Supporting Information

Cysteine Directed Fluorescent Gold Nanoclusters for the Sensing of Pyrophosphate and Alkaline Phosphatase

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Figure S1. UV-Vis absorption spectrum of Au NPs.

The value of the extinction coefficients (ϵ) of ~3 nm Au NPs at $\lambda = 450$ nm is 1.49*10⁶ M⁻¹cm⁻¹ according to the literature (Anal. Chem. 2007, 79, 4215-4221). The absorbance (A) at $\lambda = 450$ nm of Au NPs (the NPs was diluted six times by water) is 0.1972. According to the Beer-Lambert law A = ϵ bc (b = 1 cm), the concentration of as prepared Au NPs was calculated to be 0.79 μ M.



Figure S2. TEM image of Au NPs before centrifugation separation. The size is in the range of 8 - 34 nm.



Figure S3. UV-Vis absorption spectrum of Au NCs.



Figure S4. (a) UV-Vis absorption spectrum of Au NPs in the absence of cysteine. (b) Emission spectra of Au NPs with (red line) and without (black line) the addition of cysteine.



Figure S5. Fourier transform infrared (FT-IR) spectra of cysteine and the Au NCs.



Figure S6. Emission spectra of the Au NCs in 10 mM buffer solutions of different pH values of 3–12.



Figure S7. Emission intensity changes of (a) rhodamine 6G and (b) fluorescein as a function of irradiation time. The sample solution was under continuous excitation of 410 nm with a 150 W Xenon lamp.

The results show that our Au NCs have similar photo-stability with those traditional fluorescent dyes under the same experiment conditions. However, literature report has shown that the fluorescence of the dyes diminished rapidly under the continuous excitation due to the severe photobleaching (J. Am. Chem. Soc., 2011, 133, 14192–14195). This is because the irradiation intensity is different.



Figure S8. Relative emission intensity $[(I_{re}-I_q)/I_{re}]$ of the Au NCs as a function of the Cu²⁺ concentration (5, 10, 25, 50, 75, and 100 μ M). I_{re} and I_q were the maximum emission intensity of the sample solutions with or without the addition of PPi. Conditions: 10mM buffer, 50 μ M PPi.



Figure S9. Relative emission intensity $[(I-I_0)/I_0]$ of the Au NCs in the presence of different ions. I_0 and I were the maximum emission intensity of the sample solutions without and with the addition of the ions.

The experiment was conducted under the conditions used for the assay of ALP activity (10 mM buffer, 100 μ M Cu²⁺, 80 μ M PPi and interference ions). The results show that PPi has the most striking effects on the fluorescence intensity. Although our method also responses to ATP, its possible interference could be ruled out using pyrophosphatase according to the literature (Anal. Chem., 2013, 85, 2516–2522).



Figure S10. (a) Changes in emission spectrum of the Au NCs with reaction time. (b) Maximum emission intensity changes as a function of reaction time. Conditions: 10 mM buffer, 100 μ M Cu²⁺, 80 μ M PPi, 50 mU/mL ALP.



Figure S11. Emission intensity of the Au NCs with the addition of different Pi ions. The Pi concentrations were: (1) 0 μ M, (2) 1 μ M, (3) 10 μ M, (4) 50 μ M, (5) 100 μ M, (6) 500 μ M and (7) 1 mM, respectively. Conditions: 10 mM Tris-HCl, 100 μ M Cu²⁺, 80 μ M PPi, 25 mU/mL ALP.