Electrostatic Interactions Influence Protein Adsorption – but not Desorption – at the Silica-Aqueous Interface

Supplementary Information

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Additional Materials and Methods

For circular dichroism (CD) measurements, lyophilized BSA (Aldrich CAS A2153) was reconstituted to a concentration of 10 uM in 10mM CP. CD spectra were measured using a Chirascan™-plus CD spectrometer (Applied Photophysics). The instrument and sample chamber were purged under a nitrogen stream for several hours before experiments were performed. Quartz cuvettes with a volume of ~170 μl and 1 mm path length were used to measure the CD signal from 190–260 nm with 0.5 nm steps, for 0.5 seconds per step at 20° C. Each sample was measured 10 times and the resulting CD spectra were averaged and smoothed between each two adjacent points. BSA CD spectra were then normalized by subtracting the CD spectra measured with 10mM CP at the respective pH values. Each experimental condition was measured three separate times.

Additional Results

Cumulative probabilities of surface residence times for fluorescently labeled BSA on FS are shown in Figure S1. Characteristic surface residence times were calculated by fitting the cumulative probabilities to a three population exponential mixture model as discussed in the text (best fit parameters are given in Table S1). The data presented in Table S1 are presented in the main text as Figure 2.

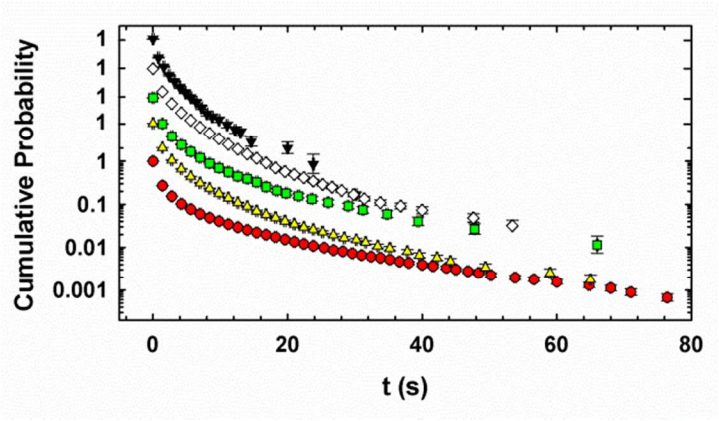

Figure 1: Cumulative residence time distributions of fluorescently labeled BSA on FS for 2.6 (red circles), 3.7 (yellow triangles), 4.7 (green squares), 5.7 (open diamonds), 7.4 (black triangles). The curves represent the mean between three replicate experiments. Error bars represent 65% confidence expected from Poisson statistics. For clarity, the graphs have been offset vertically.

Table 1: Population fractions and characteristic residence time fit from Figure 1 for each pH.

Population	1		2		3	
pH	a_1	τ_1(s)	a_2	τ_2(s)	a_3	τ_3(s)
2.6	0.53(8)	0.39(7)	0.35(4)	1.9(7)	0.10(3)	9(4)
3.7	0.5(1)	0.4(1)	0.36(8)	1.8(6)	0.10(3)	7(3)
4.7	0.62(2)	0.47(3)	0.33(1)	2.3(2)	0.05(1)	9.0(3)
5.7	0.46(5)	0.39(4)	0.39(3)	1.6(2)	0.15(2)	4.9(5)
7.4	0.64(8)	0.36(7)	0.40(7)	1.9(3)	0.06(4)	7(4)

Cumulative squared displacement distributions for fluorescently labeled BSA on FS are shown in Figure S2. Diffusion coefficients were calculated by fitting the cumulative probabilities to four population Gaussian mixture model discussed in the text are shown in Table S2. The data presented in Table S2 are presented in the main text as Figure 3.

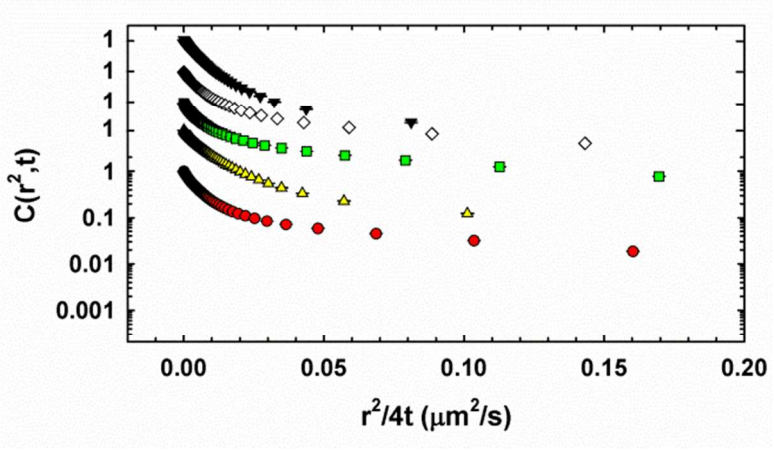

Figure 2: Cumulative squared displacement distributions of fluorescently labeled BSA on FS for 2.6 (red circles), 3.7 (yellow triangles), 4.7 (green squares), 5.7 (open diamonds), 7.4 (black triangles). The curves represent the mean between three replicate experiments. Error bars represent 65% confidence expected from Poisson statistics. For clarity, the graphs have been offset vertically.

Table 2: Mode fractions and diffusion coefficients fits from Figure 2 for each pH. Numbers in parentheses represent the uncertainty in the least significant digit.

Population	1		2		3		4	
pH	b_1	D_1 ($\mu m^2/s$)	b_2	D_2 ($\mu m^2/s$)	b_3	D_3 ($\mu m^2/s$)	b_4	D_4 ($\mu m^2/s$)
2.6	0.2(1)	0.0011(3)	0.61(1)	0.0058(5)	0.21(7)	0.015(3)	0.03(2)	0.38(9)
3.7	0.10(4)	0.0009(1)	0.49(3)	0.0051(6)	0.38(4)	0.014(3)	0.03(2)	0.11(2)
4.7	0.15(3)	0.00095(6)	0.60(4)	0.0052(3)	0.17(2)	0.025(5)	0.07(2)	0.17(1)
5.7	0.10(1)	0.00105(3)	0.63(2)	0.0052(3)	0.20(2)	0.020(1)	0.07(1)	0.149(6)
7.4	0.10(8)	0.0010(2)	0.64(3)	0.0054(2)	0.23(4)	0.0162(4)	0.04(1)	0.31(3)

Circular dichroism (CD) spectroscopy measurements were conducted using unlabeled BSA at pH 2.6, 4.7, and 7.4, shown in Figure S3. BSA structure appeared to remain mostly unchanged between pH 7.4 and 4.7 conditions, but partial loss of secondary structure was apparent at pH 2.6. This was indicated by the decrease in CD signal at 191 and 210 nm indicating a loss of α-helical structure, though not as dramatic as a fully denatured BSA indicated by the red line. As measured by the change in peak intensity at 191 nm, BSA retained 63±1% of its structure at low pH, assuming that BSA loses all of its α-helical structure at 75°C. Despite the partially unfolded state observed at pH 2.6, no dramatic changes were observed between 4.7 and 2.6 pH for adsorption rate, residence time, or diffusion, suggesting that BSA interfacial dynamics were largely unaffected by these changes in secondary structure.

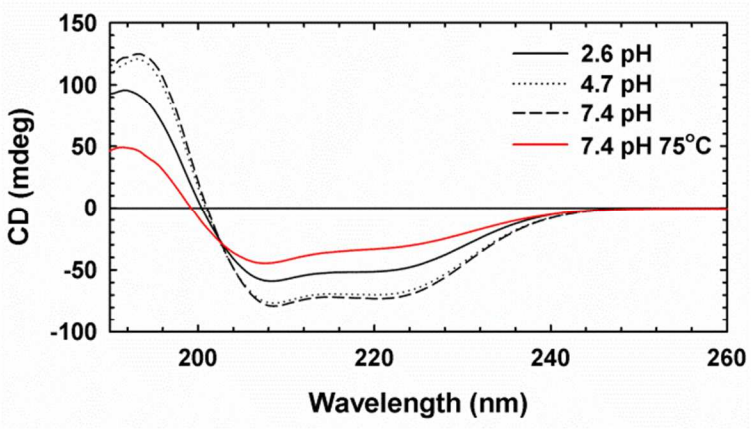

Figure 3: Representative CD spectra of BSA in 10 mM CP normalized to CD spectra of 10 mM CP at each respective pH. The black solid line represents BSA data captured at 2.6 pH, the dotted line at 4.7 pH, and the dashed line at 7.4 pH. The data in black are measurements captured at 20°C. The red solid line represents BSA data captured at 7.5 pH at 75°C.