# Model beyond Protein Corona: Thermodynamics and Stoichiometries of the Interactions between Ultrasmall Gold Nanoclusters and Proteins

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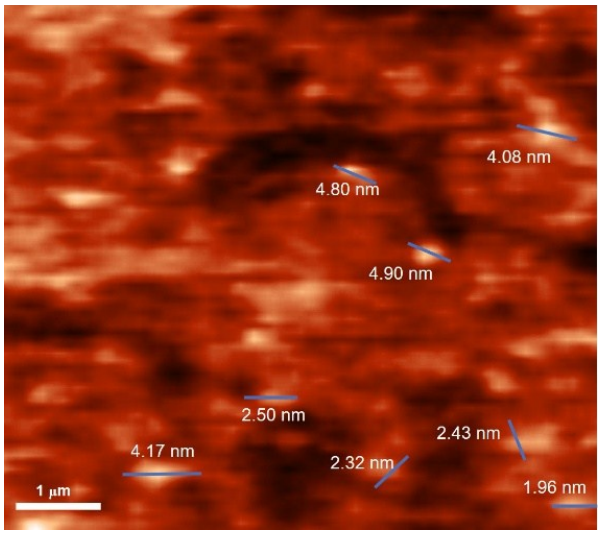


**Fig. S1** AFM topography image of the DHLA-AuNCs. The particles with heights of ~ 2 nm are corresponded to one AuNCs, while the particle with a height of ~4 nm may be corresponded to the aggregated AuNCs.

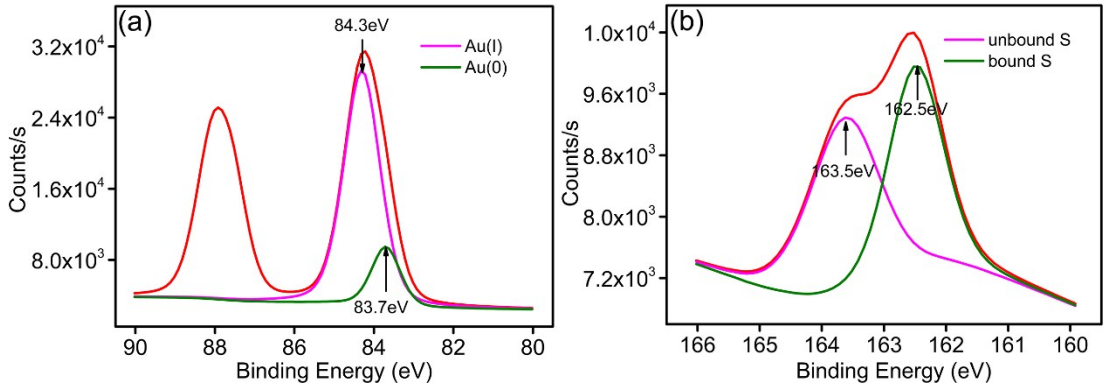


**Fig. S2** XPS spectra of Au 4f (a) and S 2p (b) species of the DHLA-AuNCs.

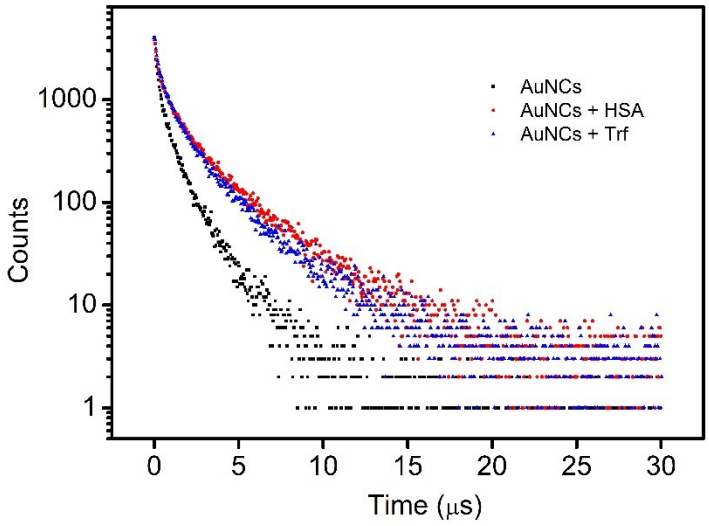


**Fig. S3** Fluorescence lifetimes of DHLA-AuNCs in the absence (black dots) and presence of HSA (red dots) and Trf (blue dots).

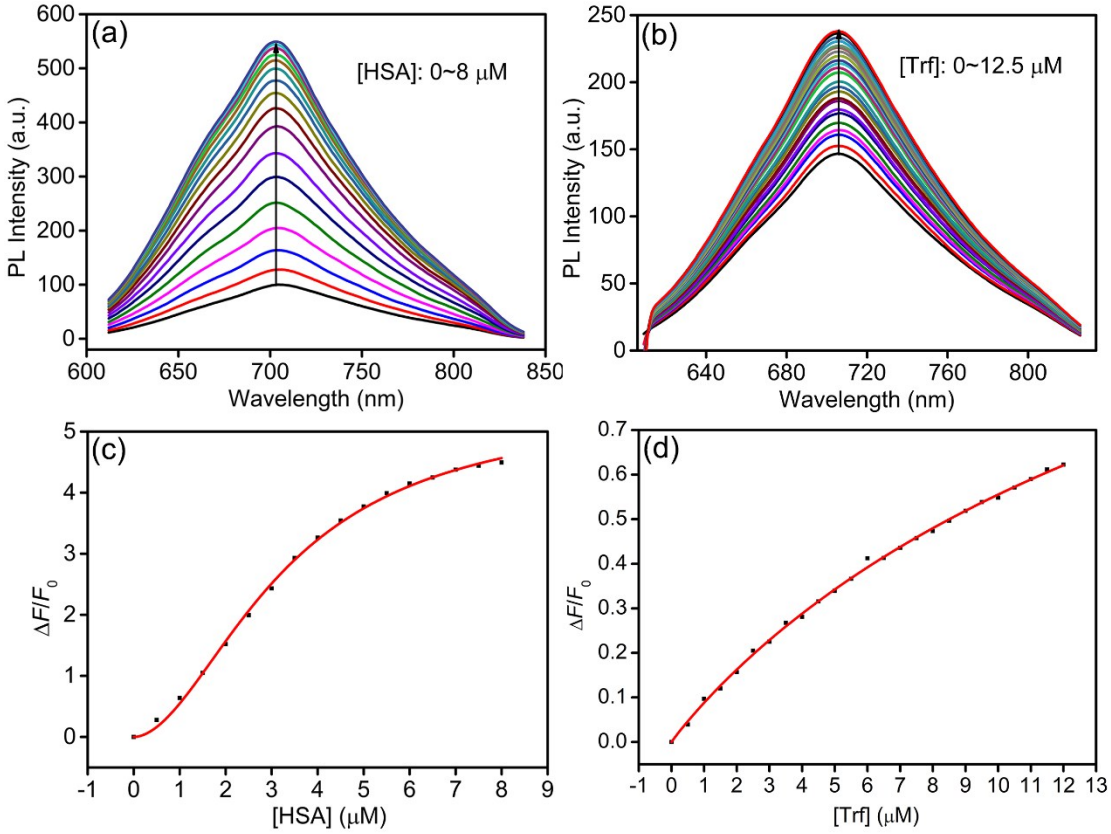


**Fig. S4** Fluorescence emission spectra of DHLA-AuNCs in the presence of different concentration of HSA (a) Trf (b), Ex = 575 nm, $T$ = 298 K; The correlation curves of $I$ and the concentration of HSA (c) and Trf (d) fitted by the Hill equation.

**Table S1** Parameters for the luminescence decay of DHLA-AuNCs in the absence and presence of two proteins, respectively.

| | $\tau_1$ (μs) | $\alpha_1$ | $\tau_2$ (μs) | $\alpha_2$ | $\tau_3$ (μs) | $\alpha_3$ | $\tau_{av}$ (μs) | $\chi^2$ |
|---|---|---|---|---|---|---|---|---|
| AuNCs | 0.138 | 6.6% | 0.658 | 30.9% | 1.969 | 62.5% | 0.955 | 0.997 |
| AuNCs-HSA | 0.155 | 32.5% | 0.905 | 52.4% | 3.141 | 15.1% | 2.083 | 0.999 |
| AuNCs-Trf | 1.186 | 52.2% | 0.954 | 36.0% | 3.006 | 11.8% | 1.770 | 0.998 |

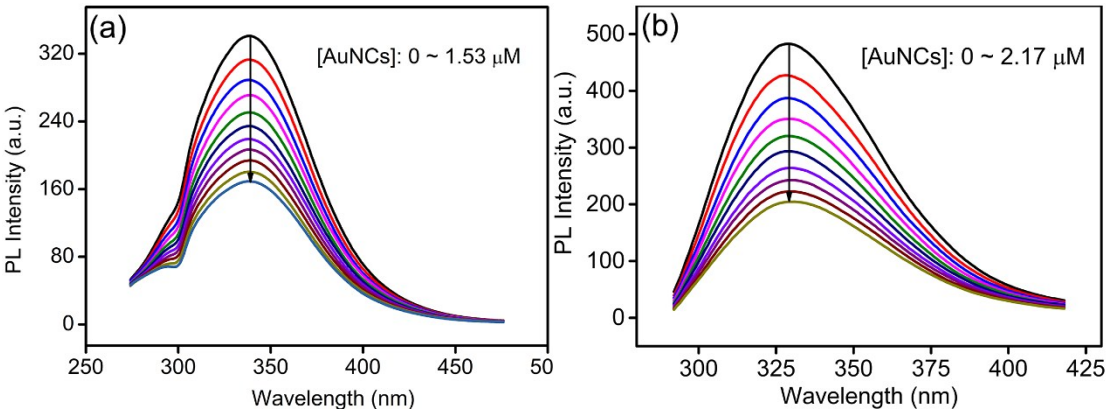


**Fig. S5** Fluorescence emission spectra of HSA (a), Trf (b) in the presence of different concentrations of Au NCs at 298 K. [HSA] = [Trf] = 2 μM.

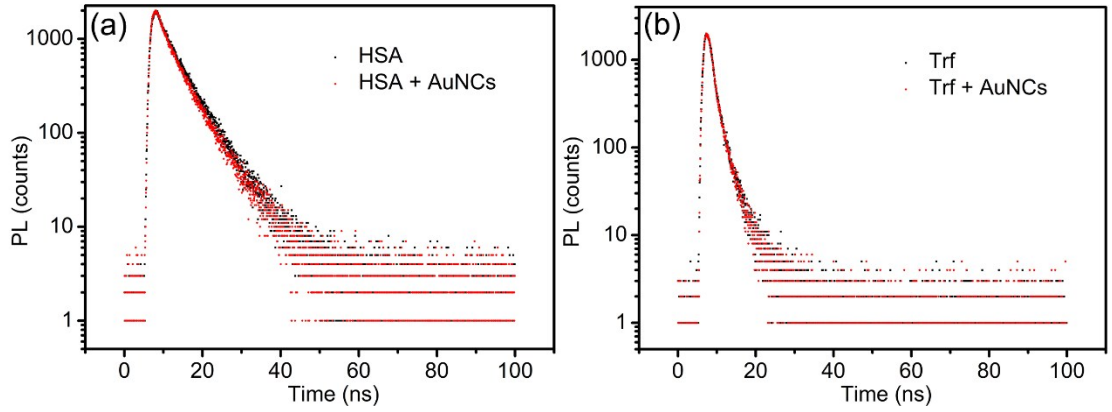


**Fig. S6** Fluorescence lifetimes of HSA (a) and Trf (b) in the absence (black dots) and presence of DHLA-AuNCs (red dots).

**Table S2** Parameters for the luminescence decay of two proteins in the absence and

presence of DHLA-AuNCs, respectively.

| | $\tau$ (ns) | $\chi^2$ |
|---|---|---|
| HSA | 5.02 | 1.086 |
| HSA-AuNCs | 4.48 | 0.941 |
| Trf | 1.78 | 1.014 |
| Trf-AuNCs | 1.75 | 1.187 |

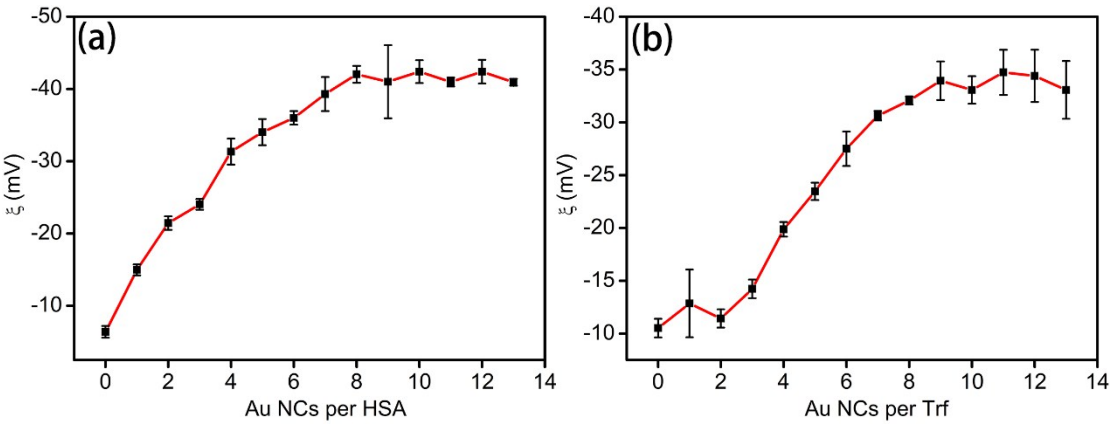


**Fig. S7** *Zeta* potential of AuNCs-HSA system (a) AuNCs-Trf system (b) at different ratios of DHLA-AuNCs to protein.

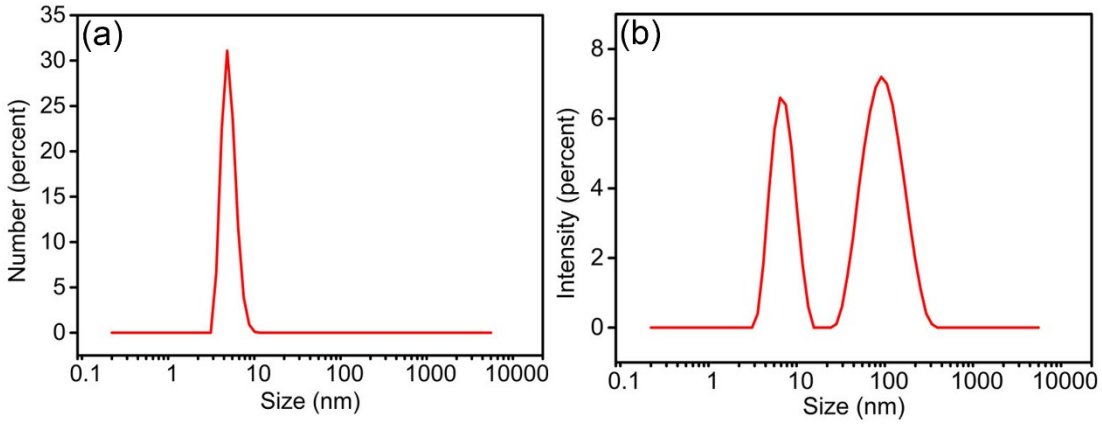


**Fig. S8** Hydrodynamic size distribution of HSA in the absence (a) and presence (b) of five equivalents of the DHLA-AuNCs.

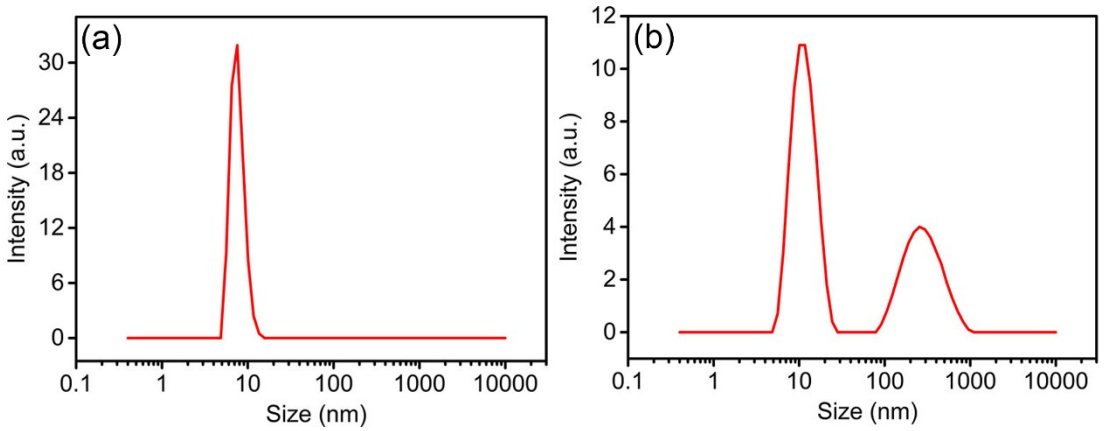


**Fig. S9** Hydrodynamic size distribution of Trf in the absence (a) and presence (b) of five equivalents of the DHLA-AuNCs.

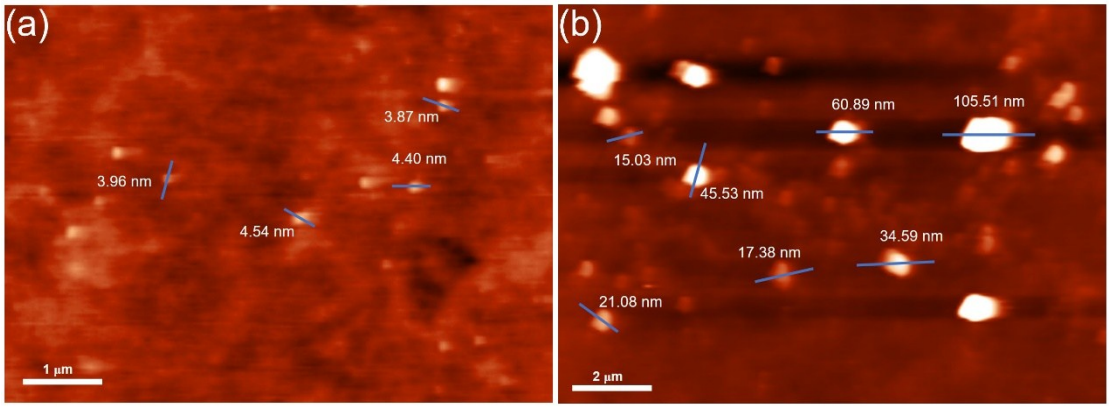


**Fig. S10** AFM topographic images of the HSA in the absence (a) and presence (b) of four equivalents of the DHLA-AuNCs.

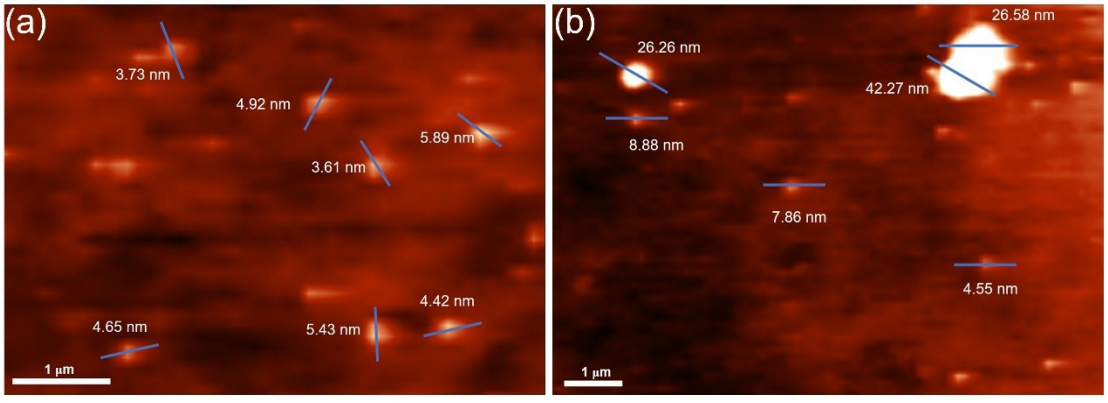


**Fig. S11** AFM topographic images of the Trf in the absence (a) and presence (b) of four equivalents of the DHLA-AuNCs.

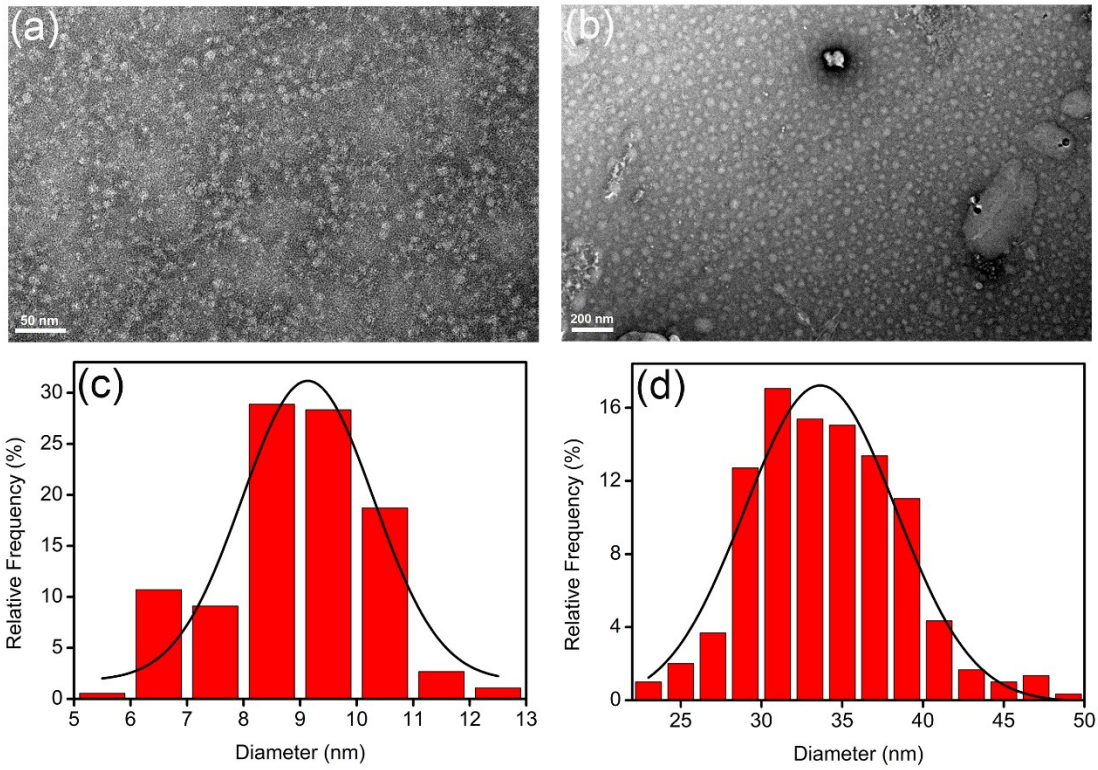


**Fig. S12** TEM images of negative stained HSA in the absence (a) and presence (b) of four equivalents of the DHLA-AuNCs. White components represent HSA. The corresponding particle size histograms of HSA in the absence (c) and presence (d) of the DHLA-AuNCs.

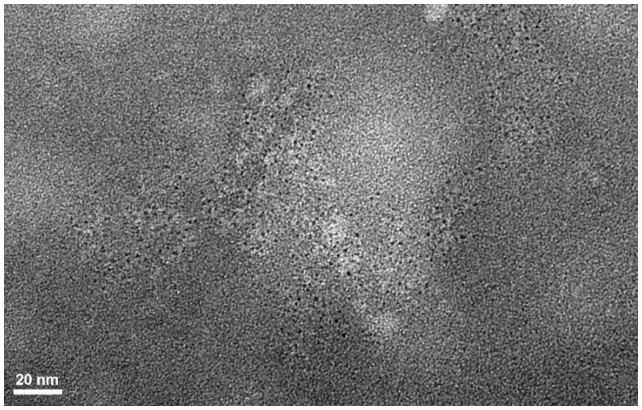


**Fig. S13** TEM image of the negative stained HSA in the presence of four equivalents of the DHLA-AuNCs. White components represent HSA while black dots represent AuNCs.

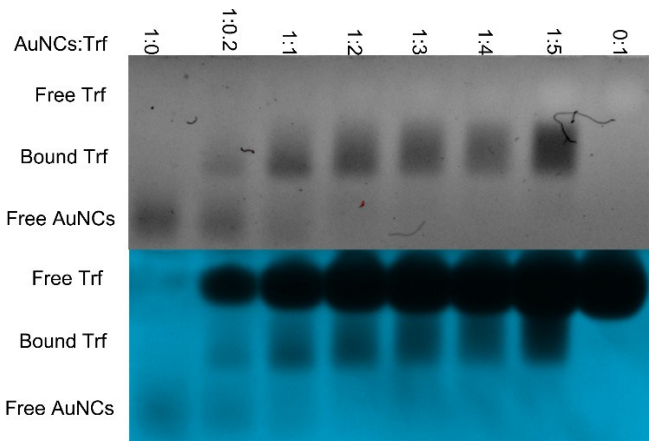


**Fig. S14** AGE images of AuNCs-Trf system at different ratios before (upper) and after (bottom) staining with Coomassie brilliant blue.

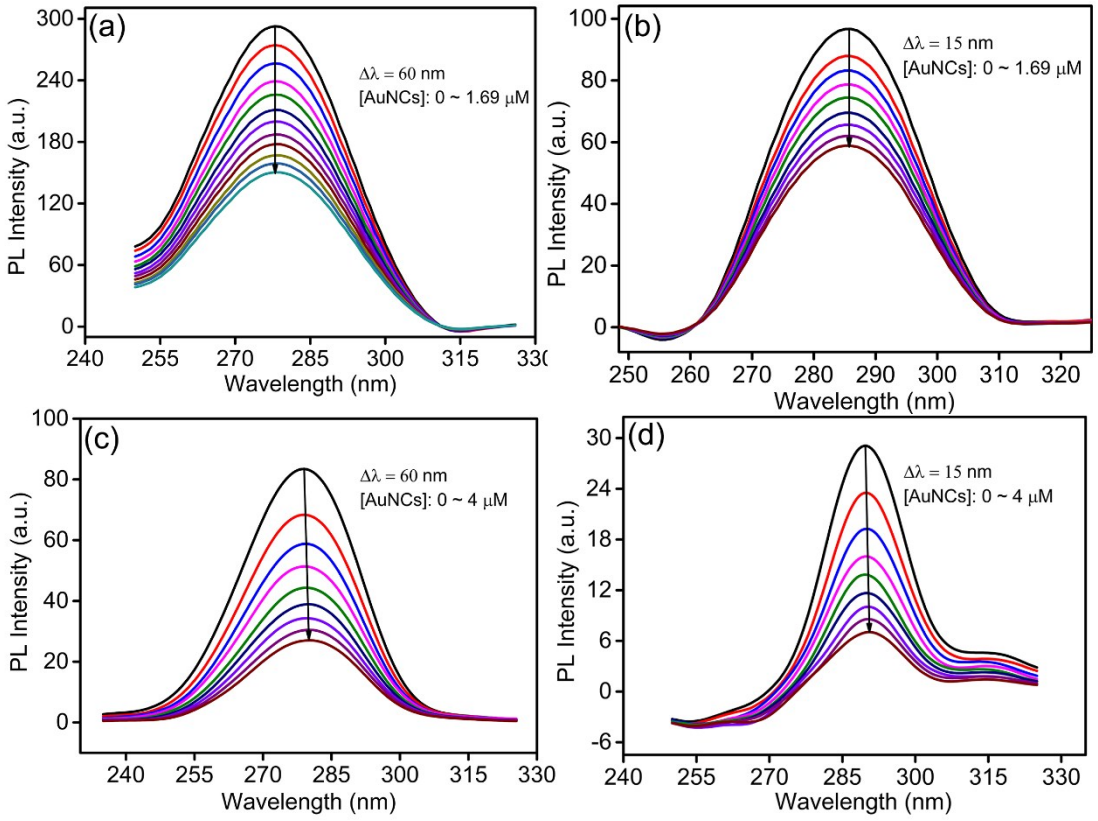


**Fig. S15** Synchronous fluorescence spectra of HSA (a, b) and Trf (c, d) in the absence and presence of DHLA-AuNCs. [HSA] = [Trf] = 2 μM; (a) and (c) are $\Delta\lambda$ = 60 nm; (b) and (d) are $\Delta\lambda$ = 15 nm.

**Table S3** Contents of secondary structures of HSA at different concentrations of DHLA-AuNCs.

| [AuNCs] (μM) | α-helix | β-sheet | β-turn | Random Coil |
|---|---|---|---|---|
| 0 | 0.540 | 0.095 | 0.171 | 0.269 |
| 2.5 | 0.526 | 0.098 | 0.193 | 0.218 |
| 5 | 0.540 | 0.050 | 0.179 | 0.266 |
| 10 | 0.511 | 0.081 | 0.092 | 0.357 |
| 20 | 0.503 | 0.066 | 0.116 | 0.312 |