Supporting Information

Effects of Surface Compositional and Structural Heterogeneity on Nanoparticle-Protein Interactions: Different Protein Configurations

Rixiang Huang[1], Randy P. Carney[2], Kaoru Ikuma[3], Francesco Stellacci[2], and Boris L.T. Lau[3*]

[1] Department of Geology, Baylor University, One Bear Place #97354

Waco, Texas, USA, 76798

[2]Institute of Materials, Ecole Polytechnique Fédérale de Lausanne,

Lausanne 1015, Switzerland

[3] Department of Civil and Environmental Engineering, 18B Marston Hall, University of Massachusetts Amherst, 130 Natural Resources Road, Amherst, MA, USA, 01003

*Corresponding author:

Email: borislau@engin.umass.edu,

Phone: 413-545-5423; fax: 413-545-2840

Contents:

Particle synthesis

0.9 mmol of gold salt ($HAuCl_4$) was dissolved in 150 mL ethanol and the reaction vessel was put in an ice bath. All solvents were purged with nitrogen gas for at least 30 minutes prior to use in this protocol. 0.75 mmol of the desired thiol ligand mixture (66MPA-34OT or 66MPA-34brOT) was added while stirring the solution. After 5 minutes, 1 g of sodium borohydride ($NaBH_4$) was dissolved in 150 mL ethanol and subsequently added drop wise to the gold-thiol mixture over a period of 1 h. The resulting solution was heavily stirred for 2 h and transferred to a refrigerator overnight for particle precipitation. The supernatant was removed and the pellet was washed via centrifugation with ethanol, methanol, and acetone, in order to remove unbound ligands. 30 mg aliquots of NPs were dissolved in ultrapure water (MilliQ) and then transferred to centrifugal filter units (Millipore Amicon Ultra, regenerated cellulose, 3,000 Da MWCO) and spun for 15 minutes at 4000 g. This washing procedure was repeated for each aliquot up to 5 times. The resulting NPs were crashed out with a non-solvent, acetone, under ultracentrifugation at 300,000 g for 30 minutes. The pellet was dried overnight under high vacuum.

Particle characterization

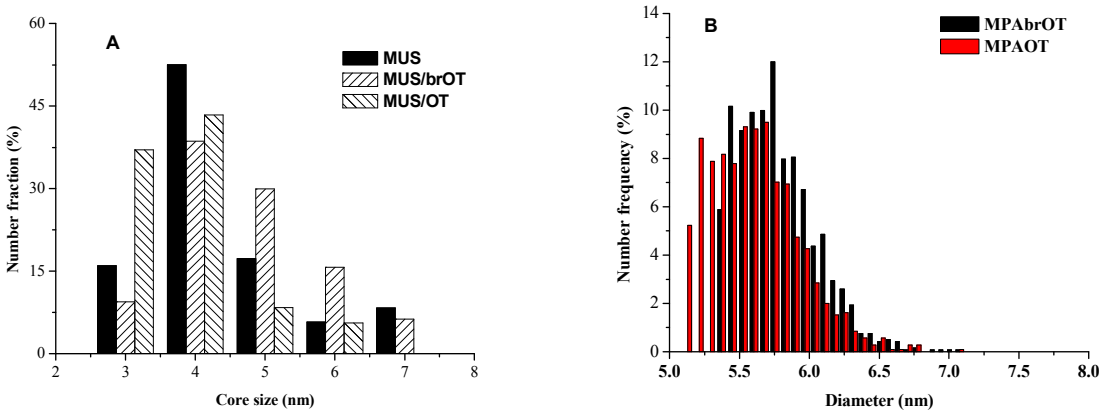

Figure S1. Size distribution of the MUS-type (number frequency was based on random counting of more than 130 particles) (A) and MPA-type AuNPs (number frequency was based on random counting of more than 1000 particles).

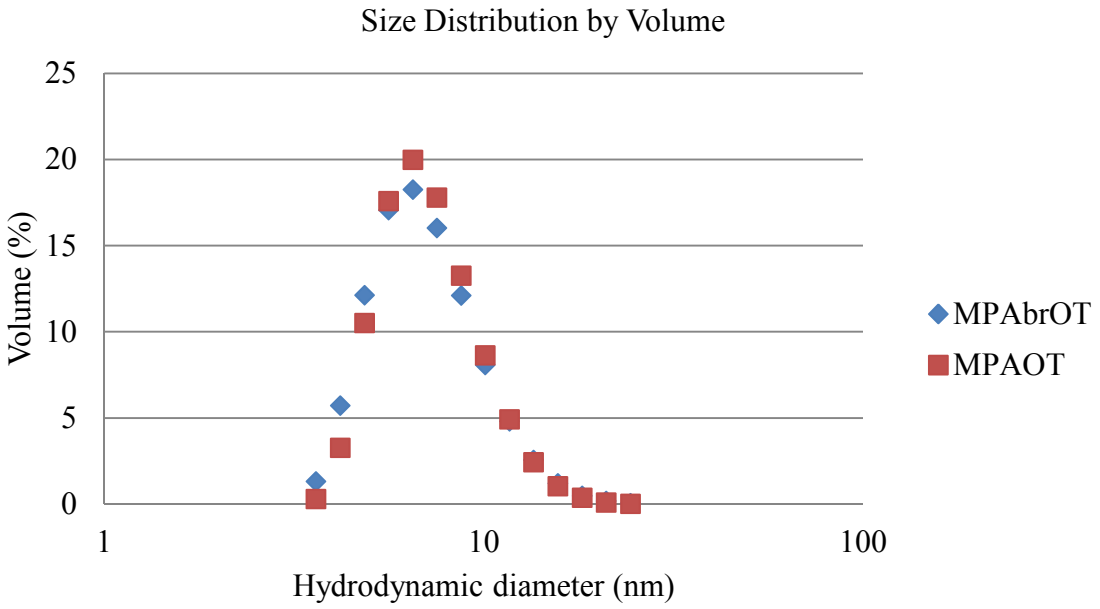

Figure S2. Hydrodynamic size distribution of the two types of AuNPs measured by dynamic light scattering.

Characterization of BSA-AuNPs interaction

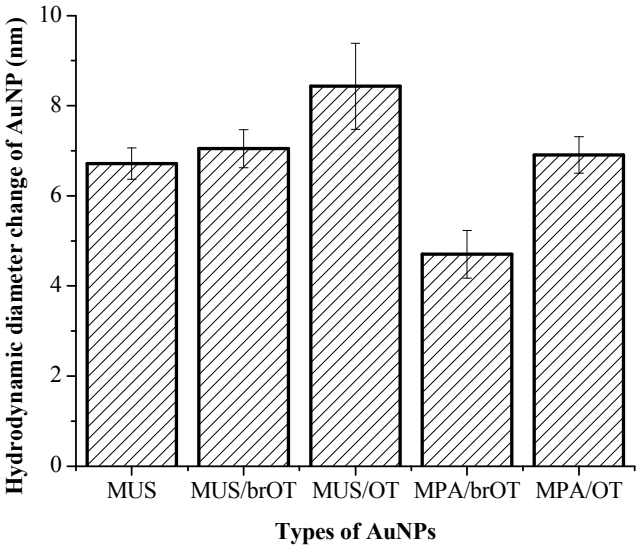

Figure S3. Maximum ΔD_h of MUS-type AuNPs due to the adsorption of BSA.

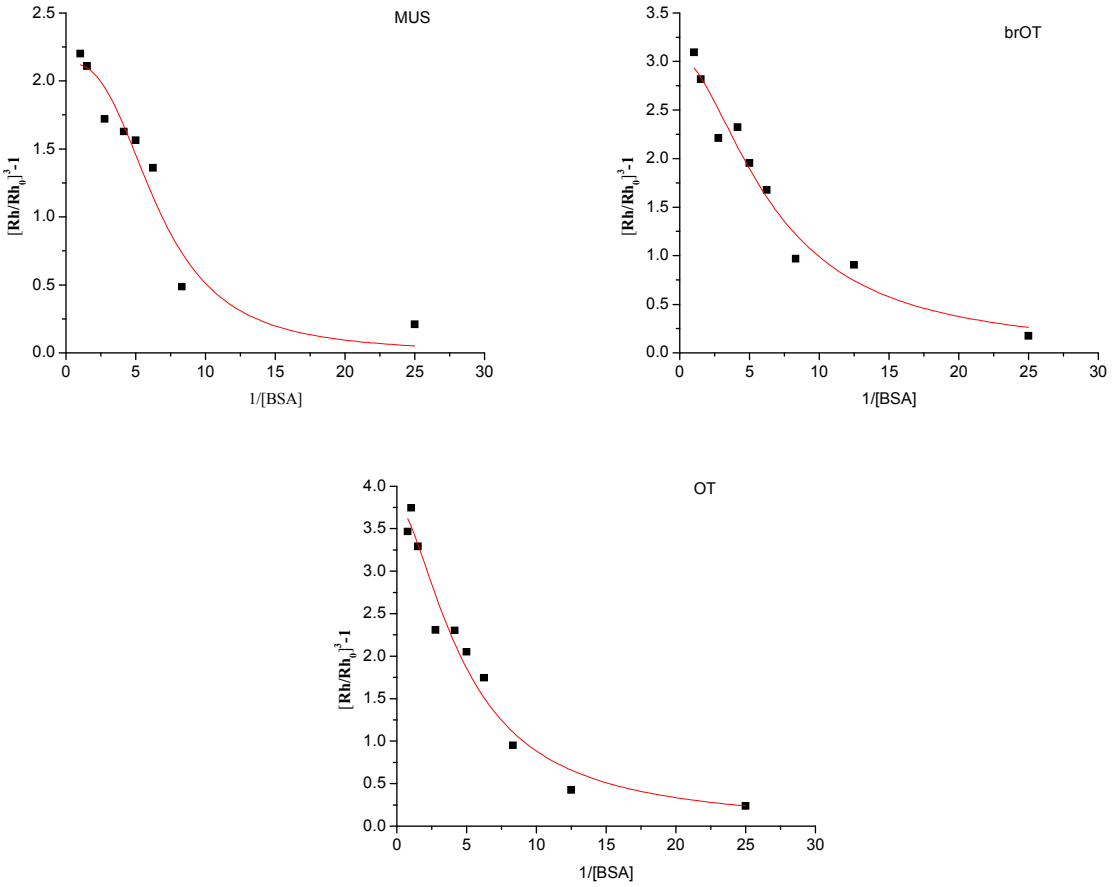

Figure S4. Fitting of the DLS results for the MUS-type AuNPs.

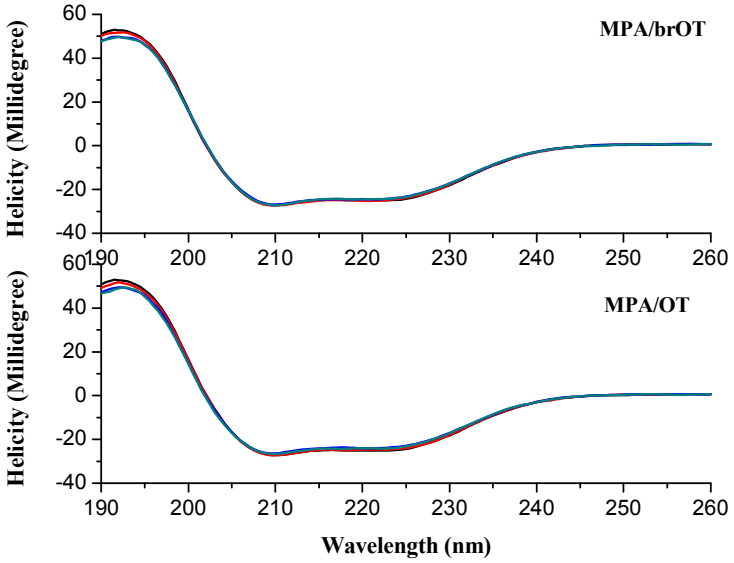

Figure S5. The secondary structure of BSA following the addition of MPA-type AuNP at pH 7.4. BSA concentration -1.5 μM, AuNP-0.025, 0.05, 0.1 μM.

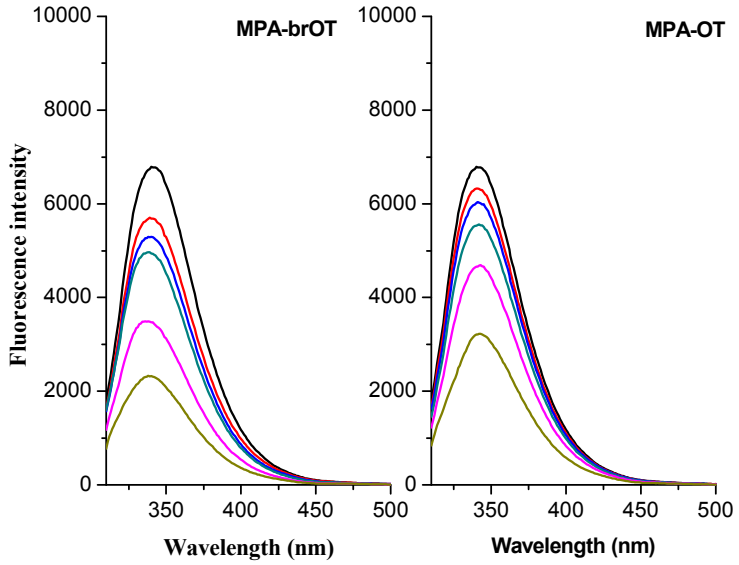

Figure S6. Tryptophan fluorescence emission of BSA (0.12 μM) in the presence of varying AuNP concentration (0~0.01 μM).

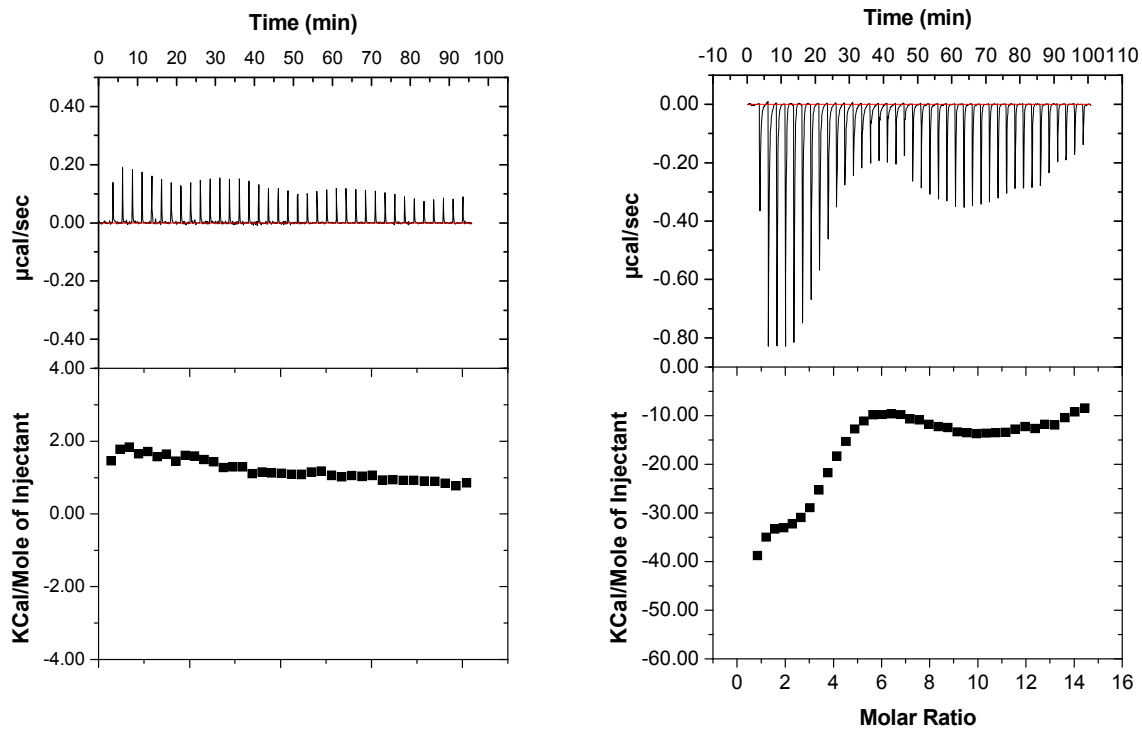

Figure S7. ITC data from the titration of 500 µM of BSA into 10 mM 7.4 phosphate buffer (left) and into 7.03 µM MUS AuNP. Heat flow versus time during injection of proteins at 25 ℃ and heat evolved per injection of proteins was present above and below respectively.

Esterase activity

Esterase activity of BSA in the absence and presence of AuNPs was determined using 4-nitrophenyl acetate (Sigma Aldrich) as the substrate. The formation of 4-nitrophenol was monitored at 400 nm by using a UV–visible spectrophotometer (Agilent 8453, CA, USA). The 2 ml reaction mixture (10 mM phosphate buffer at pH 7.4) contained 100 μM 4-nitrophenyl acetate, 2.5 μM BSA and various concentrations of AuNPs, the reaction was initiated by the addition of 4-nitrophenyl acetate. Adsorption due to 4-nitrophenol formation was calculated from the adsorption of the mixture at 5 min subtracted the adsorption before substrate addition. Enzyme activity was defined as moles of 4-nitrophenol formed per min. A molar extinction coefficient for p-nitrophenol of $\varepsilon = 17\ 700\ M^{-1} \cdot cm^{-1}$ was used for the p-nitrophenol calculation.

Evaluation of the effects of AuNPs complexation on BSA functionality was done using the well-established esterase activity assay. The catalytic function of Tyr-411 in BSA is considered to be responsible for the esterase activity of serum albumin, and factors that influence the local environment of this site will affect the catalytic activity [1].

Results and discussion

It is showed that the esterase activity of BSA was inhibited by AuNPs adsorption, and the degree of inhibition increased as AuNPs concentration increased (Figure S7). Overall, MPA-brOT inhibited more than MPA-OT. Two possible factors may contribute to the difference in the inhibition between MPA-brOT and MPA-OT: 1) the different affinity of BSA to these AuNPs; 2) the different conformations of the complex BSA between the two types of AuNPs. Considering the larger size increase in BSA-MPA-OT complexation, the number of BSA on MPA-OT should not be less than that in BSA-MPA-brOT. Therefore, the activity difference is more likely due to different conformations of BSA on the surface of AuNPs. First of all, side-on orientation adopted by BSA on MPA-brOT lead to tighter interaction and higher degree of alteration of protein structure (possibly tertiary, if not secondary at pH 7.4), compared to end-on orientation on MPA-OT. Secondly, side-on orientation is more likely to shield the active site (Tyr-411) from interaction with the substrates than the end-on orientation, due to large contact area (similar to fluorescence quenching).

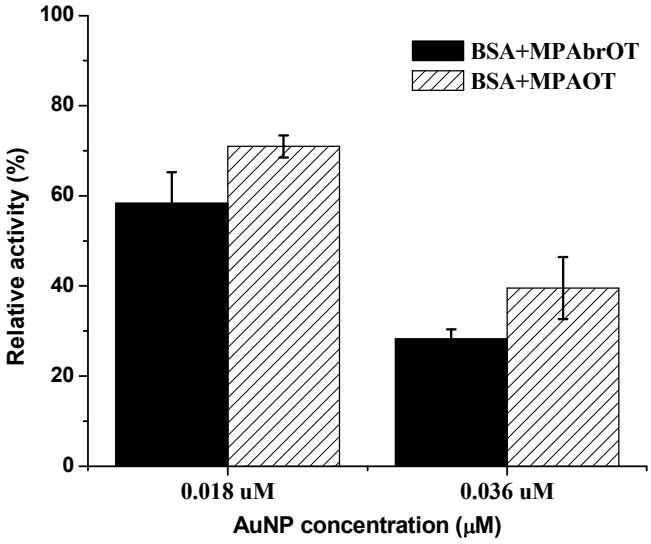

Figure S8. Effect of MPA/brOT and MPA/OT on the esterase activity of BSA, the activity was expressed as the activity relative to that of free BSA without the presence of AuNPs.

Reference

1. Sakurai, Y.; Ma, S. F.; Watanabe, H.; Yamaotsu, N.; Hirono, S.; Kurono, Y.; Kragh-Hansen, U.; Otagiri, M., Esterase-Like Activity Of Serum Albumin: Characterization Of Its Structural Chemistry Using P-Nitrophenyl Esters As Substrates. *Pharm Res* 2004, 21, 285-292.