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Supporting Information

The Determination of Cystatin C in Serum Based on Label-Free and Near-Infrared Light Emitted PbS@BSA QDs

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Experimental section

Materials and reagents

$Pb(NO_3)_2$, Na_2S, cystatin C, Na_2HPO_4, NaH_2PO_4, thrombin, glucose oxidase (GOx) and lysozyme were purchased from Sigma-Aldrich. BSA, papain, Tris were purchased from Sangon (Shanghai, China). HCL, L-Cysteine, Ethylene diamine tetraacetie acid disodium salt (EDTA-Na_2) were purchased from Sinopharm (Shanghai, China). Cystatin C detection kit was purchased from CHANG ZHOU IFCAN BIO-TECHNOLOGY AND SCIENCE CO, LTD (Jiangsu, China). All the chemicals were of analytical grade and used without further purification. Ultrapure water was obtained from a Millipore Milli-Q system (18.2 MΩ•cm) was used in all runs. Papain storage solution was a mixture of 40 mM EDTA-Na_2, 50 mM Na_2HPO_4 and 50 mM L-Cysteine. Cystatin C was dissolved in the PBS (pH=7.0, 20 mM).

Apparatus

The fluorescence spectra of PbS@BSA QDs were obtained by a PTI ASOC-10 Fluorescence System (Photo Technology International, Birmingham, NJ, USA), the excitation wavelength was 450 nm. The UV-vis absorption spectra were measured on a Hitachi U-4100 UV/vis spectrometer (Kyoto, Japan) using a quartz cuvette having 1 cm path length. Transmission electron microscopy (TEM) measurements were conducted on a JEOL 1230 electron microscope. The fluorescence imaging experiments were conducted by Lumina XR imager under the emission filters of ICG (NIR light) and DsRed (visible light), the excitation filter of GFP. The pH values were calibrated with a model 868 pH meter (Orion).

Synthesis of NIR PbS@BSA QDs

The PbS@BSA QDs were prepared according to our previous reports. For a typical synthesis, 95 μL of 50 mM Tris-HCl (pH 7.5, 25 °C) containing 5 mg/mL BSA was mixed with 12 μL of 10 mM $Pb(NO_3)_2$ and incubated at room temperature for 10 min. Next, 6 μL of 10 mM Na_2S was quickly injected into the solution followed by intense agitation on a vortex mixer for 10 s.

Procedure for detection of papain and cystatin C

To investigate the papain digestion leaded quenching of NIR fluorescence of PbS@BSA QDs, aliquots of 100 μL of freshly prepared AuNCs-PbS-QDs were added to 1.5 mL microcentrifuge tubes, then 10 μL different concentrations of papain were added to the tubes and incubated in a mixing apparatus for 2 h at 35 °C. Finally, the fluorescence spectra of the mixture were measured with an excitation wavelength at 450 nm. For the detection of cystatin C, 5 μL different concentration of cystatin C was first mixed with 5 μL papain (15 μg/mL) and incubated for 0.5 h, the 10 μL mixture solution were added to the tubes and incubated in a mixing apparatus for 2 h at 35 °C. The fluorescent spectra of the resulting solutions were measured with excitation at 450 nm. Both slit width of excitation and emission were 20 nm.

Selectivity measurements

The selectivity of this NIR QDs sensor for the papain enzyme was measured with 50 μg/mL thrombin, glucose oxidase, and lysozyme. The influence of interferent or in coexistence with target to the assay results were inspected at the same time. The selectivity for the cystatin C was measured with different kinds of interferents (10 μM) including cations, molecules, sugars, amino acids, haemoglobin, DNA and urea.

Detection of cystatin C in the serum sample

For the recovery experiments, serum sample was taken from a healthy volunteer, and it was separated by centrifugation on Amicon Ultra centrifugal filter device with a 10000 molecular weight (MW) cutoff according to the manufacturer's instructions. The treated serum samples were stored at 4 °C. Cystatin C with different concentrations were spiked in 55 μL the serum samples, which was then mixed with 5 μL papain (15 μg/mL) incubated for 0.5 h, the 60 μL of resulting sample containing different concentration of cystatin C and papain was added to 50 μL solution of PbS@BSA (0.5 mg/mL BSA). After reacted for 2 h at 35 °C, the fluorescence spectra

were measured. The concentration value of cystatin C was calculated through the established calibration curve.

For the comparison of our method with the traditional detection kit, five serum samples of healthy volunteers were used. 55 μL of the serum samples without treatment was mixed with 5 μL papain (15 μg/mL) incubated for 0.5 h, the 60 μL of resulting samples was added to 50 μL solution of PbS@BSA (0.5 mg/mL BSA). After reacted for 2 h at 35 °C, the fluorescence spectra were measured. The concentration value of cystatin C was calculated through the established calibration curve. Cystatin C assay by detection kit was carried out following the manufacture protocol.

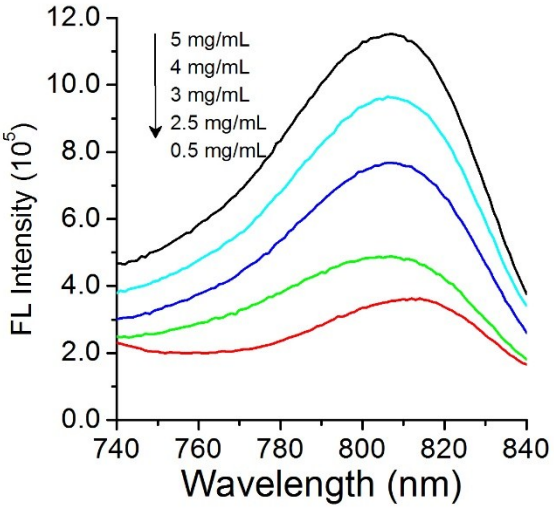

Fig. S1. The relationship between the NIR fluorescent intensity of as prepared PbS@BSA QDs and the concentration of BSA.

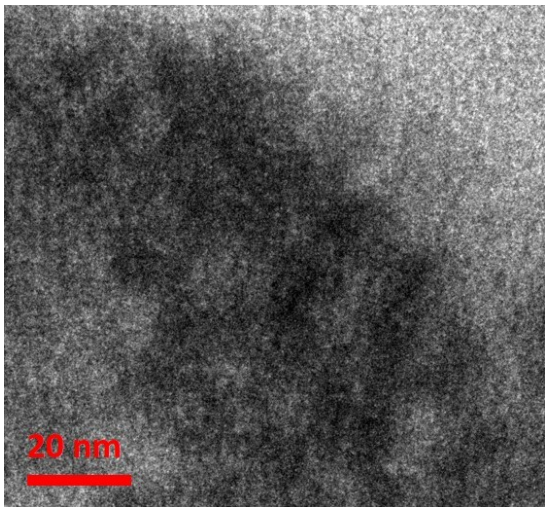

Fig. S2. The TEM image of PbS@BSA after incubated with papain for 2 h.

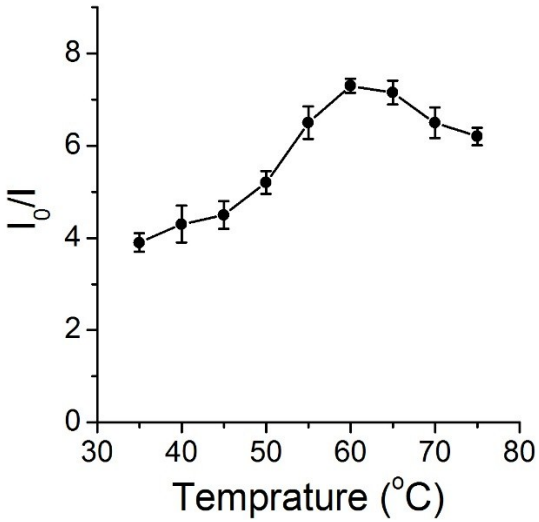

Fig. S3. The temperature-dependent NIR fluorescence quenching of PbS@BSA QDs (5 mg/mL BSA) after incubating with 40 μg/mL papain for 3 h.

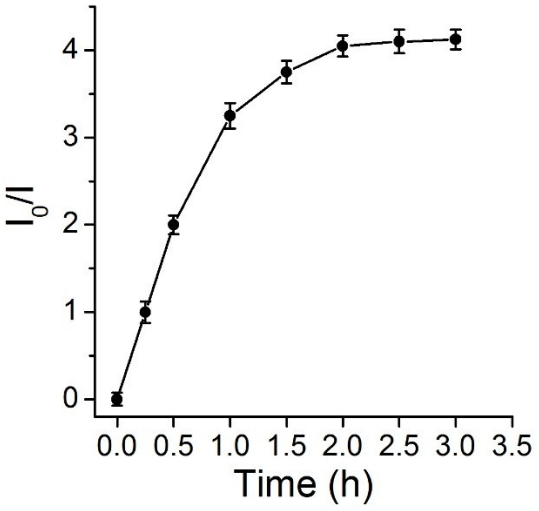

Fig. S4. The NIR fluorescence quenching of PbS@BSA QDs after incubating with 40 μg/mL papain for different time in the water bath of 35 °C.

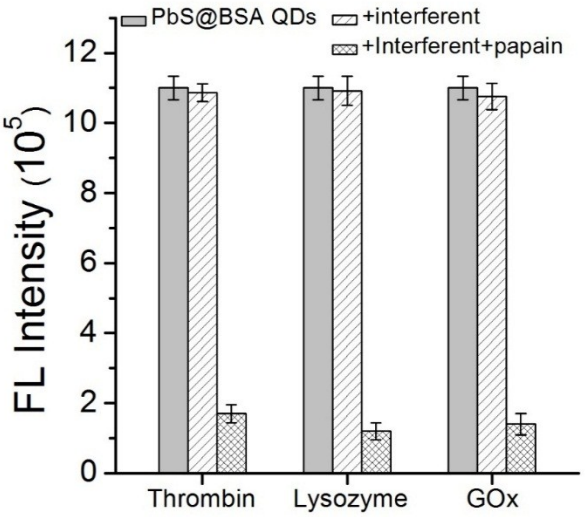

Fig. S5. The NIR fluorescent intensity at 813 nm of PbS@BSA QDs after incubated with 50 μg/mL interferents in the absence or presence of 40 μg/mL papain for 2 h.

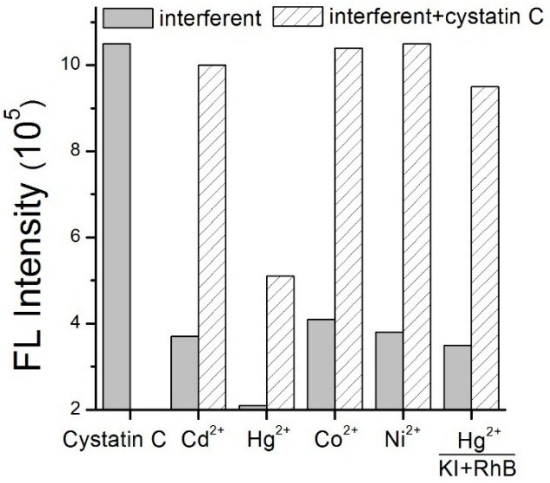

Fig. S6. Fluorescence responses of the PbS@BSA QDs towards 10 μM Cd^{2+}, Hg^{2+}, Co^{2+}, Ni^{2+}, and Hg^{2+} pretreatment with KI and RhB.

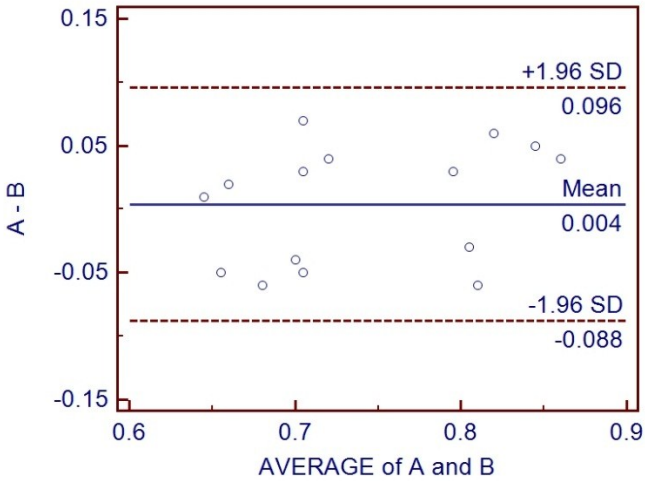

Fig. S7. Bland-altman plot of the measured results of two methods, “A” represent the cystatin C detection kit and “B” represent proposed method in this manuscript.