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Supporting Information

A Colloidal Au Nanoparticles-Based "Turn on" Fluorescence Imaging for In-gel Proteins Detection

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EXPERIMENTAL SECTION

Reagents

All reagents were of analytical reagent grade. Auric chloride acid ($AuCl_3$•HCl•$4H_2O$), sodium citrate, acetic acid (HAc), sodium hydroxide (NaOH), hydrochloric acid (HCl) and sodium acetate (NaAc) were purchased from Beijing Reagents Company (Beijing, China). Bovine serum albumin (BSA), acrylamide (Acry), aminoacetic acid (glycine), glycerine, bromophenol blue, ammoniumpersulfate, N, N'-methylenebisacrylamide (Bis), N, N, N', N'-tetramethylethylenediamine (TEMED), and tris(hydroxymethyl)aminomethane (Tris) were purchased from Dingguo Biotech Company (Beijing, China). Water was deionized and further purified with a Milli-Q water purification system (Millipore, Milford, MA). Human serum samples were obtained from the Affiliated Hospital of the Beijing Normal University (Beijing, China).

Instruments

The electrophoresis systems were made up of DYCZ-21 and DYCZ-24D vertical electrophoresis tanks, together with the electrophoresis instruments of DYY-6B and DYY-6C. These were purchased from Liuyi Instrument Factory (Beijing, China). The images were formed by using the fluorescence bioimaging system (UVP EC3 Imaging System, UVP Inc., USA). High-resolution transmission electron microscopy (HRTEM, JEOL JEM-2010) was used for the characterization of Au NPs. The

fluorescence spectra were recorded using LS-55 luminescence spectrometer (PerkinElmer Co. Ltd.). The fluorescence emissions of in-gel proteins were measured by Laser Confocal Raman Microspectroscopy (JR LabRam HR800, HORIBA Jobin Yvon, France).

Preparation of colloidal Au NPs

The colloidal Au NPs were synthesized according the reports (Z. X. Wang and L. N. Ma, ***Coordin. Chem. Rev.***, 2009, **253**, 1607-1618). In brief, 1.67 mL aqueous $HAuCl_4$ solution (0.4%，m/v) was added to ultrapure water (50 mL). Sodium citrate solution (1.94 mL, 0.1 M) was introduced after $HAuCl_4$ solution was boiling. Then the color of this solution changed from gold to purple, and finally turned to dark wine. The colloidal Au NPs were obtained after stirring for 25 min, and at last were stored at 4 ºC.

Fluorescence imaging via colloidal Au NPs

1 mL of prepared Au NPs solution (0.4%，m/v) was added into 10 mL HAc-NaAc solution (pH 3.2) to act as the incubating imaging solution. For FL imaging, the gels with in-gel proteins were incubated in the imaging solution for shaking about 2 h. The gel was then washed by water. At last, the images were obtained by the excitation at 365 nm in fluorescence bioimaging system. For quantitative analysis, the relative intensities (represented the total pixel density of the protein dots with the background

intensity subtracted) were measured using imaging software (UVP EC3 Imaging System, UVP Inc., USA) and the values exported to Origin 6.0 (Microcal Software, Inc., USA).

Preparation of polyacrylamide gel and gel electrophoresis

The gel (4%, m/v) solution was prepared by diluting 0.7 mL of gel stock solution, 1.25 mL of Tris-HCl (0.5 M, pH 6.8), 40 μL of $(NH_4)_2S_2O_8$ (10%, w/v), and 4 μL of TEMED to 5.0 mL of water. Gel stock solution (30%, w/v) was obtained by adding 29.2 g of acrylamide and 0.8 g of Bis into 100 mL of H_2O and filtrated. The in-gel proteins were mixed in the solution before solidification.

The native 1-D PAGE was performed in a vertical discontinuous gel system, including separating (7.5%, m/v) and stacking (4.0%, m/v) gels. The voltage was set at 120 V when the serum sample was in the stacking gel, and tuned to 90 V for about 3 h after it entered into the separating gel. For the native 2-D PAGE, samples were first subjected to isoelectric focusing (IEF) employing column gels, which was run at 200 V for 30 min, and then kept at 400 V for 16 h. After IEF, gels were transferred onto the second dimension-slab gels (7.5%, m/v), and electrophoresis for 3 h at 100 V. Sample dilution solution was prepared with 6.67% glycerine and 0.05% bromophenol blue, and the loading volume for each channel was 15 μL.

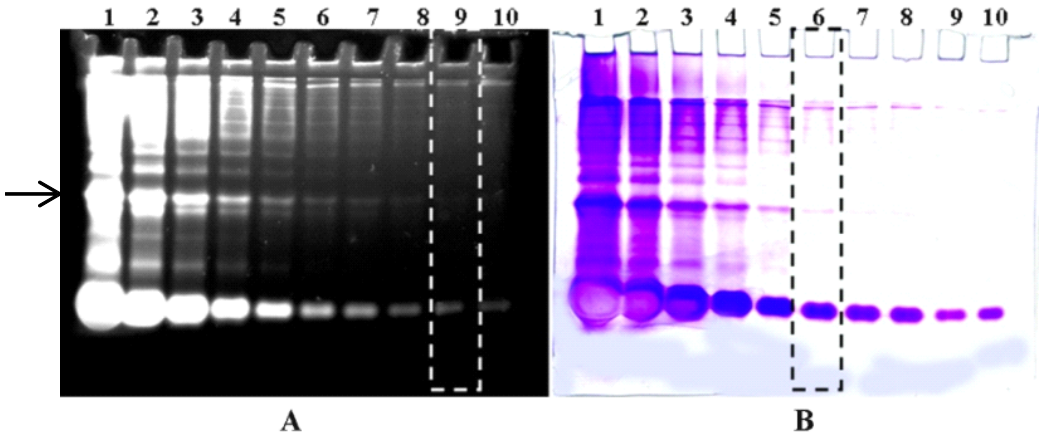

Figure S1. Sensitivity comparison of (A) Au NPs-based FL imaging and (B) CBB-R250 staining. Dilution ratio of the serum samples (from left to right): 1/2，1/5，1/10，1/20，1/40，1/80，1/100，1/200，1/300 and 1/400. Loading volume: 15 μL.

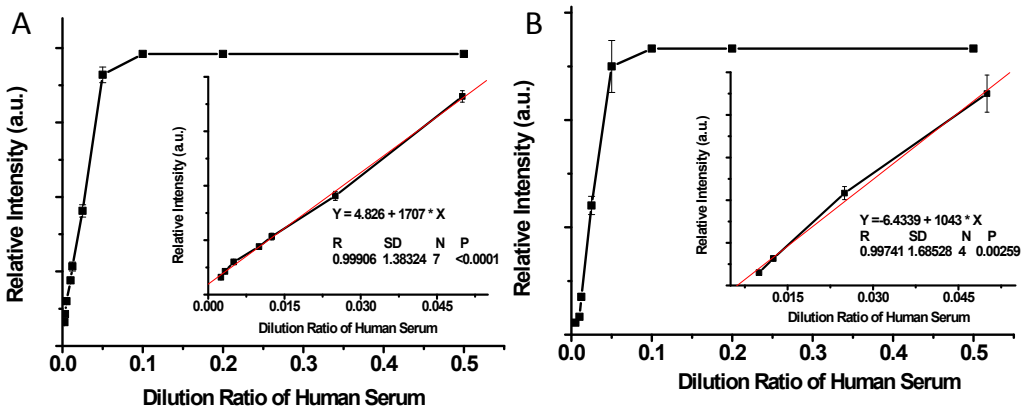

Figure S2. The relative signal intensity of transferrin as a function of dilution ratio of human serum for (A) Au NPs-based FL imaging and (B) CBB-R250 staining method. The insets show the linearity for each staining method.

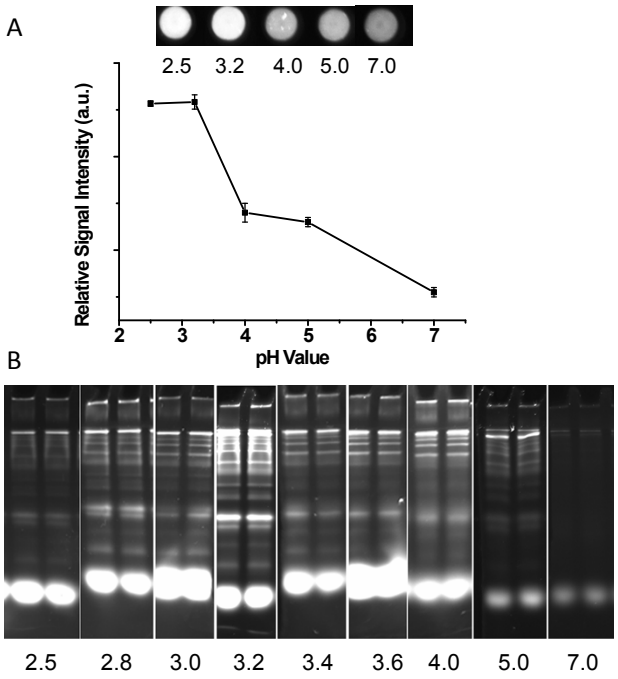

Figure S3. Effect of pH for Au NPs-based imaging. (A) Imaging for BSA in the buffer with different pH values. (B) Au NPs-based FL imaging for human serum proteins after PAGE at different pH values.

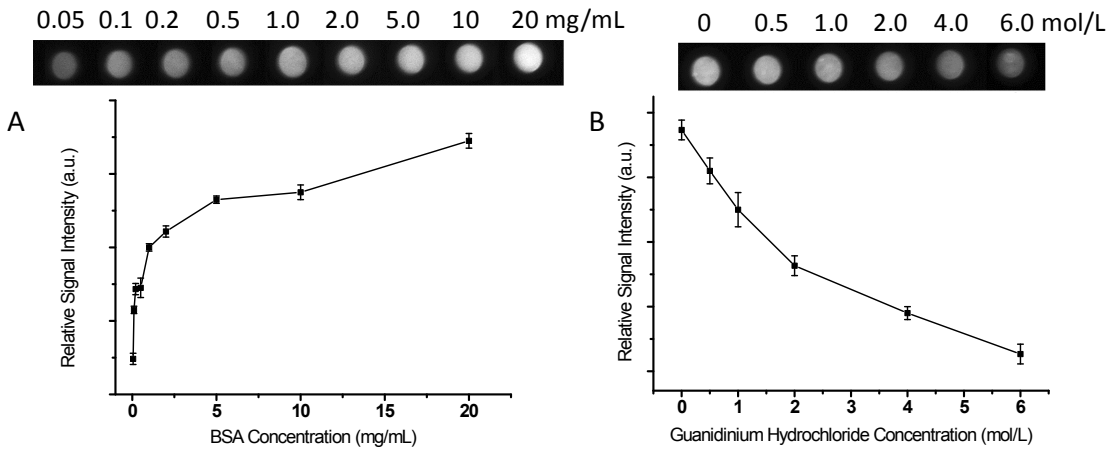

Figure S4. The effect of BSA (A) and guanidinium hydrochloride (B) concentration for Au NPs-based FL imaging. The insets are images of in-gel proteins by Au NPs-based FL imaging.

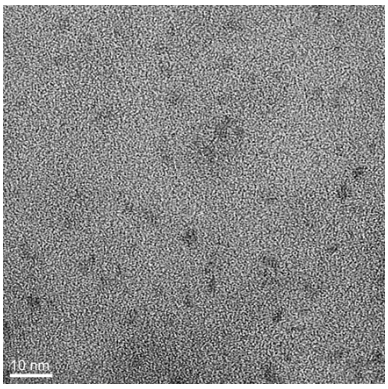

Figure S5. HRTEM images of Au NPs releasing from the gel after Au NPs-based FL imaging.

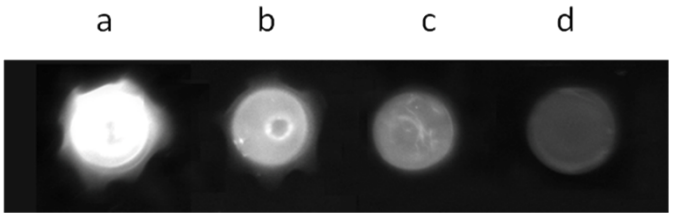

Figure S6. Images of HSA (2 mg/mL) obtained by Au NPs-based FL imaging on different gels. Percentage of the crosslinking regent (C%) of gels were (a) 0.67, (b) 0.67, (c) 4.67 and (d) 6.67, respectively. The pore size decreases with the increase of C%. The gel of (a) was pierced by the needle before the incubation.

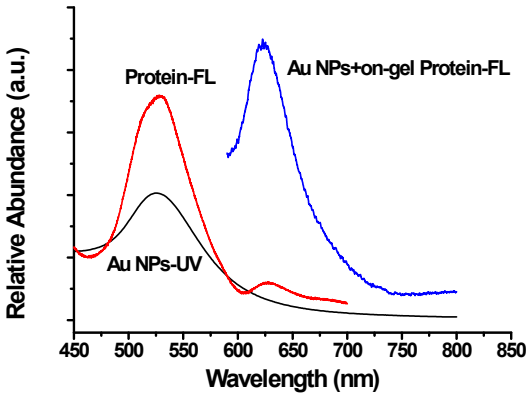

Figure S7. The spectra of the UV absorption of Au NPs, FL emission of in-gel HSA and the in-gel HSA after incubated by Au NPs.