Higher-order assembly of BSA gold nanoclusters using supramolecular host-guest chemistry: a 40 % absolute fluorescence quantum yield

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Reagents and Materials. All the following compounds are commercially available and used without further purification. Bovine Serum Albumin (Sigma-Aldrich), Lysozyme Protein (SRL) terachloroauric acid trihydrate (Sigma-Aldrich), sodium hydroxide (SRL, India), glycoluril (Sigma-Aldrich), paraformaldehyde (Sigma-Aldrich), β-cyclodextrin (SRL), hydrochloride acid (SRL, India), sodium dihydrogen phosphate (SRL, India) sodium monohydrogen phosphate (SRL, India). Milli-Q quality water was used throughout the experiment.

Instrumentation. UV-vis spectra and Fluorescence data were collected using Perkin Elmer Lambda 750 and Spectromax Me 5, respectively. Turbidity measurement has been performed in Perkin Almer Lambda 750 at 550 nm. Time-resolved fluorescence measurements were performed by DeltaPro™ Horiba Scientific using 280 nm NanoLED and 415 nm Delta Diode source. The absolute fluorescence quantum yields were measured by Quanta-ϕ Horiba Instrument coupled with Fluorolog spectrophotometer at 405 nm excitation in an integrated sphere. Atomic Force Microscopic (AFM) images were recorded in a Park systems NX-10 AFM on drop-casting the sample on a glass slit. High-Resolution Transmission-electron Microscopic images were
recorded in a TEM-T20 instrument working at 200 kV. The samples for HRTEM were prepared by dropping the samples with an appropriate concentration on the carbon-coated copper grid and drying under ambient conditions. MALDI-TOF MS experiments were conducted in Rapiflex Maldi TOF (Bruker Daltonics). DLS study and Zeta potential measurements were performed in a Brookhaven Pals Zeta Potential Analyzer instrument. NMR spectra were recorded in a Bruker 400 MHz spectrometer, and the chemical shifts(δ) in the 1H NMR spectra are reported in ppm relative to proton resonance resulting from incomplete deuteration of the solvents D2O (4.79 ppm). Liquid Chromatography-Mass Spectrometry (LC-MS) experiments were carried out in the Waters LC-MS system.

EXPERIMENTAL METHODS

Synthesis. Synthesis of BSA-Au NC. BSA-Au NCs were synthesized according to the reported procedure. In a typical synthesis process, 5 mL of 10 mM tetrachloroauric acid trihydrate (HAuCl4.3H2O) was added to 250 mg of BSA in 5 mL MQ-water under vigorous stirring for 5 min. Then 0.5 mL of 1 M NaOH was added to the above solution to make the pH ~12. The mixture was allowed to incubate at 37 °C for 12 h until the solution turned golden brown color. The mixture was stored at 4 °C for further use.

Synthesis of Lys-Au NC. We have synthesized Lys-Au NC according to the literature procedure. To a 5 mL of 10 mg/mL lysozyme aqueous solution, 5 mL of 4 mM HAuCl4 was added and diluted to 15 mL. After 5 minutes of mixing, a 0.5 mL of 1 M NaOH solution was added to make the pH of the solution ~ 12. The reaction mixture was incubated at 37 °C overnight.

Synthesis of CB7. In a typical synthesis process, glycoluril (10 gm, 70.4 mmol) was added to a 250 mL round-bottomed flask equipped with a magnetic stir. A 37 % HCl (14.2 mL) was added to the flask. Then paraformaldehyde (4.22 gm, 140.7 mmol) was added in a stirring condition. The viscous product was allowed to stir for 30 min until it was set as a gel. Then heated it at 100 °C and refluxed at the same temperature for 18 h. Then the mixture was allowed to cool for 24 h to get the crystalline products. The purification was carried out according to the previously reported literature. The 1H NMR and LC-MS have been shown in Figures S3 and S4, respectively.

1H NMR (400 MHz, D2O): δ = 4.24, 4.27 (d, 14H), 5.55 (s, 14H), 5.80, 5.83 (d, 14H). LC-MS: m/z = 658.49

UV-visible and Turbidity measurement. UV spectrophotometer (Perkin Elmer, Lambda 750) was employed for the turbidity measurement at 550 nm where the system doesn’t have any absorption. Turbidity is defined as 100 - % T, where T is transmittance. Turbidity was measured at different ratios of CB7 to BSA-Au NC. A pH-
dependent turbidity study of BSA-Au NC@CB7 and Lys-Au NC@CB7 have done at different pH at 550 nm.

**Fluorescence Study of Au\textsubscript{25}@BSA with CB7.**

To study the interaction of Au\textsubscript{25}@BSA with CB7, 40 µL of cluster solution was taken and diluted to 200 µL by adding a different volume of CB7 (10 mM in PB of 5 mM). Time-dependent fluorescence studies were carried out by mixing CB7 and incubating at 37 °C for 15 min, 6 h, 12 h, and 24 h. The absolute fluorescence quantum yield of BSA-Au NC was measured in an integrated sphere with different molar ratios of CB7 to BSA-Au NC. The fluorescence lifetime was also measured in the same way.

**DLS study and Zeta potential measurement.**

To know the hydrodynamic size and surface charge of the system, DLS study and Zeta potential measurements were performed respectively at different molar ratios of CB7 to BSA-Au NC.

Figure S1: Optical photograph of (a) BSA and (b) BSA-Au NC under visible light, (c) BSA and (d) BSA-Au NC under UV light. (e) UV-vis spectra of BSA and BSA-Au NC. (f) Fluorescence spectra of BSA and BSA-Au NC excited at 365 nm. (g) HRTEM image of BSA-Au NC.
Figure S2: Comparison between MALDI-TOF MS of BSA and BSA-Au NC.

Figure S3: $^1$H-NMR (400 MHz, D$_2$O) of CB7.
Figure S4: LC-MS of CB7.

Figure S5: AFM images of (a) BSA-Au NC and (b) BSA-Au NC@CB7 (CB7/NC = 112).
Figure S6: Confocal image of BSA-Au NC in (a) fluorescence mode, (b) bright field mode, and (c) merged of bright field and fluorescence mode.

Figure S7: Confocal image of BSA-Au NC@CB7 (CB7/NC = 28) in (a) fluorescence mode, (b) bright field mode, and (c) merged of bright field and fluorescence mode.
Figure S8: Confocal image of BSA-Au NC@CB7 (CB7/NC = 56) in (a) fluorescence mode, (b) bright field mode, and (c) merged of bright field and fluorescence mode.

Figure S9: Confocal image of BSA-Au NC@CB7 (CB7/NC = 84) in (a) fluorescence mode, (b) bright field mode, and (c) merged of bright field and fluorescence mode.
Figure S10: Confocal image of BSA-Au NC@CB7 (CB7/NC = 112) in (a) fluorescence mode, (b) bright field mode, (c) merged of bright field and fluorescence mode, and (d) 3D view of the same system.

Figure S11: Concentration-dependent fluorescence study of BSA-Au NC in the presence of CB7 (0.0001 mM to 8 mM ).
Figure S12: Time-dependent fluorescence study of BSA-Au NC@CB7 (BSA-Au NC concentration 71 µM each time).

Figure S13: Encapsulation study of BSA-Au NC in the presence of CB7 of 8 mM. It shows a 10 nm blue shift.
Figure S14: Scattering study of BSA-Au NC@CB7 (a) excited at 365 nm and (b) excited at 375 nm. In each case, the emission peak appeared at 645nm.

Figure S15: UV-visible spectra of BSA-Au NC with different concentrations of CB7.
Figure S16: Absolute fluorescence quantum yield of BSA-Au NC (slit width = 2).

Figure S17: Absolute fluorescence quantum yield of CB7/NC = 28 (slit width = 2).
Figure S18: Absolute fluorescence quantum yield of CB7/NC = 56 (slit width = 2).

Figure S19: Absolute fluorescence quantum yield of CB7/NC = 84 (slit width = 2).
Figure S20: Absolute fluorescence quantum yield of CB7/NC = 112 (slit width = 2).

Figure S21: Lifetime measurements were performed at 280 nm excitation for (a) BSA and 415 nm excitation for (b) BSA-Au NC, (c) CB7/NC = 28, (d) CB7/NC = 56, (e) CB7/NC = 84 (f) CB7/NC = 112.
Figure S22: FRET study of BSA, BSA-Au NC, and BSA-Au NC@CB7 systems at 295 nm excitation.

Figure S23: (a) A UV-vis spectrum of Lys-Au NC, and (b) A normalized fluorescence spectrum of Lys-Au NC (Excited at 365 nm).
Figure S24: pH-dependent turbidity study of (a) BSA-Au NC@CB7 and (b) Lys-Au NC@CB7.

Figure S25: Zeta potential plot of BSA-Au NC@CB7 and Lys-Au NC@CB7.

### Table 1.

| System        | Quantum Yield (\(\phi_f\)) % | Lifetime (\(\tau\)) (\(\mu\)s) |
|---------------|-------------------------------|----------------------------------|
| BSA-Au NC     | 13.45                         | 1.35                             |
| CB7/NC = 28   | 24.92                         | 1.69                             |
| CB7/NC = 56   | 26.82                         | 1.71                             |
| CB7/NC = 84   | 35.81                         | 1.78                             |
| CB7/NC = 112  | 40.56                         | 1.82                             |
Table 2.

| System    | Zeta potential (mV) at pH ~ 9 |
|-----------|-------------------------------|
| CB7/NC = 0 | -26                           |
| CB7/NC = 28| -21                           |
| CB7/NC = 56| -19                           |
| CB7/NC = 112| -18                          |

Table 3.

| System    | Zeta potential (mV) at pH ~ 9 |
|-----------|-------------------------------|
| CB7/NC = 0 | -18                           |
| CB7/NC = 44| -15                           |
| CB7/NC = 88| -0.01                         |
| CB7/NC = 132| +2.4                         |

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