Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Tailoring the Protein Conformation to Synthesize Different-Sized Gold Nanoclusters

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1. Experimental Section

Chemicals. Hydrogen tetrachloroaurate hydrate ($HAuCl_4 \cdot 3H_2O$) was purchased from *Alfa Aesar*. Bovine serum albumin (BSA) and sodium phosphate (anhydrous, monobasic, and dibasic) were purchased from *Sigma-Aldrich*. Carbon monoxide (CO, 99.9%) was purchased from *Singapore Oxygen Air Liquide Pte Ltd (SOXAL)*. All chemicals were used as received. All glassware were washed with *Aqua Regia* ($HCl:HNO_3$ volume ratio = 3:1), and rinsed with ethanol and ultrapure water. Ultrapure water with a specific resistance of 18.2 MΩ was used throughout the experiment.

Synthesis of BSA-Au$_n$ (n = 4, 8, 10, 13) NCs. In a typical synthesis of BSA-Au NCs, 0.04 mM BSA solution was prepared in phosphate buffer (PB, 100 mM, pH 7.4) in five 20-mL disposable glass vials. 5 mM $HAuCl_4$ were subsequently introduced to these five vials at the molar ratio of Au/BSA of 5:1, 8:1, 12:1, 24:1, and 47:1. The total volume of the reaction solution was ~10 mL. The reaction solutions were then saturated with 1 bar CO for 2 min and sealed airtight. The pale yellow solution gradually changed to brown in 5–30 min (the solution color changed faster as the concentration of Au ions increased), indicating the formation of Au NCs. The reaction was allowed to proceed at room temperature for 12 h. The reaction solutions were then centrifuged at 14,000 rpm to remove BSA-Au complexes or large particles, and the supernatants were subjected to ultrafiltration using a Millipore cellulose membrane with a molecular-weight cut-off (MWCO) of 10 kDa to remove small molecular impurities. The final products were stored at 4 °C for further characterizations.

Synthesis of BSA-Au$_{25}$ NCs. The synthesis of BSA-Au_{25} NCs is similar to that of BSA-Au_{13} NCs (the molar ratio of Au/BSA was kept as 24:1) except the phosphate buffer (pH 7.4) was replaced by a NaOH solution at pH 11.

Material characterizations. UV-vis spectra were recorded on a Shimadzu UV-1800 spectrometer. The matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were carried out on a Bruker Daltonics Autoflex II TOF/TOF system. The MALDI-TOF samples were prepared by mixing the samples (2 μL) with the matrix solution [2 μL, saturated 2,5-dihydroxybenzoic acid (DHB) solution in 50% acetonitrile], followed by a recrystallization in air prior to the measurement. The data were collected in both linear positive and negative mode. Far-UV circular dichroism (CD) spectra were measured on a

Jasco Model J-800 spectropolarimeter with a protein concentration of 0.0086 mM. The helical content was estimated from the mean residue ellipticity (MRE) θ at 222 nm according to the equation: % helix = $(MRE_{222} - 4000)/(33000 - 4000)\times 100$.[1] MRE was determined by MRE = $\theta/10nlC_p$, where θ is the ellipticity directly obtained from the spectropolarimeter, n is the number of amino acid residues in BSA (583), l is the optical path length (cm), and Cp is the molar fraction of the proteins (mol/L).

2. Supporting Figures

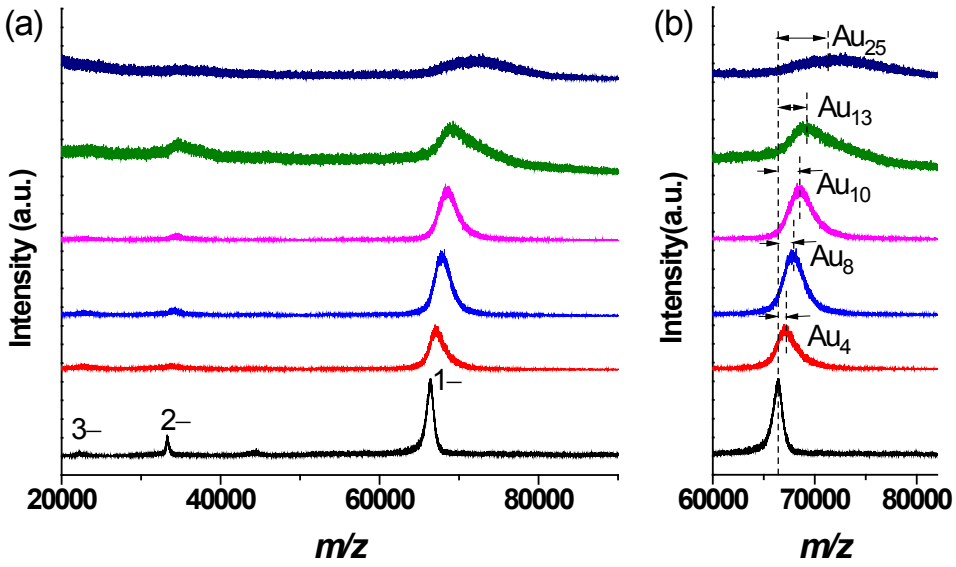

Figure S1. (a) Wide range (20–90 kDa) and (b) zoomed-in (60–82 kDa) MALDI-TOF mass spectra (linear negative mode) of pure BSA and BSA-Au_n NCs (n = 4, 8, 10, 13, and 25) [BSA (black), BSA-Au_4 (red), BSA-Au_8 (blue), BSA-Au_{10} (magenta), BSA-Au_{13} (green), and BSA-Au_{25} (navy)].

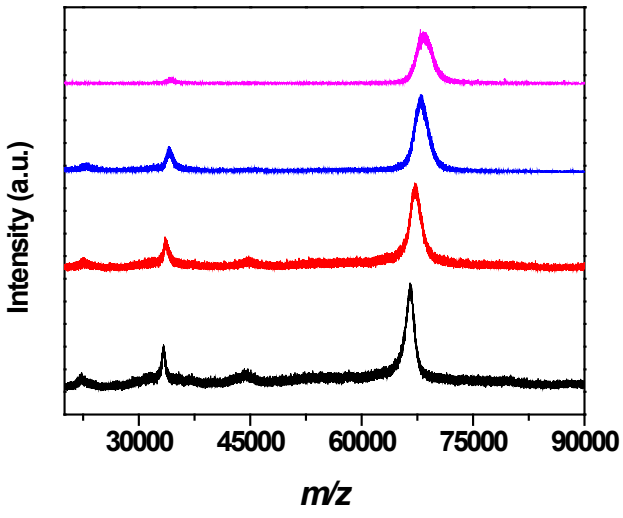

Figure S2. Expanded MALDI-TOF spectra of Figure 2b: BSA (black), BSA-Au_4 (red), BSA-Au_8 (blue), and BSA-Au_{10} (magenta).

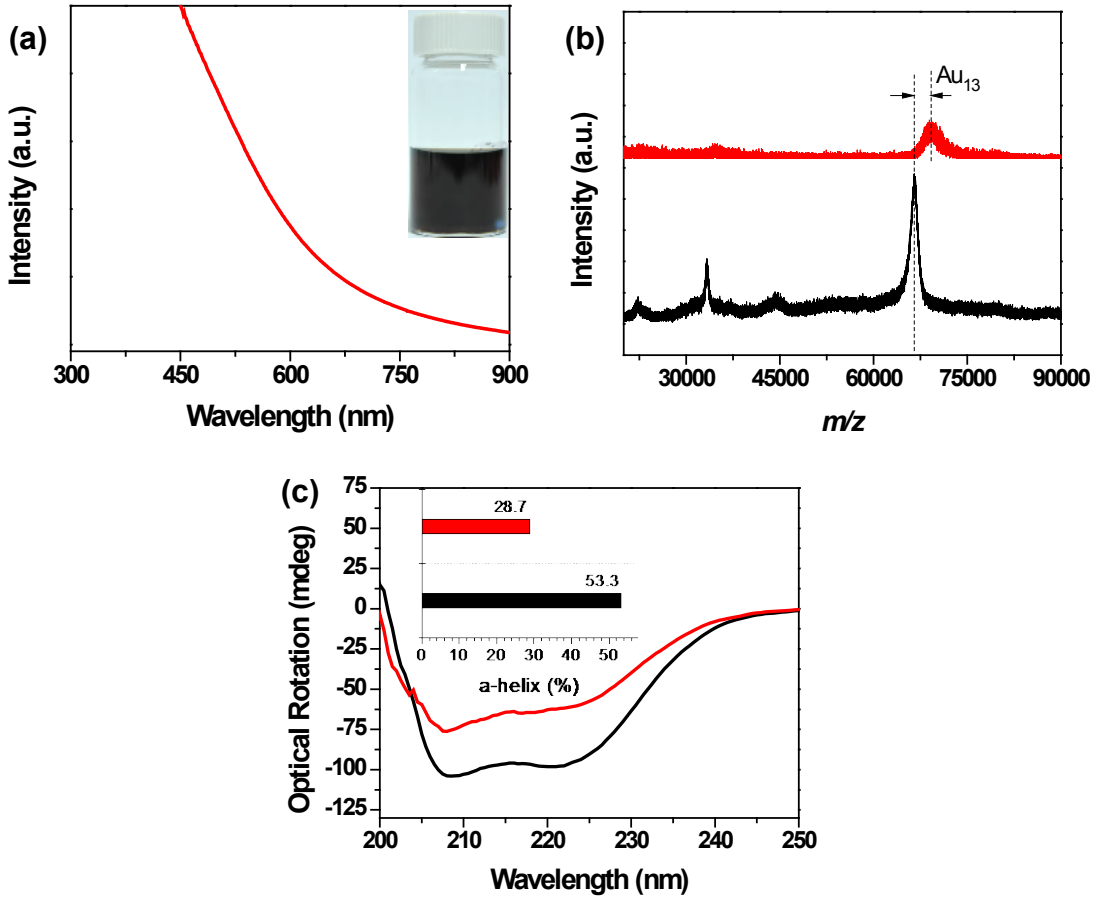

Figure S3. (a) UV-vis, (b) MALDI-TOF, and (c) far-UV CD spectra of pure BSA (black) and BSA-Au NCs synthesized with an initial Au/BSA ratio of 47:1 (red). The inset in (a) is a digital photo of as-synthesized BSA-Au NCs, and the inset in (d) is the calculated α-helix content of the NCs.

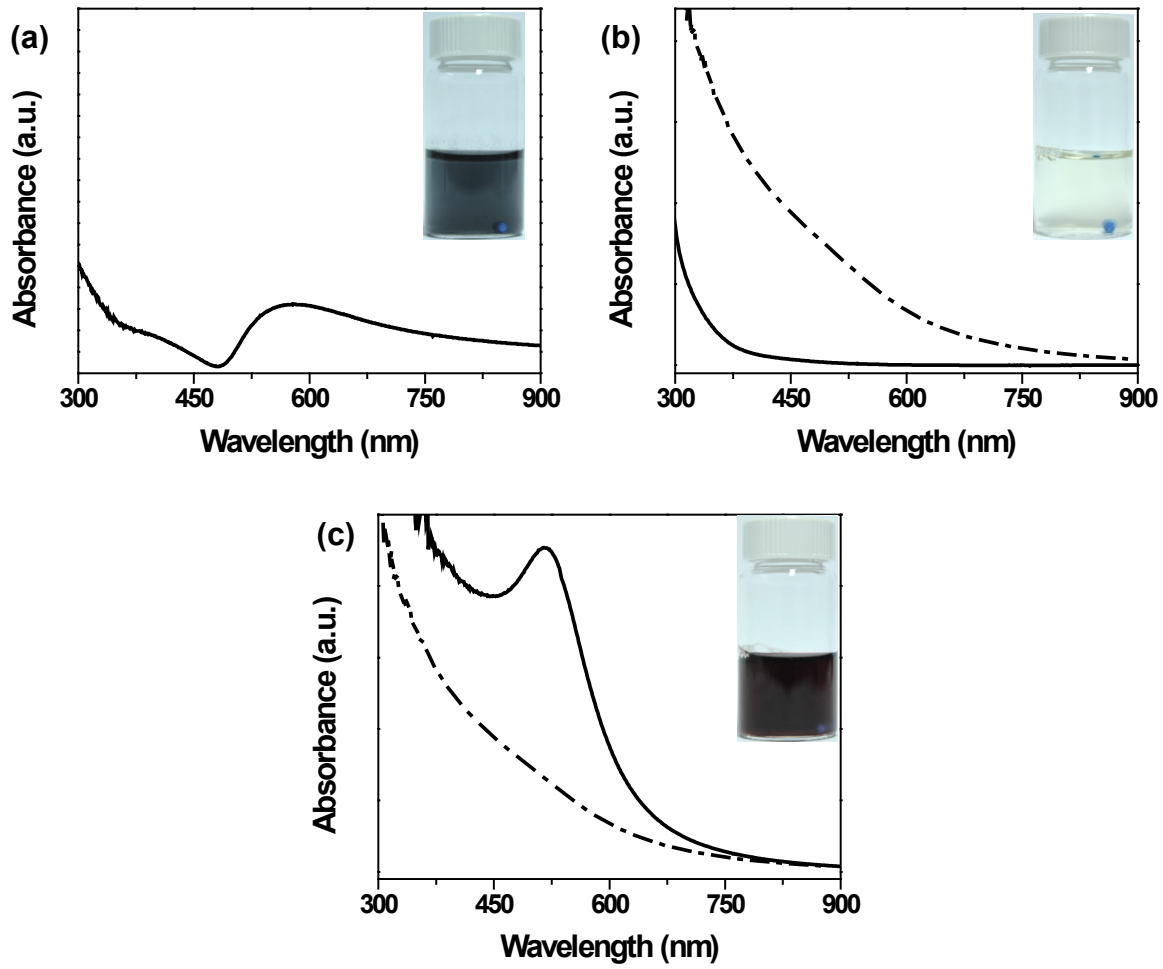

Figure S4. UV-vis spectra and digital photos (inset) of the reaction solutions in the absence of (a) BSA, (b) CO (b), and (c) PB. The dash line in (b) and (c) is the UV-vis spectrum of BSA-Au_{13} NCs synthesized in PB at pH 7.4. The initial molar ratio of Au/BSA was kept as 24:1 for all samples.

References

(1) Chen, Y.-H.; Yang, J. T.; Chau, K. H. *Biochemistry* **1974,** 13, (16), 3350-3359.