Luminescent Silver Clusters with Covalent Functionalization of Graphene

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Supporting Information

ABSTRACT: Brightly luminescent, atomically precise subnanometer clusters of silver protected by glutathione were covalently functionalized by solution phase thiolated graphene using ligand exchange. A hybrid molecule was formed, which was obtained in a nearly pure form by phase transfer from the aqueous to the organic phase. The resulting hybrid exhibited properties of both of these nanoscale materials. UV-vis, luminescence, XPS, EDAX, TEM, and Raman spectroscopy were used to characterize the hybrid structure, which showed



the spectroscopic characteristics of both the constituents. The luminescence of the cluster was retained in the hybrid, along with features characteristic of graphene. Atomically precise clusters with covalently linked graphene will be useful for new applications in the areas of drug delivery, sensors, and catalysis.

INTRODUCTION

Graphene, the two-dimensional analogue of carbon, has been the most fascinating material of the recent past.¹⁻³ Quantum clusters (QCs) are the emerging category of noble metal based materials with atomically precise cores and well-defined features in optical absorption and emission.^{4–13} QCs contain only a few atoms in their core, which leads to discrete energy levels in them rather than continuous density of states, making them brightly luminescent with distinct emission features.⁸ The fascinating properties of both of these materials have made them important candidates for applications in diverse areas ranging from chemistry to biology.^{14,15} The large surface areas of graphenic materials allow them to capture active molecules and clusters.^{16,17} Noble metal QCs, due to their luminescence, are excellent reporters of chemical and physical changes of the medium.^{18,19} As a result, creation of a covalently bonded hybrid of the two would enhance the properties of both these nanoscale materials. Analogous structures have been made in the case of large plasmonic nanoparticles to create new reporter species.²⁰ In this article, we report the first synthesis of such a QC-graphene hybrid molecule exhibiting the characteristic features of both the constituents. Functionalization of QCs with diverse molecules has been achieved to impart additional properties such as fluorescence resonance energy transfer (FRET),^{19,21} receptor mediated transfer²² and antigen-antibody interaction.⁸ This functionalization chemistry, extending to graphene and such other carbon forms will extend the scope of cluster research.

Ag and Au atoms have been well-known to have an affinity toward sulfur,²⁰ and hence, solution phase graphene, which typically contains the carboxylic acid group, was functionalized to form thiolated graphene. Thiol protected Ag QCs were bound to this by ligand exchange of thiol functionalities to form a covalently bound graphene cluster hybrid molecule. The hybrid was characterized in the aqueous phase and was later phase transferred to toluene to separate unbound graphenes from the hybrid.

EXPERIMENTAL SECTION

Synthesis of Solution Phase Graphene. The synthesis of graphite oxide from graphite powder was achieved by a method originally proposed by Kovtyukhova et al.²³ The pure, ion-free dispersion of graphite oxide (GO) obtained by this method was reduced to graphene (GRN) by a hydrothermal reduction technique first reported by Loh and colleagues,²⁴ which avoids the possibility of interferences due to chemical reduction at a later stage (see Supporting Information S1 for experimental details). The absorption spectrum of GO shows a raise in background at 1000 nm, but no distinct UV peaks were seen (Figure S2A in the Supporting Information).

Synthesis of Glutathione Functionalized Graphene (GRN–GSH). To 10 mL of 0.05% graphene dispersion, 5 mg of 1-(3-(dimethylamino)propyl)-3-ethyl-carbodiimide hydrochloride (EDC) was added and stirred for 6 h, to activate the carboxylate groups on the chemically synthesized graphene sheets. To this mixture, an equimolar quantity of reduced glutathione (GSH) was added and stirred overnight. A black precipitate of the functionalized graphene settled at the bottom of the reaction tube. The product was centrifuged around 12 000 rpm and washed several times with triply distilled water to

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remove excess EDC and unreacted glutathione. After several washing steps, the black product was redispersed in water to make a 0.05% dispersion of GRN-GSH, which shows the characteristic feature of graphene (Figure S2A in the Supporting Information).

Synthesis of Silver QCs. Silver quantum clusters (Ag_{QC}) protected with glutathione (GSH) was synthesized by a gel mediated route proposed by Chakraborty et al.²⁵ In this method, the silver precursor was uniformly dissolved in the liquid form of the gel, which was left undisturbed until it was set. Once the gel was formed, sodium borohydride solution was added to reduce the Ag precursor, and the clusters were formed within the gel matrix. These were washed and extracted from the gel and used in further experiments (see S1 in Supporting Information for details of the synthesis).

Synthesis of Graphene Protected Ag_{QC}. Clusters were protected with GRN–GSH by stirring 1 mL of its solution with 4 mL of the cluster solution for 4 h at room temperature, followed by the addition of methanol to precipitate the clusters. The GRN–GSH attached clusters, formed through ligand exchange via their thiol functionalities, precipitated, and settled at the bottom. This mixture was centrifuged at a low speed of 1500 rpm, and the supernatant was discarded. The precipitate containing graphene functionalized Ag_{QC}, referred to as hybrid molecules (GRN–Ag_{QC}), was dried and suspended in water.

The procedure involved in synthesizing the $GRN-Ag_{QC}$ hybrid molecule is shown in Scheme 1.

Scheme 1. (From Left to Right) Solution Phase Graphene Sheets with Free Carboxylate Groups, Coupled via Amide Linkage to GSH Forming Thiolated Graphene, Which Are Attached to Ag_{QCs} by Ligand Exchange Yielding Graphene– Cluster Hybrid Molecules; Relative Sizes of Atoms Are Not to Scale



A method of directly coupling the carboxylate groups on functionalized graphene with the glutathione on the clusters was not used because the coupling agent EDC used to facilitate the formation of the amide linkage between the amine and acid is available as a hydrochloride salt, which completely hydrolyses to form an ionic solution. When added in the presence of clusters, this can cause aggregation and instability of the cluster solution. Further, centrifugation and repeated washing is required to remove excess EDC and HCl. Clusters might not retain their inherent properties to their full extent after this process.

RESULTS AND DISCUSSION

Figure 1I(a) shows the absorption spectrum of $Ag_{25}SG_{18}$ (SG, glutathione thiolate) synthesized by the proposed method. The



Figure 1. (I) Absorbance spectrum of (a) freshly prepared Ag_{QC} in aqueous phase, (b) GRN–GSH, and (c) GRN– Ag_{QC} . (II) Photographs of Ag_{QC} in aqueous phase and in toluene phase, respectively (a and b). Images a^{I} , and b^{I} are the corresponding photographs under UV-light. Similar photographs of GRN– Ag_{QC} before and after phase transfer (c, d and c^{I} , d^{I} , respectively). The top layer is toluene and the bottom layer is water. (III) Raman spectra of (d) GRN and (e) GRN–GSH.

spectrum shows well-defined features due to the molecular transitions of the cluster, quite unlike plasmonic nanoparticles. The absorption spectrum of GRN–GSH in Figure 1I(b) shows the graphene feature at 258 nm. The spectrum is characterized by large scattering due to the suspended sheets, which manifests as a raise in background at 1000 nm. Figure 1III(e,f) (e,f) is the Raman spectra of graphene and GRN–GSH under 633 nm excitation. The spectra show D and G bands characteristic of the solution phase functionalized graphenes. There is no shift in either of these peaks when graphene is functionalized with thiol. Figure 1II shows photographs of Ag_{OC} in the aqueous phase and toluene phase, respectively (a and b). Photographs a^I and b^I are the corresponding images under UV irradiation. Cluster in the aqueous phase does not exhibit visible emission, and the emission intensity enhances upon phase transfer to organic phase.¹³ This makes it possible to capture the red emission. Photographs c and d are of GRN-Ag_{OC} before and after phase transfer, respectively, and c¹ and d¹ are the corresponding images under UV irradiation. GRN-Ag_{OC} shows photoluminescence under UV irradiation in both the aqueous and organic phases; however, the luminescence intensity was reduced compared to the parent Ag_{OC}. As in the case of the parent cluster, the luminescence is not observable visibly in the aqueous phase. Luminescence of Ag_{OC} was quenched as graphene binds to the clusters and transfers to the organic phase.

The absorption spectrum of GRN– Ag_{QC} (Figure 1I(c)) shows the features of both the clusters and GRN–GSH. The characteristic band of the cluster at 478 nm exists in the hybrid but is red-shifted. The 330 and 550 nm bands are observable as shoulders but the 640 nm band is not seen. This band is highly sensitive to chemical transformations. The presence of GRN is apparent in the absorption spectrum as the baseline shifted up significantly along with the presence of the feature at 260 nm, as in the case of the parent graphene and GRN–GSH.

The addition of graphene does not quench cluster fluorescence until high concentrations of graphene are added.

Figure 2I shows the quenching of cluster luminescence by the addition of GRN-GSH. In this measurement, the concen-



Figure 2. (I) Luminescence spectra of Ag_{QC} with increasing concentration of GRN–GSH. Cluster concentration is kept constant. (II) (a and b) Luminescence spectra of phase transferred Ag_{QC} and GRN– Ag_{QC} , respectively.

tration of clusters was kept constant, while the concentration of GRN-GSH was increased stepwise. The luminescence intensity decreases with the increase of GRN-GSH, but no peak shift is observed. Luminescence spectra of Agoc and graphene, as well as Ag_{OC} and GRN-GSH mixtures with concentrations of the two varied systematically are shown (Figure S3 in the Supporting InformationI). Figure 2II(a,b) shows the luminescence spectra of phase transferred Ag_{OC} and GRN-Ag_{OC}, respectively; the spectrum on the left side being the corresponding excitation spectra. Luminescence intensity of GRN-Ag_{OC} is decreased compared to the intensity of emission from Ag_{OC}, consistent with the quenching observed in Figure 1II. There is no shift seen in the emission maximum; however, the addition of excess TOABr during phase transfer can often cause a red shift in the emission spectrum by almost 10 nm, when compared with the emission peak of the cluster in the aqueous phase. This must not be mistaken as the formation of a different cluster while transferring to the organic phase. At similar ratios of graphene and Ag_{QC} in the two phases, quenching was a little more in the aqueous phase. This could be attributed to the presence of some unbound GRN-GSH before phase transfer. Low concentrations of composite were used in luminescence measurements and inner filter effect can be assumed to have only minimal effect on quenching (see also below).

In the method of cluster synthesis used, size tuning of clusters has not been reported yet. Hence, the effect of changing the size of the clusters has not been explored. However, with regard to cluster concentration, a higher concentration of the clusters (with respect to graphene) increases the intensity of fluorescence emission in a regular manner. There is no significant difference when graphene is bound at the same Ag₂₅/graphene ratio. The graphene sheet surface is covered by the clusters as seen from the TEM image. It is also equally likely that the clusters attach to either side of the sheets. Therefore, it is more probable that the clusters on the graphene surface will absorb the incident light at 450 nm as they are covering the surface and they have a higher absorption coefficient at the excitation wavelength. Moreover, in the aqueous phase there is a larger background raise arising due to graphene. This might also be due to the presence of unbound graphene sheets as they have not been separated. After phase transfer to toluene, this background in the absorption spectrum is negligible (Figure S2B, Supporting Information). Though the absorption and scattering of graphene sheets cannot be

completely avoided, they may be neglected for the reasons mentioned above.

Despite careful separation of unbound graphenes by precipitation through low speed centrifugation, there might still be a possibility that unbound GRN-GSH might be present with the clusters due to the high binding ability of graphene sheets. In order to eliminate this possibility, phase transfer of the functionalized graphene-cluster hybrid was done, from aqueous to organic phase, to ensure that the unbound free graphene is separated from GRN-Ag_{OC}. This was done by stirring GRN-GSH with the cluster solution for 4 h, followed by phase transfer of the material into toluene via the phase transfer reagent, tetra-octyl-ammonium bromide (TOABr). The phase transfer occurred within a few minutes of stirring with TOABr, just as in the case of clusters,¹³ and was marked by the organic layer turning orange due to the color of the clusters that have transferred from the aqueous phase. GRN-GSH when stirred in the absence of clusters does not transfer to the organic phase by this method even after several hours of vigorous stirring. This control experiment was validated by taking the Raman spectrum of the organic phase after stirring the aqueous GRN-GSH with TOABr in toluene overnight. No graphene signature was seen in the Raman measurement of the toluene phase. If the interaction between the graphenes and Ag_{OCs} was electrostatic, there would have been an equal chance of detecting organic phase graphene in the control study. Moreover, Ag atoms are known to have a strong affinity toward sulfur as opposed to oxygen due to soft-soft interaction of Ag and S. In the event of electrostatic binding, there would be no preferential binding of $\mbox{Ag}_{\mbox{QCs}}$ to GRN–GSH compared to graphene sheets having carboxylate functionalities used in the control. Although standard techniques for the observation of covalent functionalization such as infrared and Raman spectroscopies were used, these were not successful in showing the distinct presence of a new functionality. This is expected as glutathione and graphene have the very same functionalities (such as amide, C-S, etc. bonds). Thus, indirect evidence was obtained through XPS, EDAX, TEM, and Raman characterization.

Figure 3I shows the X-ray photoelectron survey spectrum of the phase transferred GRN-Ag_{OC} and the inset (Figure 3III) is an expanded view of the silver 3d region. Bromine is present in the sample due to the use of TOABr during phase transfer. From the XPS spectra, it is seen that there is a large amount of carbon in the phase transferred sample, strongly indicating that GRN-GSH has been transferred to the organic phase. It is therefore likely that the GRN-GSH is covalently bound to the clusters. Figure 3II shows the transmission electron microscopic (TEM) image of GRN-Ag_{QC}, phase transferred to toluene (method of phase transfer will be discussed later). Agoc are preferentially anchored on the edge of the GRN-GSH sheet (marked by the rectangles) where a larger density of dots due to the clusters can be seen. The clusters anchored on the graphene sheets are less susceptible to electron beam-induced aggregation unlike in the case of unbound clusters, which aggregate easily during TEM measurements.^{26,27} Clusters may come out from the hybrid molecule during electron impact, which may get aggregated.

Figure 4I shows the energy dispersive analysis of X-rays (EDAX) of phase transferred $\text{GRN}-\text{Ag}_{\text{QC}}$. The elemental analysis of the sample shown in its inset gave the following: silver (0.35%), sulfur (0.31%), and a large amount of carbon (78%) due to the presence of graphene sheets. The Ag/S



Figure 3. (I) XPS survey spectrum of the phase transferred GRN– Ag_{QC} hybrid. (II) TEM image of a GRN–GSH sheet, with Ag_{QCs} preferentially anchored on the edge (marked by the rectangles). (III) Expanded XPS spectrum showing the Ag 3d features.



Figure 4. (I) EDAX spectrum of phase transferred GRN– Ag_{QC} . The inset table gives the elemental analysis data. Si, Ca, Al, and Sn are impurities from the substrate. (II) Raman spectra of GRN– Ag_{QC} in the aqueous phase (a) and after phase transfer to toluene (b).

atomic ratio expected for Ag₂₅SG₁₈ is 1.39, while the observed is 1.12. The increased sulfur content, due to GSH functionalization of graphene and the presence of such free GSH (unbound to clusters) are the possible reasons for this reduced ratio. Si, Ca, Al, and Sn are signatures of the substrate on which the sample was drop casted for the measurement, and these can be disregarded. From both the XPS and EDAX spectra, it is seen that there is a large amount of carbon in the organic phase. The Raman spectrum of the phase transferred sample was measured to confirm that this is the graphene that is bound to the clusters. The spectrum of the organic phase was compared with the spectrum before phase transfer (Figure 4II). Both the spectra contain the D (1350 cm⁻¹) and G (1580 cm⁻¹) bands characteristic of the graphene sheets, confirming that GRN-GSH was bound to the clusters and was transferred to the organic phase. All Raman spectra were obtained with 633 nm laser excitation, in order to minimize the fluorescence emission from the clusters present in the sample. The spectrum under 532 nm excitation shows the emission feature of the cluster as expected (Figure S4 in the Supporting Information). Ligand exchange of clusters without core change has been reported in several cases.^{7,28} The exact matching of the luminescence

features before and after phase transfer and the absence of phase transfer of the parent GRN–GSH indicate that the GRN–Ag_{QC} hybrid with its inherent properties has been phase transferred. While the presence of glutathione in GRN–GSH could be confirmed by electrospray ionization mass spectrometry (ESI MS) shown in Figure S5 in the Supporting Information, the integral GRN–Ag_{QC} hybrid could not be observed. Integral features of the clusters are difficult to be seen even in soft ionization methods.⁸

CONCLUSIONS

We report for the first time the attachment of silver quantum clusters to graphene through a covalent linker. The luminescence of the cluster is retained even after attachment to the graphene sheets. The resulting water-dispersible hybrid molecule can act as a multimodal material exhibiting the characteristics of both graphene and the cluster. Variation in the functional groups, length, and properties of the linker molecules can potentially lead to an array of different nanocomposite structures. With variation in this linker molecule, the optical properties of the hybrid can be changed by the nature of the bonds the linker makes, as this will affect the ligand field and hence the energy states. Composites of this kind could be used in drug delivery applications. Graphene has been shown to be biocompatible in past reports. Due to its high adsorption property, it can act as a carrier and the attached luminescent cluster is an in-built tracking system for locating the drug within the bloodstream. Clusters bound to graphene could potentially be catalysts in metal catalyzed reactions involving organics or biomolecules as the composite is suspendable in both organic and aqueous phases. Luminescence of the QCs is sensitive to the physical and chemical changes in their medium. This property enables this hybrid to be useful in sensor applications. Our results provide a gateway for further experimental and theoretical exploration on the electronic, optical, catalytic, and biological properties of this new material.

ASSOCIATED CONTENT

S Supporting Information

All experimental details and other results presented in the 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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