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Facile synthesis of red-emitting lysozyme-stabilized Ag nanoclusters†

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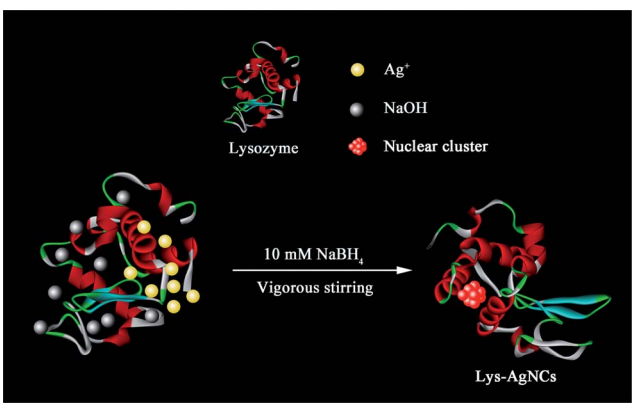
A facile approach was developed to prepare positively charged and red-emitting lysozyme-stabilized Ag nanoclusters (Lys-AgNCs) using $NaBH_4$ as a reducing agent at room temperature. The Lys-AgNCs can be applied in the highly selective detection of Hg^{2+}.

Noble metal nanoclusters, due to their unique physical, electrical and optical properties, have attracted a great deal of attention in recent years for their application in catalysis, chemical sensors, electronic devices and biological imaging.[1–4] Metal nanoclusters, consisting of several to tens of metal atoms, possess sizes approaching the Fermi wavelength of electrons and they exhibit a marked photoluminescence property due to discrete energy levels and quantum confinement.[5,6] Among metal nanoclusters, Au nanoclusters (AuNCs) and Ag nanoclusters (AgNCs) are attractive candidates due to their ultrafine size, bright fluorescence and low toxicity.[7,8] Fluorescent AuNCs have some intrinsic characteristics such as relative ease of preparation and high chemical stability in ambient conditions. However, AgNCs are not stable in aqueous solution because of their high surface energy, resulting in aggregation and the formation of larger nanoparticles.[9] To avoid aggregation, various stabilizers such as dendrimers,[10] polymers,[11,12] DNA,[13,14] peptides,[15–17] as well as small molecules containing thiols[18–20] are applied as capping agents for AgNCs.

In recent years, proteins used as templates have played an important role in the synthesis of noble metal nanoclusters due to their milder reaction conditions, size control and highly specific or multiple functions.[21,22] Various noble metal nanoclusters have been obtained using proteins as templates. Xie's group[23] first reported a simple route for directly preparing red-emitting fluorescent AuNCs using bovine serum albumin (BSA) as both a reducing agent and a capping agent under alkaline conditions (pH 12). Inspired by this finding, several other proteins, such as lysozyme (Lys),[24,25] lactotransferrin,[26] insulin,[27] pepsin,[28] as well as horseradish peroxidase,[29] have also been applied as templates for the preparation of fluorescent AuNCs. Compared with the synthesis of fluorescent AuNCs, there have been only a few reports concerning the preparation of fluorescent AgNCs using proteins as capping agents. For example, the synthesis of fluorescent AgNCs conjugated to α-chymotrypin *via* $NaBH_4$ reduction,[30] the synthesis of fluorescent AgNCs confined in denatured BSA as a stabilizing agent using $NaBH_4$,[31] and the synthesis of Ag_{15}@BSA using $NaBH_4$ (ref. 32) have been reported. However, Ag_{15}@BSA is less stable and the stability could be enhanced by the use of poly(vinyl pyrrolidone) as a stabilizing agent.[32] Ease of preparation and high stability under ambient conditions still remain as big challenges in the synthesis of AgNCs using a protein template.

Lys (pI = 11.3) is a familiar small enzyme consisting of 129 amino acid residues with free carboxylic groups, amino groups and four disulfide bonds, and has been used to prepare Au nanoparticles,[33–35] Ag nanoparticles[36] and AuNCs.[24,25] In our study, we developed a facile approach to synthesize fluorescent AgNCs with red emission using Lys as a capping agent and $NaBH_4$ as a reducing agent at room temperature. The AgNCs were water soluble, had a positive surface charge, and had good stability, which was kept through adjusting the pH using acetic acid. To the best of our knowledge, this is the first report of the synthesis of fluorescent AgNCs with positive surface charge using a protein as capping agent. Under vigorous stirring in an aqueous alkaline solution (~pH 12), the Lys-stabilized Ag nanoclusters (Lys-AgNCs) were prepared using $NaBH_4$ as a reducing agent in the presence of Ag^+ and Lys (see Scheme 1). An alkaline medium played an important role in preparing the Lys-AgNCs. In



Scheme 1 Schematic illustration of the preparation process for Lys-AgNCs.

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the alkaline solution, the disulfide bonds in the Lys molecule can be cleaved,[37] and the broken disulfide bonds exhibited the excellent ability of stabilizing the nucleated cluster. Fig. S1† shows that characteristic surface plasmon resonance absorption appeared at around 425 nm without the addition of NaOH, due to the generation of large Ag nanoparticles. The as-prepared Lys-AgNCs were characterized by UV–visible absorption and fluorescence spectra as indicated in Fig. 1. It can be observed that the UV–visible absorption spectrum of Lys-AgNCs exhibited a peak at 480 nm due to quantum confinement effects, but no characteristic surface plasmon resonance peak for larger Ag nanoparticles at around 425 nm. The fluorescence spectra of Lys-AgNCs show an emission peak at 605 nm with two excitation maxima at 430 nm and 480 nm. The inset of Fig. 1 shows that the solution of Lys-AgNCs was orange–yellow in color under visible light, while it exhibited a bright red emission under UV light at 365 nm. The lifetime of Lys-AgNCs was obtained by numerical fitting of the emission at 605 nm as shown in Fig. S2.† The fluorescence intensity decay shows three components at 2.15 ns (34%), 7.38 ns (37.5%) and 0.13 ns (28.5%). According to the integrating sphere approach,[38] the absolute quantum yield of the fluorescent AgNCs was 1.3%.

A transmission electron microscope (TEM) was used to characterize the morphology of Lys-AgNCs, and their size distribution was observed using dynamic light scattering (DLS). As shown in Fig. 2(a–c), the TEM image indicates that Lys-AgNCs were spherical in shape and well dispersed with an average diameter of 1.5 nm, while the hydrodynamic diameter measured using DLS was about 7 nm. The zeta potential of Lys-AgNCs determined at neutral pH was found to be ~+30 mV, revealing that the surface charge of Lys-AgNCs was positive due to the capping agent Lys (pI = 11.3). The AgNCs could maintain excellent stability due to a strong electrostatic repulsion.

The surface chemistry of Lys-AgNCs was studied using Fourier transform infrared (FT-IR) spectroscopy and X-ray photoelectron spectroscopy (XPS). The FT-IR spectra as shown in Fig. S3† show that there was no S–H stretching band for the natural Lys, since natural Lys only contains four disulfide bonds without a free hydrosulfide group. However, an S–H stretching band around 2471 cm^{-1} appeared after the natural Lys was incubated in a solution of pH about 12, since the alkali could cleave the disulfide bonds of Lys and release free hydrosulfide groups.[37] The S–H stretching band at

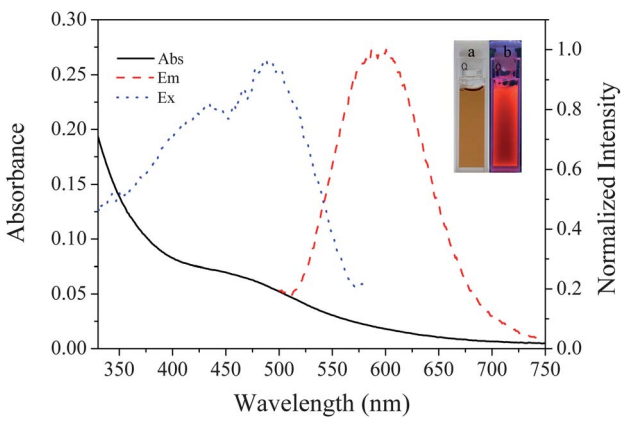

Fig. 1 UV–visible absorption spectrum and fluorescence spectra of the as-prepared Lys-AgNCs. Inset: photographs of Lys-AgNCs under visible light (a) and UV light at 365 nm (b).

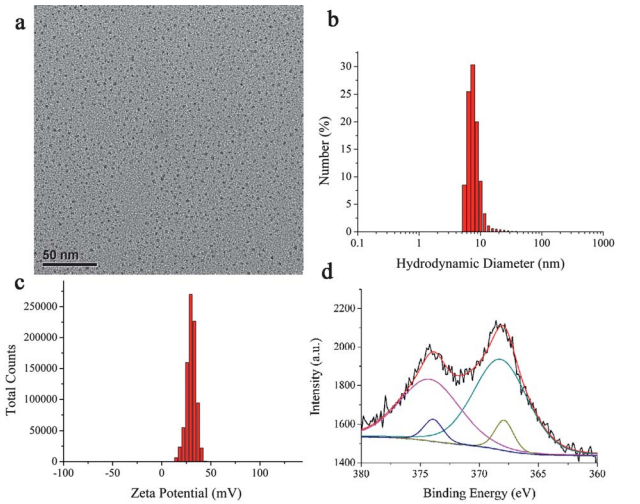

Fig. 2 (a) TEM image of Lys-AgNCs; (b) hydrodynamic diameter measured using DLS; (c) zeta potential of Lys-AgNCs at neutral pH; and (d) Ag 3d XPS spectrum of Lys-AgNCs deposited on a silica wafer.

2471 cm^{-1} in the FT-IR spectra disappeared after the formation of Lys-AgNCs, indicating that Lys was modified on the surface of AgNCs through Ag–S interactions. The XPS survey spectrum of Lys-AgNCs suggested the presence of the expected elements (see Fig. S4†). As shown in Fig. 2d, two fitting peaks at 368.2 eV and 374.2 eV were observed in the Ag 3d XPS spectrum of Lys-AgNCs, corresponding to Ag $3d_{5/2}$ and Ag $3d_{3/2}$, respectively, indicating the existence of Ag(0) in the nuclear cluster. The composition of the nuclear cluster was studied using matrix-assisted laser desorption ionization time-of-flight mass spectrometry. As shown in Fig. S5,† a marked mass peak around m/z 14.3 kDa corresponded with the molecular weight of Lys. Unfortunately, no mass shift was observed between Lys-AgNCs and Lys. The reason might have been that the grown clusters detached from the Lys capping agent during ionization since Lys is a small protein and laser irradiation might have cleaved the S–C and Ag–S bonds.[4,22]

The influences of the Lys-AgNCs synthesis conditions, including the concentrations of $NaBH_4$ and Lys, were investigated. The amount of $NaBH_4$ played an important role in the Lys-AgNCs synthesis. No characteristic absorption band could be found for AgNCs in the presence of only Ag^+ and Lys in a solution of pH about 12 (see Fig. S1†). Similarly, no emission peak around 500–750 nm could be found in the corresponding emission spectrum. After the addition of 20 mM $NaBH_4$, a broad absorption band appeared around 480 nm, and an emission peak at 605 nm was simultaneously demonstrated, as shown in Fig. S6.† Increasing the amount of 20 mM $NaBH_4$ to 10 μL caused the broad absorption band around 480 nm to gradually increase, while the corresponding emission intensity at 605 nm also gradually increased. After that, a new absorption peak at around 425 nm appeared, corresponding to the surface plasmon resonance absorption of large Ag nanoparticles. A TEM image also showed that large Ag nanoparticles were generated as shown in Fig. S7a,† revealing that an excess amount of the reducing agent could lead to the generation of large Ag nanoparticles. The concentrations of Lys and Ag^+ were crucial in the preparation of Lys-AgNCs. At a constant Ag^+ concentration, Lys-AgNCs could be obtained with different concentrations of Lys. As indicated in Fig. S8,† a high concentration of Lys (15 to 30 mg mL^{-1}) was required for the effective protection of the

AgNCs. A lower Lys concentration (≤ 5 mg mL^{-1}) led to the aggregation of the AgNCs and the generation of large nanoparticles without fluorescence.

The as-prepared Lys-AgNCs were not stable under the synthesis conditions. As shown in Fig. S9a,† the fluorescence intensity of the freshly prepared Lys-AgNCs gradually decreased as the incubation time under the synthesis conditions increased, and they exhibited little fluorescence after incubation for 6 h. A TEM image (Fig. S7b†) shows that the average diameter of Lys-AgNCs remained almost constant after incubation for 6 h, indicating that the fluorescence quenching did not result from the aggregation of the nuclear cluster. The reason might be that the alkali could etch the nuclear cluster and generate the formation of a non-fluorescent precipitate of AgOH under the synthesis conditions. To obtain more stable Lys-AgNCs, the alkaline solution was neutralized by the drop-wise addition of 1 M acetic acid, and then the resulting solution was dialyzed in membrane tubing with a molecular weight cut-off of 12 kDa against ultrapure water at room temperature for one day. The dialyzed Lys-AgNCs were more stable compared with freshly prepared Lys-AgNCs. As shown in Fig. S9b,† at room temperature, only a 23% decrease in the fluorescence intensity was observed after 2 weeks of storage in a dark place.

Hg^{2+} is a highly toxic contaminant, which can accumulate in the human body and cause brain damage and various chronic diseases.[39,40] It is essential therefore that environmental monitoring should be able rapidly to detect Hg^{2+} concentration. Due to the $5d^{10}(Hg^{2+})$–$4d^{10}(Ag^{+})$ metallophilic interaction,[31] Lys-AgNCs could be considered as a fluorescent probe using the fluorescence quenching characteristics for the determination of Hg^{2+}. To evaluate the sensitivity of Lys-AgNCs towards Hg^{2+}, different concentrations of Hg^{2+} (1 to 50 μM) were added to 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer solution (pH 7) containing Lys-AgNCs. As shown in Fig. 3a, the fluorescence intensity of Lys-AgNCs at 605 nm gradually decreased with increasing Hg^{2+} concentration. A linear correlation existed between the value of $(F_0 - F)/F_0$ and the Hg^{2+} concentration in the range from 1 to 15 μM with a correlation coefficient (R^2) of 0.9926. The limit of detection (at a signal-to-noise ratio of 3) for Hg^{2+} was 0.6 μM. To test the selectivity of Lys-AgNCs in the determination of Hg^{2+}, the effect of the metal ions Cu^{2+}, Pb^{2+}, Cd^{2+}, Cr^{3+}, Zn^{2+}, Na^{+}, K^{+}, Al^{3+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Ba^{2+}, Pd^{2+}, Fe^{3+} and Fe^{2+} was studied. As indicated in Fig. 3b, Hg^{2+} could quench the fluorescence intensity of Lys-AgNCs in a significant manner, while the other metal ions (except Cu^{2+}) presented only a slight quenching effect. In order to exclude the interference of Cu^{2+}, 2,6-pyridinedicarboxylic acid (PDCA) was used as an efficient chelating reagent.[40] Fig. 3b shows that the effect of Cu^{2+} could be obviously reduced in the presence of 1 mM PDCA. These results revealed that Lys-AgNCs were highly selective towards Hg^{2+}.

In conclusion, we have developed a facile approach for the preparation of Lys-AgNCs with positive charge and red emission using $NaBH_4$ as a reducing agent at room temperature. To the best of our knowledge, this is the first report concerning the synthesis of fluorescent AgNCs with positive surface charge using a protein as a capping agent. The Lys-AgNCs were characterized using UV–visible spectroscopy, FT-IR spectroscopy, TEM, DLS and XPS, and the experimental conditions were optimized to derive Lys-AgNCs with a higher fluorescence emission. The AgNCs exhibited excellent water solubility, large Stokes shifts, were well dispersed and had good stability, which was kept through adjusting the pH using acetic acid.

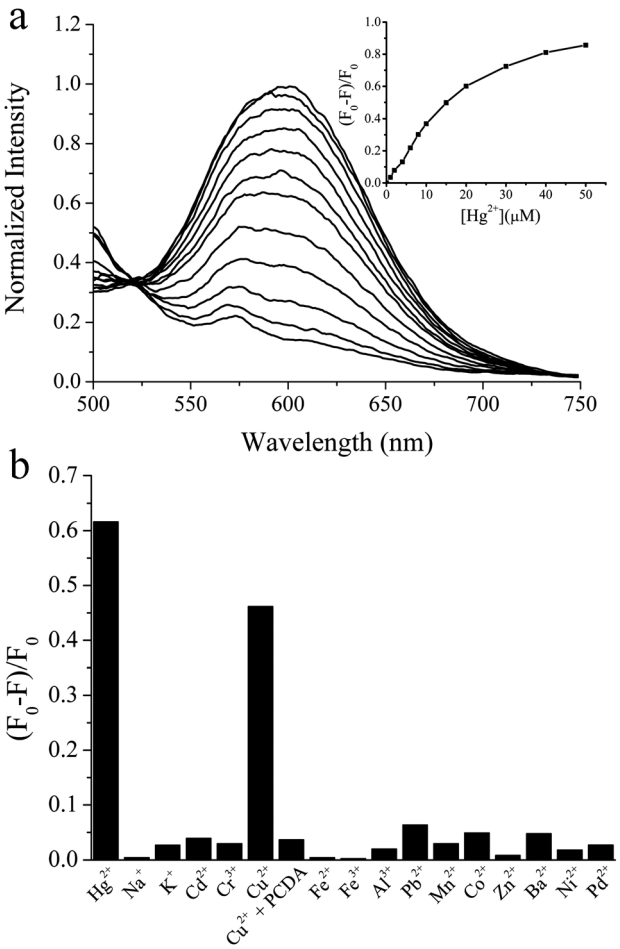

Fig. 3 (a) Emission spectra of Lys-AgNCs in the presence of various concentrations of Hg^{2+} in 20 mM HEPES buffer solution (pH 7) (from top: 0, 1, 2, 4, 6, 8, 10, 15, 20, 30, 40 and 50 μM). Inset: the relationship between relative fluorescence intensity and Hg^{2+} concentration. (b) Selectivity of Lys-AgNCs towards Hg^{2+} over other metal ions in 20 mM HEPES buffer solution (pH 7). The Hg^{2+} concentration was 20 μM, while the concentration of the other metal ions was 50 μM.

The Lys-AgNCs could be applied in the highly selective determination of Hg^{2+} based on their fluorescence quenching due to the $5d^{10}(Hg^{2+})$–$4d^{10}(Ag^{+})$ metallophilic interaction.

Acknowledgements

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