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Fluorescent Ag Clusters via a Protein-Directed Approach as a Hg(II) Ion Sensor

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Fluorescent Ag Clusters via a Protein-Directed Approach as a Hg(II) Ion Sensor

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S Supporting Information

ABSTRACT: Proteins have proven to be particularly attractive as effective ligands in the synthesis of nano- and subnanoscaled materials because of their multiple chelating and functional groups imparting unique functionalities. However, protein-directed fluorescent metal cluster synthesis is still a challenge but a promising area of research. Here, we report on the synthesis of new water-soluble, stable, fluorescent Ag clusters via a facile, green method using denatured bovine serum albumin (dBSA) as a stabilizing agent. The dBSA with its 35 free cysteine residues could contribute to polyvalent interactions with the Ag clusters and serve as effective stabilizing agents for these clusters. The as-prepared Ag clusters showed high fluorescence emission at ~637 nm and were stable even in 1 M NaCl. The fluorescent Ag clusters were then used in the detection of Hg^{2+} with high sensitivity and selectivity. The detection limit was 10 nM in the linear range from 10 nM to 5 μM.
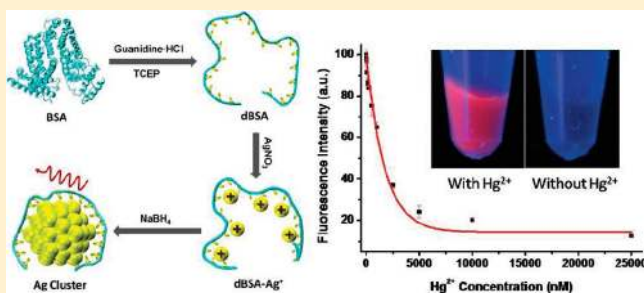
$\underset{\text{M}}{\text{etal}}$ nanoparticles of size smaller than 2 nm, known as metal clusters consisting of a few to several hundred metal atoms, have received much attention in recent years due to their unique physical, electrical, and optical properties for use in bioconjugation, catalysis, nanodevices, imaging, and sensing.[1−14] Among the various metal clusters reported to date, Au and Ag based clusters are of special interest due to their ultrasmall size, nontoxicity, and high fluorescence.[1−4,15,16] While Au clusters have been extensively studied because of their intrinsic characteristics such as ease of preparation and chemical stability, Ag clusters, especially the fluorescent ones, are emerging as promising probes.

To date, few compounds have been employed to synthesize Ag clusters.[16−28] Polyelectrolytes such as poly(amidoamine) dendrimer, poly(methacrylic acid), and poly(acrylic acid) have been successfully used to create water-soluble fluorescent Ag nanoclusters.[22,23] Several thiolate stabilized Ag clusters have also been reported recently.[17−20] For example, Ag_7 clusters were synthesized by using 2,3-dimercaptosuccinic acid as an effective polyvalence (i.e., polydentate) ligand.[20] Biomolecules were also explored for this purpose due to their inherent biocompatibility.[24−28] Highly fluorescent and emission tunable Ag clusters encapsulated by DNA have been prepared by Dickson and co-workers.[25,26,28] However, these DNA-encapsulated Ag clusters were not stable under high salt concentration, which would prevent their further applications.

Proteins can play an important role in directing the synthesis of functional nanomaterials under mild conditions because the amine, carboxyl, and thiol gro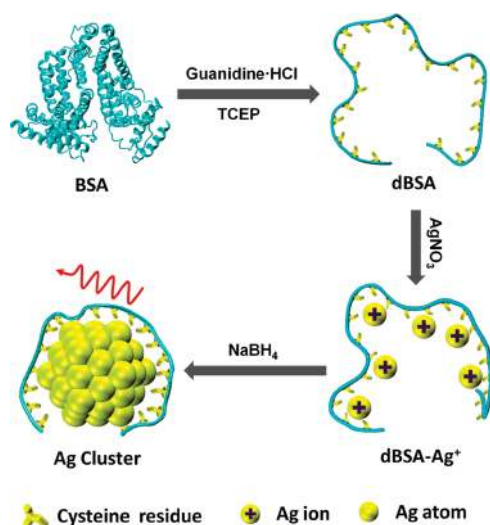ups in proteins can serve as effective stabilizing agents in such formulations.[29−35] For example, Xie and co-workers used native bovine serum albumin (BSA) to direct the synthesis of fluorescent Au clusters and further used them as fluorescent probes for sensitive and selective Hg^{2+} and Cu^{2+} ions sensing.[31,32] Recently, near-infrared light emitting luciferase-PbS quantum dots have also been reported,[33] and denatured human serum albumin (dHSA) as a polyvalence ligand has also been used for coating quantum dots to improve their hydrophilicity and stability.[34] Similar to the biomineralization process and Xie's protein-directed approach, we hypothesize that denatured BSA (dBSA) can provide the scaffolds necessary to interact with and sequester the inorganic ions during the formation of metal clusters.[31] Inspired by the protein-directed inorganic nanomaterial synthesis,[31−34] here we develop a new and facile approach to synthesize water-soluble, highly stable fluorescent Ag clusters, which are stabilized by dBSA via a wet-chemistry method (Scheme 1). The dBSA with its 35 cysteine residues can act as a polyvalent ligand to stabilize the metal core and lead to the formation of stable fluorescent Ag clusters in aqueous solutions.

It is known that Hg^{2+} is a highly toxic contaminant that exists in water, soil, and food.[36] Mercury can accumulate in organisms and interact with the thiol groups in protein to cause serious damage to the central nervous system and constitute a serious threat to human health and natural environment. Detecting

Scheme 1. Schematic of the Denatured Protein Directed Synthesis of Fluorescent Ag Clusters

collected with an Applied Biosystems Voyager-DE STR MALDI-TOF-MS instrument. High-resolution electrospray ionization mass spectrometry (ESI-MS) data were collected with a Micromass Q-TOF Ultima instrument.

Synthesis of dBSA Coated Ag Clusters. dBSA was prepared according to a reported method with some modifications.[34] Native BSA solution (100 μL, 50 mg/mL) was mixed with guanidine hydrochloride solution (40 μL, 6 M) and incubated for 15 min in an ice-bath. TCEP (200 μL, 10 mM) was then added, the mixture was further incubated for 45 min in an ice-bath to complete the BSA denaturation, and the excess saline (i.e., guanidine, TCEP, and chloride ion) in the denatured BSA solution was removed by washing with water and centrifugation. The final concentration of the dBSA was adjusted to ~50 mg/mL. Guanidine-denatured BSA or TCEP-denatured BSA was obtained according to the above approaches by using guanidine or TCEP alone as denaturing reagents, respectively.

Inspired by Xie's work,[31] the dBSA directed approach was employed to synthesize Ag clusters as follows. AgNO$_3$ solution (50 mM, 20 μL) was incubated with the dBSA solution (100 μL, 50 mg/mL) for 1 h in an ice-bath, then NaBH$_4$ (100 mM, 20 μL) was added, and the mixture was incubated overnight at 4 °C. The dBSA coated Ag clusters were obtained by further purification via centrifugation.

To examine the influence of different ligands on the synthesis of Ag clusters, reagents (including 100 μL of 50 mg/mL native BSA, 100 μL of 50 mg/mL guanidine-denatured BSA, 100 μL of 50 mg/mL TCEP-denatured BSA, 100 μL of 10 mM TCEP, and 100 μL of 2.4 M guanidine hydrochloride, respectively) other than dBSA were reacted with AgNO$_3$ and NaBH$_4$, under conditions otherwise identical for the preparation of dBSA coated Ag clusters.

To test for the long-term storage stability of the Ag clusters, as-prepared Ag cluster solution was lyophilized and stored. The long-term storage stability was studied by redispersing the lyophilized Ag clusters in water at different storage times and recording the time-dependent fluorescent intensity.

Hg^{2+} Detection Using dBSA Coated Ag Clusters. A typical Hg^{2+} detection procedure was conducted as follows. Hg^{2+} solution at different concentrations was obtained by serial dilution of the stock solution. The as-prepared Ag clusters solutions were diluted 10 times for Hg^{2+} detection. Typically, 40 μL of Hg^{2+} solutions with various concentrations were first mixed with 36 μL of PDCA (100 mM), followed by the addition of 4 μL of the as-prepared Ag cluster solution. After mixing for about 10 min, 320 μL of water was added to bring the final volume to 400 μL for fluorescence spectra measurements at room temperature.

To examine the influence of PDCA on the selectivity of the sensing system, 40 μL of 0.1 mM Hg^{2+} or 1 mM Cu^{2+} was mixed with dBSA coated Ag cluster solution directly and the fluorescent spectra was evaluated. To evaluate the selectivity of Hg^{2+} fluorescence detection by using dBSA coated Ag clusters, other metal ions such as Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+} were also tested and the response recorded and analyzed.

Hg^{2+} is undoubtedly a significant step in environment and health monitoring, and consequently, a variety of sensor platforms for Hg^{2+} detection constituting small fluorescent organic molecules, various polymers, liposomes, DNAzymes, proteins, oligonucleotides, inorganic materials, etc. have been developed.[37−43] Recently, metal fluorescent clusters formed from gold have been employed as probes to detect Hg^{2+}.[32,35,44,45] However, until now, none explored the protein-directed formation of Ag clusters and their application. Here we demonstrate the synthesis of dBSA coated Ag clusters with unique fluorescence in the red region of the visible spectrum and further use these clusters as sensitive and selective probes for label-free Hg^{2+} detection based on the specific fluorescence quenching of Ag clusters due to the 5d^{10}(Hg^{2+})−4d^{10}(Ag$^+$) metallophilic interaction. A detailed comparison between the developed sensor and those available in the literature is also provided (Tables S1 and S2 in the Supporting Information).

■ EXPERIMENTAL SECTION

Chemicals and Materials. Lyophilized bovine serum albumin, guanidine hydrochloride, silver nitrate (AgNO$_3$), sodium borohydride (NaBH$_4$), and 2,6-pyridinedicarboxylic acid (PDCA) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Tris-(2-carboxyethyl)phosphine hydrochloride (TCEP) was purchased from Calbiochem. Water (>18.2 MΩ cm) used for the experiments was purified by a Purelab system.

Instrumentation. Centrifugal filter devices were purchased from Millipore (5000 MWCO, 0.1 mL, catalog no. UFV5BCC00). Fluorescence emission spectra were recorded on a Cary Eclipse spectrophotometer (Varian). Absorption spectra were obtained with a Jasco V570 UV/visible/NIR spectrophotometer (Jasco, Inc., Easton, MD). X-ray photoelectron spectroscopy (XPS) spectra were recorded with a Kratos AXIS X-ray photoelectron spectrometer. Element analysis was performed with a Perkin-Elmer Elan DRCe inductively coupled plasma mass spectrometer (ICPMS). Matrix-assisted laser desorption/ionization-time-of-flight-mass spectrometry (MALDI-TOF-MS) data were

■ RESULTS AND DISCUSSION

Synthesis and Characterization of the dBSA Coated Ag Clusters. BSA was chosen as the model protein in this study because it is widely used and commercially available and has
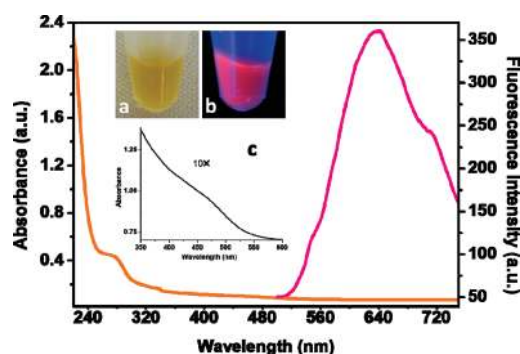
Figure 1. Absorption (yellow) and fluorescence emission (pink) spectra of the as-prepared Ag clusters. The excitation wavelength was 400 nm. Inset: the photographs of the as-prepared Ag clusters under visible (a) and UV light (b) and the magnified absorption spectrum (c).

Figure 2. Absorption $(---)$ and fluorescence emission $(—)$ spectra of the as-prepared Ag products with $NaBH_4$ in the presence of dBSA, guanidine-denatured BSA, TCEP-denatured BSA, BSA, TCEP, and guanidine, respectively.

Figure 3. Fluorescence intensity of dBSA coated Ag clusters measured at 637 nm for different concentrations of NaCl.

proven to work in the synthesis of fluorescent Au clusters.[31,32] BSA consists of 35 potential thiol groups as noted earlier, due to the 17 disulfide bonds and 1 free cysteine.[46] Hence, if these 35 cysteine residues could be completely liberated, they could act as chelating groups for sequestering metal ions (i.e., Ag^+) and as polyvalent ligands for passivating the surface of metallic materials (i.e., Ag clusters in this report) (see Scheme 1). The dBSA with 35 liberated cysteine residues was obtained by treating native BSA with protein denaturants such as guanidine and TCEP. The dBSA coated Ag clusters were then prepared by first sequestering Ag^+ with dBSA and then reducing Ag^+ to Ag^0 clusters with $NaBH_4$ (Scheme 1). The inset in Figure 1 shows that the formed Ag clusters solutions were light yellow in color under visible light (a), while it emitted a red fluorescence under UV light (b), possibly due to the quantum effect of the metal atom clusters.[4] From the optical absorption and photoemission spectra of these as-prepared Ag clusters (Figure 1), it could be concluded that no apparent surface plasmon resonance absorption peak in the range between 400 and 500 nm could be observed to indicate the existence of small Ag nanoparticles, while the fluorescence emission spectrum showed a peak centered at 637 nm. The photoluminescence quantum yield of the Ag cluster was ~1.2%, calculated with Rhodamine B as a reference with a 514 nm excitation source. To further confirm the formation of the dBSA coated Ag clusters, transmission electron microscopic images were obtained. As shown in Figure S1 in the Supporting Information, the as-prepared Ag clusters were approximately spherical in shape and about 1 nm in diameter.

To exclude the possibility that the observed fluorescence was from a transformed (i.e., oxidized or reduced) protein cage, we did the following experiments. First, oxidized dBSA was prepared by reacting dBSA with $Ce(NH_4)_2(NO_3)_6$, and reduced dBSA was synthesized by reacting dBSA with $NaBH_4$, respectively. Fluorescent emission of the products was then measured. As shown in Figure S2 in the Supporting Information, none of these products gave fluorescence emission, which further confirmed that the fluorescence was due to the Ag clusters.

Though MALDI-TOF-MS and ESI-MS are usually used to characterize the composition of metal clusters, conclusive evidence on the composition of the as-prepared Ag clusters was not possible (Figures S3 and S4 in the Supporting Information). Elemental analysis was therefore used to identify the composition of the as-prepared dBSA-capped Ag clusters (Table S3 in the Supporting Information). Since one BSA molecule has 35 Cys and 5 Met
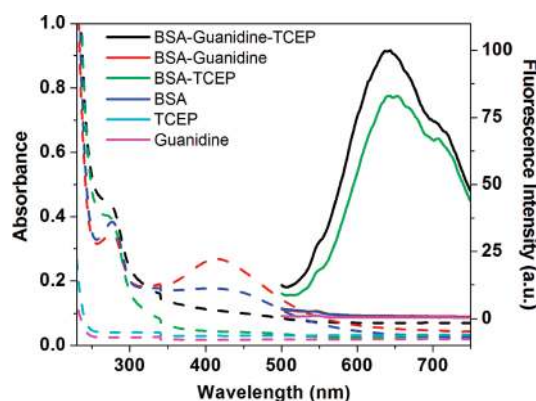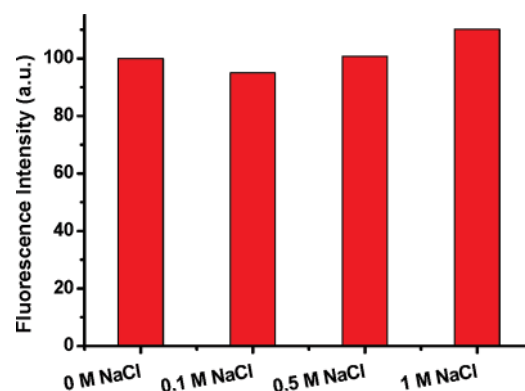
residues, a total of 40 S atoms are present in one molecule. Hence the Ag to BSA ratio can be calculated as Ag/dBSA = (1.16/107.9)/(0.4/(32.07 × 40)) = 34.48/1. On the basis of these calculations, an approximate composition of $Ag_{34}dBSA$ could be assigned to the as-prepared dBSA-capped Ag clusters. To further characterize the as-prepared Ag clusters, XPS spectra were collected. As shown in Figure S5 in the Supporting Information, the Ag 3d XPS spectrum could be deconvoluted into two distinct components: 369.202 eV (Ag 3d$^{5/2}$) and 375.202 eV (Ag 3d$^{3/2}$), typical of Ag^+, and 368.212 eV (Ag 3d$^{5/2}$) and 374.212 eV (Ag 3d$^{3/2}$), typical of Ag^0. The Ag^0 could be assigned to the core of the Ag clusters, while the Ag^+ could be assigned to the Ag atoms at the surface of Ag clusters. The above results indicated the successful synthesis of highly fluorescent Ag clusters.

To further validate the formation mechanism of the Ag clusters in Scheme 1, control experiments of Ag^+ reduction with $NaBH_4$ in the presence of several other ligands (i.e., guanidine hydrochloride-denatured BSA, TCEP-denatured BSA, native BSA, guanidine hydrochloride, and TCEP) were also performed. As shown in Figure 2, in the presence of either guanidine or TCEP, neither fluorescence emission spectra between 500 and 700 nm nor absorption spectra between 350 and 550 nm were observed, indicating that neither guanidine nor TCEP was effective in the formation of Ag particles or clusters. While, in the presence of native BSA, there was also no fluorescence
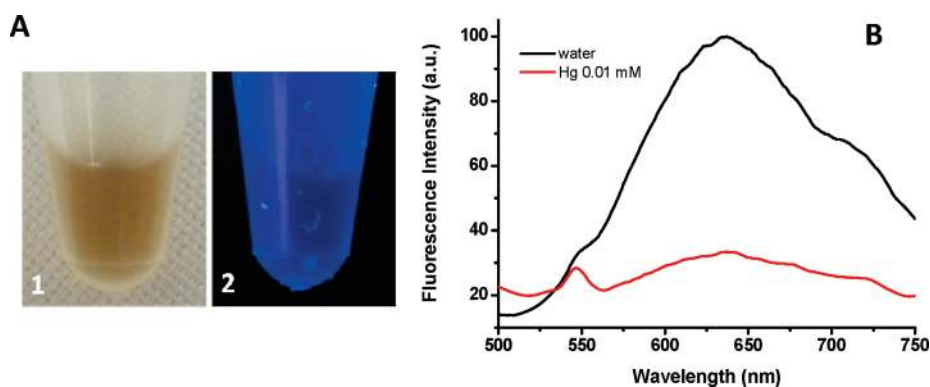
Figure 4. (A) Photographs of dBSA protected Ag clusters in the presence of 1 mM Hg^{2+} under visible (1) and UV light (2). (B) Fluorescence emission spectra of the as-prepared dBSA coated Ag clusters in the absence and presence of 0.01 mM Hg^{2+}.
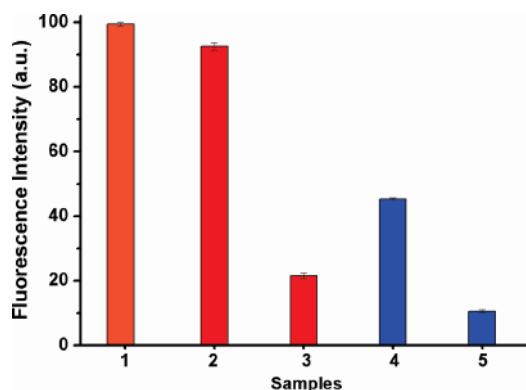

Figure 5. Fluorescence response of the dBSA coated Ag clusters to Hg^{2+} and Cu^{2+} in the presence and absence of PDCA at 637 nm. Samples 1−5 are Ag clusters alone (1), Ag clusters and Cu^{2+}/PDCA (2), Ag clusters and Hg^{2+}/PDCA (3), Ag clusters and Cu^{2+} (4), and Ag clusters and Hg^{2+} (5), respectively. Concentrations of Cu^{2+} and Hg^{2+} are 0.1 and 0.01 mM, respectively.

Figure 6. (A) Fluorescence emission spectra of dBSA coated Ag clusters in the absence and presence of different metal ions with PDCA. (B) Selectivity of the dBSA coated Ag clusters to different metal ions. The fluorescence intensities were recorded at 637 nm. For panel B, the concentration of Hg^{2+} is 0.01 mM; concentrations of the other ions are 0.1 mM.

emission; however, a weak and broad absorption peak could be noted between 350 and 550 nm, which indicated the absence of Ag clusters and signal the presence of Ag nanoparticles (larger than 2 nm) that could be formed with native BSA. Besides using the above untreated agents to participate in the Ag reaction, we further, respectively, adopted guanidine-denatured BSA and TCEP-denatured BSA to study the mechanism of Ag clusters formation. When Ag^+ was reduced by $NaBH_4$ in the presence of guanidine-denatured BSA, a higher absorption spectrum without fluorescence was observed, denoting the formation of Ag nanoparticles. When Ag^+ was reduced by $NaBH_4$ in the presence of TCEP-denatured BSA, a fluorescent emission peak centered at ∼640 nm was noted, which was similar to but weaker than the dBSA coated Ag clusters. Taken together, our results show that only dBSA (denatured by guanidine and TCEP) could stabilize fluorescent Ag clusters more effectively than the TCEP-denatured BSA while the Ag products stabilized by native BSA and guanidine-denatured BSA exhibited no fluorescent property (Figure 2).

The results obtained with different ligands could be explained as follows. When native BSA was used, the cysteine residues were buried within the intact structure of the protein and not accessible for the formation of Ag clusters. When BSA was treated with guanidi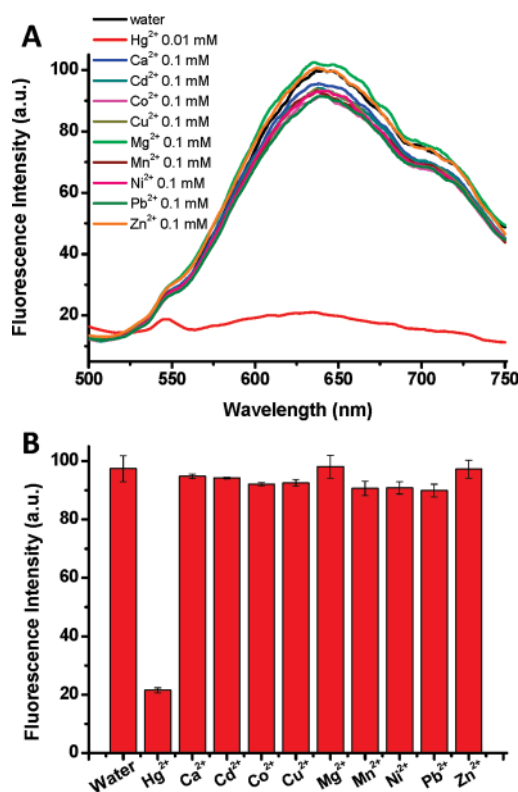ne, the tertiary and secondary structure of the protein was denatured due to the breakage of the hydrogen bonds to result in a decrease in the hydrophobic interaction. Although the guanidine-denatured BSA was looser than native BSA, most of cysteine residues were still not available for the interaction to form Ag clusters because of its existence as disulfide bonds (there are 17 disulfide bonds in cysteines). When BSA was treated with TCEP, only some of the disulfide bonds were reduced by TCEP due to the steric hindrance of the native BSA. These free cysteine residues could act as polyvalent ligands to form Ag clusters with weak fluorescence. If BSA was treated
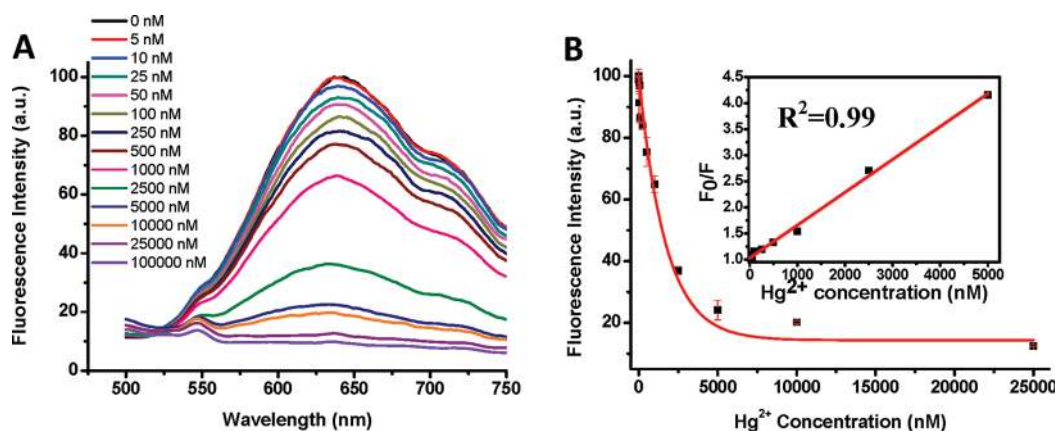
Figure 7. (A) Fluorescence emission spectra of the as-prepared dBSA coated Ag clusters in the absence and presence of Hg^{2+} with different concentration with PDCA. (B) The relative fluorescence intensity at 637 nm against Hg^{2+} concentration. The inset is the Stern–Volmer plot of fluorescence quenching of the dBSA coated Ag clusters by Hg^{2+}.

with guanidine and TCEP, all of the 35 cysteine residues were exposed and the dBSA can be considered as a linear polymer with thiol groups.[34] The resulting denatured dBSA could act as an effective polyvalent ligand to react with Ag^+ to further stabilize the fluorescent Ag clusters, as demonstrated above (also see Scheme 1).

To explore its potential application in sensing, one of the primary requirements is water-solubility and stability under high ionic strength environments. While biomolecules stabilized fluorescent metal clusters are inherently water-soluble, their stability in high ionic strength environments is still a challenge. We envision that the polyvalent interactions between the ligand and the metal core should provide enhanced stability for the as-prepared clusters. The normalized fluorescent spectra of the dBSA coated Ag clusters in the absence and presence of various concentrations of NaCl are shown in Figure 3. At 637 nm, the fluorescence intensities of the as-prepared Ag clusters at different concentrations of NaCl were almost the same as the one without NaCl, although a slight increase was noted for the clusters within 1 M NaCl. Our results indicate that the dBSA coated Ag clusters were highly stable even under high ionic conditions, as demonstrated by its performance in the presence of a high concentration of NaCl, which imparts a significant advantage in a range of applications. The as-prepared Ag clusters could be lyophilized and then stored in a solid form for a longer-term (Figure S7 in the Supporting Information). As shown in Figure S8 in the Supporting Information, the lyophilized Ag clusters were stable for at least 1 month and were ready for use by simply redissolving them when needed.

Detecting Hg^{2+} Using Ag Clusters as Fluorescent Probes. On the basis of the specific and strong $d^{10}-d^{10}$ metallophilic interaction $(5d^{10}(Hg^{2+})-5d^{10}(Au^+))$, sensitive and selective Hg^{2+} sensors were developed using Au clusters as fluorescent probes.[32,35] As discussed above, since the surface of the as-prepared Ag clusters were covered by Ag^+, we reasoned that Hg^{2+} would also quench its fluorescence via the same $d^{10}-d^{10}$ metallophilic interaction $(5d^{10}(Hg^{2+})-4d^{10}(Ag^+)$ in this case). When a certain amount of Hg^{2+} was added to the dBSA coated Ag clusters, the original light yellow color of the Ag clusters turned brown (Figures 1a and 4A,1); meanwhile the fluorescent emission under UV lamp illumination was much weaker (Figures 1b and 4A,2). Correspondingly, this phenomenon

reflected an apparent decrease in emission at 637 nm in the fluorescence spectra of Ag clusters (Figure 4B). The proposed sensing mechanism was further supported by the following experiment. As shown in Figure S9 in the Supporting Information, the XPS data showed that the Hg ions were still in the +2 valence state after its quenching reaction. Thus, we attributed the fluorescence quenching to $5d^{10}(Hg^{2+})-4d^{10}(Ag^+)$ metallophilic interaction, which was consistent with previous reports.[32,35]

To study the selectivity of the as-prepared Ag clusters, several divalent metal ions (Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}) were tested as a control. Among these ions, Cu^{2+} could also quench the fluorescence of the as-prepared Ag clusters to a certain degree at higher concentrations and interfere with Hg^{2+} detection (Figure 5). The reason for this mechanism to be hypothesized is as follows. In the presence of dBSA, amino acid residues (e.g., tryptophan and tyrosine) in the protein could reduce Cu^{2+} to Cu^+.[47] The Cu^+ could react with Ag^+ at the surface of the metal clusters via the $3d^{10}(Cu^+)-4d^{10}$-(Ag^+) metallophilic interactions to induce fluorescence quenching, as reported previously.[32,35,48] Therefore, 2,6-pyridinedicarboxylic acid (PDCA),[38] an efficient chelating reagent for certain divalent metal ions, was chosen as the masking ligand to minimize the interference of Cu^{2+} and improve the selectivity of Hg^{2+} detection (Figure 5). As shown in Figure 6, in the presence of PDCA, all of the other ions including Cu^{2+} at the concentration of 0.1 mM could not quench the fluorescence of Ag clusters, while only 0.01 mM Hg^{2+} could effectively quench the fluorescence effectively. These results indicate that in the presence of PDCA, the as-prepared Ag clusters display excellent selectivity toward Hg^{2+} ions.

On the basis of the above results, the sensitivity and linearity of the dBSA coated Ag clusters–Hg^{2+} system was evaluated by varying the Hg^{2+} concentration in the presence of PDCA. As shown in Figure 7A, the fluorescence emission intensity was sensitive and proportionately decreased with an increasing concentration of Hg^{2+} as noted from the relationship between the fluorescent intensity at 637 nm and Hg^{2+} concentration (Figure 7B). The linear relation (range was 10 and 5000 nM) with an r^2 of 0.996 could be described by the Stern–Volmer equation, $F_0/F = 1 + K_{SV}[Q]$, where F_0 and F denote the fluorescent intensity at 637 nm with and without Hg^{2+}, respectively (inset of Figure 7B). Under the current experimental

condition, the lowest Hg^{2+} concentration that could be detected was 10 nM, which meets the limit of detection requirement of Hg^{2+} in drinking water by the U.S. Environmental Protection Agency (Table S2 in the Supporting Information).[38,41,49]

■ CONCLUSIONS

In summary, a convenient approach for the synthesis of stable, water-soluble, fluorescent Ag clusters was demonstrated by using guanidine and TCEP denatured BSA as the stabilizing agent. Because of the polyvalent interactions between the exposed thiol groups in dBSA and the metal core, the fabricated dBSA coated Ag clusters are about 1 nm in size. They possess excellent fluorescence emission (noted at 637 nm) and high stability even when exposed to high ionic conditions (up to 1 M NaCl). The Ag clusters could be lyophilized for longer-term storage. The dBSA coated Ag clusters were further used for sensitive and selective detection of Hg^{2+} via a specific $d^{10}-d^{10}$ metallophilic interaction between Hg^{2+} and Ag^+ onto the dBSA coated Ag clusters. The as-prepared clusters were not only biocompatible but were found to be highly stable and water-soluble. The protein capping could also avail numerous functional groups, which would facilitate further modifications for bioimaging.

■ ASSOCIATED CONTENT

⑤ 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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■ ACKNOWLEDGMENT

■ REFERENCES

(1) Whetten, R. L.; Khoury, J. T.; Alvarez, M. M.; Murthy, S.; Vezmar, I.; Wang, Z. L.; Stephens, P. W.; Cleveland, C. L.; Luedtke, W. D.; Landman, U. *Adv. Mater.* **1996**, *8*, 428–433.

(2) Whetten, R. L.; Shafigullin, M. N.; Khoury, J. T.; Schaaff, T. G.; Vezmar, I.; Alvarez, M. M.; Wilkinson, A. *Acc. Chem. Res.* **1999**, *32*, 397–406.

(3) Templeton, A. C.; Wuelfing, M. P.; Murray, R. W. *Acc. Chem. Res.* **2000**, *33*, 27–36.

(4) Zheng, J.; Nicovich, P. R.; Dickson, R. M. *Annu. Rev. Phys. Chem.* **2007**, *58*, 409–431.

(5) Peyser, L. A.; Vinson, A. E.; Bartko, A. P.; Dickson, R. M. *Science* **2001**, *291*, 103–106.

(6) Jadzinsky, P. D.; Calero, G.; Ackerson, C. J.; Bushnell, D. A.; Kornberg, R. D. *Science* **2007**, *318*, 430–433.

(7) Heaven, M. W.; Dass, A.; White, P. S.; Holt, K. M.; Murray, R. W. *J. Am. Chem. Soc.* **2008**, *130*, 3754–3755.

(8) Muhammed, M. A. H.; Verma, P. K.; Pal, S. K.; Kumar, R. C. A.; Paul, S.; Omkumar, R. V.; Pradeep, T. *Chem.—Eur. J.* **2009**, *15*, 10110–10120.

(9) Lei, Y.; Mehmood, F.; Lee, S.; Greeley, J.; Lee, B.; Seifert, S.; Winans, R. E.; Elam, J. W.; Meyer, R. J.; Redfern, P. C.; Teschner, D.; Schlogl, R.; Pellin, M. J.; Curtiss, L. A.; Vajda, S. *Science* **2010**, *328*, 224–228.

(10) Schmid, G. *Chem. Soc. Rev.* **2008**, *37*, 1909–1930.

(11) Yu, J. H.; Choi, S.; Dickson, R. M. *Angew. Chem., Int. Ed.* **2009**, *48*, 318–320.

(12) Maretti, L.; Billone, P. S.; Liu, Y.; Scaiano, J. C. *J. Am. Chem. Soc.* **2009**, *131*, 13972–13980.

(13) Huang, C. C.; Chen, C. T.; Shiang, Y. C.; Lin, Z. H.; Chang, H. T. *Anal. Chem.* **2009**, *81*, 875–882.

(14) Guo, W. W.; Yuan, J. P.; Dong, Q. Z.; Wang, E. K. *J. Am. Chem. Soc.* **2010**, *132*, 932–934.

(15) Jin, R. C. *Nanoscale* **2010**, *2*, 343–362.

(16) Xu, H. X.; Suslick, K. S. *Adv. Mater.* **2010**, *22*, 1078–1082.

(17) Cathcart, N.; Mistry, P.; Makra, C.; Pietrobon, B.; Coombs, N.; Jelokhani-Niaraki, M.; Kitaev, V. *Langmuir* **2009**, *25*, 5840–5846.

(18) Mrudula, K. V.; Rao, T. U. B.; Pradeep, T. *J. Mater. Chem.* **2009**, *19*, 4335–4342.

(19) Bakr, O. M.; Amendola, V.; Aikens, C. M.; Wenseleers, W.; Li, R.; Dal Negro, L.; Schatz, G. C.; Stellacci, F. *Angew. Chem., Int. Ed.* **2009**, *48*, 5921–5926.

(20) Wu, Z.; Lanni, E.; Chen, W.; Bier, M. E.; Ly, D.; Jin, R. *J. Am. Chem. Soc.* **2009**, *131*, 16672–16674.

(21) Zhang, J. G.; Xu, S. Q.; Kumacheva, E. *Adv. Mater.* **2005**, *17*, 2336–2340.

(22) Shang, L.; Dong, S. J. *Chem. Commun.* **2008**, 1088–1090.

(23) Zheng, J.; Dickson, R. M. *J. Am. Chem. Soc.* **2002**, *124*, 13982–13983.

(24) Yu, J.; Patel, S. A.; Dickson, R. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 2028–2030.

(25) Petty, J. T.; Zheng, J.; Hud, N. V.; Dickson, R. M. *J. Am. Chem. Soc.* **2004**, *126*, 5207–5212.

(26) Vosch, T.; Antoku, Y.; Hsiang, J. C.; Richards, C. I.; Gonzalez, J. I.; Dickson, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 12616–12621.

(27) Gwinn, E. G.; O'Neill, P.; Guerrero, A. J.; Bouwmeester, D.; Fygenson, D. K. *Adv. Mater.* **2008**, *20*, 279–283.

(28) Richards, C. I.; Choi, S.; Hsiang, J. C.; Antoku, Y.; Vosch, T.; Bongiorno, A.; Tzeng, Y. L.; Dickson, R. M. *J. Am. Chem. Soc.* **2008**, *130*, 5038–5039.

(29) Meldrum, F. C.; Wade, V. J.; Nimmo, D. L.; Heywood, B. R.; Mann, S. *Nature* **1991**, *349*, 684–687.

(30) Dickerson, M. B.; Sandhage, K. H.; Naik, R. R. *Chem. Rev.* **2008**, *108*, 4935–4978.

(31) Xie, J. P.; Zheng, Y. G.; Ying, J. Y. *J. Am. Chem. Soc.* **2009**, *131*, 888–889.

(32) Xie, J. P.; Zheng, Y. G.; Ying, J. Y. *Chem. Commun.* **2010**, *46*, 961–963.

(33) Ma, N.; Marshall, A. F.; Rao, J. H. *J. Am. Chem. Soc.* **2010**, *132*, 6884–6885.

(34) Wu, Y. Z.; Chakrabortty, S.; Gropeanu, R. A.; Wilhelmi, J.; Xu, Y.; Er, K. S.; Kuan, S. L.; Koynov, K.; Chan, Y.; Weil, T. *J. Am. Chem. Soc.* **2010**, *132*, 5012–5014.

(35) (a) Wei, H.; Wang, Z. D.; Yang, L. M.; Tian, S. L.; Hou, C. J.; Lu, Y. *Analyst* **2010**, *135*, 1406–1410. (b) Lin, Y.-H.; Tsen, W.-L. *Anal. Chem.* **2010**, *82*, 9194–9200.

(36) Valko, M.; Morris, H.; Cronin, M. T. D. *Curr. Med. Chem.* **2005**, *12*, 1161–1208.

(37) Chen, Y. Q.; Bai, H.; Hong, W. J.; Shi, G. Q. *Analyst* **2009**, *134*, 2081–2086.

(38) Li, D.; Wieckowska, A.; Willner, I. *Angew. Chem., Int. Ed.* **2008**, *47*, 3927–3931.

(39) Yigit, M. V.; Mishra, A.; Tong, R.; Cheng, J. J.; Wong, G. C. L.; Lu, Y. *Chem. Biol.* **2009**, *16*, 937–942.

(40) Huang, C. C.; Yang, Z.; Lee, K. H.; Chang, H. T. *Angew. Chem., Int. Ed.* **2007**, *46*, 6824–6828.

(41) Wang, Z. D.; Lee, J. H.; Lu, Y. *Chem. Commun.* **2008**, 6005–6007.

(42) Chen, P.; He, C. A. *J. Am. Chem. Soc.* **2004**, *126*, 728–729.

(43) Yoon, S.; Miller, E. W.; He, Q.; Do, P. H.; Chang, C. J. *Angew. Chem., Int. Ed.* **2007**, *46*, 6658–6661.

(44) Guo, W. W.; Yuan, J. P.; Wang, E. K. *Chem. Commun.* **2009**, 3395–3397.

(45) Hu, D. H.; Sheng, Z. H.; Gong, P.; Zhang, P. F.; Cai, L. T. *Analyst* **2010**, *135*, 1411–1416.

(46) Hirayama, K.; Akashi, S.; Furuya, M.; Fukuhara, K. *Biochem. Biophys. Res. Commun.* **1990**, *173*, 639–646.

(47) Wiechelman, K. J.; Braun, R. D.; Fitzpatrick, J. D. *Anal. Biochem.* **1988**, *175*, 231–237.

(48) Hermann, H. L.; Boche, G.; Schwerdtfeger, P. *Chem.—Eur. J.* **2001**, *7*, 5333–5342.

(49) http://water.epa.gov/drink/contaminants/basicinformation/mercury.cfm#four

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