# Gold Nanoparticle Sensor for Homocysteine Thiolactone-Induced Protein Modification

*Arther T. Gates,[1] Sayo O. Fakayode,[2] Mark Lowry,[1] Gabriela M. Ganea,[1] Abitha Murugeshu,[1] James W. Robinson,[1] Robert M. Strongin,[3*] and Isiah M. Warner[1*]*

[1]Department of Chemistry and Department of Biological Engineering, Louisiana State University, Baton Rouge, LA 70803

[2]Department of Chemistry, Winston-Salem State University, Winston-Salem, NC 27110

[3]Department of Chemistry, Portland State University, Portland, OR 97207

[*]Correspondence to: Isiah M. Warner Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, USA and Robert M. Strongin, Portland State University, Portland, OR 97207

E-mail: Isiah M. Warner, iwarner@lsu.edu and Robert M. Strongin, strongin@pdx.edu

Tel: (225) 578-3971

**Supporting Information Table of Contents**

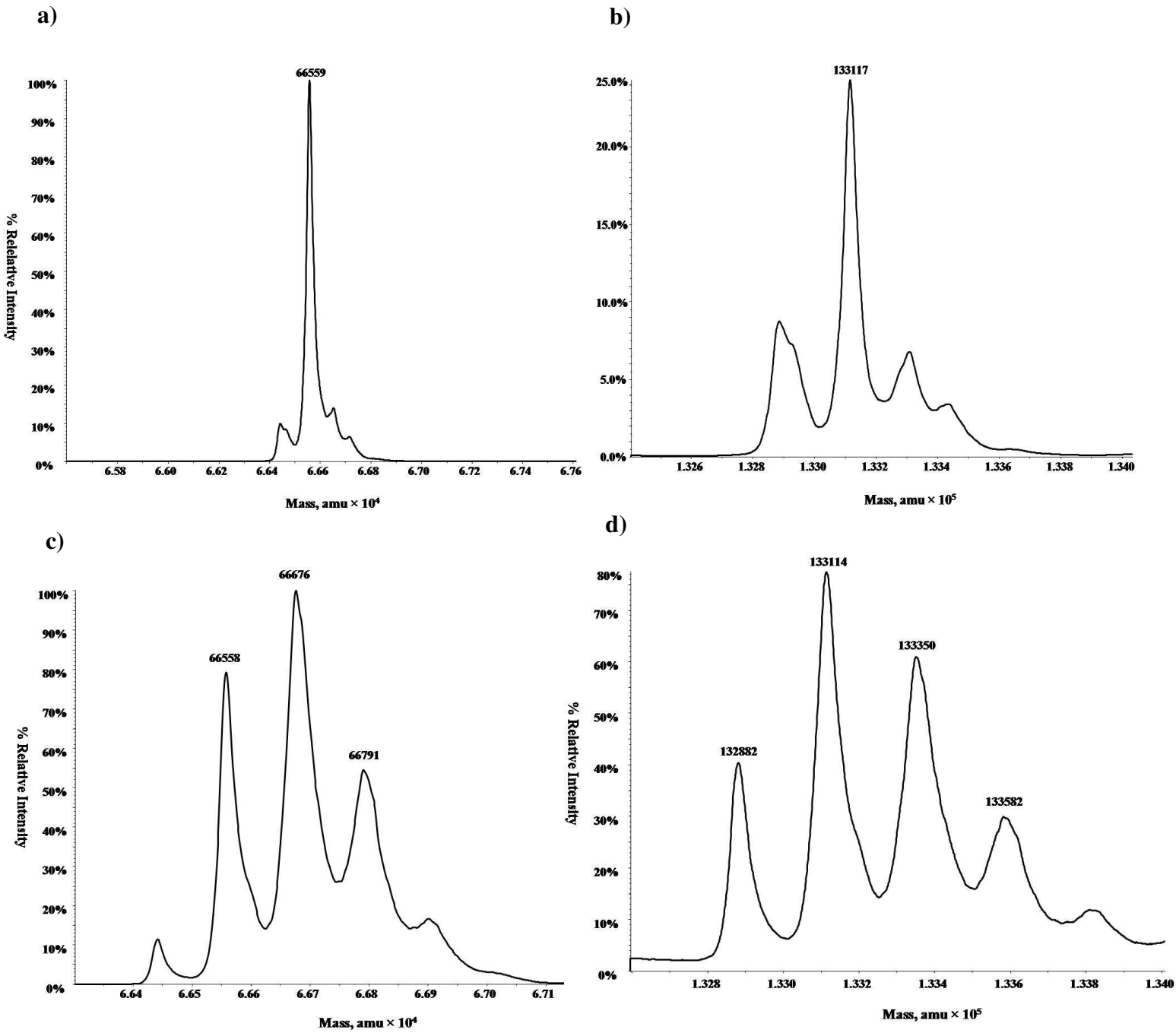


Figure S1. Mass spectra for a) the monomeric, b) the dimeric mass regions, respectively, of an unmodified HSA (control sample). Mass spectra for c) the monomeric and d) dimeric mass regions, respectively, of an HTL-modified HSA sample illustrate the extent of modification produced in the protein reaction mixture.

Discussion of mass spectrometry data

Electrospray ionization mass spectrometry (ESI-MS) was employed to verify HTPM and assess the extent of modification obtained for the protein modification protocol used in study. Accordingly, fresh protein reaction mixtures were prepared as described in the text and diluted with water to obtain a 1 mg/mL solution. The resultant solution was diluted 2-fold with a 1:1 (acetonitrile:water) prior to injection onto a liquid chromatography-guard column interface. The total elapsed time from the initiation of the modification reaction to MS analysis was 6 hr. Note that the predominant species in the control sample is monomeric HSA (66559 amu). A small signal corresponding to naturally occurring dimeric HSA, ~25% relative intensity, was also detected in the control sample. In contrast, the signal due to the unmodified HSA species (66558 amu) is greatly diminished in the modified sample. The differences in amu between consecutive species in the monomeric region of the mass spectra for the modified sample are ~117 amu, which is consistent with the formation protein homocystamide formation via dipeptide bond formation, which results in the loss of $H_2O$ (18 amu). Substantial signals from singularly and doubly modified HSA species are detected at 66676 and 66791 amu, respectively. A smaller signal corresponding to triply modified HSA is also observed. Additionally, there is a substantial increase in the signals corresponding to dimeric HSA species in the protein reaction mixture due to modification-induced oligomerization.

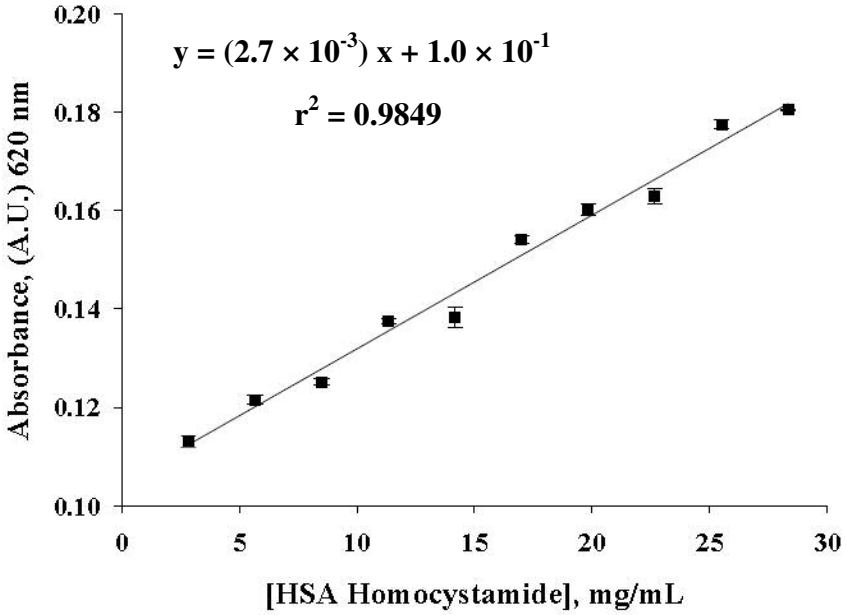


Figure S2. Plot of the colorimetric signal produced by the GNP sensor signal at 620 nm in response to HSA-homocystamide (2.8 – 28 mg/mL), assuming complete modification. Each data point is the average of triplicate measurements. Additional experimental parameters are provided in the experimental section of the text in the main document.

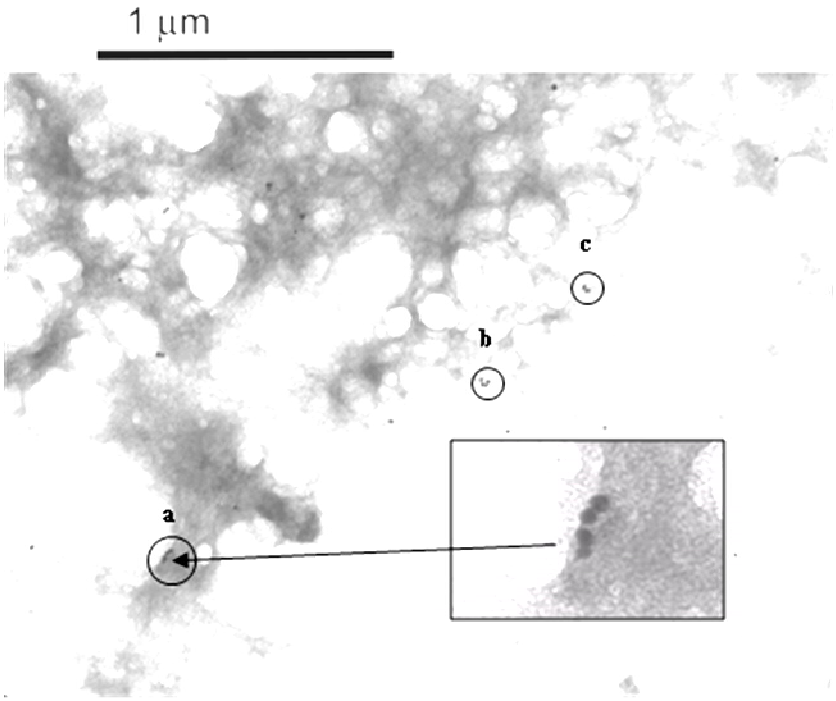


Figure S3. Transmission electron micrograph (TEM) images of the GNP sensor in the presence of HSA-homocystamide. The circled regions a) and b) highlight regions within the oligomeric protein network containing 4-GNP clusters at 33,000 × magnification. The inset shows a) at 100,000 × magnification. Region c) contains a 2-GNP cluster ( 33,000 × magnification). Note that region b) is shown at 100,000 × magnification in the manuscript (inset of Figure 3a).

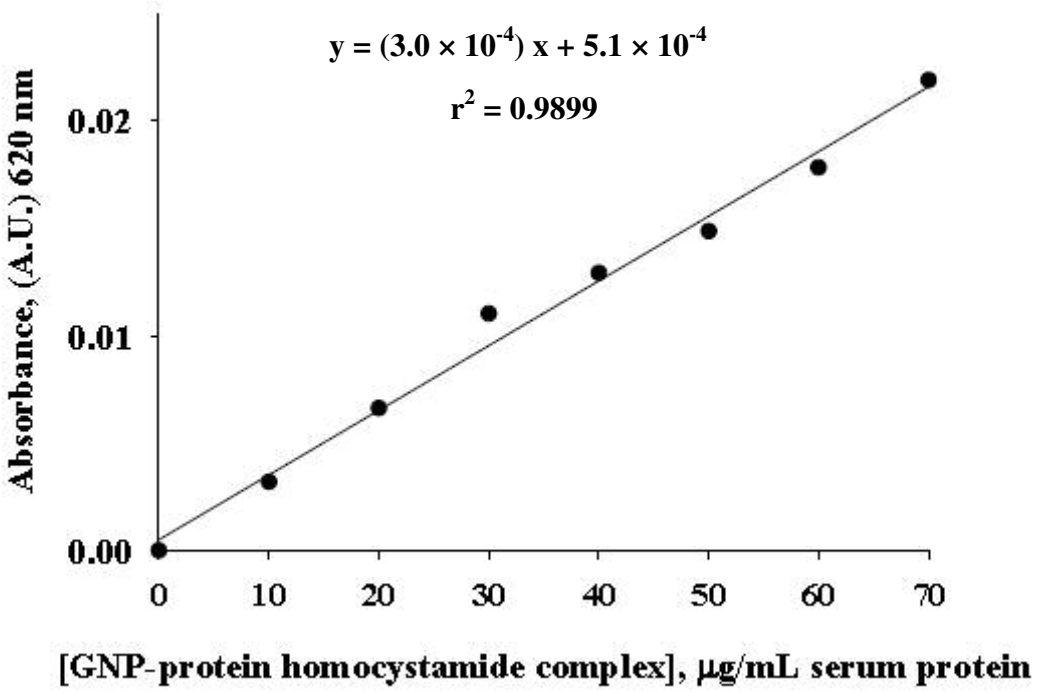


Figure S4. The dilutional stability of the sensor-protein homocystamide complex was evaluated by serial dilution of a sensor solution containing the GNP-protein complex (2.3 mg/mL sera homocystamide and 0.7 mM GNP). The concentration of the sensor complex in reported in unit of µg/mL serum protein, assuming complete modification. Deviation from linearity is indicative of a weakly bound complex; while linearity is indicative of a tightly bound complex. The calibration sensitivity is 0.30 AU · $(ng/mL)^{-1}$. This data suggest that the colorimetric complex resulting from modification-induced nanoparticle assembly is irreversible under the conditions used in this study. Each data point is the average of 5 measurements. Additional experimental parameters are provided in the experimental section of the text in the main document.