Supporting Information

Electrostatic Interactions and Protein Competition Reveal a Dynamic Surface in Gold Nanoparticle-Protein Adsorption

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Mathematical Relationship Between Apparent Binding Capacity and pH

In this model, we assumed that titratable residues in the unbound state possess model-compound pK_a values. This is justified by the fact that all residues involved in binding are solvent exposed and have minimal interactions with other protein residues. The model compound pK_a values were: Asp (3.6), Glu (4.3), Lys (10.5), and Arg (12.5). In the bound state, residues involved in binding exhibit shifted pK_a^b values induced by binding. The reference state, containing M negatively charged (deprotonated) acidic residues at the binding site, has the intrinsic association constant, K_{in}. At any other pH, the protein has the apparent association constant, K_{app}, given by:

$$K_{app} = K_{in} \cdot \frac{\prod_{i=1}^{M}\left[1+10^{-\left(pH-pK_{a,i}^{b}\right)}\right]}{\prod_{i=1}^{M}\left[1+10^{-\left(pH-pK_{a,i}^{f}\right)}\right]} \quad (1)$$

We assume that 15 nm AuNPs have N independent binding sites for a particular protein. If the total concentrations of AuNPs and protein are $[Au]_t$ and $[P]_t$, respectively, the concentration of bound protein $[P]_{bound}$ is given by:

$$[P]_{bound} = \frac{1}{2}\left[([Au]_t N + [P]_t + K_d) - \sqrt{([Au]_t N + [P]_t + K_d)^2 - 4[P]_t[Au]_t N}\right] \quad (2)$$

In NMR binding measurements described here and previously,[1] we observe a plot of $[P]_{bound}$ vs. $[Au]$. This plot is observed to have a near-constant initial slope, which corresponds to the partial derivative of $[P]_{bound}$ with respect to $[Au]$.

The mathematical expression for $\frac{\partial[P]_{bound}}{\partial[Au]}$ is:

$$\frac{\partial[P]_{bound}}{\partial[Au]_t} = \frac{N}{2}\left[1 + \frac{[P]_t - [Au]_t N - K_d}{\sqrt{([P]_t + [Au]_t N + K_d)^2 - 4[P]_t[Au]_t N}}\right] \quad (3)$$

In the expression above, K_d is the apparent dissociation constant for AuNP adsorption. In other words, $K_d = K_{app}^{-1}$ from equation (1). The complete expression, combining equations (1) and (3) descries the apparent number of proteins bound as a function of pH. At low pH, when all acidic residues are protonated (neutral charge), this value approaches the total number of proteins bound per nanoparticle, N. At higher pH, the slope diminishes as binding decreases. Thus, the slope given by the expression in (3) can be thought of as the apparent maximum adsorption, and at low pH, (3) represents the true adsorption maximum (N).

Under the conditions of our experiments ($N \approx 200$, $pK_a^f \approx 3$, $pK_a^b \approx 5.2$, $K_{in} \approx 10^7$, $[P]_t \approx 20 \times 10^{-6}$ M, and $[Au]$ from $10 - 200 \times 10^{-9}$ M), the slope of $[P]_{bound}$ with respect to $[Au]$ ($\frac{\partial[P]_{bound}}{\partial[Au]}$) is expected to be approximately constant (within 5-10%) over the range of $[Au]$ values used. Moreover, the apparent number of proteins bound is expected to have a sigmoid shape near the shifted pK_a values. Using the parameters above, and assuming a single titratable site, the plot of $\frac{\partial[P]_{bound}}{\partial[Au]}$ vs. pH is shown below.

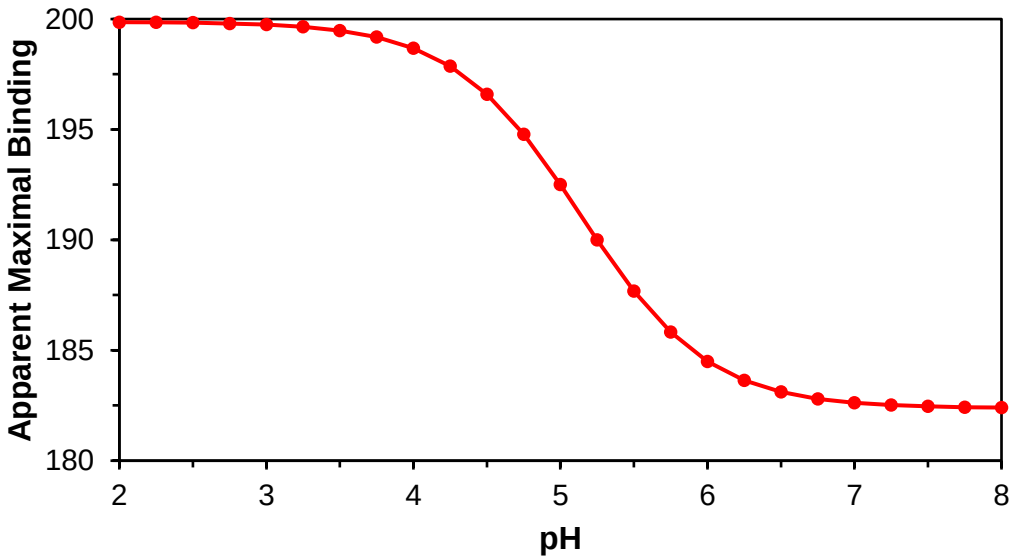

Figure S1. Model of maximal binding using parameters given above.

As described in the text, in this work we optimized values of pK_a^b and K_{in} based on the observed behavior of the apparent maximum adsorption as a function of pH.

Table S1. Apparent Adsorption Capacity for GB3 Lysine Variants

Variant	Binding Capacity[1]	*p*-value
Wild-Type	200 ± 5	-
K4A	154 ± 4	0.00024
K10A	184 ± 6	0.024
K13A	146 ± 7	0.00041
K19A	171 ± 7	0.0043
K28A	185 ± 5	0.021
K31A	188 ± 3	0.023
K50A	148 ± 8	0.00067

[1]Error bars represent the standard deviation of three separate measurements.
[2]Calculated from an unpaired *t*-test, compared to Wild-Type GB3.

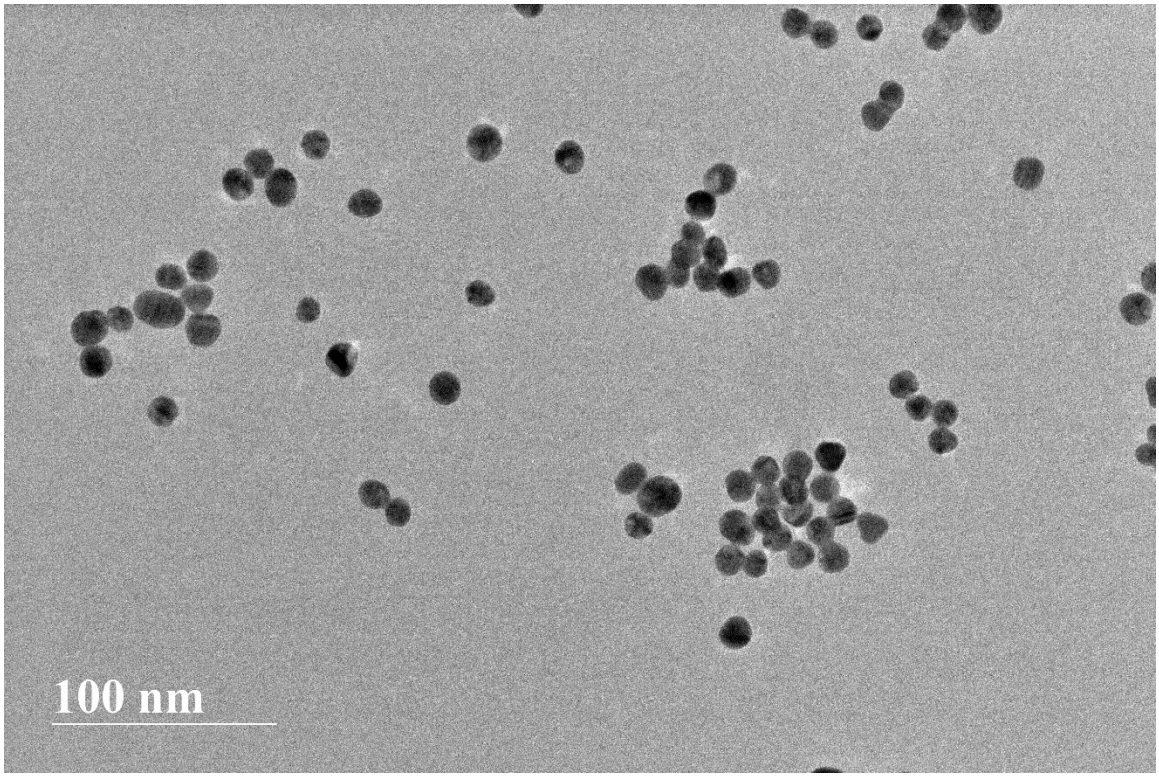

Figure S6. TEM image of 15 nm, citrate-coated AuNPs used in this study.

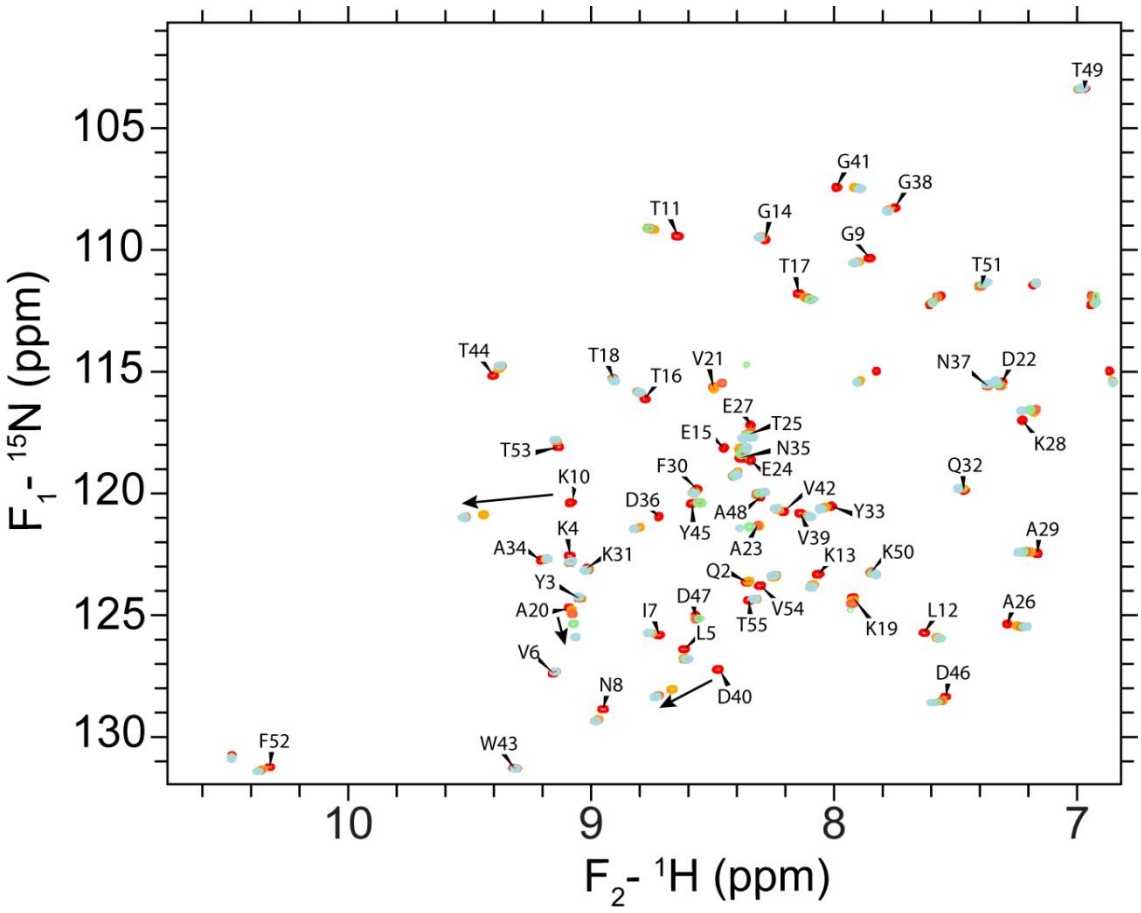

Figure S3. ^{1}H-^{15}N HSQC spectra of GB3 at different pH conditions. Overlaid spectra of WT GB3 at pH 4.6, 5.5, 6.6, 7.3 and 8.3 denoted as red ,orange, coral, green and blue. The arrows indicate the chemical shift change vs. pH. This data suggest that the overall structure of GB3 is unchanged and that the protein is not unfolding under conditions measured here.

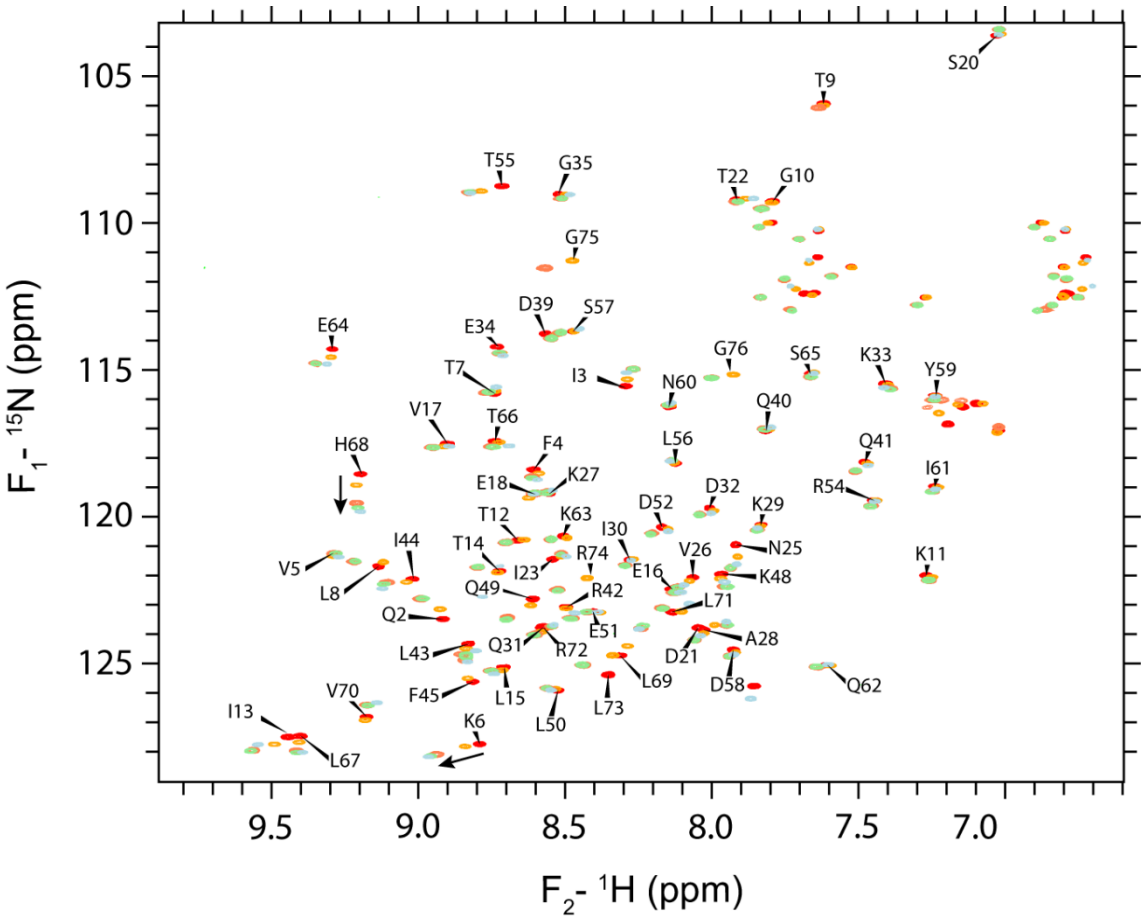

Figure S4. ^{1}H-^{15}N HSQC spectra of ubiquitin at different pH conditions. Overlaid spectra of WT ubiquitin at pH 4.6, 5.5, 6.6, 7.3 and 8.3 denoted as red ,orange, coral, green and blue. The arrows indicate the chemical shift change vs. pH. This data suggest that the overall structure of ubiquitin is unchanged and that the protein is not unfolding under conditions measured here.

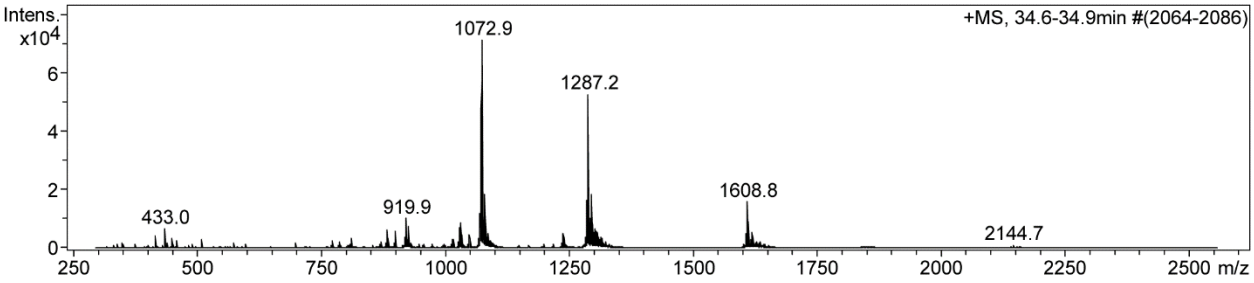

Figure S5. ESI mass spectrum of lysine-methylated GB3. The observed peaks correspond to a deconvolved mass of 6432.1 Da. The expected mass if all seven lysine residues are methylated (including methylation of the N-terminus) is 6431.8.

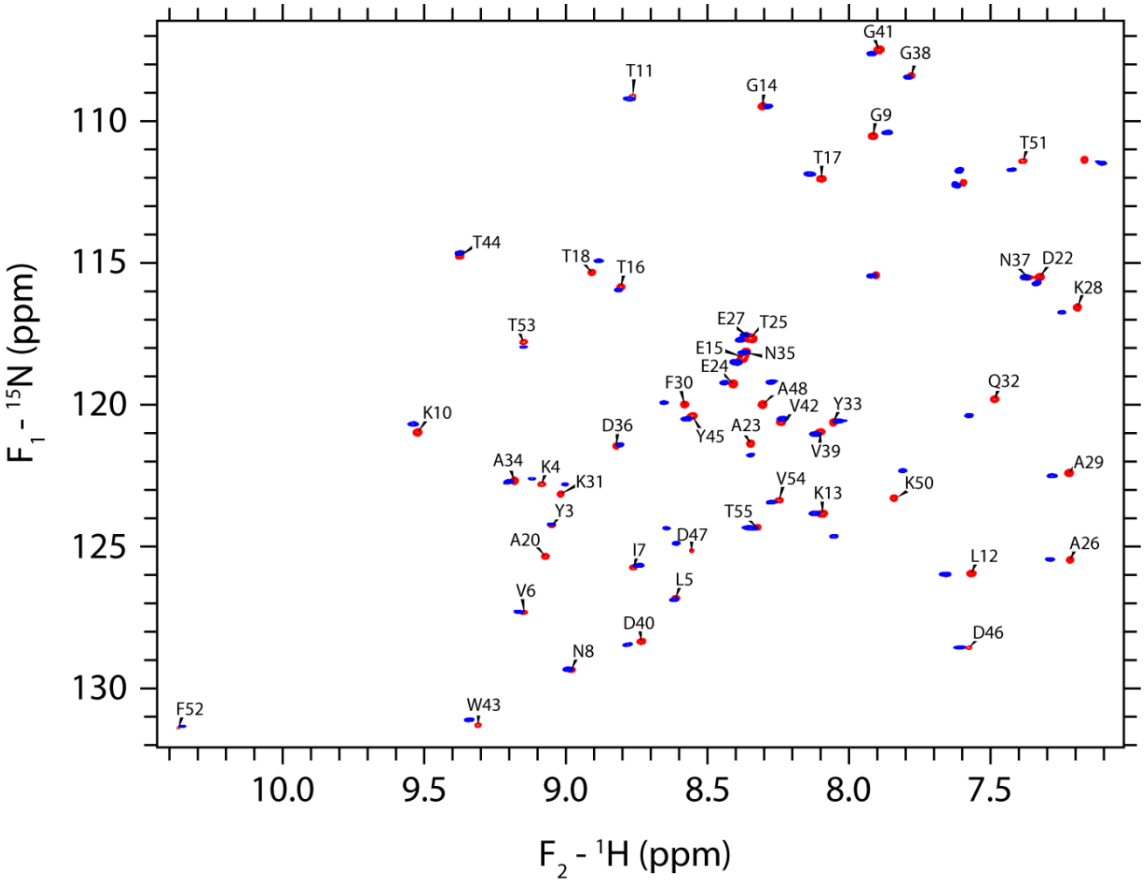

Figure S6. A comparison of the WT GB3 and methylated GB3 proteins. The ^{1}H-^{15}N HSQC spectra of WT GB3 (red) and methylated GB3 (blue) are overlaid at pH 7.0. The conformation of GB3 is unchanged by methylation and most of the non-Lys peaks exhibit very small chemical shift perturbations upon methylation.

References

(1) Wang, A.; Vangala, K.; Vo, T.; Zhang, D.; Fitzkee, N. C. A Three-Step Model for Protein-Gold Nanoparticle Adsorption. *The Journal of Physical Chemistry C* **2014**, *118*, 8134.