# Kinetics of the Fluorophore Formation in Bovine Serum Albumin-Gold Complex

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## Supporting Information

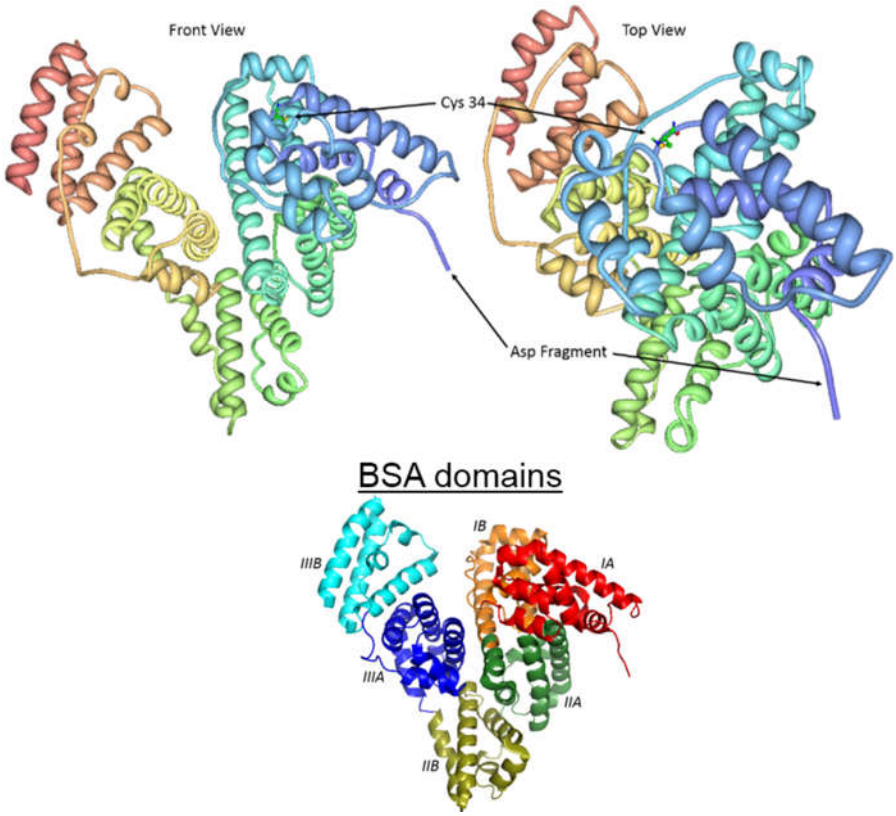


**Figure S1.** Structure of bovine serum albumin (BSA) at pH = 7. Locations of the "Asp fragment" (Asp1-Thr2-His3-) at the N-terminus, and Cys34, are indicated. The commonly used domain names are also shown. PDB ID: 4F5S.

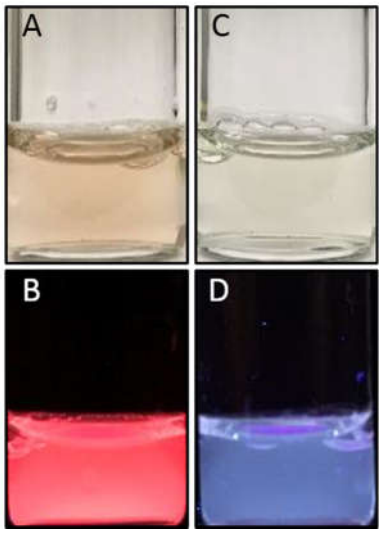


**Figure S2.** Dithiothreitol (DTT), a Cys-Cys disulfide bond-cleaving agent, readily quenched the red fluorescence of BSA-Au complex. (A) BSA-Au complex was prepared using the standard protocol (BSA : Au = 1 : 13). (B) The UV ($\lambda$ = 365 nm)-excited red fluorescence of the BSA-Au complex. (C) BSA-Au complex was treated with 10 mM DTT and 1% SDS and was incubated for 30 minutes at 37$^{o}$C, following the commonly-used protocol for protein gel electrophoresis sample preparation. (D) The red fluorescence of the DTT-treated BSA-Au complex was readily quenched. The blue fluorescence is from the aromatic residues of BSA at pH = 12. We note that this blue fluorescence is identical to that of BSA at pH = 12, without the addition of Au (Dixon and Egusa, *J. Am. Chem. Soc.* **2018**, 140, 2265 – 2271).

The quenching of red fluorescence in this compound was also observed by others (Shu et al., *Anal. Chem.* **2016**, 88, 11193 – 11198) using another disulfide bond-cleaving agent, tris(2-carboxyethyl)phosphine (TCEP) at room temperature. They did not observe the quenching of red fluorescence by the simple addition of DTT at room temperature. TCEP is a strong reducer that cleaves all disulfide bonds of proteins readily. It is well-known that DTT is a milder agent that does not cleave the internal disulfide bonds of proteins, unless 1% SDS and incubation at 37$^{o}$C is added to the protocol.

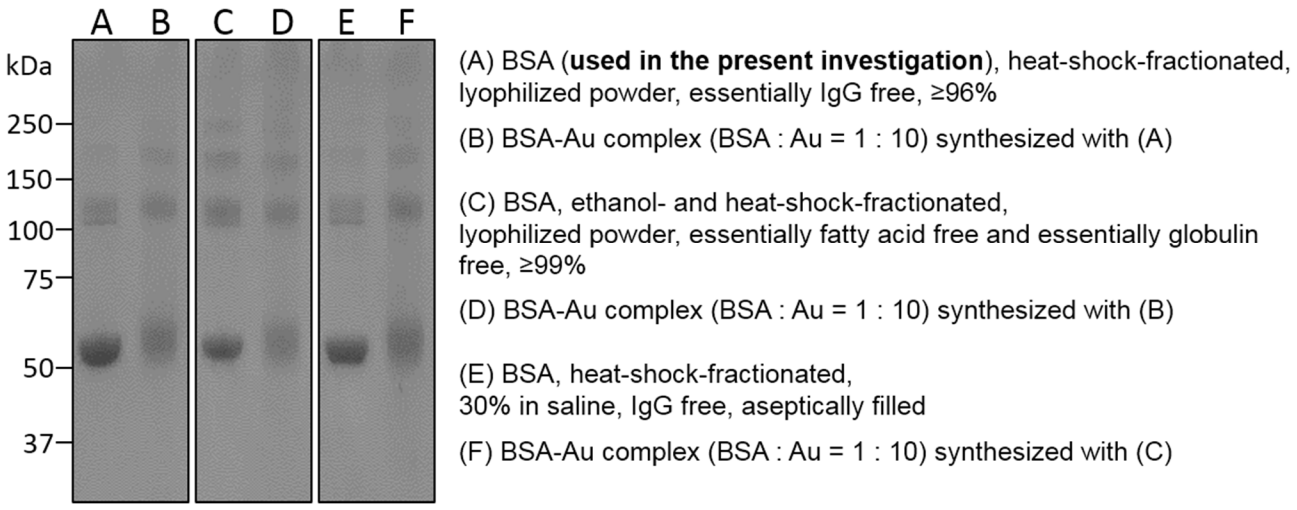


**Figure S3.** Comparison of the commercially available BSA's with different purities (all are fraction V), and the synthesized BSA-Au complex compounds. All BSA's, used as received, showed aggregation with the present gel electrophoresis protocol (without denaturing agent). Dimer (66.4 × 2 = 132.8 kDa) and trimer (66.4 × 3 = 199.2 kDa) bands were consistently observed with all the commercial BSA's we tested.

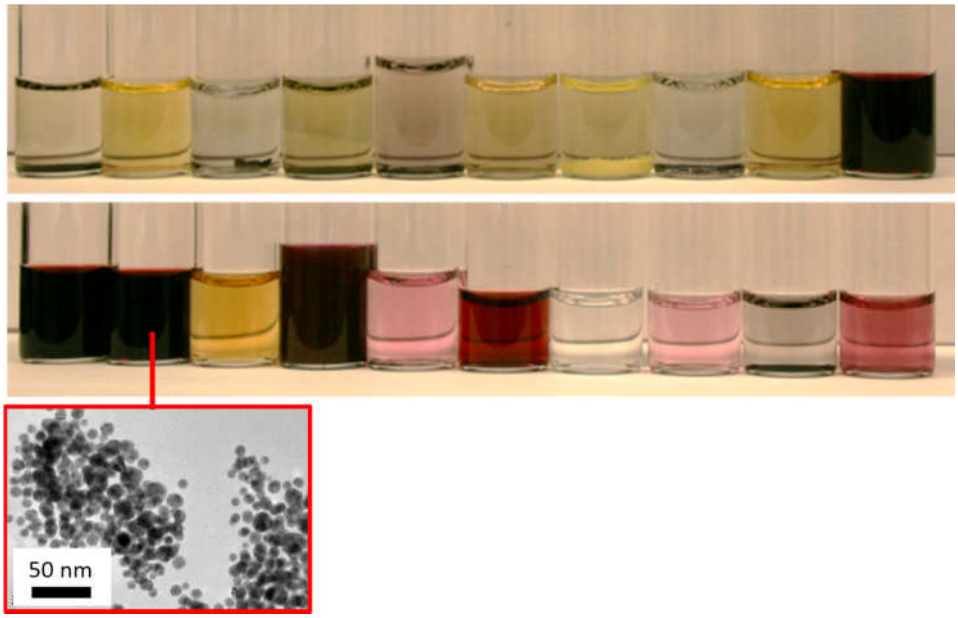


**Figure S4.** Reactivity of individual amino acid residues in the synthesis of BSA-Au complex. $HAuCl_4$ was mixed with the twenty common amino acids individually, followed by the pH adjustment to 12, mimicking the synthesis of BSA-Au complex with BSA-to-Au molar ratio = 1 : 13. The total number of amino acid residues in BSA is 583. An individual amino acid (with *X* residues in BSA) was mixed with $HAuCl_4$, with the amino acid-to-Au molar ratio = $^{X}/_{583}$ : 13. For example, there are 35 Cysteine residues in BSA: therefore, Cysteine was mixed with $HAuCl_4$ with the Cys-to-Au molar ratio = $^{35}/_{583}$ : 13.

Top, left to right: Isoleucine, Leucine, Proline, Valine, Aspartic Acid, Glutamic Acid, Arginine, Methionine, Alanine, Glycine.

Bottom, left to right: Tryptophan, Tyrosine, Histidine, Lysine, Serine, Phenylalanine, Cysteine, Threonine, Asparagine, Glutamine.

The ruby-red color of the solution indicate the formation of Au nanoparticles. TEM image of Tyr-Au nanoparticles shown as a representative.

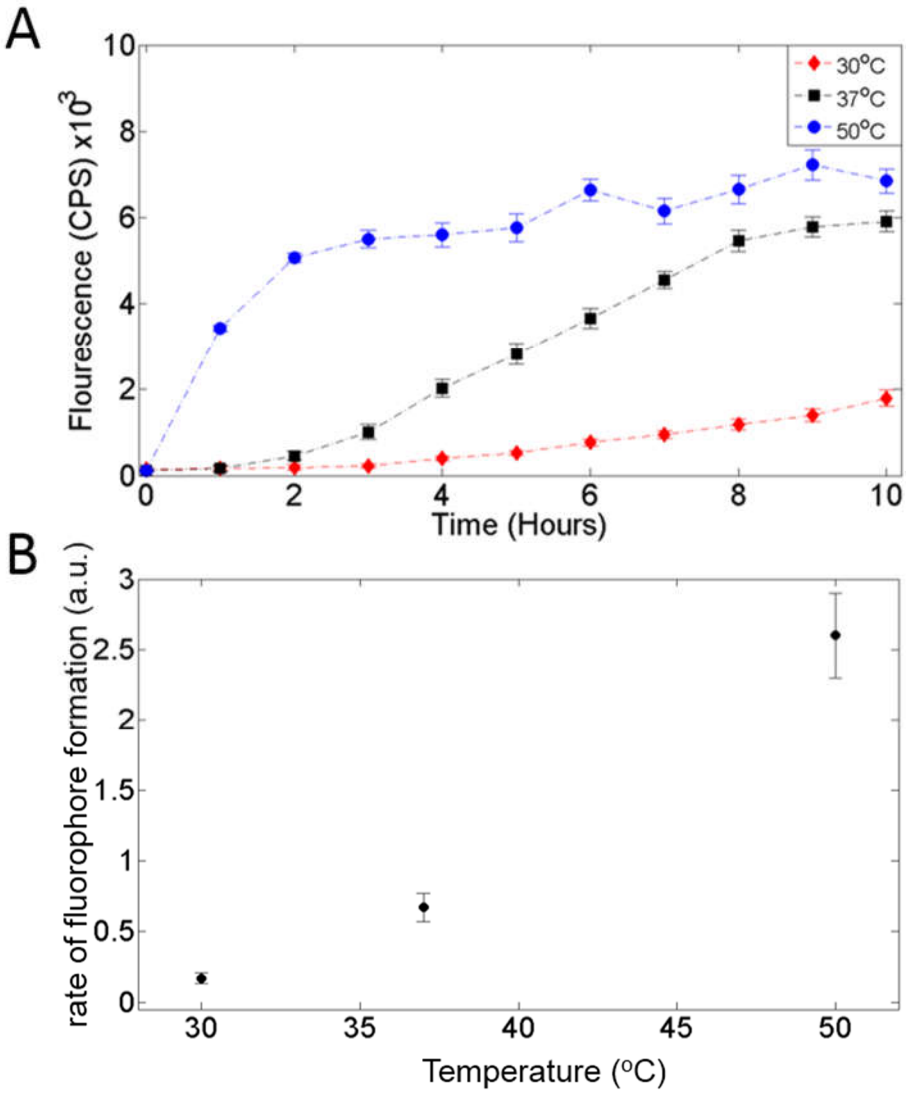


**Figure S5.** The temperature-dependent kinetics of the red fluorophore formation in BSA-Au complex, at the BSA-to-Au molar ratio of 1 : 7, and at a pH of 11.5. The red fluorescence peak of the BSA-Au complex is plotted over the time course.

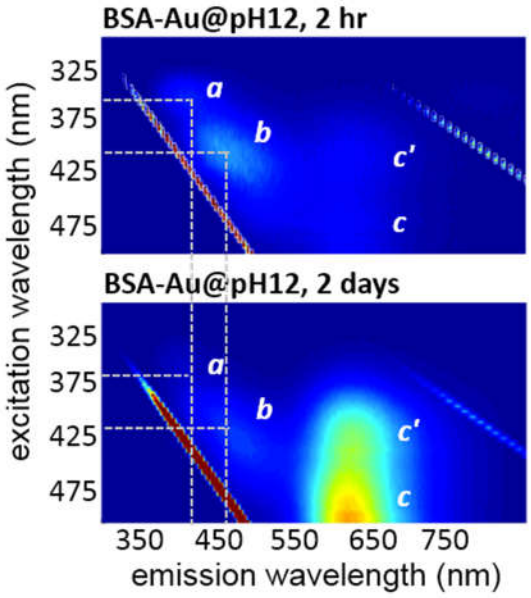


**Figure S6.** The cascaded energy transfer within BSA-Au complex. The peaks at $\lambda_{ex}$ /$\lambda_{em}$ = 370/400 nm (peak *a*); 400/460 nm (peak *b*); 400/640 nm (peak *c'*); 470/640 nm (peak *c*) suggest the possible energy transfer pathways, $a \rightarrow b$, $a \rightarrow c'$, and $b \rightarrow c$.