

Electronic Supplementary Information on

Facile synthesis of red-emitting lysozyme-stabilized Ag nanoclusters

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Experimental Section

Materials

Silver nitrate (ACS, 99.9+%) was purchased from Alfa Aesar; Lys (egg white) was ordered from Sangon Biotech Co., Ltd.; and NaBH₄ (96%) and other chemicals were obtained from Sinopharm Chemical Reagent Co., Ltd. Cellulose membrane dialysis tubing (avg. flat width 35 mm, M.W.>12.000) was purchased from Sigma. All the reagents were used as received without further purification. Ultra-pure water with a resistivity 18.2 MΩ·cm obtained from a Millipore purification system was used for the experiments.

Synthesis of Lys-AgNCs

Lys-AgNCs were prepared using a procedure modified from that used by Mathew et al.^{S1} for the synthesis of Ag₁₅@BSA. In a typical synthesis, 1 mL of 10 mM silver nitrate solution was added to a 5 mL water solution containing 75 mg Lys with vigorous stirring at room temperature. The mixture solution was left to incubate for 5 minutes under vigorous stirring before the drop-wise addition of NaOH solution (1 M, 0.3 mL). After that, 10 mM NaBH₄ was added drop-wise to the solution and the reaction mixture was allowed to proceed for another 15 minutes under vigorous stirring. To collect clean and stable Lys-AgNCs, 0.5 mL 1M acetic acid was introduced to the as-prepared Lys-AgNCs solution and the resulting solution was dialyzed in membrane tubing with a molecular weight cut-off of 12 KDa against ultrapure water at room temperature. After three changes of ultrapure water, a clean solution containing Lys-AgNCs was obtained.

Characterization

UV-Vis absorption and fluorescence spectra were recorded with a UV2550 UV-Vis spectrophotometer (Shimadzu, Japan) and an F-4500 spectrophotometer (Hitachi, Japan),

respectively. Fourier transform infrared (FT-IR) spectra were recorded with a Nicolet 330 spectrophotometer (Thermo Electron Corp., USA). The fluorescence lifetime was determined on a FluoroMax-4 spectrofluorometer (Horiba Jobin Yvon, France). The Absolute quantum yield was measured using the integrating sphere approach^{S2} on a FluoroMax-4 spectrofluorometer equipped with an F-3018 integrating sphere accessory (Horiba Jobin Yvon, France). Ultra-pure water was used to be blank reference for Lys-AgNCs. The background excitation and fluorescence emission spectra of blank reference were obtained before the excitation and fluorescence emission spectra of Lys-AgNCs were recorded. Horiba Jobin Yvon Quantum Yield and Colour Calculator v.3 software was then used to calculate the quantum yield. Transmission electron microscope (TEM) images were collected using a TECNAI F-30 (Philips-FEI, Netherlands). The samples for TEM were prepared by dropping the dialyzed Lys AgNCs on amorphous carbon films supported on a copper grid and dried. Dynamic light scattering (DLS) and zeta potential of the Lys-AgNCs were determined using a Nano-ZS (Malvern Instruments, UK). X-ray photoelectron spectroscopy (XPS) measurements were performed on a PHI Quantum 2000 XPS system (Physical Electronics, USA). Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrum analysis was conducted with a Bruker Daltonics - microflex MALDI TOF-MS (Bruker Daltonics, Germany). Mass spectra were collected in positive mode and the MALDI samples were prepared by mixing Lys or dialyzed Lys-AgNCs with an equal volume of sinapic acid.

Fluorescence detection of Hg²⁺

Hg (NO₃)₂ aqueous solution with different concentrations was freshly prepared before use. For quenching studies, the same amount of Lys-AgNCs and different concentration Hg(NO₃)₂ were mixed in 20 mM HEPES buffer solution (pH 7) and equilibrated for 10 min before the spectral measurements. The fluorescence spectra were recorded by operating the fluorescence spectrophotometer with an excitation wavelength of 480 nm.

Selectivity measurements

To investigate the cation selectivity of Lys-AgNCs to Hg²⁺, the following inorganic salts were used: sodium nitrate, potassium nitrate, chromic nitrate, copper nitrate, iron (III) nitrate, lead acetate, zinc acetate, cadmium chloride, nickelous chloride, aluminum chloride, cobalt chloride, barium chloride, palladium nitrate and manganese chloride. The solutions of Lys-AgNCs were mixed with an appropriate volume of each inorganic salts solution in 20 mM HEPES buffer solution (pH 7) and equilibrated for 10 min before spectral measurement. The fluorescence spectra

were recorded by operating the fluorescence spectrophotometer with an excitation wavelength of 480 nm.

References

S1. A. Mathew, P. R. Sajanalal and T. Pradeep, *J. Mater. Chem.*, 2011, **21**, 11205-11212.

S2. J. C. de Mello, H. F. Wittmann and R. H. Friend, *Adv. Mater.*, 1997, **9**, 230-232.

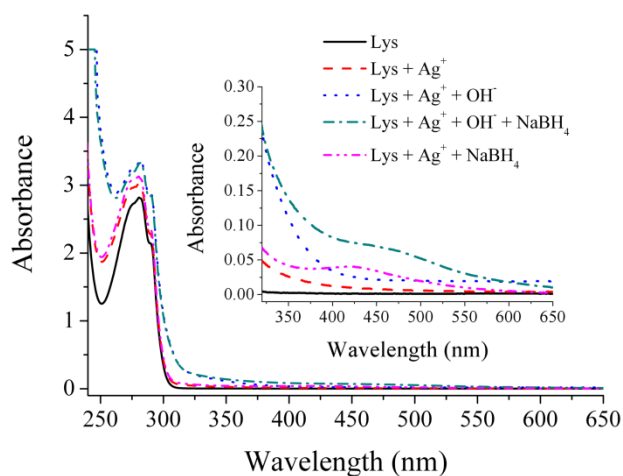


Fig. S1 UV-Vis absorption spectra of natural Lys and Lys AgNCs prepared under different conditions. The inset is an enlarged view of the spectra.

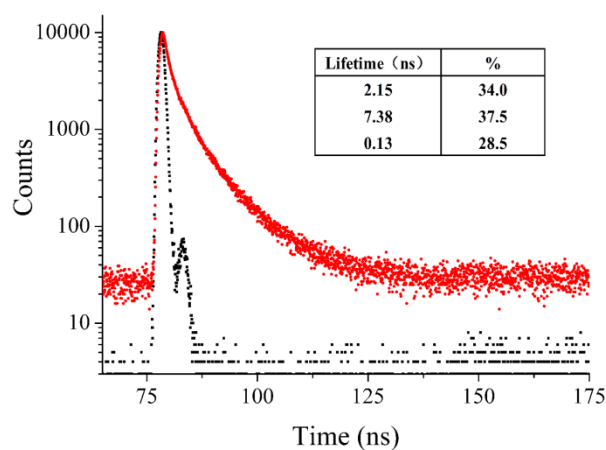


Fig. S2 Time-resolved decay of Lys-AgNCs. Lifetime values obtained are given in the inset.

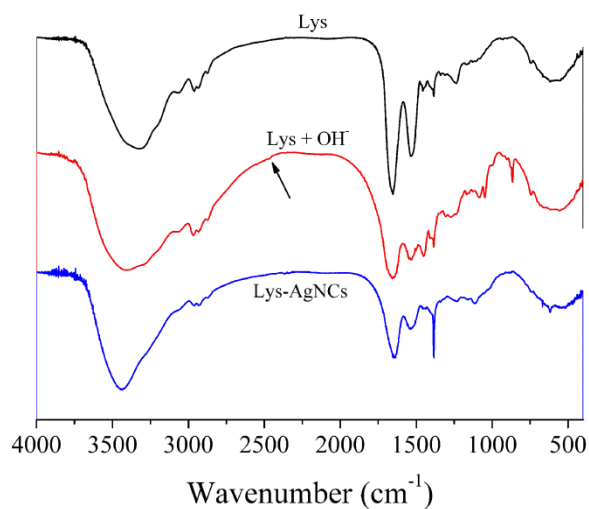


Fig. S3 FT-IR spectra of natural Lys, Lys incubated in a solution of pH about 12 and Lys-AgNCs.

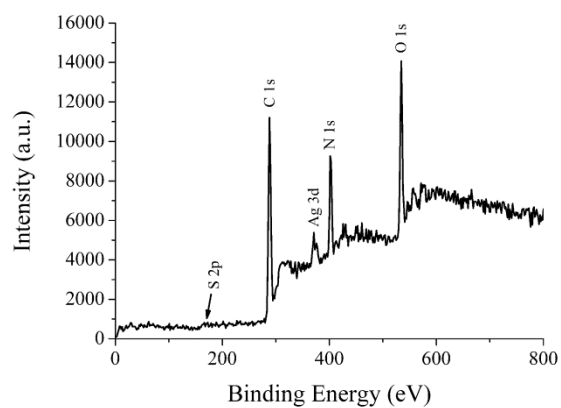


Fig. S4 XPS survey spectrum of Lys-AgNCs.

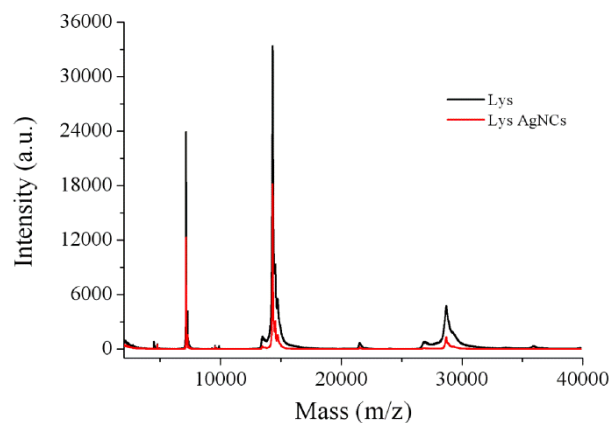


Fig. S5 MALDI-TOF mass spectra of the pure Lys and Lys-AgNCs

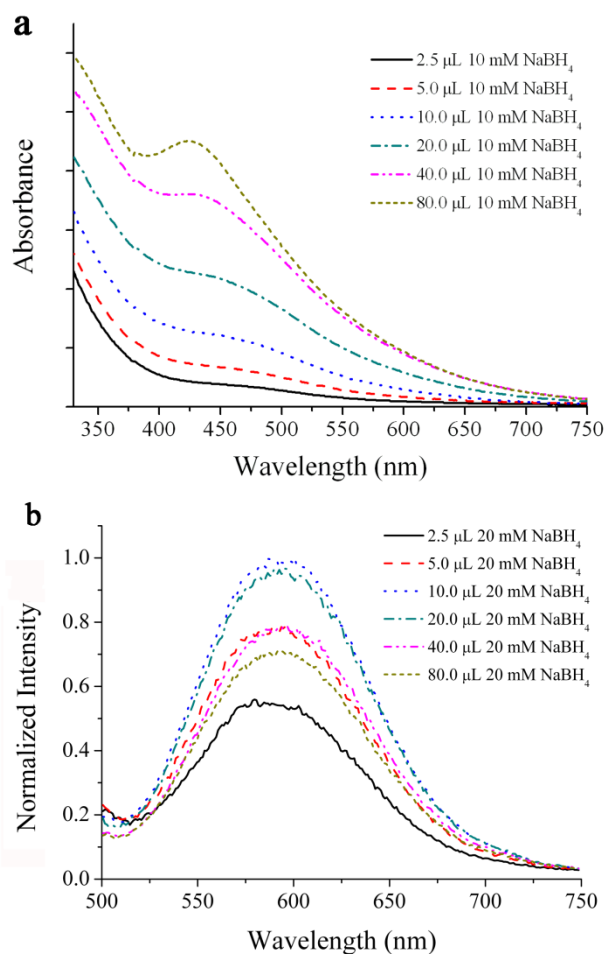


Fig. S6 UV-Vis absorption spectra (a) and emission spectra (b) of Lys AgNCs prepared by addition of different volumes of 20 mM NaBH₄ solution.

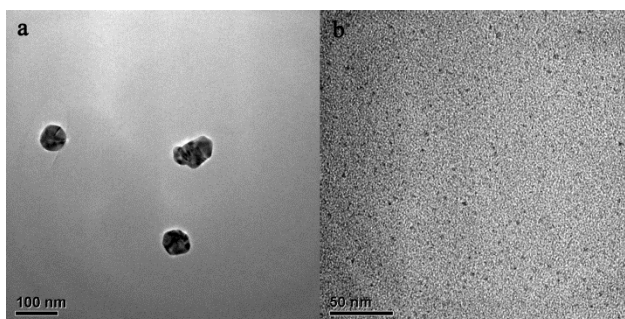


Fig. S7 (a) TEM images of Lys-AgNCs prepared by the addition of 80 μL 20 mM NaBH₄ solution. (b) TEM images of Lys-AgNCs after incubation for 6 h under the synthesis conditions and dialyzed by addition of acetic acid.

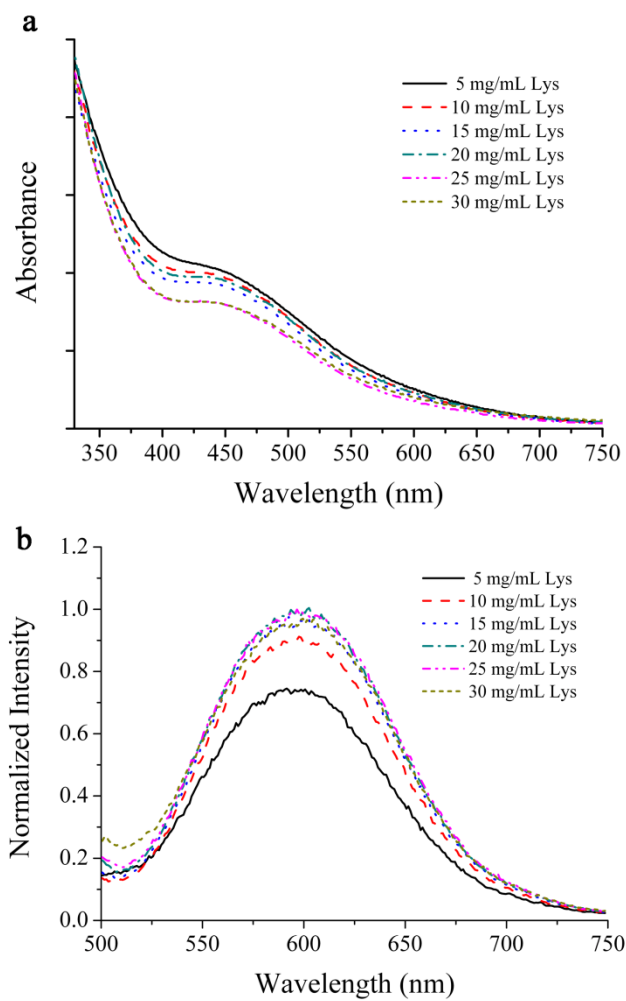


Fig. S8 UV-Vis absorption spectra (a) and emission spectra (b) of Lys AgNCs prepared in the presence of different concentrations of Lys.

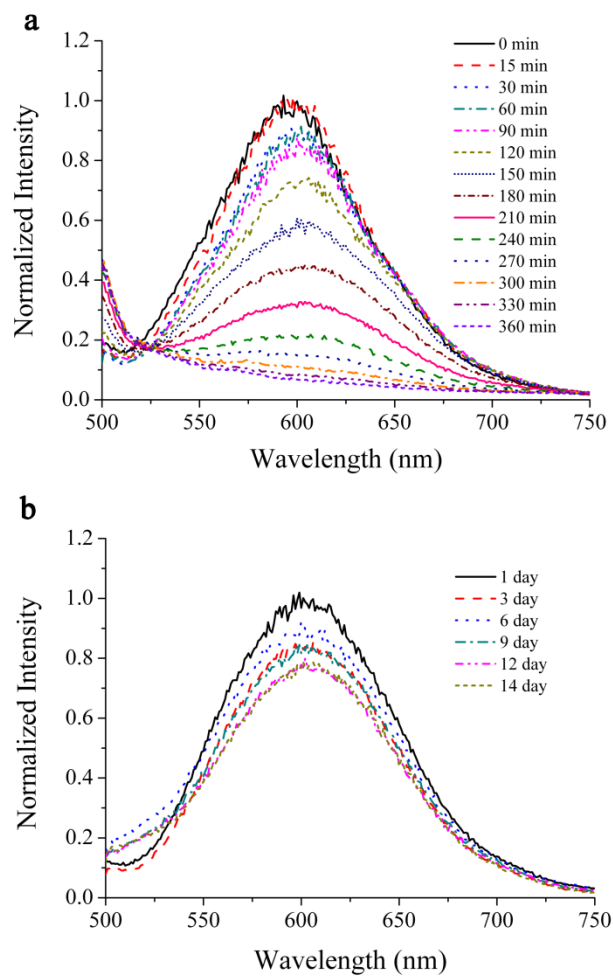


Fig. S9 Emission spectra of the freshly prepared AgNCs (a) and the dialyzed Lys-AgNCs (b) at different storage times.