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Supporting Information

Serum Albumin adsorbed on Au Nanoparticles: Structural Changes Over Time Induced by S-Au Interaction

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1. Experimental procedures

Materials

Human serum albumin (HSA) was purchased from Sigma. The stock solution of HSA was prepared in 0.1M phosphate buffer solution (pH 7.4). The $HAuCl_4.3H_2O$ and trisodium citrate were purchased from Chinese Sinopharm Chemical Reagent Co., Ltd. All other chemicals were of analytical grade or better.

Preparation of Gold Nanoparticles (AuNPs)

AuNPs were prepared using the published method[1]. All glass used in the experiment was cleaned in aqua regia, and rinsed thoroughly in H_2O prior to use. A 50 mL aqueous solution containing $HAuCl_4$ (10^{-2}% by weight) was heated to boiling with vigorous stirring, and 0.5 mL of Na_3-citrate (1% by weight) was added to the vortex of the solution rapidly.

Preparation of HSA-AuNPs Bioconjugates

HSA-AuNPs were prepared by mixing HSA and AuNPs in buffer and then incubated for different time. After incubation the bioconjugates were centrifuged (11,000 rpm, 15 min) and redissolved in buffer before the spectra were obtained[2a, 2b] All measurements in the following discussion were performed at ambient temperature.

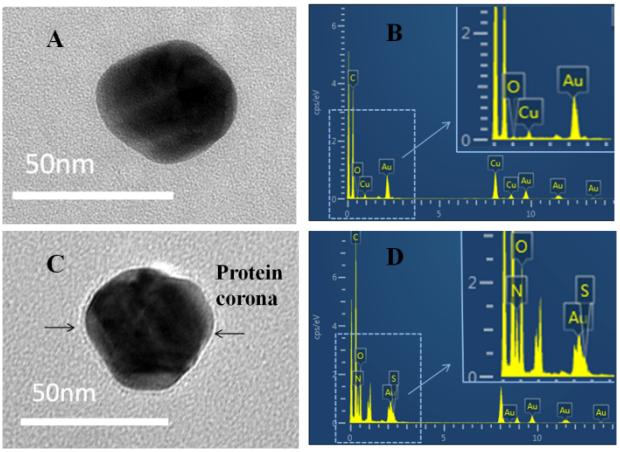

Figure S1. TEM image and EDX spectrum of AuNPs (A and B) and HSA-AuNP bioconjugates (C and D).

2. DLS Measurement

The HSA-AuNPs size of the nanoparticles was determined by DLS (Nicomp380/ZLS, Santa Barbara, CA) at a wavelength of 635 nm and a scattering angle of 90° at 25°C.

The sizes of two sets of AuNPs with nominal diameters of 40 nm and 70 nm were examined by DLS before and after different incubation times with HSA. DLS measurements before incubation revealed hydrodynamic diameters of 52.62 nm and 78.50 nm for the two sets (Figure. S1). After incubation with HSA for 1 h, the hydrodynamic particle diameters increased to 75.94 nm and 97.76 nm, respectively.

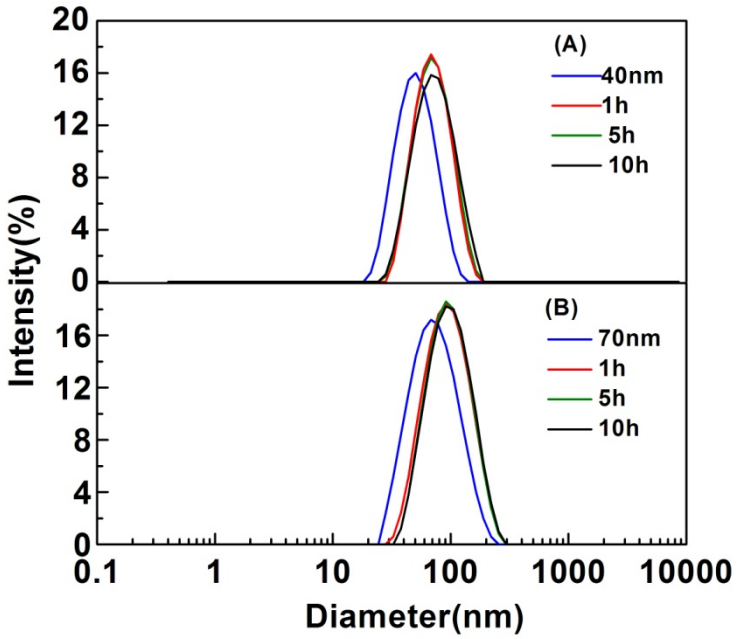

Figure S2. Size distribution of 40 nm AuNPs and HSA-AuNPs (A) and 70 nm AuNPs and HSA-AuNPs (B) measured by DLS as a function of incubation time. The hydrodynamic diameters showed obvious changes when AuNPs were incubated with HSA, but no obvious changes were observed when AuNPs were incubated with HSA for 10 h or more.

3. Fourier Transform Infrared (FT-IR) Measurements

HSA was mixed with AuNPs and incubated at room temperature for 20 min. After centrifugation for 15 min at 10,000 rpm, the supernatant was removed and the HSA-AuNPs was resuspended in PBS buffer. The procedure was repeated three times to remove all unbound HSA[3]. The sample was injected immediately into the demountable liquid flow cell with CaF_2 windows and then collected at 1.5 h intervals to observe changes in structure over time. The time from sample preparation to acquisition of the first spectrum was about 1 h.

FT-IR spectra were recorded with an ABB Bomem (Quebec, Canada) MB-3000 Fourier transform infrared spectrometer purged constantly with dry air. For each spectrum, a 128-scan interferogram was collected in single-beam mode at 4 cm^{-1} resolution to ensure a good signal-to-noise ratio at room temperature. A reference spectrum was recorded under identical conditions without protein. All spectra were processed using a previously established protocol[4]. Second-derivative spectra were

obtained using a seven-point Savitsky–Golay derivative function and baseline - corrected[5]. The secondary structure content of the protein was calculated by curve-fitting analysis of the inverted second-derivative amide I band from 1600 to 1700 cm^{-1}[6].

We investigated the structural changes in HSA over time after the addition of 70 nm AuNPs and obtained similar results as HSA absorbed on 40 nm AuNPs. The intensities of the bands assigned to α-helix (1658, 1665 cm^{-1}) decreased, whereas the intensities of bands assigned to β-structures (β-sheet at 1630, 1640cm^{-1}; β-turn at 1683, 1689 cm^{-1}) and random coil (1649 cm^{-1}) increased (Figure S3 and Figure S4). The FT-IR results show that the structure of HSA absorbed on AuNPs evolved over time and reached a relatively stable value for approximately 8-9 h.

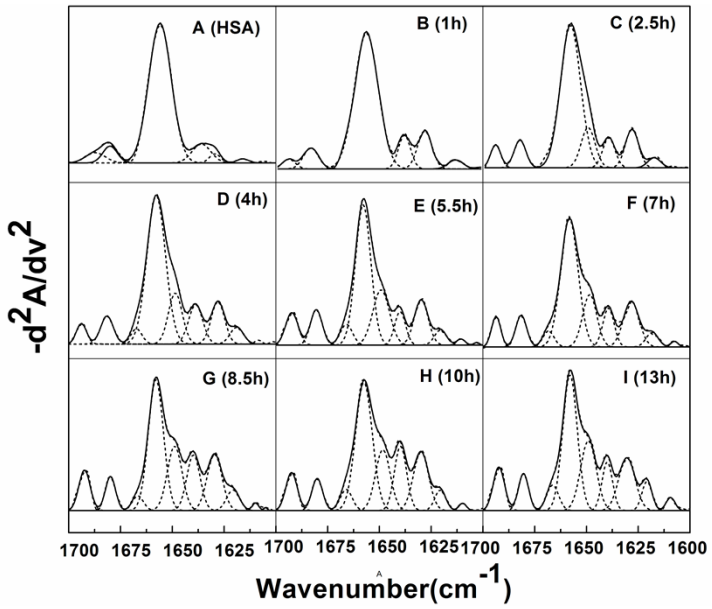

Figure S3. Curve-fitted inverted second-derivative amide I spectrum of 70 nm HSA-AuNPs at different incubation times.

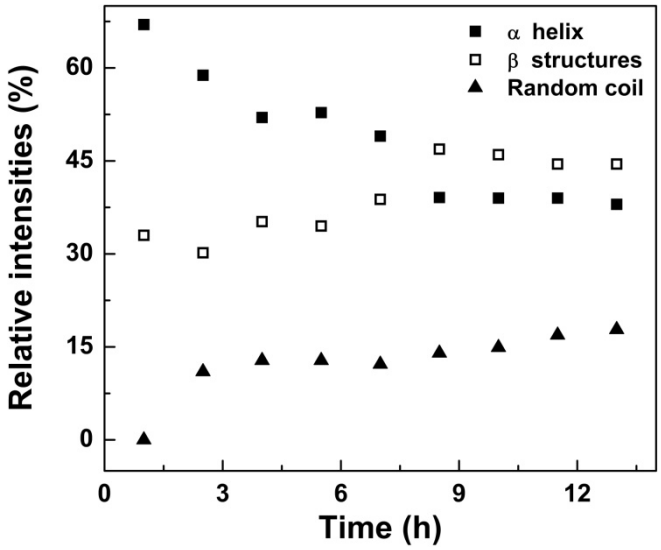

Figure S4. Effects of 70 nm AuNPs on the relative amount of α-helix structure (■), β-structure (β-sheet and β-turn, □), and random coil (▲).

4. Fluorescence

HSA-AuNPs were redissolved and then incubated with different concentrations of guanidine hydrochloride (GnHCl) for 30 min at room temperature before fluorescence measurements. The samples from different incubation times were performed in the same way. Fluorescence was measured using a Varian Cary Eclipse fluorescence spectrometer (Pal Alto, CA, USA) with a 1-cm quartz cuvette at room temperature (25 ± 1°C). The excitation and emission wavelengths were 280 and 340 nm, respectively.

The data in Figure S5 indicate that HSA was significantly stabilized in the presence of AuNPs (70 nm) relative to the protein dissolved in buffer and that the HSA-AuNPs was stabilized with extended incubation time.

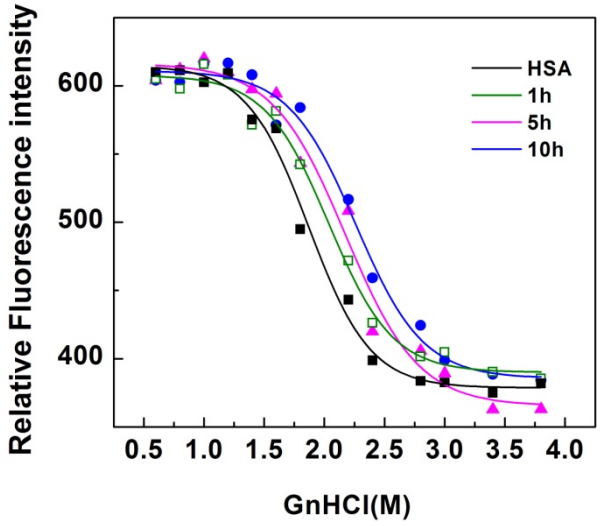

Figure S5. Fluorescence emission intensity of HSA adsorbed to 70 nm AuNPs as a function of the concentration of guanidinium hydrochloride (GnHCl) at incubation times of 1 h (□), 5 h (▲), and 10 h (●).

5. Raman Spectroscopy

HSA-AuNPs were incubated as a function of time. After centrifugation for 15 min at 10,000 rpm, the supernatant was removed and HSA-AuNPs resuspended in PBS. Raman and SERS measurements were performed with a Jobin Yvon XploRA Raman microscope using a 785 nm laser excitation source. The excitation light intensity in front of the objective was 8 mW with a spectral collection time of 30 s for SERS experiments. The integration time for the measurements was set to 150s.

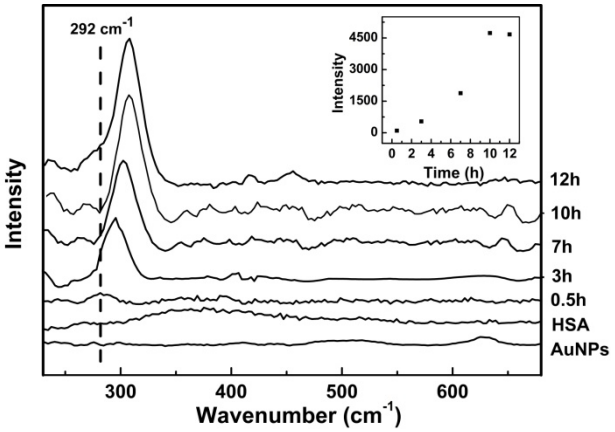

Figure S6. Raman spectra of raw HSA, AuNPs, and SERS spetra of HSA-AuNPs as a function of incubation time.

6. The FT-IR spectra of 24 h

We collected the FT-IR spectra of 24 h and longer incubation time. There are no changes after 10 h incubation (Figure S7).

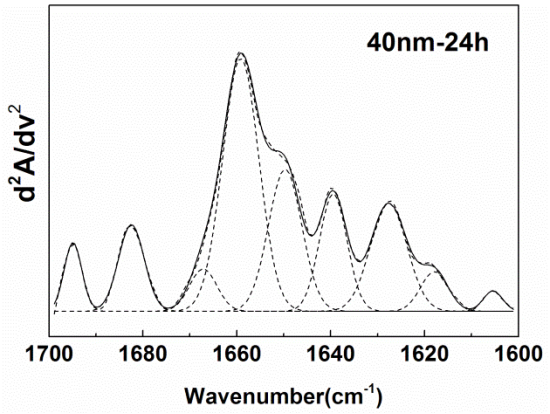

Figure S7. Curve-fitted inverted second-derivative amide I spectra of HSA absorbed on 40 nm AuNPs after 24 h incubation.

References

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