Fluorescence Visual Gel-separation of Dansylated BSA-protected Gold-Nanoclusters

(Electronic Supporting Information)

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Experimental Section

All chemicals were obtained from commercial suppliers and used without further purification. The molecular weights of the BSA, dBSA, AuNC@BSA and AuNC@dBSA were determined by using an Autoflex III MALDI-TOF/TOF mass spectrometer (Sinapinic acid was used as the matrix for MALDI and tetrafluoroacetic acid was added to enhance the ionization). The elemental analysis was performed by using an ELAN 9000/DRC ICP-MS system. Fluorescence spectra were measured on a Shimadzu RF-5301PC fluorescence spectrophotometer. Infrared (IR) spectra were recorded with a Bruker Vertex 80V FT-IR spectrophotometer. Circular Dichroism (CD) spectra were detected with a Bio-Logic MOS-450 spectrophotometer.

(1) Synthesis and characterization of AuNC@dBSA

The synthesis of the AuNC@dBSA, involves two proposed routes. In the first route, the BSA is modified with dansyl chlorine firstly. The reaction was easily performed in the H2O and DMSO mix solution in the presence of NaHCO3. The NaHCO3 (50 mg) was added to the BSA aqueous (10 mL, 100 mg/mL) under vigorous stirring at room temperature. Then the dansyl chlorine solution (4.5 mg in 0.5 mL DMSO) was added to the solution. The reaction was completed in ~4 hours, as confirmed by time-course measurements of the fluorescence evolution. And then the solution were dialyzed with a 10 kDa cut-off dialysis bag extensively against doubly distilled water for more than 24 hours with a water change every 4 h to remove all small molecular impurity. And then it was subjected to freeze-drying to obtain dBSA in the powder form. The labeling ratio was determined by measuring both the concentrations of protein and probe in dBSA based on the early reported method.1,2 By following this procedure, we have obtained a mole ratio of dansyl/BSA = 0.96.

Then the aqueous HAuCl4 solution (5 mL, 10 mM) was added to the dBSA solution (5 mL, 50 mg/mL, 37 °C) under stirring. Two minutes later, NaOH solution (0.5 mL, 1 M) was introduced, and the mixture was incubated at 37 °C for 12 h. The color of the solution changed from light yellow to light brown, and then to deep brown. The aqueous solution was isolated for subsequent conversion.

In the second route, the synthesis procedure of AuNC@BSA was same as
previously reported. The HAuCl₄ aqueous (20 mL, 10 mM) was added to BSA aqueous (20 mL, 50 mg/mL, 37 °C) under vigorous stirring. Two minutes later, NaOH solution (2 mL, 1 M) was introduced, and the mixture was incubated at 37 °C for 12 hours. And the solution after synthesis were dialyzed with a 10 kDa cut-off dialysis bag extensively against doubly distilled water for more than 24 hours with a water change every 4 h to remove the residual NaOH and other salts.

Then the AuNC@BSA and residual BSA were modified with dansyl chloride. The NaHCO₃ (50 mg) was added to the dialyzed solution (mentioned above) under vigorous stirring at room temperature. Then the dansyl chloride solution (4.5 mg in 2 mL DMSO) was added to the solution. The reaction was completed in ~4 hours, and then the solution were dialyzed extensively against doubly distilled water for more than 24 hours with a water change every 4 h to remove all small molecular impurity. The aqueous solution was isolated for subsequent conversion.

(2) Separation of AuNC@dBSA in gel column.

The gel column for the separation of the mixture of AuNC@dBSA was prepared with the commercially supplied Sephadex G-100 and G-75 gel (supplied by Pharmacia). The gel (25 g) was soaked in water for 3 d, and then the supernatant (including the suspended ultrafine gel) was discarded. The remaining gel was washed until no any gel was suspended in the supernatant. Separately, a glass column (45 mm inner diameter) was filled with a little water and then the gel suspension described above was poured into the column until it reached about 2 cm in height. The column was opened for the continuous addition of the gel suspension, followed by washing until no change in height (40 cm) was observed. Under the separation, an aqueous solution of the AuNC@dBSA was added to the gel column and eluted with water. Colored fractions were collected separately for further characterization and investigations.

(3) Measurements of fluorescence spectra

To reduce the fluctuation in the excitation intensity during measurement, the lamp was kept on for 1 h and the samples were stock-still for 3 min prior to the experiment. Samples for emission measurement were contained in 1 cm × 1 cm quartz cuvettes (4 mL volume). All spectroscopic measurements of AuNC@BSA were performed in distilled water. A fixed excitation wavelength at 350 nm was used.

References

**Fig. S1** The fluorescence spectrum ($\lambda_{ex}=350$ nm) in monitoring the separation process of AuNC@dBSA from dBSA by using the aqueous gel column packed with G-100. The spectra were recorded for samples collected with an 0.5h interval started from AuNC@dBSA flow out.
**Fig. S2** Fluorescence photographs of the gel column (packed with Sephadex G-75) separation procedure of AuNC@dBSA under the 365 nm irradiation. The photos represent the elution at the indicated times.
Fig. S3 The fluorescence spectral monitoring on the synthesis processes of dansyl-modified BSA and Au\textsubscript{NC}@dBSA in first route (\(\lambda\text{ex}=350\) nm) and the following isolation.

Fig. S4 The fluorescence spectral monitoring on the synthesis processes of direct modifying the Au\textsubscript{NC}@BSA by dansyl chloride (in second route) and the following isolation (\(\lambda\text{ex}=350\) nm).
**Fig. S5** The FT-IR spectra in monitoring the products obtained in the first (top) and second (bottom) synthesis routes of AuNC@dBSA.
**Fig. S6** The Circular Dichroism (CD) spectra in monitoring the synthesis of AuNC@dBSA. No much secondary structure changes occurred after modifying BSA with dansyl chloride.

**Fig. S7** The fluorescence spectra of AuNCs@dBSA in the application as a sensor for Hg$^{2+}$ detection in aqueous solution. Compared to the typical method the proposed separation protocol can improve the response sensitivity of AuNCs@dBSA to Hg$^{2+}$ obviously.
**Fig. S8** The fluorescence intensity of AuNC@BSA before and after gel column separation in the same mass concentration (2 mg/mL).

**Fig. S9** MALDI-MS spectra of the AuNC@BSA before (top) and after (bottom) gel column separation.