Supporting Information

pH-sensitive gold nanocluster: preparation and analytical applications for urea, urease, and urease inhibitor

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Experimental Section

Materials

All chemicals and solvents were of analytical grade and commercially available. $HAuCl_4 \cdot 4H_2O$, N-acetyl-L-cysteine, urea, and Rhodamine 6G were obtained from Aladdin Reagent Company (Shanghai, China). Urease was purchased from Sigma-Aldrich (Shanghai, China). C_2H_5OH, bromothymol blue, and NaOH were brought from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Deionized water was used throughout experiments.

Preparation of NAC-AuNCs

All glassware used in the following procedures was cleaned in a bath of freshly prepared solution of HNO_3-HCl (1:3, v/v), rinsed thoroughly in water and dried in air prior to use. AuNCs were synthesized in a blending manner. Typically, NaOH (0.6 mL, 0.5 M) and $HAuCl_4$ (0.4 mL, 20 mg/mL) were added into an aqueous solution of N-acetyl-L-cysteine (4 mL, 0.08 M). The mixture was incubated at 37 °C for 2.5 h, obtaining a colorless solution. The solution after synthesis was dialyzed for more than 24 h to remove all small-molecular impurity. The resulting solution of AuNCs was stored in the dark at 4 °C for later use.

Characterization

The UV-vis absorption spectrum and the photoluminescence spectrum were measured by UV-2450 UV-vis spectrophotometer (Shimadzu, Japan) and Cary Eclipse fluorescence spectrophotometer (Agilent, USA), respectively. Time decay

measurements were conducted on a F900 time-correlated single photo counting fluorescence lifetime spectrometer (Edinburgh Analytical Instruments, UK). The TEM and HRTEM images were collected with a JEM-2100 microscope (JEOL, Japan). X-ray photoelectron spectroscopy (XPS) was performed using an ESCALAB 250 XI electron spectrometer (Thermo, USA) using monochromatic Al Kα radiation (1486.6 eV) for analysis of the surface composition and chemical states of the product. Binding energy calibration was based on C 1s at 284.8 eV. The Fourier transform infrared spectroscopy (FTIR) was measured at the wavenumbers ranging from 400 to 4000 cm^{-1} using a Nicolet Avatar 360 FTIR spectrophotometer. Dynamic light scattering (DLS) and zeta-potential experiments were carried out on a Zetasizer Nano-ZS. All measurements were performed at room temperature under ambient conditions.

NAC-AuNCs for Urea Detection

The reaction solution contained 200 μL NAC-AuNCs, 200 μL of 10 U/mL urease (pH=6), and 50 μL of various concentrations urea (pH=6). The solution was incubated at 25 °C for 40 min. The change of fluorescence intensity of the solution was recorded by Cary Eclipse fluorescence spectrophotometer.

For real sample analysis, human urine samples were collected and were adjusted to pH=6. The reaction solution contained 200 μL NAC-AuNCs, 200 μL of 10 U/mL urease (pH=6), and 50 μL of diluted sample solution (pH=6). The solution was incubated at 25 °C for 40 min before measuring.

NAC-AuNCs for Urease Detection and Its Inhibitor Screening

The solution contained 200 μL of 1 M urea (pH=6), and 50 μL of various concentrations urease (pH=6). The solution was incubated at 25 °C for 40 min. After that, 200 μL NAC-AuNCs was introduced and the mixed solution was incubated at 25 °C for 3 min. Finally, the change of fluorescence intensity of the mixed solution was recorded by Cary Eclipse fluorescence spectrophotometer.

In the inhibition assay for urease, the solution (pH=6) containing 0.3 U/mL urease, 1 M urea, and different concentrations of inhibitors was incubated at 25 °C for 40 min. After that, 200 μL NAC-AuNCs was introduced and the mixed solution was incubated at 25 °C for 3 min. Finally, the fluorescence intensity of the mixed solution

was recorded by Cary Eclipse fluorescence spectrophotometer. Inhibition efficiency defined in eq 1 was then calculated for each inhibitor:

$$\text{Inhibition Efficiency (\%)} = [(F_{\text{inhibitor}} - F_{\text{no inhibitor}})/(F_0 - F_{\text{no inhibitor}})] \times 100 \quad (1)$$

where F_0 is the fluorescence intensity of the reaction mixture at 650 nm in the absence of both urease and inhibitors, $F_{\text{no inhibitor}}$ is the fluorescence intensity of the reaction mixture at 650 nm with urease but without any inhibitor, and $F_{\text{inhibitor}}$ is the fluorescence intensity of the reaction mixture at 650 nm with both urease and a specific inhibitor. IC_{50}, which was defined as the concentration of an inhibitor which achieves 50% inhibition efficiency, was determined from the plot of inhibition efficiency versus inhibitor concentration.

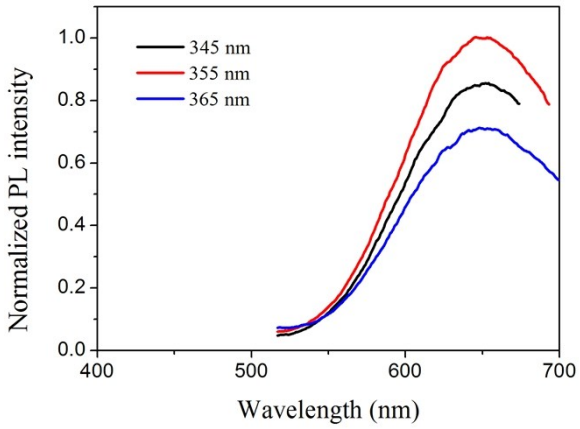

Fig. S1 Fluorescence emission spectra of aqueous solution of NAC-AuNCs upon excitation at 345, 355, and 365 nm.

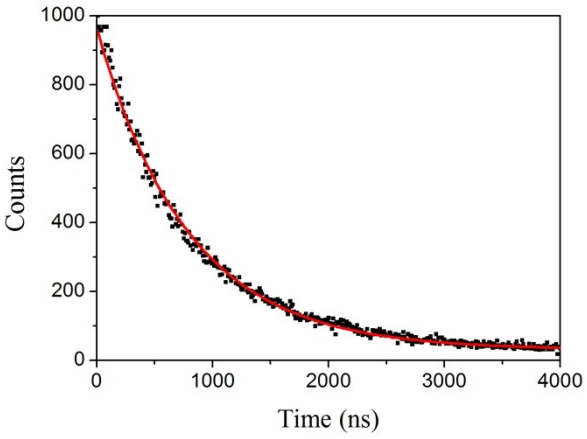

Fig. S2 Fluorescence decay of the aqueous NAC-AuNCs (excitation at 355 nm)

collected at 650 nm. The solid line is the best-fit curve.

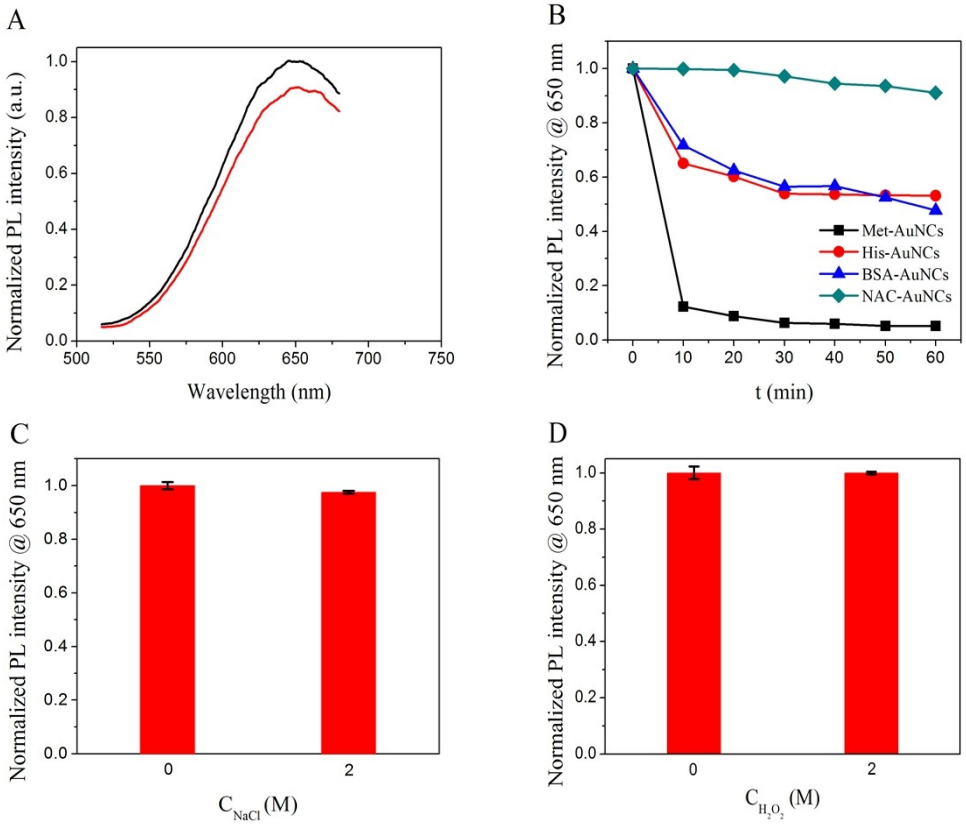

Fig. S3 (A) The fluorescence spectra of as-prepared NAC-AuNCs before and after storing in dark at 4 °C for one month. (B) Photostability of NAC-AuNCs irradiated by a 64 W UV lamp for various times. BSA-templated AuNCs (BSA-AuNCs), histidine-stabilized AuNCs (His-AuNCs), and methionine-protected AuNCs (Met-AuNCs) were prepared according to previous study.[1] (C) Effect of salt on the fluorescence intensitiy of AuNCs. (D) Effect of H_2O_2 on the fluorescence intensitiy of AuNCs.

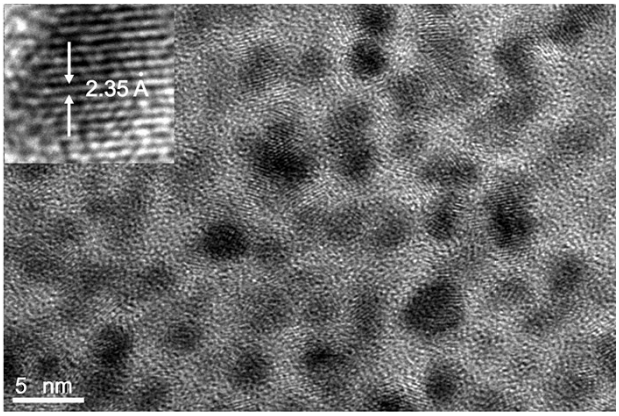

Fig. S4 Typical TEM image of NAC-AuNCs. The inset shows a close-up displaying the crystalline structure of an individual nanocluster.

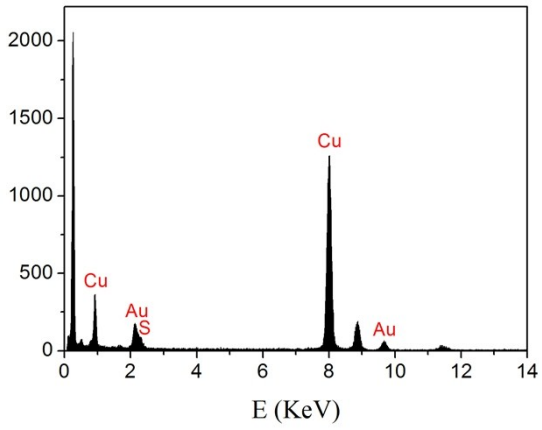

Fig. S5 Energy-dispersive X-ray spectroscopy (EDS) spectra of NAC-AuNCs sample.

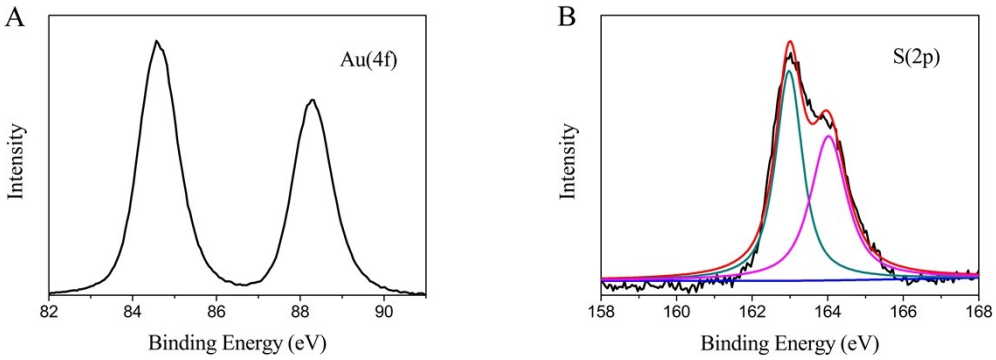

Fig. S6 XPS spectra showing the binding energy of Au(4f) (A) and S(2p) (B) of NAC-AuNCs.

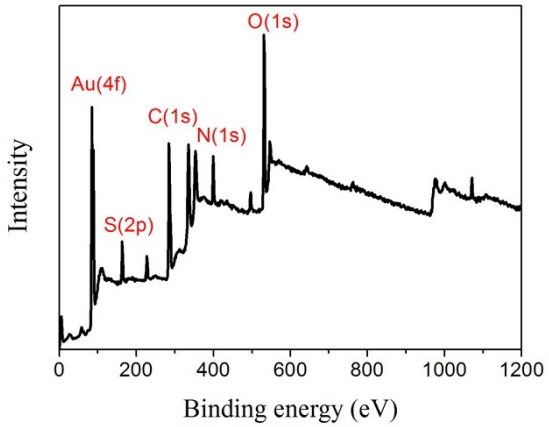

Fig. S7 The whole XPS spectrum of NAC-AuNCs. C(1s), N(1s), O(1s), S(2p) and Au(4f) core-level photoemission spectra all appeared on the XPS spectrum.

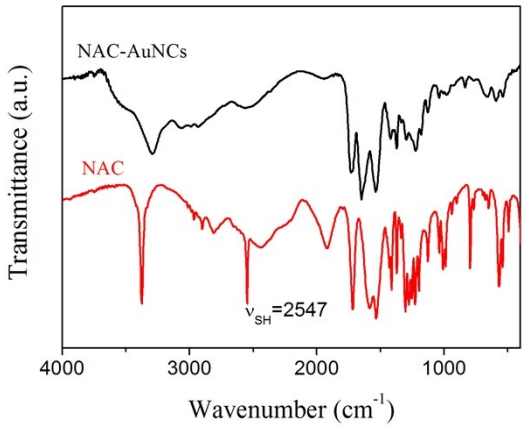

Fig. S8 Fourier transform infrared (FTIR) spectra of pure NAC and NAC-AuNCs.

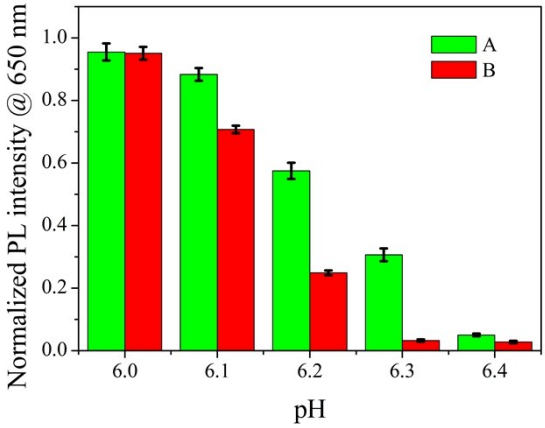

Fig. S9 The pH response of NAC-AuNCs in solution without (A) and with 50 mM NaCl (B).

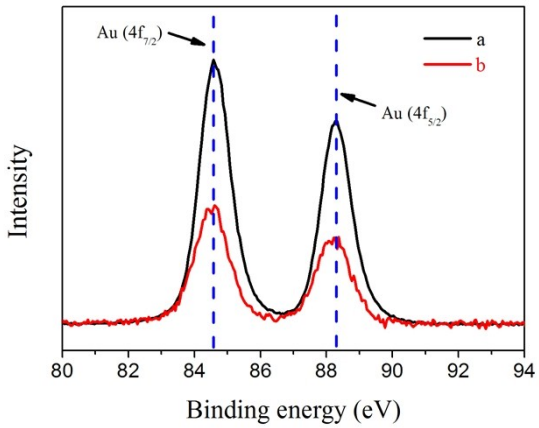

Fig. S10 XPS spectra showing the binding energy of Au(4f) of NAC-AuNCs before (a) and after (b) the addition of pH 6.50 buffer solution.

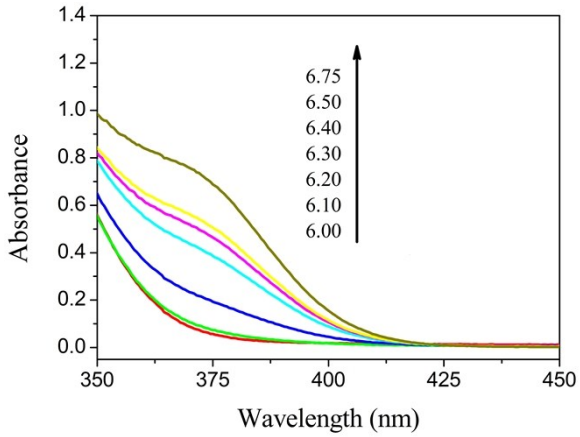

Fig. S11 UV-vis spectra of NAC-AuNCs in buffer solution with different pH values.

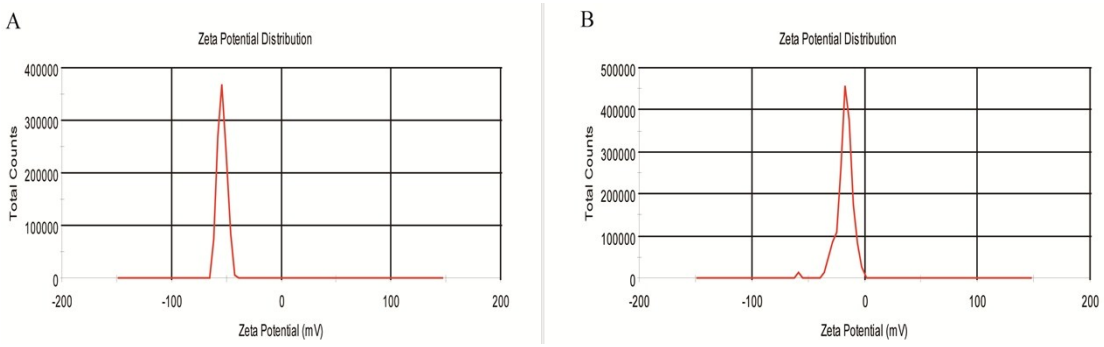

Fig. S12 Zeta potential of NAC-AuNCs before (A) and after (B) the addition of pH 6.50 buffer solution.

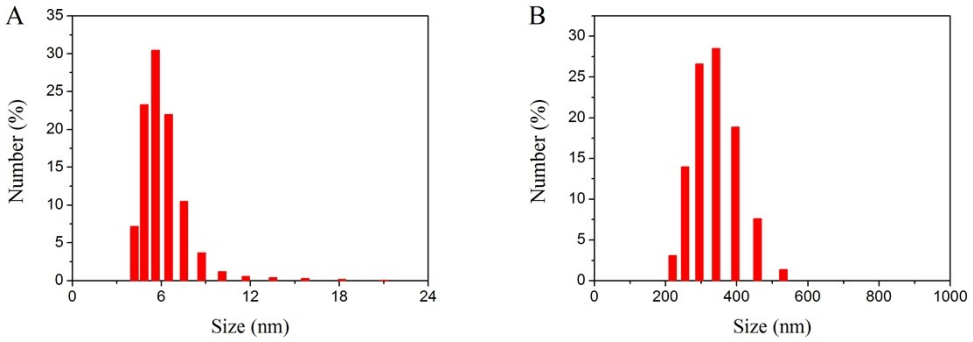

Fig. S13 DLS of NAC-AuNCs before (A) and after (B) the addition of pH 6.50 buffer solution.

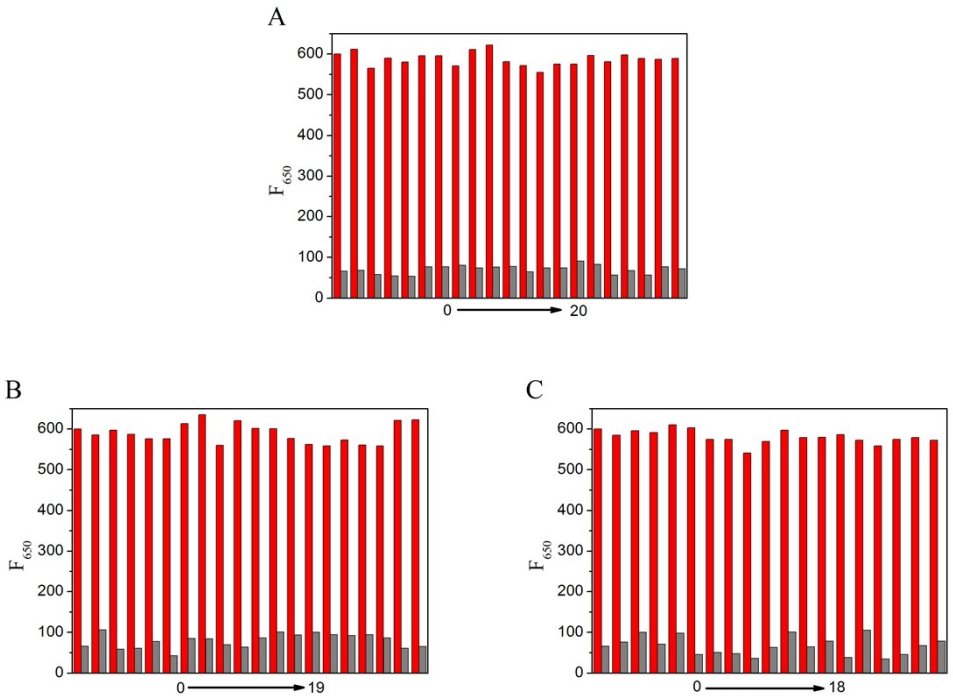

Fig. S14 (A) Fluorescence intensity of NAC-AuNCs (excitation at 355 nm) in the presence of different molecules; (0) NAC-AuNCs alone (red) and after addition of urea (black); (1-20) NAC-AuNCs in presence of glutathione, ascorbic acid, adenosine triphosphate, glucose, lactose, maltose, creatinine, creatine, uric acid, L-arginine, L-cysteine, L-methionine, L-serine, L-isoleucine, L-valine, L-histidine, L-aspartic acid, L-phenylalaine, L-leucine, and L-alanine (red) and after addition of urea (black). The concentration of urea is 0.55 mM and that of all the molecules is 100 μM. (B) Fluorescence intensity of NAC-AuNCs (excitation at 355 nm) in the presence of different cations; (0) NAC-AuNCs alone (red) and after addition of urea (black); (1-19) NAC-AuNCs in presence of Ni^{2+}, Mg^{2+}, Fe^{3+}, Cd^{2+}, Mn^{2+}, NH_4^+, Cu^{2+}, Ag^+, Fe^{2+}, Al^{3+}, Pb^{2+}, Zn^{2+}, Ba^{2+}, Co^{2+}, Hg^+, Cr^{3+}, Ca^{2+}, Na^+, and K^+ (red) and after addition of urea (black). (C) Fluorescence intensity of NAC-AuNCs (excitation at 355 nm) in the presence of different anions; (0) NAC-AuNCs alone (red) and after addition of urea (black); (1-18) NAC-AuNCs in presence of $S_2O_3^{2-}$, NO_2^-, SO_3^{2-}, F^-, SCN^-, S^{2-}, $H_2PO_4^-$, BrO_7^{2-}, IO_3^-, BrO_3^-, SO_4^{2-}, NO_3^-, $S_2O_8^{2-}$, ClO_4^-, I^-, Br^-, CO_3^{2-}, Ac^- (red) and after addition of urea (black). The concentration of urea is 0.55 mM and that of all the ions is 10 μM.

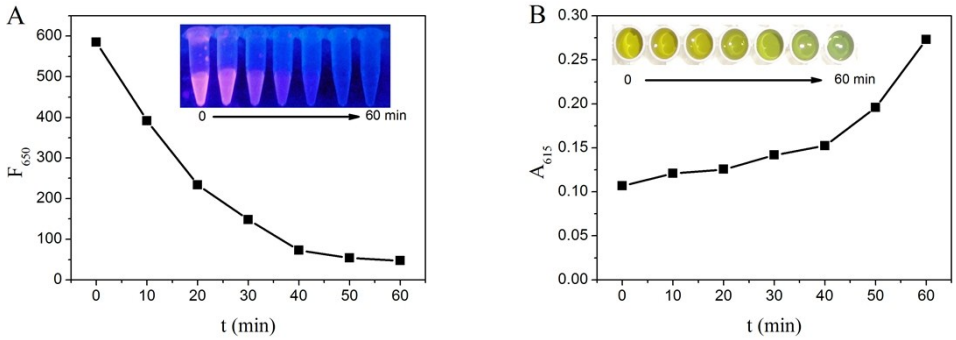

Fig. S15 Comparison of the analyzing time between the proposed method (A) and the conventional bromothymol blue method (B) in the detection of H. pylori in human gastric tissue. Inset: the corresponding photographs.

Table S1 The PL lifetime of NAC-AuNCs in buffer solution with different pH values.

pH	6.00	6.15	6.30
τ (ns)	816	824	856

Table S2 Analysis of urea in human urine samples by NAC-AuNCs.

Sample	The proposed method（mM, n=3）	The standard method（mM, n=3）	F-test between the two methods	T-test between the two methods
1	416±9.20	417±6.30	2.132	0.155
2	364±13.5	347±5.90	5.236	2.425
3	465±5.45	474±5.10	1.142	2.088

$F_{0.05, 2, 2}$=19.00, $t_{0.05, 4}$=2.776

1 (a)J. Xie, Y. Zheng, J. Y. Ying, *J. Am. Chem. Soc.* 2009, **131**, 888-889; (b)X. Yang, M. Shi, R. Zhou, X. Chen, H. Chen, *Nanoscale* 2011, **3**, 2596-2601. (c)H. Deng, L. Zhang, S. He, A. Liu, G. Li, X. Lin, X. Xia, W. Chen. *Biosens. Bioelectron.* 2015, **65**, 397-403.