster are lost and subsequently a new peak around 450 nm along with a hump at 430 nm started appearing, due to selenolate. As time progresses, the baseline of the spectrum started increasing because of the low solubility of selenolate in acetonitrile. During the reduction of this selenolate 1 with NaBH<sub>4</sub>, the color changes in the reverse order and finally the solution becomes clear and attains wine red color which confirms the formation of the Ag<sub>44</sub> cluster. The corresponding UV/Vis spectra are given in Fig. 1C where the spectra also change in the reverse order. The sharp selenolate peak disappears and all the features of Ag44 started

appearing with time. The reaction time is controlled by the concentration of the cluster, the amount of  $H_2O_2$  and NaBH<sub>4</sub>. Constant stirring is also important for this case. For more clarity, these reversible cycles are shown by selecting the intensity of the 516 nm peak for five consecutive cycles (Fig. S1, ESI†).

Similar experiments have been tried for Ag44(SPh)30 which is the thiol analogue of Ag44(SePh)30. For this cluster, upon addition of H<sub>2</sub>O<sub>2</sub> (all concentrations were kept constant) thiolates were formed, as expected. The UV/Vis spectra (Fig. S2A, ESI<sup>†</sup>) clearly show a sharp peak near 350 nm corresponding to thiolates and the features of the  $Ag_{44}(SPh)_{30}$  cluster have been lost completely. Reduction of this degraded thiolate (thiolate 1) has resulted in a broad hump near 400 nm and the cluster feature did not develop suggesting that the system was not reversible. We have studied Ag44(4-FTP)30 (Fig. S2B, ESI<sup>†</sup>) and Ag44(3-FTP)30 (Fig. S2C, ESI<sup>†</sup>) also but they both do not show the reversible formation. The 'Ag<sub>44</sub>(4-FTP)<sub>30</sub> derived thiolate' form nanoparticles by reduction. The plasmonic feature can be seen in the optical spectrum (Fig. S2B, ESI<sup>†</sup>) while a hump at 480 nm can be observed in the case of 'Ag44(3-FTP)30derived thiolate' under the same conditions. Degradation to thiolate is not reversible in the larger cluster, Ag152(PET)60 (Fig. S3, ESI<sup>†</sup>). In the cluster literature, the only case of reversibility was observed by Anand et al.45 who reported that the reversible transformation of human serum albumin protected  $Ag_9$  to the  $Ag_{14}$  cluster. Note that the cluster core changes during this transformation. Therefore, the reversibility seen in the case of Ag<sub>44</sub>(SePh)<sub>30</sub> is unprecedented. As Au clusters are chemically different in most of their properties,46 a similar study was not attempted on them.

To find the reason for this unique transformation, a detailed characterization of selenolate 1 was performed.



**Fig. 2** MALDI MS data of the  $Ag_{44}(SePh)_{30}$  cluster (black trace) and selenolate **1** (red trace). Inset shows the ESI mass spectra of the  $Ag_{44}$  cluster (a) and selenolate **1** (b), respectively. All the peaks are marked.

Initially, matrix assisted laser desorption ionization mass spectrometry (MALDI MS) was performed for the cluster as well as for selenolate 1 (Fig. 2).  $Ag_{44}(SePh)_{30}$  shows a peak centered around m/z 9500 using DCTB as the matrix<sup>19,33,34</sup> and as expected, selenolate 1 does not show any feature. Electrospray ionization mass spectrometry (ESI MS) shows 3, 4 features (Fig. 2a) of  $Ag_{44}(SePh)_{30}$  which confirm the purity of the cluster. As selenolates have very less solubility, a methanolacetonitrile mixture was used for the ESI measurement. Some selenolate species such as  $Ag_3(SePh)_2$ ,  $Ag_2(SePh)_2$  and Ag(SePh)<sub>2</sub> have been observed in the negative ion mode. For confirmation, ESI-MS was also taken for the reversibly formed  $Ag_{44}$  cluster which shows the same feature as depicted in Fig. 2A.

We thought that comparison of selenolate 1 with the as-synthesized selenolate (selenolate 2) and similar thiolate samples (synthesis procedures are given in ESI<sup>†</sup>) might be useful to understand this phenomenon. UV/Vis spectroscopy for both the cases have been compared (Fig. S4, ESI<sup>†</sup>) and it was found that for the case of selenolate, the spectra are comparable (Fig. S4A, ESI<sup>†</sup>) but drastic differences are seen for thiolates (Fig. S4B, ESI<sup>†</sup>). As-synthesized thiolate (thiolate 2) shows a sharp peak at 237 nm and a broad hump at 277 nm whereas thiolate 1 shows four broad humps at 240, 280, 360 and 420 nm, respectively. Selenolate 1 shows a peak at 464 nm along with a hump at 430 nm and similar two peaks at 430 and 450 nm were observed for selenolate 2. So, selenolates 1 and 2 might be the same as it appears in absorption spectroscopy. To understand it better, laser desorption ionization mass spectrometric (LDI MS) analysis was attempted for the thiolates and selenolates (Fig. 3). It is important to mention here that LDI MS has been used as these thiolates and selenolates have very less solubility, so it was difficult to perform electrospray ionization (ESI) or matrix assisted laser desorption ionization (MALDI) mass analysis. Here also similar trends have been observed in absorption spectroscopy. Systematic

Ag<sub>2</sub>S losses have been observed for both the thiolates (Fig. 3A) but the loss started from higher mass (m/z 4500) for thiolate 2 while it started from m/z 1800 for thiolate 1, that too with much lower intensity (an expanded view of a peak at m/z 1375 is shown in the inset of Fig. 3A). Interestingly, in the lower mass, intensity gradually increases for thiolate 2 and some shifts have also been observed. The data suggest that thiolates 1 and 2 are different in nature. Selenolates show similar features and systematic Ag<sub>2</sub>Se losses have been seen (Fig. 3B) from almost the same mass region and the isotope pattern is also similar (inset of Fig. 3B). The reason for the complicated fine structure of selenolates compared to thiolates is because of the isotopes of Se. Selenium has six isotopes namely, <sup>74</sup>Se (0.89%), <sup>76</sup>Se (9.37%), <sup>77</sup>Se (7.63%), <sup>78</sup>Se (23.77%), <sup>80</sup>Se (49.61%), and <sup>82</sup>Se (8.73%), which contribute significantly to the fine mass spectral features compared to sulfur which has only one predominant isotope, namely <sup>32</sup>S (95%). Raman spectroscopy was used to confirm that selenolates are the same but thiolates are different in nature (Fig. S5, ESI<sup>†</sup>). Although detailed peak assignments have not been done, many are assigned based on the literature.47 From a comparative study, we can see that Raman features for selenolates 1 and 2 (Fig. S5A, ESI<sup>†</sup>) are the same but differences are there for thiolates (Fig. S5, ESI<sup> $\dagger$ </sup>). The peak at 1070 cm<sup>-1</sup> (ring deformation mode<sup>47</sup>) for thiolate **1** has been split for thiolate **2** along with some more additional peaks near 1020  $\text{cm}^{-1}$  (ring breathing mode<sup>47</sup>). Differences were observed for thiolates in SEM images (Fig. S6, ESI<sup>†</sup>). As-synthesized thiolates appear amorphous whereas degraded ones show crystalline nature. For the case of selenolates, both show porous structures (Fig. S6, ESI<sup>†</sup>). From the SEM/EDAX analysis, the ratio of Ag : Se for the degraded selenolate species is found to be 1:0.68 (3:2.04) which is very close to the atomic ratios present in the cluster (Fig. S7, ESI<sup>†</sup>). Non-stoichiometry in thiolates was found earlier<sup>48-51</sup> and a similar case is expected for selenolates also. Parikh et al. have shown the existence of silver rich thiolates



**Fig. 3** Comparative LDI mass spectra of thiolates (A) and selenolates (B). Insets for both the cases show an extended view of some selected peaks centred at m/z 1377 (for thiolates) and m/z 1656 (for selenolates). True ion intensities are compared and not relative abundances.

using elemental analysis.<sup>52</sup> Li *et al.*<sup>48,49</sup> and Sun *et al.*<sup>50</sup> have shown the crystal structures of metal rich thiolates. McLauchlan and Ibers have shown sulphur and selenium rich silver thiolates.<sup>53</sup> So it is not difficult to rationalize the existence of non-stoichiometric thiolates or selenolates in our experiments. In detail such thiolates may be structures with sulphide cores with thiolate shells, although the authors refer to them as thiolates.

From all these data it is confirmed that degraded and assynthesized selenolates are the same in nature and because of that formation of Ag44SePh30 is reversible. Another reason for the reversibility could be the higher stability of this selenolate protected Ag<sub>44</sub> cluster compared to the thiol protected one. To confirm this, the cluster has been synthesized with different concentrations of benzeneselenol and surprisingly, all of them resulted in Ag44 clusters with distinct optical and mass spectral features (Fig. S8, ESI<sup>†</sup>). The cluster has been synthesized at different temperatures and surprisingly, in all the cases (from 0 to 60 °C) they formed (Fig. S9, ESI<sup>†</sup>) which suggests the high stability of the system. Formation time of the cluster reduced drastically with increase in temperature, which is expected (inset of Fig. S9, ESI<sup>†</sup>). For the thiol case, irreversibility has been observed because of the different nature of the thiolates and may be upon reduction, thiolates have many possibilities to make diverse clusters or bigger nanoparticles.

In summary, we have synthesized selenolate and thiolate analogues of  $Ag_{44}$  clusters using similar synthetic methodologies. Unusual reversible formation of the  $Ag_{44}$  cluster from selenolate was observed. This phenomenon has not been seen for the corresponding thiolates. Several characterization techniques have been used to understand the reversibility. It has been found that degraded selenolates and as-synthesized selenolates are the same in nature but they are different in the case of thiolates, which is responsible for this unusual property. This opens up a new possibility of making clusters from the degraded materials, which may be economical for precious metals like gold and silver. The most important aspect of this finding appears to be that cluster synthesis is proven to be reversible, at least in the limited case of selenolates. This suggests that clusters may be treated just as molecules in their chemistry.

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Supporting information for the paper

### **Reversible Formation of Ag<sub>44</sub> from Selenolates**

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### Materials and methods:

### 1. Chemicals

Silver trifluoroacetate (99%), sodium borohydride (NaBH<sub>4</sub>, 98%), benzeneselenol (97%), 4flurothiophenol (4-FTP, 98%), 3-flurothiophenol (3-FTP), 2-phenylethanethiol (PETH, 98%) and thiophenol (98%) are form Aldrich and hydrogenperoxide (H<sub>2</sub>O<sub>2</sub>, 30%), toluene, ethanol, and acetonitrile (AR grade) are from Rankem Chemicals, India. All the chemicals were commercially available and were used without further purification.

### 2. Synthesis of thiolated Ag<sub>44</sub> cluster

The synthesis procedure is exactly same as selenolate protected  $Ag_{44}$  cluster. Here same concentrations of thiophenol, 4-FTP and 3-FTP have been used, instead of benzeneselenol.

### 3. Synthesis of Ag<sub>152</sub> cluster

Ag<sub>152</sub><sup>1</sup> clusters have been synthesized using reported procedures.

### 4. Oxidation and reduction procedure

About 5 mL of the Ag<sub>44</sub> cluster was taken in which  $5\mu$ L of 30% H<sub>2</sub>O<sub>2</sub> was added and it was monitored with time with constant stirring. After the complete oxidation, 1.5 mg of NaBH<sub>4</sub> (s) was added and stirring was continued. After a few minutes a deep pink colored cluster was formed. Similar strategy has been followed for all the clusters.

### 5. Synthesis of thiolates and selenolates

For synthesizing *thiolate 2* and *selenolate 2*, same ratio of silver:ligand was taken as described for the cluster (solution A). Then the settled thiolate/selenolate was isolated from the solution which was used for characterization.

### 6. Instrumentation

UV-Vis spectra were measured with a Perkin Elmer Lambda 25 spectrometer in the range of 200-1100 nm. Matrix-assisted desorption ionization mass spectrometry (MALDI MS) studies were conducted using a Voyager-DE PRO Biospectrometry Workstation from Applied Biosystems. DCTB (trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile) was used as matrix (at 1:100 ratio of sample to matrix). A pulsed nitrogen laser of 337 nm was used for the MALDI MS studies. Mass spectra were collected in negative ion mode and were averaged for 200 shots. ESI MS measurements was done on LTQ XL mass spectrometer from Thermo Scientific, San Jose, CA. Methanol-acetonitrile mixture was used for selenolates and acetonitrile for Ag<sub>44</sub> cluster. Raman spectra and images were done with a WITec GmbH, Alpha-SNOM alpha 300 S confocal Raman microscope having a 532 nm laser as the excitation source.





Fig. S1. Reversible cycles during the oxidation and reduction of  $Ag_{44}(SePh)_{30}$  cluster considering the 516 nm peak intensity of the cluster.



Fig. S2. Comparative UV/Vis spectra (black traces) of  $Ag_{44}(SPh)_{30}$  (A),  $Ag_{44}(4-FTP)_{30}$  (B) and  $Ag_{44}(3-FTP)_{30}$  (C). Red and blue traces show (in A, B and C) the UV/Vis spectra for  $H_2O_2$  oxidation and NaBH<sub>4</sub> reduction of cluster and thiolates, respectively.



Fig. S3. UV/Vis spectra (black trace) of  $Ag_{152}(PET)_{60}$ . Red and blue traces show the UV/Vis spectra for  $H_2O_2$  oxidation and NaBH<sub>4</sub> reduction of cluster and thiolates, respectively.



Fig. S4. Comparative UV/Vis spectra of degraded and as-synthesized selenolates (A) and thiolates (B). Inset shows an expanded view in the lower wavelength regions.



Fig. S5. Raman spectra of selenolates (A) and thiolates (B).



Fig. S6. SEM images of thiolates (as-synthesized (A) and degraded (B)) and selenolates (assynthesized (C) and degraded (D)). Looking at the circled regions, some similarity can be noticed.



Fig. S7. SEM/EDAX spectrum of degraded selenolates.



Fig. S8. UV/Vis spectra of  $Ag_{44}(SePh)_{30}$  cluster synthesized using different concentrations of benzeneselenol. The corresponding amounts of benzeneselenol are mentioned. Inset shows the corresponding ESI mass spectra.



Fig S9. UV/Vis spectra of  $Ag_{44}(SePh)_{30}$  cluster synthesized at different temperatures. The spectra have been normalized. Inset shows the formation time for each case.

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### Isolation and Tandem Mass Spectrometric Identification of a Stable Monolayer Protected Silver–Palladium Alloy Cluster

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**Supporting Information** 

**ABSTRACT:** A selenolate-protected Ag–Pd alloy cluster was synthesized using a one-pot solution-phase route. The crude product upon chromatographic analyses under optimized conditions gave three distinct clusters with unique optical features. One of these exhibits a molecular peak centered at m/z 2839, in its negative ion mass spectrum assigned to Ag<sub>5</sub>Pd<sub>4</sub>(SePh)<sub>12</sub><sup>-</sup>, having an exact match with the corresponding calculated spectrum. Tandem mass spectrometry of the molecular ion peak up to MS<sup>9</sup> was performed. Complex isotope distributions in each of the mass peaks confirmed the alloy composition. We find the Ag<sub>3</sub>Pd<sub>3</sub><sup>-</sup> core to be highly stable. The composition was further supported by scanning electron microscopy, energy-dispersive spectroscopy, and X-ray photoelectron spectroscopy.



SECTION: Physical Processes in Nanomaterials and Nanostructures

**N** oble metal clusters with monolayer protection are becoming one of the most fascinating areas of contemporary chemical research. They have been used in several applications<sup>1-12</sup> because of their unique optical properties, especially photoluminescence.<sup>13–16</sup> Among them, gold clusters have been studied extensively. Crystal structures of several gold clusters such as  $Au_{23}$ ,<sup>17</sup>  $Au_{25}$ ,<sup>15,18</sup>  $Au_{28}$ ,<sup>19</sup>  $Au_{30}$ ,<sup>20</sup>  $Au_{36}$ ,<sup>21</sup>  $Au_{38}$ ,<sup>22</sup> and  $Au_{102}$ ,<sup>23</sup> have been reported. Silver clusters have been studied to a lesser extent,<sup>24–26</sup> although reports exist on  $Ag_{7,8}$ ,<sup>27</sup>  $Ag_{9}$ ,<sup>28</sup>  $Ag_{14}$ ,<sup>29</sup>  $Ag_{16}$ ,<sup>30</sup>  $Ag_{32}$ ,<sup>31</sup>  $Ag_{44}$ ,<sup>32,33</sup> and  $Ag_{152}$ ,<sup>44</sup> with detailed characterization, dominated by mass spectrometry. Reports of mixed ligand (phosphine and thiol)-protected  $Ag_{14}$ ,<sup>29</sup>  $Ag_{16}$ ,<sup>30</sup> and  $Ag_{32}$ ,<sup>30</sup> are also there. Recently, crystal structure of the very first complete thiol-protected core, namely,  $Ag_{44}$ ,<sup>35,36</sup> has been solved. A limited number of studies exist on other metal clusters like Pt,<sup>37</sup> Cu,<sup>38</sup> and so on. However, synthesis of atomically precise alloy clusters of various compositions is a challenge, and very few reports exist in the literature;<sup>39–41</sup> examples include PdAu<sub>24</sub>,<sup>42</sup> Pd<sub>2</sub>Au<sub>36</sub>,<sup>43</sup> PdAu<sub>10</sub>,<sup>44</sup> and  $Ag_7Au_6$ ,<sup>45</sup> all of which have thiol protection. Selenolate-protected single metal clusters such as  $Au_{25}$ ,<sup>46</sup>  $Au_{18}$ ,<sup>47</sup>  $Au_{38}$ ,<sup>48</sup>  $Au_{24}$ ,<sup>49</sup> and  $Ag_{44}$ ,<sup>33</sup> have been reported, whereas only one alloy, namely, Cu<sub>n</sub>Au<sub>25-n</sub> (n = 1-9),<sup>50</sup> is known with such monolayers. So far, to the best of our knowledge, there is no report on Ag–Pd alloy clusters.

We report the very first selenolate protected Ag–Pd alloy cluster. The crude cluster was synthesized from silver trifluoroacetate(s) and sodium tetracholopalladate(s) in the presence of benzeneselenol (l) (details are in Supporting Information 1). Solid NaBH<sub>4</sub> was added, and stirring was continued. The color change of orange to yellowish brown indicated the conversion of selenolates to clusters (Figure S2 in the Supporting Information). Several analytical methods were used to characterize the clusters (Supporting Information 1).

The crude clusters show the presence of molecule-like features in absorption spectroscopy (Figure 1). The spectrum gives peaks near 400 (3.1), 381 (3.25), 326 (3.8), 282 (4.4), and 248 (4.99) nm (eV) and a small hump at 590 (2.1) nm (eV). The crude cluster was subjected to HPLC for purification using acetonitrile as the solvent in the isocratic mode. Several parameters such as the pump pressure, solvents, flow rate, temperature, and so on were optimized to achieve the isolation process. Slower flow rate leads to longer time for the cluster to come out, but isolation becomes easier as expected (Figure S3 in the Supporting Information). The best separation was observed with 0.25 mL/min flow rate at room temperature. The corresponding chromatogram gives three well-separated peaks (Figure 1) at retention times of 6.44, 7.69, and 10.70 min, which have been marked as clusters 1, 2, and 3, respectively. Characteristic UV/vis features were observed for different isolated species (Figure 1a and Figure S4 in the Supporting Information). Cluster 1 shows peaks at 3.27 (379), 3.94 (315), 4.4 (282), and 4.8 (258) eV (nm) and a small hump at 1.93 eV (642 nm), whereas cluster 2 has peaks at 3.1 (400), 3.8 (326), 4.47 (277), and 4.9 (253) eV (nm) and a small hump at 2.1 (590) eV (nm). Compared with clusters 1 and 2, cluster 3 shows a different nature, and it has multiple humps at 1.9 (653), 2.8 (443), 3.3 (376), 3.8 (326), 4.4 (282)

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Figure 1. HPLC chromatogram of the crude cluster in acetonitrile at room temperature (30 °C) using the isocratic mode. Flow rate was optimized at 0.25 mL/min. The UV/vis detector was used for analysis. Three distinct peaks correspond to clusters 1–3. Inset: (a) UV/vis spectra of as-synthesized (i) crude and (ii) cluster 2; inset of inset shows the corresponding photographs of the cluster solutions (marked as i' and ii', respectively). (b) HRTEM image of cluster 2. (c) Size distribution. A few particles are marked with yellow circles.

eV (nm) and 4.9 (253) eV (nm). Each cluster has its own characteristic color, which makes them distinguishable from each other as well as from the crude cluster (Figure 1a and Figure S4 in the Supporting Information).

Electrospray ionization mass spectrometry (ESI MS) is known to be the best tool for precise characterization of clusters.<sup>28,51</sup> The assignment can be made based on the molecular ion peak and various charged species of the parent ion. Comparison with the corresponding calculated spectrum can be useful. Observation of good isotopic distribution can also be a strong support for predicting the elements present in the cluster as well as the composition. Here the crude as well as all isolated clusters have been subjected to ESI MS. First, the ESI MS of the crude cluster was analyzed in the negative ion mode with acetonitrile as the solvent. No characteristic peaks were observed in the positive ion mode. Only negative ions were detected. The crude cluster shows a peak centered at m/z2839 (Figure S5A in the Supporting Information) along with some selenolate fragments at lower mass regions. For cluster 1 (Figure S5B in the Supporting Information), many peaks were observed, but isotope distribution was not seen, and they appeared broad. Cluster 3 (Figure S5D in the Supporting Information) does not show significant peaks in both negative and positive modes. For cluster 2, the spectrum shows (Figure 2 and Figure S5C in the Supporting Information) a molecular ion peak at m/z 2839 along with some fragments at m/z 2604 and 2204, which have been assigned as [Ag<sub>5</sub>Pd<sub>4</sub>(SePh)<sub>12</sub>]<sup>-</sup>, [Ag<sub>5</sub>Pd<sub>4</sub>(SePh)<sub>10</sub>Se]<sup>-</sup>, and [Ag<sub>3</sub>Pd<sub>3</sub>(SePh)<sub>10</sub>]<sup>-</sup>, respectively. The fragments are due to (SePh+Ph) and Ag<sub>2</sub>Pd(SePh)<sub>2</sub> losses, respectively (Figure 2). The isotope distribution of the molecular ion peak shows an exact match with the corresponding calculated one (Figure 2a). Differences between the spectra are very small and are hardly noticeable. Absence of selenolate fragments in the lower mass region confirms the purity of the isolated cluster (Figure S5 in the Supporting Information). The fragment at m/z 2204 appears with reasonable intensity, which contains the unusual metallic core of Ag<sub>3</sub>Pd<sub>3</sub> protected with ligands and the absence of fragments from this species, suggesting its stability.



**Figure 2.** ESI MS data of cluster 2. All peaks are assigned to their compositions. Inset: (a) Calculated (red trace) and experimental (black trace) mass spectra of  $[Ag_5Pd_4(SePh)_{12}]$ . (b) Cartoon representation of the  $Ag_5Pd_4$  cluster with its ligands.

We believe that the peak at m/z 2839 is the molecular ion and others are fragments due to the following reason. Even when the cluster is stable, fragmentation is common under ESI conditions. For example, the stable  $Au_{25}(SR)_{18}$  cluster shows a fragment in the mass spectrum due to the  $Au_4(SR)_4$  loss.<sup>52</sup> Even the stable  $Ag_{44}(SR)_{30}$  cluster, for which the crystal structure was determined recently,<sup>35,36</sup> shows several fragments due to  $Ag_m(SR)_n$  losses. It is important to mention here that for this cluster the molecular peak with 4- charge has much lower intensity compared with the fragments seen in the lower mass range  $(m/z \ 100-1500)$ .<sup>32</sup> Au<sub>25</sub> and Ag<sub>44</sub> are the most stable clusters reported so far, for the case of gold and silver, respectively. They have closed-shell electronic configuration consisting of 8 and 18 number of electrons. These clusters show stable species in the gas phase along with their fragments. So, it is clear that fragmentation is likely under ESI conditions for clusters. However, the peak at m/z 2839 is very likely to be an intact ion as no other ion was seen in the higher mass region, until m/z 20 000. Spectrum was also measured at various ESI conditions, which ensured that no other higher mass molecules are present in the solution. No higher charge state was detected. All of these suggest that the peak at m/z2839 is the molecular ion and the cluster in solution state itself is negatively charged. We have focused only on cluster 2 in this report because it gives a well-defined and fully assignable mass spectrum. Subsequent studies were done only on this cluster. The cluster is stable for more than one month under ambient conditions.

For a detailed understanding of the fragmentation pattern and for structural elucidation, tandem mass spectra were measured for each peak present in the mass spectrum of cluster 2. The most intense and closest peak obtained in  $MS^2$  was subjected to further fragmentation to get the MS<sup>3</sup> data, and subsequent MS<sup>n</sup> measurements were performed until reasonable intensities were seen. Although there is no particular pattern followed for each fragmentation, we could see four different types of losses overall; they correspond to -SePh, -Ph, Ag, and Pd. Sometimes, simultaneous losses of -SePh and -Ph as well as Ag/Pd and -SePh were seen. The MS/MS data for the peak at m/z 2839 shows (Figure 3) an initial loss of m/z 312, followed by a loss of m/z 624 that correspond to 2 and 4 SeR (R =  $C_6H_5$ ) moieties, respectively. MS<sup>3</sup> was performed with the fragment at m/z 2525 (assigned to  $Ag_5Pd_4(SePh)_{10}$ , as previously discussed. Interestingly, all of the fragments seen in Figure 2 for the parent clusters are also

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**Figure 3.** MS/MS data of m/z 2839. Repeated MS/MS was done with the most intense fragments closer to the parent ion (marked by gray star in all traces, except the last one). Multiple losses were seen for each case, and they are indicated on the arrow. Up to MS<sup>9</sup> was achievable with reasonable intensity. Spectra have been shifted vertically for clarity.

present here but with low intensity. Repeated MS/MS data were taken with the most intense and the closest fragment of the parent ions, and, surprisingly,  $MS^9$  was achievable. This is the maximum limit of MS/MS studies of clusters reported so far. The fragmentation pattern observed is shown in Figure 3. In  $MS^9$ , after fragmentation, the stable species generated is of composition  $[Ag_3Pd_3(SePh)_3Se]^-$ , which has a  $Ag_3Pd_3^-$  core. More importantly, here also we did not see any further fragmentation, suggesting that the  $Ag_3Pd_3^-$  core is highly stable.

MS/MS of the fragment at m/z 2604 was also studied (Figure S6 in the Supporting Information), and for this case, successive ligand losses were seen and the metal core remains intact throughout.  $MS^2$  of  $[Ag_5Pd_4(SePh)_{10}Se]^-$  produces two new peaks due to consecutive losses of two units of (SeR+R), part of the ligand. Mass difference for the fragments obtained from MS<sup>3</sup> of the peak at m/z 2374 corresponds to the losses of two units of selenium and one R. Similar trends have been observed for MS<sup>4</sup> and MS<sup>5</sup> also. In this process, five units of SeR and two units of R have been lost from the parent species, and the cluster was left with a probable composition,  $[Ag_5Pd_4(SePh)_3Se_3]^-$ . Because of poor intensity, beyond MS<sup>5</sup> was not achievable. Tandem MS of the peak at m/z 2204 due to  $[Ag_3Pd_3(SePh)_{10}]^-$  also shows similar fragmentation pattern, as seen for the previous case (Figure S7 in the Supporting Information). It loses a total of seven units of SeR and one unit of R keeping the metal part intact. Finally, it gives the daughter ion having a probable composition, [Ag<sub>3</sub>Pd<sub>3</sub>(SePh)<sub>2</sub>Se]<sup>-</sup>, which further supports the high stability of the Ag<sub>3</sub>Pd<sub>3</sub><sup>-</sup> core. Detailed fragmentation patterns have been illustrated in Figure S7 in the Supporting Information. Because silver and palladium are close in their atomic masses, the presence of Pd in the cluster ions should be ensured. This was thought to be accomplished by tandem mass spectrometry, utilizing the isotope distribution of elements ( $^{107}$ Ag (51.8%),  $^{109}$ Ag (48.2%),  $^{102}$ Pd (1.0%),  $^{104}$ Pd (11.2%),  $^{105}$ Pd (22.3%),  $^{106}$ Pd (27.3%),  $^{108}$ Pd (26.5%), and <sup>110</sup>Pd (11.7%); abundances are given as percentages). The parent ion at m/z 2839 was selected that contains an envelope

of 31 detectable peaks with unit mass difference, ranging from m/z 2823 to 2853. Initially, MS/MS was taken for the peak at m/z 2839 with the given isotope width of 30. This corresponds to the parent ion selection with a width of 15 mass units on either side of m/z 2839. As expected, it will generate the corresponding fragments with similar isotope width (one of the fragments at m/z 1904 has been shown in Figure 4A). In the second case, each of the 31 peaks of the parent ion (ranging from 2823 to 2853) has been selected for MS/MS studies with an isotope width of 1. Here each peak was chosen with a width of 0.5 mass unit on each side. In most cases, several (more than 6) peaks were seen in the MS/MS spectrum with varying intensities which are expected from the isotope pattern of palladium. One such MS/MS spectrum taken with unit isotope width is shown in Figure S8 in the Supporting Information. The cumulative envelope shown in Figure 4A can be generated by accumulating all of the individual MS/MS spectra taken with unit isotope width (Figure 4B). A computational analysis has been done (Table S1, details of the method used are in Supporting Information 1) to predict the assignment of each peak responsible for this MS/MS data (with a maximum at m/z1905) through a home-built software. As can be seen, although each peak is composed of multiple combinations of Pd and Ag isotopes, a complete analysis of the positions and intensities is possible, confirming the alloy composition (Figure S9 in the Supporting Information). The mass assignment is complicated as the number of combinations contributing to the peaks is ~465 million (see Computational details in Supporting Information 1 for a discussion), and suitable truncation has to be done to relate to the observed mass peaks. Isotope combinations of the peaks of maximum abundances are shown in Figure S9 in the Supporting Information. Other ions have also been investigated by tandem MS.

It is also important to mention here that this composition is one in which ligands are more than the core atoms and such structures are less common. However, recent crystal structures of silver clusters (such as  $[Ag_{14}(SC_6H_3F_2)_{12}(PPh_3)_8]^{29}$  and  $[Ag_{16}(DPPE)_4(SC_6H_3F_2)_{14}])^{53}$  suggest that such ligand-excess

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**Figure 4.** MS/MS spectrum showing a fragment derived from m/z 2839. "A" and "B" are the MS/MS data derived from m/z 2839 with the isotope width of 30 and 1, respectively. Trace B is composed of a number of MS/MS spectra. Each peak selected for MS/MS studies (m/z 2823 to 2853, shown on top of the spectra) and the resulting fragments is shown using specific colors.

structures are also possible. It was also shown that silver can coordinate with three sulfur ligands. It is important to note that Pd(II) can coordinate with four ligands. So, it is not unusual to see the existence of such clusters.

HRTEM of cluster 2 (Figure 1b) shows the presence of highly monodisperse clusters with an average diameter of 0.85 nm (Figure 1c). Complete absence of bigger particles suggests the high purity of the cluster. The composition was further supported with SEM/EDAX data, where the Ag:Pd:Se ratio matches well with the calculated one (Figure S10 in the Supporting Information). Precise composition was already confirmed by HRESI MS data. The EDAX data were to support the suggestion. XPS survey spectrum shown peaks (Figure S11A in the Supporting Information) for all expected elements. The Ag  $3d_{5/2}$  peak could be fitted to two components (Figure S11B in the Supporting Information) with maxima at 367.5 and 368.0 eV binding energy (BE). The Pd  $3d_{5/2}$  peak appeared at a BE of 336.6 eV, which lies in b etween the values of Pd(II) and Pd(0) (Figure S11D in the Supporting Information).<sup>54</sup> The Se 3d peak came at 54.3 eV BE (Figure S11C in the Supporting Information), as expected for the selenolate species. It is important to mention here that Ag(0) and Ag(I) have only 0.5 eV<sup>55</sup> difference in binding energy, so it is difficult to predict the exact charge on silver from photoemission data. However, the data suggest that Ag is present in two oxidation states with 3:2 ratio, whereas Pd is closer to its Pd(II). Although it is difficult to propose a structure based on this information alone, it is likely that distinct silver forms exist in the cluster.

Outer electron count of the  $Ag_5Pd_4(SePh)_{12}$  system would suggest a 33 electron species (taking s<sup>1</sup> and d<sup>10</sup> valence shells for Ag and Pd, respectively, and subtracting one electron each for the ligands, which form the selenolates, and the entity as a whole may be stabilized by a negative change, making the system a closed shell. This may be the reason for the larger intensity of the negative ion. However, a clear rationalization of the data would require the structure.

In summary, selenolate-protected Ag–Pd alloy clusters have been synthesized using one-pot solution-phase synthesis. The crude cluster was purified using HPLC, which gave three different clusters with characteristic optical absorption spectra. ESI MS data suggest that only cluster **2** shows interesting features for which the molecular ion peak appeared at m/z2839, assigned as  $[Ag_5Pd_4(SePh)_{12}]^-$ . Systematic MS/MS was carried out for the molecular ion peak as well as other fragments. Interestingly, up to MS<sup>9</sup> is achievable with reasonable intensity, which is the maximum limit for a monolayer-protected cluster reported so far. The result is also supported by several other characterization measurements. It is hoped that single-crystal studies will be possible once its stability is improved.

#### EXPERIMENTAL METHODS

Materials used in the synthesis are listed in the Supporting Information.

Synthesis of  $Ag_SPd_4$  Cluster. One-pot solution phase route was followed to synthesize the crude cluster. Silver trifluoroacetate (10 mg) and sodium tetracholopalladate (7 mg) were dissolved in 36 mL of acetonitrile taken in a conical flask and kept for string. Then, 5  $\mu$ L of benzeneselenol was added and the color changed from faint yellow to orange, confirming the formation of selenolate. Finally, 10.2 mg of sodium borohydride (s) was added and stirring was continued for 2 h. The color changed to yellowish brown, which confirmed the formation of the crude cluster. It was then centrifuged at 5000 rpm, and the centrifugate was collected for HPLC purification.

Materials, methods, and computational details are given in Supporting Information 1.

### ASSOCIATED CONTENT

#### **S** Supporting Information

Details of materials, XPS, SEM/EDAX, ESI MS, and MS/MS data of  $Ag_5Pd_4$  clusters. Photographs, UV/vis, and ESI MS data of crude and isolated clusters, individual peak assignments of a MS/MS fragment, and a table of assignments of an MS/MS fragment. This material is available free of charge via the Internet at http://pubs.acs.org.

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#### Notes

The authors declare no competing financial interest.

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# Isolation and Tandem Mass Spectrometric Identification of a Stable Monolayer Protected Silver-Palladium Alloy Cluster

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### **Supporting information 1.**

### Materials and methods:

### 1. Chemicals:

Silver trifluoroacetate (CF<sub>3</sub>COOAg, Aldrich, 98%), sodium tetrachloropalladate (Na<sub>2</sub>PdCl<sub>4</sub>, Aldrich, 98%), benzeneselenol (PhSeH, Aldrich, 97%), sodium borohydride (NaBH<sub>4</sub>, Aldrich, 99.99%), acetonitrile (HPLC grade, Aldrich) were used for synthesis. All the chemicals were commercially available and were used without further purification.

### 2. Instrumentation

The UV/Vis measurements were carried out at ambient temperature using a double-beam spectrometer (Jasco V-630). The absorbances of raw spectral data were corrected [I(E)] using the following equation and plotted in terms of energy (1239.8/ Wavelength in nm = Energy (eV)).

$$I(E) = \frac{I(w)}{\partial E/\partial w} \propto I(w)w^2$$

HPLC measurement was done using a Shimadzu HPLC system equipped with a normal phase column (Shimadzu) and a UV/Vis detector. Isocratic mode was used. ESI MS measurements were done using LTQ XL mass spectrometer from Thermo Scientific, San Jose, CA. Acetonitrile was used as the solvent and the spectra were collected in negative ion mode. High resolution transmission electron microscopy of clusters was carried out with a JEOL 3010 instrument. The samples were drop casted on carbon-coated copper grids and allowed to dry under ambient conditions. For reduced beam-induced damage, the samples were imaged at 200 keV. Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) studies were performed with a FEI QUANTA-200 SEM. For measurements, samples were drop casted on an indium tin oxide (ITO) coated glass and dried in vacuum. X-ray photoelectron spectroscopy (XPS) measurements were conducted using an Omicron ESCA Probe spectrometer with polychromatic MgK $\alpha$  X-rays (h $\nu$  = 1253.6 eV). The samples were spotted as drop-cast films on a sample stub. Constant analyzer energy of 20 eV was used for the measurements.

### **3.** Computation

We have developed a novel algorithm to combinatorially enumerate the possible number of isotope combinations appearing in high resolution mass spectra and tandem mass spectra ( $MS/MS^n$ ) of any given species. We apply this algorithm to this an alloy cluster fragment,  $Ag_5Pd_4Se_6C_{36}H_{30}^-$ , derived from m/z 2839. Possible combinations for this cluster fragment are more than 465 million and therefore, it is a resource and time consuming task.

Briefly, the methodology is as follows. Let us consider a molecule or ion with k different elements. Let each of the distinct elements be denoted by  $E_i$ , where  $1 \le i \le k$  and each  $E_i$  has  $n_i$  number of atoms and  $m_i$  number of isotopes. The number of possible combinations  $N_i$  for each  $E_i$  will be,

$$N_{i} = \binom{n_{i} + m_{i} - 1}{m_{i}} = \frac{(n_{i} + m_{i} - 1)!}{(n_{i})!(m_{i} - 1)!}$$

So the total number of isotope combinations for the molecule will be the product N where,

$$N = \prod_{1 \le i \le k} N_i$$

which is the total number of peaks in the high resolution mass spectrum. The total combination for  $Ag_5Pd_4Se_6C_{36}H_{30}$  are,

$$N_{i} = {\binom{5+2-1}{2}} {\binom{4+6-1}{6}} {\binom{6+6-1}{6}} {\binom{36+2-1}{2}} {\binom{30+2-1}{2}}$$
  
= 465230304 \approx 465 million

We perform the calculations based on our algorithm. We have executed it three times to select peaks whose contributions in the mass spectral abundances were more than or equal to 1.0, 0.5, and  $10^{-4}$  and the number of peaks computed were 29255, 46555 and 1027865, respectively in each of these cases. We have used natural abundances and masses of isotopes for these elements. We have performed these calculations on a desktop computer with an i7 processor (2.2 GHz) and 8 Gb RAM. We have shown part of the mass spectrum and number of isotopes from the third run (i.e. peaks with abundance greater then equal to  $10^{-4}$ ) in the table below (TableS1).

**Supporting information 2.** 



**Figure S2.** Photographs at different stages of solution state synthesis - i) Silver trifluoroacetate in acetonitrile, ii) addition of sodium tetrachloropalladate to the mixture, iii) addition of benzeneselenol, iv) just after the addition of sodium borohydride, v) reaction mixture after 5 minutes, vi) after 30 minutes, and vii) after 2 hours. After the synthesis, the cluster was centrifuged (viii) and the centrifugate was collected (ix) which contains the crude cluster.



*Figure S3.* Chromatogram under different flow rates: 1 (A), 0.75 (B), 0.5 (C), and 0.25 (D) mL/min, respectively. Best separation came at 0.25 mL/min flow rate. Chromatograms have been shifted vertically for clarity.

**Supporting information 4.** 



*Figure S4.* UV/Vis absorption spectra of as-synthesized crude (a), isolated 1(b), isolated 2 (c), and isolated 3 clusters (d), respectively. Inset shows the corresponding photographs of cluster solutions which are marked with a', b', c' and d', respectively. Concentration in d is small which gives a faint appearance.

## Supporting information 5.



*Figure S5. ESI MS spectra of crude cluster (A), isolated cluster* 1(B)*, cluster* 2(C)*, and cluster* 3(D)*, respectively. Spectra have been shifted vertically for clarity.* 

## **Supporting information 6.**



*Figure S6. MS/MS* data of *m/z* 2604. *Repeated MS/MS* spectra were done with peaks marked with grey star. Spectra have been shifted vertically for clarity.

**Supporting information 7.** 



*Figure S7. MS/MS* data of *m/z* 2204. *Repeated MS/MS* were done with the peaks marked with grey star. *Spectra have been shifted vertically for clarity.* 

## Supporting information 8.



*Figure S8.* Comparative MS/MS spectra of m/z 2839 collected with a given isotope width of 30(A) and 1(B), respectively. Spectra have been shifted vertically for clarity.

## Table S1

**Table S1:** Individual peak assignment for MS/MS fragment derived from m/z 2839. The abundances  $\geq 20$  are shown here. Essential details of the calculation are discussed in S1.

							A	.g5Pd	4(SeC <sub>6</sub>	H <sub>5</sub> ) <sub>6</sub> <sup>-</sup>									-	
					Pd (iso	otopes)	)		Ag (ise	otopes)		5	Se (iso	otopes	5)		H (isot	opes)	C (iso	topes)
ID	Mass	Abundance	102	104	105	106	108	110	107	109	74	76	77	78	80	82	1	2	12	13
1	1891.88059	25.98811606	0	1	2	1	0	0	3	2	0	1	0	2	3	0	30	0	36	0
2	1891.881595	20.41977997	0	1	1	2	0	0	3	2	0	1	1	1	3	0	30	0	36	0
3	1891.882355	21.2803997	0	1	1	1	1	0	4	1	0	1	1	1	3	0	30	0	36	0
4	1891.882801	28.41727798	0	1	1	1	1	0	3	2	0	1	1	2	2	0	30	0	36	0
5	1892.87899	31.80721952	0	1	1	2	0	0	3	2	0	1	0	2	3	0	30	0	36	0
6	1892.87975	33.14777856	0	1	1	1	1	0	4	1	0	1	0	2	3	0	30	0	36	0
7	1892.880197	29.50977272	0	1	1	1	1	0	3	2	0	1	0	3	2	0	30	0	36	0
8	1892.88129	21.16214787	0	1	2	1	0	0	3	2	0	0	1	2	3	0	30	0	36	0
9	1892.881313	24.27819074	0	1	1	1	1	0	3	2	0	2	0	1	3	0	30	0	36	0
10	1892.88155	21.40385391	0	0	1	2	1	0	3	2	0	2	0	2	2	0	30	0	36	0
11	1892.882644	20.46560533	0	0	2	2	0	0	3	2	0	1	1	1	3	0	30	0	36	0
12	1892.883404	21.32815643	0	0	2	1	1	0	4	1	0	1	1	1	3	0	30	0	36	0
13	1892.88385	28.48105105	0	0	2	1	1	0	3	2	0	1	1	2	2	0	30	0	36	0
14	1893.87815	20.28501541	0	1	0	2	1	0	4	1	0	1	0	2	3	0	30	0	36	0
15	1893.878685	21.97572105	0	1	2	1	0	0	3	2	0	0	0	3	3	0	30	0	36	0
16	1893.87969	25.9006494	0	1	1	2	0	0	3	2	0	0	1	2	3	0	30	0	36	0
17	1893.879802	27.11969789	0	1	2	1	0	0	3	2	0	1	0	1	4	0	30	0	36	0
18	1893.880039	31.87860017	0	0	2	2	0	0	3	2	0	1	0	2	3	0	30	0	36	0
19	1893.880249	24.14328066	0	1	2	1	0	0	2	3	0	1	0	2	3	0	30	0	36	0
20	1893.88045	26.99226792	0	1	1	1	1	0	4	1	0	0	1	2	3	0	30	0	36	0
21	1893.880799	33.22216765	0	0	2	1	1	0	4	1	0	1	0	2	3	0	30	0	36	0
22	1893.880897	24.02983627	0	1	1	1	1	0	3	2	0	0	1	3	2	0	30	0	36	0
23	1893.881009	25.16083245	0	1	2	0	1	0	3	2	0	1	0	2	3	0	30	0	36	0
24	1893.881246	29.57599753	0	0	2	1	1	0	3	2	0	1	0	3	2	0	30	0	36	0
25	1893.881804	26.10382961	0	0	1	2	1	0	4	1	0	1	1	1	3	0	30	0	36	0
26	1893.882014	39.53950808	0	1	1	1	1	0	3	2	0	1	1	1	3	0	30	0	36	0
27	1893.88225	34.8583576	0	0	1	2	1	0	3	2	0	1	1	2	2	0	30	0	36	0
28	1893.882362	24.33267501	0	0	2	1	1	0	3	2	0	2	0	1	3	0	30	0	36	0
29	1893.88246	26.40000208	0	1	1	1	1	0	2	3	0	1	1	2	2	0	30	0	36	0
30	1894.876638	22.65916333	0	1	1	2	0	0	4	1	0	0	0	2	4	0	30	0	36	0
31	1894.877085	26.89639302	0	1	1	2	0	0	3	2	0	0	0	3	3	0	30	0	36	0
32	1894.877845	28.02997853	0	1	1	1	1	0	4	1	0	0	0	3	3	0	30	0	36	0
33	1894.878202	33.19217838	0	1	1	2	0	0	3	2	0	1	0	1	4	0	30	0	36	0
34	1894.878439	26.0111104	0	0	1	3	0	0	3	2	0	1	0	2	3	0	30	0	36	0
35	1894.878649	29.54929961	0	1	1	2	0	0	2	3	0	1	0	2	3	0	30	0	36	0
36	1894.878962	34.59110842	0	1	1	1	1	0	4	1	0	1	0	1	4	0	30	0	36	0
37	1894.879199	40.66107666	0	0	1	2	1	0	4	1	0	1	0	2	3	0	30	0	36	0

38	1894.879409	61.58939103	0	1	1	1	1	0	3	2	0	1	0	2	3	0	30	0	36	0
39	1894.879646	36.19847794	0	0	1	2	1	0	3	2	0	1	0	3	2	0	30	0	36	0
40	1894.879856	27.41494318	0	1	1	1	1	0	2	3	0	1	0	3	2	0	30	0	36	0
41	1894.880414	24.19647908	0	1	0	2	1	0	3	2	0	1	1	1	3	0	30	0	36	0
42	1894.880502	22.08359604	0	1	2	1	0	0	3	2	0	0	1	1	4	0	30	0	36	0
43	1894.880739	25.95877474	0	0	2	2	0	0	3	2	0	0	1	2	3	0	30	0	36	0
44	1894.880762	29.78110202	0	0	1	2	1	0	3	2	0	2	0	1	3	0	30	0	36	0
45	1894.880972	22.55473893	0	1	1	1	1	0	2	3	0	2	0	1	3	0	30	0	36	0
46	1894.881499	27.05284303	0	0	2	1	1	0	4	1	0	0	1	2	3	0	30	0	36	0
47	1894.881709	20.48849003	0	1	2	0	1	0	3	2	0	0	1	2	3	0	30	0	36	0
48	1894.881946	24.08376319	0	0	2	1	1	0	3	2	0	0	1	3	2	0	30	0	36	0
49	1894.881969	20.72250181	0	0	1	1	2	0	3	2	0	2	0	2	2	0	30	0	36	0
50	1894.883063	39.62824127	0	0	2	1	1	0	3	2	0	1	1	1	3	0	30	0	36	0
51	1894.883509	26.45924804	0	0	2	1	1	0	2	3	0	1	1	2	2	0	30	0	36	0
52	1895.877362	21.16827123	0	1	0	2	1	0	4	1	0	1	0	1	4	0	30	0	36	0
53	1895.877687	22.71001424	0	0	2	2	0	0	4	1	0	0	0	2	4	0	30	0	36	0
54	1895.877809	37.69005949	0	1	0	2	1	0	3	2	0	1	0	2	3	0	30	0	36	0
55	1895.877897	34.39889108	0	1	2	1	0	0	3	2	0	0	0	2	4	0	30	0	36	0
56	1895.878134	26.95675297	0	0	2	2	0	0	3	2	0	0	0	3	3	0	30	0	36	0
57	1895.878344	20.41571616	0	1	2	1	0	0	2	3	0	0	0	3	3	0	30	0	36	0
58	1895.878894	28.09288243	0	0	2	1	1	0	4	1	0	0	0	3	3	0	30	0	36	0
59	1895.878902	27.02842274	0	1	1	2	0	0	3	2	0	0	1	1	4	0	30	0	36	0
60	1895.879104	21.27616462	0	1	2	0	1	0	3	2	0	0	0	3	3	0	30	0	36	0
61	1895.879139	21.18087218	0	0	1	3	0	0	3	2	0	0	1	2	3	0	30	0	36	0
62	1895.879251	33.26666711	0	0	2	2	0	0	3	2	0	1	0	1	4	0	30	0	36	0
63	1895.879349	24.06202306	0	1	1	2	0	0	2	3	0	0	1	2	3	0	30	0	36	0
64	1895.879461	25.19453415	0	1	2	1	0	0	2	3	0	1	0	1	4	0	30	0	36	0
65	1895.879662	28.16757281	0	1	1	1	1	0	4	1	0	0	1	1	4	0	30	0	36	0
66	1895.879698	29.61561312	0	0	2	2	0	0	2	3	0	1	0	2	3	0	30	0	36	0
67	1895.879899	33.11035378	0	0	1	2	1	0	4	1	0	0	1	2	3	0	30	0	36	0
68	1895.880011	34.66873658	0	0	2	1	1	0	4	1	0	1	0	1	4	0	30	0	36	0
69	1895.880109	50.15230027	0	1	1	1	1	0	3	2	0	0	1	2	3	0	30	0	36	0
70	1895.880221	26.25639247	0	1	2	0	1	0	3	2	0	1	0	1	4	0	30	0	36	0
71	1895.880346	29.47645536	0	0	1	2	1	0	3	2	0	0	1	3	2	0	30	0	36	0
72	1895.880458	61.7276078	0	0	2	1	1	0	3	2	0	1	0	2	3	0	30	0	36	0
73	1895.880556	22.32401456	0	1	1	1	1	0	2	3	0	0	1	3	2	0	30	0	36	0
74	1895.880668	23.37472397	0	1	2	0	1	0	2	3	0	1	0	2	3	0	30	0	36	0
75	1895.880905	27.47646684	0	0	2	1	1	0	2	3	0	1	0	3	2	0	30	0	36	0
76	1895.881226	20.63057421	0	1	1	1	1	0	3	2	0	1	1	0	4	0	30	0	36	0
77	1895.881463	48.50155996	0	0	1	2	1	0	3	2	0	1	1	1	3	0	30	0	36	0
78	1895.881673	36.73269115	0	1	1	1	1	0	2	3	0	1	1	1	3	0	30	0	36	0
79	1895.881909	32.38384456	0	0	1	2	1	0	2	3	0	1	1	2	2	0	30	0	36	0
80	1895.882021	22.60535549	0	0	2	1	1	0	2	3	0	2	0	1	3	0	30	0	36	0
81	1895.882192	20.87360848	0	1	1	1	1	0	3	2	0	1	1	1	2	1	30	0	36	0
82	1895.882223	25.27286248	0	0	1	1	2	0	4	1	0	1	1	1	3	0	30	0	36	0

83	1895 882669	33 74870626	0	0	1	1	2	0	3	2	0	1	1	2	2	0	30	0	36	0
84	1895 882764	24 66066618	0	1	1	1	1	0	3	2	0	1	0	2	3	0	30	0	35	1
85	1896.876297	42,10128496	0	1	1	2	0	0	3	2	0	0	0	2	4	0	30	0	36	0
86	1896.876534	21,99516521	0	0	1	3	0	0	3	2	0	0	0	3	3	0	30	0	36	0
87	1896.876744	24.98708117	0	1	1	2	0	0	2	3	0	0	0	3	3	0	30	0	36	0
88	1896.877057	43.87570156	0	1	1	1	1	0	4	1	0	0	0	2	4	0	30	0	36	0
89	1896.877294	34,38327258	0	0	1	2	1	0	4	1	0	0	0	3	3	0	30	0	36	0
90	1896.877504	52 0803922	0	1	1	1	1	0	3	2	0	0	0	3	3	0	30	0	36	0
91	1896.877651	27,14369345	0	0	1	3	0	0	3	2	0	1	0	1	4	0	30	0	36	0
92	1896.877741	22.95725242	0	0	1	2	1	0	3	2	0	0	0	4	2	0	30	0	36	0
93	1896.877861	30.8359435	0	1	1	2	0	0	2	3	0	1	0	1	4	0	30	0	36	0
94	1896.878098	24.16464268	0	0	1	3	0	0	2	3	0	1	0	2	3	0	30	0	36	0
95	1896.87838	23.36365388	0	1	1	2	0	0	3	2	0	1	0	1	3	1	30	0	36	0
96	1896.878411	42.43155266	0	0	1	2	1	0	4	1	0	1	0	1	4	0	30	0	36	0
97	1896.878509	30.69105164	0	1	0	2	1	0	3	2	0	0	1	2	3	0	30	0	36	0
98	1896.878621	64.27113355	0	1	1	1	1	0	3	2	0	1	0	1	4	0	30	0	36	0
99	1896.878858	75.54928442	0	0	1	2	1	0	3	2	0	1	0	2	3	0	30	0	36	0
100	1896.878946	22.98405858	0	0	3	1	0	0	3	2	0	0	0	2	4	0	30	0	36	0
101	1896.879068	57.21730463	0	1	1	1	1	0	2	3	0	1	0	2	3	0	30	0	36	0
102	1896.87914	24.34834723	0	1	1	1	1	0	4	1	0	1	0	1	3	1	30	0	36	0
103	1896.879305	33.6288329	0	0	1	2	1	0	2	3	0	1	0	3	2	0	30	0	36	0
104	1896.879377	21.46570445	0	0	1	2	1	0	4	1	0	1	0	2	2	1	30	0	36	0
105	1896.879587	32.51413326	0	1	1	1	1	0	3	2	0	1	0	2	2	1	30	0	36	0
106	1896.879618	39.3667065	0	0	1	1	2	0	4	1	0	1	0	2	3	0	30	0	36	0
107	1896.879828	29.81440334	0	1	1	0	2	0	3	2	0	1	0	2	3	0	30	0	36	0
108	1896.879951	27.08907898	0	0	2	2	0	0	3	2	0	0	1	1	4	0	30	0	36	0
109	1896.880065	35.04616635	0	0	1	1	2	0	3	2	0	1	0	3	2	0	30	0	36	0
110	1896.880073	22.47882779	0	1	0	2	1	0	2	3	0	1	1	1	3	0	30	0	36	0
111	1896.880161	20.51593336	0	1	2	1	0	0	2	3	0	0	1	1	4	0	30	0	36	0
112	1896.880398	24.11602221	0	0	2	2	0	0	2	3	0	0	1	2	3	0	30	0	36	0
113	1896.880421	27.66701144	0	0	1	2	1	0	2	3	0	2	0	1	3	0	30	0	36	0
114	1896.880684	27.27995702	0	1	1	1	0	1	3	2	0	1	0	2	3	0	30	0	36	0
115	1896.880711	28.2307855	0	0	2	1	1	0	4	1	0	0	1	1	4	0	30	0	36	0
116	1896.880833	23.42622893	0	1	0	1	2	0	3	2	0	1	1	1	3	0	30	0	36	0
117	1896.880921	21.3806056	0	1	2	0	1	0	3	2	0	0	1	1	4	0	30	0	36	0
118	1896.881069	20.65275732	0	0	0	2	2	0	3	2	0	1	1	2	2	0	30	0	36	0
119	1896.881158	50.26485032	0	0	2	1	1	0	3	2	0	0	1	2	3	0	30	0	36	0
120	1896.881181	28.83307572	0	0	1	1	2	0	3	2	0	2	0	1	3	0	30	0	36	0
121	1896.881605	22.37411334	0	0	2	1	1	0	2	3	0	0	1	3	2	0	30	0	36	0
122	1896.882275	20.67687263	0	0	2	1	1	0	3	2	0	1	1	0	4	0	30	0	36	0
123	1896.882722	36.81512538	0	0	2	1	1	0	2	3	0	1	1	1	3	0	30	0	36	0
124	1896.883241	20.9204523	0	0	2	1	1	0	3	2	0	1	1	1	2	1	30	0	36	0
125	1896.883464	20.08120415	0	1	1	1	1	0	3	2	0	0	1	2	3	0	30	0	35	1
126	1896.883813	24.71600878	0	0	2	1	1	0	3	2	0	1	0	2	3	0	30	0	35	1
127	1897.875457	26.85004307	0	1	0	2	1	0	4	1	0	0	0	2	4	0	30	0	36	0

128	1897.875667	20.33489934	0	2	0	1	1	0	3	2	0	0	0	2	4	0	30	0	36	0
129	1897.875904	31.87096101	0	1	0	2	1	0	3	2	0	0	0	3	3	0	30	0	36	0
130	1897.877021	39.33117062	0	1	0	2	1	0	3	2	0	1	0	1	4	0	30	0	36	0
131	1897.87711	28.71735778	0	1	2	1	0	0	3	2	0	0	0	1	5	0	30	0	36	0
132	1897.877258	30.82194273	0	0	0	3	1	0	3	2	0	1	0	2	3	0	30	0	36	0
133	1897.877346	42.1957672	0	0	2	2	0	0	3	2	0	0	0	2	4	0	30	0	36	0
134	1897.877468	35.01453058	0	1	0	2	1	0	2	3	0	1	0	2	3	0	30	0	36	0
135	1897.877556	31.95699449	0	1	2	1	0	0	2	3	0	0	0	2	4	0	30	0	36	0
136	1897.877781	20.49441847	0	1	0	1	2	0	4	1	0	1	0	1	4	0	30	0	36	0
137	1897.877793	25.04315631	0	0	2	2	0	0	2	3	0	0	0	3	3	0	30	0	36	0
138	1897.878018	24.09073194	0	0	0	2	2	0	4	1	0	1	0	2	3	0	30	0	36	0
139	1897.878076	24.2130473	0	1	2	1	0	0	3	2	0	0	0	2	3	1	30	0	36	0
140	1897.878106	43.97416588	0	0	2	1	1	0	4	1	0	0	0	2	4	0	30	0	36	0
141	1897.878228	36.49026616	0	1	0	1	2	0	3	2	0	1	0	2	3	0	30	0	36	0
142	1897.878316	33.303866	0	1	2	0	1	0	3	2	0	0	0	2	4	0	30	0	36	0
143	1897.878351	22.10313565	0	0	1	3	0	0	3	2	0	0	1	1	4	0	30	0	36	0
144	1897.878465	21.44674712	0	0	0	2	2	0	3	2	0	1	0	3	2	0	30	0	36	0
145	1897.878553	52,1972692	0	0	2	1	1	0	3	2	0	0	0	3	3	0	30	0	36	0
146	1897.878561	25.10973841	0	1	1	2	0	0	2	3	0	0	1	1	4	0	30	0	36	0
147	1897.87891	30.90514445	0	0	2	2	0	0	2	3	0	1	0	1	4	0	30	0	36	0
148	1897.879111	34,55205408	0	0	1	2	1	0	4	1	0	0	1	1	4	0	30	0	36	0
149	1897 879321	52 33604578	0	1	1	1	1	0	3	2	0	0	1	1	4	0	30	0	36	0
150	1897 879429	23 41608578	0	0	2	2	0	0	3	2	0	1	0	1	3	1	30	0	36	0
151	1897 879558	61 51985487	0	0	1	2	1	0	3	2	0	0	1	2	3	0	30	0	36	0
152	1897 87967	64.41536859	0	0	2	1	1	0	3	2	0	1	0	1	4	0	30	0	36	0
153	1897 879768	46 59210612	0	1	1	1	1	0	2	3	0	0	1	2	3	0	30	0	36	0
154	1897 87988	24.39251275	0	1	2	0	1	0	2	3	0	1	0	1	4	0	30	0	36	0
155	1897 880005	27 38399093	0	0	1	2	1	0	2	3	0	0	1	3	2	0	30	0	36	0
156	1897 880117	57 34570971	0	0	2	1	1	0	2	3	0	1	0	2	3	0	30	0	36	0
157	1897 880189	24 40298894	0	0	2	1	1	0	4	1	0	1	0	1	3	1	30	0	36	0
158	1897 880287	26 47628994	0	1	1	1	1	0	3	2	0	0	1	2	2	1	30	0	36	0
150	1897 880318	32 05634691	0	0	1	1	2	0	4	1	0	0	1	2	3	0	30	0	36	0
160	1897 880528	24 27789728	0	1	1	0	2	0	3	2	0	0	1	2	3	0	30	0	36	0
161	1897.880636	32.58710035	0	0	2	1	1	0	3	2	0	1	0	2	2	1	30	0	36	0
162	1897.880675	25.30671424	0	0	1	2	1	0	3	2	0	1	1	0	4	0	30	0	36	0
163	1897 880765	28 53812692	0	0	1	1	2	0	3	2	0	0	1	3	2	0	30	0	36	0
164	1897 880859	20.85322074	0	1	1	1	1	0	3	2	0	0	0	3	3	0	30	0	35	1
165	1897.880877	29.88131179	0	0	2	0	2	0	3	2	0	1	0	2	3	0	30	0	36	0
166	1897.881122	45.05854799	0	0	1	2	1	0	2	3	0	1	1	1	3	0	30	0	36	0
167	1897.881384	22.21409521	0	1	1	1	0	1	3	2	0	0	1	2	3	0	30	0	36	0
168	1897.881641	25.60483482	0	0	1	2	1	0	3	2	0	1	1	1	2	1	30	0	36	0
169	1897.881733	27.34117775	0	0	2	1	0	1	3	2	0	1	0	2	3	0	30	0	36	0
170	1897 881882	46 95760251	0	0	1	1	2	0	3	2	0	1	1	1	3	0	30	0	36	0
171	1897 881076	25 73444781	0	1	1	1	1	0	3	2	0	1	0	1	4	0	30	0	35	1
171	1807 802212	30 2502606	0	0	1	2	1	0	2	2	0	1	0	2	2	0	30	0	35	1
1/2	1071.002213	20.4204090	U	U	1	4	1	U	,	4	U	1	U	4	3	U	50	U	33	1

173	1897 882328	31 35296476	0	0	1	1	2	0	2	3	0	1	1	2	2	0	30	0	36	0
174	1897.882423	22.91006333	0	1	1	1	1	0	2	3	0	1	0	2	3	0	30	0	35	1
175	1897.882738	21 4829283	0	0	1	2	0	1	3	2	0	1	1	1	3	0	30	0	36	0
176	1897.883498	22.38835588	0	0	1	1	1	1	4	1	0	1	1	1	3	0	30	0	36	0
177	1897.883944	29.8968131	0	0	1	1	1	1	3	2	0	1	1	2	2	0	30	0	36	0
178	1897.884513	20.12626969	0	0	2	1	1	0	3	2	0	0	1	2	3	0	30	0	35	1
179	1898.87551	35.14757672	0	1	1	2	0	0	3	2	0	0	0	1	5	0	30	0	36	0
180	1898 875746	34,42932729	0	0	1	3	0	0	3	2	0	0	0	2	4	0	30	0	36	0
181	1898 875956	39,1126135	0	1	1	2	0	0	2	3	0	0	0	2	4	0	30	0	36	0
182	1898.876193	20.43378003	0	0	1	3	0	0	2	3	0	0	0	3	3	0	30	0	36	0
183	1898.87627	36.62891972	0	1	1	1	1	0	4	1	0	0	0	1	5	0	30	0	36	0
184	1898.876476	29.63468798	0	1	1	2	0	0	3	2	0	0	0	2	3	1	30	0	36	0
185	1898.876506	53.82059801	0	0	1	2	1	0	4	1	0	0	0	2	4	0	30	0	36	0
186	1898.876716	81.52213685	0	1	1	1	1	0	3	2	0	0	0	2	4	0	30	0	36	0
187	1898.876953	63.88496943	0	0	1	2	1	0	3	2	0	0	0	3	3	0	30	0	36	0
188	1898.877163	48.38332732	0	1	1	1	1	0	2	3	0	0	0	3	3	0	30	0	36	0
189	1898.877236	30.88368269	0	1	1	1	1	0	4	1	0	0	0	2	3	1	30	0	36	0
190	1898.87731	25.21682632	0	0	1	3	0	0	2	3	0	1	0	1	4	0	30	0	36	0
191	1898.8774	21.32757092	0	0	1	2	1	0	2	3	0	0	0	4	2	0	30	0	36	0
192	1898.877476	21.23949988	0	1	1	0	2	0	4	1	0	0	0	2	4	0	30	0	36	0
193	1898.877682	27.49416391	0	1	1	1	1	0	3	2	0	0	0	3	2	1	30	0	36	0
194	1898.877713	33.2887447	0	0	1	1	2	0	4	1	0	0	0	3	3	0	30	0	36	0
195	1898.877721	32.02741001	0	1	0	2	1	0	3	2	0	0	1	1	4	0	30	0	36	0
196	1898.877833	26.8278581	0	1	1	1	1	0	3	2	0	1	0	0	5	0	30	0	36	0
197	1898.877923	25.21125462	0	1	1	0	2	0	3	2	0	0	0	3	3	0	30	0	36	0
198	1898.877958	25.09833757	0	0	0	3	1	0	3	2	0	0	1	2	3	0	30	0	36	0
199	1898.878039	21.7051229	0	1	1	2	0	0	2	3	0	1	0	1	3	1	30	0	36	0
200	1898.87807	78.83887253	0	0	1	2	1	0	3	2	0	1	0	1	4	0	30	0	36	0
201	1898.87816	22.22645075	0	0	1	1	2	0	3	2	0	0	0	4	2	0	30	0	36	0
202	1898.878168	28.51236588	0	1	0	2	1	0	2	3	0	0	1	2	3	0	30	0	36	0
203	1898.87828	59.70867654	0	1	1	1	1	0	2	3	0	1	0	1	4	0	30	0	36	0
204	1898.878517	70.18621793	0	0	1	2	1	0	2	3	0	1	0	2	3	0	30	0	36	0
205	1898.878589	29.86716023	0	0	1	2	1	0	4	1	0	1	0	1	3	1	30	0	36	0
206	1898.878605	21.35247417	0	0	3	1	0	0	2	3	0	0	0	2	4	0	30	0	36	0
207	1898.878727	26.57779119	0	1	1	1	1	0	1	4	0	1	0	2	3	0	30	0	36	0
208	1898.878779	23.06811023	0	1	1	1	0	1	3	2	0	0	0	3	3	0	30	0	36	0
209	1898.878799	45.23983035	0	1	1	1	1	0	3	2	0	1	0	1	3	1	30	0	36	0
210	1898.87883	41.08082266	0	0	1	1	2	0	4	1	0	1	0	1	4	0	30	0	36	0
211	1898.878928	29.71405878	0	1	0	1	2	0	3	2	0	0	1	2	3	0	30	0	36	0
212	1898.879036	39.88380889	0	0	1	2	1	0	3	2	0	1	0	2	2	1	30	0	36	0
213	1898.87904	31.11259045	0	1	1	0	2	0	3	2	0	1	0	1	4	0	30	0	36	0
214	1898.879246	30.20603121	0	1	1	1	1	0	2	3	0	1	0	2	2	1	30	0	36	0
215	1898.879277	73.14431269	0	0	1	1	2	0	3	2	0	1	0	2	3	0	30	0	36	0
216	1898.879365	22.25240359	0	0	3	0	1	0	3	2	0	0	0	2	4	0	30	0	36	0
217	1898.879487	27.69794878	0	1	1	0	2	0	2	3	0	1	0	2	3	0	30	0	36	0

218	1898 87961	25 16608881	0	0	2	2	0	0	2	3	0	0	1	1	4	0	30	0	36	0
210	1898 879724	32 55832121	0	0	1	1	2	0	2	3	0	1	0	3	2	0	30	0	36	0
220	1898 879796	20 78238345	0	0	1	1	2	0	4	1	0	1	0	2	2	1	30	0	36	0
221	1898 879896	28 46778856	0	1	1	1	0	1	3	2	0	1	0	1	4	0	30	0	36	0
222	1898 880133	33 46325069	0	0	1	2	0	1	3	2	0	1	0	2	3	0	30	0	36	0
223	1898.880282	28.73603396	0	0	0	2	2	0	3	2	0	1	1	1	3	0	30	0	36	0
224	1898.880343	25.34341687	0	1	1	1	0	1	2	3	0	1	0	2	3	0	30	0	36	0
225	1898 88037	52 45349651	0	0	2	1	1	0	3	2	0	0	1	1	4	0	30	0	36	0
226	1898.880492	21.76325589	0	1	0	1	2	0	2	3	0	1	1	1	3	0	30	0	36	0
227	1898.880817	46.6966665	0	0	2	1	1	0	2	3	0	0	1	2	3	0	30	0	36	0
228	1898.88084	26.78628331	0	0	1	1	2	0	2	3	0	2	0	1	3	0	30	0	36	0
229	1898.880893	34.87360545	0	0	1	1	1	1	4	1	0	1	0	2	3	0	30	0	36	0
230	1898.881103	26.41155005	0	1	1	0	1	1	3	2	0	1	0	2	3	0	30	0	36	0
231	1898.881336	26.53570711	0	0	2	1	1	0	3	2	0	0	1	2	2	1	30	0	36	0
232	1898.88134	31.04618818	0	0	1	1	1	1	3	2	0	1	0	3	2	0	30	0	36	0
233	1898.881577	24.33238089	0	0	2	0	2	0	3	2	0	0	1	2	3	0	30	0	36	0
234	1898.881908	20.90001881	0	0	2	1	1	0	3	2	0	0	0	3	3	0	30	0	35	1
235	1898.882108	20.752487	0	1	0	1	1	1	3	2	0	1	1	1	3	0	30	0	36	0
236	1898.882433	22.26394731	0	0	2	1	0	1	3	2	0	0	1	2	3	0	30	0	36	0
237	1898.882456	25.54222581	0	0	1	1	1	1	3	2	0	2	0	1	3	0	30	0	36	0
238	1898.882676	20.95558557	0	1	1	1	1	0	3	2	0	0	1	1	4	0	30	0	35	1
239	1898.882913	24.63282359	0	0	1	2	1	0	3	2	0	0	1	2	3	0	30	0	35	1
240	1898.883025	25.79220016	0	0	2	1	1	0	3	2	0	1	0	1	4	0	30	0	35	1
241	1898.883472	22.9614773	0	0	2	1	1	0	2	3	0	1	0	2	3	0	30	0	35	1
242	1899.87467	22.41532414	0	1	0	2	1	0	4	1	0	0	0	1	5	0	30	0	36	0
243	1899.874906	21.95726143	0	0	0	3	1	0	4	1	0	0	0	2	4	0	30	0	36	0
244	1899.875116	49.88804299	0	1	0	2	1	0	3	2	0	0	0	2	4	0	30	0	36	0
245	1899.875353	26.06323652	0	0	0	3	1	0	3	2	0	0	0	3	3	0	30	0	36	0
246	1899.875563	29.60851625	0	1	0	2	1	0	2	3	0	0	0	3	3	0	30	0	36	0
247	1899.875876	25.99532161	0	1	0	1	2	0	4	1	0	0	0	2	4	0	30	0	36	0
248	1899.876113	20.37128062	0	0	0	2	2	0	4	1	0	0	0	3	3	0	30	0	36	0
249	1899.876323	30.85640792	0	1	0	1	2	0	3	2	0	0	0	3	3	0	30	0	36	0
250	1899.87647	32.16400039	0	0	0	3	1	0	3	2	0	1	0	1	4	0	30	0	36	0
251	1899.876559	35.22645369	0	0	2	2	0	0	3	2	0	0	0	1	5	0	30	0	36	0
252	1899.87668	36.53914308	0	1	0	2	1	0	2	3	0	1	0	1	4	0	30	0	36	0
253	1899.876769	26.67877992	0	1	2	1	0	0	2	3	0	0	0	1	5	0	30	0	36	0
254	1899.876917	28.63396531	0	0	0	3	1	0	2	3	0	1	0	2	3	0	30	0	36	0
255	1899.877005	39.20038866	0	0	2	2	0	0	2	3	0	0	0	2	4	0	30	0	36	0
256	1899.877199	27.68483125	0	1	0	2	1	0	3	2	0	1	0	1	3	1	30	0	36	0
257	1899.87723	25.13969734	0	0	0	2	2	0	4	1	0	1	0	1	4	0	30	0	36	0
258	1899.877288	25.26733859	0	1	2	1	0	0	3	2	0	0	0	1	4	1	30	0	36	0
259	1899.877319	36.71112107	0	0	2	1	1	0	4	1	0	0	0	1	5	0	30	0	36	0
260	1899.87744	38.07913555	0	1	0	1	2	0	3	2	0	1	0	1	4	0	30	0	36	0
261	1899.877525	29.70119312	0	0	2	2	0	0	3	2	0	0	0	2	3	1	30	0	36	0
262	1899.877529	27.80319381	0	1	2	0	1	0	3	2	0	0	0	1	5	0	30	0	36	0

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263	1899.877677	44.76117478	0	0	0	2	2	0	3	2	0	1	0	2	3	0	30	0	36	0
264	1899.877735	22.49421987	0	1	2	1	0	0	2	3	0	0	0	2	3	1	30	0	36	0
265	1899.877765	81.70508599	0	0	2	1	1	0	3	2	0	0	0	2	4	0	30	0	36	0
266	1899.877887	33.89990776	0	1	0	1	2	0	2	3	0	1	0	2	3	0	30	0	36	0
267	1899.877975	30.93970268	0	1	2	0	1	0	2	3	0	0	0	2	4	0	30	0	36	0
268	1899.87801	20.5340859	0	0	1	3	0	0	2	3	0	0	1	1	4	0	30	0	36	0
269	1899.878212	48.4919075	0	0	2	1	1	0	2	3	0	0	0	3	3	0	30	0	36	0
270	1899.878285	30.95299078	0	0	2	1	1	0	4	1	0	0	0	2	3	1	30	0	36	0
271	1899.878495	23.44226972	0	1	2	0	1	0	3	2	0	0	0	2	3	1	30	0	36	0
272	1899.878525	21.28716482	0	0	2	0	2	0	4	1	0	0	0	2	4	0	30	0	36	0
273	1899.878534	21.84595062	0	1	1	1	1	0	3	2	0	0	1	0	5	0	30	0	36	0
274	1899.878731	27.55586536	0	0	2	1	1	0	3	2	0	0	0	3	2	1	30	0	36	0
275	1899.87877	64.19856963	0	0	1	2	1	0	3	2	0	0	1	1	4	0	30	0	36	0
276	1899.878882	26.88806425	0	0	2	1	1	0	3	2	0	1	0	0	5	0	30	0	36	0
277	1899.878972	25.26783284	0	0	2	0	2	0	3	2	0	0	0	3	3	0	30	0	36	0
278	1899.87898	48.62083266	0	1	1	1	1	0	2	3	0	0	1	1	4	0	30	0	36	0
279	1899.879088	21.75383278	0	0	2	2	0	0	2	3	0	1	0	1	3	1	30	0	36	0
280	1899.879217	57.15270468	0	0	1	2	1	0	2	3	0	0	1	2	3	0	30	0	36	0
281	1899.879289	24.32085726	0	0	1	2	1	0	4	1	0	0	1	1	3	1	30	0	36	0
282	1899.879293	21.34114727	0	0	0	2	1	1	4	1	0	1	0	2	3	0	30	0	36	0
283	1899.879329	59.84267267	0	0	2	1	1	0	2	3	0	1	0	1	4	0	30	0	36	0
284	1899.879427	21.6423209	0	1	1	1	1	0	1	4	0	0	1	2	3	0	30	0	36	0
285	1899.879499	36.83883731	0	1	1	1	1	0	3	2	0	0	1	1	3	1	30	0	36	0
286	1899.879503	32.32546632	0	1	0	1	1	1	3	2	0	1	0	2	3	0	30	0	36	0
287	1899.87953	33.45215335	0	0	1	1	2	0	4	1	0	0	1	1	4	0	30	0	36	0
288	1899.879736	32.47742389	0	0	1	2	1	0	3	2	0	0	1	2	2	1	30	0	36	0
289	1899.87974	25.33501228	0	1	1	0	2	0	3	2	0	0	1	1	4	0	30	0	36	0
290	1899.879776	26.63743615	0	0	2	1	1	0	1	4	0	1	0	2	3	0	30	0	36	0
291	1899.879828	23.11987888	0	0	2	1	0	1	3	2	0	0	0	3	3	0	30	0	36	0
292	1899.879848	45.341356	0	0	2	1	1	0	3	2	0	1	0	1	3	1	30	0	36	0
293	1899.879861	21.55000689	0	0	1	2	1	0	4	1	0	0	0	2	4	0	30	0	35	1
294	1899.879946	24.59680023	0	1	1	1	1	0	2	3	0	0	1	2	2	1	30	0	36	0
295	1899.879977	59.56148408	0	0	1	1	2	0	3	2	0	0	1	2	3	0	30	0	36	0
296	1899.880071	32.64182628	0	1	1	1	1	0	3	2	0	0	0	2	4	0	30	0	35	1
297	1899.880089	31.18241224	0	0	2	0	2	0	3	2	0	1	0	1	4	0	30	0	36	0
298	1899.880187	22.5544663	0	1	1	0	2	0	2	3	0	0	1	2	3	0	30	0	36	0
299	1899.880295	30.27381853	0	0	2	1	1	0	2	3	0	1	0	2	2	1	30	0	36	0
300	1899.880308	25.57982598	0	0	1	2	1	0	3	2	0	0	0	3	3	0	30	0	35	1
301	1899.880334	23.51024995	0	0	1	2	1	0	2	3	0	1	1	0	4	0	30	0	36	0
302	1899.880424	26.51227223	0	0	1	1	2	0	2	3	0	0	1	3	2	0	30	0	36	0
303	1899.880536	27.76010756	0	0	2	0	2	0	2	3	0	1	0	2	3	0	30	0	36	0
304	1899.880596	23.18134757	0	1	1	1	0	1	3	2	0	0	1	1	4	0	30	0	36	0
305	1899.880781	20.92997368	0	0	1	2	1	0	1	4	0	1	1	1	3	0	30	0	36	0
306	1899.880833	27.24915718	0	0	1	2	0	1	3	2	0	0	1	2	3	0	30	0	36	0
307	1899.880945	28.53167498	0	0	2	1	0	1	3	2	0	1	0	1	4	0	30	0	36	0

308	1899.881043	20.6371687	0	1	1	1	0	1	2	3	0	0	1	2	3	0	30	0	36	0
309	1899.881094	24.5011218	0	0	1	1	2	0	3	2	0	1	1	0	4	0	30	0	36	0
310	1899.8813	23.78720766	0	0	1	2	1	0	2	3	0	1	1	1	2	1	30	0	36	0
311	1899.881392	25.40029168	0	0	2	1	0	1	2	3	0	1	0	2	3	0	30	0	36	0
312	1899.881425	31.56743531	0	0	1	2	1	0	3	2	0	1	0	1	4	0	30	0	35	1
313	1899.881541	43.62419245	0	0	1	1	2	0	2	3	0	1	1	1	3	0	30	0	36	0
314	1899.881593	28.39761042	0	0	1	1	1	1	4	1	0	0	1	2	3	0	30	0	36	0
315	1899.881635	23.90761973	0	1	1	1	1	0	2	3	0	1	0	1	4	0	30	0	35	1
316	1899.881803	21.50695057	0	1	1	0	1	1	3	2	0	0	1	2	3	0	30	0	36	0
317	1899.881872	28.10287391	0	0	1	2	1	0	2	3	0	1	0	2	3	0	30	0	35	1
318	1899.88204	25.28094085	0	0	1	1	1	1	3	2	0	0	1	3	2	0	30	0	36	0
319	1899.88206	24.78975226	0	0	1	1	2	0	3	2	0	1	1	1	2	1	30	0	36	0
320	1899.882152	26.47082193	0	0	2	0	1	1	3	2	0	1	0	2	3	0	30	0	36	0
321	1899.882632	29.28730821	0	0	1	1	2	0	3	2	0	1	0	2	3	0	30	0	35	1
322	1899.883157	41.59811802	0	0	1	1	1	1	3	2	0	1	1	1	3	0	30	0	36	0
323	1899.883603	27.77450847	0	0	1	1	1	1	2	3	0	1	1	2	2	0	30	0	36	0
324	1899.883725	21.00261337	0	0	2	1	1	0	3	2	0	0	1	1	4	0	30	0	35	1
325	1900.874959	28.74276696	0	0	1	3	0	0	3	2	0	0	0	1	5	0	30	0	36	0
326	1900.875169	32.6525327	0	1	1	2	0	0	2	3	0	0	0	1	5	0	30	0	36	0
327	1900.875405	31.9852701	0	0	1	3	0	0	2	3	0	0	0	2	4	0	30	0	36	0
328	1900.875688	30.92504987	0	1	1	2	0	0	3	2	0	0	0	1	4	1	30	0	36	0
329	1900.875719	44.93125565	0	0	1	2	1	0	4	1	0	0	0	1	5	0	30	0	36	0
330	1900.875925	24.23447105	0	0	1	3	0	0	3	2	0	0	0	2	3	1	30	0	36	0
331	1900.875929	68.05743726	0	1	1	1	1	0	3	2	0	0	0	1	5	0	30	0	36	0
332	1900.876135	27.53099099	0	1	1	2	0	0	2	3	0	0	0	2	3	1	30	0	36	0
333	1900.876165	100	0	0	1	2	1	0	3	2	0	0	0	2	4	0	30	0	36	0
334	1900.876375	75.73507158	0	1	1	1	1	0	2	3	0	0	0	2	4	0	30	0	36	0
335	1900.876448	32.22842866	0	1	1	1	1	0	4	1	0	0	0	1	4	1	30	0	36	0
336	1900.876612	59.3499253	0	0	1	2	1	0	2	3	0	0	0	3	3	0	30	0	36	0
337	1900.876685	37.88379928	0	0	1	2	1	0	4	1	0	0	0	2	3	1	30	0	36	0
338	1900.876822	22.4743542	0	1	1	1	1	0	1	4	0	0	0	3	3	0	30	0	36	0
339	1900.876895	57.382645	0	1	1	1	1	0	3	2	0	0	0	2	3	1	30	0	36	0
340	1900.876925	52.10731882	0	0	1	1	2	0	4	1	0	0	0	2	4	0	30	0	36	0
341	1900.877131	33.72600986	0	0	1	2	1	0	3	2	0	0	0	3	2	1	30	0	36	0
342	1900.877135	39.4635152	0	1	1	0	2	0	3	2	0	0	0	2	4	0	30	0	36	0
343	1900.87717	26.19117641	0	0	0	3	1	0	3	2	0	0	1	1	4	0	30	0	36	0
344	1900.877282	32.90867872	0	0	1	2	1	0	3	2	0	1	0	0	5	0	30	0	36	0
345	1900.877341	25.54241771	0	1	1	1	1	0	2	3	0	0	0	3	2	1	30	0	36	0
346	1900.877372	61.85130959	0	0	1	1	2	0	3	2	0	0	0	3	3	0	30	0	36	0
347	1900.87738	29.7538593	0	1	0	2	1	0	2	3	0	0	1	1	4	0	30	0	36	0
348	1900.877492	24.92341139	0	1	1	1	1	0	2	3	0	1	0	0	5	0	30	0	36	0
349	1900.877582	23.42156679	0	1	1	0	2	0	2	3	0	0	0	3	3	0	30	0	36	0
350	1900.877617	23.31666545	0	0	0	3	1	0	2	3	0	0	1	2	3	0	30	0	36	0
351	1900.877729	73.2422859	0	0	1	2	1	0	2	3	0	1	0	1	4	0	30	0	36	0
352	1900.877781	23.83890433	0	0	1	2	0	1	4	1	0	0	0	2	4	0	30	0	36	0

353	1900.877819	20.64864715	0	0	1	1	2	0	2	3	0	0	0	4	2	0	30	0	36	0
354	1900.877899	22.54378467	0	1	0	2	1	0	3	2	0	0	1	1	3	1	30	0	36	0
355	1900.87793	20.47127969	0	0	0	2	2	0	4	1	0	0	1	1	4	0	30	0	36	0
356	1900.877939	27.73504882	0	1	1	1	1	0	1	4	0	1	0	1	4	0	30	0	36	0
357	1900.877991	36.10882252	0	1	1	1	0	1	3	2	0	0	0	2	4	0	30	0	36	0
358	1900.878011	23.60483786	0	1	1	1	1	0	3	2	0	1	0	0	4	1	30	0	36	0
359	1900.87814	31.00787666	0	1	0	1	2	0	3	2	0	0	1	1	4	0	30	0	36	0
360	1900.878176	32.60193148	0	0	1	2	1	0	1	4	0	1	0	2	3	0	30	0	36	0
361	1900.878228	28.29674383	0	0	1	2	0	1	3	2	0	0	0	3	3	0	30	0	36	0
362	1900.878248	55.49392116	0	0	1	2	1	0	3	2	0	1	0	1	3	1	30	0	36	0
363	1900.878377	36.44906762	0	0	0	2	2	0	3	2	0	0	1	2	3	0	30	0	36	0
364	1900.878438	21.43055919	0	1	1	1	0	1	2	3	0	0	0	3	3	0	30	0	36	0
365	1900.878458	42.02836091	0	1	1	1	1	0	2	3	0	1	0	1	3	1	30	0	36	0
366	1900.878489	76.32918284	0	0	1	1	2	0	3	2	0	1	0	1	4	0	30	0	36	0
367	1900.878587	27.60472745	0	1	0	1	2	0	2	3	0	0	1	2	3	0	30	0	36	0
368	1900.878695	37.05255085	0	0	1	2	1	0	2	3	0	1	0	2	2	1	30	0	36	0
369	1900.878699	28.90398063	0	1	1	0	2	0	2	3	0	1	0	1	4	0	30	0	36	0
370	1900.878936	67.9519695	0	0	1	1	2	0	2	3	0	1	0	2	3	0	30	0	36	0
371	1900.878988	29.48934904	0	0	1	1	1	1	4	1	0	0	0	3	3	0	30	0	36	0
372	1900.879008	28.91639442	0	0	1	1	2	0	4	1	0	1	0	1	3	1	30	0	36	0
373	1900.879024	20.67275765	0	0	3	0	1	0	2	3	0	0	0	2	4	0	30	0	36	0
374	1900.879198	22.3337796	0	1	1	0	1	1	3	2	0	0	0	3	3	0	30	0	36	0
375	1900.879218	21.89985201	0	1	1	0	2	0	3	2	0	1	0	1	3	1	30	0	36	0
376	1900.879345	34.92031693	0	0	1	2	0	1	3	2	0	1	0	1	4	0	30	0	36	0
377	1900.879455	38.6141816	0	0	1	1	2	0	3	2	0	1	0	2	2	1	30	0	36	0
378	1900.879555	26.44692702	0	1	1	1	0	1	2	3	0	1	0	1	4	0	30	0	36	0
379	1900.879583	21.89497654	0	0	2	1	1	0	3	2	0	0	1	0	5	0	30	0	36	0
380	1900.879696	23.60529959	0	0	1	0	3	0	3	2	0	1	0	2	3	0	30	0	36	0
381	1900.879792	31.08777302	0	0	1	2	0	1	2	3	0	1	0	2	3	0	30	0	36	0
382	1900.879941	26.69613031	0	0	0	2	2	0	2	3	0	1	1	1	3	0	30	0	36	0
383	1900.880029	48.72994584	0	0	2	1	1	0	2	3	0	0	1	1	4	0	30	0	36	0
384	1900.880074	20.0382015	0	1	1	1	0	1	3	2	0	1	0	1	3	1	30	0	36	0
385	1900.880105	36.39208175	0	0	1	1	1	1	4	1	0	1	0	1	4	0	30	0	36	0
386	1900.880203	26.32265827	0	1	0	1	1	1	3	2	0	0	1	2	3	0	30	0	36	0
387	1900.880315	27.56156916	0	1	1	0	1	1	3	2	0	1	0	1	4	0	30	0	36	0
388	1900.880476	21.69088984	0	0	2	1	1	0	1	4	0	0	1	2	3	0	30	0	36	0
389	1900.880548	36.92150974	0	0	2	1	1	0	3	2	0	0	1	1	3	1	30	0	36	0
390	1900.880552	64.79602001	0	0	1	1	1	1	3	2	0	1	0	2	3	0	30	0	36	0
391	1900.880762	24.53665607	0	1	1	0	1	1	2	3	0	1	0	2	3	0	30	0	36	0
392	1900.880789	25.39186824	0	0	2	0	2	0	3	2	0	0	1	1	4	0	30	0	36	0
393	1900.880995	24.65199951	0	0	2	1	1	0	2	3	0	0	1	2	2	1	30	0	36	0
394	1900.880999	28.8422921	0	0	1	1	1	1	2	3	0	1	0	3	2	0	30	0	36	0
395	1900.88112	32.71507993	0	0	2	1	1	0	3	2	0	0	0	2	4	0	30	0	35	1
396	1900.881236	22.60508225	0	0	2	0	2	0	2	3	0	0	1	2	3	0	30	0	36	0
397	1900.881557	25.45625986	0	0	0	2	1	1	3	2	0	1	1	1	3	0	30	0	36	0

398	1900 881645	23 23337034	0	0	2	1	0	1	3	2	0	0	1	1	4	0	30	0	36	0
399	1900.882092	20.68348191	0	0	2	1	0	1	2	3	0	0	1	2	3	0	30	0	36	0
400	1900.882115	23.72904311	0	0	1	1	1	1	2	3	0	2	0	1	3	0	30	0	36	0
401	1900.882125	25.70539289	0	0	1	2	1	0	3	2	0	0	1	1	4	0	30	0	35	1
402	1900.882572	22.88419722	0	0	1	2	1	0	2	3	0	0	1	2	3	0	30	0	35	1
403	1900.882684	23.96127237	0	0	2	1	1	0	2	3	0	1	0	1	4	0	30	0	35	1
404	1900.882852	21.55521572	0	0	2	0	1	1	3	2	0	0	1	2	3	0	30	0	36	0
405	1900.883332	23.84868321	0	0	1	1	2	0	3	2	0	0	1	2	3	0	30	0	35	1
406	1901.874329	41.64822571	0	1	0	2	1	0	3	2	0	0	0	1	5	0	30	0	36	0
407	1901.874565	40.7971339	0	0	0	3	1	0	3	2	0	0	0	2	4	0	30	0	36	0
408	1901.874775	46.34660784	0	1	0	2	1	0	2	3	0	0	0	2	4	0	30	0	36	0
409	1901.875012	24.2130685	0	0	0	3	1	0	2	3	0	0	0	3	3	0	30	0	36	0
410	1901.875089	21.70177375	0	1	0	1	2	0	4	1	0	0	0	1	5	0	30	0	36	0
411	1901.875295	35.11571178	0	1	0	2	1	0	3	2	0	0	0	2	3	1	30	0	36	0
412	1901.875325	31.88743894	0	0	0	2	2	0	4	1	0	0	0	2	4	0	30	0	36	0
413	1901.875535	48.29994942	0	1	0	1	2	0	3	2	0	0	0	2	4	0	30	0	36	0
414	1901.875772	37.85034239	0	0	0	2	2	0	3	2	0	0	0	3	3	0	30	0	36	0
415	1901.875982	28.6659839	0	1	0	1	2	0	2	3	0	0	0	3	3	0	30	0	36	0
416	1901.876129	29.88075345	0	0	0	3	1	0	2	3	0	1	0	1	4	0	30	0	36	0
417	1901.876218	32.72581037	0	0	2	2	0	0	2	3	0	0	0	1	5	0	30	0	36	0
418	1901.876391	22.09704701	0	1	0	2	0	1	3	2	0	0	0	2	4	0	30	0	36	0
419	1901.876648	22.63992932	0	0	0	3	1	0	3	2	0	1	0	1	3	1	30	0	36	0
420	1901.876737	30.99445079	0	0	2	2	0	0	3	2	0	0	0	1	4	1	30	0	36	0
421	1901.876858	25.71955002	0	1	0	2	1	0	2	3	0	1	0	1	3	1	30	0	36	0
422	1901.876889	46.71017839	0	0	0	2	2	0	3	2	0	1	0	1	4	0	30	0	36	0
423	1901.876947	23.47366949	0	1	2	1	0	0	2	3	0	0	0	1	4	1	30	0	36	0
424	1901.876978	68.21016939	0	0	2	1	1	0	3	2	0	0	0	1	5	0	30	0	36	0
425	1901.877099	35.37598704	0	1	0	1	2	0	2	3	0	1	0	1	4	0	30	0	36	0
426	1901.877151	23.02835747	0	1	0	1	1	1	4	1	0	0	0	2	4	0	30	0	36	0
427	1901.877184	27.59277509	0	0	2	2	0	0	2	3	0	0	0	2	3	1	30	0	36	0
428	1901.877188	25.8295103	0	1	2	0	1	0	2	3	0	0	0	1	5	0	30	0	36	0
429	1901.877336	41.58368398	0	0	0	2	2	0	2	3	0	1	0	2	3	0	30	0	36	0
430	1901.877424	75.90503359	0	0	2	1	1	0	2	3	0	0	0	2	4	0	30	0	36	0
431	1901.877497	32.30075458	0	0	2	1	1	0	4	1	0	0	0	1	4	1	30	0	36	0
432	1901.877598	27.33462591	0	1	0	1	1	1	3	2	0	0	0	3	3	0	30	0	36	0
433	1901.877618	26.80353585	0	1	0	1	2	0	3	2	0	1	0	1	3	1	30	0	36	0
434	1901.877707	24.4629996	0	1	2	0	1	0	3	2	0	0	0	1	4	1	30	0	36	0
435	1901.877855	23.63021906	0	0	0	2	2	0	3	2	0	1	0	2	2	1	30	0	36	0
436	1901.877871	22.52479037	0	0	2	1	1	0	1	4	0	0	0	3	3	0	30	0	36	0
437	1901.877944	57.51142114	0	0	2	1	1	0	3	2	0	0	0	2	3	1	30	0	36	0
438	1901.877983	26.79756869	0	0	1	2	1	0	3	2	0	0	1	0	5	0	30	0	36	0
439	1901.878096	28.89085705	0	0	0	1	3	0	3	2	0	1	0	2	3	0	30	0	36	0
440	1901.878154	21.77815798	0	1	2	0	1	0	2	3	0	0	0	2	3	1	30	0	36	0
441	1901.878184	39.55207785	0	0	2	0	2	0	3	2	0	0	0	2	4	0	30	0	36	0
442	1901.878193	20.29515783	0	1	1	1	1	0	2	3	0	0	1	0	5	0	30	0	36	0

	1001 07020	25 50052011	0	0				0		2	0	0		2			20	0	26	
443	1901.87839	25.59973911	0	0	2	1	1	0	2	3	0	0	0	3	2	1	30	0	36	0
444	1901.878429	59.64126376	0	0	1	2	1	0	2	3	0	0	1	1	4	0	30	0	36	0
445	1001.878505	22.27038948	0	0	0	2	1	1	4	2	0	1	0	1	4	0	30	0	36	0
440	1001 878621	24.97934304	0	0	2	0	2	0	2	2	0	1	0	2	2	0	30	0	30	0
447	1001 878620	23.47412800	0	1	1	1	1	0	1	3	0	0	1	3	3	0	30	0	30	0
448	1901.878639	22.5846769	0	1	1	1	1	0	1	4	0	1	1	1	4	0	30	0	36	0
449	1901.878715	33./3299083	0	1	0	1	1	1	3	2	0	1	0	1	4	0	30	0	36	0
450	1901.878876	26.54//8412	0	0	1	2	1	0	1	4	0	0	1	2	3	0	30	0	36	0
451	1901.878948	45.188/533	0	0	1	2	1	0	3	2	0	0	1	1	3	1	30	0	36	0
452	1901.878952	39.65237857	0	0	0	2	1	1	3	2	0	1	0	2	3	0	30	0	36	0
453	1901.878988	27.79729086	0	0	2	1	1	0	1	4	0	1	0	1	4	0	30	0	36	0
454	1901.87904	36.18985668	0	0	2	1	0	1	3	2	0	0	0	2	4	0	30	0	36	0
455	1901.87906	23.65781101	0	0	2	1	1	0	3	2	0	1	0	0	4	1	30	0	36	0
456	1901.879158	34.22373466	0	1	1	1	1	0	2	3	0	0	1	1	3	1	30	0	36	0
457	1901.879162	30.03075729	0	1	0	1	1	1	2	3	0	1	0	2	3	0	30	0	36	0
458	1901.879189	62.1549269	0	0	1	1	2	0	3	2	0	0	1	1	4	0	30	0	36	0
459	1901.879284	27.25050066	0	1	1	1	1	0	3	2	0	0	0	1	5	0	30	0	35	1
460	1901.879395	30.17192775	0	0	1	2	1	0	2	3	0	0	1	2	2	1	30	0	36	0
461	1901.879399	23.53653919	0	1	1	0	2	0	2	3	0	0	1	1	4	0	30	0	36	0
462	1901.879487	21.47865291	0	0	2	1	0	1	2	3	0	0	0	3	3	0	30	0	36	0
463	1901.879507	42.12267949	0	0	2	1	1	0	2	3	0	1	0	1	3	1	30	0	36	0
464	1901.87952	40.04044489	0	0	1	2	1	0	3	2	0	0	0	2	4	0	30	0	35	1
465	1901.879636	55.33335403	0	0	1	1	2	0	2	3	0	0	1	2	3	0	30	0	36	0
466	1901.879708	23.54664775	0	0	1	1	2	0	4	1	0	0	1	1	3	1	30	0	36	0
467	1901.879712	20.66179132	0	0	0	1	2	1	4	1	0	1	0	2	3	0	30	0	36	0
468	1901.87973	30.3246596	0	1	1	1	1	0	2	3	0	0	0	2	4	0	30	0	35	1
469	1901.879748	28.96884594	0	0	2	0	2	0	2	3	0	1	0	1	4	0	30	0	36	0
470	1901.879967	23.76397414	0	0	1	2	1	0	2	3	0	0	0	3	3	0	30	0	35	1
471	1901.880045	28.43564762	0	0	1	2	0	1	3	2	0	0	1	1	4	0	30	0	36	0
472	1901.880155	31.44356517	0	0	1	1	2	0	3	2	0	0	1	2	2	1	30	0	36	0
473	1901.880247	22.3839003	0	0	2	0	1	1	3	2	0	0	0	3	3	0	30	0	36	0
474	1901.88025	22.97626635	0	1	1	1	1	0	3	2	0	0	0	2	3	1	30	0	35	1
475	1901.880255	21.53575808	0	1	1	1	0	1	2	3	0	0	1	1	4	0	30	0	36	0
476	1901.880267	21.9489989	0	0	2	0	2	0	3	2	0	1	0	1	3	1	30	0	36	0
477	1901.88028	20.86400228	0	0	1	1	2	0	4	1	0	0	0	2	4	0	30	0	35	1
478	1901.880492	25.31480343	0	0	1	2	0	1	2	3	0	0	1	2	3	0	30	0	36	0
479	1901.880604	26.5062783	0	0	2	1	0	1	2	3	0	1	0	1	4	0	30	0	36	0
480	1901.880727	24.76553953	0	0	1	1	2	0	3	2	0	0	0	3	3	0	30	0	35	1
481	1901.880753	22.76184463	0	0	1	1	2	0	2	3	0	1	1	0	4	0	30	0	36	0
482	1901.880805	29.63410713	0	0	1	1	1	1	4	1	0	0	1	1	4	0	30	0	36	0
483	1901.881015	22.44341224	0	1	1	0	1	1	3	2	0	0	1	1	4	0	30	0	36	0
484	1901.881084	29.32653712	0	0	1	2	1	0	2	3	0	1	0	1	4	0	30	0	35	1
485	1901.881123	20.08317053	0	0	2	1	0	1	3	2	0	1	0	1	3	1	30	0	36	0
486	1901.8812	20.26370668	0	0	1	1	2	0	1	4	0	1	1	1	3	0	30	0	36	0
487	1901.881252	52.76346133	0	0	1	1	1	1	3	2	0	0	1	2	3	0	30	0	36	0

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488	1901.881364	27.62342188	0	0	2	0	1	1	3	2	0	1	0	1	4	0	30	0	36	0
489	1901.881603	22.22001292	0	0	1	2	1	0	3	2	0	1	0	1	3	1	30	0	35	1
490	1901.881699	23.48630616	0	0	1	1	1	1	2	3	0	0	1	3	2	0	30	0	36	0
491	1901.881719	23.0299859	0	0	1	1	2	0	2	3	0	1	1	1	2	1	30	0	36	0
492	1901.881811	24.59172037	0	0	2	0	1	1	2	3	0	1	0	2	3	0	30	0	36	0
493	1901.881844	30.56254439	0	0	1	1	2	0	3	2	0	1	0	1	4	0	30	0	35	1
494	1901.882291	27.2082709	0	0	1	1	2	0	2	3	0	1	0	2	3	0	30	0	35	1
495	1901.882369	21.70469746	0	0	1	1	1	1	3	2	0	1	1	0	4	0	30	0	36	0
496	1901.882816	38.6451652	0	0	1	1	1	1	2	3	0	1	1	1	3	0	30	0	36	0
497	1901.883335	21.96038522	0	0	1	1	1	1	3	2	0	1	1	1	2	1	30	0	36	0
498	1901.883576	20.13695944	0	0	1	0	2	1	3	2	0	1	1	1	3	0	30	0	36	0
499	1901.883907	25.94461468	0	0	1	1	1	1	3	2	0	1	0	2	3	0	30	0	35	1
500	1902.874618	26.70238536	0	0	1	3	0	0	2	3	0	0	0	1	5	0	30	0	36	0
501	1902.875137	25.28969518	0	0	1	3	0	0	3	2	0	0	0	1	4	1	30	0	36	0
502	1902.875141	23.67360442	0	1	1	1	1	0	3	2	0	0	0	0	6	0	30	0	36	0
503	1902.875347	28.72975312	0	1	1	2	0	0	2	3	0	0	0	1	4	1	30	0	36	0
504	1902.875378	83.48338241	0	0	1	2	1	0	3	2	0	0	0	1	5	0	30	0	36	0
505	1902.875584	22.5141228	0	0	1	3	0	0	2	3	0	0	0	2	3	1	30	0	36	0
506	1902.875588	63.22619943	0	1	1	1	1	0	2	3	0	0	0	1	5	0	30	0	36	0
507	1902.875824	92.90123457	0	0	1	2	1	0	2	3	0	0	0	2	4	0	30	0	36	0
508	1902.875897	39.53334629	0	0	1	2	1	0	4	1	0	0	0	1	4	1	30	0	36	0
509	1902.876034	35.17940825	0	1	1	1	1	0	1	4	0	0	0	2	4	0	30	0	36	0
510	1902.876107	59.88121621	0	1	1	1	1	0	3	2	0	0	0	1	4	1	30	0	36	0
511	1902.876138	43.50095223	0	0	1	1	2	0	4	1	0	0	0	1	5	0	30	0	36	0
512	1902.876271	27.56840666	0	0	1	2	1	0	1	4	0	0	0	3	3	0	30	0	36	0
513	1902.876344	70.38903447	0	0	1	2	1	0	3	2	0	0	0	2	3	1	30	0	36	0
514	1902.876348	32.94547731	0	1	1	0	2	0	3	2	0	0	0	1	5	0	30	0	36	0
515	1902.876554	53.30918564	0	1	1	1	1	0	2	3	0	0	0	2	3	1	30	0	36	0
516	1902.876584	96.81668496	0	0	1	1	2	0	3	2	0	0	0	2	4	0	30	0	36	0
517	1902.87679	31.33187953	0	0	1	2	1	0	2	3	0	0	0	3	2	1	30	0	36	0
518	1902.876794	36.66209283	0	1	1	0	2	0	2	3	0	0	0	2	4	0	30	0	36	0
519	1902.876829	24.33192624	0	0	0	3	1	0	2	3	0	0	1	1	4	0	30	0	36	0
520	1902.876941	30.57256881	0	0	1	2	1	0	2	3	0	1	0	0	5	0	30	0	36	0
521	1902.877031	57.4606302	0	0	1	1	2	0	2	3	0	0	0	3	3	0	30	0	36	0
522	1902.877104	36.6778386	0	0	1	1	2	0	4	1	0	0	0	2	3	1	30	0	36	0
523	1902.877204	30.14486639	0	1	1	1	0	1	3	2	0	0	0	1	5	0	30	0	36	0
524	1902.877314	27.77798732	0	1	1	0	2	0	3	2	0	0	0	2	3	1	30	0	36	0
525	1902.877388	34.02149391	0	0	1	2	1	0	1	4	0	1	0	1	4	0	30	0	36	0
526	1902.87744	44.29327286	0	0	1	2	0	1	3	2	0	0	0	2	4	0	30	0	36	0
527	1902.87746	28.95512651	0	0	1	2	1	0	3	2	0	1	0	0	4	1	30	0	36	0
528	1902.87755	32.65240472	0	0	1	1	2	0	3	2	0	0	0	3	2	1	30	0	36	0
529	1902.877558	20.94345428	0	1	0	2	1	0	2	3	0	0	1	1	3	1	30	0	36	0
530	1902.877589	38.03614313	0	0	0	2	2	0	3	2	0	0	1	1	4	0	30	0	36	0
531	1902.87765	33.54554191	0	1	1	1	0	1	2	3	0	0	0	2	4	0	30	0	36	0
532	1902.87767	21.92918579	0	1	1	1	1	0	2	3	0	1	0	0	4	1	30	0	36	0

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533	1902.877701	31.8610918	0	0	1	1	2	0	3	2	0	1	0	0	5	0	30	0	36	0
534	1902.877799	28.80670023	0	1	0	1	2	0	2	3	0	0	1	1	4	0	30	0	36	0
535	1902.877887	26.28802436	0	0	1	2	0	1	2	3	0	0	0	3	3	0	30	0	36	0
536	1902.877907	51.55453787	0	0	1	2	1	0	2	3	0	1	0	1	3	1	30	0	36	0
537	1902.878036	33.86163381	0	0	0	2	2	0	2	3	0	0	1	2	3	0	30	0	36	0
538	1902.878148	70.9107532	0	0	1	1	2	0	2	3	0	1	0	1	4	0	30	0	36	0
539	1902.87817	25.41665153	0	1	1	1	0	1	3	2	0	0	0	2	3	1	30	0	36	0
540	1902.8782	46.16007381	0	0	1	1	1	1	4	1	0	0	0	2	4	0	30	0	36	0
541	1902.878318	21.82614499	0	1	0	1	2	0	3	2	0	0	1	1	3	1	30	0	36	0
542	1902.87841	34,95936494	0	1	1	0	1	1	3	2	0	0	0	2	4	0	30	0	36	0
543	1902 878595	31 56410929	0	0	1	1	2	0	1	4	0	1	0	2	3	0	30	0	36	0
544	1902 878647	54 79193865	0	0	1	1	1	1	3	2	0	0	0	3	3	0	30	0	36	0
545	1902 878667	53 77737482	0	0	1	1	2	0	3	2	0	1	0	1	3	1	30	0	36	0
546	1002.878706	22 52585264	0	0	0	1	2	0	2	2	0	0	1	2	2	0	20	0	26	0
540	1002.878757	20.74825608	0	1	1	0	1	1	2	2	0	0	0	2	2	0	20	0	26	0
547	1902.070037	20.74855098	0	1	1	0	1	1	2	3	0	1	0	3	2	1	30	0	30	0
548	1902.8/88//	20.34523289	0	1	1	0	2	0	2	3	0	1	0	1	3	1	30	0	30	0
549	1902.878908	24.63312816	0	0	1	0	3	0	3	2	0	1	0	1	4	0	30	0	36	0
550	1902.879004	32.44140555	0	0	1	2	0	1	2	3	0	1	0	1	4	0	30	0	36	0
551	1902.879114	35.87305143	0	0	1	1	2	0	2	3	0	1	0	2	2	1	30	0	36	0
552	1902.879242	20.34070352	0	0	2	1	1	0	2	3	0	0	1	0	5	0	30	0	36	0
553	1902.879355	21.92961475	0	0	1	0	3	0	2	3	0	1	0	2	3	0	30	0	36	0
554	1902.879415	27.46880683	0	1	0	1	1	1	3	2	0	0	1	1	4	0	30	0	36	0
555	1902.879523	24.58007392	0	0	1	2	0	1	3	2	0	1	0	1	3	1	30	0	36	0
556	1902.879652	32.28896995	0	0	0	2	1	1	3	2	0	0	1	2	3	0	30	0	36	0
557	1902.879688	22.63536064	0	0	2	1	1	0	1	4	0	0	1	1	4	0	30	0	36	0
558	1902.879764	67.61738646	0	0	1	1	1	1	3	2	0	1	0	1	4	0	30	0	36	0
559	1902.879862	24.45407451	0	1	0	1	1	1	2	3	0	0	1	2	3	0	30	0	36	0
560	1902.879974	25.60503802	0	1	1	0	1	1	2	3	0	1	0	1	4	0	30	0	36	0
561	1902.880207	34.30053837	0	0	2	1	1	0	2	3	0	0	1	1	3	1	30	0	36	0
562	1902.880211	60.19630254	0	0	1	1	1	1	2	3	0	1	0	2	3	0	30	0	36	0
563	1902.880283	25.61603497	0	0	1	1	1	1	4	1	0	1	0	1	3	1	30	0	36	0
564	1902.880333	27.31165529	0	0	2	1	1	0	3	2	0	0	0	1	5	0	30	0	35	1
565	1902.880448	23.58935907	0	0	2	0	2	0	2	3	0	0	1	1	4	0	30	0	36	0
566	1902.88073	34.20696964	0	0	1	1	1	1	3	2	0	1	0	2	2	1	30	0	36	0
567	1902.880779	30.39271314	0	0	2	1	1	0	2	3	0	0	0	2	4	0	30	0	35	1
568	1902.880971	31.36667928	0	0	1	0	2	1	3	2	0	1	0	2	3	0	30	0	36	0
569	1902.881216	23.64917969	0	0	0	2	1	1	2	3	0	1	1	1	3	0	30	0	36	0
570	1902.881299	23.02782889	0	0	2	1	1	0	3	2	0	0	0	2	3	1	30	0	35	1
571	1902.881304	21.58408788	0	0	2	1	0	1	2	3	0	0	1	1	4	0	30	0	36	0
572	1902.881784	23.88062735	0	0	1	2	1	0	2	3	0	0	1	1	4	0	30	0	35	1
573	1902.881976	24.64590691	0	0	0	1	2	1	3	2	0	1	1	1	3	0	30	0	36	0
574	1902.882064	22.49377897	0	0	2	0	1	1	3	2	0	0	1	1	4	0	30	0	36	0
575	1902.882511	20.02506152	0	0	2	0	1	1	2	3	0	0	1	2	3	0	30	0	36	0
576	1902.882544	24.88710926	0	0	1	1	2	0	3	2	0	0	1	1	4	0	30	0	35	1
577	1902.882991	22.15572113	0	0	1	1	2	0	2	3	0	0	1	2	3	0	30	0	35	1
			~						_	-						. ~		~		-

578	1902 884607	21 12672466	0	0	1	1	1	1	3	2	0	0	1	2	3	0	30	0	35	1
579	1903 873778	34 05882731	0	0	0	3	1	0	3	2	0	0	0	1	5	0	30	0	36	0
580	1903 873988	38 69171586	0	1	0	2	1	0	2	3	0	0	0	1	5	0	30	0	36	0
581	1903 874224	37 90104106	0	0	0	3	1	0	2	3	0	0	0	2	4	0	30	0	36	0
582	1903.874434	21.52828543	0	1	0	2	1	0	1	4	0	0	0	2	4	0	30	0	36	0
583	1903.874507	36.64472994	0	1	0	2	1	0	3	2	0	0	0	1	4	1	30	0	36	0
584	1903.874538	26.6207126	0	0	0	2	2	0	4	1	0	0	0	1	5	0	30	0	36	0
585	1903.874744	28,71670864	0	0	0	3	1	0	3	2	0	0	0	2	3	1	30	0	36	0
586	1903.874748	40.32243148	0	1	0	1	2	0	3	2	0	0	0	1	5	0	30	0	36	0
587	1903.874954	32.62292977	0	1	0	2	1	0	2	3	0	0	0	2	3	1	30	0	36	0
588	1903.874984	59.2476489	0	0	0	2	2	0	3	2	0	0	0	2	4	0	30	0	36	0
589	1903.875194	44.8712493	0	1	0	1	2	0	2	3	0	0	0	2	4	0	30	0	36	0
590	1903.875431	35.16343537	0	0	0	2	2	0	2	3	0	0	0	3	3	0	30	0	36	0
591	1903.875504	22.44526039	0	0	0	2	2	0	4	1	0	0	0	2	3	1	30	0	36	0
592	1903.875714	33.99786804	0	1	0	1	2	0	3	2	0	0	0	2	3	1	30	0	36	0
593	1903.875744	20.5815742	0	0	0	1	3	0	4	1	0	0	0	2	4	0	30	0	36	0
594	1903.87605	20.52842947	0	1	0	2	0	1	2	3	0	0	0	2	4	0	30	0	36	0
595	1903.87619	23.7267319	0	0	2	1	1	0	3	2	0	0	0	0	6	0	30	0	36	0
596	1903.876191	24.43029783	0	0	0	1	3	0	3	2	0	0	0	3	3	0	30	0	36	0
597	1903.876307	21.03277385	0	0	0	3	1	0	2	3	0	1	0	1	3	1	30	0	36	0
598	1903.876396	28.79422743	0	0	2	2	0	0	2	3	0	0	0	1	4	1	30	0	36	0
599	1903.876548	43.3943324	0	0	0	2	2	0	2	3	0	1	0	1	4	0	30	0	36	0
600	1903.8766	28.24798068	0	0	0	2	1	1	4	1	0	0	0	2	4	0	30	0	36	0
601	1903.876637	63.36808946	0	0	2	1	1	0	2	3	0	0	0	1	5	0	30	0	36	0
602	1903.87681	42.78725678	0	1	0	1	1	1	3	2	0	0	0	2	4	0	30	0	36	0
603	1903.877047	33.53031087	0	0	0	2	1	1	3	2	0	0	0	3	3	0	30	0	36	0
604	1903.877067	32.87884357	0	0	0	2	2	0	3	2	0	1	0	1	3	1	30	0	36	0
605	1903.877083	35.25835665	0	0	2	1	1	0	1	4	0	0	0	2	4	0	30	0	36	0
606	1903.877156	60.01559955	0	0	2	1	1	0	3	2	0	0	0	1	4	1	30	0	36	0
607	1903.877257	25.39420494	0	1	0	1	1	1	2	3	0	0	0	3	3	0	30	0	36	0
608	1903.877277	24.90081571	0	1	0	1	2	0	2	3	0	1	0	1	3	1	30	0	36	0
609	1903.877308	30.14883084	0	0	0	1	3	0	3	2	0	1	0	1	4	0	30	0	36	0
610	1903.877366	22.72642864	0	1	2	0	1	0	2	3	0	0	0	1	4	1	30	0	36	0
611	1903.877397	33.0194124	0	0	2	0	2	0	3	2	0	0	0	1	5	0	30	0	36	0
612	1903.877514	21.95276524	0	0	0	2	2	0	2	3	0	1	0	2	2	1	30	0	36	0
613	1903.877603	53.42882025	0	0	2	1	1	0	2	3	0	0	0	2	3	1	30	0	36	0
614	1903.877642	24.89527215	0	0	1	2	1	0	2	3	0	0	1	0	5	0	30	0	36	0
615	1903.877755	26.83996288	0	0	0	1	3	0	2	3	0	1	0	2	3	0	30	0	36	0
616	1903.877843	36.74436862	0	0	2	0	2	0	2	3	0	0	0	2	4	0	30	0	36	0
617	1903.878088	27.70373517	0	0	1	2	1	0	1	4	0	0	1	1	4	0	30	0	36	0
618	1903.878161	23.57818733	0	0	1	2	1	0	3	2	0	0	1	0	4	1	30	0	36	0
619	1903.878164	41.37893354	0	0	0	2	1	1	3	2	0	1	0	1	4	0	30	0	36	0
620	1903.878253	30.21251645	0	0	2	1	0	1	3	2	0	0	0	1	5	0	30	0	36	0
621	1903.878363	27.84032571	0	0	2	0	2	0	3	2	0	0	0	2	3	1	30	0	36	0
622	1903.878374	31.33836494	0	1	0	1	1	1	2	3	0	1	0	1	4	0	30	0	36	0

623	1903.878402	25.94451766	0	0	1	1	2	0	3	2	0	0	1	0	5	0	30	0	36	0
624	1903.878607	41.9809097	0	0	1	2	1	0	2	3	0	0	1	1	3	1	30	0	36	0
625	1903.878611	36.83754922	0	0	0	2	1	1	2	3	0	1	0	2	3	0	30	0	36	0
626	1903.878699	33.62082364	0	0	2	1	0	1	2	3	0	0	0	2	4	0	30	0	36	0
627	1903.878719	21.9783985	0	0	2	1	1	0	2	3	0	1	0	0	4	1	30	0	36	0
628	1903.878733	33.42711773	0	0	1	2	1	0	3	2	0	0	0	1	5	0	30	0	35	1
629	1903.878848	57.74269444	0	0	1	1	2	0	2	3	0	0	1	1	4	0	30	0	36	0
630	1903.878893	23.74432654	0	1	0	1	1	1	3	2	0	1	0	1	3	1	30	0	36	0
631	1903.878924	21.56145282	0	0	0	1	2	1	4	1	0	1	0	1	4	0	30	0	36	0
632	1903.878943	25.31605154	0	1	1	1	1	0	2	3	0	0	0	1	5	0	30	0	35	1
633	1903.87913	20.93319481	0	0	0	2	1	1	3	2	0	1	0	2	2	1	30	0	36	0
634	1903.879179	37.19806763	0	0	1	2	1	0	2	3	0	0	0	2	4	0	30	0	35	1
635	1903.879219	25.47369069	0	0	2	1	0	1	3	2	0	0	0	2	3	1	30	0	36	0
636	1903.879295	25.70268451	0	0	1	1	2	0	1	4	0	0	1	2	3	0	30	0	36	0
637	1903.879367	43.75025292	0	0	1	1	2	0	3	2	0	0	1	1	3	1	30	0	36	0
638	1903.879371	38.39011844	0	0	0	1	2	1	3	2	0	1	0	2	3	0	30	0	36	0
639	1903.879459	35.03781953	0	0	2	0	1	1	3	2	0	0	0	2	4	0	30	0	36	0
640	1903.879462	23.97670538	0	1	1	1	1	0	3	2	0	0	0	1	4	1	30	0	35	1
641	1903.879608	20.05877992	0	0	1	0	3	0	3	2	0	0	1	1	4	0	30	0	36	0
642	1903.879699	28.18408256	0	0	1	2	1	0	3	2	0	0	0	2	3	1	30	0	35	1
643	1903.879704	26.4170677	0	0	1	2	0	1	2	3	0	0	1	1	4	0	30	0	36	0
644	1903.879814	29.21146023	0	0	1	1	2	0	2	3	0	0	1	2	2	1	30	0	36	0
645	1903.879906	20.79491972	0	0	2	0	1	1	2	3	0	0	0	3	3	0	30	0	36	0
646	1903.879909	21.3452351	0	1	1	1	1	0	2	3	0	0	0	2	3	1	30	0	35	1
647	1903.879926	20.39089095	0	0	2	0	2	0	2	3	0	1	0	1	3	1	30	0	36	0
648	1903.879939	38.76583139	0	0	1	1	2	0	3	2	0	0	0	2	4	0	30	0	35	1
649	1903.880223	20.0155778	0	0	1	2	0	1	3	2	0	0	1	1	3	1	30	0	36	0
650	1903.880386	23.00749197	0	0	1	1	2	0	2	3	0	0	0	3	3	0	30	0	35	1
651	1903.880464	55.06090275	0	0	1	1	1	1	3	2	0	0	1	1	4	0	30	0	36	0
652	1903.880674	20.85020705	0	1	1	0	1	1	2	3	0	0	1	1	4	0	30	0	36	0
653	1903.880911	49.01790697	0	0	1	1	1	1	2	3	0	0	1	2	3	0	30	0	36	0
654	1903.880983	20.85916188	0	0	1	1	1	1	4	1	0	0	1	1	3	1	30	0	36	0
655	1903.881023	25.66249995	0	0	2	0	1	1	2	3	0	1	0	1	4	0	30	0	36	0
656	1903.881262	20.64266632	0	0	1	2	1	0	2	3	0	1	0	1	3	1	30	0	35	1
657	1903.88143	27.85476824	0	0	1	1	1	1	3	2	0	0	1	2	2	1	30	0	36	0
658	1903.881503	28.39298106	0	0	1	1	2	0	2	3	0	1	0	1	4	0	30	0	35	1
659	1903.881671	25.54191706	0	0	1	0	2	1	3	2	0	0	1	2	3	0	30	0	36	0
660	1903.882002	21.938936	0	0	1	1	1	1	3	2	0	0	0	3	3	0	30	0	35	1
661	1903.882022	21.51267991	0	0	1	1	2	0	3	2	0	1	0	1	3	1	30	0	35	1
662	1903.882028	20.1639319	0	0	1	1	1	1	2	3	0	1	1	0	4	0	30	0	36	0
663	1903.882994	20.40146899	0	0	1	1	1	1	2	3	0	1	1	1	2	1	30	0	36	0
664	1903.883119	27.07430237	0	0	1	1	1	1	3	2	0	1	0	1	4	0	30	0	35	1
665	1903.883566	24.10286735	0	0	1	1	1	1	2	3	0	1	0	2	3	0	30	0	35	1
666	1904.87459	29.03947975	0	0	1	2	1	0	3	2	0	0	0	0	6	0	30	0	36	0
667	1904.874796	23.49443904	0	0	1	3	0	0	2	3	0	0	0	1	4	1	30	0	36	0

668	1904 8748	21,99307077	0	1	1	1	1	0	2	3	0	0	0	0	6	0	30	0	36	0
669	1904.875037	77.55709292	0	0	1	2	1	0	2	3	0	0	0	1	5	0	30	0	36	0
670	1904.875247	29.36895992	0	1	1	1	1	0	1	4	0	0	0	1	5	0	30	0	36	0
671	1904.875319	24.99543236	0	1	1	1	1	0	3	2	0	0	0	0	5	1	30	0	36	0
672	1904.875483	43.15319692	0	0	1	2	1	0	1	4	0	0	0	2	4	0	30	0	36	0
673	1904.875556	73.45393353	0	0	1	2	1	0	3	2	0	0	0	1	4	1	30	0	36	0
674	1904.875766	55.63038913	0	1	1	1	1	0	2	3	0	0	0	1	4	1	30	0	36	0
675	1904.875797	80.82584335	0	0	1	1	2	0	3	2	0	0	0	1	5	0	30	0	36	0
676	1904.876003	65.39228202	0	0	1	2	1	0	2	3	0	0	0	2	3	1	30	0	36	0
677	1904.876007	30.60675516	0	1	1	0	2	0	2	3	0	0	0	1	5	0	30	0	36	0
678	1904.876213	24.7624458	0	1	1	1	1	0	1	4	0	0	0	2	3	1	30	0	36	0
679	1904.876243	89.9438956	0	0	1	1	2	0	2	3	0	0	0	2	4	0	30	0	36	0
680	1904.876285	21.07490496	0	1	1	1	1	0	3	2	0	0	0	1	3	2	30	0	36	0
681	1904.876316	38.27487533	0	0	1	1	2	0	4	1	0	0	0	1	4	1	30	0	36	0
682	1904.876526	28.98750423	0	1	1	0	2	0	3	2	0	0	0	1	4	1	30	0	36	0
683	1904.876653	36.97752237	0	0	1	2	0	1	3	2	0	0	0	1	5	0	30	0	36	0
684	1904.87669	26.69081743	0	0	1	1	2	0	1	4	0	0	0	3	3	0	30	0	36	0
685	1904.876763	68.14832975	0	0	1	1	2	0	3	2	0	0	0	2	3	1	30	0	36	0
686	1904.876863	28.00495303	0	1	1	1	0	1	2	3	0	0	0	1	5	0	30	0	36	0
687	1904.876973	25.80609316	0	1	1	0	2	0	2	3	0	0	0	2	3	1	30	0	36	0
688	1904.877003	31.24490162	0	0	1	0	3	0	3	2	0	0	0	2	4	0	30	0	36	0
689	1904.877099	41.14899732	0	0	1	2	0	1	2	3	0	0	0	2	4	0	30	0	36	0
690	1904.877119	26.89967	0	0	1	2	1	0	2	3	0	1	0	0	4	1	30	0	36	0
691	1904.877209	30.3344871	0	0	1	1	2	0	2	3	0	0	0	3	2	1	30	0	36	0
692	1904.877248	35.33604655	0	0	0	2	2	0	2	3	0	0	1	1	4	0	30	0	36	0
693	1904.87736	29.59934763	0	0	1	1	2	0	2	3	0	1	0	0	5	0	30	0	36	0
694	1904.877382	26.52335049	0	1	1	1	0	1	3	2	0	0	0	1	4	1	30	0	36	0
695	1904.877413	38.53599094	0	0	1	1	1	1	4	1	0	0	0	1	5	0	30	0	36	0
696	1904.877566	23.94740108	0	0	1	2	1	0	1	4	0	1	0	1	3	1	30	0	36	0
697	1904.877619	31.1776071	0	0	1	2	0	1	3	2	0	0	0	2	3	1	30	0	36	0
698	1904.877623	29.18526032	0	1	1	0	1	1	3	2	0	0	0	1	5	0	30	0	36	0
699	1904.877767	26.7732739	0	0	0	2	2	0	3	2	0	0	1	1	3	1	30	0	36	0
700	1904.877807	32.93848258	0	0	1	1	2	0	1	4	0	1	0	1	4	0	30	0	36	0
701	1904.877829	23.61238306	0	1	1	1	0	1	2	3	0	0	0	2	3	1	30	0	36	0
702	1904.877859	85.7665569	0	0	1	1	1	1	3	2	0	0	0	2	4	0	30	0	36	0
703	1904.877879	28.03339361	0	0	1	1	2	0	3	2	0	1	0	0	4	1	30	0	36	0
704	1904.877977	20.27675815	0	1	0	1	2	0	2	3	0	0	1	1	3	1	30	0	36	0
705	1904.878008	24.55022191	0	0	0	1	3	0	3	2	0	0	1	1	4	0	30	0	36	0
706	1904.878069	32.47768163	0	1	1	0	1	1	2	3	0	0	0	2	4	0	30	0	36	0
707	1904.878306	50.90238745	0	0	1	1	1	1	2	3	0	0	0	3	3	0	30	0	36	0
708	1904.878326	49.91339451	0	0	1	1	2	0	2	3	0	1	0	1	3	1	30	0	36	0
709	1904.878339	23.72302221	0	0	0	2	2	0	3	2	0	0	0	2	4	0	30	0	35	1
710	1904.878379	32.49163027	0	0	1	1	1	1	4	1	0	0	0	2	3	1	30	0	36	0
711	1904.878455	21.85580755	0	0	0	1	3	0	2	3	0	0	1	2	3	0	30	0	36	0
712	1904.878567	22.88448018	0	0	1	0	3	0	2	3	0	1	0	1	4	0	30	0	36	0

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713	1904.878589	24.60755944	0	1	1	0	1	1	3	2	0	0	0	2	3	1	30	0	36	0
714	1904.878619	22.34532662	0	0	1	0	2	1	4	1	0	0	0	2	4	0	30	0	36	0
715	1904.878825	28.92563744	0	0	1	1	1	1	3	2	0	0	0	3	2	1	30	0	36	0
716	1904.878864	33.69490533	0	0	0	2	1	1	3	2	0	0	1	1	4	0	30	0	36	0
717	1904.878976	28.22464066	0	0	1	1	1	1	3	2	0	1	0	0	5	0	30	0	36	0
718	1904.879066	26.52386932	0	0	1	0	2	1	3	2	0	0	0	3	3	0	30	0	36	0
719	1904.879074	25.51886067	0	1	0	1	1	1	2	3	0	0	1	1	4	0	30	0	36	0
720	1904.879182	22.83519213	0	0	1	2	0	1	2	3	0	1	0	1	3	1	30	0	36	0
721	1904.879311	29.99685172	0	0	0	2	1	1	2	3	0	0	1	2	3	0	30	0	36	0
722	1904.879423	62.81738681	0	0	1	1	1	1	2	3	0	1	0	1	4	0	30	0	36	0
723	1904.87987	27.96155411	0	0	1	1	1	1	1	4	0	1	0	2	3	0	30	0	36	0
724	1904.879942	47.59522547	0	0	1	1	1	1	3	2	0	1	0	1	3	1	30	0	36	0
725	1904.879992	25.37286494	0	0	2	1	1	0	2	3	0	0	0	1	5	0	30	0	35	1
726	1904.880071	31.26111032	0	0	0	1	2	1	3	2	0	0	1	2	3	0	30	0	36	0
727	1904.880183	32.73245602	0	0	1	0	2	1	3	2	0	1	0	1	4	0	30	0	36	0
728	1904.880389	31.77869711	0	0	1	1	1	1	2	3	0	1	0	2	2	1	30	0	36	0
729	1904.880511	24.03051307	0	0	2	1	1	0	3	2	0	0	0	1	4	1	30	0	35	1
730	1904.88063	29.14003229	0	0	1	0	2	1	2	3	0	1	0	2	3	0	30	0	36	0
731	1904.880958	21.39313733	0	0	2	1	1	0	2	3	0	0	0	2	3	1	30	0	35	1
732	1904.881635	22.89635179	0	0	0	1	2	1	2	3	0	1	1	1	3	0	30	0	36	0
733	1904.881723	20.89699836	0	0	2	0	1	1	2	3	0	0	1	1	4	0	30	0	36	0
734	1904.882203	23.12043175	0	0	1	1	2	0	2	3	0	0	1	1	4	0	30	0	35	1
735	1904.883819	22.04663042	0	0	1	1	1	1	3	2	0	0	1	1	4	0	30	0	35	1
736	1905.873437	31.64107105	0	0	0	3	1	0	2	3	0	0	0	1	5	0	30	0	36	0
737	1905.873956	29.96709962	0	0	0	3	1	0	3	2	0	0	0	1	4	1	30	0	36	0
738	1905.874166	34.04340652	0	1	0	2	1	0	2	3	0	0	0	1	4	1	30	0	36	0
739	1905.874197	49.46194131	0	0	0	2	2	0	3	2	0	0	0	1	5	0	30	0	36	0
740	1905.874403	26.67817686	0	0	0	3	1	0	2	3	0	0	0	2	3	1	30	0	36	0
741	1905.874407	37.46003665	0	1	0	1	2	0	2	3	0	0	0	1	5	0	30	0	36	0
742	1905.874643	55.04179728	0	0	0	2	2	0	2	3	0	0	0	2	4	0	30	0	36	0
743	1905.874716	23.42257821	0	0	0	2	2	0	4	1	0	0	0	1	4	1	30	0	36	0
744	1905.874853	20.84297228	0	1	0	1	2	0	1	4	0	0	0	2	4	0	30	0	36	0
745	1905.874926	35.47821274	0	1	0	1	2	0	3	2	0	0	0	1	4	1	30	0	36	0
746	1905.875163	41.70384801	0	0	0	2	2	0	3	2	0	0	0	2	3	1	30	0	36	0
747	1905.875373	31.58443914	0	1	0	1	2	0	2	3	0	0	0	2	3	1	30	0	36	0
748	1905.875403	38.24107306	0	0	0	1	3	0	3	2	0	0	0	2	4	0	30	0	36	0
749	1905.875813	23.58236974	0	0	0	2	1	1	4	1	0	0	0	1	5	0	30	0	36	0
750	1905.875849	22.04242686	0	0	2	1	1	0	2	3	0	0	0	0	6	0	30	0	36	0
751	1905.87585	22.69604829	0	0	0	1	3	0	2	3	0	0	0	3	3	0	30	0	36	0
752	1905.876023	35.7202492	0	1	0	1	1	1	3	2	0	0	0	1	5	0	30	0	36	0
753	1905.876207	20.15693526	0	0	0	2	2	0	1	4	0	1	0	1	4	0	30	0	36	0
754	1905.876259	52.48544559	0	0	0	2	1	1	3	2	0	0	0	2	4	0	30	0	36	0
755	1905.876296	29.43486872	0	0	2	1	1	0	1	4	0	0	0	1	5	0	30	0	36	0
756	1905.876368	25.05152624	0	0	2	1	1	0	3	2	0	0	0	0	5	1	30	0	36	0
757	1905.876469	39.74988978	0	1	0	1	1	1	2	3	0	0	0	2	4	0	30	0	36	0

758	1905.876706	31,15007275	0	0	0	2	1	1	2	3	0	0	0	3	3	0	30	0	36	0
759	1905.876726	30.54485159	0	0	0	2	2	0	2	3	0	1	0	1	3	1	30	0	36	0
760	1905.876815	55,75523292	0	0	2	1	1	0	2	3	0	0	0	1	4	1	30	0	36	0
761	1905.876967	28.00863606	0	0	0	1	3	0	2	3	0	1	0	1	4	0	30	0	36	0
762	1905.876989	30.11753692	0	1	0	1	1	1	3	2	0	0	0	2	3	1	30	0	36	0
763	1905.877019	27.34875847	0	0	0	1	2	1	4	1	0	0	0	2	4	0	30	0	36	0
764	1905.877056	30.67544177	0	0	2	0	2	0	2	3	0	0	0	1	5	0	30	0	36	0
765	1905.877229	20.7126018	0	1	0	0	2	1	3	2	0	0	0	2	4	0	30	0	36	0
766	1905 877262	24 81801682	0	0	2	1	1	0	1	4	0	0	0	2	3	1	30	0	36	0
767	1905.877334	21,12220053	0	0	2	1	1	0	3	2	0	0	0	1	3	2	30	0	36	0
768	1905.877466	32.46293544	0	0	0	1	2	1	3	2	0	0	0	3	3	0	30	0	36	0
769	1905.877486	21.22147093	0	0	0	1	3	0	3	2	0	1	0	1	3	1	30	0	36	0
770	1905.877575	29.05255697	0	0	2	0	2	0	3	2	0	0	0	1	4	1	30	0	36	0
771	1905.87782	21.90442712	0	0	1	2	1	0	2	3	0	0	1	0	4	1	30	0	36	0
772	1905.877823	38.44154011	0	0	0	2	1	1	2	3	0	1	0	1	4	0	30	0	36	0
773	1905.877912	28.06780077	0	0	2	1	0	1	2	3	0	0	0	1	5	0	30	0	36	0
774	1905.878022	25.86400629	0	0	2	0	2	0	2	3	0	0	0	2	3	1	30	0	36	0
775	1905.878061	24.10277721	0	0	1	1	2	0	2	3	0	0	1	0	5	0	30	0	36	0
776	1905.878342	29.1262318	0	0	0	2	1	1	3	2	0	1	0	1	3	1	30	0	36	0
777	1905.878392	31.05420505	0	0	1	2	1	0	2	3	0	0	0	1	5	0	30	0	35	1
778	1905.878431	26.58287327	0	0	2	1	0	1	3	2	0	0	0	1	4	1	30	0	36	0
779	1905.878507	26.821838	0	0	1	1	2	0	1	4	0	0	1	1	4	0	30	0	36	0
780	1905.878552	22.0587725	0	1	0	1	1	1	2	3	0	1	0	1	3	1	30	0	36	0
781	1905.87858	22.82761935	0	0	1	1	2	0	3	2	0	0	1	0	4	1	30	0	36	0
782	1905.878583	40.06171173	0	0	0	1	2	1	3	2	0	1	0	1	4	0	30	0	36	0
783	1905.878672	29.25075687	0	0	2	0	1	1	3	2	0	0	0	1	5	0	30	0	36	0
784	1905.878878	23.66537314	0	0	2	1	0	1	2	3	0	0	0	2	3	1	30	0	36	0
785	1905.878911	29.41128178	0	0	1	2	1	0	3	2	0	0	0	1	4	1	30	0	35	1
786	1905.879026	40.64452509	0	0	1	1	2	0	2	3	0	0	1	1	3	1	30	0	36	0
787	1905.87903	35.66489398	0	0	0	1	2	1	2	3	0	1	0	2	3	0	30	0	36	0
788	1905.879118	32.55056691	0	0	2	0	1	1	2	3	0	0	0	2	4	0	30	0	36	0
789	1905.879121	22.27465531	0	1	1	1	1	0	2	3	0	0	0	1	4	1	30	0	35	1
790	1905.879152	32.36302727	0	0	1	1	2	0	3	2	0	0	0	1	5	0	30	0	35	1
791	1905.879358	26.18336065	0	0	1	2	1	0	2	3	0	0	0	2	3	1	30	0	35	1
792	1905.879549	20.26682527	0	0	0	1	2	1	3	2	0	1	0	2	2	1	30	0	36	0
793	1905.879598	36.01393595	0	0	1	1	2	0	2	3	0	0	0	2	4	0	30	0	35	1
794	1905.879638	24.66278287	0	0	2	0	1	1	3	2	0	0	0	2	3	1	30	0	36	0
795	1905.879677	22.983352	0	0	1	1	1	1	3	2	0	0	1	0	5	0	30	0	36	0
796	1905.880118	27.28689442	0	0	1	1	2	0	3	2	0	0	0	2	3	1	30	0	35	1
797	1905.880123	51.15225841	0	0	1	1	1	1	2	3	0	0	1	1	4	0	30	0	36	0
798	1905.88057	22.76912037	0	0	1	1	1	1	1	4	0	0	1	2	3	0	30	0	36	0
799	1905.880642	38.75683781	0	0	1	1	1	1	3	2	0	0	1	1	3	1	30	0	36	0
800	1905.880883	26.65407037	0	0	1	0	2	1	3	2	0	0	1	1	4	0	30	0	36	0
801	1905.881089	25.87742358	0	0	1	1	1	1	2	3	0	0	1	2	2	1	30	0	36	0
802	1905.881214	34.34131095	0	0	1	1	1	1	3	2	0	0	0	2	4	0	30	0	35	1

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803	1905.88133	23.72875629	0	0	1	0	2	1	2	3	0	0	1	2	3	0	30	0	36	0
804	1905.881661	20.3815424	0	0	1	1	1	1	2	3	0	0	0	3	3	0	30	0	35	1
805	1905.882778	25.15236115	0	0	1	1	1	1	2	3	0	1	0	1	4	0	30	0	35	1
806	1906.874249	26.9780352	0	0	1	2	1	0	2	3	0	0	0	0	6	0	30	0	36	0
807	1906.874696	36.02574841	0	0	1	2	1	0	1	4	0	0	0	1	5	0	30	0	36	0
808	1906.874768	30.66091411	0	0	1	2	1	0	3	2	0	0	0	0	5	1	30	0	36	0
809	1906.874978	23.22106525	0	1	1	1	1	0	2	3	0	0	0	0	5	1	30	0	36	0
810	1906.875009	28.11506162	0	0	1	1	2	0	3	2	0	0	0	0	6	0	30	0	36	0
811	1906.875215	68.23961109	0	0	1	2	1	0	2	3	0	0	0	1	4	1	30	0	36	0
812	1906.875425	25.84065915	0	1	1	1	1	0	1	4	0	0	0	1	4	1	30	0	36	0
813	1906.875456	75.08820632	0	0	1	1	2	0	2	3	0	0	0	1	5	0	30	0	36	0
814	1906.875662	30.37511866	0	0	1	2	1	0	1	4	0	0	0	2	3	1	30	0	36	0
815	1906.875734	25.8517573	0	0	1	2	1	0	3	2	0	0	0	1	3	2	30	0	36	0
816	1906.875902	41.77949471	0	0	1	1	2	0	1	4	0	0	0	2	4	0	30	0	36	0
817	1906.875975	71.11566342	0	0	1	1	2	0	3	2	0	0	0	1	4	1	30	0	36	0
818	1906.876185	26.9297493	0	1	1	0	2	0	2	3	0	0	0	1	4	1	30	0	36	0
819	1906.876216	26.08430071	0	0	1	0	3	0	3	2	0	0	0	1	5	0	30	0	36	0
820	1906.876312	34.35257479	0	0	1	2	0	1	2	3	0	0	0	1	5	0	30	0	36	0
821	1906.876422	63.31063967	0	0	1	1	2	0	2	3	0	0	0	2	3	1	30	0	36	0
822	1906.876662	29.02689935	0	0	1	0	3	0	2	3	0	0	0	2	4	0	30	0	36	0
823	1906.876831	32.53515121	0	0	1	2	0	1	3	2	0	0	0	1	4	1	30	0	36	0
824	1906.877041	24.64052005	0	1	1	1	0	1	2	3	0	0	0	1	4	1	30	0	36	0
825	1906.877072	71.60082268	0	0	1	1	1	1	3	2	0	0	0	1	5	0	30	0	36	0
826	1906.877182	21.99298457	0	0	1	0	3	0	3	2	0	0	0	2	3	1	30	0	36	0
827	1906.877278	28.96438191	0	0	1	2	0	1	2	3	0	0	0	2	3	1	30	0	36	0
828	1906.877282	27.11346715	0	1	1	0	1	1	2	3	0	0	0	1	5	0	30	0	36	0
829	1906.877426	24.87270199	0	0	0	2	2	0	2	3	0	0	1	1	3	1	30	0	36	0
830	1906.877518	79.6781902	0	0	1	1	1	1	2	3	0	0	0	2	4	0	30	0	36	0
831	1906.877538	26.04336876	0	0	1	1	2	0	2	3	0	1	0	0	4	1	30	0	36	0
832	1906.877591	33.90638993	0	0	1	1	1	1	4	1	0	0	0	1	4	1	30	0	36	0
833	1906.877667	22.80745925	0	0	0	1	3	0	2	3	0	0	1	1	4	0	30	0	36	0
834	1906.877801	25.67902869	0	1	1	0	1	1	3	2	0	0	0	1	4	1	30	0	36	0
835	1906.877965	23.64447318	0	0	1	1	1	1	1	4	0	0	0	3	3	0	30	0	36	0
836	1906.877985	23.18507986	0	0	1	1	2	0	1	4	0	1	0	1	3	1	30	0	36	0
837	1906.877998	22.03898051	0	0	0	2	2	0	2	3	0	0	0	2	4	0	30	0	35	1
838	1906.878038	60.3702513	0	0	1	1	1	1	3	2	0	0	0	2	3	1	30	0	36	0
839	1906.878248	22.86072652	0	1	1	0	1	1	2	3	0	0	0	2	3	1	30	0	36	0
840	1906.878278	41.5181686	0	0	1	0	2	1	3	2	0	0	0	2	4	0	30	0	36	0
841	1906.878484	26.87227429	0	0	1	1	1	1	2	3	0	0	0	3	2	1	30	0	36	0
842	1906.878523	31.30298304	0	0	0	2	1	1	2	3	0	0	1	1	4	0	30	0	36	0
843	1906.878635	26.22103963	0	0	1	1	1	1	2	3	0	1	0	0	5	0	30	0	36	0
844	1906.878725	24.64100205	0	0	1	0	2	1	2	3	0	0	0	3	3	0	30	0	36	0
845	1906.879042	23.71751853	0	0	0	2	1	1	3	2	0	0	1	1	3	1	30	0	36	0
846	1906.879082	29.17906393	0	0	1	1	1	1	1	4	0	1	0	1	4	0	30	0	36	0
847	1906.879154	24.83381505	0	0	1	1	1	1	3	2	0	1	0	0	4	1	30	0	36	0

				1														1		
848	1906.879283	32.62229034	0	0	0	1	2	1	3	2	0	0	1	1	4	0	30	0	36	0
849	1906.879601	44.21655205	0	0	1	1	1	1	2	3	0	1	0	1	3	1	30	0	36	0
850	1906.879614	21.01540592	0	0	0	2	1	1	3	2	0	0	0	2	4	0	30	0	35	1
851	1906.87973	29.04195742	0	0	0	1	2	1	2	3	0	0	1	2	3	0	30	0	36	0
852	1906.879842	30.40885574	0	0	1	0	2	1	2	3	0	1	0	1	4	0	30	0	36	0
853	1906.88017	22.32464331	0	0	2	1	1	0	2	3	0	0	0	1	4	1	30	0	35	1
854	1906.880361	23.04005975	0	0	1	0	2	1	3	2	0	1	0	1	3	1	30	0	36	0
855	1906.883478	20.48159184	0	0	1	1	1	1	2	3	0	0	1	1	4	0	30	0	35	1
856	1907.873615	27.83980551	0	0	0	3	1	0	2	3	0	0	0	1	4	1	30	0	36	0
857	1907.873856	45.95075411	0	0	0	2	2	0	2	3	0	0	0	1	5	0	30	0	36	0
858	1907.874302	25.5672546	0	0	0	2	2	0	1	4	0	0	0	2	4	0	30	0	36	0
859	1907.874375	43.51972864	0	0	0	2	2	0	3	2	0	0	0	1	4	1	30	0	36	0
860	1907.874585	32.95969764	0	1	0	1	2	0	2	3	0	0	0	1	4	1	30	0	36	0
861	1907.874616	31.92494126	0	0	0	1	3	0	3	2	0	0	0	1	5	0	30	0	36	0
862	1907.874822	38.74338966	0	0	0	2	2	0	2	3	0	0	0	2	3	1	30	0	36	0
863	1907.875062	35.52642898	0	0	0	1	3	0	2	3	0	0	0	2	4	0	30	0	36	0
864	1907.875472	43.81662525	0	0	0	2	1	1	3	2	0	0	0	1	5	0	30	0	36	0
865	1907.875582	26.9175221	0	0	0	1	3	0	3	2	0	0	0	2	3	1	30	0	36	0
866	1907.875682	33.1845525	0	1	0	1	1	1	2	3	0	0	0	1	5	0	30	0	36	0
867	1907.875918	48.75962692	0	0	0	2	1	1	2	3	0	0	0	2	4	0	30	0	36	0
868	1907.875991	20.74925295	0	0	0	2	1	1	4	1	0	0	0	1	4	1	30	0	36	0
869	1907.876027	23.27317716	0	0	2	1	1	0	2	3	0	0	0	0	5	1	30	0	36	0
870	1907.876201	31.42892315	0	1	0	1	1	1	3	2	0	0	0	1	4	1	30	0	36	0
871	1907.876232	22.83166862	0	0	0	1	2	1	4	1	0	0	0	1	5	0	30	0	36	0
872	1907.876438	36.94399839	0	0	0	2	1	1	3	2	0	0	0	2	3	1	30	0	36	0
873	1907.876474	25.89864986	0	0	2	1	1	0	1	4	0	0	0	1	4	1	30	0	36	0
874	1907.876648	27.97956362	0	1	0	1	1	1	2	3	0	0	0	2	3	1	30	0	36	0
875	1907.876678	50.81466851	0	0	0	1	2	1	3	2	0	0	0	2	4	0	30	0	36	0
876	1907.877125	30.1584678	0	0	0	1	2	1	2	3	0	0	0	3	3	0	30	0	36	0
877	1907.877234	26.9901841	0	0	2	0	2	0	2	3	0	0	0	1	4	1	30	0	36	0
878	1907.878001	27.05862892	0	0	0	2	1	1	2	3	0	1	0	1	3	1	30	0	36	0
879	1907.87809	24.69581745	0	0	2	1	0	1	2	3	0	0	0	1	4	1	30	0	36	0
880	1907.878239	21.2071402	0	0	1	1	2	0	2	3	0	0	1	0	4	1	30	0	36	0
881	1907.878242	37.21782479	0	0	0	1	2	1	2	3	0	1	0	1	4	0	30	0	36	0
882	1907.878331	27.17431425	0	0	2	0	1	1	2	3	0	0	0	1	5	0	30	0	36	0
883	1907.87857	27.32344387	0	0	1	2	1	0	2	3	0	0	0	1	4	1	30	0	35	1
884	1907.878761	28.19905208	0	0	0	1	2	1	3	2	0	1	0	1	3	1	30	0	36	0
885	1907.878811	30.06565187	0	0	1	1	2	0	2	3	0	0	0	1	5	0	30	0	35	1
886	1907.87885	25.73665667	0	0	2	0	1	1	3	2	0	0	0	1	4	1	30	0	36	0
887	1907.879297	22.91202976	0	0	2	0	1	1	2	3	0	0	0	2	3	1	30	0	36	0
888	1907.87933	28.47502802	0	0	1	1	2	0	3	2	0	0	0	1	4	1	30	0	35	1
889	1907.879336	21.35181775	0	0	1	1	1	1	2	3	0	0	1	0	5	0	30	0	36	0
890	1907.879777	25.34986179	0	0	1	1	2	0	2	3	0	0	0	2	3	1	30	0	35	1
891	1907.879782	23.76053979	0	0	1	1	1	1	1	4	0	0	1	1	4	0	30	0	36	0
892	1907.879855	20.22219945	0	0	1	1	1	1	3	2	0	0	1	0	4	1	30	0	36	0

802	1007 000201	26 00559091	0	0	1	1	1	1	2	2	0	0	1	1	2	1	20	0	26	0
893	1907.880301	28 66028705	0	0	1	1	1	1	2	2 2	0	0	0	1	5	0	30	0	30	1
805	1907.880427	24.76106044	0	0	1	0	2	1	2	2	0	0	1	1	1	0	20	0	26	0
895	1907.880342	31 90350184	0	0	1	1	1	1	2	3	0	0	0	2	4	0	30	0	35	1
890	1907.881393	24 1725172	0	0	1	1	1	1	3	2	0	0	0	2	3	1	30	0	35	1
898	1908 874427	28.48436774	0	0	1	2	1	0	2	3	0	0	0	0	5	1	30	0	36	0
899	1908.874668	26.11923935	0	0	1	1	2	0	2	3	0	0	0	0	6	0	30	0	36	0
900	1008 874874	31 60772058	0	0	1	2	1	0	1	1	0	0	0	1	4	1	30	0	36	0
900	1008 875115	3/ 8780353/	0	0	1	1	2	0	1	4	0	0	0	1	5	0	30	0	36	0
901	1008 875187	20 68/88062	0	0	1	1	2	0	3	2	0	0	0	0	5	1	30	0	36	0
903	1908 875393	24.01660169	0	0	1	2	1	0	2	3	0	0	0	1	3	2	30	0	36	0
904	1908 875634	66.06732929	0	0	1	1	2	0	2	3	0	0	0	1	4	1	30	0	36	0
905	1908 875875	24 23263739	0	0	1	0	3	0	2	3	0	0	0	1	5	0	30	0	36	0
906	1908 876081	29.40818294	0	0	1	1	2	0	1	4	0	0	0	2	3	1	30	0	36	0
907	1908 876153	25.02881442	0	0	1	1	2	0	3	2	0	0	0	1	3	2	30	0	36	0
908	1908 876284	23.02001442	0	0	1	1	1	1	3	2	0	0	0	0	6	0	30	0	36	0
909	1908 876394	22 95060927	0	0	1	0	3	0	3	2	0	0	0	1	4	1	30	0	36	0
910	1908 87649	30 22555714	0	0	1	2	0	1	2	3	0	0	0	1	4	1	30	0	36	0
911	1908 876731	66 51804823	0	0	1	1	1	1	2	3	0	0	0	1	5	0	30	0	36	0
912	1908 876841	20 43175419	0	0	1	0	3	0	2	3	0	0	0	2	3	1	30	0	36	0
913	1908 877177	37.01101119	0	0	1	1	1	1	1	4	0	0	0	2	4	0	30	0	36	0
914	1908 87725	62 99890969	0	0	1	1	1	1	3	2	0	0	0	1	4	1	30	0	36	0
915	1908 87746	23 85613467	0	1	1	0	1	1	2	3	0	0	0	1	4	1	30	0	36	0
916	1908 877491	34 66077146	0	0	1	0	2	1	3	2	0	0	0	1	5	0	30	0	36	0
917	1908.877697	56.08470877	0	0	1	1	1	1	2	3	0	0	0	2	3	1	30	0	36	0
918	1908 877937	38 5708912	0	0	1	0	2	1	2	3	0	0	0	2	4	0	30	0	36	0
919	1908 878457	29 224238	0	0	1	0	2	1	3	2	0	0	0	2	3	1	30	0	36	0
920	1908.878701	22,03386752	0	0	0	2	1	1	2	3	0	0	1	1	3	1	30	0	36	0
921	1908.878813	23 07092078	0	0	1	1	1	1	2	3	0	1	0	0	4	1	30	0	36	0
922	1908.878942	30,30651047	0	0	0	1	2	1	2	3	0	0	1	1	4	0	30	0	36	0
923	1908.87926	20.53886137	0	0	1	1	1	1	1	4	0	1	0	1	3	1	30	0	36	0
924	1908.879461	22.96251519	0	0	0	1	2	1	3	2	0	0	1	1	3	1	30	0	36	0
925	1908.88002	21.40449995	0	0	1	0	2	1	2	3	0	1	0	1	3	1	30	0	36	0
926	1908.880033	20.34641934	0	0	0	1	2	1	3	2	0	0	0	2	4	0	30	0	35	1
927	1909.873515	21.34440893	0	0	0	2	2	0	1	4	0	0	0	1	5	0	30	0	36	0
928	1909.874034	40.43036519	0	0	0	2	2	0	2	3	0	0	0	1	4	1	30	0	36	0
929	1909.874275	29.65866457	0	0	0	1	3	0	2	3	0	0	0	1	5	0	30	0	36	0
930	1909.874794	28.08957238	0	0	0	1	3	0	3	2	0	0	0	1	4	1	30	0	36	0
931	1909.875131	40.70618581	0	0	0	2	1	1	2	3	0	0	0	1	5	0	30	0	36	0
932	1909.875241	25.00671034	0	0	0	1	3	0	2	3	0	0	0	2	3	1	30	0	36	0
933	1909.875577	22.64914769	0	0	0	2	1	1	1	4	0	0	0	2	4	0	30	0	36	0
934	1909.87565	38.55262432	0	0	0	2	1	1	3	2	0	0	0	1	4	1	30	0	36	0
935	1909.87586	29.19785762	0	1	0	1	1	1	2	3	0	0	0	1	4	1	30	0	36	0
936	1909.875891	42.42180403	0	0	0	1	2	1	3	2	0	0	0	1	5	0	30	0	36	0
937	1909.876097	34.3214306	0	0	0	2	1	1	2	3	0	0	0	2	3	1	30	0	36	0
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938	1909.876337	47.20745438	0	0	0	1	2	1	2	3	0	0	0	2	4	0	30	0	36	0
939	1909.87641	20.08873886	0	0	0	1	2	1	4	1	0	0	0	1	4	1	30	0	36	0
940	1909.876857	35.76795453	0	0	0	1	2	1	3	2	0	0	0	2	3	1	30	0	36	0
941	1909.877953	22.50747978	0	0	0	1	1	2	3	2	0	0	0	2	4	0	30	0	36	0
942	1909.87842	26.19726752	0	0	0	1	2	1	2	3	0	1	0	1	3	1	30	0	36	0
943	1909.878509	23.90967178	0	0	2	0	1	1	2	3	0	0	0	1	4	1	30	0	36	0
944	1909.878989	26.45365258	0	0	1	1	2	0	2	3	0	0	0	1	4	1	30	0	35	1
945	1909.880086	26.63412245	0	0	1	1	1	1	2	3	0	0	0	1	5	0	30	0	35	1
946	1909.880605	25.22504372	0	0	1	1	1	1	3	2	0	0	0	1	4	1	30	0	35	1
947	1909.881052	22.45656691	0	0	1	1	1	1	2	3	0	0	0	2	3	1	30	0	35	1
948	1910.874846	27.57762058	0	0	1	1	2	0	2	3	0	0	0	0	5	1	30	0	36	0
949	1910.875293	30.68868228	0	0	1	1	2	0	1	4	0	0	0	1	4	1	30	0	36	0
950	1910.875812	23.25207759	0	0	1	1	2	0	2	3	0	0	0	1	3	2	30	0	36	0
951	1910.875943	23.13813191	0	0	1	1	1	1	2	3	0	0	0	0	6	0	30	0	36	0
952	1910.876053	21.32139935	0	0	1	0	3	0	2	3	0	0	0	1	4	1	30	0	36	0
953	1910.87639	30.89804401	0	0	1	1	1	1	1	4	0	0	0	1	5	0	30	0	36	0
954	1910.876462	26.29681035	0	0	1	1	1	1	3	2	0	0	0	0	5	1	30	0	36	0
955	1910.876909	58.52676487	0	0	1	1	1	1	2	3	0	0	0	1	4	1	30	0	36	0
956	1910.87715	32.2002846	0	0	1	0	2	1	2	3	0	0	0	1	5	0	30	0	36	0
957	1910.877356	26.05169342	0	0	1	1	1	1	1	4	0	0	0	2	3	1	30	0	36	0
958	1910.877428	22.17216213	0	0	1	1	1	1	3	2	0	0	0	1	3	2	30	0	36	0
959	1910.877669	30.49672796	0	0	1	0	2	1	3	2	0	0	0	1	4	1	30	0	36	0
960	1910.878116	27.1496779	0	0	1	0	2	1	2	3	0	0	0	2	3	1	30	0	36	0
961	1910.87912	21.3324601	0	0	0	1	2	1	2	3	0	0	1	1	3	1	30	0	36	0
962	1911.874453	26.09555953	0	0	0	1	3	0	2	3	0	0	0	1	4	1	30	0	36	0
963	1911.875309	35.81586395	0	0	0	2	1	1	2	3	0	0	0	1	4	1	30	0	36	0
964	1911.87555	39.41037967	0	0	0	1	2	1	2	3	0	0	0	1	5	0	30	0	36	0
965	1911.875996	21.92815397	0	0	0	1	2	1	1	4	0	0	0	2	4	0	30	0	36	0
966	1911.876069	37.32537283	0	0	0	1	2	1	3	2	0	0	0	1	4	1	30	0	36	0
967	1911.876516	33.22887134	0	0	0	1	2	1	2	3	0	0	0	2	3	1	30	0	36	0
968	1911.877612	20.90972658	0	0	0	1	1	2	2	3	0	0	0	2	4	0	30	0	36	0
969	1911.880264	23.43437704	0	0	1	1	1	1	2	3	0	0	0	1	4	1	30	0	35	1
970	1912.876121	24.43006147	0	0	1	1	1	1	2	3	0	0	0	0	5	1	30	0	36	0
971	1912.876568	27.18604356	0	0	1	1	1	1	1	4	0	0	0	1	4	1	30	0	36	0
972	1912.877087	20.59821235	0	0	1	1	1	1	2	3	0	0	0	1	3	2	30	0	36	0
973	1912.877328	28.33183678	0	0	1	0	2	1	2	3	0	0	0	1	4	1	30	0	36	0
974	1913.875728	34.67573216	0	0	0	1	2	1	2	3	0	0	0	1	4	1	30	0	36	0

# **Supporting information 9.**



**Figure S9.** Computed (red trace) and experimental (black trace) mass spectrum of the MS/MS fragment at m/z 1905. All the peaks have been assigned considering the highest abundance from a bunch of peaks contributing to the spectrum. For example, although the peak at m/z 1893 is due to 9 species, only one assignment is given here. However, it may be noted that this number itself is due to the abundance cut-off discussed in Supporting information 1.

# **Supporting information 10.**



*Figure S10. SEM image (inset) and EDAX spectrum of the cluster 2 showing the presence of all the expected elements. The elements O, Si, Sn and Ca are from the substrate (conducting glass) used.* 

# **Supporting information 11.**



*Figure S11. XPS survey spectrum of cluster 2* (*A*). *Expanded XPS spectra for Ag* (*B*), *Se* (*C*) *and Pd* (*D*) *regions, respectively.* 



# Approaching Sensitivity of Tens of Ions Using Atomically Precise Cluster–Nanofiber Composites

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Supporting Information

**ABSTRACT:** A new methodology has been demonstrated for ultratrace detection of  $Hg^{2+}$ , working at the limit of a few tens of metal ions. Bright, red luminescent atomically precise gold clusters, Au@BSA (BSA, bovine serum albumin), coated on Nylon-6 nanofibers were used for these measurements. A green emitting fluorophore, FITC (fluorescein isothiocyanate), whose luminescence is insensitive to  $Hg^{2+}$  was precoated on the fiber. Exposure to mercury quenched the red emission completely, and the green emission of the fiber appeared which was observed under dark field fluorescence microscopy. For the sensing experiment at the limit of sensitivity, we have used individual nanofibers. Quenching due to  $Hg^{2+}$  ions was fast and uniform. Adaptation of such sensors to pH paper-like test-strips would make affordable water quality sensors at ultralow concentrations a reality.



N oble metal nanoparticles (NM NPs) and their atomically precise analogs called quantum clusters (QCs) (also known as monolayer protected clusters, nanomolecules, and quantum dots) are new paradigms in the ultralow detection of various species due to their unique electronic structure and versatile surface functionalization.<sup>1–13</sup> While limits of sensitivity have reached zeptomole levels<sup>14</sup> with such systems, adaptation of these materials into useful devices has not been advancing significantly. A combination of ultralow sensitivity with affordability and adaptability to devices in the field would make cluster-based sensors available as consumer products. One of the major expanding nanoscale platforms for these applications is electrospun nanofibers<sup>15-22</sup> which enables extremely small quantities of materials distributed over large areas with high uniformity. This platform also enables measurements using optical techniques (in the visible window) at single fiber level, as they are of submicrometer dimensions, larger than the diffraction limit of visible light.

Atomically precise clusters of noble metals, due to their discrete energy states, show inherent luminescence.<sup>23</sup> In a few such clusters, emission occurs in the visible region of the electromagnetic spectrum, observable even to the naked eye.<sup>24,25</sup> Cluster luminescence has been shown to be sensitive to many environmental factors such as chemical contamination, pH, temperature, etc.<sup>23,26</sup> Molecular luminescence of the clusters is enhanced upon trapping the clusters in cavities as well as confining them in containers.<sup>27,28</sup> The nonradiative relaxation channels are blocked by such methods resulting in enhanced emission.<sup>27,29</sup>

Such clusters confined to micrometer ( $\mu$ m) dimensions can be useful in optical microscopy-based detection. An application toward this was demonstrated recently using mesoflowers in which zeptomole detection of the explosive trinitrotoluene (TNT) was accomplished.<sup>14</sup> Confining clusters on nanofibers is a more versatile strategy in the optical microscopy context as it enables easy attachment on other substrates besides allowing direct observation. In this communication, we demonstrate single fiber-based detection of mercuric ion nearly at the limit of single ion concentration. Adaptation of such sensors to pH paper-like test-strips would make affordable water quality sensors at ultralow concentrations a reality.

Electrospinning has become a simple and versatile technique for the preparation of nanofibers which possesses many advantages including controlled morphology and large surface area-to-volume ratio.<sup>30</sup> They have found applications in tissue engineering, filtration, electronic devices, catalyst supports, and sensing devices.<sup>31</sup> Electrospun nanofibers could act as suitable substrates to load clusters.

Mercury in both organic and inorganic forms is one of the most hazardous environmental contaminants. Different approaches have been used for the determination of trace levels of mercury.<sup>32–36</sup> Fluorescence-based techniques have great importance in the determination of ionic mercury.<sup>9,14</sup> However, as we understand the implications of even trace levels of heavy

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Figure 1. (a) SEM image of a fiber mat. (b) Visible light photograph of the Au@BSA/N6 fiber mat (inset: Au@BSA cluster solution under visible light). (c) Photograph of the same mat under UV light (inset: Au@BSA cluster solution under UV light). (d) Optical image of a few Au@BSA/FITC/N6 fibers prepared on a glass slide. (e) Fluorescence image of FITC/N6 fibers. Green emission is due to FITC. (f) Fluorescence image of Au@BSA/FITC/N6 fiber. As red luminescence dominates over green, the fibers showed only red color. (g), (h), and (i) are the corresponding images of a single fiber.

metals, methods for ultrasensitive, field-implementable solutions have to be developed, working at the limit of single ions.

The appproach used in the present experiment is as follows. The red luminescence of atomically precise gold clusters is quenched by Hg<sup>2+</sup> ions in water. The quenching is even more prominent on nanofibers as the cluster emission is enhanced on them as nonradiative relaxation channels are reduced. As the disappearnce of cluster luminescence (due to Hg<sup>2+</sup> exposure) will lead to a nonluminescent or black fiber mat, a fluorophore, whose luminescence is insensitive to Hg<sup>2+</sup>, is precoated on the fiber. The fluorophore used here is fluorescein isothiocyanate (FITC) which emits in green. As a result, when the luminescence of gold clusters disappear, that of FITC becomes visible. Although the fiber has emissive clusters and has FITC anchored on it initially, due to the dominance of the fluorescence of the former, the fiber appears red initially in the luminesence image. Exposure to mercury quenches the red emission completely, and the green emission of the fiber mat appears. In the in-between concentrations, intermediate colors are seen. The detection can happen down to a single fiber level with a simple fluorescence microscope, which requires just a few Hg<sup>2+</sup> ions for observable color change, enhancing detection limits.

#### EXPERIMENTAL SECTION

**Chemicals.** Nylon-6 (N6, Molecular weight 10 031 g/mol), fluorescein isothiocyanate (FITC), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. Formic acid and divalent acetates of mercury, zinc, copper, nickel, manganese,

lead, and cadmium were purchased from Merck. Deionized water was used for all experiments. All chemicals were used directly without any additional purification.

**Synthesis of Au@BSA Cluster.** The Au@BSA nanoclusters with red luminescence were prepared following a reported method<sup>37</sup> by adding 10 mL of aqueous solution of HAuCl<sub>4</sub> (6 mM) to 10 mL of BSA (25 mg/mL in water) under vigorous stirring for 5 min. The pH of the solution was adjusted around 11.0 with the addition of NaOH (1 mL, 1 M). The reactions were kept for 24 h. The solution turned from pale yellow to dark orange, with deep red emission, indicating the formation of Au@BSA nanoclusters. The sample was stored at 4 °C.

Synthesis of N6 Fiber Mat. N6 fiber mat was prepared by slight modification of an earlier report.<sup>30</sup> 4.5 g of N6 polymer was dissolved in 10 mL of formic acid, and the mixture was stirred at 60 °C for 15 h to obtain a transparent homogeneous solution. The prepared N6 solution was loaded into a 2 mL plastic syringe equipped with a needle of 0.8 mm outer diameter and 0.6 mm inner diameter. A high voltage was applied between the needle and the collector. The syringe was fixed on an electric syringe pump set to maintain a constant flow rate of 0.1 mL/h. Positive potential was applied to the needle by a high-voltage power supply. The voltage used for electrospinning was 28 kV. The distance between the needle tip and the collector was 16 cm. Nanofibers were collected on an aluminum sheet mounted on a cylindrical drum collector, which rotated at a speed of 2500 rpm. The electrospun fiber mat was prepared by spinning the respective solutions for 1 h on aluminum sheets.



**Figure 2.** Images for the sensing experiment which was done using the Au@BSA/N6 single fiber. Fluorescence images (a, b, c, and d) of single fibers before the addition of  $Hg^{2+}$  ion solution. (a<sub>1</sub>, b<sub>1</sub>, c<sub>1</sub>, and d<sub>1</sub>) Flourescence images of the respective fibers (a, b, c, and d) after the addition of 1 ppb and 100, 50, and 20 ppt of  $Hg^{2+}$  ion solutions, respectively. The red color was quenched completely in 1 ppb, but in the case of 20 ppt, a faint red color can be seen upon closer examination. Optical images of the corresponding fibers are shown in a<sub>2</sub>, b<sub>2</sub>, c<sub>2</sub>, and d<sub>2</sub>, respectively. It shows that fiber morphology is intact after the addition of the  $Hg^{2+}$  ion solution and only luminescence of the fiber was quenched.

**Synthesis of Au@BSA/N6 Fiber Mat.** The above prepared fiber mat was dipped in an as-synthesized aqueous Au@BSA cluster solution for 10 min. Then, it was washed three times with water to remove the excess loosely bound clusters. After water washing, the clusters were present only on the fibers. It is shown in the case of the single fiber experiment (Figures 2 and 4i).

**Synthesis of Au@BSA/FITC/N6 Multiple and Single Fiber.** 4.5 g of N6 polymer and 3 mg of FITC were dissolved in 10 mL of formic acid, and the mixture was stirred at 60 °C for 15 h to obtain a transparent homogeneous solution. Electrospining of the solution was done in the same way. The FITC/N6 fibers were collected on a glass slide instead of an aluminum sheet. To examine the FITC/N6 single fibers, they were collected on the glass slide only for 2–3 s. The fibers show green luminescence under a microscope due to FITC. The Au@BSA cluster solution was added to the FITC/N6 fibers on the glass side drop by drop and dried in air. Then, the slide was carefuly washed 2–3 times with water to remove the excess cluster present on the slide and dried in air to get red luminescent Au@BSA/FITC/N6 fibers.

**Sensing Experiment.** For the single fiber sensing experiment, we have placed the glass slide containing the fiber under the microscope. About 2.5  $\mu$ L of Hg<sup>2+</sup> ion solution was added on the fiber. A fluorescence image of the seleted fiber was taken before and after the addition of Hg<sup>2+</sup>.

UV-vis spectra were recorded using a PerkinElmer Lambda 25 spectrophotometer. Scanning electron microscopic (SEM) images and energy-dispersive analysis of X-ray (EDAX) studies were performed using a FEI QUANTA-200 SEM. The photoexcitation and luminescence (PL) studies were done using a NanoLog HORIBA JOBINYVON spectrofluorimeter. Luminescence spectra of fiber mats were collected using 488 nm LASER excitation in a RAMAN spectrometer. X-ray photoelectron spectroscopy (XPS) measurements were conducted using an Omicron ESCA Probe spectrometer with unmonochromatized Al K $\alpha$  X-rays (energy = 1486.6 eV). Fluorescence imaging measurements were done with the Cytoviva HSI system containing an Olympus BX-41 microscope equipped with a Dage high resolution camera and a Specim V10E spectrometer. Dark field fluorescence microscopy was used with an excitation band at  $492 \pm 18$  nm, and emission was collected using a triple pass emission filter DAPI/FITC/ TEXAS RED (DAPI, 452-472 nm; FITC, 515-545 nm; TEXAS RED, 600-652 nm). Further details of the experiment are presented in the Supporting Information.

#### RESULTS AND DISCUSSION

The materials used in this study, namely, red luminescent gold clusters protected by BSA (Au@BSA; BSA, bovine serum albumin), were synthesized by a reported procedure, and details are presented in the Experimental Section.<sup>37</sup> In view of the properties already known of this material, we list only the essential details here. The Au@BSA cluster contains a core of 25 atoms of Au protected with the protein, and BSA shows an optical absorption feature at 520 nm.<sup>37</sup> The photoluminescence spectrum (Figure S1, Supporting Information) shows an emission maximum at 670 nm when excited at 365 nm, at room temperature.

#### **Analytical Chemistry**

A sensor platform was developed using electrospun nanofibers prepared by N6. Preparation of the fibers was discussed in detail in the Experimental Section. The SEM images of the N6 fiber mat showed high uniformity (Figure 1a). The fiber diameters range from 180 to 260 nm (distribution has been given in Figure S2, Supporting Information). The N6 fiber mat was dipped in an as-synthesized Au@BSA cluster solution for 10 min for adsorption of the clusters on the fibers, and the mat was washed with water three times to remove the excess cluster solution. The sample was labeled as a Au@BSA/N6 fiber mat. The mat appears white in visible light (Figure 1b) and red in UV light (Figure 1c). The solution of Au@BSA in visible light and UV light is shown in the insets of Figure 1b,c, respectively.

In order to observe individual fibers, electrospinning was performed on glass slides for short periods. FITC incorporated N6 nanofiber, labeled as FITC/N6, was produced by the electrospinning of a solution which contains N6 and FITC. The FITC/N6 fibers on the glass slides were coated with the Au@ BSA cluster and washed 2-3 times carefully to remove the excess cluster present on the slides. The cluster coated fibers are labeled as Au@BSA/FITC/N6. Details of the preparation of FITC/N6 and Au@BSA/FITC/N6 fibers are given in the Experimental Section. The optical image of a collection of Au@ BSA/FITC/N6 fibers is shown in Figure 1d. Fluorescence images of FITC/N6 and Au@BSA/FITC/N6 fibers are shown in Figure 1e,f, respectively. FITC/N6 fibers showed a green color due to the green luminescence of FITC (Figure 1e). As red luminescence dominates over green, the Au@BSA/FITC/ N6 fibers showed only red color (Figure 1f). The same images for single fibers are shown in Figure 1g-i. For the sensing experiment at the limit of sensitivity, we have used these individual fibers.

The concentration dependence (mercuric ion) of luminescence quenching of individual Au@BSA/N6 fibers is shown in Figure 2. The quenching due to Hg<sup>2+</sup> ions was performed with various concentrations ranging from 1 ppb to 20 ppt. These experiments were performed by dropping 2.5  $\mu$ L of the appropriate quantity of aqueous Hg<sup>2+</sup> solution on the fiber and measuring the fluorescence image. Before adding Hg<sup>2+</sup>, Au@ BSA/N6 was observed under dark field fluorescence microscopy with an excitation at 492 ± 18 nm and emission was collected using a triple pass emission filter, DAPI/FITC/ TEXAS RED (DAPI, 452–472 nm; FITC, 515–545 nm; TEXAS RED, 600–652 nm), which showed red luminescence (Figure 2a–d). Details of the dark field microscopy setup are given in the Experimental Section of the Supporting Information.

These fibers were exposed to  $Hg^{2+}$  at various concentrations (1 ppb and 100, 50, and 20 ppt of  $Hg^{2+}$  solution); the quenching of red luminescence was observed as shown in Figure  $2a_1-d_1$ ). The fibers were very sensitive to  $Hg^{2+}$ ; the red color was quenched completely in 1 ppb, but even in the case of 20 ppt, a faint red color could be seen. The optical image of the fibers after the quenching experiments is shown in Figure  $2a_2-d_2$ . These images showed that there is no physical change in fiber dimension even after the quenching experiment.

The quenching experiments and fluorescence response of an ensemble of Au@BSA/FITC/N6 fibers on glass slides were conducted with various metal ions. Ions such as  $Mn^{2+}$ ,  $Ni^{2+}$ ,  $Cu^{2+}$ ,  $Zn^{2+}$ ,  $Pb^{2+}$ , and  $Cd^{2+}$ , chosen as they could be present in water with  $Hg^{2+}$ , did not induce any color change under florescence microscopy. Thus, the luminescence quenching is specific to  $Hg^{2+}$  as shown in Figure 3. About 5  $\mu$ L of 20 ppm of

Letter



**Figure 3.** Images of sensing experiments using Au@BSA/FITC/N6 fiber mats with various metal ions. In the case of  $Hg^{2+}$  ion, the red luminescence of the cluster disappered and a green color can be seen. For other metal ions, there is no change in red luminescence. It confirms that Au@BSA/FITC/N6 is selective to the  $Hg^{2+}$  ion. Inset: The variation of fluorescence intensity with different concentrations of the  $Hg^{2+}$  ion. It confirms that the limit of detection for mercuric ion is 1 ppt. The reduction in signal was more than three times the noise of the luminescence intensity of the parent fibers.

different metal ions was taken, and their quenching effect on Au@BSA/FITC/N6 fibers was investigated. It was first tested with water, and no quenching was observed. For other metal ions, quenching was not observed and the entire mat showed red luminescence. However, for  $Hg^{2+}$ , red luminescence quenched and green luminescence was visible due to FITC. Therefore, it confirms that Au@BSA/FITC/N6 is very sensitive and selective to Hg2+. Emission spectra of mats under various conditions were measured. After measuring the spectrum of the Au@BSA/N6 fiber mats, the mats were dipped in different M<sup>2+</sup> ion solutions (20 ppm). The mats were dried for 20 min, and then, the emission spectra of each mat was measured at 488 nm excitation. Variation in emission intensity is shown in Figure S3, Supporting Information. A large decrease in intensity was observed for  $Hg^{2+}$  compared to other  $M^{2+}$  ions. The sensing capability and limit of detection estimated from the luminecence spectra are shown in inset of Figure 3. Fluorescence spectra of Au@BSA/N6 fiber mats after exposure to varying concentrations of Hg<sup>2+</sup> were measured. Fluorescence intensities of blank and Hg<sup>2+</sup> treated fiber mats are compared in the inset of Figure 3. The experiment showed that the mats are sensitive even down to 1 ppt.

In Figure 4i, we examine the sensitivity limit at a single fiber level. A fixed volume (2.5  $\mu$ L) of varying concentrations of Hg<sup>2+</sup> (100, 20, and 10 ppt) was exposed to Au@BSA/FITC/ N6 fibers separately. Red luminescence due to the cluster disappears, and the green luminescence due to FITC appears at 100 and 20 ppt Hg<sup>2+</sup>. At 10 ppt, however, the decrease in red emission is barely observable. It did not quench completely, and as a result, a faint green luminescence is seen. An amount of 2.5  $\mu$ L of 10 ppt Hg<sup>2+</sup> translates to 7.5 × 10<sup>7</sup> ions. From



**Figure 4.** (i) (a, c, e) Fluorescence images of the Au@BSA/FITC/N6 single fiber showing red emission. Quenching experiment at (b) 100 ppt (green emission), (d) 20 ppt (green emission), and (f) 10 ppt  $Hg^{2+}$  (faint red emission). At 20 ppt, the fluorescence due to FITC is visible, and at 10 ppt, luminescence of Au@BSA is still observable. (ii) XPS spectra of the Au 4f region for the Au@BSA/N6 fiber mat before (down) and after (top)  $Hg^{2+}$  exposure. It shows that gold oxidizes due to the addition of the  $Hg^{2+}$  ion solution which may be responsible for the quenching of red luminescence of the Au@BSA cluster.

experiments conducted on glass substrates, we know that 2.5  $\mu$ L of solution covers an area of  $8.34 \times 10^{-6}$  m<sup>2</sup> (details of calculation are given in Supporting Information, Section S4). The surface area of a 15  $\mu$ m long fiber (typical length examined in an image) of 200 nm diameter is 9.41  $\times 10^{-12}$  m<sup>2</sup>. The number of ions present in this area is ~80 which amounts to extremely low levels of detection. The level of sensitivity observed is extremely unusual. At this limiting concentration, the quenching is not complete, and therefore, the underlying green fluorescence does not appear distinctly, as mentioned earlier.

The response of a single fiber to the analyte is fast. As shown in the video (Supporting Information), the luminescence disappears as the  $Hg^{2+}$  solution wets the fiber from the top to the bottom. Response of the fiber is uniform as shown in Figure S5a, Supporting Information. Luminescence of one-half of Au@BSA/N6 fibers disappears as  $Hg^{2+}$  is exposed to the fibers (Figure S5b, Supporting Information). This is because the solution was exposed only to one-half of the fibers. The optical image of the fibers after  $Hg^{2+}$  addition shows that the fibers are intact (inset of Figure S5b, Supporting Information) although luminescence disappears. The data suggest that the fiber is uniformly exposed to the  $Hg^{2+}$  solution during wetting. We have observed the same changes for the Au@BSA/FITC/ N6 fiber also (Figure S6, Supporting Information).

To demonstrate a real life application of our methodology, we have tested surface water from a well. We have added 2.5  $\mu$ L of well water on a Au@BSA/N6 single fiber, and no quenching of luminescence was observed. We repeated the same experiment by adding 1 ppb of Hg<sup>2+</sup> ions on the fibers. We have seen quenching of red luminescence of Au@BSA. It implies that the red luminescence of the fiber is selective to Hg<sup>2+</sup> and not to other species in the natural water. The results are presented in Figure S7, Supporting Information.

To understand the reason behind the quenching of red luminescence on the addition of mercuric ion, we performed XPS analysis. XPS spectra in the Au 4f region of the Au@BSA/N6 fiber mat before and after addition of Hg<sup>2+</sup> are shown in Figure 4ii. In the case of the cluster, Au is nearly in the zero oxidation state (with a binding energy (BE) of 84.1 eV for Au  $4f_{7/2}$ ). The BE for Au is increased on treatment with mercuric

ion (at 85.3 eV). Data suggest that mercuric ion  $(Hg^{2+})$  oxidizes the Au core which is responsible for quenching of the red luminescence.  $Hg^{2+}$  uptake leads to  $Hg^{0}$  implying its reduction on the fiber surface. The peak of Hg  $4f_{7/2}$  is seen at 101.8 eV (Figure S8, Supporting Information).

#### CONCLUSION

In conclusion, we demonstrated a methodology for ultratrace  $Hg^{2+}$  detection using atomically precise clusters of gold coated on single nanofibers. The fibers present a highly sensitive, selective, and cost-effective way of sensing  $Hg^{2+}$  ions. The data show the possibility of sensing single ions at a single fiber level, when smaller lengths are examined. Electrospun nanofibers could be mass-produced at low-cost, allowing these novel sensing materials for field applications. Additional experiments are required to understand the reason for enhancement of emission of clusters on nanofibers.

#### ASSOCIATED CONTENT

#### **S** Supporting Information

Figures S1–S8 and a video. This material is available free of charge via the Internet at http://pubs.acs.org.

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#### Notes

The authors declare no competing financial interest.

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# Supporting information for the paper:

Approaching sensitivity of tens of ions using atomically precise cluster-nanofiber composites

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#### Video S1

Response of the Au@BSA/N6 fiber with time upon exposure to 1 ppm  $Hg^{2+}$  ion solution. (video file is uploaded separately).

#### Experimental section and analytical method

Dark-field microscopy: Dark-field imaging of the fibers was done using an Olympus BX-51 microscope having a 100 W quartz halogen light source mounted on a CytoViva microscope setup. Here, a broadband white light was shown on the fibers from an oblique angle via a dark field condenser. The scattered/emitted light from the fiber was collected by 10x objective and imaged by a true-color charge-coupled device (CCD) camera or by a spectrophotometer. Spectral analysis was performed with a hyperspectral image analysis software. For fluorescence imaging, a mercury lamp light source was used. Light after passing through a specific excitation (band pass) filter falls on the sample. The emitted light was passed through a 460-500 nm band pass filter and fluorescence (if any) emitted by the clusters was imaged.

# **S1. Supporting Information 1**

Excitation and emission spectra of Au@BSA cluster



**Figure S1.** Excitation (black line) and emission (red line) spectra of as-synthesized aqueous Au@BSA cluster.

# S2. Supporting Information 2

Size distribution of N6 nanofiber



Figure S2. SEM image of the fiber mat and the diameter distribution of fibers.

# **S3.** Supporting Information 3

Selective detection of mercuric ion of Au@BSA/N6 fiber mat



**Figure S3.** Effect of different metal ions on the luminescence intensity of Au@BSA/N6 fiber mats, measured in a fluorescence spectrometer with 488 nm excitation.

# **S4. Supporting Information 4**

### **Details of the calculation**

To get the area of water drop,  $2.5 \ \mu$ L of cluster solution was spotted on a glass slide and dried. The diameters of three different drops were measured using vernier caliper as shown in the following figure.





Radius = 0.163 cm

$$= 0.163 \times 10^{-2} \text{ m}$$

Area of water droplet =  $\pi r^2$ 

$$= 3.14 \times (0.163)^2 \times 10^{-4}$$

$$= 8.34 \times 10^{-6} \text{ m}^2$$

 $10 \text{ ppt} = 4.985 \times 10^{-11} \text{ molar Hg}^{2+}$ 

No. ions per liter =  $4.985 \times 10^{-11} \times 6.023 \times 10^{23}$ 

$$= 2.99 \times 10^{13}$$

Hence,

2.5 
$$\mu$$
L contains = 2.5 × 10<sup>-6</sup> × 2.99 × 10<sup>13</sup>

$$=7.49 \times 10^7 \text{ Hg}^{2+} \text{ ions}$$

Surface area of a fiber =  $2\pi rh$ 

 $= 2 \times 3.14 \times 100 \times 10^{-9} \times 15 \times 10^{-6}$ 

$$= 9.41 \times 10^{-12} \text{ m}^2$$

 $8.34\times 10^{\text{-6}}\,m^2$  (2.5  $\mu L$  water drop) contains  $7.49\times 10^7\,Hg^{2\text{+}}$  ions

Hence,

$$9.41 \times 10^{-12} \text{ m}^2$$
 (single fiber) contains =  $9.41 \times 10^{-12} \times 7.49 \times 10^7 / 8.34 \times 10^{-6}$ 

$$= 84 \text{ Hg}^{2+} \text{ ions}$$

Fiber radius = 100 nm Fiber length = 15 μm

# **S5. Supporting Information 5**

Uniform response of Au@BSA/N6 fibers by exposure of mercuric ions



**FigureS5.** [a] Fluorescence image of Au@BSA/N6 fibers (red emission). [b] Same sample after  $Hg^{2+}$ exposure.  $Hg^{2+}$  solution quenched half of the fibers as the amount of  $Hg^{2+}$  solution is less. Inset of b: Optical image of the Au@BSA/N6 nanofibers.

# **S6.** Supporting Information 6

Uniform response of Au@BSA/FITC/N6 fibers by exposure of mercuric ions



**Figure S6.** Fluorescence image of Au@BSA/FITC/N6 fiber after the addition of  $Hg^{2+}$  solution.

# **S7. Supporting Information 7**

# Analysis of real water sample



**Figure S7. A)** Luminescence image of a single fiber of Au@BSA/N6. B) Luminescence image of the same fiber after addition of well water on it. There is no change of red luminescence. C) Luminescence image of the same fiber after addition of 1 ppb Hg<sup>2+</sup> containing well water. The red luminescence is quenched.

# **S8.** Supporting Information 8





**Figure S8.** XPS spectrum of C1s and Hg 4f regions. Hg 4f data is given only after treatment with  $Hg^{2+}$  ions as the parent fibers do not have mercury.

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# Antimicrobial silver: An unprecedented anion effect

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Silver is an indispensable metal but its use has to be minimised for sustainable growth. Much of the silver lost during use is unrecoverable; an example being its use as an antimicrobial agent, a property known since ages. While developing methods to create an affordable drinking water purifier especially for the developing world, we discovered that 50 parts per billion (ppb) of  $Ag^+$  released continuously from silver nanoparticles confined in nanoscale cages is enough to cause antimicrobial activity in conditions of normal water. Here we show that the antibacterial and antiviral activities of  $Ag^+$  can be enhanced ~1,000 fold, selectively, in presence of carbonate ions whose concentration was maintained below the drinking water norms. The protective layers of the organisms were affected during the carbonate-assisted antimicrobial activity. It is estimated that ~1,300 tons of silver can be saved annually using this new way to enhance its antimicrobial activity.

ilver exhibits unique optical, catalytic, sensing and antimicrobial properties due to which its industrial demands, pegged at 16,000 to 24,000 tons per year<sup>1</sup>, are estimated to grow at 12.5% annually<sup>2</sup>. Silver, as an antimicrobial agent is expected to have an annual demand of 3125 tons/year for medicine and 2800 tons/ year in the fields of 'food, hygiene and water purification'<sup>3</sup>. Of the silver consumed in industrial processes, only 36% is recycled and the remaining is let out to the environment<sup>4</sup>. With increasing demands, production from the mines might not be sufficient. Silver is traditionally used as a disinfectant in delivering safe portable water to world<sup>5</sup> against severe gastroenteritis-causing pathogens, which are the second leading cause of death in children under the age of five. United Nations Children's Fund (UNICEF) claims that about 9% of global child deaths are due to diarrhoea. World Health Organization (WHO) estimates that gastroenteritis infection is responsible for 760,000 child deaths every year. Among the developing countries, India accounts for the highest number of diarrhoeal deaths (>24%), with 100,000 child deaths annually<sup>6</sup>. Biochemical properties of silver in the ionic form, against pathogens like bacteria, virus, fungi and protozoa have been widely studied and the reaction products silver may produce are less harmful compared to other disinfectants which produce disinfection by-products (DBPs). Complexity of contaminants, growth of population, emergence of resistive pathogens and their impact on the environment have led to the demand for advanced technologies for clean and safe drinking water<sup>7</sup>. Several trihalomethanes, haloacetic acids, haloacetonitriles, haloketones and other DBPs were found to cause colon, rectal and bladder cancers and adverse reproductive disorders, because of which the US EPA initiated a rule in the year 1992 to evaluate the need for additional controls for microbial pathogens and DBPs. The goal of this rule was to develop an approach that would reduce the level of exposure from disinfectants and DBPs, simultaneously controlling microbial pathogens<sup>8</sup>. Such technologies to reach the masses should not only be efficient but also affordable and thus silver ion-based commercial water purification units are evolving<sup>9</sup>. Therefore, it is appropriate to reduce the consumption of silver in view of the anticipated crisis in silver production and the current study points to a direction in this context.

In this work, we demonstrate the effectiveness of a synergetic combination of  $Ag^+$  with the anion,  $CO_3^{2-}$  which enhances the antimicrobial activity. The concentration of silver used in water purification is differently stated by authors, but generally up to a maximum of 1 ppm<sup>10</sup>. For a specific silver ion concentration, the antimicrobial performance is in the order, seawater  $\leq$  high organic matter-containing water  $\leq$  high divalent cation-containing water  $\leq$  synthetic water<sup>11</sup> due to the speciation of  $Ag^+$ . Active-silver, the concentration of effective ionic silver available for interaction with microbes in test water conditions varies with water quality. In this work, we initially focused on experiments to optimize the concentration of carbonate below the drinking water norms (secondary standard, United States Environmental Protection Agency, US EPA) while reducing the active-silver concentration, maintaining effective antimicrobial activity. The results were confirmed by further experiments and the mode of action was analysed. *Escherichia coli* and MS2 bacteriophage, the surrogates for water borne pathogens

(bacteria and viruses, respectively) were used for antimicrobial testing. The  $Ag^+$  ion treatment was aimed for cent percent removal of coliforms and coliphages<sup>12</sup>.

#### Results

Effect of speciation on the antimicrobial property of Ag<sup>+</sup>. Initially, in the natural drinking water, spiked with an input bacterial load of 10<sup>5</sup> colony forming units (CFU)/mL (typical natural drinking water sources contain a maximum of 10<sup>2</sup>-10<sup>3</sup> CFU/mL, in rural India), certain quantity of Ag<sup>+</sup> (in the range of 10 to 100 ppb) was added separately and the microbial survival was tested after a contact time of 1 h. A concentration of 50 ppb was found to be the active Ag<sup>+</sup> input concentration needed for maximum antibacterial activity (up to 99%), in natural drinking water. Even at an enhanced concentration of 1 ppm Ag<sup>+</sup>, the residual microbial concentration was not diminished. It is important to note that chloride ions invariably present in natural water (1-250 ppm) leads to speciation of Ag<sup>+</sup> and thus reduces the availability of silver ions. Silver chloride complexes [AgCl (aq), (AgCl)Ag<sup>+</sup>, (AgCl)Cl<sup>-</sup>,  $(AgCl)_n$  and their aggregates) due to their negative charge are nearly 300 times less in toxicity to bind with bacterial surface when compared to free silver ions<sup>13</sup>. With increasing concentrations of chloride ions (up to 200 ppm as in natural waters) at a fixed silver ion concentration, the speciation increases leading to a drop in the performance of bactericidal activity. At 200 ppm of Cl<sup>-</sup>, silver exists as 0.16% of [AgCl<sub>3</sub>]<sup>2-</sup>, 31% of [AgCl<sub>2</sub>]<sup>-</sup>, 63% of AgCl (aq), and only 5.8% of input silver remains as Ag<sup>+</sup> (Supplementary Fig. S1). With Ag<sup>+</sup> alone, no significant antiviral activity was observed for MS2 bacteriophage in tap water. Silver in the ionic form continue to be available during the period of antimicrobial activity in the test water conditions (Supplementary Fig. S2).

Synergy of anions with silver's antimicrobial property. The residual microbial concentration (to the extent of 1% or 10<sup>3</sup> CFU/ mL of E. coli and essentially all of the input viral load) still present is a challenge for reliable water purification. To address this economically and sustainably, the above method was improved upon. To 50 ppb Ag<sup>+</sup>, various anions, namely NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, SiO<sub>4</sub><sup>2-</sup>, PO<sub>4</sub><sup>3</sup>, I<sup>-</sup>, Br<sup>-</sup> and CO<sub>3</sub><sup>2-</sup> (or species derived from them, at prevalent conditions), as many of them can also be present in natural waters, were added at varying amounts to achieve a cent percent potential system. Among these, SiO42- and CO32- were promising compared to other anions, and the latter was more significant in this property. Therefore, the experiments were designed to reduce the Ag<sup>+</sup> concentration to a minimum at acceptable CO<sub>3</sub><sup>2-</sup> levels, sufficient to kill bacterial and viral cells in the natural drinking water. It is important to note that antimicrobial activity due to carbonate alone was insignificant even at 100 mM (10,000 ppm)  $Na_2CO_3$ . However, we found that in presence of  $CO_3^{2-}$ , 50% lower concentration of silver i.e., 25 ppb Ag<sup>+</sup>, was enough to obtain a complete (100,000 times) reduction in the input bacterial load (Supplementary Fig. S3). Therefore, while 50 ppb Ag<sup>+</sup> showed a 100 times reduction (from 10<sup>5</sup> CFU/mL to 10<sup>3</sup> CFU/mL), a combination of 25 ppb  $Ag^+$  + 20 ppm  $CO_3^{2-}$  showed 100,000 times reduction (from 105 CFU/mL to 100 CFU/mL) resulting in 1,000 times enhanced antimicrobial effect.

In order to explain the carbonate's promotional activity, a combined Ag<sup>+</sup> (25  $\pm$  10 ppb) and CO<sub>3</sub><sup>2-</sup> (20  $\pm$  5 ppm) release device was prepared, using silver nanoparticle (AgNP)-loaded biopolymer composite<sup>14</sup> and a sodium carbonate pellet, respectively. The output water from the device was spiked with *E. coli* and MS2 bacteriophage at required concentrations following which the water was tested for microbial survival (see Methods). In presence of CO<sub>3</sub><sup>2-</sup> (~20 ppm) the combination was found to possess a significant antimicrobial activity at concentrations of Ag<sup>+</sup> as low as 20  $\pm$  10 ppb within a contact time of 15 min. Thus CO<sub>3</sub><sup>2-</sup> not only minimizes the concentration of silver required, but also increases the kinetics of the reaction (Supplementary Fig. S4). The combination was also tested for antibacterial activity against a gram positive organism, *Staphylococus aureus* at similar bacterial load used for *E. coli*. Pour plate experiments showed that the antibacterial property of the combination was similar for both gram positive and gram negative bacteria. In order to confirm the bactericidal activity of this combination, the water was given a standing time of 24 h and tested for bacterial viability. The overnight standing experiments showed that the combination acted as bactericidal agent and not as bacteriostatic (Supplementary Fig. S5). The combination was tested for cytotoxicity on human rhabdomyosarcoma RD cells and no such effect was seen.

Antiviral property of the combination. Considering the fact that the major water purification technique used for virus removal is the addition of chlorine which produces harmful DBPs, we propose that the  $Ag^{+} + CO_{3}^{2-}$  combination, in addition could also serve as an alternative to reduce pathogenic viruses by a safer method. Here, we tested the combination for antiviral property on MS2 phage. A sequential time interval study of 50 ppb Ag+ alone and a combination of 50 ppb Ag<sup>+</sup> and 20 ppm CO<sub>3</sub><sup>2-</sup> was performed (see Methods). We observed that while 50 ppb Ag<sup>+</sup> alone (even after 1-2 h contact) was unable to affect the phage, the combination of 50 ppb Ag<sup>+</sup> and 20 ppm CO<sub>3</sub><sup>2-</sup> was found to achieve effective antiviral property within a contact time of 15 min (Supplementary Fig. S6). Moreover, by this study, we proved that this combination can handle virus at concentrations likely in ground water (10<sup>3</sup> plaque forming units (PFU)/mL) but can also efficiently control comparatively higher concentrations ( $\sim 10^6$  PFU/mL) that can prevail in non-portable water (Supplementary Fig. S7).

Compromised cell membrane/viral capsid. A defective outer membrane/capsid is suspected as the reason for enhanced activity of the combination. In order to confirm this, we conducted detailed dark field fluorescence imaging of bacteria and transmission electron microscopy of the viruses. Fluorescent staining experiments using dark field microscopy can distinguish between intact and membrane-permeable cells using LIVE/DEAD Baclight<sup>TM</sup> bacterial viability kit. The two stains used were the membrane impermeant, propidium iodide (PI), causing red fluorescence of membrane permeabilized cells and SYTO 9, a nucleic acid binding green fluorescent dye that could enter into all the cells regardless of whether they were intact or permeabilized. Defective PI fluorescing bacteria were observed as red when they were treated with  $Ag^+$  + CO<sub>3</sub><sup>2-</sup> whereas untreated and Ag<sup>+</sup>-treated bacteria (partly) show SYTO 9 emission (green) (Fig. 1 i, ii). Membrane permeabilization is seen in all the bacteria upon treatment with  $Ag^{+} + CO_{3}^{2-}$  (Fig. 1 C, F). We note that this happens for both E. coli and S. aureus, showing the effectiveness of the composition for gram negative and gram positive bacteria. Similarly, uranyl acetate stain enters defective viral capsids. Therefore, distinct difference in contrast is observed for carbonate-treated and untreated viruses under transmission electron microscopy (TEM)15. Dense dark centred capsids (defective capsids) which had taken up the uranyl acetate stain were seen in the  $Ag^+ + CO_3^{2-}$  treated virus whereas untreated/ Ag<sup>+</sup> treated viruses were found to be rigid which did not show the stain (Fig. 1 iii). These experiments prove that there is a significant damage to the outer protective layer of the bacterial/viral membrane as a result of  $Ag^+ + CO_3^{2-}$  treatment.

**Uptake of silver by bacteria/virus.** Enhancement that carbonate creates on silver's antimicrobial performance was confirmed by studying the changes in  $Ag^+$  uptake by bacteria and viruses, using inductively coupled plasma mass spectrometry (ICP MS). In these experiments, the bacteria and viruses, after treatment with  $Ag^+$  and  $Ag^+ + CO_3^{2-}$  at various incubation times were separated carefully and the filtrates were analyzed accurately for their silver content.





Figure 1 | The fluorescence microscopy and HRTEM images demonstrating the defective outer membrane/capsid in microbes caused due to  $Ag^+ + CO_3^{2-}$  treatment. Line (a): Fluorescence microscopy images of *E. coli*: ([i] Input bacteria, [ii] 50 ppb Ag<sup>+</sup>-treated bacteria and [iii] 50 ppb Ag<sup>+</sup> + 20 ppm  $CO_3^{2-}$ -treated bacteria after staining with syto9 and propidium iodide. Line (b): Similar fluorescence microscopy images of *S. aureus*: [iv] Input bacteria, [v] 50 ppb Ag<sup>+</sup>-treated bacteria and [vi] 50 ppb Ag<sup>+</sup> + 20 ppm  $CO_3^{2-}$ -treated bacteria. Line (c) TEM images of MS2 bacteriophage: [vii] Input viruses, [viii] 50 ppb Ag<sup>+</sup>-treated viruses and [ix] 50 ppb Ag<sup>+</sup> + 20 ppm  $CO_3^{2-}$ -treated viruses after staining with 0.2% uranyl acetate. Images (vii) and (viii) appear the same as they are not damaged, while image (ix) appears dark as damage in the capsid allowed the stain to pass through it.

Appropriate blanks were conducted. The bacteria and viruses showed intake of Ag<sup>+</sup> up to 90% (±5%) in presence of CO<sub>3</sub><sup>2-</sup> within a contact time of 10 min, whereas in its absence, the intake was less than 50% (Fig. 2). ICP MS results showed that the rate of uptake is an order of magnitude larger with carbonate (k<sub>(Ag</sub>+) = 0.0001, k<sub>(Ag</sub>+<sub>+CO3)</sub> = 0.001 (both in min<sup>-1</sup>), assuming a first order process.

Effect of pH change on  $Ag^+$ . Considering that the increase in the pH (from 7.0 to 8.5) due to the addition of  $CO_3^{2-}$  might be the reason behind this enhancement in the antibacterial effect of  $Ag^+$ , we performed experiments in natural drinking water with an adjusted pH of 8.5 using NaOH. The treated output was examined for microbial viability. Results of several experiments concluded that the NaOH-induced pH change did not enhance the antimicrobial activity of  $Ag^+$ .

**Target of carbonate ions.** The enhanced effect due to carbonate is suspected to be due to the peripheral proteins of the membrane. These peripheral proteins are associated with the membrane lipids and other proteins via electrostatic forces or hydrophobic interactions. Thus, conditions like high salt concentrations or an alkaline environment disrupt the interactions leading to the detachment of peripheral proteins<sup>16</sup>. The distinctive property of carbonate in the removal of peripheral membrane proteins of low molecular weight has been reported in animal cells<sup>17</sup> and *E. coli* K-12 cells<sup>18</sup>. Therefore, in presence of  $CO_3^{2-}$ , a disturbed membrane, free of peripheral proteins is suspected, which allows the penetration/ cellular mobility of Ag<sup>+</sup>, by increasing the bioavailability of silver ions. Fig. 3 is a schematic representation of this likely mechanism. In

Fig. 3(i), bacteria are subjected to Ag<sup>+</sup> ions at a concentration  $\leq 100$  ppb. Ag<sup>+</sup> ions bind to negatively charged membrane proteins in a non-selective way and sufficient ions are unavailable to enter into cells to cause cell damage. In Fig. 3(ii), bacteria are subjected to Ag<sup>+</sup> at concentrations as low as 25 ppb along with 20 ppm CO<sub>3</sub><sup>2-</sup>. In presence of CO<sub>3</sub><sup>2-</sup>, several membrane-bound peripheral proteins are removed and thus Ag<sup>+</sup> binds directly to the cell surface. As a result, increased number of Ag<sup>+</sup> enter into the exposed cells and cause sufficient damage in them.

To verify this possibility, the treated bacteria were tested for the presence of low molecular weight membrane proteins/peptides using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF MS) (see Supplementary Note 1). Specific peaks at a set of m/z values (7,000-10,000) were observed in the control and Ag<sup>+</sup>-treated bacterial cells whereas such peaks were completely absent in the case of carbonate-treated cells (Supplementary Fig. S8). The peaks obtained in the range of m/z 7,000-10,000 correspond to several proteins in E. coli which include both mature and immature proteins and peptides that are released from membrane bound proteins<sup>19</sup>. It is clear that carbonate, by acting upon membrane proteins/peptides, removes them. Removal of these membrane proteins increases the active-silver (as they offer a barrier for penetration by binding it) that leads to the total bioavailability of silver, in accordance with the uptake studies presented above. Separation of specific proteins responsible for the enhanced activity due to carbonate was difficult due to their reduced concentrations.

#### Discussion

In conclusion, the proposed combination of  $Ag^+ + CO_3^{2-}$  yielded more than 100,000 times reduction in the case of bacteria and 1,000





Figure 2 | ICP MS measurement of silver intake by bacteria and viruses. Concentration of silver in bacteria (a) and viruses (b) upon various treatments. Input concentration of silver is 50 ppb in the input water. Upon incubation with bacteria and viruses, Ag<sup>+</sup> concentration in the solution decreases while that in the organisms increase. These are plotted with time of incubation, measured by careful filtration through 100 kDa ultrafiltration membranes. After incubation with Ag<sup>+</sup>; filtered water (i) and bacteria/viruses (ii). After incubation with Ag<sup>+</sup> + CO<sub>3</sub><sup>2-</sup>; filtered water (iii) and bacteria/viruses (iv). Blank measurements without bacteria and viruses; filtered water (v); concentration on the membrane (vi). Measurements show that silver ion uptake is nearly quantitative; some loss of Ag<sup>+</sup> on the membrane as shown by trace (vi) is unavoidable as all membranes pick a small amount of silver. Sum of (i) and (ii) at 225 min does not give exactly 50 ppb due to this reason.

times reduction in the case of virus at concentrations as low as 25 ppb Ag<sup>+</sup> (Supplementary Fig. S9). In the absence of  $CO_3^{2^-}$ , even double the concentration of silver (50 ppb) resulted in only 100 times reduction in the case of bacteria and negligible reduction in the case of virus. The mechanism behind the enhancement was found to be the disturbance caused to the cell by carbonate which targets the peripheral membrane proteins. Thus  $CO_3^{2^-}$  exposed cells were disturbed and a lower concentration of Ag<sup>+</sup> was sufficient to cause complete cell damage. By reducing the Ag<sup>+</sup> concentration for antimicrobial activity, the unrecoverable release of metal ions into the environment is halved<sup>20</sup>. This work leads to a new paradigm in the field of affordable water purification by reducing the cost of antimicrobial treatment, particularly in the developing world, without disinfection by-products.

#### Methods

Testing protocol for antibacterial and antiviral efficacy. Natural drinking water condition was used for all the testing (Supplementary Table S1). Flasks containing 100 mL of water were spiked separately with 50 ppb Ag<sup>+</sup>, 50 ppb Ag<sup>+</sup> + 20 ppm  $CO_3^{2^-}$ , 20 ppm  $CO_3^{2^-}$ , respectively and a bacterial load of  $\sim 1 \times 10^5$  CFU/mL of *Escherichia coli* (ATCC 10536) was introduced into it. In the case of antiviral testing,  $\sim 1 \times 10^3$  F-specific bacteriophage MS2 (ATCC 15597-B1) was used. Thereafter, the water was shaken gently and left for a contact time of 1 h and subsequently the viable microbial count was measured by conventional pour plate technique (for bacteria) and double layer plaque assay (for virus using *E. coli host C-3000* (ATCC 15597). Viable microbial counts were evaluated after an incubation period of 20–24 h at 37°C. In the case of gram positive organism, *Staphylococcus aureus* (ATCC 9144) was used at the same concentration. Corresponding control and blank experiments were maintained for each trial.

**ICP MS Analysis.** To flasks containing 100 mL of water, 50 ppb Ag<sup>+</sup>, 50 ppb Ag<sup>+</sup> + 20 ppm  $\text{CO}_3^{2^-}$  and 20 ppm  $\text{CO}_3^{2^-}$  were spiked and bacteria/virus were added. 10 mL sample was collected at varying contact times and the cells were separated using polyvinylidene difluoride (PVDF) ultrafiltration membrane with molecular-weight cut-off of 100 kDa. The cells retained by the ultrafiltration membrane were suspended in 1 mL of millipore water and digested using HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub>. Concentration of Ag<sup>+</sup> present in both cells/viruses and the filtrate were measured using Perkin Elmer NexION 300X ICP MS. Careful standardization was performed.

Dark field Fluorescent Microscopic Analysis. Fluorescent microscopy imaging was performed using a Cytoviva microscopy system. For sample preparation, LIVE/ DEAD Baclight<sup>TM</sup> bacterial viability kit (Molecular Probes, Eugene, OR)<sup>13</sup> was used. At each time point, 1 mL of the sample (50 ppb Ag<sup>+</sup>, 50 ppb Ag<sup>+</sup> + 20 ppm CO<sub>3</sub><sup>2-</sup> treated and control bacteria) was mixed with 2 µL of PI-SYTO 9 mix (1:1) and incubated in dark for 15 min. 0.5–1 µL sample was spotted on a 1 mm thick ultrasonically cleaned glass slide (SCHOTT) and it was covered with a 0.145 mm thick cleaned glass coverslip (SCHOTT). Imaging was performed using 100X oil (Cargille) immersion objective.



Figure 3 | Schematic representation of suspected mechanism behind the unprecedented enhancement of antimicrobial property of silver in presence of carbonate. (a) Healthy cells treated up to 100 ppb  $Ag^+$  showed inadequate cell damage. (b) Healthy cells after exposed to 20 ppm  $CO_3^{2-}$  were damaged completely with 25 ppb  $Ag^+$ .



**Transmission Electron Microscopy Analysis.** Transmission electron microscopy (TEM) was carried out using a JEOL 3010 instrument with an ultra-high resolution (UHR) polepiece. TEM specimens were prepared by dropping one or two drops of the sample onto a paraffin tape and the carbon-coated copper grid was placed over it for 5 sec. After removing the excess sample, the grid was placed on a drop of 2% solution uranyl acetate in water for 5 sec and excess stain was blotted away. The grid was dried under ambient conditions<sup>15</sup>. Measurements were carried out at 100 kV.

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#### Author contributions

T.P., J.R.S., A.C., M.U.S., S.A. and A designed the experiments, J.R.S., A.C., M.U.S., S.A. and A performed the experiments, T.P., J.R.S., A.C., M.U.S., S.A. and A analysed the data, wrote the paper.

#### Additional information

Supplementary information accompanies this paper at http://www.nature.com/ scientificreports

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# **Supplementary Information for**

# Antimicrobial silver: An unprecedented anion effect

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**Speciation diagram of silver ion in pure water:** The speciation diagram prepared by varying  $[Cl^-]$  and keeping  $[Ag_{total}] = 50$  ppb, pH = 7, temperature = 25°C. The graph shows the number of complexes formed due to speciation of 50 ppb Ag<sup>+</sup> at different chloride ion concentration. This speciation graph infers that higher the Cl<sup>-</sup> concentration, lower is the Ag<sup>+</sup> concentration. The speciation diagram is prepared using simulations run on Visual MINTEQ software version 3.1 (freeware, available at, http://vminteq.lwr.kth.se).



**Stability of silver ions in the test water:** Concentration of silver ions in ppb available in solution as a function of time. Experiments have been done to assess the concentration after centrifugation to ensure that the estimation is for the species in solution.



Carbonate-supported antimicrobial activity of silver [A] Bacteria: *Escherichia coli* (ATCC 10536) [B] Virus: F-specific bacteriophage MS2 (ATCC 15597-B1) on *E. coli* host C-3000 (ATCC 15597): (A) Graph represents the optimization study of silver concentration on bacteria in which  $Ag^+$  at 50 ppb reduced the input concentration ( $10^5$  CFU/mL) by 2 log whereas in the presence of 20 ppm of  $CO_3^{2-}$ ,  $Ag^+$  at 20 ppb reduced the viable bacterial count to zero. (B) Graph represents the optimization study of silver concentration on virus in which  $Ag^+$  at 50 ppb did not reduce the input concentration ( $10^3$  PFU/mL), whereas in the presence of 20 ppm or less of  $CO_3^{2-}$ ,  $Ag^+$  reduced the viable viral count to zero.



**Rate of antibacterial efficiency of Ag**<sup>+</sup> **in the absence and presence of CO**<sub>3</sub><sup>2-</sup>: Enhancement in the rate of antimicrobial property obtained by Ag<sup>+</sup> + CO<sub>3</sub><sup>2-</sup>. In experimental water, Ag<sup>+</sup> showed 100 times reduction, CO<sub>3</sub><sup>2-</sup> individually showed no significant antimicrobial property, whereas their combination was 100% effective. This reduction was obtained within a contact time of 15 minutes in the case of Ag<sup>+</sup> + CO<sub>3</sub><sup>2-</sup>, but was not observed by Ag<sup>+</sup> treatment even after a longer contact time.



Comparison of the antibacterial activity of  $Ag^+$  and  $Ag^+$ +  $CO_3^{2-}$  against gram positive bacteria, *S. aureus* (ATCC 9144): Graph represents the comparison between the antimicrobial effects obtained by  $Ag^+$  and  $CO_3^{2-}$  individually and in combination. While  $CO_3^{2-}$  individually showed no antimicrobial activity against gram positive *S. aureus*,  $Ag^+$  showed a 3 log reduction and the combination showed a 5 log reduction of the input bacteria (10<sup>5</sup> CFU/mL) after a contact time of 1 h. On 24 h standing time, the results show that this activity was bactericidal and was not bacteriostatic.



Rate of virus killing efficiency for  $Ag^+$  in the absence and presence of  $CO_3^{2-}$ : Graph represents the enhancement in the rate of antiviral property obtained by  $Ag^+ + CO_3^{2-}$ . Neither  $Ag^+$  nor  $CO_3^{2-}$  individually showed a significant antiviral property for MS2 bacteriophage while their combination was 100% effective. This reduction was obtained within a contact time of 15 minutes in the case of  $Ag^++CO_3^{2-}$ , but was not observed by  $Ag^+$  treatment even after a longer contact time.



Efficiency of  $Ag^+$ +  $CO_3^{2-}$  on higher concentration of viruses: Graph represents the antiviral property obtained by  $Ag^+$  +  $CO_3^{2-}$  against higher viral load: Input concentrations of (1) 10<sup>6</sup>, (2) 10<sup>5</sup>, (3) 10<sup>4</sup> and (4) 10<sup>3</sup>. The output virus concentration after treatment with  $[Ag^+] = 50$  ppb and  $[CO_3^{2-}] = 20$  ppm. Studies were conducted in synthetic challenge water for a contact time of 1 h.



**Supplementary Figure S8** 

Mass spectra of the peripheral membrane proteins/peptides using MALDI TOF MS: Mass spectra of the peripheral membrane proteins/peptides using MALDI TOF MS (a) Control – The bacteria without any treatment, (b) 50 ppb Ag<sup>+</sup> treatment, (c) 100 ppm Ag<sup>+</sup> treatment, (d) Bacteria after  $CO_3^{2^-}$  treatment. The  $CO_3^{2^-}$  treatment should have removed the peripheral proteins/peptides on the membrane which had eluted away with the supernatant and thus showed no peak when the peripheral protein-free cells, subjected to sonication were measured under MS. Inset shows MALDI TOF spectrum of region m/z 6,000 to 12,000.



A quantitative expression of enhanced antimicrobial activity observed in the presence and absence of  $CO_3^{2-}$ : A quantitative expression of the enhanced antimicrobial activity observed in the presence and absence of  $CO_3^{2-}$ . A 5 log reduction in the case of bacteria and a 3 log reduction in the case of virus were observed on 25 ppb Ag<sup>+</sup> + 20 ppm  $CO_3^{2-}$  treatment. Bacterial counts in most of the measurements were zero for 25 ppb Ag<sup>+</sup> + 20 ppm  $CO_3^{2-}$  treatment, but in a few cases counts of 1 or 2 were also seen in 1 h treatment which went to zero in 24 h treatment.
#### Supplementary Table S1

#### Physicochemical characteristics of influent natural water:

(Note: All parameters are expressed in mg  $L^{-1}$ , except for pH and conductivity)

Parameters	Value
Total coliforms (CFU/mL)	$1-2 \ge 10^3$
р Н @25°С	7.8
Conductivity (µS/cm)	640.000
Fluoride	0.573
Chloride	86.340
Nitrate	1.837
Sulphate	32.410
Silicate	15.870
Lithium	ND
Sodium	53.740
Ammonium	ND
Potassium	2.330
Magnesium	14.340
Calcium	28.720

ND-not detected

#### **Supplementary Note 1**

#### **MALDI MS – Sample Preparation:**

The peripheral membrane proteins that are suspected to be affected by the carbonate treatment were separated from the cells and analyzed under mass spectrometry (MALDI TOF MS). Here, the cells were initially treated with 50 ppb  $Ag^+$ , 20 ppm  $CO_3^{2-}$  and 100 ppm  $Ag^+$ . After treatment, the cells were separated from the solution and were subjected to sonication for the removal of peripheral proteins/peptides. In the case of control and  $Ag^+$  treatment, the peripheral proteins/peptides were not affected during the reaction time. Thus, these cells on sonication, released low molecular weight proteins into the solution and were detected by MALDI MS. Whereas  $CO_3^{2-}$  treatment removed the proteins and the cells when separated and processed did not contain these proteins to be detected by MALDI MS.

For MALDI TOF MS analysis, an Applied Biosystems Voyager De Pro instrument was used with sinapic acid as the matrix. A pulsed nitrogen laser of 337 nm was used for ionizing the sample. Spectra were collected in the negative mode and an average of 250 shots were used for each spectrum. The matrix was prepared by dissolving 10 mg of sinapic acid in a 1:3 mixture of acetonitrile: 0.1% trifluoroacetic acid (overall volume of 1 mL). While preparing samples for analysis, 5  $\mu$ L of the supernatant solution, without dilution, was mixed thoroughly with 100  $\mu$ L of the matrix mixture. 2.5  $\mu$ L of the resulting mixture was used for spotting.





#### Optical rotation by plasmonic circular dichroism of isolated gold nanorod aggregates

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## Optical rotation by plasmonic circular dichroism of isolated gold nanorod aggregates

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We show that plasmonic chirality in single gold nanorod (GNR) aggregates leads to the rotation of polarization of the scattered light. 3D glasses in conjunction with linearly polarized dark field scattering microspectroscopy were used to study the chirality of single GNR aggregates. Using this hetero-polarizer setup, we not only detect but also quantify their chirality. A polar mapping strategy was used for providing direct evidence for the emergence of light of different polarization angles when chiral GNR aggregates were excited with circularly polarized light of different handedness. Further, we have developed a methodology to eliminate fluctuations in the scattering intensity by averaging and normalizing the data. This allows calculation of plasmonic circular dichroism scattering spectra with high accuracy. © 2014 AIP Publishing LLC. [http://dx.doi.org/10.1063/1.4902318]

Diverse combinations of chiral molecules and arrangements of nanoparticles exhibit plasmonic circular dichroism (PCD).<sup>1-6</sup> The origin of PCD may be different in each of these combinations. Chiral molecules which otherwise exhibit circular dichroism (CD) signals in the UV range, if inserted within the plasmonic hot spots, exhibit giant enhancement in the same.<sup>7</sup> This enhancement arises from the coulomb interaction of the molecular dipoles parallel to the axis of the dimers of nanoparticles.<sup>7</sup> But for nanocrystals such as Au-twisters or pyramid-like and tetrahedral crystals of gold, chirality is intrinsic and CD is induced by mixing of different plasmon harmonics.<sup>8</sup> In some cases, when nanoclusters of gold or silver, composed of a few atoms, are stabilized with chiral ligands, the resultant protected clusters exhibit chiroptical properties<sup>5,9</sup> due to the transfer of ligand chirality to the metal core either in its structure or in its electronic states as a result of the perturbation of the electronic field of the ligands.<sup>5</sup> It has been shown that a variety of plasmonic nanoparticle (PNP) assemblies formed on helical molecular templates exhibit plasmonic chirality.<sup>3,4,6,10,11</sup> Interestingly, assemblies of gold nanorods (GNRs) as well as dimers of GNRs also exhibit PCD.<sup>1,12</sup> Auguié et al. have shown by theoretical calculations that when inter-particle distance is comparable to the wavelength of incident light, the phase of electromagnetic field coupling two dipoles becomes distance dependent. This leads to retardation effect and gives rise to asymmetric CD line shape.<sup>1</sup> Ma *et al.* have shown the possibility of PCD measurements of such single particles using correlated transmission electron microscopy (TEM) and scattering spectroscopy.<sup>12</sup> Although such advanced measurements are necessary for testing the validity of concepts; simplification of methods is required for their routine use. For such simplification, new techniques for signal detection as well as understanding of novel phenomena associated with the system under investigation are equally important.

In this work, we show that there is optical rotation involved in the PCD of GNR aggregates and it can be detected using recently developed hetero-polarizer setup. These aggregates were formed as a result of interaction between GNRs of size  $\sim 30/10$  nm (length/diameter) and L-glutathione, a tripeptide (GSH). Details of the method of preparation are provided in the supplementary material.<sup>13</sup> TEM images of few such GNR aggregates are shown in



FIG. 1. A hetero-polarizer setup to sense and quantify PCD signals at single particle level, consisting of 3D glasses and linear polarizers on an upright DFSMS setup. First two images ((a) and (b)) at the bottom left show a single GNR as seen in DFSMS along with its polar map, respectively. Scale bar is 1  $\mu$ m. Next four images ((c)–(f)) are TEM images of single isolated GNR aggregates. Scale bar is 20 nm. GNRs aligned with off-axis tilt, even minor, are likely to be chiral.<sup>1,12</sup> The symbols and codes for polarizers will be used in the next figures.

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Fig. 1 (see below). Few more images are shown in the supplementary material.<sup>13</sup> In dark field scattering microspectroscopy (DFSMS) studies, GNR aggregation was confirmed by changes in their scattering spectrum while analyzing the PCD data. From the previous studies, it has been observed that single particle spectra of end-to-end or side-by-side arrangements of GNRs give rise to a single prominent plasmonic band (in contrast to two peaks in the ensemble spectra in solution) but other arrangements give rise to two plasmonic bands.<sup>14,15</sup> Hence, existence of two plasmonic bands confirms the presence of GNR aggregates.

To sense and quantify PCD signals at single particle level, a simple hetero-polarizer based technique was developed. This technique utilizes regular 3D glasses as circular polarizers for illumination of the samples in linearly polarized-DFSMS (PDFSMS). Details of the experimental setup are shown in Fig. 1. Please note that although 3D glasses themselves are analyzers of circularly polarized light (CPL) (quarter wave plate + linear polarizer), to provide uniform linearly polarized input to the quarter wave plate, LCP/RCP (left/right handed circular polarizers) were placed after Down-POL (downside polarizer, Fig. 1) in the imaging setup. Recent studies from our group have shown that when scattering from isolated GNRs was analyzed with a linear analyzer having a minute tilt, their polarization patterns can be mapped (referred as polar maps, Fig. 1 inset).<sup>16</sup> Such maps are formed due to refraction of light rays when they pass through an analyzer. This technique provides in situ mapping of the polarization patterns of nanostructures which selectively scatter light of specific polarization. Isolated rod shaped particles exhibit "figure 8" shaped polar map, while isotropic spherical particle exhibits a filled circle. For this purpose, scattered light from the sample was passed from linear analyzer (Top-POL, topside polarizer) whenever polar mapping was required. Since polar maps, being projections of maximum intensity values from a set of images captured at various analyzer angles, they span a larger area than the actual GNR image. Hence, size of the polar map depends on the extent of shift in GNR image which occurs due to refraction of scattered light through analyzer with minute angular tilt.<sup>16</sup> Decrease in the scattering intensity due to polarizers in the optical path was taken care of by increasing the exposure time of the camera. Note that the diffraction limited images of single GNRs are spherical in shape and are red in color due to the excitation of longitudinal plasmon.

Previous reports using ensemble CD spectroscopy have shown that when GNRs are treated with GSH, they exhibit chiral response.<sup>17</sup> Chirality of GNR aggregates can be attributed to the arrangements in which longitudinal axis of GNRs are crossed<sup>1,12</sup> as in TEM images (Figs. 1(c)-1(f)). In the case of single particle spectroscopic measurements, different arrangements of aggregates can settle on the glass substrate with different orientations. Hence, when such aggregates having different orientations were studied, some of them exhibit strong chirality whereas others do not. Also due to randomness in the orientation and arrangements of GNR aggregates, there can be variations in the PCD signals. Due to the same reason, results from single particle spectroscopic measurements cannot be compared with ensemble measurements. Such measurements performed on a chiral and non-chiral GNR aggregate (referred as CGNR and NCGNR, respectively) are discussed below.

Scattering spectra of CGNR and NCGNR confirm that these are indeed aggregates and not single GNRs (Fig. 2(a)).<sup>14,15</sup> Figure 2(b) shows the polar plots of CGNR and NCGNR. Polar plots are calculated by integrating the grayscale intensity over



FIG. 2. (a) Scattering spectra of GNR aggregates. Colors correspond to the encircled GNR aggregates shown in the image (d). Chiral GNR aggregate is encircled with green color and nonchiral GNR aggregate is encircled in red color. (b) Polar plots for two gold nanorod aggregates are shown in corresponding color. (c) Polar profile of GNR aggregates when orientation of Top-POL was kept constant and Down-POL was rotated. (d)-(h) Scale bar is 1 µm. Combinations of polarizers for each measurement ((d)-(h)) are shown below the images. Further details are provided in Fig. 1. (Multimedia view) [URL: http:// dx.doi.org/10.1063/1.4902318.1]

This article is copyrighted as indicated in the article. Reuse of AIP content is subject to the terms at: http://scitation.aip.org/termsconditions. Downloaded to IP 14.139.160.234 On: Thu. 20 Nov 2014 05:32:12 the image spot of the particle as reported previously.<sup>16</sup> Fig. 2(c)shows that when both Top-POL and Down-POL are parallel  $(0^{\circ}/180^{\circ})$ , relative scattering intensity of CGNR and NCGNR can be correlated with the one observed when only Down-POL was used (Fig. 2(b)). At other angles, changes in the scattering intensity follow the same trend, which are due to changes in the transmittance when Down-POL is rotated with respect to Top-POL. These data are used to quantify chirality of particles as described in the subsequent discussion. Images of CGNR and NCGNR nanoaggregates captured with unpolarized white light illumination are shown in Fig. 2(d). For unpolarized illumination, both these aggregates exhibit intense scattering signals in DFSMS images. When circular polarizers (LCP/RCP) were placed after Down-POL, both the aggregates exhibit intense scattering spots similar to the case of unpolarized illumination (Figs. 2(e) and 2(f)). This is because CPL also allows excitation of plasmon resonances along all the axes. Then to check whether polarization of scattered light has any dependence on the handedness of illumination, PDFSMS measurements were performed. In these measurements, samples were illuminated with circularly polarized light generated by LCP/RCP and subsequent scattering signal was detected through Top-POL. By comparing Figs. 2(g) and 2(h), we see that CGNR exhibits substantial change in the scattering intensity whereas NCGNR remains almost constant. This was attributed to the strong chirality of CGNR. Please also note that this can be observed only when Top-POL is in the optical path (compare Figs. 2(e) and 2(f), where the experiment is the same as Figs. 2(g) and 2(h), except that Top-POL is not in the optical path). Repeatability of these measurements is shown in Fig. 3(a). See multimedia view of Fig. 2(g) showing images of CGNR and NCGNR captured upon alternate RCP and LCP illumination along with few more chiral and non-chiral GNR aggregates. It implies that this behavior is indeed due to chirality of nanoaggregates and not due to rotation of loosely bound aggregates or other random fluctuations. From this data, we see that CGNR consistently goes through substantial change (% change) in the scattering intensity when illuminated alternatively with RCP and LCP light. But NCGNR scattering intensity remains almost constant upon illumination with LCP/RCP.

From Fig. 3(a), we see that scattering intensity of CGNR drops to  $\sim$ 50% when illumination changes from RCP to LCP. These results suggest that one can illuminate sample with LCP/RCP and follow the changes in the linear polarization of the scattered light ( $\Phi$ ) by monitoring the transmitted light intensity through Top-POL. This difference in transmission is interpolated to obtain angular change in polarization of scattered light (Fig. 3(b)). Note that Fig. 3(b) is an alternate representation of Fig. 2(c). The curves in Fig. 3(b) may be fitted with the following equation:

$$\% I = C + a\cos^4(\theta - \beta),\tag{1}$$

where,  $\theta$  is the angular position of Down-POL with respect to Top-POL, *C* is the constant baseline, *a* is a multiplier which helps in matching the amplitude of  $\cos^4(\theta)$  with actual signal, and  $\beta$  indicates shift in  $\theta$ . Fig. 3(b) marks the parameter  $\Phi$  for CGNR, for which it comes out as ~60° where scattering intensity changes from 100% to 50%. This parameter is a good measure of the PCD of GNR aggregates and takes



FIG. 3. (a) Percentage changes in the variation of the scattering intensity for CGNR and NCGNR plotted against the handedness of circularly polarized illumination. Odd numbers on X scale presents RCP illumination and even numbers present LCP illumination. (b) Polar profile of CGNR and NCGNR when Top-POL was kept constant and Down-POL was rotated.

values from 0–90 in units of degrees. Thus, chirality may be quantitated as

$$\Phi = \left| \cos^{-4} \left( \frac{\mathscr{H}_{LCP < 90} - C}{a} \right) - \cos^{-4} \left( \frac{\mathscr{H}_{RCP < 90} - C}{a} \right) \right|,\tag{2}$$

where,  $\% I_{LCP<90}$  and  $\% I_{RCP<90}$  are scattering intensities collected through Top-POL upon illuminating the sample with LCP and RCP light, respectively. Percentage values of  $I_{LCP<90}$  and  $I_{RCP<90}$  used in this formula are for  $\theta$  values less than 90°, each percentage maximum is determined amongst  $I_{LCP<90}$  and  $I_{RCP<90}$ . Maximum change in linear polarization occurs between 0° and 90°.

To provide direct evidence of optical rotation, we use the polar mapping technique reported before.<sup>16</sup> Figures 4(a)-4(c) show the results of mapping done over two GNR aggregates with unpolarized, LCP and RCP illumination, respectively. Corresponding PCD spectra are shown in Fig. 4(d). For aggregate "1," polar map is symmetric throughout the observation, but for aggregate "2" it exhibits complex asymmetric polar maps when illuminated with light of different polarizations. This can be attributed to excitation of multiple plasmon axes. Polar map of aggregate "2" with LCP illumination shows selection of specific polarization in scattered light (Fig. 4(b)) and this subsequently changes when illumination was changed to RCP. Polar plots determined by integration of the scattering intensity over the image area of individual GNR aggregates are given in the supplementary material<sup>13</sup> to further support the mapping data.

The results discussed above show that quantification of chirality can be done by PDFSMS. Although such quantification is possible by detecting optical rotation through Top-



FIG. 4. Polar maps of CGNR (green) and NCGNR (red) aggregates upon three different dark field illuminations: (a) Unpolarized, (b) LCP, and (c) RCP. Two aggregates are designated as "1" and "2" in (a). For nonchiral aggregate "1" map remains almost same whereas for chiral aggregate "2" map changes drastically when the type of illumination is changed. (d) Corresponding PCDS spectra are shown for GNR aggregates, "1" and "2" in corresponding colors.

POL, there can be minute differences between images when same chiral GNR aggregate is observed at RCP/LCP illumination without Top-POL (Figs. 2(e) and 2(f)). Hence, for the determination of single particle PCD scattering (PCDS) spectra, more sensitive measurements or noise removal technique is required. Supplementary material<sup>13</sup> describes this methodology. Briefly, it is based on simple normalization of the spectroscopic data which helps in the reduction of quantitative variations in the scattering intensity but conserves the qualitative changes due to chirality. Normalization leads to noise removal as PCDS involves wavelength dependent changes in the scattering intensity and not an overall increase in scattering intensity. PCDS spectra determined using such calculations are shown in Fig. 4(d).

In summary, we have shown that there is optical rotation involved in the plasmonic circular dichroism of single GNR aggregates. This was detected efficiently using a heteropolarizer-based setup. It is as simple composed of a combination of regular DFSMS along with 3D glasses. Observations have shown that the differential scattering property was greatly amplified when the detection was done through a linear analyzer. The major benefits of this methodology are that it is simple, easy to perform, and quick measurements can be done over a large area of the sample. Cases in which ensemble spectroscopy cannot resolve the complexity of various components contributing the CD spectra, this kind of single particle measurements will be useful. We believe that the phenomena of chirality dependent optical rotation will be of prime importance in the development of new sensing and characterization techniques in materials science and biology.

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## Simple and Efficient Separation of Atomically Precise Noble Metal Clusters

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#### **Supporting Information**

**ABSTRACT:** There is an urgent need for accessible purification and separation strategies of atomically precise metal clusters in order to promote the study of their fundamental properties. Although the separation of mixtures of atomically precise gold clusters  $Au_{25}L_{18}$ , where L are thiolates, has been demonstrated by advanced separation techniques, we present here the first separation of metal clusters by thin-layer chromatography (TLC), which is simple yet surprisingly efficient. This method was successfully applied to a binary mixture of  $Au_{25}L_{18}$  with different ligands, as well as to a binary mixture of different cluster cores,  $Au_{25}$  and  $Au_{144}$ , protected with the same ligand. Importantly, TLC even enabled the challenging separation of a multicomponent



mixture of mixed-monolayer-protected  $Au_{25}$  clusters with closely similar chemical ligand compositions. We anticipate that the realization of such simple yet efficient separation technique will progress the detailed investigation of cluster properties.

tomically precise clusters of noble metals protected with A tomically precise clusters of notice incluse precises monolayers are some of the most fascinating molecules of contemporary chemical science.<sup>1–3</sup> Most of the reports on such clusters are concerned with gold, though a few analogues of silver and copper have also appeared in the literature.<sup>4–10</sup> Molecules such as  $Au_{25}SR_{18}$ ,<sup>11–13</sup>  $Au_{38}SR_{24}$ ,<sup>14</sup> and  $Au_{144}SR_{60}$ <sup>15</sup> are some of the most stable species in this family of materials. Catalytic and biological applications of such materials are fast evolving.<sup>2,16–18</sup> Along with this development, we have begun to explore the complex chemistry of these systems.<sup>19,20</sup> The different chemically nonequivalent environments at their surfaces offer different possibilities for ligand exchange. The possibility of core rearrangement, reduction in size and chirality of the core contribute to the diversity of their chemistry.<sup>21-23</sup> Whereas some synthesis protocols may produce directly atomically precise metal clusters without the need for separation, other synthesis protocols always yield a mixture of slightly different atomically precise metal clusters and their separation often is a challenge.<sup>24–26</sup> Efficient methods to isolate the chemically varying species would enhance the growth of science in this area. In this article, we present a simple yet effective way of separating atomically precise clusters, which helps to expand the exploration of their diverse properties.

Although high-pressure liquid chromatography (HPLC),<sup>27,28</sup> polyacrylamide gel electrophoresis (PAGE),<sup>24,29</sup> and solvent extraction are used extensively in separating clusters,<sup>30,31</sup> the

simplest of chromatographic techniques, namely, thin-layer chromatography (TLC) has not been attempted for the separation of such clusters. In the following, we show that differently functionalized clusters of the same core, varying cores with the same chemical functionality and even mixedmonolayer-protected clusters of the same core having only slight structural differences are well separable by a simple TLC methodology. Even though TLC has been used in organic chemistry for a long time, the realization of its applicability to metal clusters adds a new tool in the toolbox of cluster science and further emphasizes the analogous nature of metal clusters and small organic molecules.

#### **EXPERIMENTAL SECTION**

**Chemicals and Materials.** Gold(III) chloride trihydrate ( $\geq$ 99,9%), butanethiol (99%), hexanethiol (99%), phenylethanethiol ( $\geq$ 99%), sodium borohydride (95%), tetraoctylammonium bromide (TOABr, 98%), and trans-2-[3-(4-*tert*butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB, > 98%) were purchased from Sigma-Aldrich. Tetrathiolated calix[4]arene (25,26,27,28-tetrakis(4-mercapto-*n*-butoxy)calix-

Received: August 22, 2014 Accepted: November 14, 2014 Published: November 14, 2014 [4]arene) was synthesized according to a reported method.<sup>32</sup> All the chemicals were used as received without further purification. All solvents (dichloromethane (DCM), *n*-hexane, tetrahydrofuran (THF) and methanol) were purchased from Sigma or Rankem and were of analytical grade. Silica gel 60 F<sub>254</sub> TLC plates were purchased from Merck. The water used in syntheses was Milli-Q grade with a resistivity of 18.2 M $\Omega$ ·cm.

Synthesis of Au<sub>25</sub>L<sub>18</sub> and Mixed Monolayer Au<sub>25</sub>Calix<sub>0-3</sub>BT<sub>6-18</sub> Clusters. Au<sub>25</sub>BT<sub>18</sub> (BT- butanethiolate, C4HoS-) and the calixarene-functionalized Au25 clusters were synthesized by a reported method.<sup>32</sup> Briefly,  $HAuCl_4 \cdot 3H_2O$  (80) mg) was dissolved in 15 mL of THF and 130 mg of TOABr was added to this vigorously stirred solution. The stirring was continued for 15 min after which the solution was orange-red in color. To this solution, 110  $\mu$ L of butanethiol in 500  $\mu$ L of THF (in case of  $Au_{25}BT_{18}$ ) or a mixture of 110  $\mu$ L of butanethiol and 4.4 mg of calixarene tetrathiol in 500  $\mu$ L of THF (in case of  $Au_{25}Calix_{0-3}BT_{6-18}$ ) was rapidly added under vigorous stirring (1200 rpm). The stirring was continued for 2 h during which the solution turned colorless. After that, 78 mg of NaBH<sub>4</sub> dissolved in 5 mL of ice-cold H<sub>2</sub>O was rapidly added to the reaction solution under vigorous stirring, and the stirring was continued for 5 h in the case of Au<sub>25</sub>BT<sub>18</sub> and 15 h in the case of  $Au_{25}Calix_{0-3}BT_{6-18}$ . The solvent was removed from the reaction mixture by rotary vacuum evaporation, and clusters were purified by centrifugal washing with methanol (4 times, 3000 RCF). In the case of Au<sub>25</sub>BT<sub>18</sub>, methanol/water mixture (3:1 v/v) was used in centrifugal washing. Subsequently, the product was dissolved to THF, and the white insoluble matter consisting most likely of Au(I)-thiolates was removed by centrifugation. In case of Au<sub>25</sub>Calix<sub>0-3</sub>BT<sub>6-18</sub>, the clusters in THF were further purified by size-exclusion chromatography (stationary phase Bio-Rad Bio-Beads S-X1 200-400 Mesh) to remove a small amount of larger clusters. Au<sub>25</sub>PET<sub>18</sub> (PETphenylethanethiolate, PhCH<sub>2</sub>CH<sub>2</sub>S-) and Au<sub>25</sub>HT<sub>18</sub> (HThexanethiolate, C<sub>6</sub>H<sub>13</sub>S-) were synthesized following the same procedure, and 136 and 144 µL thiol were added for the synthesis of Au<sub>25</sub>PET<sub>18</sub> and Au<sub>25</sub>HT<sub>18</sub>, respectively.

Synthesis of Au<sub>25</sub>PET<sub>18</sub> and Au<sub>144</sub>PET<sub>60</sub> Mixture. A crude mixture of Au<sub>25</sub>PET<sub>18</sub> and Au<sub>144</sub>PET<sub>60</sub> was synthesized in a single batch according to a reported method.  $^{30}$  HAuCl\_4  $\cdot 3H_2O$ (120 mg) was dissolved in 15 mL of methanol, and 193 mg of TOABr was added to this vigorously stirred solution. The stirring was continued for 15 min after which the solution was orange-red in color. To this solution, 216 µL of phenylethanethiol was added, and the mixture was stirred for 15 min. After that, 115 mg of NaBH<sub>4</sub> dissolved in 6 mL of ice-cold H<sub>2</sub>O was rapidly added to the reaction mixture. The stirring was continued for 5 h at room temperature, after which the precipitated clusters were collected by centrifugation and washed with methanol 5 times. The crude product containing Au<sub>25</sub>PET<sub>18</sub> and Au<sub>144</sub>PET<sub>60</sub> was dissolved in DCM and precipitate consisting of insoluble Au-thiolates was removed. DCM solution was rotary evaporated to dryness and spotted on the TLC plate for separation.

**TLC Separation.** The cluster samples  $(1 \ \mu L)$  were pipetted to TLC plates and dried in air. After drying, the plate was eluted with DCM/hexane mixture (the optimal solvent ratio varies with cluster system). After the separation, the bands were cut from the TLC plate, and the clusters from each band were individually extracted with DCM. The solids (pieces of TLC plate) were removed from these extracts by centrifugation. The

retention factors  $(R_f)$  for all separated clusters are given in the Supporting Information (Table S-1).

**Characterization.** Absorption spectra of clusters were recorded in UV-vis range with PerkinElmer Lambda 25 UV-vis absorption spectrophotometer. Clusters were dissolved to DCM and spectra recorded in quartz cells with 10 mm path length. Matrix-assisted laser desorption ionization (MALDI) mass spectra of clusters were collected using either of two different linear time-of-flight mass spectrometers: Voyager DE PRO (Applied Biosystems) or Autoflex II (Bruker Daltoniks). Both mass spectrometers were equipped with UV N<sub>2</sub> lasers (337 nm) and provided similar results. DCTB in DCM (12.5 mg/mL) was used as the matrix. The measurements were performed in positive ion mode. For each measurement, typically 500 scans were acquired.

#### RESULTS AND DISCUSSION

Separation of Binary Mixture of  $Au_{25}L_{18}$ , L = BT/HT/ **PET.** In Figure 1, we show the separation of  $Au_{25}HT_{18}$  (HThexanethiolate, C<sub>6</sub>H<sub>13</sub>S-) and Au<sub>25</sub>BT<sub>18</sub> (BT- butanethiolate,  $C_4H_9S_-$ ) from a mixture of two, even though the polarity difference of BT and HT is very small. A mixture of clusters was spotted on the TLC plate and then eluted using DCM/hexane mixture. For this system, the best separation occurred at a DCM/hexane ratio of 40:60 (unless otherwise noted, the solvent ratios are expressed as volume ratios throughout the manuscript). A photograph of the two separated bands is shown in Figure 1A. After optimization of the solvent mixture, preparative scale separation was performed by simultaneously eluting multiple spots of this mixture. The separated bands 1 and 2 were cut off from the plate and individually extracted using DCM. The UV-vis spectra of band 1 (red) and band 2 (blue) show characteristic absorption features originating from the  $Au_{25}$  core (Figure 1).

To verify the identity of these bands, we performed MALDI MS using DCTB as the matrix which is known to enhance intact ionization for this system at threshold laser powers.<sup>33</sup> MALDI MS data of bands 1 (red) and 2 (blue) are shown in



**Figure 1.** UV-vis spectra of the TLC separated materials. (A) Photograph of the TLC plate used for cluster separation. Bands 1 and 2 are due to two separated clusters. The base shows the location where the mixture was spotted. The level of liquid is marked with a dashed line. (B) MALDI MS data of TLC separated materials confirming that bands 1 (red trace) and 2 (blue trace) are pure  $Au_{25}HT_{18}$  and  $Au_{25}BT_{18}$ , respectively. The fragmented product,  $Au_{21}L_{14}$  is shown with an asterisk (\*) in each trace.



**Figure 2.** (A) UV–vis spectra of the two bands separated by TLC. Red trace matches well with the previously reported UV–vis spectrum of  $Au_{144}PET_{60}$  cluster,<sup>15</sup> and blue trace matches with the UV–vis spectrum of  $Au_{25}PET_{18}$  cluster.<sup>11</sup> The red trace has been vertically shifted for clarity. Inset: photograph of the TLC plate used for cluster separation (solvent front marked as a dashed line). (B) MALDI MS data of crude (black trace) and isolated clusters (blue and red trace) confirming that bands 1 and 2 consist of isolated  $Au_{25}PET_{18}$  and  $Au_{144}PET_{60}$ , respectively. The fragmented product,  $Au_{21}L_{14}$ , is shown with an asterisk (\*).

Figure 1B, thus confirming that bands 1 and 2 correspond to  $Au_{25}HT_{18}$  and  $Au_{25}BT_{18}$ , respectively. We have also separated BT and PET protected  $Au_{25}$  using the same methodology. Both the clusters were spotted on the same spot of the TLC plate and then eluted immediately using DCM/hexane mixture. For this system, the best separation occurs at a DCM/hexane ratio of 60:40. Details about this separation are given in Supporting Information (Figure S-1). Figure S-1A shows the photograph of two separated bands on the TLC plate. MALDI MS data (S-1B) confirms that bands 1 (black) and 2 (red) correspond to  $Au_{25}PET_{18}$ , respectively.

Analogously to TLC separation of small organic molecules, the choice of solvent polarity plays a key role in deciding the extent of separation of clusters. In 100% DCM, all the Au<sub>25</sub> clusters elute along with the solvent front, and thus, no separation occurred in either BT-HT or BT-PET system. In both systems, a slight decrease of solvent polarity reduces the movement of clusters on the TLC plate, resulting in a clear separation of the two bands. For the BT-HT system, we have decreased the polarity of the solvent mixture more than that used in the BT-PET system. In the former case, the movement of clusters on the TLC plate should be slow to get a better separation as the polarity difference between BT and HT is very small in comparison to BT-PET system.

It is worth noting that, based on the UV–vis spectra in Figure 1, the charge of the separated clusters is neutral. Au<sub>25</sub>L<sub>18</sub> clusters have two significantly stable oxidation states, namely, Au<sub>25</sub>L<sub>18</sub><sup>-</sup> and Au<sub>25</sub>L<sub>18</sub><sup>0</sup>, which can be converted from one to another by electrochemistry<sup>34</sup> and by exposure to oxidizing or reducing agents.<sup>35</sup> Fresh Au<sub>25</sub>L<sub>18</sub> synthesis products are typically negative charged. However, these clusters easily oxidize to Au<sub>25</sub>L<sub>18</sub><sup>0</sup> due to exposure to air and ambient light.<sup>36,37</sup> When separating freshly prepared Au<sub>25</sub>PET<sub>18</sub> by TLC, we observed a rapid oxidation during drying and elution of the clusters on the TLC plate (Figure S-2). If drying and elution times are decreased, thus leading to only partial oxidation, even separation of Au<sub>25</sub>L<sub>18</sub><sup>-</sup> and Au<sub>25</sub>L<sub>18</sub><sup>0</sup> can be realized with TLC, since Au<sub>25</sub>L<sub>18</sub><sup>-</sup> elutes slower than Au<sub>25</sub>L<sub>18</sub><sup>0</sup> likely due to the attached TOA<sup>+</sup> (Figure S-3).

**Separation of Different Cluster Nuclearities.** We have also separated a mixture of two different cluster cores,  $Au_{25}$  and  $Au_{144}$ , protected by the same ligand, PET. The mixture of

clusters was dissolved in a minimum amount of DCM and spotted on a TLC plate. The sample was eluted with a DCM/ hexane mixture (60:40), and two separate bands were observed (Figure 2A, inset). The UV-vis spectra of those two isolated bands were measured after extracting them in DCM (Figure 2A). Blue and red traces correspond to bands 1 and 2, respectively, and these traces match with those of Au<sub>25</sub>PET<sub>18</sub> and Au<sub>144</sub>PET<sub>60</sub>.<sup>11,15</sup> To confirm the purity of each band, we performed MALDI MS measurements of the crude and isolated bands using DCTB as the matrix. The mass spectrum of the crude product contains both Au<sub>25</sub>PET<sub>18</sub> and Au<sub>144</sub>PET<sub>60</sub> (Figure 2B). As ionization efficiency of  $Au_{25}$  is considerably higher than that of the bigger cluster Au144, the intensity of MALDI MS peak for the former is higher. Expanded spectrum shows the presence of Au144. Blue and red traces confirm that bands 1 and 2 contain pure Au<sub>25</sub> and Au<sub>144</sub>, respectively. Due to its reduced size, Au25 elutes faster on the TLC plate. As differently sized clusters can be separated by TLC, we foresee this method to be applicable in monitoring cluster synthesis (see further discussion below).

**Separation of Mixed-Monolayer-Protected Clusters.** To further explore the potential of TLC separation, we continued our study with mixed-monolayer-protected Au<sub>25</sub> clusters, in which the monolayer consisted of BT and tetrathiolate of 25,26,27,28-tetrakis (4-mercapto-*n*-butoxy)calix-[4]arene (Calix, Figure 3 inset). MALDI MS measurements confirmed that the clusters contained 0–3 Calix moieties (Figure 3), which is in agreement with our earlier electrospray ionization mass spectrometric study.<sup>32</sup> The MALDI MS data shows that the crude product is composed of several different clusters varying slightly in their monolayer composition (Au<sub>25</sub>Calix<sub>0–3</sub>BT<sub>6–18</sub>).

Notably, the tetrathiolated Calix ligands are bound to Au<sub>25</sub> surface predominantly in tetradentate or bidentate manner leading to the absence of odd numbers of BT ligands on the clusters. Peak positions and molecular compositions are discussed in Supporting Information (Tables S-2 and S-3). This cluster mixture was subjected to TLC with DCM/hexane mixture as the eluent. For this system, the optimal DCM/ hexane ratio was found to be 30:70. Even though the clusters elute more slowly in such low polarity media, we observed undesired smearing of bands with higher DCM content. In



**Figure 3.** MALDI MS data of  $Au_{25}Calix_{0-3}BT_{6-18}$  cluster mixture. Peak spacings of 773 m/z (1 Calix) and 178.4 m/z (2 BT) have been marked for clarity. The compositions of the labeled peaks (1–13) are interpreted in Table S-2. Insets show the structures of the ligands in the mixed-monolayer-protected  $Au_{25}$  clusters.

order to achieve a greater separation between the bands, the same TLC plate was eluted several times. Four distinct bands could be separated in this manner, in addition to the immobile base band (Figure 4A). To confirm the identity of these bands, they were extracted and analyzed by MALDI MS using DCTB as the matrix (Figure 4B).

From the MALDI MS data of bands 1–5, it is evident that the fastest eluting clusters are  $Au_{25}BT_{18}$  (band 1, blue), followed by  $Au_{25}Calix_1BT_{16}$  (band 2, green) and  $Au_{25}Calix_1BT_{14}$  (band 3, magenta). Surprisingly, even clusters having such minor differences in composition could be separated by TLC.  $Au_{25}Calix_1BT_{16}$  is less polar than  $Au_{25}Calix_1BT_{14}$  based on the difference in conformation and binding of Calix on the cluster surface, and therefore, it elutes faster (see further discussion below). Furthermore, band 4 is composed of clusters having exclusively two Calix moieties  $(Au_{25}Calix_2BT_{10-16})$ , whereas the majority of clusters in the immobile base band 5 have three Calix units attached. It is worthwhile to mention that no separation of  $Au_{25}Calix_{0-3}BT_{6-18}$  clusters could be achieved by size-exclusion chromatography.

Bands 4 and 5 from the TLC run still contain multiple mixed-monolayer compositions. These bands were extracted, combined, and subjected to another TLC run with a slightly higher polarity eluent (DCM/hexane 35:65). Three bands could be extracted from the second run, containing two bands composed of  $Au_{25}Calix_2BT_nclusters$  (band 1: n = 12-14, band 2: n = 10-12) and one band composed of various  $Au_{25}Calix_{2-4}BT_{6-12}$  clusters (Figure 5). Thus,



**Figure 5.** Further TLC separation of  $Au_{25}Calix_{0-3}BT_{6-18}$  clusters. Bands 4 and 5 from first TLC run (DCM/hexane 30:70) were combined and subjected to another run with DCM/hexane 35:65 (photograph on the left). Three bands could be extracted and subjected to MALDI MS analysis (right).

 $Au_{25}Calix_2BT_{10-16}$  clusters could be separated into two fractions (bands 1 and 2), which were not separable in the first TLC run because they were retained near the base band. Thus, it is possible to separate more products by running the TLC of third band again by tuning the polarity of the eluent. It is also noteworthy that separation can reveal new cluster compositions. Note that clusters having four Calix moieties were not observed in MALDI MS of the crude



Figure 4. (A) Photograph of the TLC plate used for cluster separation. The faint bands observed between bands 1 and 2 are caused by sticking of the clusters to the TLC plate upon drying between runs. (Note that the TLC plates were eluted repeatedly. See also Supporting Information, Figure S-4.) (B) MALDI MS data of  $Au_{25}Calix_{0-3}BT_{6-18}$  crude product before TLC (black trace) and bands 1–5 separated by TLC.

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 $Au_{25}Calix_{0-3}BT_{6-18}$  product because of suppression due to more abundant species (Figure 3).

On the basis of the TLC data, we can also derive qualitative information about the polarity of clusters. As seen from the clusters containing only one Calix unit, those having more BT ligands are less polar. Based on the results from the second TLC separation, this rule seems to hold also for clusters with two and three Calix moieties. Clusters having more BT ligands have more Calix ligands bound with two thiolates. On the other hand, clusters with a smaller amount of BT have more Calix ligands bound with four thiolates. These two binding modes explain the polarity differences of the clusters: Binding with four thiolates exposes the polar ether groups of Calix, whereas two thiolate binding causes pinching of the calixarene cone, thus shielding the polar oxygen atoms.

Purification of Clusters from Excess Thiol and Monitoring Cluster Synthesis. Excess thiol is a common impurity in thiolate-protected cluster samples despite rigorous washing of clusters after synthesis. In Figure 6, we show that

# Visible light UV light

Figure 6. Photographs of TLC separation of small amount of thiol present in methanol-washed  $Au_{25}PET_{18}$ . (A) and (B) are the photographs of same TLC plate under visible and UV light, respectively.

TLC method can be used to remove this small amount of excess thiol typically present after the synthesis. Initially, the sample was eluted with DCM/hexane mixture (70:30) to get a band. Then the sample was further eluted with 100% hexane three times. The TLC of a four-times-methanol-washed  $Au_{25}PET_{18}$  cluster revealed a fast moving colorless band due to excess phenylethanethiol, which is visible under UV light (Figure 6).

We have also shown that it is possible to monitor the progress of cluster synthesis by TLC. In Supporting Information (Figure S-5), we have presented the time-dependent TLC of synthesis of  $Au_{25}PET_{18}$ . In this experiment, aliquots of the raw cluster mixture were precipitated by addition of water and further washed with methanol to quickly remove most of the excess thiol. Thereafter, the clusters were spotted to a TLC plate and eluted with hexane to completely remove the remaining thiol. Subsequently, the clusters were eluted with DCM/hexane 60:40.

Initially, a mixture of clusters is formed as shown in Figure S-5A after 1 and 3 h of reaction. The fraction of larger clusters decreases as the reaction proceeds and after 8 h, and TLC of the sample shows a single band (Figure S-5A, right). It implies that the synthesis of  $Au_{25}PET_{18}$  was completed after 8 h. The bands after 1 h of reaction were individually extracted with DCM and further purified by another TLC run. The bands were analyzed by MALDI MS (Figure S-5B). These data show that the top band (band 1) consists of  $Au_{25}PET_{18}$  clusters, as expected. Slightly below the  $Au_{25}PET_{18}$  band, we observed a band mainly composed of  $Au_{38}PET_{24}$  and  $Au_{40}PET_{24}$  clusters. Moreover, bands 3 and 4 were found to contain multiple larger clusters in the size range of 12 000–15 000 m/z and 14 000–21 000 m/z, respectively. In addition, band 3 produced a strong MALDI MS signal of  $Au_{67}PET_{35}$  cluster.<sup>38</sup> This experiment further validates that clusters of different nuclearities can be separated by TLC. We foresee TLC as a highly applicable tool in monitoring cluster synthesis that advances the understanding of reaction pathways leading to specific clusters and provides a straightforward way for optimization of synthetic methods.

#### CONCLUSIONS

In summary, we have shown the surprisingly efficient TLC separation of atomically precise clusters of gold varying in ligand structure, core size, and mixed-monolayer composition. The data presented show that simple, inexpensive chromatographic tools can be used for the isolation of monolayer-protected clusters, although they are chemically complex. We anticipate that such a simple, broadly applicable methodology will enhance the detailed investigation and understanding of chemical and photophysical properties of well-defined cluster systems.

#### ASSOCIATED CONTENT

#### **S** Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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#### Notes

The authors declare no competing financial interest.

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#### Communication: Vacuum ultraviolet photoabsorption of interstellar icy thiols

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### Communication: Vacuum ultraviolet photoabsorption of interstellar icy thiols

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Following the recent identification of ethanethiol in the interstellar medium (ISM) we have carried out Vacuum UltraViolet (VUV) spectroscopy studies of ethanethiol ( $CH_3CH_2SH$ ) from 10 K until sublimation in an ultrahigh vacuum chamber simulating astrochemical conditions. These results are compared with those of methanethiol ( $CH_3SH$ ), the lower order thiol also reported to be present in the ISM. VUV spectra recorded at higher temperature reveal conformational changes in the ice and phase transitions whilst evidence for dimer production is also presented. © 2014 AIP Publishing LLC. [http://dx.doi.org/10.1063/1.4903840]

#### I. INTRODUCTION

The identification of molecular species within any environment is often through spectroscopy, molecules having a distinctive "spectral fingerprint." Spectroscopy is therefore used to identify molecules in planetary atmospheres and in the ISM.<sup>1</sup> The impending high resolution studies possible with next generation of telescopes (ALMA, JWST) have highlighted the need to expand the "spectroscopic database" both for larger molecular targets and into new regions of the electromagnetic spectrum (e.g., THz). However, an even greater challenge is to study the spectroscopy of molecules when they are in the condensed phase.<sup>1</sup> In the ISM, it is now recognised that many molecular species are synthesised on the surface of dust grains and remain within the icy mantles on these grains until desorbed or sputtered (e.g., during star or planetary formation processes).<sup>2-6</sup> Spitzer has revealed the presence of molecular ices in the dense clouds of the ISM<sup>7-9</sup> but it is the advent of ALMA and then JWST that is expected to reveal the chemical complexity of such ices. It remains a great challenge to study the spectroscopy of molecules in the condensed phase and to date most studies are constrained to the IR region through FTIR and Raman spectroscopy. There are fewer studies of VUV spectra of molecules in the condensed phase and until recently almost no THZ studies. VUV spectra are important since they reveal the electronic state spectroscopy of molecules in the condensed phase, excitation of which can subsequently lead to local chemistry with the (photo)dissociation products synthesizing other chemical species. In the condensed phase many electronic states are "quenched" (e.g., Rydberg states) whilst the adiabatic energies of the valence states are shifted in energy (in the case of water by upwards of 1 eV) thus the chemistry and physics of condensed phase molecular solids are therefore, distinctly different from their corresponding gas phase.<sup>10–13</sup> Accordingly, we have commenced an authoritative study of the VUV spectroscopy of molecular solids with particular focus on those species found in the ISM and on planetary surfaces.

Since the detection of methanol (CH<sub>3</sub>OH) in Sgr A and Sgr B2<sup>14</sup> and hydrogen sulphide (H<sub>2</sub>S) in Sgr B2, and other sources,<sup>15</sup> it was long expected that a molecule containing thiol group could the synthesized in the complex chemical regions of the ISM. In 1979, Linke *et al.*<sup>16</sup> reported the first detection of methanethiol in Sgr B2. However, the first report on the detection of the higher order thiol, ethanethiol, has only been made recently<sup>17</sup> in Orion KL, 30 years after the first observations of methanethiol, although the necessary precursors were detected earlier, ethylene in IRC +10216,<sup>18</sup> ethanol in Sgr B2<sup>19</sup> and hydrogen sulfide.

In this paper, we report the first condensed phase VUV spectra of two of the simplest thiols now identified in the ISM, ethanethiol, and methanethiol. By studying such spectra as a function of temperature we can provide some insight into the structure of these molecular solids and hence their likely reactivity within the ISM.

#### **II. EXPERIMENTAL METHODOLOGY**

The present experiments were carried out at the National Synchrotron Radiation Research Center (NSRRC) in Taiwan. The VUV light source was the synchrotron radiation dispersed with a 6-m cylindrical grating monochromator. Four gratings were equipped in the monochromator among which the grating 450 grooves/mm was used for our measurements to cover the spectral range (107–240 nm; 11.6– 5.2 eV). The minimum wavelength cutoff (107 nm) in these spectra was determined by the window material (Lithium fluoride; LiF) used as both the entrance and substrate window. The apparatus resolution was determined by the slit width (0.25  $\times$  0.25 mm) of the monochromator. Spectra were

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FIG. 1. VUV spectrum of methanethiol ice, formed at 10 K, compared with the gas phase spectrum.

recorded over the whole wavelength range in 0.5 nm steps. Samples were deposited on a precooled LiF window, kept at 10 K, that was enclosed in a vacuum chamber at pressures of the order of  $10^{-9}$  mbar. After passing through the sample the VUV light irradiated a glass window coated with sodium salicylate which converts transmitted VUV light into visible light which as detected by a photomultiplier tube operating in photon counting mode (placed outside the chamber). Further details on the experimental setup can be found in our earlier publication.<sup>20</sup>

Methanethiol and ethanethiol were purchased from Sigma Aldrich and Alfa Aesar with purity >99.5% and 97%, respectively. The vapour from samples of methanethiol and ethanethiol were allowed to form a uniform pure icy film of methanethiol and ethanethiol on the LiF substrate. Spectra were accumulated before and after deposition to obtain the incident (Io) and transmitted (It) intensities from which absorbance spectra of the ice films were derived using the Beer-Lambert law. The temperature dependence of the VUV photoabsorption spectra were recorded by annealing the sample to higher temperatures, which included, but were not limited to, 30 K, 50 K, 70 K, 90 K, 100 K, 120 K, 130 K and until sublimation.

#### **III. RESULTS AND DISCUSSION**

#### A. Methanethiol

The VUV spectrum of methanethiol recorded at 10 K was found to have strong absorption from 107 nm to 210 nm (11.6–5.9 eV) with two prominent, broad peaks at 8.6 eV and 6.2 eV and a third weak, broad peak at 7.2 eV (Figure 1). The gas phase photoabsorption spectrum of methanethiol in the VUV region contains three Rydberg series (4*s*, 4*p*, 4*d*, and 5*p*) 9.5–6.25 eV region. The vibrational structures of lowerenergy Rydberg members are mainly assigned in terms of v<sub>5</sub>, v<sub>6</sub>, v<sub>7</sub>, and v<sub>8</sub> of the excited state<sup>21</sup> which disappeared as expected in the ice phase.<sup>10,13</sup> The broad peaks observed in the ice phase could be attributed as due to the absorption from first two triplet excited states of the molecule energetically shifted, which are not allowed otherwise in gas phase.

A spectrum recorded at 30 K (Figure 2(a)) was found to be similar to the spectrum recorded at 10 K except for a small change was observed in the absorption region between 6.2 and 6.6 eV (Figure 2(b)). Spectra recorded by annealing the sample to 50 K were found to be similar to that observed at 30 K. Further annealing to 70 K revealed a slight decrease in the 6.2 eV peak was observed and this intensity drop was found to be even more significant in spectrum recorded at 90 K. At this temperature the absorption intensity increased beyond 8 eV. The spectrum recorded at 100 K was found to be similar to the 90 K spectra until 9 eV after which a small drop in intensity was observed (Figure 2(a)). The spectrum recorded at 120 K was found to be similar to those at lower temperatures but with significantly reduced absorption intensity. No absorption peaks in the spectrum recorded at 150 K.

It is widely known<sup>10,12</sup> that dimers can be formed whilst depositing the molecules at low temperatures (10 K). In the



FIG. 2. Temperature dependent VUV spectra of methanethiol at different temperature (a) 5.6–11.2 eV and (b) 5.6–8.0 eV. Methanethiol was deposited on LiF substrate at 10 K and then annealed to higher temperatures.

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FIG. 3. Temperature dependent VUV spectra of ethanethiol at different temperature (a) 5.6–11.2 eV and (b) 5.6–8.0 eV. Ethanethiol was deposited on LiF substrate at 10 K and then annealed to higher temperatures.

methanethiol spectrum, the absorption band at 6.2 eV could be due to the formation of methanethiol dimers.<sup>22</sup> Matrix isolation studies suggest that open chain dimers can be formed and there is a possibility of forming cyclic tetramers of methanethiol.<sup>23</sup> Upon annealing to higher temperatures, the changes observed in spectral shape, at lower energies, can be due to the conformational changes observed in the dimers of methanethiol due to the bonds that are made in the ice phase between the S-H...S atoms of methanethiol molecules, similar to the H-O...H interaction observed in formamide ices.<sup>13,24</sup> The spectra at 90 K and at 100 K suggest reorientation has occurred within the ice matrix of methanethiol and from this we could conclude that there is a phase change from amorphous to crystalline, ortho-rhombic structure.<sup>25</sup> Methanethiol molecules started subliming off from the substrate above 120 K and all the ice has sublimed by 150 K.

#### B. Ethanethiol

The VUV spectrum of ethanethiol recorded at 10 K was found to have a strong absorption cross section between 107 nm and 210 nm (11.6–5.9 eV) with two prominent, broad peaks at 8.6 eV and 6.4 eV and a third weak, broad peak around 7.2 eV (Figure 3(a)). To the best of our knowledge, we could not find gas phase photoabsorption spectrum of ethanethiol in the VUV region, so a comparison with the gas phase spectrum could not be presented here.

The 10 K spectrum is very similar to that of the methanethiol. Spectra recorded at 30 K and 50 K were found to be similar to the spectrum recorded at 10 K albeit with a significant increase in the absorption cross section at energies above 8 eV (Figure 3(b)). Spectra recorded by annealing the sample to 70 K were found to be similar to that observed at 50 K but at 90 K the peak at 8.6 eV intensified. At this temperature intensity increase beyond 8 eV to the higher energy side was also observed. The spectrum recorded at 110 K has similar peak positions but all decreased intensity in compar-

ison with the spectrum recorded at 90 K (Figure 3(a)). The spectrum recorded at 130 K showed further reductions in the absorption intensity but the peak at 8.6 eV became very prominent and peak at 6.4 eV was found to be very weak. Further strong reduction in the absorption intensities were noticed in those two spectra recorded at 150 K and then at 170 K, respectively.

From the VUV spectra obtained at different temperatures it is very hard to discuss whether the ethanethiol molecular ice formed from vapour deposition contains either trans or gauche conformers and/ or both forms, since the difference between trans and gauche forms is known to be only 0.3 kcal mol<sup>-1 26,27</sup> although the *gauche* conformer is the stablest form.<sup>28</sup> However, based on the Gibbs free energy difference of 1.92 kJ mol<sup>-1</sup> between *trans* and *gauche* forms, the relative abundances of trans and gauche forms were estimated to be 19% and 81%, respectively.29 However, conformerspecific ionization spectroscopy studies on ethanethiol revealed only gauche conformer of ethanethiol to be present in a molecular jet.<sup>30</sup> Infrared studies recorded under identical environmental conditions to those used in the present experiment are needed if we are to reveal the nature of ethanethiol molecules formed at 10 K.

Nevertheless, in our experiment the VUV spectra demonstrate the bonding between S-H...S atoms of ethanethiol molecules through the appearance of an absorption band at 6.4 eV, similar to that observed at 6.2 eV within methanethiol ices. At 6.4 eV significant changes were not observed in the peak by annealing to higher temperatures, this could be due to the cyclic tetramers of ethanethiol molecules that are reported being more stable in the ice matrix<sup>23</sup> than the open chain dimers in methanethiol. However, the intensity and spectral shape changes in the absorption at the higher energies suggest molecular reorientation to have taken place within the ethanethiol ices upon annealing from 10 K to 110 K. By comparing the 90 K and 110 K spectra it is evident that a phase change from amorphous to crystalline started within the ices at 110 K and is completed by further annealing and therefore the spectra at 130 K is that of a crystalline spectra of ethanethiol ice. At 150 K molecular sublimation was significant and by 170 K all the ethanethiol ice is lost from the surface.

#### **IV. CONCLUSION**

VUV spectra of methanethiol and ethanethiol were recorded over a range of temperatures, from which we can conclude that, upon deposition at lower temperatures, methanethiol and ethanethiol both form an amorphous ice that by annealing is transformed into a crystalline form at temperatures above 120 K. Absorption at longer wavelengths (lower energy region) suggests the formation of dimers which undergo rearrangement within the ice well before crystallization. In the case of ethanethiol ice, the dimers seem more stable than those in methanethiol. In order to obtain absolute VUV photoabsorption cross sections of methanethiol and ethanethiol in the condensed phase, our future laboratory simulation experiments will focus on deducing the methanethiol and ethanethiol ice density at conditions relevant to the ISM.

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#### Identification of effective substrates for the direct analysis of lipids from cell lines using desorption electrospray ionization mass spectrometry

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RATIONALE: Various disease conditions, particularly tumours, can be understood easily by studying changes in the lipid profile of cells. While lipid profiles of tissues have been recorded by desorption electrospray ionization mass spectrometric (DESI-MS) imaging, there is paucity in standardized protocols for sample preparation involving cell cultures to generate reliable results. In this study, we report a method for the direct analysis of lipids from cultured cells by incorporating them onto Whatman 42 filter paper as a substrate for reliable DESI-MS analysis.

METHODS: The WERI-RB1 cell line was spotted on commonly used substrates for DESI-MS analysis, such as glass slides, Teflon coated glass slides, thin layer chromatography (TLC) plates, and Whatman 42 filter paper. A comparison of mass spectrometric images with two different lipids was made to understand the behaviour of different surfaces when the same sample was spotted on them. Relative intensities of different lipid peaks in the WERI-RB1 cell line were compared and relative lipid abundances were also compared across two different human retinoblastoma cell lines; WERI-RB1 and Y79.

**RESULTS:** The study demonstrates that good lipid signals can be obtained by DESI-MS when the cells are spotted on Whatman 42 filter paper. Tandem mass spectrometry was performed to identify the lipids as glycerophosphocholines (PC). Better lipid images from assembly of cells were obtained with distinct boundary when they were spotted on Whatman 42 filter paper than other surfaces.

CONCLUSIONS: We demonstrate the use of a simple substrate for reliable DESI-MS analysis of cultured cells. This method has the potential to understand various interactions of cells with other external agents. The current method would help in the application of DESI-MS for biology in general and medical sciences in particular. Copyright © 2014 John Wiley & Sons, Ltd.

Desorption electrospray ionization (DESI) is an ambient mass spectrometric technique used for the analysis of surfaces.<sup>[1]</sup> In this method, a stream of charged solvent droplets is directed towards a sample surface. It forms a thin film over the surface which dissolves the analytes. Subsequent impact of droplets creates secondary droplets which contain dissolved analytes. These secondary droplets enter into the mass spectrometer through the atmospheric interface and ions are mass analyzed. Being a surface desorption ionization process, it enables imaging of analytes from a given surface by directing the spray to small segments of the surface, systematically.<sup>[2]</sup> Spatial resolution of such imaging depends primarily on the spot size of the primary droplets impacting the surface and the spot size can be controlled by various instrumental and experimental parameters of the source during the experiment.

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Being an ambient ionization process, the experiments are carried out in open atmosphere and minimal/no sample preparation is involved prior to the experiment.<sup>[3,4]</sup> As a consequence, the process is fast, simple and enables the analysis of biological samples in their almost native state without much distortion. For this reason, DESI-MS, after its introduction in late 2004, has found a wide range of applications in biology, medicine and related disciplines. They include study of molecules from plant (<sup>[5–9]</sup>) rapid identification of drug molecules from plants, <sup>[10]</sup> distribution of metabolites in leaves and petals,<sup>[11,12]</sup> direct detection of drugs from human skin<sup>[13]</sup> and animal tissues,<sup>[14]</sup> analysis of urine,<sup>[15]</sup> etc. However, the most important and promising use of DESI-MS in biology is the analysis of lipids directly from biological specimens.

Different lipids can be identified by DESI-MS in positive and negative ionization modes depending on their structures. For example, fatty acids (FA), glycerophosphoinositols (PI), glycerophosphoserines (PS), glycerophosphoethanolamines (PE), plasmenyl glycerophosphoethanolamines (plasmenyl-PE), sulfatides (ST), etc., give signals in negative ionization mode whereas glycerophosphocholines (PC), glycerophosphoethanolamines (PE), ceramides (Cer), etc., give

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signals in positive ionization mode. The ease of ionization of lipids in DESI-MS by choosing the appropriate ionization mode and solvent has been reflected in a large number of applications reported in the literature. DESI-MS has been used to identify and/or monitor changes in lipid profiles in tissue samples of mouse brain,<sup>[2,16]</sup> human brain with different degrees of tumour,<sup>[17]</sup> colorectal adenocarcinoma,<sup>[18]</sup> human prostate,<sup>[19]</sup> human lens,<sup>[20]</sup> tumorous and non-tumorous canine urinary bladder,<sup>[21]</sup> and human gastric cancer<sup>[22]</sup> as well as bacteria.<sup>[23,24]</sup> This list can be extended further. A somewhat old review presents a comprehensive discussion on lipid analysis by DESI MS.<sup>[25]</sup> Discussion about lipid analysis by other mass spectrometric methods including ambient ionization techniques can be found elsewhere.<sup>[26]</sup>

Cells contain different kinds of lipids and they play different biological roles therein. During a diseased condition, the lipid profile changes from the normal. Alteration in lipid profile has been reported in different diseases like human brain cancer,<sup>[27]</sup> colon cancer,<sup>[28,29]</sup> stomach cancer,<sup>[30]</sup> and other diseases.<sup>[31]</sup> Thus, studying the lipid profile can be used to understand the diseased condition and it can be used for diagnostic purposes. DESI-MS provides scope for rapid lipid analysis and has been used successfully to differentiate tumorous and non-tumorous tissues in many different human organs such as brain,<sup>[32]</sup> meninges,<sup>[33]</sup> stomach,<sup>[22]</sup> colon,<sup>[18]</sup> testis,<sup>[34]</sup> bladder,<sup>[35]</sup> kidney,<sup>[36]</sup> prostate,<sup>[19]</sup> etc., by lipid profiling. Although in all the above-mentioned examples direct tissues are used, study of cell lines for lipid analysis is also equally important due to its easy availability compared to tissues from subjects. Furthermore, during new drug discovery and evaluation of existing drugs for new diseases, cell lines are the primary objects where trial is carried out. From this perspective, it is important to study cell lines for rapid analysis of lipids.

In our present work, we examined different surfaces like glass slides, Teflon-coated glass slides, thin layer chromatography (TLC) plates, Whatman filter paper, etc., which are commonly used in DESI-MS, for analysis of retinoblastoma cell lines, WERI-RB1 and Y79. It is reported that in retinoblastoma, a childhood eye cancer, fatty acid synthase, a lipogenic enzyme, is over-expressed<sup>[37,38]</sup> and lipid profile changes from normal to tumor cells.<sup>[39]</sup> From this perspective, retinoblastoma cell lines are important and chosen for our work. Comparison of different surfaces is shown in terms of spectra and image quality. We show that when Whatman 42 filter paper was used as a substrate, good quality spectral data and lipid images could be obtained. Moreover, our method is completely independent of cell culture procedure; cells can be cultured, treated or made to undergo any other modifications elsewhere but still the experimental procedure will remain the same for all kinds of cells. Such reliable sample-handling procedures are highly beneficial in rapid and reliable screening of cell lines.

#### **EXPERIMENTAL**

#### Reagents

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Materials

Whatman 42 filter paper was purchased from GE Healthcare UK Limited, UK. The TLC plates (silica gel 60  $F_{254}$ ) were purchased from Merck KGaA, Germany. Teflon-coated glass slides were purchased from Prosolia, USA, and normal glass slides from Polar Industrial Corporation, India.

#### Cell culture

Retinoblastoma WERI-RB1 and Y79 cells were obtained from RIKEN BioResource Center, Ibaraki, Japan, and they were maintained in Roswell Park Memorial Institute 1640 (RPMI 1640), with 10% FBS and supplemented with antibiotic antimycotic solution (containing 100  $\mu$ g/mL penicillin, streptomycin and 250 ng/mL amphotericin B). Cells were seeded in a T25 flask and maintained in a 5% CO<sub>2</sub> environment at 37 °C till they reached 100% confluency. After that, the cells were removed from the flask by adding 1 mL of trypsin. To stop the action of trypsin, 1 mL of complete media (RPMI 1640) with 10% FBS was added, completely resuspended and collected in a centrifuge vial.

#### Spotting of cells on different surfaces

Cells collected in the centrifuge tube were washed twice with 1× phosphate-buffered saline (PBS) and centrifuged at 1500 rpm for 5 min. Then 50 µL of 1× PBS was added to the pellet to resuspend it completely. From this 10 µL was pipetted out and spotted on different surfaces (filter paper, glass slide, Teflon-coated glass slide and TLC plate) and left under the laboratory condition for drying. During the spotting, precautions were taken so that pipette tip did not touch the surface and hands did not shake. In the case of the filter paper, when the solution containing the cells contacted the surface, first it wetted the surface forming a circle. Then slowly it diffused outwards in a circular manner. Cells did not move in this process and stayed within the initially formed circle. The paper was kept drying for about 5 min. After that, the filter paper was cut into a square containing the cells and pasted on a glass slide. This glass slide was placed on the stage of the DESI mass spectrometer and a spectrum was collected. Figure 1 schematically illustrates this procedure. In the case of the TLC plate, similar events were observed. In the case of the glass slide and the Teflon-coated



**Figure 1.** Schematic of the sample preparation using cell cultures. Cell suspensions are dropped on Whatman 42 filter paper, dried, the paper is cut to the required size and mounted on a slide for DESI MS analysis. A spectrum is also shown.

Germany, and used as such without any purification. RPMI 1640, antibiotic antimycotic solution, fetal bovine serum (FBS) and trypsin were purchased from Gibco Life Technologies, USA.

HPLC grade methanol was purchased from Sigma-Aldrich,

glass slide, diffusion was absent and for the Teflon-coated glass slide initial wetting was also absent due to the hydrophobic nature of Teflon.

#### **DESI-MS** measurement

Mass spectrometric measurements were performed in a LTQ XL ion trap mass spectrometer (Thermo Scientific, San Jose, CA, USA). The DESI source used for ionization was from Prosolia. Data were acquired in the positive ion mode with a spray voltage of 5 kV. Methanol was used as spray solvent and it was sprayed at a flow rate of 5  $\mu$ L/min. Dry nitrogen was used as the nebulization gas and the pressure was kept at 150 psi. The inlet capillary of the mass spectrometer was kept very close to the surface, the spray tip was kept at 2 mm above the surface, the distance between the inlet capillary and the spray tip was 3mm and the spray angle was maintained at 60° to the surface. Tandem mass spectrometry was performed with collision-induced dissociation (CID). The following parameters were used for CID experiments; isolation width: 1 m/z, normalized collision energy: 20 (manufacturer's unit), activation Q: 0.250 (manufacturer's unit) and activation time: 30 ms. For imaging experiments by DESI-MS, a pixel size of 250  $\mu$ m × 250  $\mu$ m was chosen throughout.

#### **RESULTS AND DISCUSSION**

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#### Lipid analysis from the WERI-RB1 cell line by DESI-MS

The human retinoblastoma cell line, WERI-RB1, was tested for direct identification of lipids by DESI-MS after spotting them on Whatman 42 filter paper. Figure 2 shows the positive ion mass spectrum of the WERI-RB1 cells from the filter paper in the region of m/z 700–900. The mass spectrum was acquired in this particular region because it is rich in information about lipids. Several lipid peaks corresponding to the glycerophosphocholine (PC) class were observed in the spectrum. The inset of Fig. 2 shows the general structure of glycerophosphocholine lipids where R1 and R2 represent two similar or dissimilar hydrocarbon chains coming from saturated or unsaturated fatty acids. Identification of the lipids was made by tandem DESI-MS analysis and using the database as reference.<sup>[40]</sup> The highest abundance peak appeared at m/z 782.6 and it corresponds to [PC(34:1)+Na]<sup>+</sup>. Other prominent peaks observed in the spectrum were at *m*/*z* 754.6 [PC(32:1)+Na]<sup>+</sup>, 768.6 [PC(33:1)+Na]<sup>+</sup>, 780.6 [PC(34:2)+Na]<sup>+</sup>, 808.6 [PC(36:2)+Na]<sup>+</sup>, 810.6 [PC(36:1)+Na]<sup>+</sup>,

[PC(34:2)+Na]<sup>+</sup>, 808.6 [PC(36:2)+Na]<sup>+</sup>, 810.6 [PC(36:1)+Na]<sup>+</sup>, 832.7 [PC(38:4)+Na]<sup>+</sup>, and a few more. Beside these, several other low intensity peaks were also observed in the spectrum. DESI-MS/MS spectra of two representative lipids occurring at m/z 782.6 and 810.6 are shown in Supplementary Figs. S1 and S2, respectively (see Supporting Information).

#### Performance evaluation of different surfaces for DESI-MS analysis of cells in terms of signal intensity, noise and image quality

To evaluate the efficacy of different substrates on the DESI-MS signal intensity and image quality, four different, commonly used substrates were chosen. They were, Whatman 42 filter paper, glass slide, Teflon-coated glass slide and TLC plate. Among them, the surfaces of the glass slide and the Teflon coated glass slide are smooth and devoid of any porosity of macroscopic diameter whereas the filter paper and the TLC plate possess porous structures. When samples (WERI-RB1 cells) were spotted on these four substrates and kept under the laboratory condition to dry excess water, it took different times to dry each substrate. For example, due to the porous nature of the filter paper and the TLC plate, water diffused to peripheral regions of the initial drop spotted on the surface. This fact is illustrated in more details in the Experimental section with the help of Fig. 1. This accounted for the fast evaporation of water from the filter paper and the TLC plate and it took a maximum of 5 min to dry it completely. In the case of the glass slide, there was no evidence of diffusion and it took almost 15-20 min to dry the spot completely. For the Teflon-coated slide also, diffusion was restricted. Moreover, owing to the hydrophobic nature of Teflon, the drop spotted on this surface tended to shrink unlike a normal glass surface where it got spread. For this reason, it took more time (30-40 min) to dry the spot from the Teflon surface than from the glass surface, though both of them were smooth surfaces without any porosity to help fast evaporation. The spot size on the surface mostly depends on the volume of the drop coming out of the pipette tip and the way it is spotted. For example, if properly handled, and solution-containing cells were spotted without touching the pipette tip on the surface, it normally created a circular



Figure 2. DESI-MS spectrum of WERI-RB1 cells from Whatman 42 filter paper. Inset shows the generalized structure of glycerophosphocholine lipids.



spot containing cells with a diameter of 5–6 mm when  $10\,\mu$ L solutions were used. In case of porous surfaces (TLC plate and filter paper), the spot got spread and it formed a circle of ~10 mm with the initial inner circle of ~6 mm diameter. In this process, only the water medium was spread and not the cells; they remained in the inner circle. For the glass surface the cells did not get spread and a spot size of ~6 mm diameter was observed. However, when a Teflon surface was used, the spot size obtained was 2–2.5 mm diameter because of the obtuse contact angle of the drop on the hydrophobic Teflon surface. It is noteworthy to mention that if during the spotting the hand shook or the pipette tip with the protruding drop touched the surface, the spot shape could change from a regular, almost circular shape to an elongated shape.

After drying the spots on different substrates, they were subjected for DESI-MS imaging experiments. Figure 3 shows a comparison of the mass spectrometric images taken from different surfaces and two lipids are chosen here for detailed discussion. These two lipid ions are m/z 782.6 (highest in intensity) and 810.6 (relatively lower in intensity) (Fig. 2). The top row of Fig. 3 (Figs. 3(A), 3(B), 3(C), and 3(D)) shows the distribution of the m/z 782.6 ion over the spot from different surfaces. Spots appeared almost circular in shape as expected and were visible to the naked eye before the experiments. For the Teflon surface, during the long time of solvent evaporation, cells became more settled in one direction and this could happen if the surface was tilted minutely by a few degrees which may not be prominent to detect normally. However, the spot looked like a circle to the naked eye. Intensities obtained from the filter paper, Teflon surface and TLC plate were comparable and are evident from the images (Figs. 3(A), 3(C), and 3(D)). For the glass surface (Fig. 3(B)), however, the signal intensity was low. Almost uniform distribution is noticed from all the four surfaces (Fig. 3(A), 3(B), 3(C), and 3(D)). If we consider the signal-to-noise ratio, noise is much less for the filter paper (Fig. 3(A)) and glass slide (Fig. 3(B)). Distinct circular shapes

are obtained with sharp contrast in the outside regions where the cells are absent. For the Teflon surface (Fig. 3(C)) and the TLC plate (Fig. 3(D)), more noise is evident from the images and boundary regions of the spots do not contain sharp contrast. To understand the reason behind these observations regarding noise, we need to first group all the surfaces into two different categories depending on how solvent evaporates from these surfaces. In the group of porous surfaces there are filter paper and TLC plate. The porosity of filter paper is more than the TLC plate. When solution is spotted on the filter paper, it is absorbed very quickly and cells do not get any time to spread. However, the TLC plate is not as porous as the filter paper. After spotting solution on it, a thin film is formed before complete absorption of the solvent and this thin solvent film slowly diffuses radially. During this radial diffusion, cells also move outwards and that is the reason for more noise, the absence of sharp contrast in the boundary region and the gradient in the intensity distribution (red > yellow > green > cyan > blue, Fig. 3(D)). In the other group of non-porous surfaces we have the glass slide and the Teflon-coated slide. In both these cases, solvent does not diffuse after spotting and it evaporates slowly. For the glass slide, there is an initial spread of solvent (wetting of surface) just after spotting and for the Teflon surface that too is absent due to the hydrophobic nature of Teflon. Cells do not get any chance to spread in these cases. As a result, a good quality image is obtained from the glass slide (Fig. 3(B)). The noise associated with the Teflon can be attributed to the DESI spray. Probably during the spray, secondary droplets as splashes can go outside of the spot and contribute to the noise. This particular phenomenon cannot contribute to the noise of porous surfaces as splashing is less due to rapid absorption of solvent from the DESI spray. Considering the images (Figs. 3(A), 3(B), 3(C), and 3(D)), signal intensities obtained and time required for drying the spots, it is clear that the filter paper is the most suitable substrate than the others for cell line analysis by DESI-MS. It should also be noted that it



**Figure 3.** DESI-MS images of lipid distribution of WERI-RB1 cells spotted on different surfaces. Top and bottom rows show images without any noise reduction in BioMAP. Middle row shows images with noise reduction. The scale bars of the top row apply to all images across each column. Intensity is colour coded; from black (low) to red (high).

makes no difference which grade of filter paper is used. Filter papers other than Whatman 42 can also be used for the same purpose as long as they have no organic impurities to interfere during the DESI-MS experiments and can absorb water rapidly. Whatman 40 filter paper was also used for DESI-MS imaging experiments. Supplementary Fig. S3 (see Supporting Information) shows images of different lipids from WERI-RB1 cells spotted on Whatman 40 filter paper. Other fibrous surfaces like nanofibre mat or cloth have the potential to become suitable substrates as they can help fast evaporation of the solvent and restrict cell movement.

Noise in the images can be reduced to some extent during the processing of the images in BioMAP. To reduce noise, we need to put some cut-off value in the minimum intensity field. This will reduce noise but the overall peak intensity will also be reduced and for low intensity peaks this process will not help much to reduce noise as it will significantly reduce the peak intensities. This process was applied to reduce noise of the images and the middle row of Fig. 3 (Figs. 3(E), 3(F), 3(G), and 3(H)) shows the distribution of the same ion, m/z782.6, on different substrates. For the Teflon surface (Fig. 3(G)) and the TLC plate (Fig. 3(H)), noise are reduced to some extent and sharp boundaries appear in some regions. This noise reduction has very little effect on the filter paper (Fig. 3(E)) as it is does not produce significant noise. However, the effect of this method on peak intensity is prominently observed in the case of the glass surface (Fig. 3(F)) as signal intensity is already less here compared to the other surfaces. Overall intensity is also reduced due to the noise reduction process in BioMAP. Another interesting fact regarding the glass surface becomes prominent during this exercise. The image obtained from the glass slide (Fig. 3(F)) shows somewhat more intensity of the lipid of m/z 782.6 on the circumference of the spot. This can be attributed to the 'coffee ring effect'. When solvent evaporates from a drop situated at some surfaces, outward flow of solvent occurs which drives dispersed particles towards the edge.<sup>[41]</sup> This is the reason why the cells move towards the circumference of the spot and more signal intensity is observed. Reduction of noise during the experiment is more desirable than reduction by data processing. The bottom row of Fig. 3 (Figs. 3(I), 3(J), 3(K),

and 3(L)) shows the distribution of another ion at m/z 810.6 which is almost 50% less in intensity than the base peak at m/z 782.6. These images are made without any noise reduction in BioMAP. In this situation of comparatively low intensity peak, noise becomes much more prominent for the Teflon surface (Fig. 3(K)) and the TLC plate (Fig. 3(L)) and the data cannot be modified much by processing. For the filter paper (Fig. 3(I)) and the glass slide (Fig. 3(J)), increase in noise is small. Again the coffee ring effect is prominent for the glass slide (Fig. 3(J)).

DESI-MS imaging experiments were done in triplicate for each substrate and the data were consistent for each substrate. Almost similar intensities from each substrate were obtained. Sensitivity of the substrates has been evaluated by spotting lower numbers of cells. Approximately  $10^5$  cells in  $10 \ \mu$ L solution were required to obtain proper signals from a filter paper, Teflon surface and TLC plate. Reducing the number of cells resulted in poor signals which were not resolved properly from the background. For the glass surface,  $10^5$  cells in  $10 \ \mu$ L solution itself was not sufficient to give proper signals as expected from the earlier discussion. Considering all the facts discussed, it is clear that filter paper is a better choice as a substrate for DESI-MS analysis of cells.

#### Intensity comparison of different lipids from the WERI-RB1 cell line

After choosing the filter paper as an ideal substrate for imaging lipids derived from cells, distribution of different lipids was mapped from Whatman 42 filter paper when the WERI-RB1 cell line was spotted on them. Six major lipids shown in Fig. 2 are considered and the images are shown in Fig. 4. It is clear from the images that m/z 782.6 (Fig. 4(C)) is the most abundant ion in the WERI-RB1 cells. Next abundant ion is m/z 810.6 (Fig. 4(E)) followed by m/z 808.6 (Fig. 4(D)), 754.6 (Fig. 4(A)) as well as 780.6 (Fig. 4(B), both having almost similar abundance) and 832.7 (Fig. 4(F)). This observation is in agreement with the spectrum shown in Fig. 2. A similar pattern for lipid distribution was observed when WERI-RB1 cells were spotted on Whatman 40 filter paper (Supplementary Fig. S3, see Supporting Information).





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**Figure 6.** DESI-MS images of different lipids from WERI-RB1 and Y79 cells spotted on Whatman 42 filter paper. Scale bars apply throughout the entire rows. Intensity is colour coded; from black (low) to red (high).

#### Intensity comparison of different lipids from WERI-RB1 and Y79 cell lines

Not only different lipids of a particular cell line, but also different lipids across the cell lines can be compared by this technique. For this purpose, another human retinoblastoma cell line, Y79, was chosen. The mass spectrum of the Y79 cell line from the filter paper is shown in Fig. 5. The lipids observed were similar to that of WERI-RB1 cells and lipids identification was confirmed by tandem DESI-MS. Major lipids that appeared were m/z 754.6 [PC(32:1)+Na]<sup>+</sup>, 768.6 [PC(33:1)+Na]<sup>+</sup>, 780.6 [PC(34:2)+Na]<sup>+</sup>, 782.6 [PC(34:1)+Na]<sup>+</sup>, 808.6 [PC(36:2)+Na]<sup>+</sup>, 810.6 [PC(36:1)+Na]<sup>+</sup> and 832.7 [PC (38:4)+Na<sup>+</sup> with m/z 782.6 as the base peak. Though the relative intensities of different peaks can be readily observed from the mass spectrum, comparison across the cell lines cannot be made directly from them and images are required for that. Fig. 6 shows a comparison of four lipids from the WERI-RB1 and Y79 cell lines when both were spotted on Whatman 42 filter paper. Ion intensities are more in WERI-RB1 for m/z 782.6 (Figs. 6(B) and 6(F)), 808.6 (Figs. 6(C) and 6(G)) and 810.6 (Figs. 6(D) and 6(H)) whereas both the cell lines possess similar ion intensity of m/z 754.6 (Figs. 6(A) and 6(E)). This kind of observation can be made directly from the images.

#### CONCLUSIONS

In the present study we have successfully demonstrated that Whatman filter papers can be used as a suitable substrate for direct lipid imaging from cells reliably when they are spotted on them. Other common substrates like Teflon-coated glass slides and TLC plates give a considerable amount of noise in the images and from the glass slides the signal intensity is lower. The relative intensities of different lipids from a given cell line can be discovered easily. Moreover, lipid intensities across different cell lines can also be compared by this imaging technique. It should be noted that other surface-sensitive sampling techniques like liquid extraction surface analysis (LESA) can also be used to study lipids from cells. The method has already demonstrated its capability for studying lipids from human atherosclerotic plaques,<sup>[42]</sup> drugs and their metabolites from rat tissue,<sup>[43]</sup> etc. This method has also been used to study lipids from single cells.<sup>[44]</sup> Furthermore, electrospray ionization mass spectrometry (ESI-MS) of direct lipid extracts can also be used to study lipids from cells. These methods can produce similar data and can potentially be used as complementary techniques to DESI-MS. As both the above-mentioned methods are based on the extraction of lipids, suitable solvent/solvent mixtures have to be chosen carefully and more time will be required due to the extraction process compared to DESI-MS where the extraction step is not necessary. A very recent report also shows that lipids from a cell line can be imaged from a glass surface by DESI-MS.<sup>[45]</sup> In that study, cells were grown on the modified glass plate and imaged. Our study does not require any kind of modifications of the surface. Filter paper can be used directly. Moreover, the current method allows cells grown elsewhere by any process to be imaged by transferring them onto filter paper for imaging. The method is also advantageous in the case of cells that do not adhere properly on glass surfaces. We believe that in the foreseeable future

this method will find its own place in the field of biomedical research with respect to drug development and screening where initial trials of new drugs are carried out on cultured cells.

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#### SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website.

#### USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

#### Required software to e-Annotate PDFs: Adobe Acrobat Professional or Adobe Reader (version 7.0 or above). (Note that this document uses screenshots from Adobe Reader X) The latest version of Acrobat Reader can be downloaded for free at: http://get.adobe.com/uk/reader/



#### 3. Add note to text Tool – for highlighting a section to be changed to bold or italic.



Highlights text in yellow and opens up a text box where comments can be entered.

#### How to use it

- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.

#### 4. Add sticky note Tool – for making notes at specific points in the text.



Marks a point in the proof where a comment needs to be highlighted.

#### How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted
- Type instruction on what should be changed regarding the text into the yellow box that appears.



- Type the comment into the yellow box that appears.

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#### **USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION**



## • Drawing Markups T □

## 7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..

#### How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.





## 4. Patents, Technologies and Grants

#### Patents/Technologies/Grants

#### Patents

- Vertical growth of nanoparticles leading to micrometer long brushes by ambient electrolytic spray deposition, T. Pradeep, Depankar Sarkar, M. K. Mahita, Anirban Som, R. Graham Cooks, Anyin Li, 6669/CHE/2014, filed on December 29, 2014.
- Method of extraction of silver by glucose, T. Pradeep, Ananya Baksi, Mounika Gandi, Swathi Chaudhari, Soumabha Bag, 6730/CHE/2014, December 30, 2014.

#### Technologies

- AMRIT is undergoing installations across the country. It has now reached West Bengal, Bihar, Uttar Pradesh and Karnataka.
- A new AMRIT purifier with RFID-based delivery and internet-based data collection was displayed at Bangalore India Nano 2014.
- On-line AMRIT water purifiers attached to hand pumps have been developed recently.
- The pesticide removal technology has reached about Rs.1.5 cores in royalty earnings, translating to the production of nearly 1.5 million filter units. The technology must have reached about 5 million people so far.

#### Grants

• Soft ionization ion mobility mass spectrometry of atomically precise clusters of noble metals, Rs.608 lakhs.

## 5. Media Reports

THE HINDU . THURSDAY, AUGUST 7, 2014

Whey consumption helps diabetics Consuming whey protein before meals reduces the blood sugar spikes seen after meals. It also improves the body's insulin response.

LEDs made from perovskite cheaper
 Colourful LEDs made from a material known as
 perovskite could lead to LED displays which are both
 cheaper and easier to manufacture.

## **SCIENCE & TECHNOLOGY**

## Turning a simple optical microscope into a powerful tool

#### R. PRASAD

mall modifications to an ordinary optical microscope have helped turn it into a powerful instrument that can be used for studying complex cellular biology in real time at the nanoscale level. And such is the resolution that the modified optical microscope can study the cellular biology of a living single cell. All biological events - transport mechanism, protein synthesis - happen at the nanoscale level.

The work carried out by an IIT Madras duo - Kamalesh Chaudhari, a research scholar from the Department of Biotechnology and Prof. T. Pradeep of the Department of Chemistry - has made it possible to observe the three dimensional dynamics of tiny nanoparticles with "simpler "Simple instrumentation." optical microscopes have never been used for observing detailed dynamics of simple nanoscale objects," said Prof. Pradeep. The results of the study were published on August 5 in Scientific Reports.

Popular methods for studying biological events at nanoscale use fluorescence; the other is tagging a nanoparticle to an object of interest and studying it. "So far people have tried to label fluorescent particles/small molecules and study them. But in those cases the cell is fixed, and if the cell is live, the fluorescence of the small molecules lasts at most for 20 minutes," said Mr. Chaudhari.



Various motions of a gold nanorod in a human cell and the polar map derived from the study can unravel complex cellular biology in real time.

Optical scattering is one of the other methods used for studying biological events. But it would be useful only if an object produces intense scattering of incident light at a given wavelength. Gold nanorods are excellent scatters of light. So if you observe scattering at a specific wavelength we know 2 nanoparticle is there," Prof. Pradeep said. But it was the use of a polariser with a small

angular tilt that produces an asymmetry in the scattering pattern that has made the technique unique for studying biological events.

Since the scattered light from the nanorod will be polarised in a preferential direction, the direction of the polarisation analyser is changed to transmit light of specific polarisation.

Unlike a spherical nanoparticle, a nanorod produces two types of scattering — one is along the longitudinal axis and the other is in the transverse axis. While the longitudinal scattering is enhanced in the red region, the transverse scattering is in the green region. "With an optical microscope, we can't see nanorods, but can distinguish the scattering pattern," the senior author said. Based on the colour of the scattered light it becomes possible to say if the rod is perpendicular or horizontal with respect to the incident light.

"If a particle is spherical then polarisation has no effect because the scattering of an isotropic sphere is independent of orientation," said Prof. Pradeep. But in the case of a rod, the scattering is orientation dependent — whether the rod is vertical or horizontal with respect to the incident light.

"We get a polar pattern by varying the polarisation angle," the senior author said. In the case of nanorods, the pattern produced by scattering resembles the figure eight. Based on the polar pattern, it is possible to deduce how the nano rods move/rotate inside a cell or if they are sticking to the surface of a cell. According to him, the scattering is very different when the rod is sticking to a cell surface.

"We can see small changes as rods move up and down and also track the vertical and horizontal rotation." said Prof Pradeep. "When the rod is inside a cell, we can track the motion as a movie with an optical microscope. So tomorrow, selective delivery into an organelle inside a cell and how a particle moves inside the organelle can be studied." The team used nanorods of 30 by 10 nanometre In comparison, the size of a cell is 20 microns (20,000 nanometre). The size of a nanoparticle should be much smaller than a cell so it does not alter the chemistry inside the cell.

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S&T » SCIENCE

Nov 26, 2014

## Novel way to produce safer drinking water

R. PRASAD

aking drinking water a lot safer by killing an overwhelming number of bacteria and most viruses is now possible. A novel research to this end was carried out by a team led by Prof. T. Pradeep, Department of Chemistry, Indian Insti-tute of Technology (IIT), Madras.

In May 2013, the same team was able to achieve only 100 times reduction in bacterial load and negligible reduction in viral load through sustained release of 50 parts per billion (ppb) of silver ions in drinking water.

But in the latest study, the team was able to achieve 1,00,000 times reduction in bacterial load and 1,000 times reduction in viral load by synergistically combining silver with carbonate ions. The drastic improvement in antimicrobial performance was achieved despite the team using only 25 ppb of silver ions, half the amount used in the earlier work.

"A novel way has been found to save 1,300 tonnes of silver annually, which would have been unrecoverable otherwise, amounting to a saving of Rs.4,600 crores," Prof. Pradeep told this Correspondent.

The amount of carbonate and silver used was well below the permissible level in drinking water. Tap water was used for the experiments. The results of the study were published a couple of days ago in Nature Group's journal Scientific Reports.

came out of our earlier study easily deployable. So



was that the antibacterial activity of silver can be tuned by simple methods," Prof. Pradeep said. "Everybody was studying silver in isolation but we looked at synergistically combining silver with some other ions."

Explaining what prompted him to combine silver with another ion, he said: "It was intuition (based on chemistry). When you say there is an effect of an ion on an organism, what it means is that the ion has to get into the body of an organism. The penetration has to go through several steps. Each one is a chemical binding process. Therefore, silver can be tuned by ions or molecules."

The search for ions that can be combined with silver without causing toxicity to humans eventually led to carbonates.

"It's a very common ion in "A fundamental result that water. It is also cheap and we

stayed with carbonate," Prof. Pradeep said.

The team had earlier found that silver was able to destroy the integrity of the cell membrane and also damage the DNA. In the case of carbonates, several peripheral membrane-bound proteins get removed. "We found that the peripheral proteins of the organisms were cleaned up aftreatment with ter carbonates," he said.

As a result, more silver ions were able to penetrate the exposed cell surfaces of bacteria and virus much more effectively and quickly. Hence, a large quantity of pathogenic microorganisms was destroyed.

Antibacterial and antiviral effects were tested on E. coli and Staphylococcus aureus and MS2 bacteriophage (virus). Both bacteria and virus were destroyed within 15 minutes of contact time.

Carbonates and silver were

released into water at the same time but their contact areas with water were controlled by playing around with their sizes. "By controlling the size of the particles, one can effectively control the concentration of ions in water," he said.

Since carbonate gets dissolved more quickly than silver, carbonate particles were sand-sized while silver was nanosize. "We want more dissolved carbonates than silver in water as carbonates have to first remove the peripheral proteins of a cell," he said. The amount of dissolved ions in water is 25 parts per billion (ppb) of silver and 20 parts per million (ppm) of carbonate.

Prof. Pradeep is confident that there is scope for more improvement. "You can still make it better by controlling the activity of silver by synergizing with other ions," he said.



S&T » SCIENCE

March 27 2014

## Honey, I shrunk the mass spectrometer

IIT Madras team was able to create ions from any sample even at one volt R. PRASAD opens up.

ass spectrometers that are as small as a smart phone and require as little as one volt -a3,000-time reduction in potential – to create an electric field which would turn a sample into ions for identification of composition may soon become a reality.

The feat of shrinking the ion source that requires very little voltage was achieved by a team led by Prof. T. Pradeep of the Department of Chemistry, IIT Madras. The results were published last week in the Angewandte Chemie International journal.

Conventionally, a solution of the sample is electrosprayed at 3,000 volts to create charged droplets that become ions. The ions are, in turn, analysed to find the composition or chemical constituents in the case of a sample mixture.

The massive reduction in voltage requirement became possible by using carbon nanotube-impregnated paper to act as a substrate on which the sample was deposited. If the conventional method uses very high voltage to create a strong electric field, the sharp protrusions of the carbon nanotubes help in creating the high electric field by using very low voltage.

"One volt over a few nanometres creates an electric field equivalent to 10 million volts over a centimetre," Prof. Pradeep explained. "The whole idea was to keep the nanotubes separated from each other. Normally they get bundled."



**INNOVATIVE:** Rahul Narayanan of the Department of Chemistry, IIT Madras and the lead author of the paper performs an experiment using nanotubecoated paper. - PHOTO: SPECIAL ARRANGEMENT

Once nanotubes get bundled, they turn out to become large wire-like structures thereby increasing the voltage required to create an elecfield. "Earlier tric experiments [by others] using carbon nanotubes failed as the nanotubes were bundled," he said. In fact, standard procedures are available to disperse the nanotubes.

Incidentally, the order in which Prof. Pradeep's experiment progressed was unusual. "I had been after this method for a long time. I knew ionisation is possible and can be detected using low voltage. But the answer came first," he recalled. "I understood that by using the nanotube dispersion technique I could get ions. So the ions came first, and I looked at why this happened." And he soon figured it out. "I realised that ions were observed as the nanotubes were separated,"

he said.

"All good science is commonsense," he noted. "When you look back, [the way] many science breakthroughs [happen] look simple ... quite silly. But if you had told this [miniaturizing mass spectrometer] 20 years ago, people would not have believed you."

A few puzzles remain to be solved. The researchers are vet to decipher where the samples get charged - along the entire length of the nanotube or just at the tip. It is also not clear why molecules present in the air don't get ionised and create their own signals (technically called as noise).

Earlier, scientists succeeded in shrinking the size of the analyser and detectors to 1 cm<sup>3</sup> each. Now, by shrinking the size of the ion source, the possibility of simplifying mass spectrometry for analysing various substances

"If you have a good vacuum system and controlled electronics, we can shrink a mass spectrometer to smart-phone size... we can simplify it. That's the importance of this discovery," he emphasised.

He foresees a day not too far away when gently rubbing the nanotube-coated paper on any object – an apple or a tablet - will be sufficient to collect samples for analysis in a lab. The nanotube-coated substrate can also be reused. In all, there is a real possibility of completely rewriting the way sample testing gets done.

"So what it means [is that] you can collect samples remotely and analyse them elsewhere for disease or pollution prevention or any such thing," he noted. "In a sense, we can make a mass spectrometer reach a wider audience." The mass spectrometer is a sophisticated instrument and has been out of bounds to the common man.

Producing a nanotubecoated substrate is also quite simple. Nanotubes can be grown separately and then coated on the substrate and, behold, it is ready for sample loading.

Since samples can be collected by gently rubbing the substrate on the material, there is a possibility of some tubes breaking and sticking to the surface of the material tested. Will such broken nanotubes cause any health hazard?

"We must ensure that the substrate is holding the nanotubes firmly, so no nanotubes stick to the sample tested," he noted.

#### SCIENCE & TECHNOLOGY

## **NANOTECH ON TAP**

Indian technology offers **CLEAN WATER** at low cost

**GROUNDWATER** in the Indian state of West Bengal naturally contains arsenic, causing ailments including skin diseases and cancer. Thanks to nanotechnology, thousands of people there have gained access to arsenic-free water since 2013, with the installation of treatment tanks using porous granules developed by a team at the Indian Institute of Technology (IIT), Madras, led by chemistry professor Thalappil Pradeep. The technology has received government support for field-testing as an option for low-cost, point-of-use water treatment.

The granules are nanocomposites made from ferric oxyhydroxide and a biopolymer, chitosan. Iron oxides remove arsenic ions from water by adsorption. The team boosted their metal oxyhydroxide's activity by reducing the particle size to nanoscale, thereby increasing the surface-to-volume ratio, and anchoring the material within a network of chitosan. With this structure, which resembles sand and is made at room temperature, embedded particles don't leach into water, and the captured arsenic stays put. What goes on "in the atomic scale is not completely understood," Pradeep says, but that has not stopped the material's real-world use.

At the Ambattur industrial estate, in a suburb of the Indian city of Chennai, a facility makes about 36 kg of the ferric oxyhydroxide-chitosan nanocomposite per day. Production at the plant—run by InnoNano Research, a start-up founded by the IIT Madras team—is enabling field trials in West Bengal.

With funding from the state government, about 100 community water purifiers using the nanocomposites, typically in 600-L tanks, have been installed in the district of Murshidabad, says an InnoNano cofounder known only as Anshup.

Each one, he estimates, serves 50–100 families and lasts one to two years. In the lab, the composite reduces a 1-ppm arsenic load to less than 10 ppb, the limit set by the World Health Organization (WHO). In field trials, natural arsenic loads of up to 330 ppb, the highest found in the field according to the team, drop to less than 10 ppb.



Globally, 137 million people are exposed to arsenic levels greater than the WHO limit. And some 780 million people do not have clean drinking water, according to the Centers for Disease Control & Prevention (CDC). "Every 20 seconds, a child dies from INVENTORS The InnoNano team in front of a water treatment tank that uses the materials they developed. From left, Anshup, holding a cartridge of ferric oxyhydroxide-chitosan nanocomposite; Chaudhary; Anil Kumar; Pradeep; and Sankar.

a water-related disease, especially in the developing world," says Emmanuel I. Unuabonah, a researcher from Redeemer's University in Nigeria who also develops water treatment materials.

**TO REMOVE MICROBES,** the Ambattur plant produces smaller quantities of another material developed by the team, an aluminum oxyhydroxide-chitosan composite (*Proc. Natl. Acad. Sci. USA* 2013, DOI: 10.1073/pnas.1220222110). When impregnated with silver nanoparticles, the material kills microbes by gradually releasing Ag<sup>+</sup>, a microbicide. Team member Udhaya Sankar estimates that 120 g of the composite could continuously provide 10 L of microbe-free drinking water daily for a year.

In the lab, microbial loads of 10<sup>5</sup> colonyforming units (100 times the amount in natural drinking water) drop to zero. Lab studies also show that together, the Fe and Al composites remove both arsenic and microbes; limited field trials corroborate the lab results, says team member Amrita Chaudhary. The composites can be made to remove other contaminants, such as lead or mercury, and assembled for specific needs. The antimicrobial material is housed at the roof of a vessel fed with untreated water from the top. The vessel volume can vary from

a few liters for a household to hundreds of liters for a small community. A multilayer block of composites for specific contaminants sits behind the water tap.

InnoNano's materials join many water purification techniques, including ultraviolet radiation, chlorine treatment, and various filtration methods. "You need a basket of technologies," Pradeep says, to address the diverse needs around the world.

A powder called the P&G Purifier of Water, developed by CDC and Procter & Gamble, is perhaps the best-known water purification technology for use in impoverished or disaster-stricken areas. The product, which contains ferric sulfate and calcium hypochlorite, costs 3.5 cents per sachet. One sachet treats 10 L of water in about 30 minutes, removing metals, including arsenic, and killing microbes. For a family using 10 L of drinking water per day, treatment would cost \$12.80 per year, a month's earnings for many West Bengalis. InnoNano's

filters would deliver the same amount of drinking water for \$2.00–\$3.00 per year, Chaudhary says.

The nanocomposites stand a good chance of being used on a large scale, Redeemer's Unuabonah says. However, more evidence of their robustness is needed, and the arsenic-scavenging material needs to be tested on higher levels of contamination.

The technology is already popular in Murshidabad. The system works well, says Rajeev Kumar, a former Murshidabad district magistrate, and because community units—such as schools or offices—are responsible for operating the tanks, people have a sense of ownership. In a documentary prepared for IIT Madras, residents ask for installations in their villages. The district has ordered at least 100 more purifiers.

For its part, InnoNano wants not only to provide a purification solution, but also to maintain the installations. "Originally, we were thinking of keeping our role to materials manufacturing," Pradeep says, "but that alone is not enough."—VIRAT MARKANDEYA, *special to C&EN* 

## PAPER SPRAY IONIZATION WITH A 3-V BATTERY

Like most ambient sample ionization methods used for mass spectrometry, paper spray usually requires voltages in the kilovolt range. Such high ionization voltages can be dangerous and require large power supplies. Rahul Narayanan, Depanjan Sarkar, and Thalappil Pradeep of the Indian Institute of Technology, Madras, in Chennai, and R. Graham Cooks of Purdue University now show that they can ionize samples with potentials as low as 3 V by using paper coated with carbon nanotubes (Angew. Chem. Int. Ed. 2014, DOI: 10.1002/ anie.201311053). In a demonstration, the group used the low-voltage method to collect mass spectra of pesticides, medicines, amino acids, and other compounds. Without the nanotube coating, more than 500 V is required to produce any signal, they say. Electron micrographs of the coated paper show that nanotubes protrude from the surface. The researchers suggest that the nanotubes act as electrodes that induce an electric field between the paper tip and the mass spectrometer inlet. More recently, the team has found that they could obtain spectra by applying just 1 V, Pradeep says.-CHA




Prof. Pradeep's work on drinking water was highlighted by CNN as one of the most promising technologies which could change the world.

Visit: http://edition.cnn.com/videos/tech/2014/09/11/pkg-build-tomorrow-clean-water-technology-crane.cnn

## Acknowledgements



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