Supporting Information

Understanding Protein Structure Deformation on the Surface of Gold Nanoparticles of Varying Size

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Table S1. Characterization of AuNPs Used in this Study

AuNP Diameter (nm)[a]	TEM Diameter (nm)[b]	Au Atoms per AuNP	ε (M^{-1} cm^{-1})[c]	λ_{max} (nm)
14	14.3 ±0.2	84,800	3.9×10^8	520
30	31 ± 3	834,000	3.0×10^9	521
43	43.3 ± 0.6	2,460,000	9.1×10^9	531
86	86 ± 3	19,700,000	1.1×10^{11}	555

[a] Diameter of AuNPs referenced in the text and used for calculations.
[b] Observed average diameter of AuNPs observed by TEM.
[c] Extinction coefficient calculated at λ_{max}, calculated as described in the text. [1]

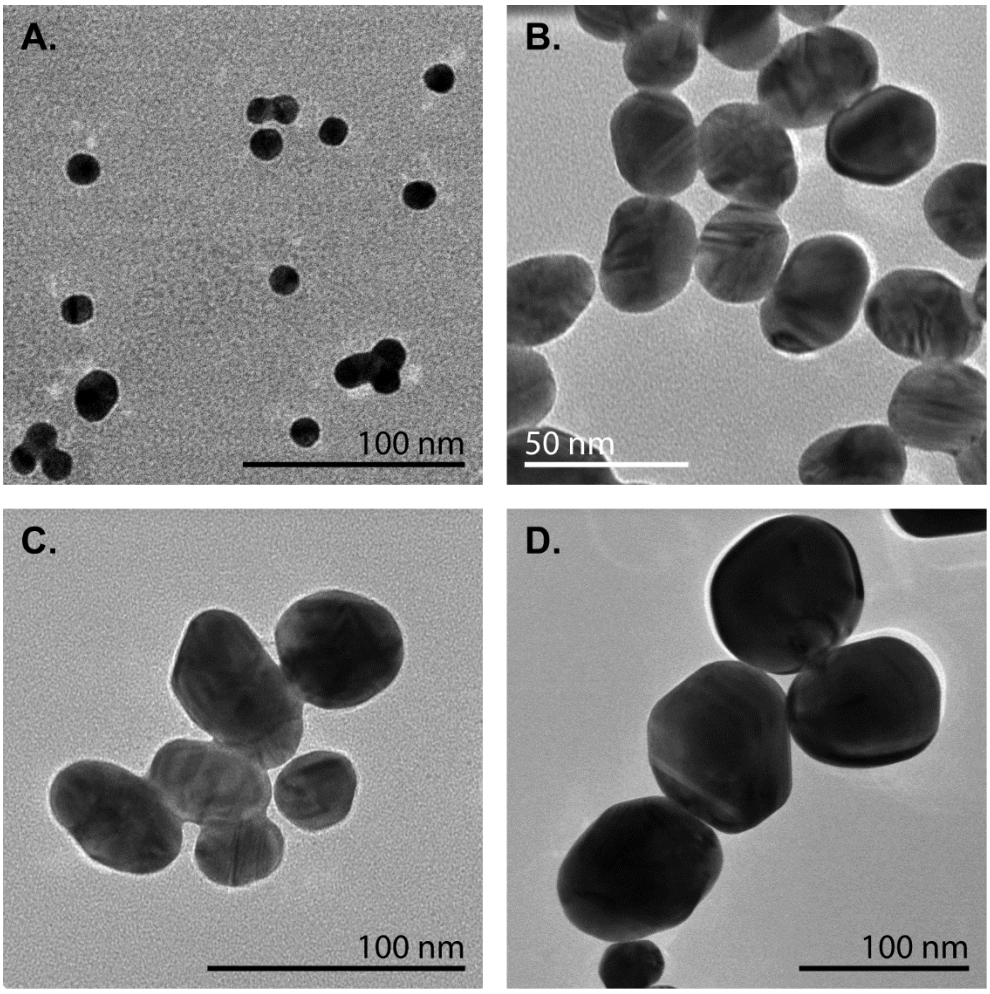

Figure S1. TEM images of synthesized nanoparticles. Citrate-coated AuNPs of size 14 nm (A), 30 nm (B), 43 nm (C) and 86 nm (D) are shown.

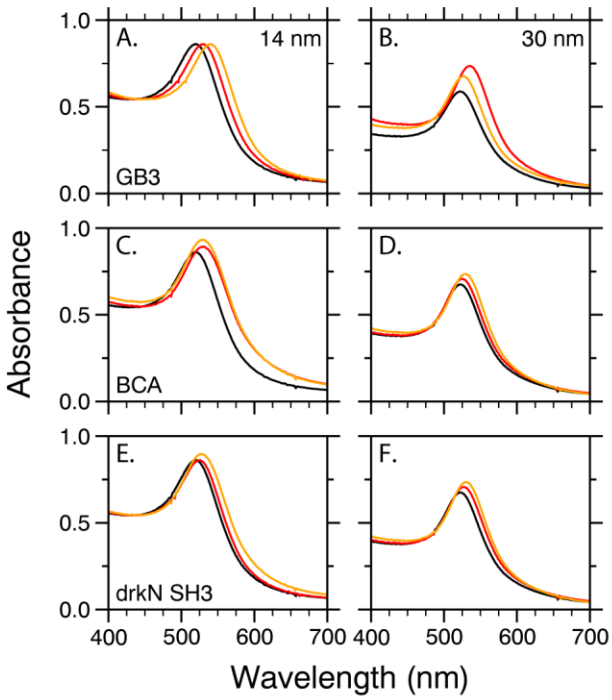

Figure S2. UV-Visible absorbance spectra for the proteins and AuNPs used in this study. Rows indicate GB3 (A-B), bovine carbonic anhydrase (C-D), and the drkN SH3 domain (E-F). Columns correspond to 14 nm (A, C, E) or 30 nm (B, D, F) AuNPs. The concentration of 14 nM AuNPs was 2.3 nM for all figures, and the concentration of 30 nM AuNPs was 0.29 nM. Different curves represent different levels of protein saturation: Black curves correspond to no added protein. Red curves correspond to protein added at half saturation, according to the maximum number of proteins bound per AuNP (see text). Yellow curves correspond to a protein concentration matching the maximum number of proteins bound per AuNP.

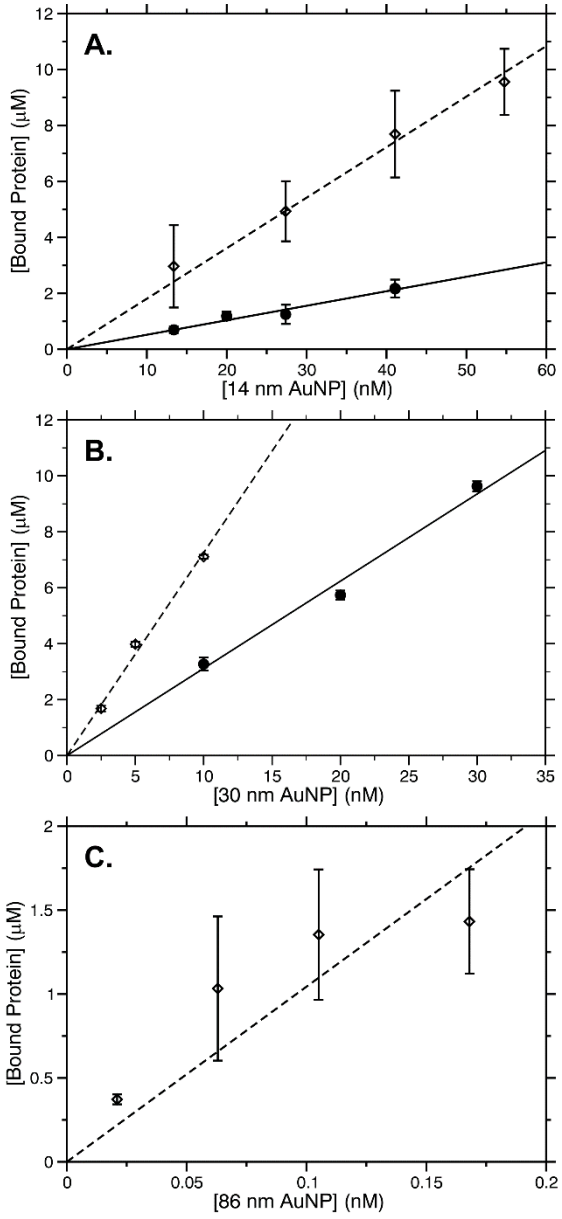

Figure S3. Complete binding data for 14 nm (A), 30 nm (B), and 86 nm (C) AuNPs. Binding was measured for either GB3 (open diamonds, dotted line) or BCA (closed circles, solid lines). In (A) and (B), a total protein concentration of 20 μM was used. In (C), 7 μM was used because of the much lower attainable concentration of AuNPs. The slope of each line represents the apparent binding capacity of each AuNP. Because of the much lower signal to noise for BCA, the binding capacity reported in the text was calculated as an average of several two-point calculations instead of a linear fit. Error bars represent the standard deviation of three separate experiments.

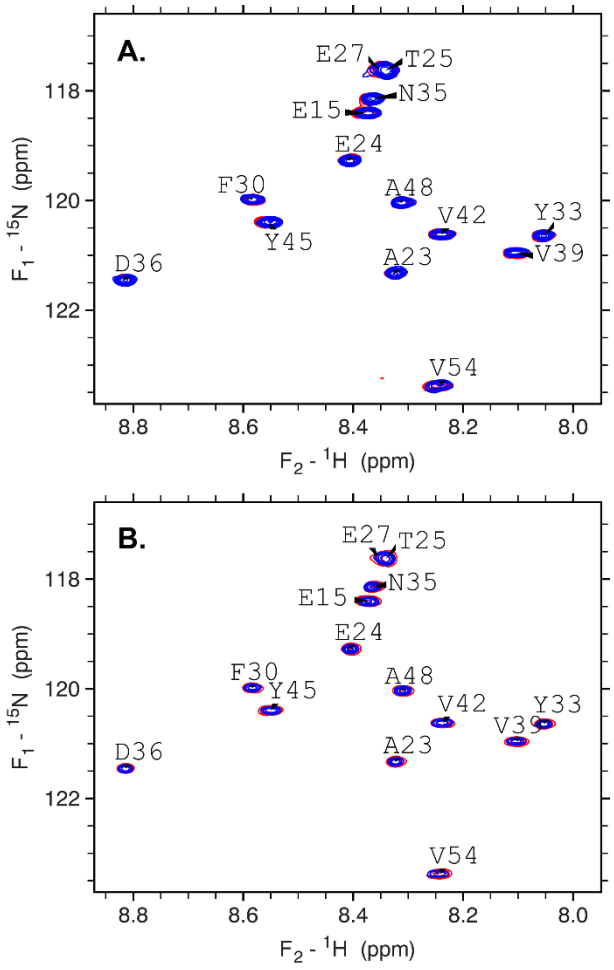

Figure S4. Two dimensional ^{15}N-^{1}H TROSY HSQC spectra of GB3 in the absence (red) or presence (blue) of 30 nm (A) and 86 nm (B) AuNPs. Contour levels are identical for spectra with and without AuNPs.

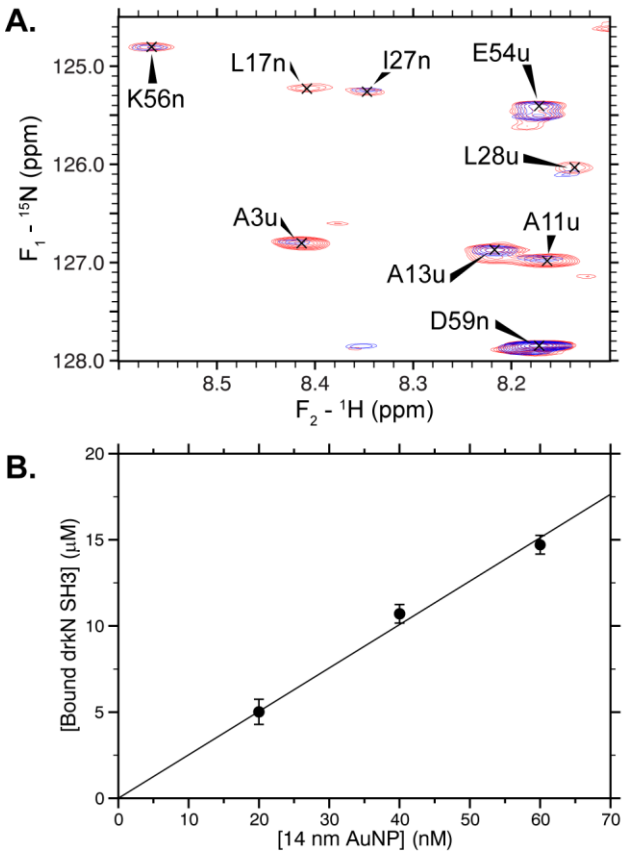

Figure S5. Apparent adsorption capacity measurement for the drkN SH3 domain on 14 nm AuNPs. (A) Region of a ^{15}N-^{1}H HSQC spectrum demonstrating that both native (n) and unfolded (u) peaks in the drkN SH3 domain exhibit similar behavior in the presence of 30 nM, 14 nm AuNPs. The red spectrum corresponds to drkN SH3 in the absence of AuNPs, and the blue spectrum corresponds to the protein in the presence of AuNPs. The protein concentration in both samples is 20 μM. (B) Bound protein concentration of drkN SH3 as 14 nm AuNP concentration is increased, as measured by 1D ^{1}H NMR spectra. Each data point represents contributions from both folded and unfolded signals. As described in the text, the folded and unfolded peaks behave identically in the presence of AuNPs.

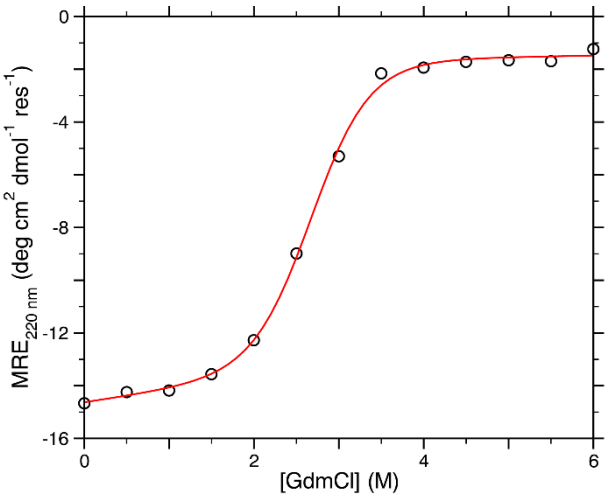

Figure S6. Guanidinium Chloride (GdmCl) unfolding experiment of GB3 at pH 7.0 and 25 °C. Folding was monitored using circular dichroism at 220 nm. The red line represents a two state fit using the linear extrapolation model,[2] assuming linear baselines for the folded and unfolded states. The equations used for the fit were:

$$K_{eq}(x) = e^{-\frac{\Delta\bar{G}^0(H_2O)-mx}{RT}}$$

$$CD(x) = (a_f x + b_f)\frac{1}{1+K_{eq}(x)} + (a_u x + b_u)\frac{K_{eq}(x)}{1+K_{eq}(x)}$$

In these equations, x is the concentration of GdmCl in molar units, a_n and b_n represent the slope and intercept of the folded state signal, a_u and b_u represent the slope and intercept of the unfolded state signal, m represents the slope of denaturant dependence of stability, and $\Delta\bar{G}^0(H_2O)$ represents the extrapolated unfolding stability at 0 M GdmCl. R and T represent the gas constant and temperature, respectively.

References

1. Liu, X.; Atwater, M.; Wang, J.; Huo, Q., Extinction Coefficient of Gold Nanoparticles with Different Sizes and Different Capping Ligands. *Colloids and Surfaces B: Biointerfaces.* **2007,** *58*, 3-7.
2. Pace, C. N.; Shaw, K. L., Linear Extrapolation Method of Analyzing Solvent Denaturation Curves. *Proteins.* **2000,** *Suppl 4*, 1-7.