Convenient purification of gold clusters by co-precipitation for improved sensing of hydrogen peroxide, mercury ions and pesticides

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Introduction

- Fluorescent nanomaterials have been studied extensively because of their unique optical and photo physical properties. Among various NCs, maximum effort has been given to the study of fluorescent Au-NCs.

- These gold clusters have been prepared using different sulfur-bearing agents such as glutathione, dendrimers and proteins. Among them, the protein directed synthesis of fluorescent clusters in aqueous solutions is of great interest due to their promising applications in chemical detection, biosensing/imaging etc.

- Bovine serum albumin (BSA) has been adopted to produce highly fluorescent Au_{25} clusters with high chemical stability, simple synthetic reproducibility and biological selectivity.

- Experimentally, largely excessive BSA is required to reduce chloroauric acid to form BSA-protected gold cluster.

- The large amount of free BSA remaining in the cluster solution will not only decrease the fluorescence of clusters but also compromise the detection sensitivity.

- There are many separation methods are known like ultracentrifugation, chromatographic technique and dialysis. But these methods are not appropriate for highly soluble protein protected clusters with extremely small size.

- So it still remains a great challenge to develop an effective separation process to remove free proteins from cluster solution.

In this paper

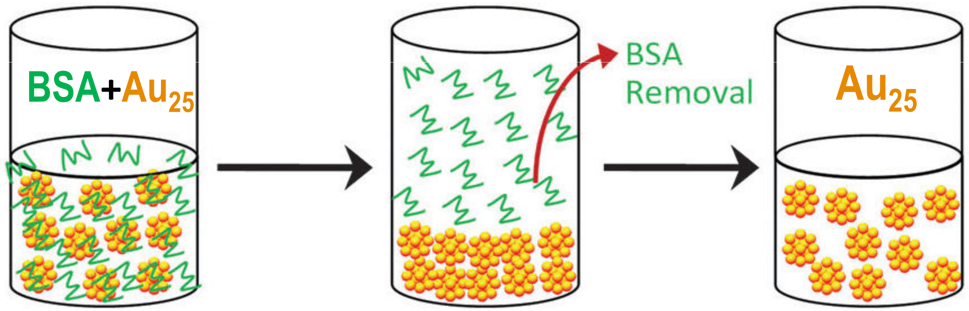
- A simple and novel method to remove free BSA protein from a very basic cluster solution via the co-precipitation of gold clusters with zinc hydroxide.

Synthesis of BSA-protected Au_{25} cluster

5 mL of HAuCl4 solution + 5 mL of BSA solution (10 mM) (50 mg/mL) —Vigorous stirring for 10 min→ Addition of 1 M NaOH

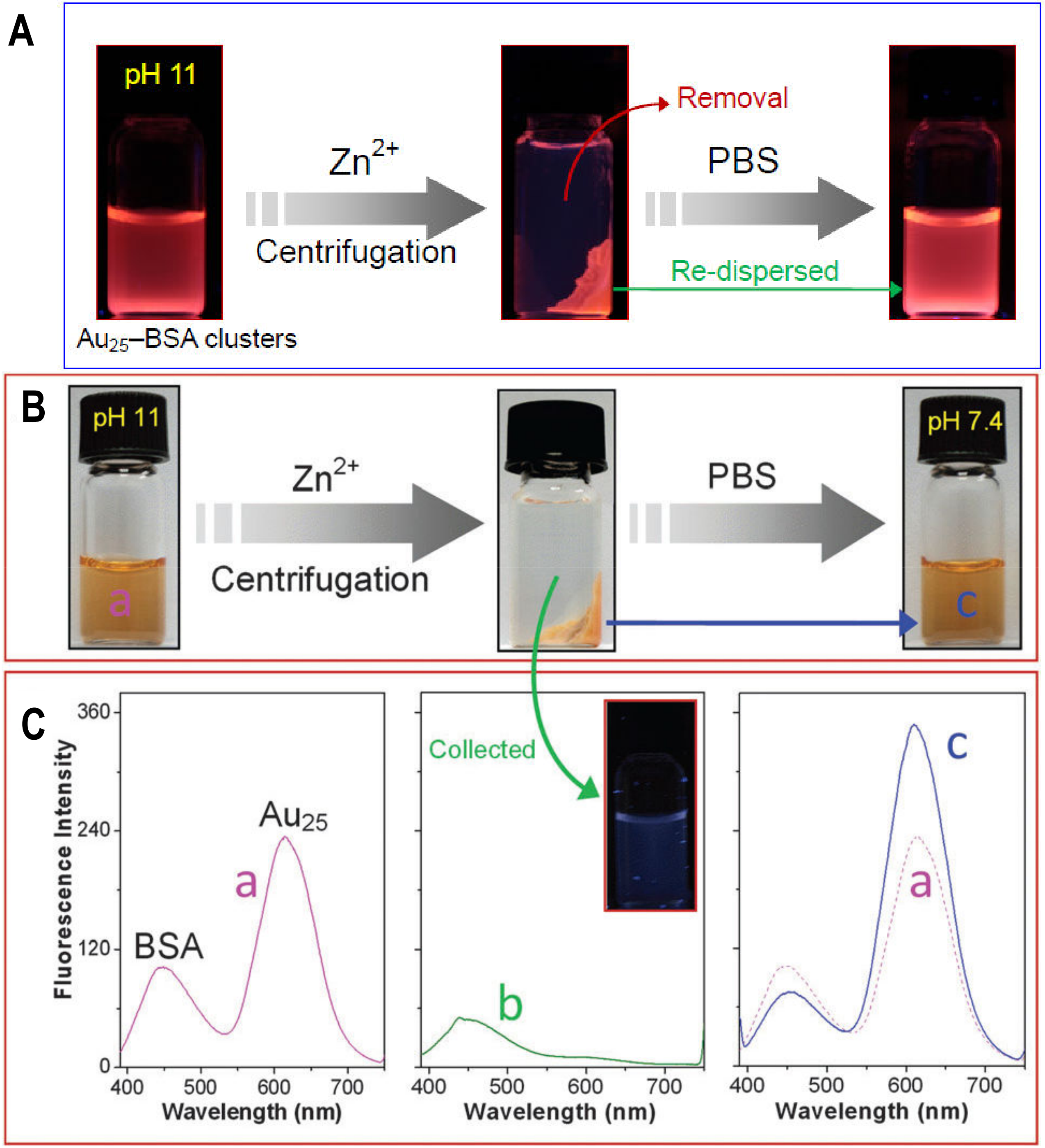
—Stirred for 24 h, 40 °C→ Clear brown solution of Au_{25} (pH 11)

Co-precipitation and purification of the as synthesized cluster



Schematic presentation of the purification of Au_{25} clusters.

To 0.8 mL of the as-synthesized cluster, same volume of 10 mM Zn^{2+} was injected rapidly. The resultant precipitate was then centrifuged at 1500 rpm for 10 min and washed with distilled water for three times. After removing the supernatant, the ppt. was dissolved into 0.8 mL of PBS solution to get a clear brown solution. The re-dispersed cluster solution in PBS buffer was dialyzed for 24 h with buffer and then with distilled H_2O for 24 h.



The ppt. of clusters only occurred at the pH > 10 indiactes co-precipitation $Zn(OH)_2$ occurs along with cluster.

A) Fluorescence images during the purification of BSA-protected Au_{25} clusters by the addition of Zn^{2+} solution.

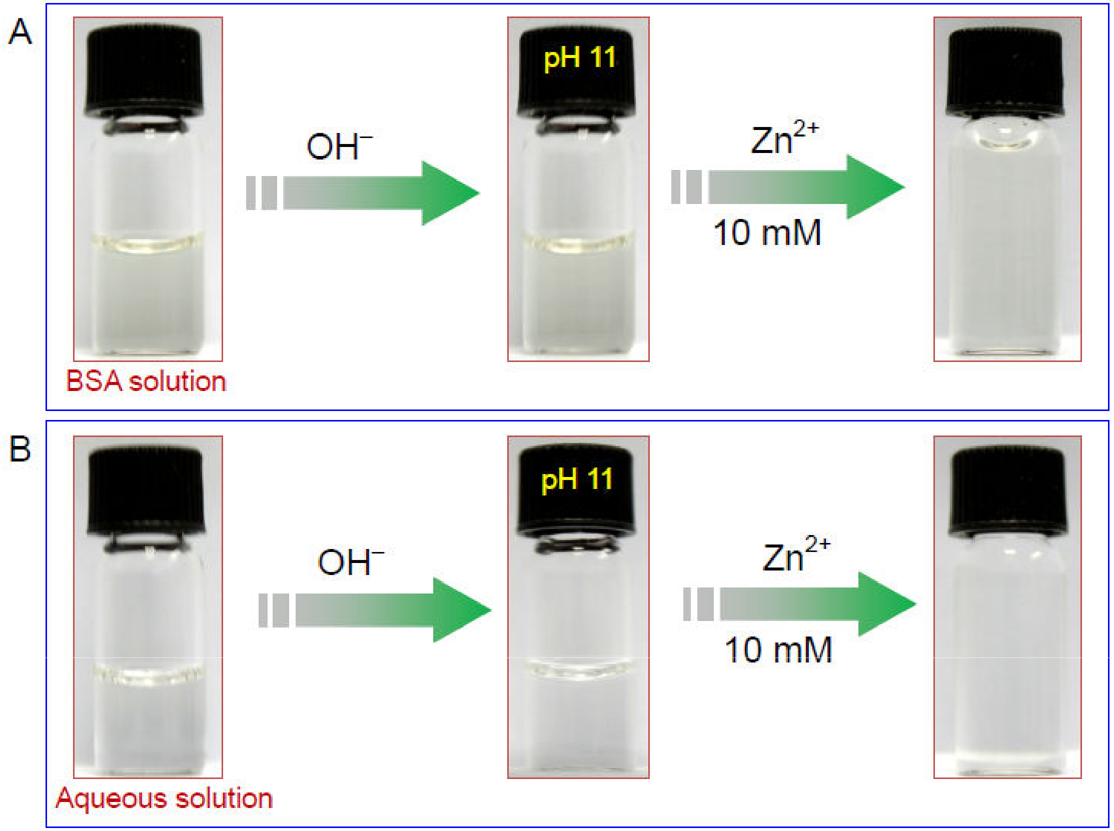
(B) Optical images of the BSA-protected Au_{25} clusters by the addition of Zn^{2+} solution.

(C) Fluorescence spectra of

(a) as synthesized clusters

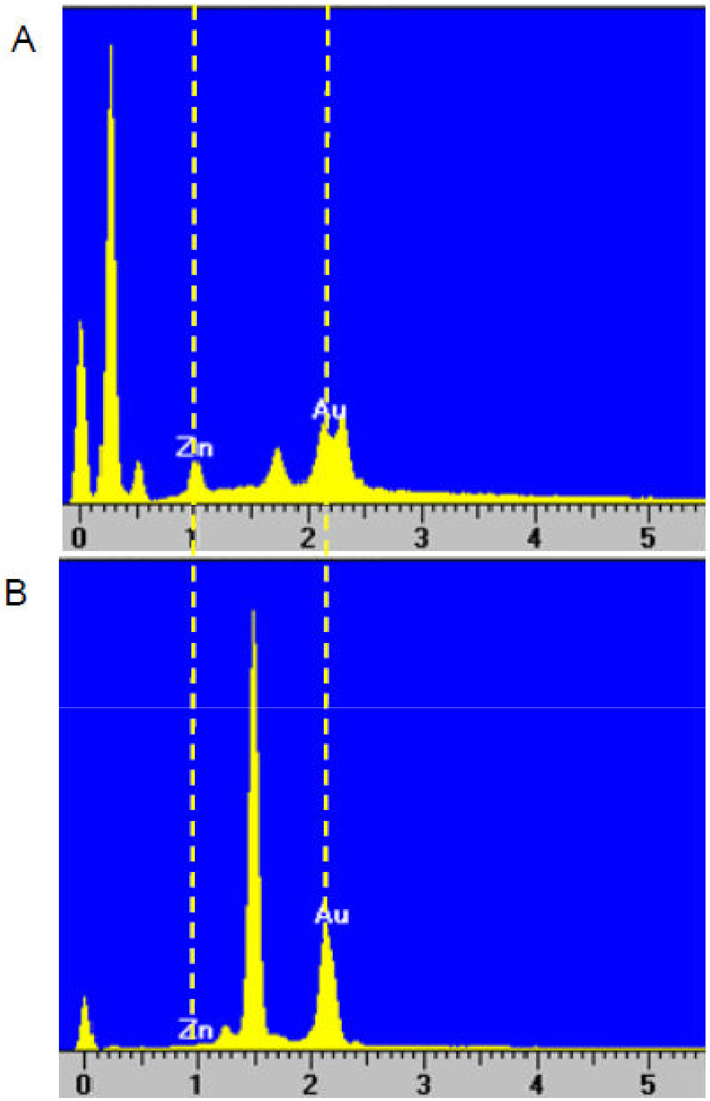
(b) the supernatant after precipitating clusters, and

(c) the purified clusters after being re-dispersed in PBS buffer.

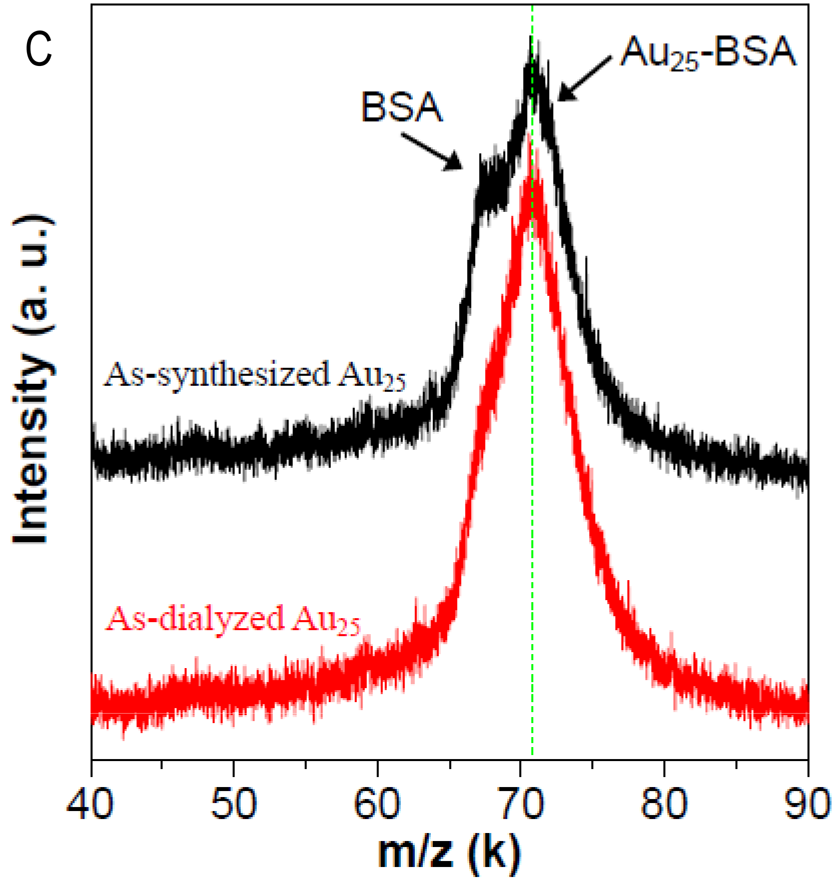


Optical images of BSA solution (A) and aqueous solution (B) after addition of 10 mM Zn^{2+} solution.

The pH of the BSA and aqueous solution was adjusted to 11 by adding 1 M NaOH. After incubating for 24 h, 10 mM Zn^{2+} was added. Both did not lead to formation of any precipitate.

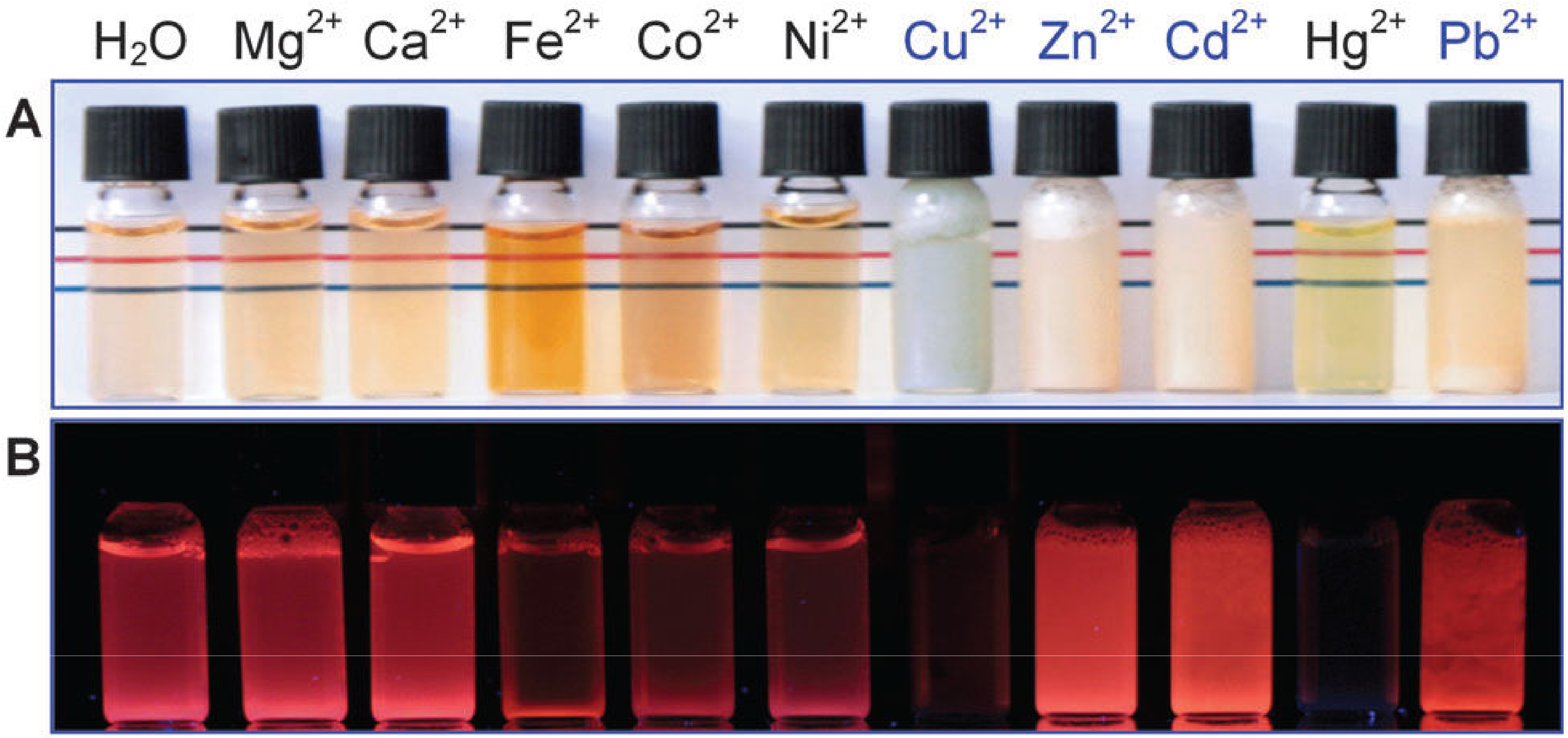


SEM EDX spectra of the co-precipitated Au_{25} clusters before dialysis (A) and after dialysis (B) This showed that Zn^{2+} ions on the surface of the dialyzed clusters were almost completely removed.

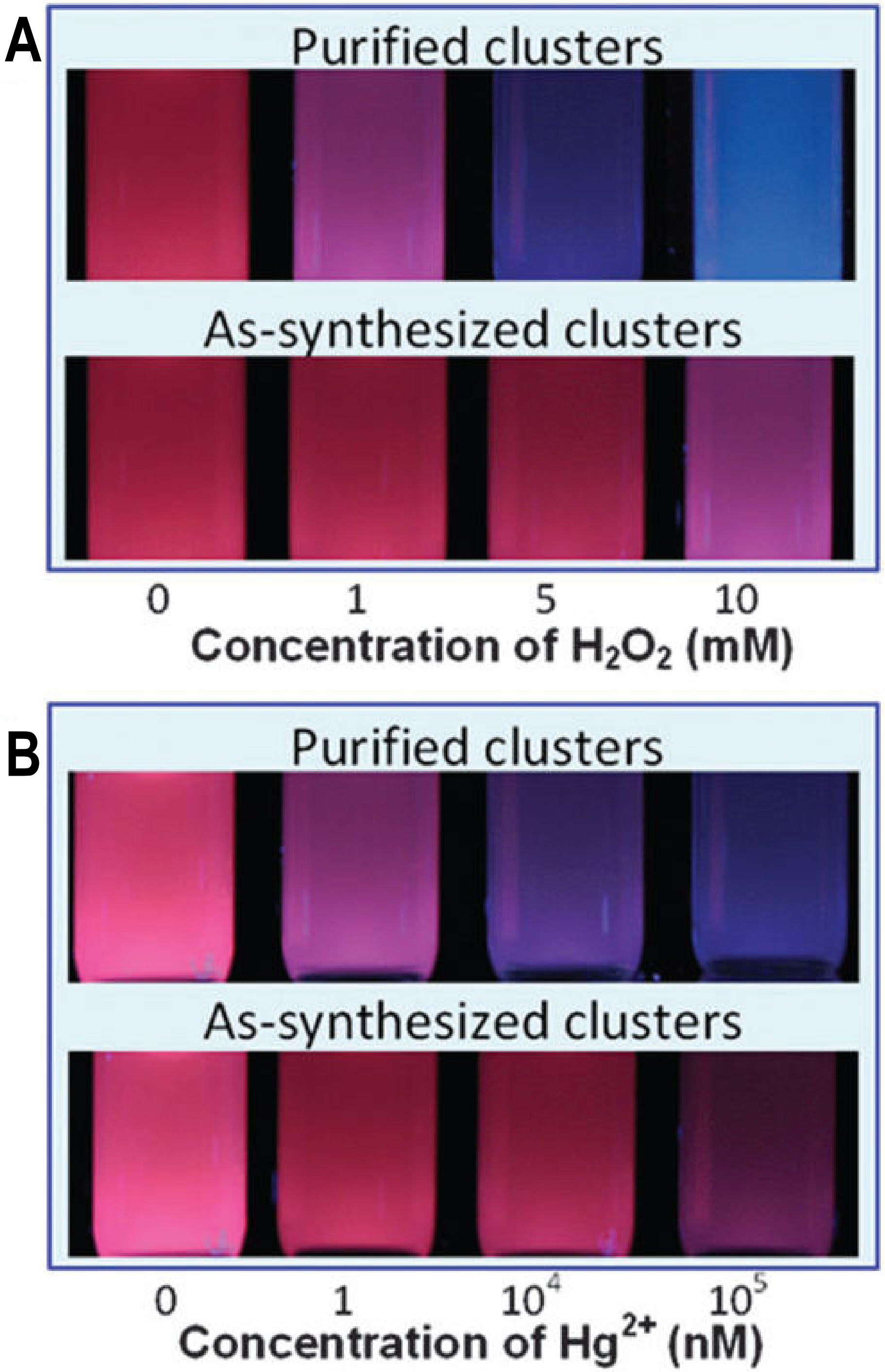


(C) MALDI spectra of the as synthesized Au_{25} clusters (black line). (~ 70.9 kDa) purified cluster (red line) without free BSA peak. This showed structure of Au_{25} cluster remained unchanged before and after purification.

The removal of free BSA is also confirmed by the absence of BSA's peak in precipitated and dialyzed clusters.



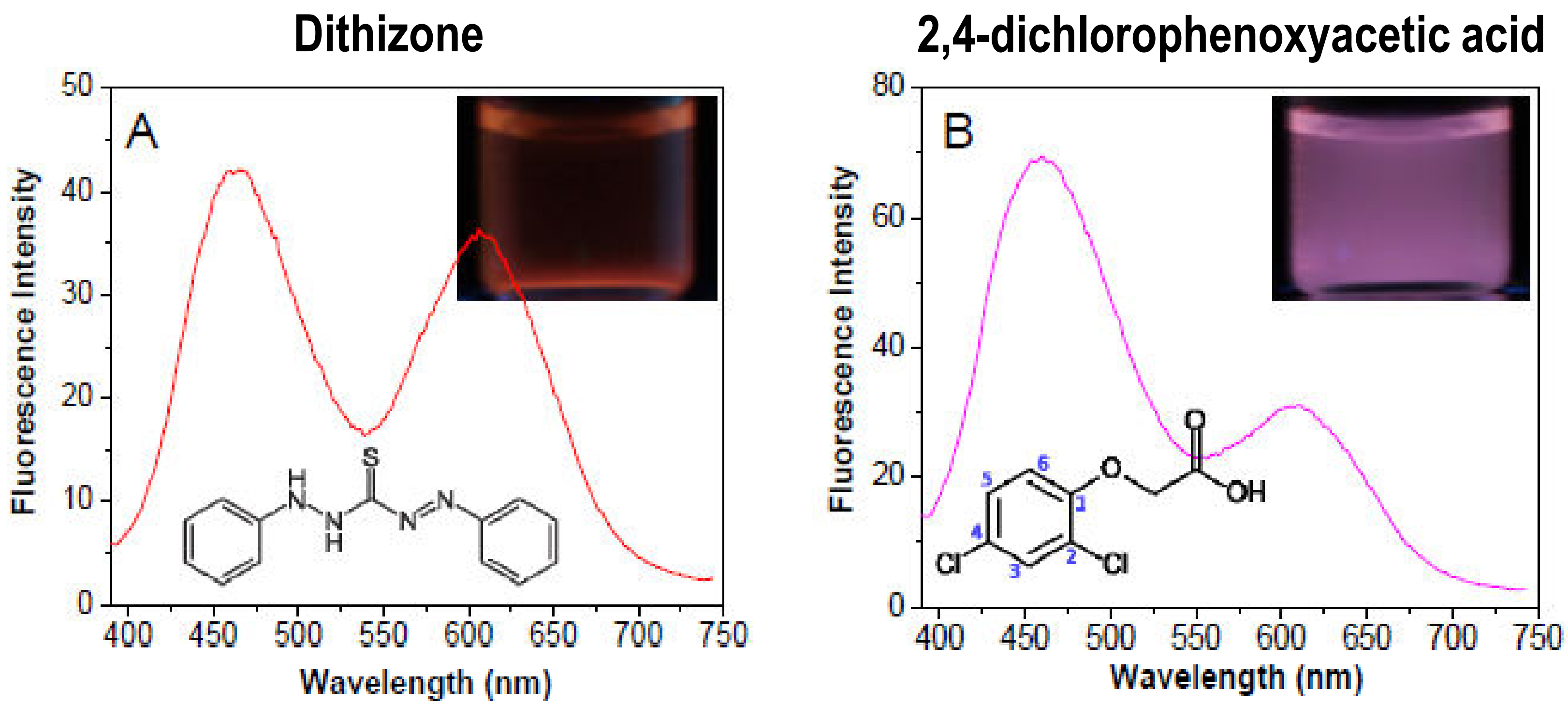
(A) Optical and (B) fluorescence images of Au_{25}-BSA clusters after adding the same volume of distilled water or solutions containing various metal ions. The addition of Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} led to the precipitation of clusters. Zn^{2+} and Cd^{2+} greatly enhanced the fluorescence of purified clusters by decreasing the content of free BSA.



Greatly improved sensitive fluorescent and visual detection of H_2O_2 (A) and Hg^{2+} (B) with the as-purified Au_{25} clusters.

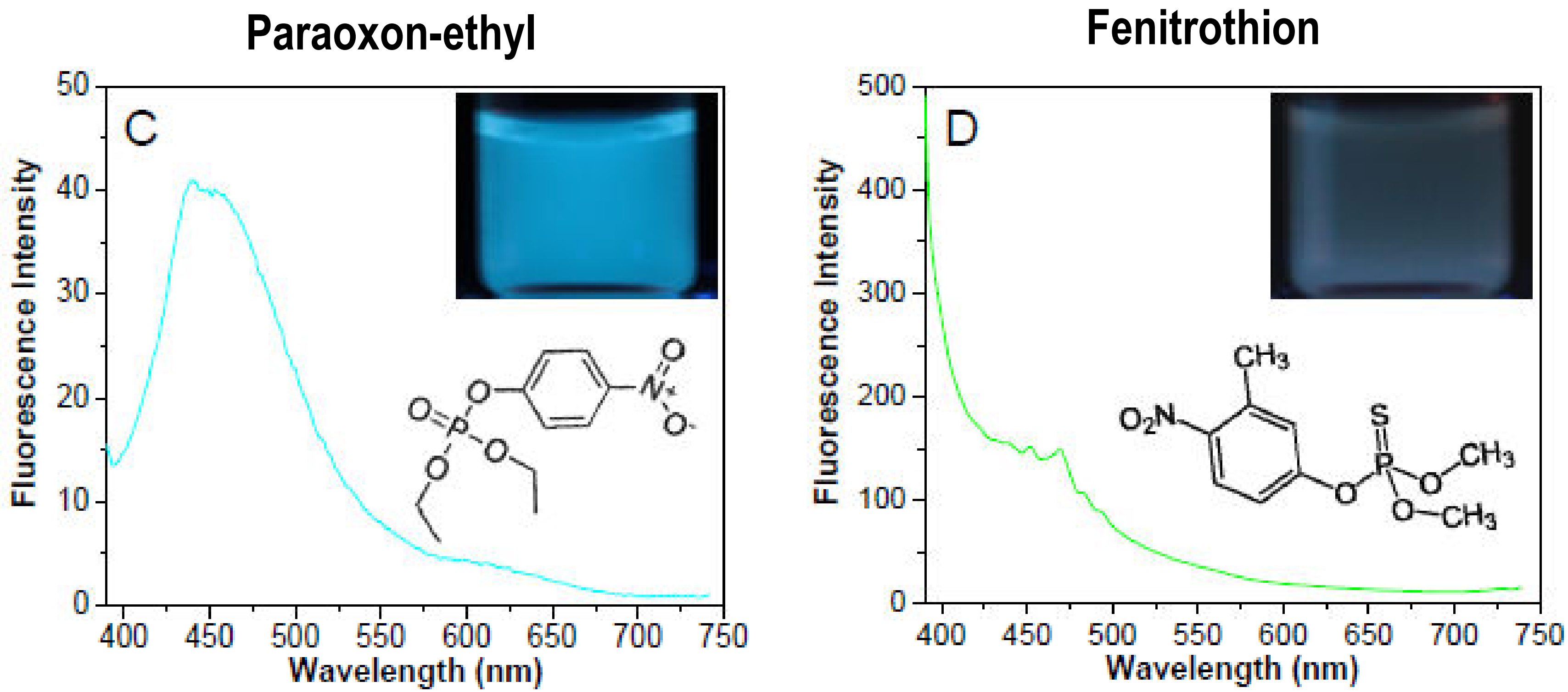
10 μL of H_2O_2 / Hg^{2+} was added to 1 mL of cluster solution.

The fluorescence response of the purified clusters is 4 times higher than that of the as-synthesized clusters for use in clear visual fluorescence detection of both Hg^{2+} and H_2O_2.



This change is attributed to their different response to the blue emission of BSA and the red emission of Au_{25} cores. **A.** Dithizone quenched the red fluorescence to exhibit blue and red emissions with almost equal intensities. **B.** 2, 4- Dichlorophenoxyacetic acid leads to the instability of clusters due to acidic environment. So it quenched red fluorescence to a larger extent to exhibit stronger blue and weaker red emissions.

The detection selectivity of purified clusters was investigated using four types of pesticides as analytes, which were dissolved in ethanol to form a 10 mM concentration. Fluorescence spectra were recorded using a 380 nm excitation wavelength and performed at room temp and before each measurement, the mixture was shaken for 2 min.



C. Paraxon-ethyl oxidizes BSA to decompose the cluster. It completely quenched the red fluorescence to exhibit blue emission.

D. Fenitrothion reacted with Au_{25} –BSA , generating a new emission.

Summary

- They developed an effective separation method for isolating BSA-protected Au_{25} clusters from free BSA in the as-synthesized cluster solution.

- Density Functional Theory (DFT) simulation shows that OH^{-} groups are stably bound to Au^{+} ions on the surface of gold clusters, and the added Zn^{2+} then interacts with the surface OH^{-} strongly, leading to the co-precipitation of clusters with zinc hydroxide for the convenient removal of free BSA.

- Various control experiments were carried out to confirm this co-precipitation mechanism.

- The quantum yields of the Au_{25} clusters before and after purification are 6% and 6.5%, respectively.

- After dialysis, the resulting purified gold clusters exhibited enhanced fluorescence, improved sensitivity, and high selectivity in fluorescence detection.

Thank you