Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Substrate Hydrolysis Triggered Formation of Fluorescent Gold Nanoclusters – A New Platform for the Sensing of Enzyme Activity

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Experimental Section

Materials: Tetrachloroauric acid tetrahydrate ($HAuCl_4 \bullet 4H_2O$) and potassium thioacetate (CH_3COSK) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Tetrakis(hydroxymethyl)phosphonium chloride (ca. 80% in water) (THPC) was obtained from TCI Development Co., Ltd. (Shanghai, China). 6-Bromo-1-hexanol (95%) was purchased from Acros Organics (New Jersey, USA). Sodium thiophosphate dodecahydrate ($Na_3PO_3S \bullet 12H_2O$) was purchased from Alfa Aesar (Tianjin, China). Esterase was purchased from Sigma–Aldrich (St. Louis, MO, USA). Alkaline phosphatase (from calf intestine) was purchased from Takara Biotechnology Co., Ltd. (Dalian, China). The ultra-pure water used throughout the experiments was purified with a Milli-Q A10 filtration system (Millipore, Billerica, MA, USA). All other reagents were of analytical grade and used without further purification.

Measurements: Fluorescence spectra were recorded on a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., USA). Excitation and emission slit widths were both of 2 nm. UV-Vis absorption spectra were collected using a Cary 50 Bio Spectrophotometer (Varian Inc., CA, USA). Quartz cuvettes with 10 mm path length and 2 mm window width were used for UV-Vis and fluorescence measurements. Transmission electron microscopy (TEM) measurements were performed on a FEI TECNAI G^2 high-resolution transmission electron microscope (the Netherlands) operated at an accelerating voltage of 200 kV. Samples for TEM measurements were obtained by dropping 15 μL sample solution onto a carbon-coated copper grid and then dried at room temperature. Fluorescence lifetime values were obtained with a FLS980 spectrofluorometer (Edinburgh instruments, UK). X-ray photoelectron spectroscopy (XPS) spectra were obtained using a VG Thermo ESCALAB 250 spectrometer (VG Scientific) operated at 120 W. ^1H NMR spectra were obtained with a Bruker AVANCE

400 (400 MHz) Fourier transform NMR spectrometer with chemical shifts reported in parts per million (ppm) relative to tetramethylsilane. Splitting patterns are reported as s (singlet), d (doublet), t (triplet), and m (multiplet). Unless otherwise specified, all spectra were taken at an ambient temperature of 22 ºC.

Synthesis of S-(6-Hydroxy-hexyl) thioacetate (substrate 1): 6-Bromo-1-hexanol (0.50 g, 2.76 mmol), CH_3COSK (0.63 g, 5.52 mmol), and dry DMF (4 mL) were stirred at room temperature for 20 h. The product was poured to water (30 mL) and extracted with ethyl acetate (3 × 10 mL). The solvent was removed by rotary evaporation and the crude product was purified by column chromatography on silica gel using a mixture of CH_2Cl_2/CH_3OH (40 : 1) as eluent to obtain the esterase substrate 1 (0.444 g, 91%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 3.64 (t, 2H, OCH_2), 2.87 (t, 2H, SCH_2), 2.32 (s, 3H, $COCH_3$), 1.60-1.55 (m, 4H, alkyl), 1.43-1.38 (m, 4H, alkyl) (Fig. S15).

Synthesis of sodium S-(6-hydroxy-hexyl) thiophosphate (substrate 2): 6-Bromo-1-hexanol (0.20 g, 1.10 mmol) and Na_3PO_3S (0.99 g, 5.50 mmol) were added to NaOH (0.1 M, 10 mL) aqueous solution. The mixture was stirred under nitrogen atmosphere at 55 ºC for 2 h, then cooled to room temperature and stirred overnight. Methanol (20 mL) was added to the reaction mixture and excess Na_3PO_3S was precipitated as a white solid. The precipitate was then removed by centrifugation and the supernatant was decanted and dried under vacuum. The residue was then suspended in acetone (10 mL) and stirred at room temperature for 30 min. The solid was collected on a funnel and washed with acetone (3 × 5 mL) and acetonitrile (3 mL), respectively. The solid was then dried overnight to afford the ALP substrate 2 (0.194 g, 68%). ^{1}H-NMR (400 MHz, D_2O): δ = 3.54-3.51 (t, 2H, OCH_2), 2.68-2.62 (m, 2H, SCH_2), 1.57-1.46 (m, 4H, alkyl), 1.33-1.28 (m, 4H, alkyl) (Fig. S16).

Preparation of the gold nanoparticles (AuNPs): A typical experimental procedure is as follows: 0.25 mL of NaOH (1 M) was added to 22.5 mL of water, followed by the addition of 0.5 mL of THPC aqueous solution [prepared by the addition of 4.5 μL of THPC (80%) to 0.5 mL of water]. The mixture was stirred at 25 ºC for 5 min, followed by rapid addition of $HAuCl_4$ (1 wt%, 0.75 mL). The solution color turned brown immediately and the mixture was stirred for another 25 min. The obtained AuNP sample solution was stored at 4 ºC before use. The molar ratio of THPC to $HAuCl_4$ was 1 : 1.5. The AuNPs concentration was calculated to be ca. 0.79 μM according to the Beer–Lambert law (the molar extinction coefficient of ~3 nm AuNPs is 1.49×10^6).[S1]

The same experimental procedures were used to prepare the AuNPs with different amounts of THPC added. In these cases, the molar ratio of THPC to $HAuCl_4$ was 1 : 1 and 1 : 2, respectively.

Enzyme triggered etching of the AuNPs: 20 μL of HCl (0.1 M) aqueous solution was introduced to 200 μL of the as-prepared AuNPs to adjust the solution pH to a neutral value (~ pH 7.0). The mixture was then combined with 80 μL of borate buffer (100 mM, pH 9.0). 50 μL of the enzyme substrate (40 mM) was introduced, followed by the addition of 40 μL of the enzyme of various concentrations. 10 μL of water was added to bring the final volume to 400 μL. The samples were incubated at 37 ºC for 4 h prior to fluorescence measurements. Final concentrations: 20 mM borate buffer, 5 mM substrate.

The esterase and ALP generated NCs are denoted as esterase-AuNCs and ALP-AuNCs, respectively. Unless specified, the following conditions were used for the assay optimizations: 5 mM substrate; 500 mU/mL esterase (or 250 mU/mL ALP); 4 h reaction time.

Selectivity of the protease activity assay: Different proteins including ALP, esterase, collagenase, AChE, lysozyme, trypsin, lipase, and BSA were added to the sample solutions of 200 μL AuNPs and 5 mM substrate 2 in 20 mM borate buffer (pH 9.0). The solutions were incubated at 37 ºC for 4 h and then the emission spectra were recorded. Protein concentrations: ALP, esterase, collagenase, AChE, lysozyme, trypsin, and lipase: 100 mU/mL each; BSA was 0.2 mg/mL. Final sample volume: 400 μL.

ALP activity assay in biological fluids: Different amounts of ALP were added to the sample solutions of 200 μL AuNPs, 5 mM substrate 2, and 2% calf serum (or 2% A549 cell lysate) in 20 mM borate buffer (pH 9.0). The solutions were incubated at 37 ºC for 4 h and then the emission spectra were recorded. Final sample volume: 400 μL.

ALP inhibition assay: 80 μL of the borate buffer was mixed with 40 μL of ALP. 10 μL of Na_3VO_4 of various concentrations was added. The solutions were incubated at 4 ºC for 20 min. 50 μL of substrate 2 and 200 μL of the AuNPs were introduced. The samples were incubated at 37 ºC for 4 h and the emission spectra were recorded. Final concentrations: 20 mM borate buffer (pH 9.0), 5 mM substrate 2, 50 mU/mL ALP. Final sample volume: 400 μL.

References:

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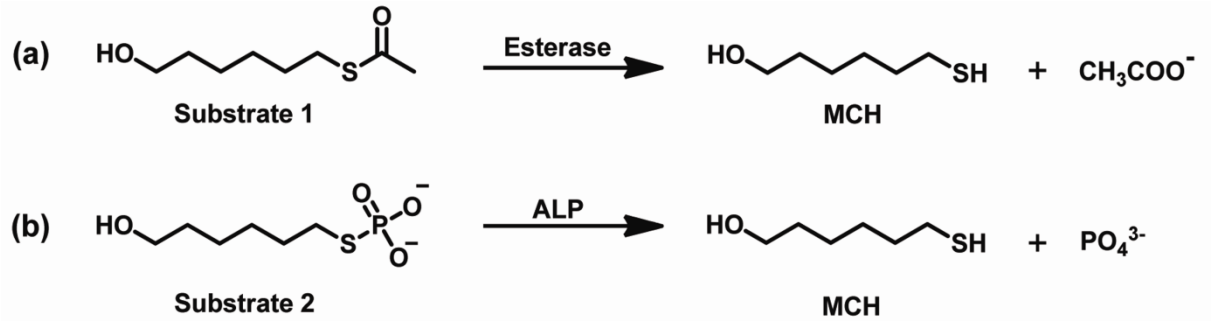

Scheme S1. Enzyme catalyzed hydrolysis of substrate 1 (**a**) and substrate 2 (**b**).

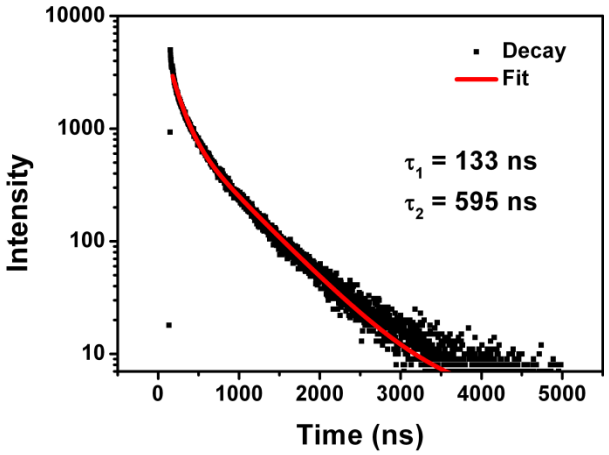

Fig. S1 Fluorescence decay profile of the esterase-AuNCs (λ_{ex} = 375 nm, λ_{em} = 505 nm, χ^2 = 1.474). The fractional weights of τ_1 and τ_2 are 31.35% and 68.65%, respectively. The intensity-weighted average lifetime was 552 ns, which was calculated according to the literature reported procedures.[S2, S3]

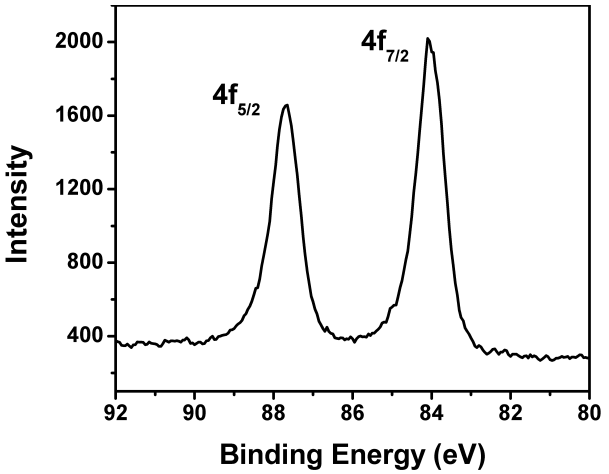

Fig. S2 XPS spectrum of Au-THPC NPs. The binding energy of Au($4f_{7/2}$) is 84.10 eV. The results suggest that more Au(I) was generated after the etching process as suggested in the literature (ref 13).

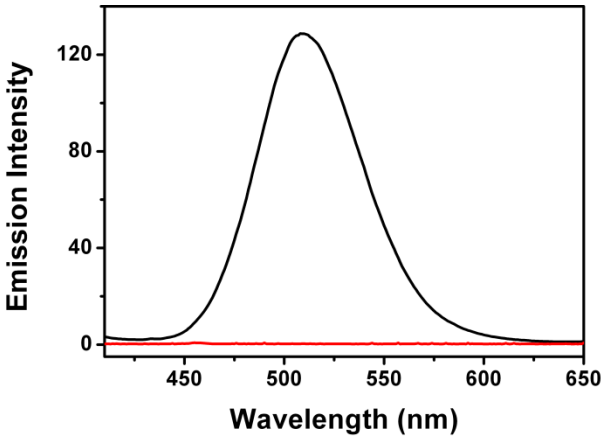

Fig. S3 Emission spectra of the esterase-AuNCs (black line) and the AuNPs mixed with 100 mU/mL esterase in the absence of substrate 1 (red line).

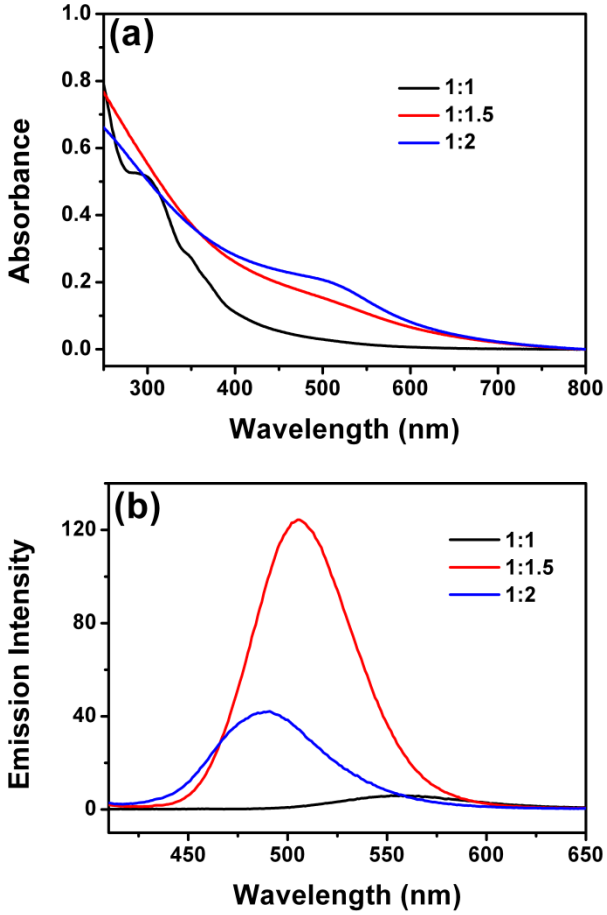

Fig. S4 (a) UV-Vis absorption spectra of the gold nanoparticles prepared with different ratios of THPC to $HAuCl_4$. The spectra was measured after six times dilution of the original solution. **(b)** Emission spectra of the esterase-AuNCs prepared with the gold nanoparticles of (a).

The amount of the reducing agent used during the synthesis of the AuNPs affected their size and consequently affected the core etching product. The surface plasma resonance (SPR) peak at 520 nm could be clearly observed in the UV-Vis absorption spectrum when the molar ratio of THPC to $HAuCl_4$ was 1 : 2. With the increase of the amount of the reducing agent (at a molar ratio of THPC to $HAuCl_4$ of 1 : 1.5), the SPR peak reduced considerably and the

emission intensity of the esterase-AuNCs was the strongest. When the molar ratio of THPC to $HAuCl_4$ was changed to 1 : 1, there was no SPR peak and the band at 304 nm appeared. The absorption spectrum is similar to those of the literature reported AuNCs, indicating that some small-sized clusters were formed.[S4-S6]

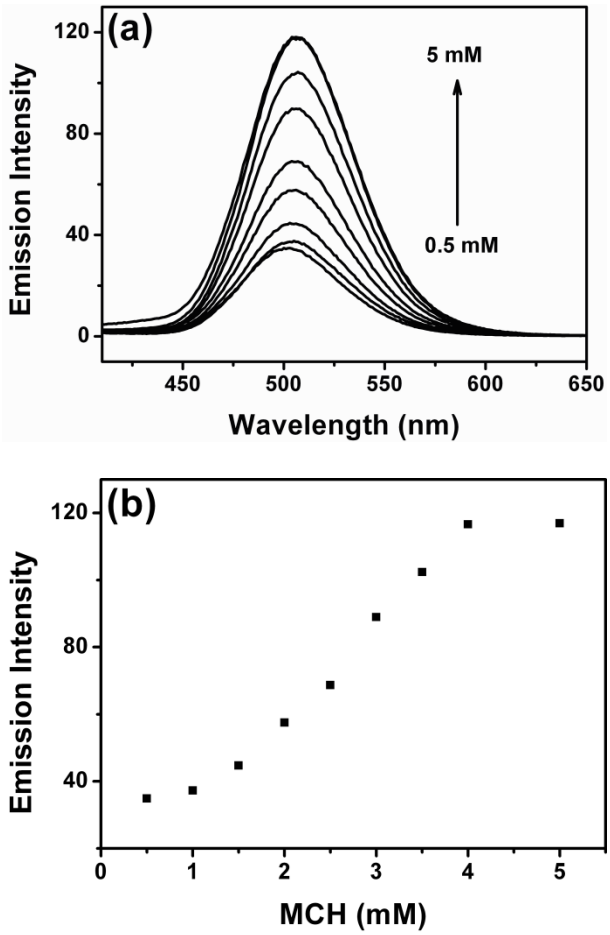

Fig. S5 (a) Changes in emission spectrum of the AuNCs as a function of the MCH concentration. **(b)** Changes in maximum emission of (a) versus MCH concentration. Conditions: 4 h reaction time.

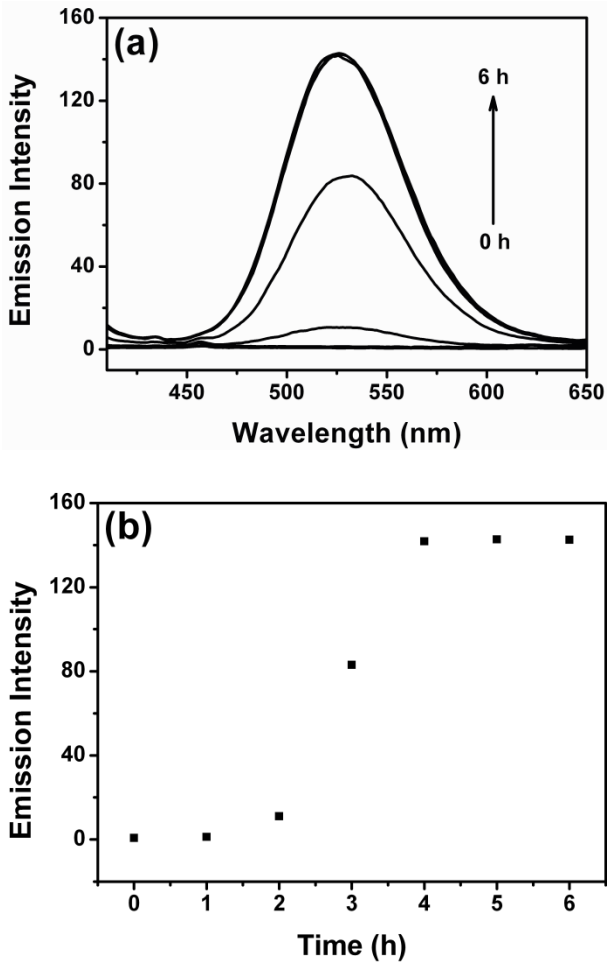

Fig. S6 (a) Changes in emission spectrum of the esterase-AuNCs as a function of the reaction time. **(b)** Changes in maximum emission of (a) versus reaction time. Conditions: 5 mM substrate; 500 mU/mL esterase.

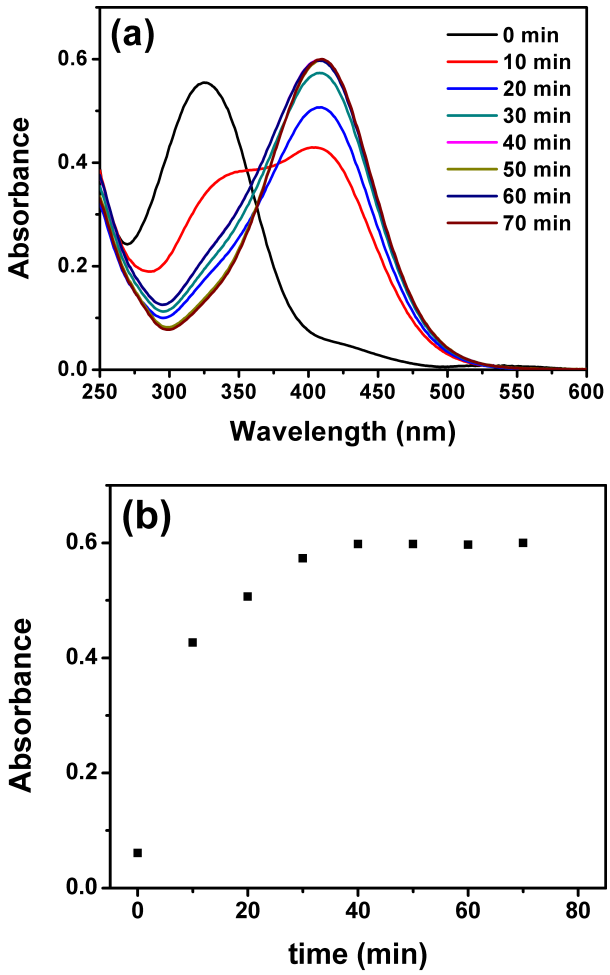

Fig. S7 (a) Changes in UV-Vis absorption spectra with the enzymatic reaction time. **(b)** Changes in UV-Vis absorption of (a) at 410 nm versus reaction time. Conditions: 5 mM substrate 1; 500 mU/mL esterase. 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) was used to quantify the concentration of the newly generated MCH.[S7]

Fig. S7 shows that the enzyme reaction completed in about 40 min. The relatively long reaction time required in the current assay is thus possibly a result of the relatively slow Au NP etching process.

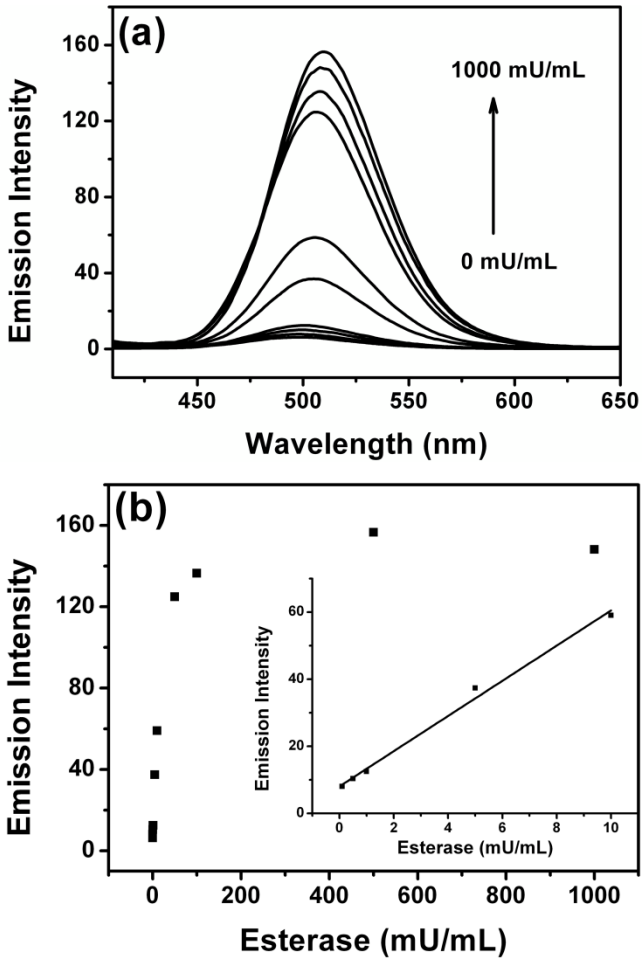

Fig. S8 (a) Changes in emission spectrum of the AuNCs upon the addition of esterase in different concentrations (0, 0.1, 0.5, 1, 5, 10, 50, 100, 500, and 1000 mU/mL). **(b)** Maximum emission intensity changes as a function of the esterase concentration. Inset: expanded linear region of the calibration curve. The linear regression equation is I = 8.6 + 5.25C (correlation coefficient R^2 = 0.991), where "I" is the maximum emission intensity and "C" is the esterase concentration in mU/mL.

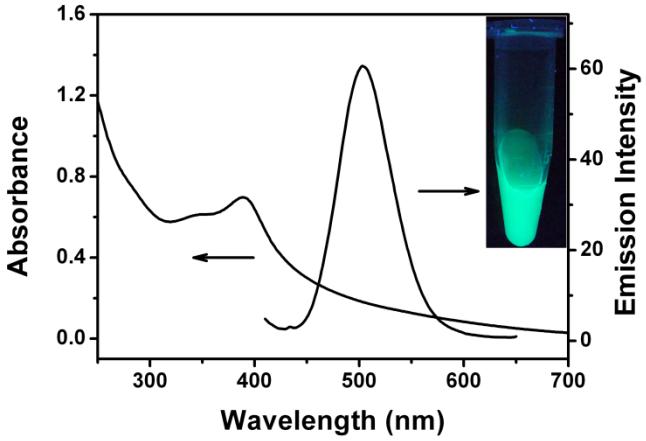

Fig. S9 UV-Vis absorption and emission spectra of the ALP-AuNCs. Inset: photograph of the AuNCs under 365 nm UV light illumination.

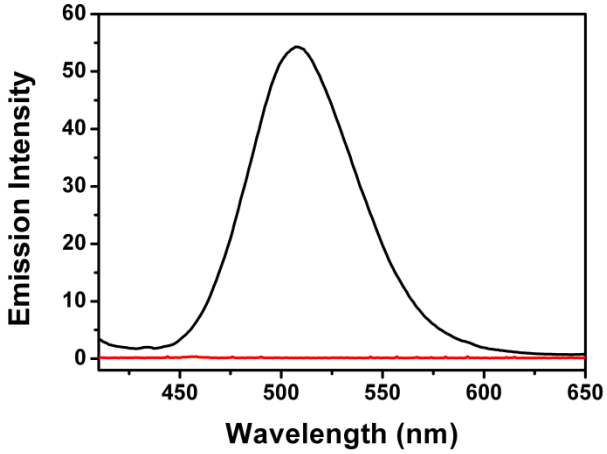

Fig. S10 Emission spectra of the ALP-AuNCs (black line) and the AuNPs mixed with 100 mU/mL ALP in the absence of substrate 2 (red line).

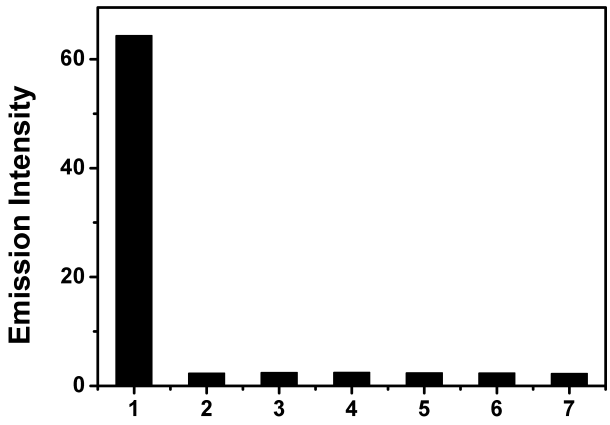

Fig. S11 Changes in emission intensity at 505 nm of the ALP-AuNCs and the thiol-containing compounds induced etching products. Sample 1: ALP-AuNCs (250 mU/mL ALP + 5 mM substrate 2). Samples 2 – 7: glutathione, cysteine, homocysteine, dithiothreitol, 2-mercaptoethanol, and thioglycolic acid, respectively, each at 5 mM concentration.

The proteins tested in the selectivity assay (Fig. 4) all contain the thiol-containing amino acid cysteine. The number of cysteines in the proteins is 5 for esterase, 5 for collagenase, 7 for AChE, 8 for lysozyme, 12 for trypsin, 14 for lipase, and 35 for BSA (the protein sequences were obtained from the database of the National Center for Biotechnology Information).

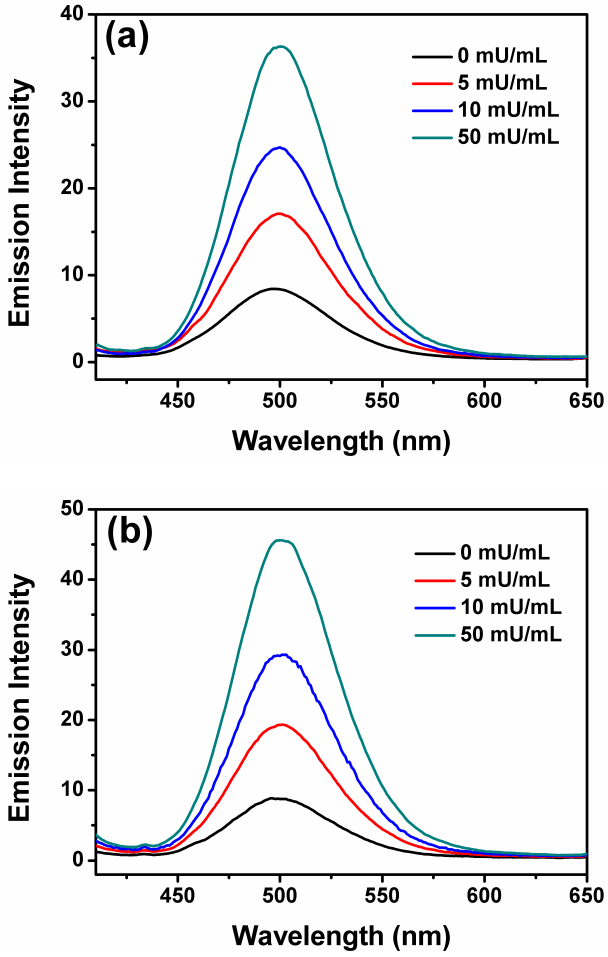

Fig. S12 Changes in emission spectrum of the ALP-AuNCs with ALP concentration in 2% calf serum **(a)** or 2% A549 cell lysate **(b)**.

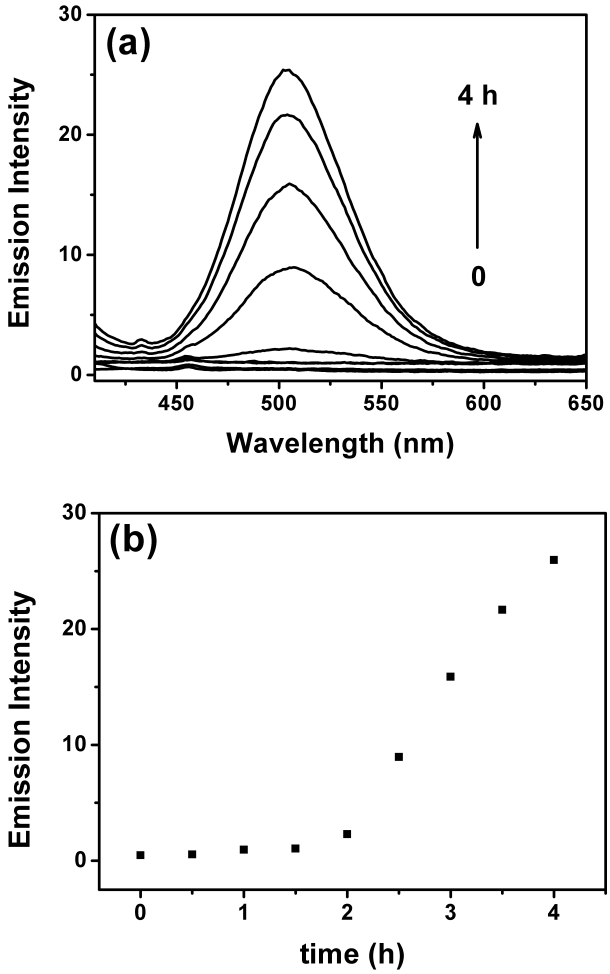

Fig. S13 (a) Changes in emission spectrum of the AuNCs as a function of reaction time. **(b)** Changes in maximum emission of (a) versus reaction time. Conditions: 50 mU/mL ALP, 3 mM Na_3VO_4.

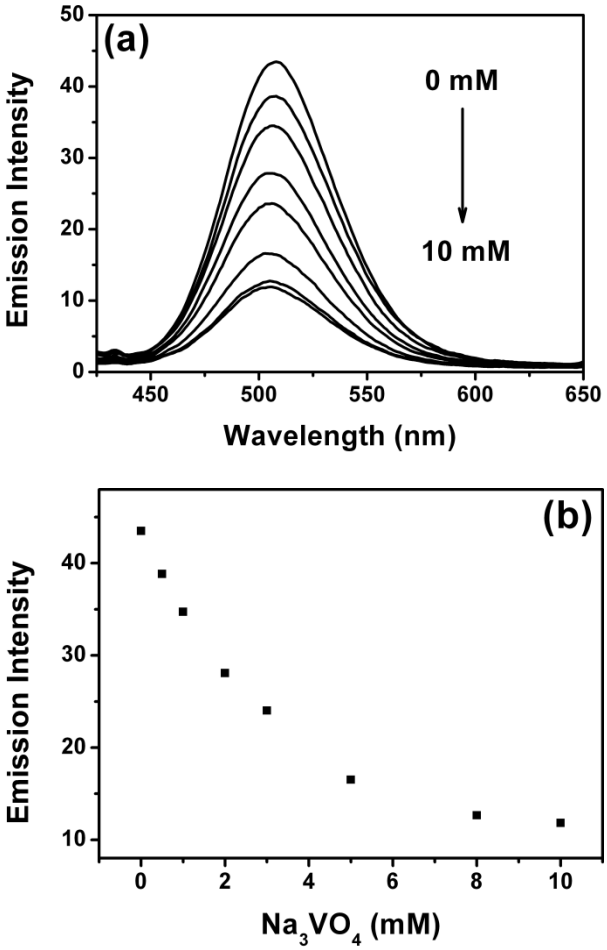

Fig. S14 (a) Changes in emission spectrum of the ALP-AuNCs as a function of the Na_3VO_4 concentration. **(b)** Maximum emission of the ALP-AuNCs of (a) versus Na_3VO_4 concentration.

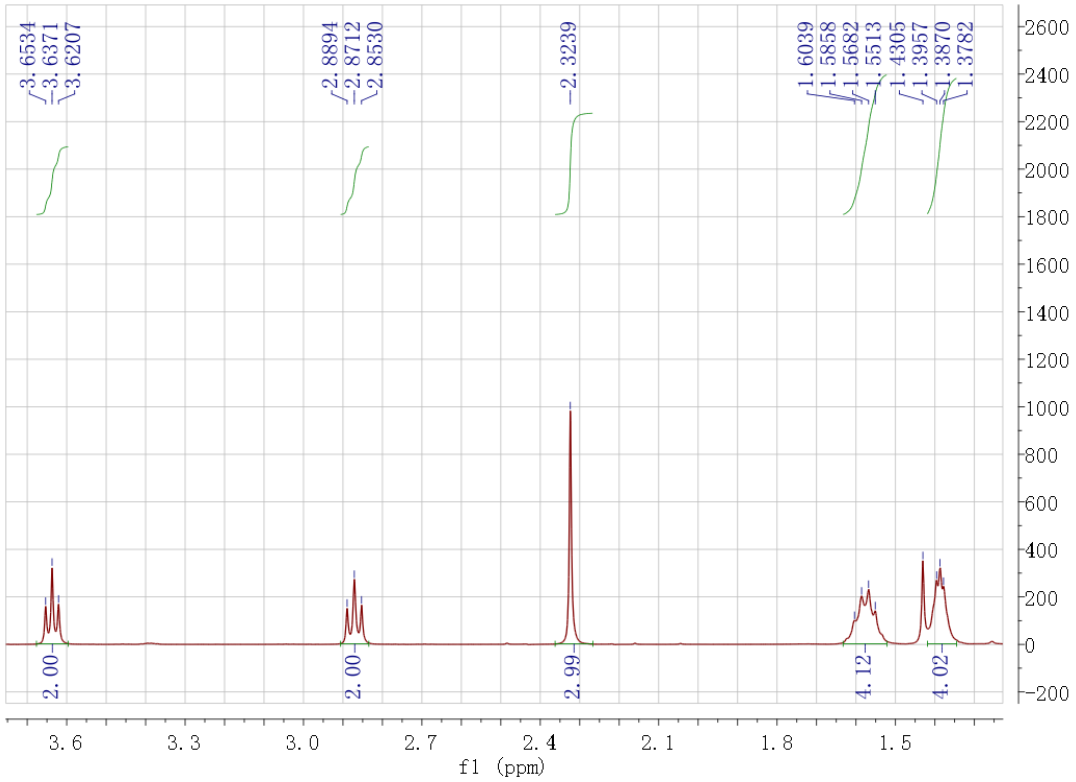

Fig. S15 ^{1}H-NMR spectrum of substrate **1**.

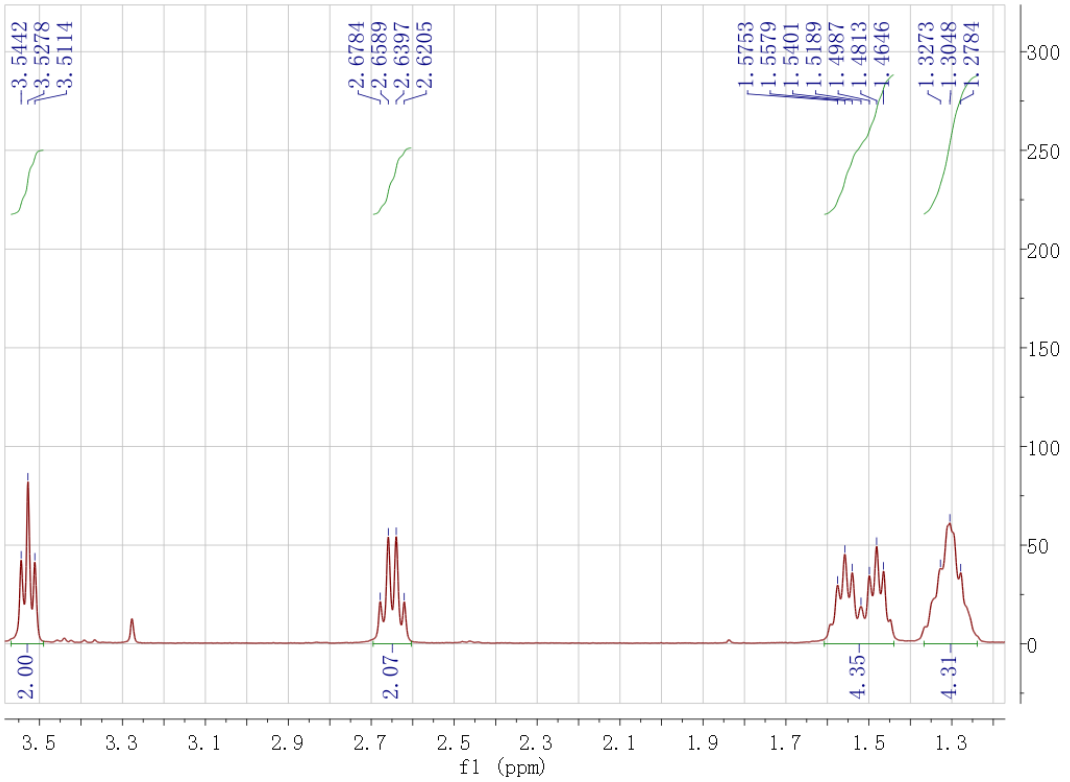

Fig. S16 ^{1}H-NMR spectrum of substrate **2**.