Supplementary Information

Ultrasmall Gold Nanoparticles Coated with Zwitterionic Glutathione Monoethyl Ester: A Model Platform for the Incorporation of Functional Peptides

Luiza L. Knittel[1], Huaying Zhao[2], Ai Nguyen[2], Antônio Miranda[1], Peter Schuck[2], Alioscka A. Sousa[1,*]

1. Department of Biochemistry, Federal University of São Paulo, São Paulo, SP, Brazil
2. National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, MD, USA

* Corresponding author: alioscka.sousa@unifesp.br

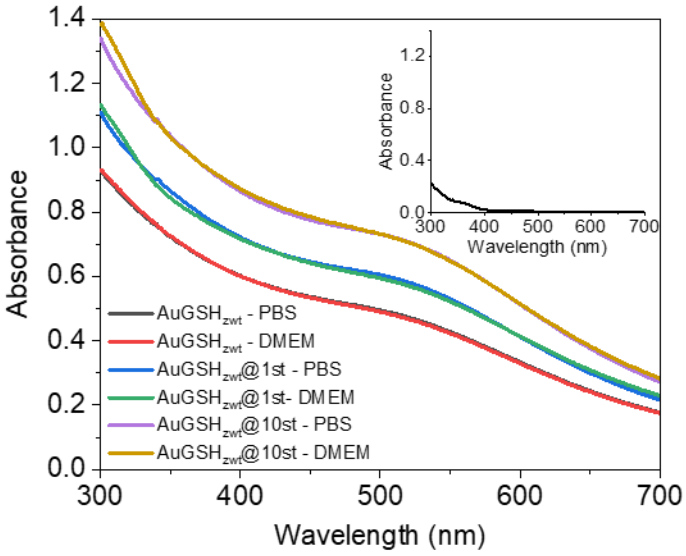

Figure S1. UV-visible spectroscopy characterization of AuNPs in DMEM. The absence of distinct spectral changes for the AuNPs in DMEM vs. PBS indicates lack of significant nanoparticle aggregation or degradation. Inset shows the absorbance spectrum of DMEM used for background subtraction. Spectra of the different AuNPs (AuGSH$_{zwt}$, AuGSH$_{zwt}$@1st and AuGSH$_{zwt}$@10st) are displaced vertically for clarity.

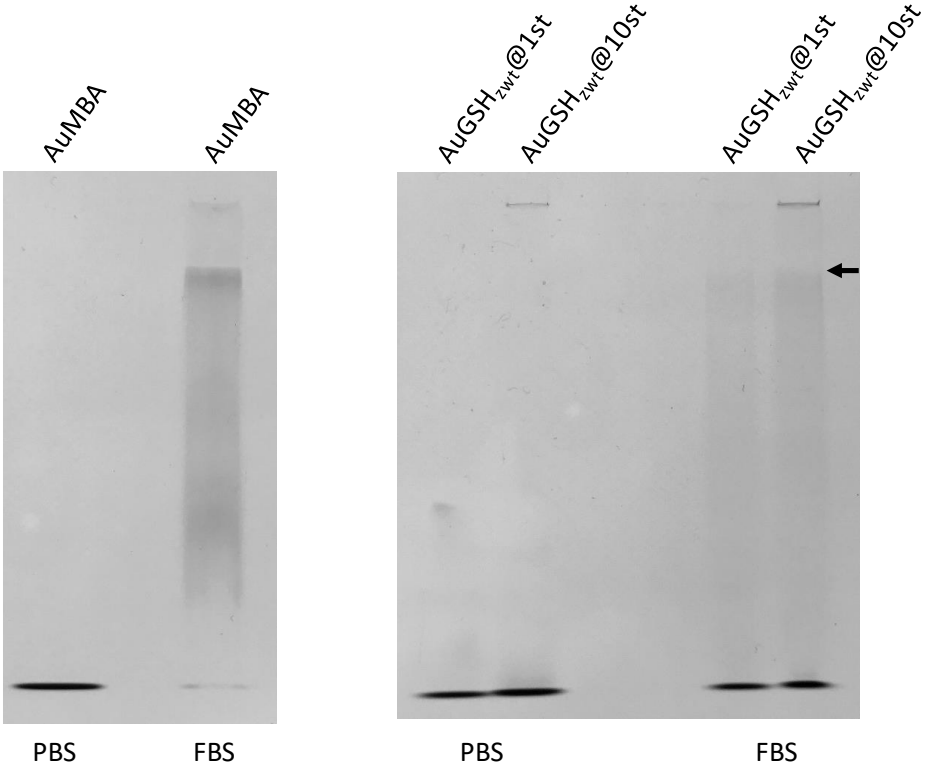

Figure S2. Native polyacrylamide gel electrophoresis characterization of ultrasmall AuNPs in biological media. Data were obtained for AuMBA, AuGSH$_{zwt}$@1st and AuGSH$_{zwt}$@10st in PBS and undiluted fetal bovine serum (FBS). The AuNPs were pre-incubated in the different media for 1 h prior to loading into the gel. The percentage of acrylamide in the running and stacking gels were 7.5 and 5%, respectively. Arrow marks the point of transition from the stacking to the running gel; no AuNP accumulation is observed at this interface.

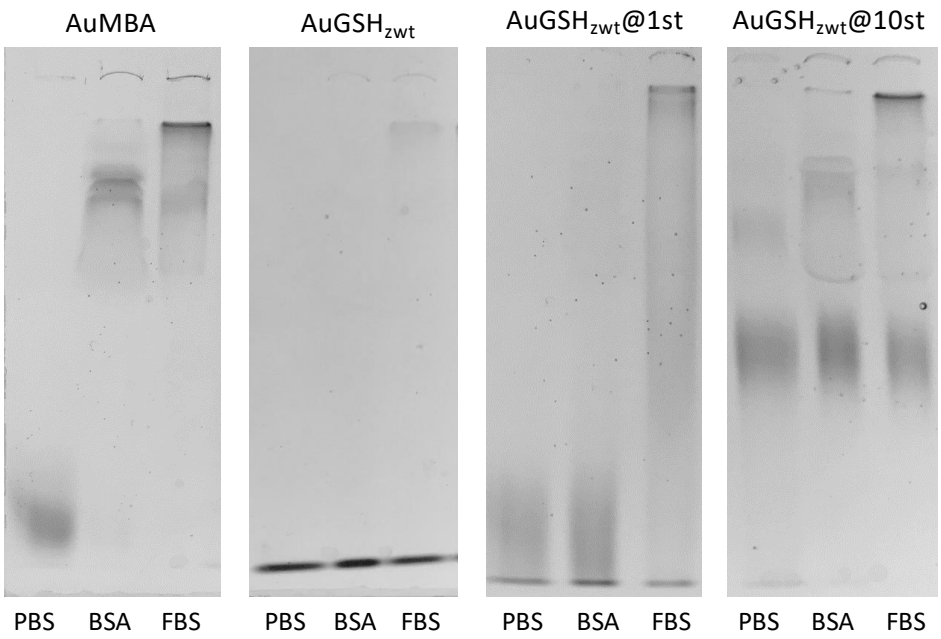

Figure S3. Native polyacrylamide gel electrophoresis characterization of ultrasmall AuNPs in biological media. Data were obtained for AuMBA, AuGSH$_{zwt}$, AuGSH$_{zwt}$@1st and AuGSH$_{zwt}$@10st in PBS, 40 mg/mL BSA and undiluted fetal bovine serum (FBS). The AuNPs were pre-incubated in the different media for 24 h prior to loading into the gel. The percentage of acrylamide in the running and stacking gels were 15 and 5%, respectively.

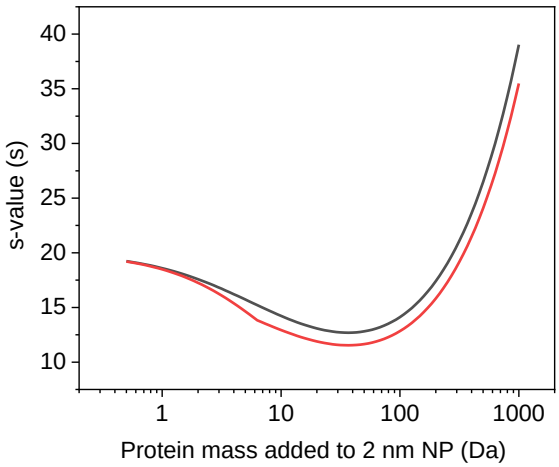

Figure S4. Theoretical s-values of an AuNP of 2 nm sedimenting at 20 S bound by increasing mass of protein. Bound protein contributes both to buoyant mass and to friction. Initially, contributions to the friction dominate, slowing sedimentation of the particle down. However, as the composite particle density decreases, the further changes to the friction diminish relative to the added buoyant mass, leading ultimately to an increase in the sedimentation rate with increasing protein mass. In the intermediate region a minimum is found, at which the s-value is insensitive to added protein mass. Calculations assume either a spherical composite particle (black line), or growth from spherical AuNP into prolate ellipsoid by added protein until an axial ratio of ~ 3:1 is reached and then keeping the frictional ratio (1.1) constant (red line). Calculations are based on a protein partial-specific volume of 0.73 ml/g, and using Stokes-Einstein and Svedberg relationships [1].

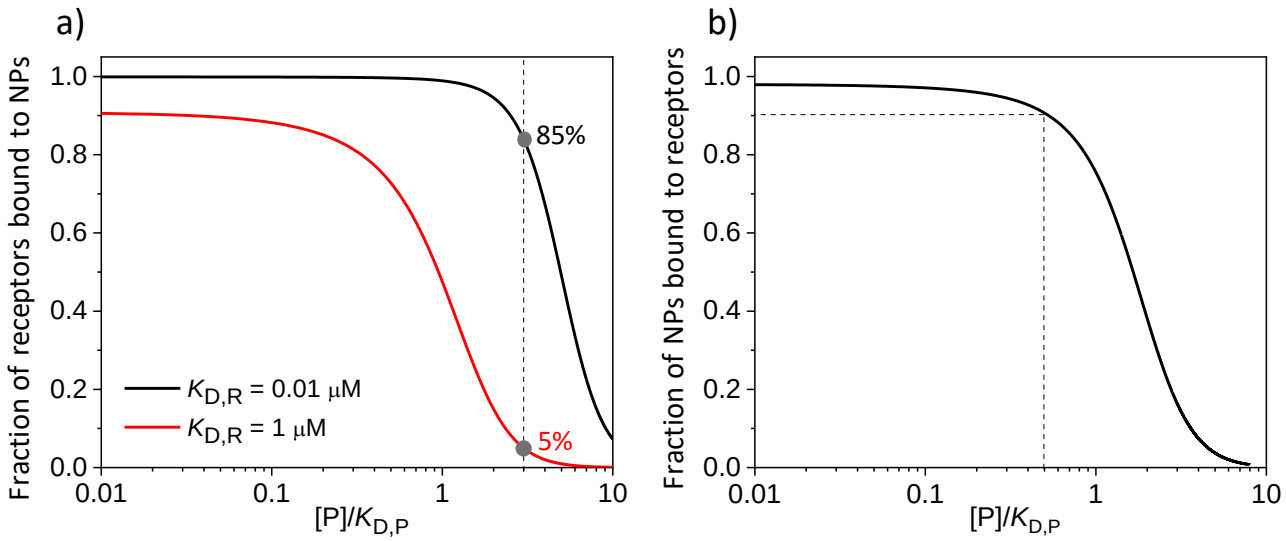

Figure S5. Competition between nonspecific NP-serum protein interactions and specific NP-receptor interactions (please refer to the main text for details). (a) Fraction of receptors bound to NPs as a function of [P]/$K_{D,P}$. The calculations assumed $K_{D,R}$ = 10 nM or 1 μM (black and red traces, respectively), [R] = 0.1 μM and [NP] = 10 μM. (b) Fraction of NPs bound to receptors as a function of [P]/$K_{D,P}$. The calculations assumed $K_{D,R}$ = 1 μM, [R] = 50 μM and [NP] = 1 μM. Dashed line: fractions higher than 90% are obtained when [P]/$K_{D,P}$ < 0.5. Calculations were done in Dynafit® [2].

References

[1] P. Schuck, H. Zhao, C.A. Brautigam, R. Ghirlando, Basic principles of analytical ultracentrifugation, CRC Press 2016.

[2] Kuzmic, P. (1996) *Anal. Biochem.* **237**, 260-273