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Electronic Supplementary Information

Lysozyme nanoparticle-encapsulated gold nanoclusters for label-free ratiometric fluorescent pH Sensing: Applications to enzyme-substrate system and cellular imaging

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ABSTRACT

In the section of supporting information, we include detail experimental information associated with synthesis of lysozyme-stabilized AuNCs and LysNPs (experimental section), culture of HeLa cells (experimental section), fluorescence lifetime decay of LysNPs, lysozyme-stabilized AuNCs, and LysNP-AuNCs (Figures S1 and S2), salt stability of LysNP-AuNCs (Figure S3), effects of the NaCl concentration and solution pH on the hydrodynamic diameter of LysNP-AuNCs (Figures S4 and S5), high-resolution Au 4f XPS spectrum of LysNP-AuNCs (Figure S6), the determination of molecular weight of LysNP-AuNCs (Figure S7), high-resolution N1s XPS spectra, high-resolution C1s XPS spectra, FT-IR spectra, and CD spectra of lysozyme, LysNPs, and LysNP-AuNCs (Figures S8–S12), CD spectra of LysNP-AuNCs at different pH conditions (Figure S13), the selectivity of LysNP-AuNCs (Figure S14), and the determination of serum urea by LysNP-AuNCs (Figure S15).

EXPERIMENTAL SECTION

Synthesis of Lysozyme-stabilized AuNCs and LysNPs. (a) Lysozyme-stabilized AuNCs. Lysozyme-stabilized AuNCs were prepared based on the previously reported study.[32] Briefly, a solution of lysozyme (25 mg/mL) was mixed with an equal volume of $HAuCl_4$ (10 mM) at ambient temperature. The pH of the mixture was adjusted to 12.0 by adding 1 M NaOH. The resulting solution was incubated at 37 ^oC for 12 h and then purified by dialyzing against 5 mM phosphate buffer (pH 12.0) for two days. **(b) LysNPs**. Briefly, 6 mL of ethanol was added dropwise to an aqueous solution of lysozyme (3 mL, 4.2 mg/mL) at ambient temperature with gentle stirring. Subsequently, the pH of the mixture was adjusted to a basic condition by injecting NaOH solution (500 µL, 1 M), following by addition of glutaraldehyde solution (50 µL, 50% w/v). The above solutions kept stirring at ambient temperature for 5 h to induce cross-linking of lysozyme. For the removal of ethanol, the resulting solution was transferred into a 15-mL screw-capped test tube and then evaporated to near dryness (approximately 500 µL) in a cold-trap centrifugal evaporator. Deionized water was added to make up a total volume of 10 mL. The as-prepared Lys-NPs solution was stored in the refrigerator at 4 ^oC for further use.

Cell Culture. Primary cultured cells were incubated in Dulbecco's Modified Eagle Medium (cat. no. 11885, Invitrogen) and cultured in a humidified incubator at 37 °C in the presence of 5% CO_2. The composition of the culture medium included 10% fetal bovine serum and 1% penicillin-streptomycin solution. The cells were dispersed in a flask of T25 and harvested to reach about 80% confluence. The attached cells were washed twice with 1× PBS and then incubated in 5 mL of trypsin-ethylenediaminetetraacetic (EDTA) solution (0.25 w/v % trypsin, 2.5 g/L EDTA) at 37 °C for 10 min. The suspended cells were collected conducted by centrifugation at 3000 rpm for 5 min. The obtained cells were washed once with 1 mL of culture media, suspended in 5 mL of culture media, immersed in a glass-bottomed culture dish, and then cultivated in a humidified incubator chamber containing 5% CO_2 for 24 h.

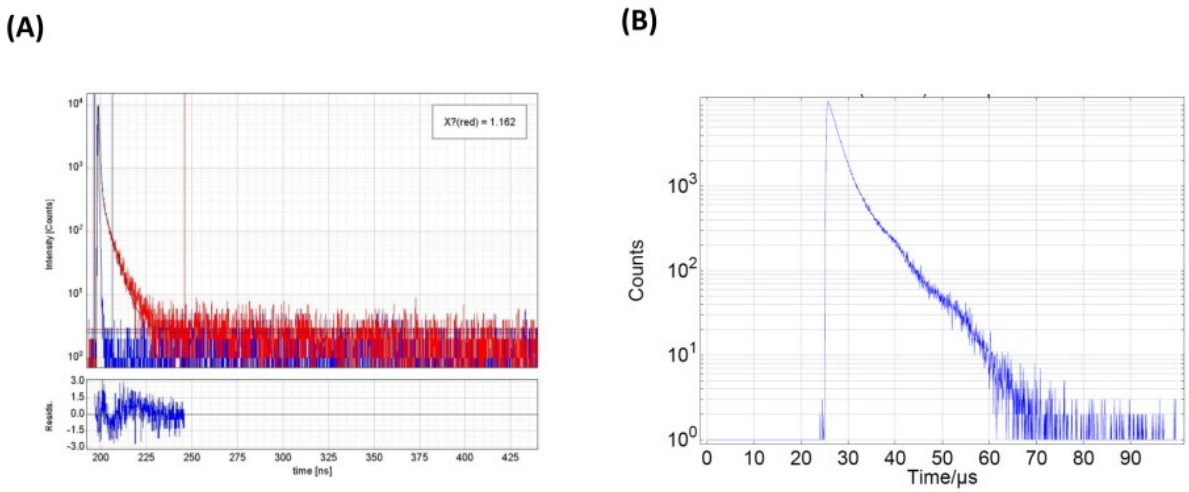

Figure S1. Fluorescence lifetime decay of (A) LysNPs and (B) lysozyme-stabilized AuNCs.

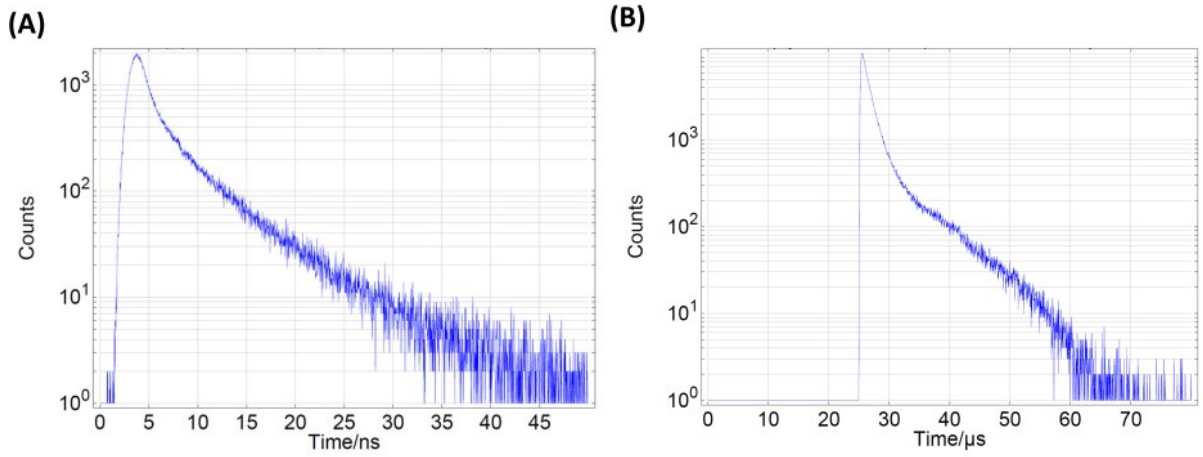

Figure S2. Fluorescence lifetime decay of LysNP-AuNCs at emission wavelength of (A) 495 and (B) 676 nm.

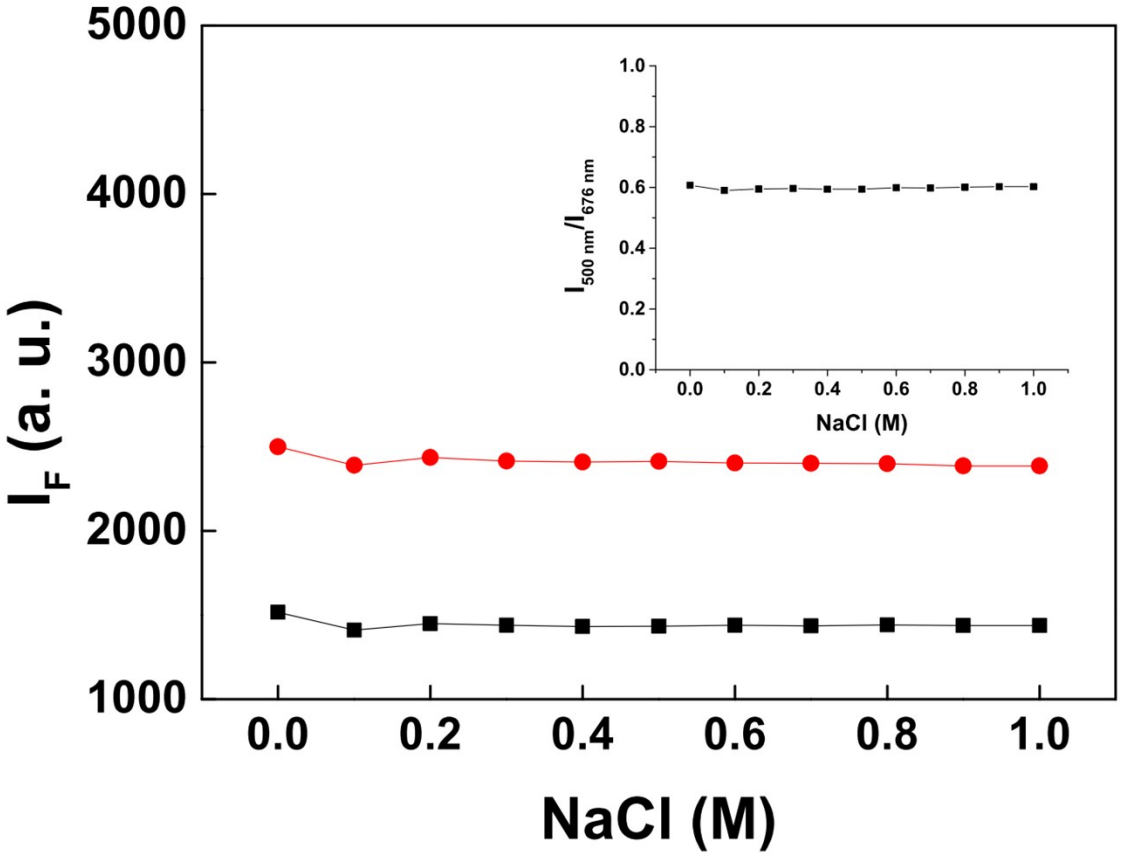

Figure S3. Effect of the NaCl concentration on the fluorescence intensity of LysNP-AuNCs at 495 nm (solid black square) and 676 nm (solid red circle). Inset: the $I_{500\ nm}/I_{676\ nm}$ value of lysNP-AuNCs as a function of the NaCl concentration. LysNP-AuNCs were prepared in 10 mM phosphate buffer at pH 7.5.

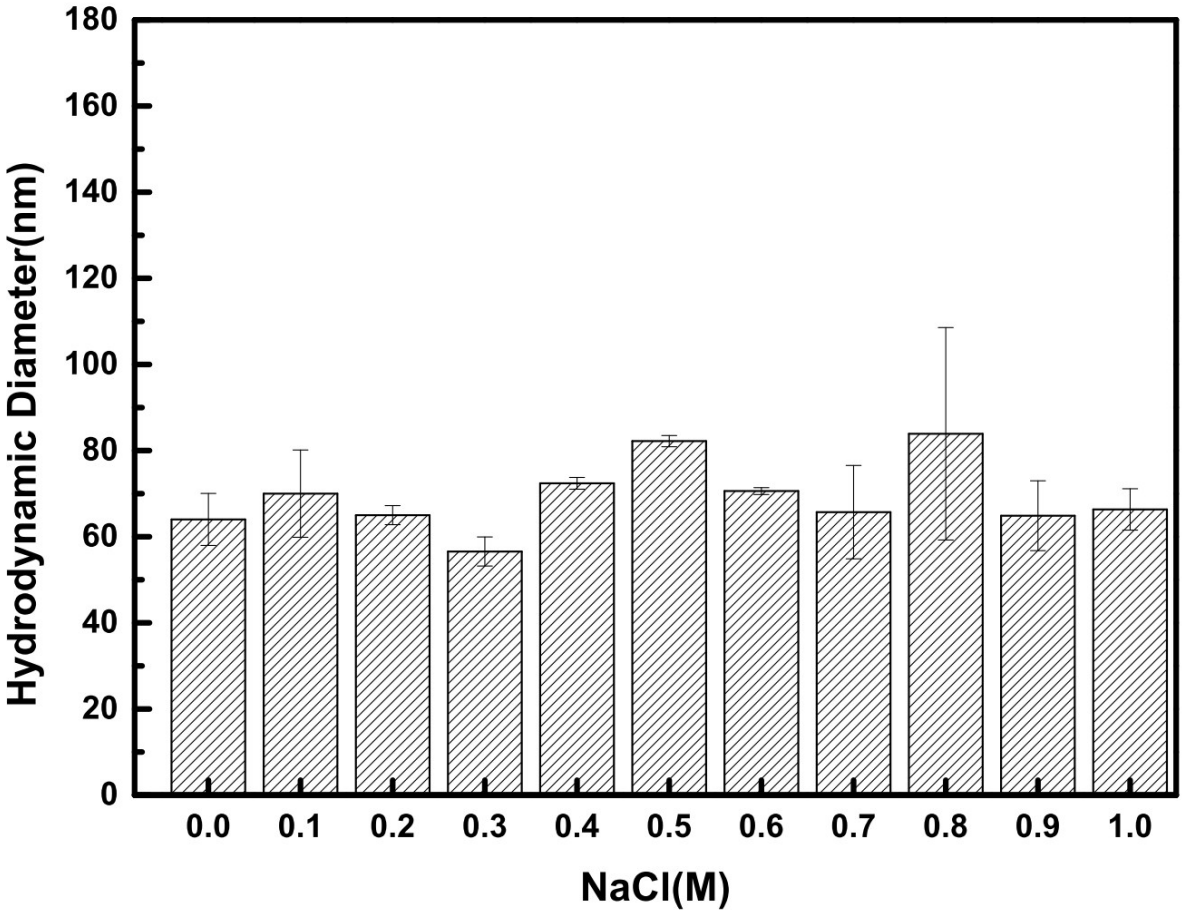

Figure S4. Effect of the NaCl concentration on the hydrodynamic diameter of LysNP-AuNCs. LysNP-AuNCs were prepared in 10 mM phosphate buffer at pH 7.5.

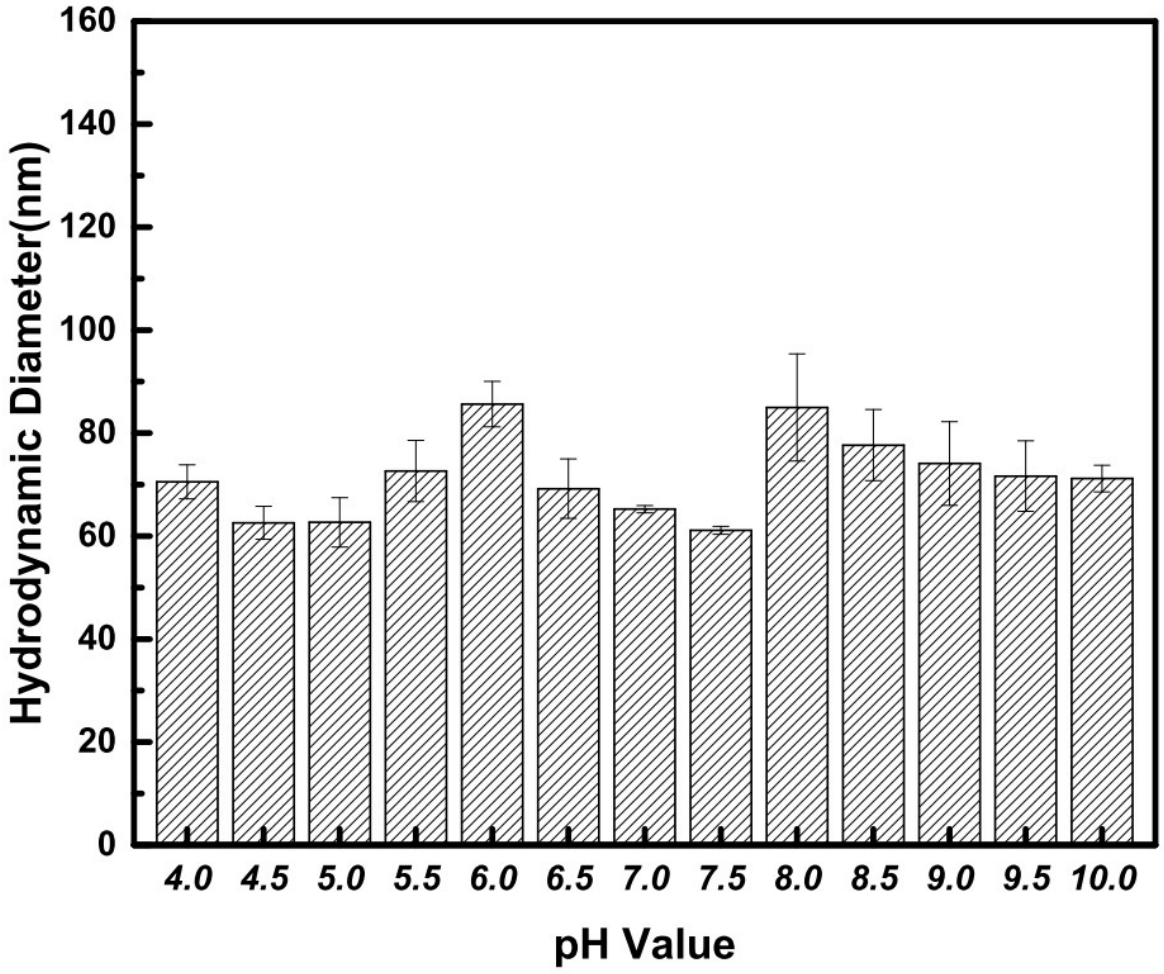

Figure S5. Effect of solution pH on the hydrodynamic diameter of LysNP-AuNCs. LysNP-AuNCs were prepared in 10 mM phosphate buffer at pH 4.0–10.0.

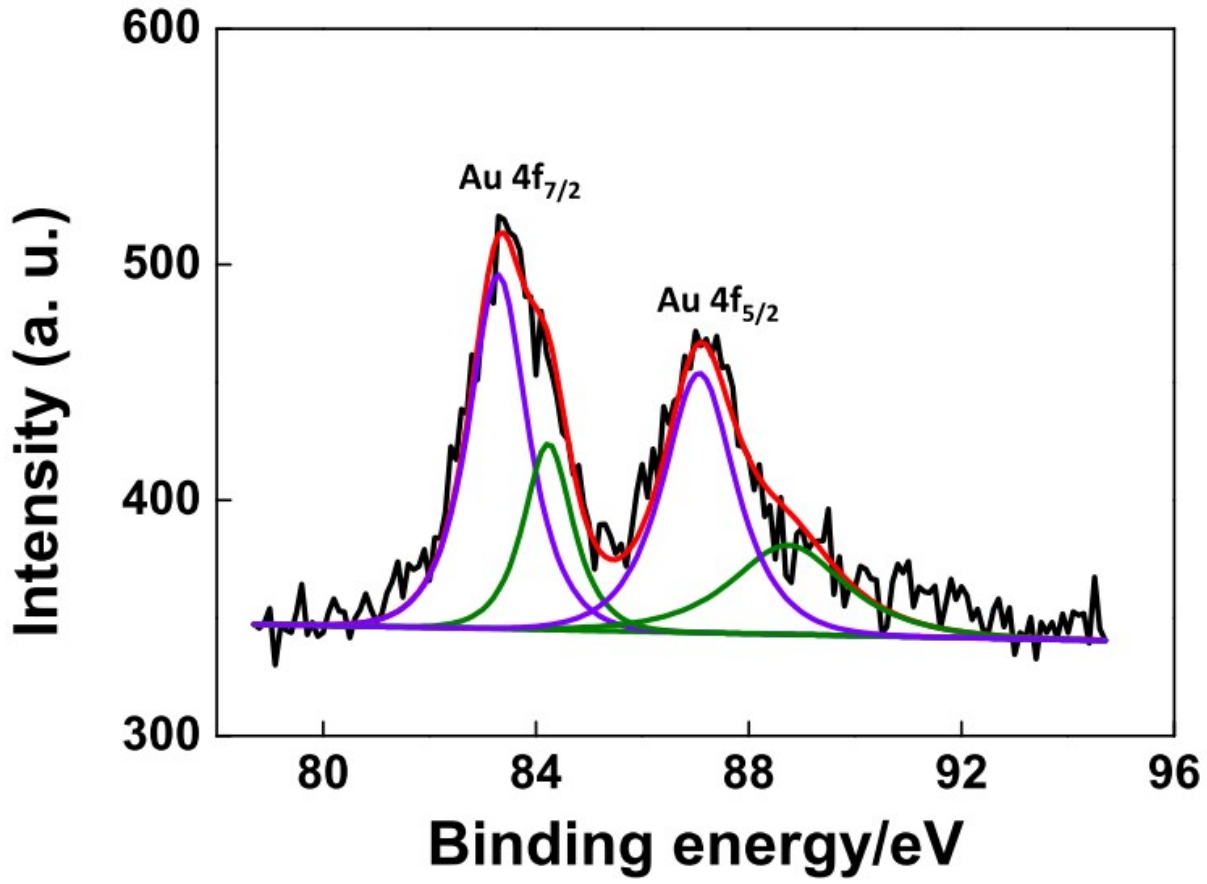

Figure S6. High-resolution Au4f XPS spectrum of Lys NPs-AuNCs. The original spectrum is in black, the fitted spectrum is in red, the A(0) $4f_{7/2}$ and $4f_{5/2}$ spectra are in purple, and the A(I) $4f_{7/2}$ and $4f_{5/2}$ spectra are in green

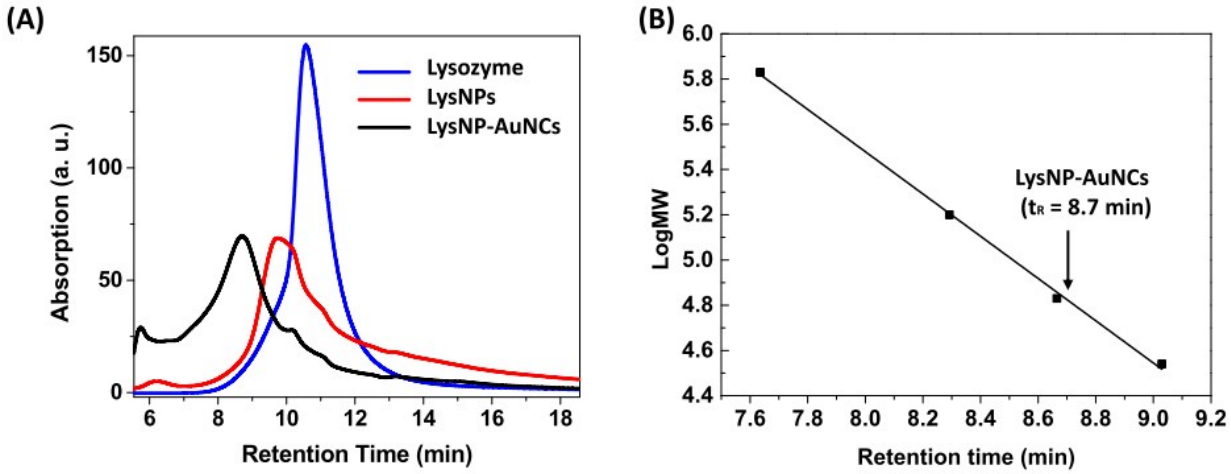

Figure S7. (A) SEC chromatograms of LysNP-AuNCs (black line), LysNPs (red line), and lysozyme (blue line). (B) A plot of the logarithm of protein molecular weight versus the retention time.

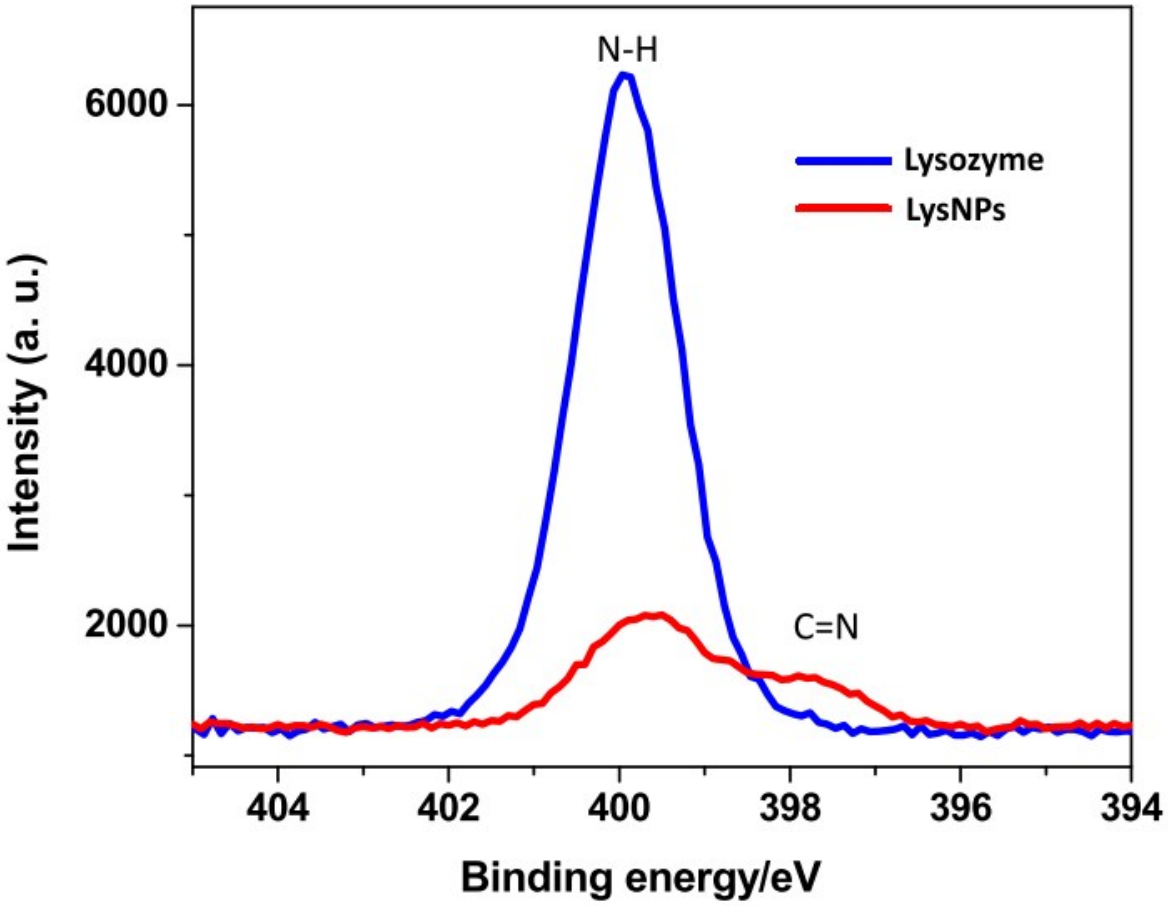

Figure S8. High-resolution N1s XPS spectrum of Lysozyme and LysNPs.

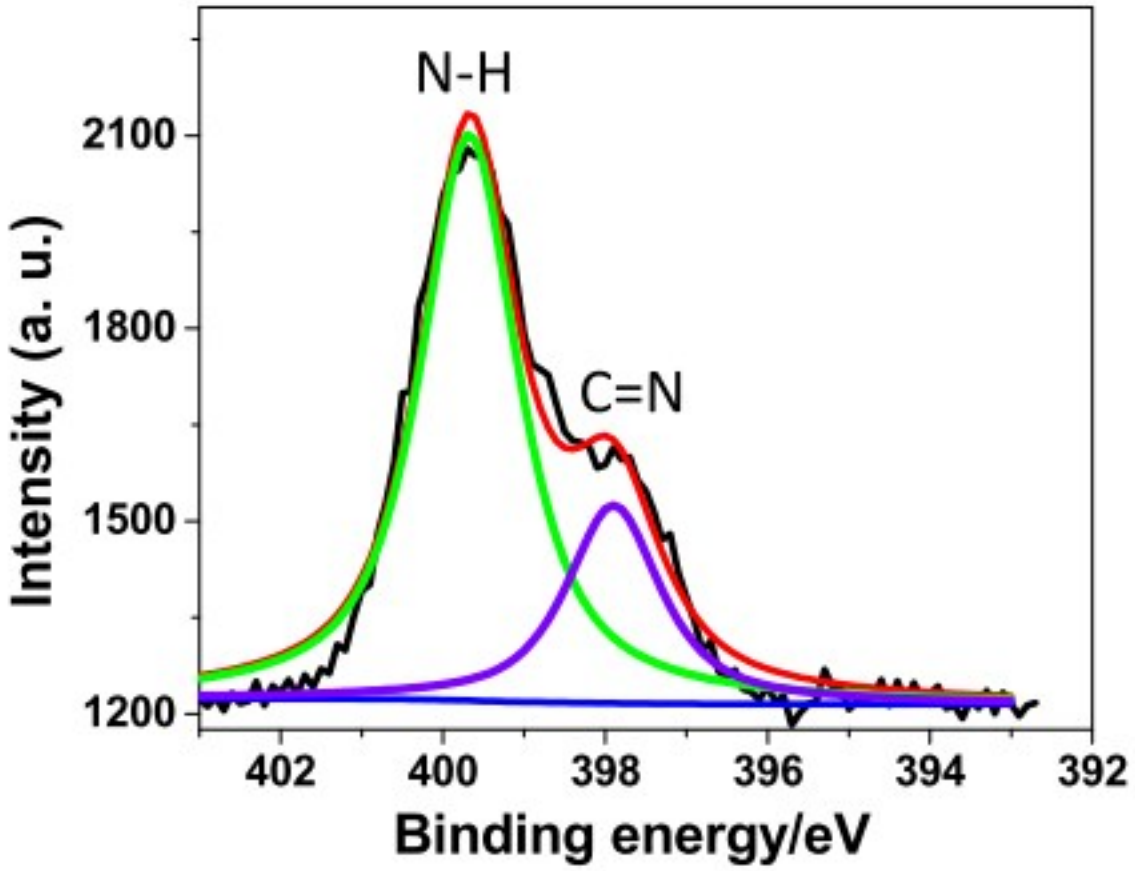

Figure S9. High-resolution N1s XPS spectra of LysNP-AuNCs. The original spectrum is in black, the fitted spectrum is in red, the deconvoluted spectra are in green (C=N) and violet (N-H bond).

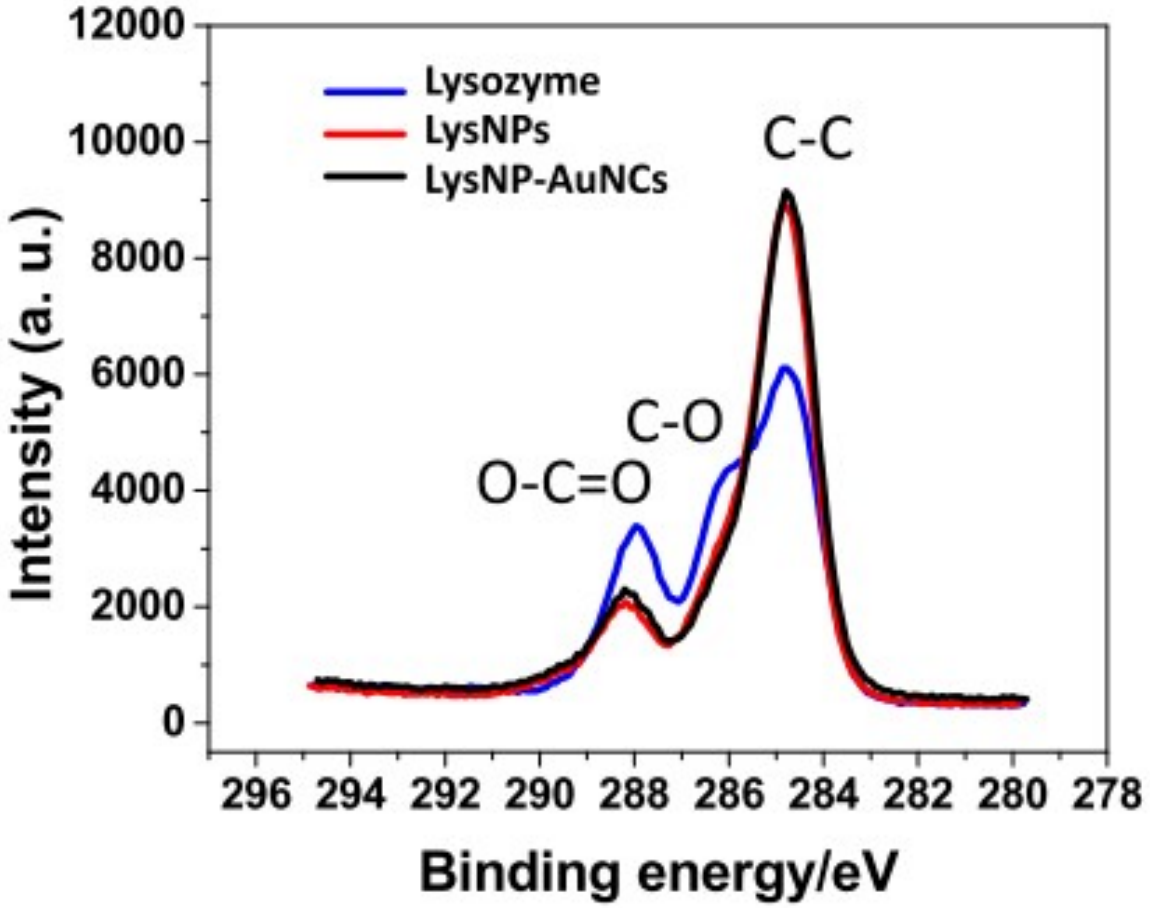

Figure S10. High-resolution C1S XPS spectra of LysNP-AuNCs (black curve), LysNPs (red curve), and lysozyme (blue line). LysNP-AuNCs, LysNPs, and lysozyme were all prepared in 10 mM phosphate buffer at pH 10.

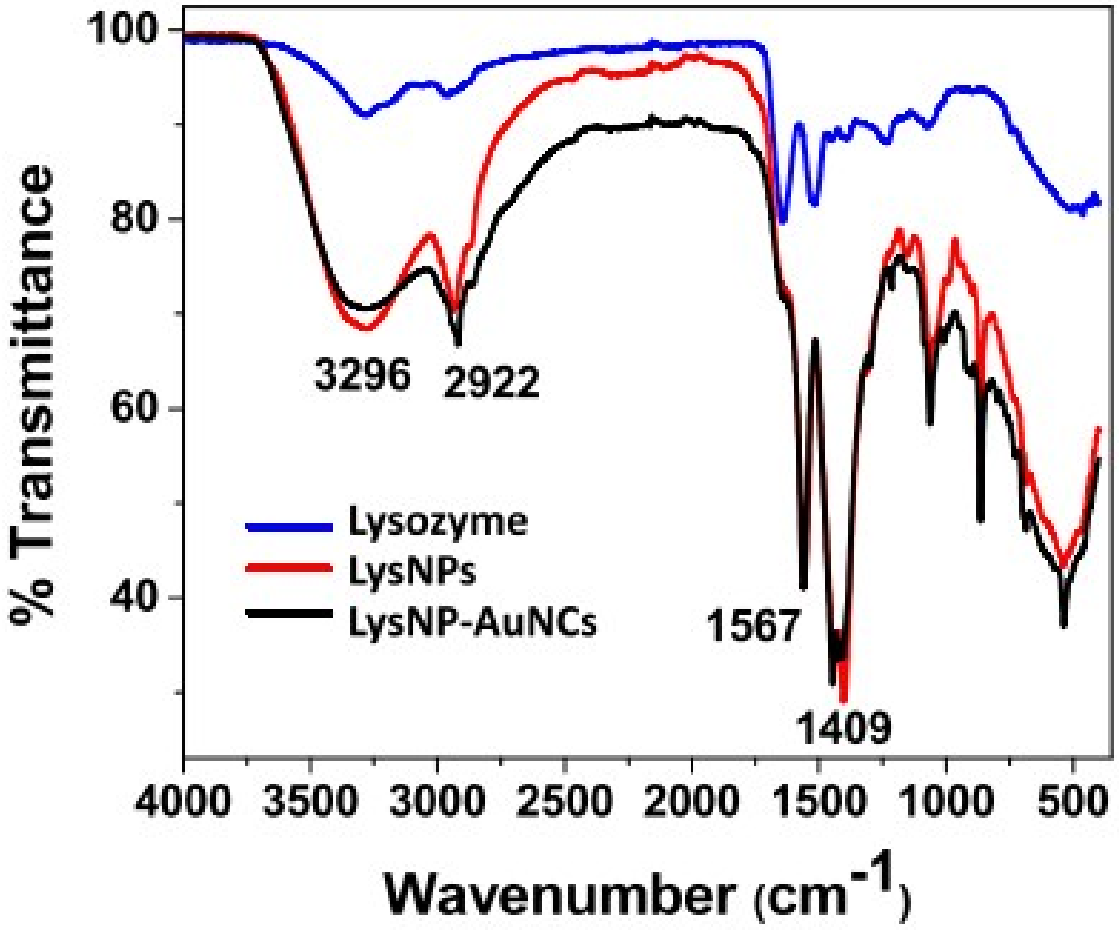

Figure S11. FT-IR spectra of LysNP-AuNCs (black curve), LysNPs (red curve), and lysozyme (blue line). LysNP-AuNCs, LysNPs, and lysozyme were all prepared in 10 mM phosphate buffer at pH 10.

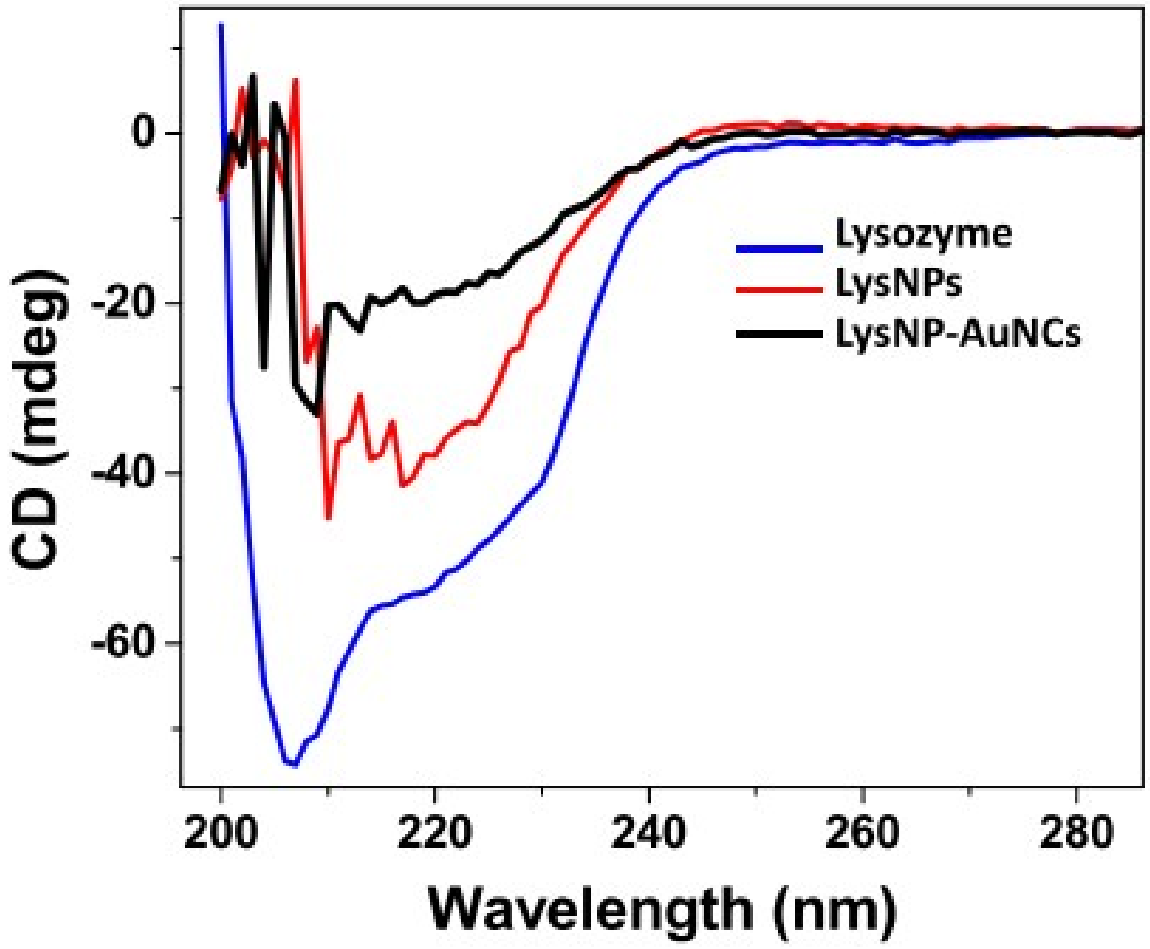

Figure S12. (B) High-resolution C1S XPS spectra, (C) FT-IR spectra, and (D) CD spectra of LysNP-AuNCs (black curve), LysNPs (red curve), and lysozyme (blue line). LysNP-AuNCs, LysNPs, and lysozyme were all prepared in 10 mM phosphate buffer at pH 10.

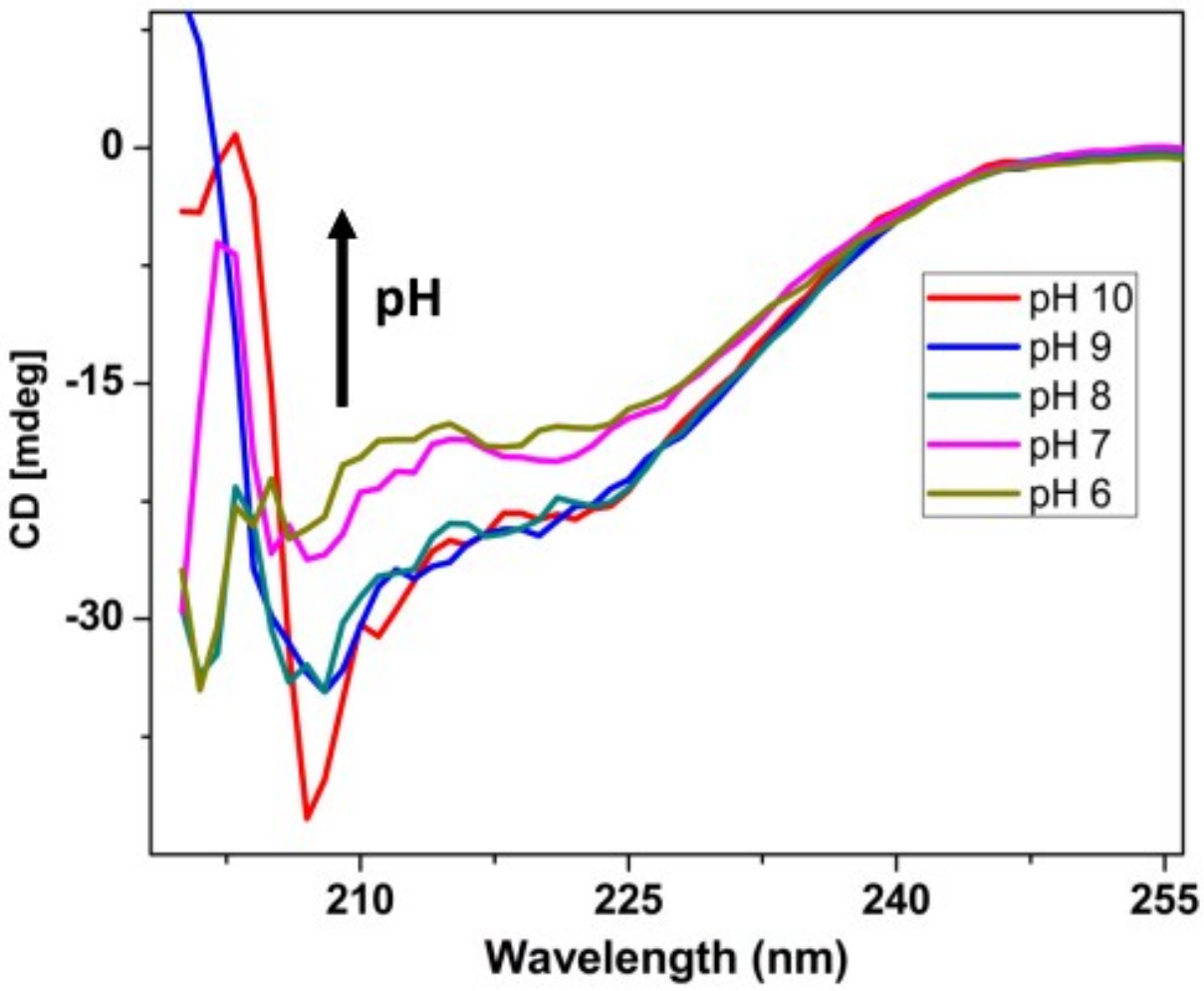

Figure S13. CD spectra of LysNP-AuNCs as a function of pH value.

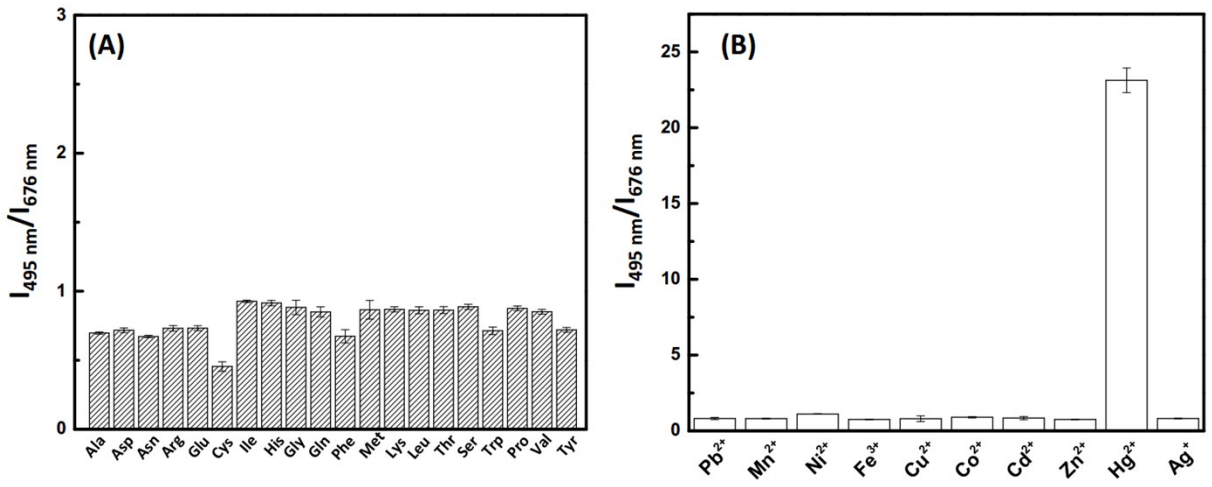

Figure S14. Effects of (A) amino acids and (B) metal ions on the $I_{495\ nm}/I_{676\ nm}$ value of LysNP-AuNCs. The concentration of amino acids and metal ions are both 100 μM. LysNP-AuNCs were prepared in 10 mM phosphate buffer at pH 7.5.

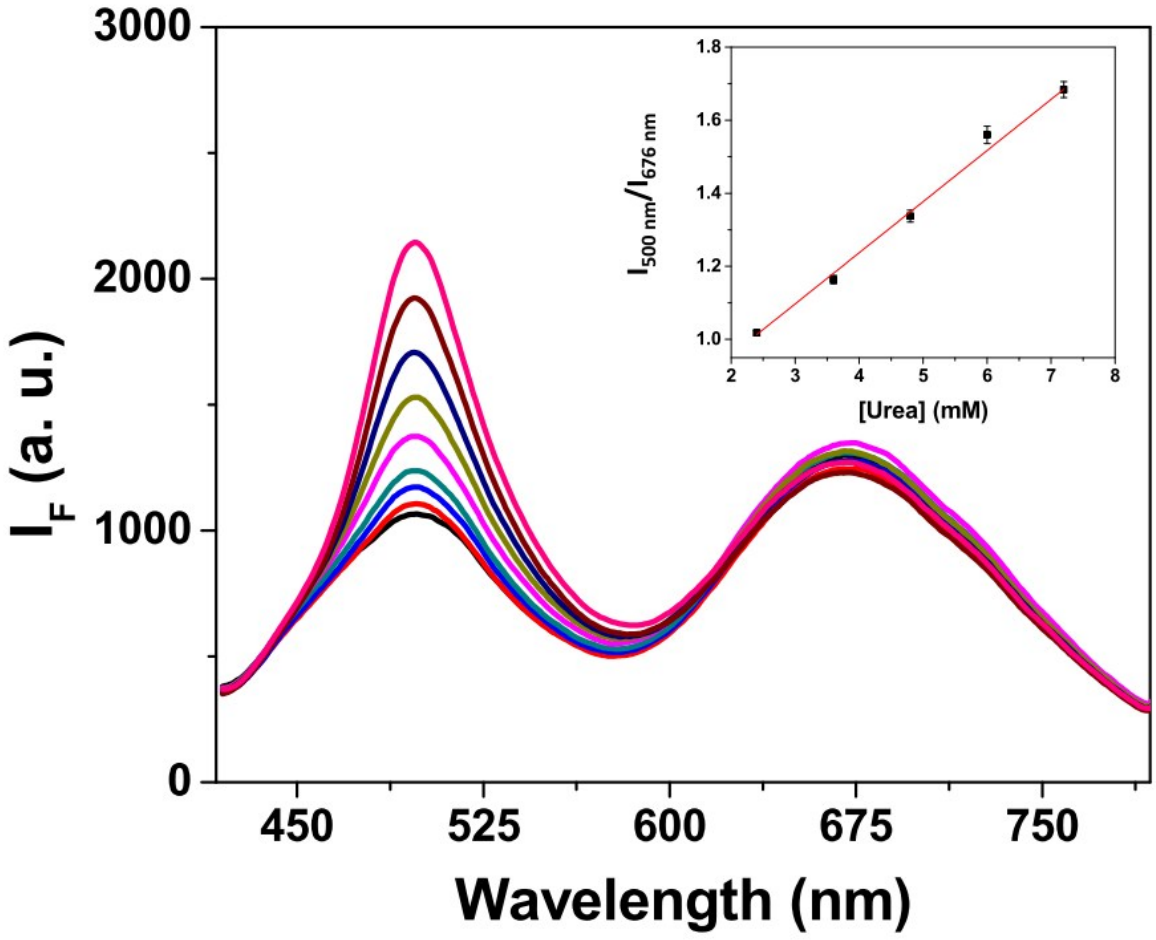

Figure S15. Fluorescence spectra of LysNP-AuNCs obtained from the incubation of a series of urea-spiked serum samples and 0.05 mg/mL urease in 10 mM phosphate buffer (pH 7.4). The arrow indicates the signal changes with increasing the concentration of urea (0, 0.8, 1.6, 2.4, 3.2, 4.0, 4.8, 5.6, 6.4, and 7.2 mM). Inset: A plot of the $I_{495\ nm}/I_{676\ nm}$ value versus the spiked concentration of urea. The error bars represent standard deviation based on three independent measurements.