Protein Adsorption Induced Bridging flocculation: The dominant entropic pathway of Nano-bio Complexation

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ELECTRONIC SUPPOTIVE INFORMATION (ESI)

Adsorption Isotherms

It is important to note that the current procedure used for the batch depletion method is slightly different from Norde's method [30] because it was designed with the purpose of mimicking the adsorption process that occurs during an isothermal titration calorimetry (ITC) experiment. Usually, in the batch depletion method, a constant adsorbent surface is exposed to protein solutions of varying concentrations to provide a spectrum of surface coverages as a function of free protein concentrations in the mixtures (Ceq). Also to ensure the equilibrium/ steady state adsorption, the adsorbent and adsorbate were incubated under mild rotation for usually 16 hours. Conversely, in the ITC test, a concentrated protein solution was titrated into the silica suspension as small volume aliquots and equilibrium time between injections and total experimental time were shorter compared to batch depletion method. In order to test any potential discrepancy that could result from the difference in equilibrium time between the traditional depletion method and the ITC method, the depletion method was conducted in both ways; first using the timeframe of ITC and second extending the equilibrium time to 16 hours. The "ITC mimic" depletion method and traditional depletion method did not show any significant differences and were quite in line with Norde's isotherm results (Figure 1A, ITC mimic depletion method (n=3) and traditional depletion method (n=3) were plotted together with error bars to depict the agreement between methods). So the two conclusions from this particular experiment were: (1) Using constant volumes of protein solutions with varying concentrations or adding a larger volume of protein solution with the same concentration do not affect the isotherm plateau as long as the mass balance is conducted in a careful manner and (2) lysozyme adsorption on hydrophilic silica reaches an equilibrium within the timeframe of the experiments that are conducted in an ITC instrument.

Table ESI 1Experimental design for ITC mimic adsorption test

	Reference Injection									
	n1	n2	n3	n4	n5	n6	n7	n8	n9	n10
	Inoculation Time									
Tube	0	5	10	15	20	25	30	35	40	45
1	S1									
2		S2								
3			S3							
4				S4						
5					S5					
6						S6				
7							S7			
8								S8		
9									S9	
10										S10

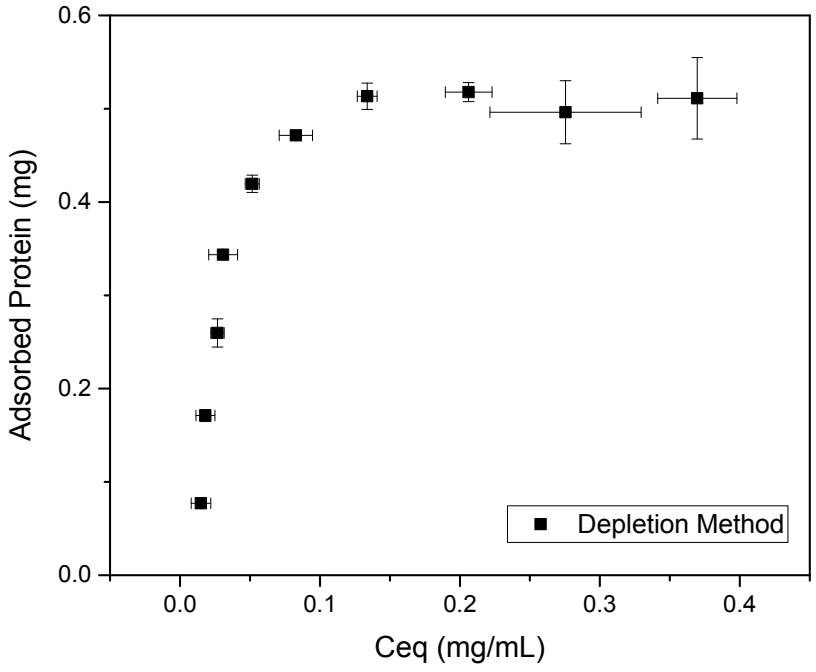

Figure ESI-1a. The raw adsorption data before normalizing the adsorbed protein amount with the surface area.

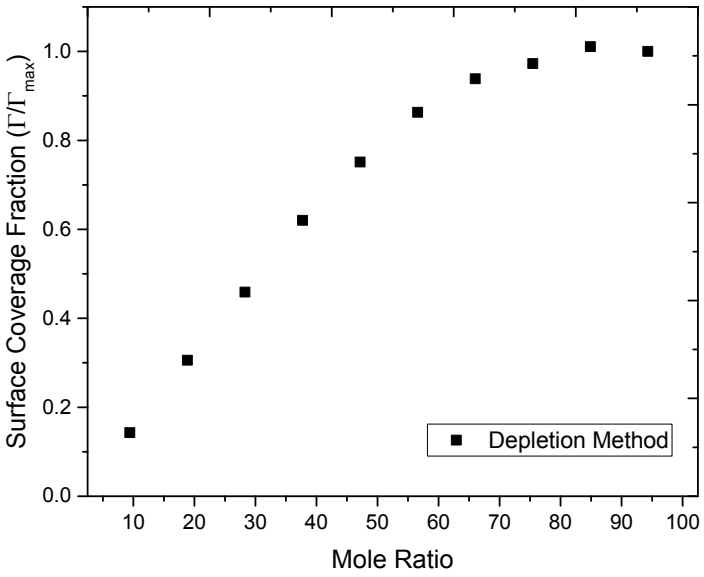

Figure ESI-1b. Surface coverage fraction was calculated by normalizing the surface coverage with respect to the maximum experimental surface coverage.

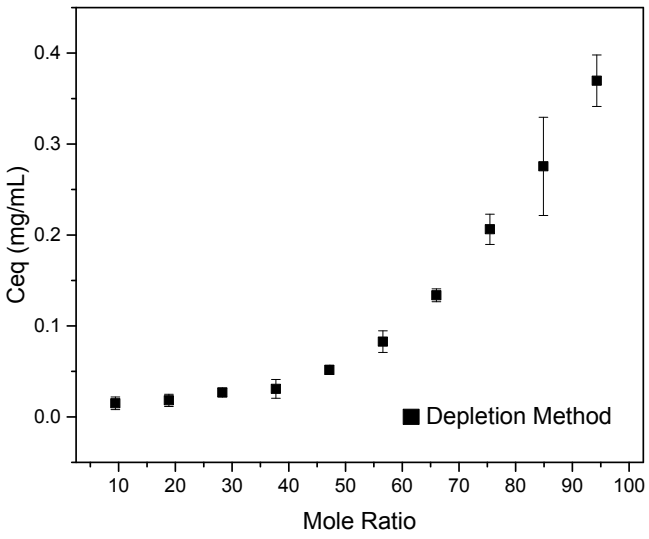

Figure ESI-1c. Molar ratio is the ratio of the total protein molarity to the theoretically calculated total molarity of silica.

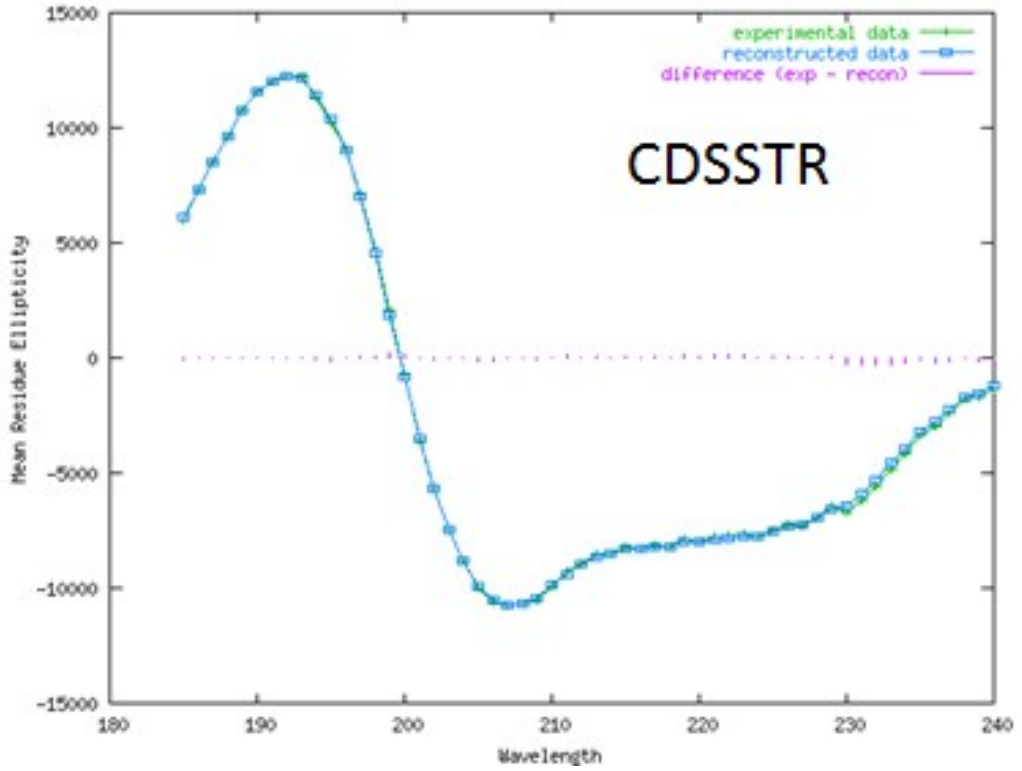

Figure ESI-2a. Comparison of different CD Deconvolution Algorithms: CDSSTR

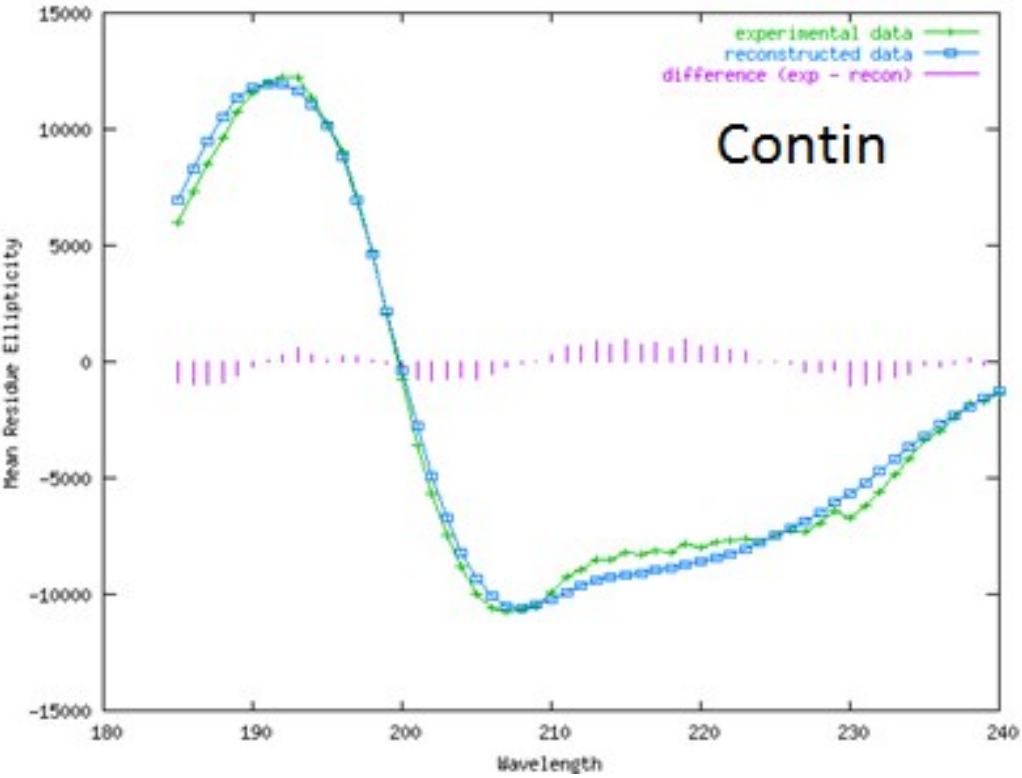

Figure ESI-2b. Comparison of different CD Deconvolution Algorithms: CONTIN

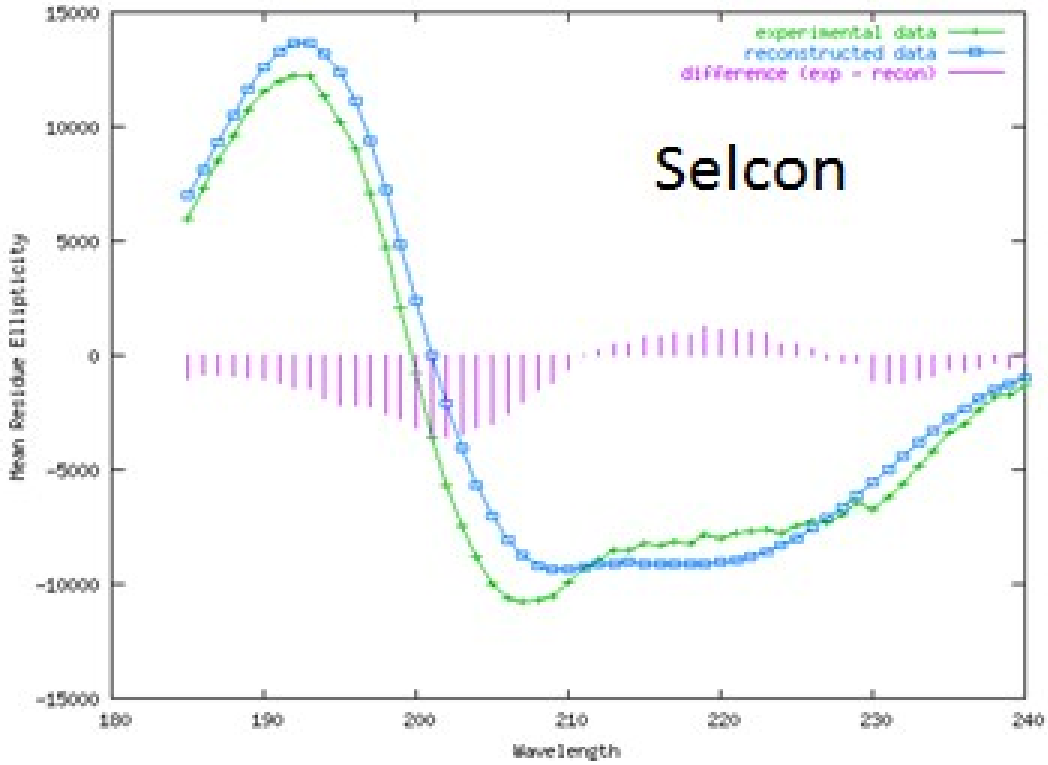

Figure ESI-2c. Comparison of different CD Deconvolution Algorithms: Selcon

Surface Coverage Calculations for Protein Adsorption

$RSi := 11$ nm $\rho si := 2.2$ g/cm^3

$Nav := 6.022 \cdot 10^{23}$ $MwLys := 14307$ g/mol

$SASi := 4\pi \cdot (RSi)^2 = 1.521 \times 10^3$ nm^2 surface area of one silica

$VSi := \pi \cdot \frac{4}{3} \cdot RSi^3 = 5.575 \times 10^3$ nm^3 volume of one silica

$mSi := VSi \cdot 2.2 \cdot 10^{-21} = 0$ g mass of one silica

$SApermass := \frac{SASi}{mSi} \cdot 10^{-18} = 123.967$ m^2/g

BET Surface area is 110-150 m^2/g since my silica is non-porous geometric surface area matches with BET quite well

$RLyslong := 2.25$ nm

$RLysshort := 1.5$ nm

Lysozyme is an ellipsoid with dimensions 4.5x3x3 nm

$CALyslong := \pi \cdot RLyslong^2 = 15.904$ nm^2 Long Cross Sectional area

$CALysshort := \pi \cdot RLysshort^2 = 7.069$ nm^2 Short Cross Sectional area

Assuming the Lysozyme does not change the conformation, Surface Area of Silica at a distance of protein radius is:

$SAforLysshort := 4 \cdot \pi \cdot (RSi + RLyslong)^2 = 2.206 \times 10^3$ nm^2

$SAforLyslong := 4 \cdot \pi \cdot (RSi + RLysshort)^2 = 1.963 \times 10^3$ nm^2

hexagonel close packing of sphres on a flat surface would yield a 0.9069 density

$HCPlong := \frac{SAforLyslong \cdot 0.9069}{CALyslong} = 111.963$ number of protein bindindg side on (long diameter)

$HCPshort := \frac{SAforLysshort \cdot 0.9069}{CALysshort} = 283.054$ number of protein bindindg side on (short diameter)

$$\text{Longmass} := \text{HCPlong} \cdot \frac{\text{MwLys}}{\text{Nav}} \cdot 1000 = 2.66 \times 10^{-15} \quad \text{mg per Silica}$$

$$\text{Longcoverage} := \frac{\text{Longmass}}{\text{SASi}} \cdot 10^{18} = 1.749 \quad \text{mg/m^2}$$

$$\text{Shortmass} := \text{HCPshort} \cdot \frac{\text{MwLys}}{\text{Nav}} \cdot 1000 = 6.725 \times 10^{-15} \quad \text{mg per Silica}$$

$$\text{Shortcoverage} := \frac{\text{Shortmass}}{\text{SASi}} \cdot 10^{18} = 4.423 \quad \text{mg/m^2}$$

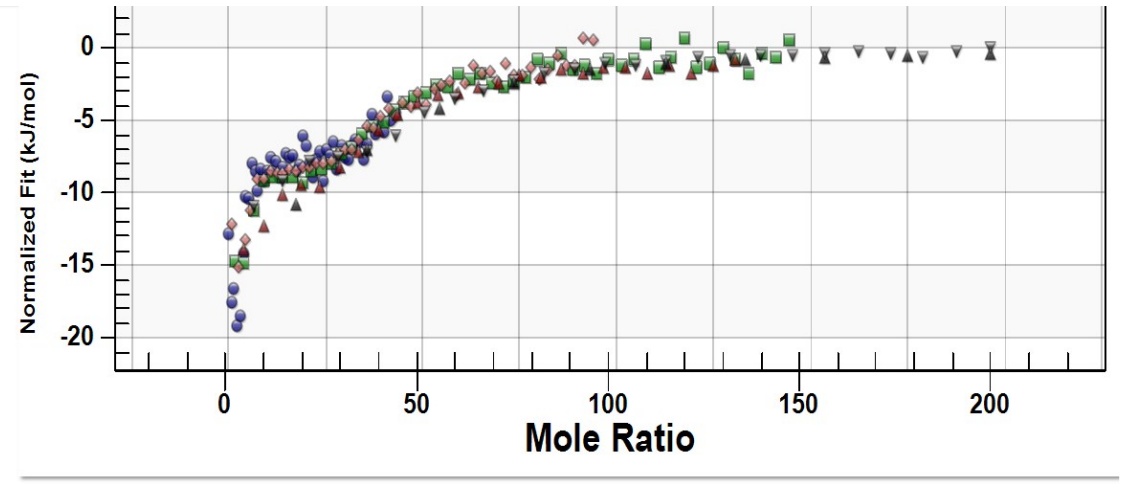

Figure ESI-3 ITC data master curve. Each color represents a different initial mole ratio of Lysozyme and Silica.