

Supplementary Information

Measuring Biotherapeutic Viscosity and Degradation On-Chip with Particle Diffusometry

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Table S1. Relative BSA Viscosity Measurements (Low Concentrations). Raw data values for the relative viscosity of low concentrations of native and denatured BSA using PD (inset on Figure 3B).

Concentration (mg/ml)	0.003 ± 0.000	0.036 ± 0.004	0.068 ± 0.007	0.127 ± 0.003	0.324 ± 0.003	0.613 ± 0.016
Native	1.00 ± 0.04	1.16 ± 0.05	1.11 ± 0.02	1.09 ± 0.02	1.10 ± 0.02	1.14 ± 0.02
Denatured	1.00 ± 0.04	1.09 ± 0.05	1.17 ± 0.09	1.07 ± 0.06	1.17 ± 0.05	1.18 ± 0.02

Table S2. Relative BSA Viscosity Measurements (High Concentrations). Raw data values for the relative viscosity of high concentrations of native and denatured BSA using PD (Figure 3B).

Concentration (mg/ml)	1.184 ± 0.011	2.914 ± 0.035	5.622 ± 0.007	8.170 ± 0.019	10.451 ± 0.079
Native	1.09 ± 0.02	1.09 ± 0.02	1.14 ± 0.04	1.10 ± 0.02	1.16 ± 0.03
Denatured	1.23 ± 0.05	1.59 ± 0.08	3.48 ± 0.42	N/A	N/A

Fig. S1. Microrheology Measurements. The microrheology tool on the Zetasizer Nano S90 was used to measure BSA protein viscosity. The viscosity of BSA solutions are relative to the buffer solution. Similar to the PD results, the relative viscosity of denatured BSA solutions increases as the concentration of the protein increases and the viscosity of solutions containing native protein remains constant as a function of concentration. $n = 3$.

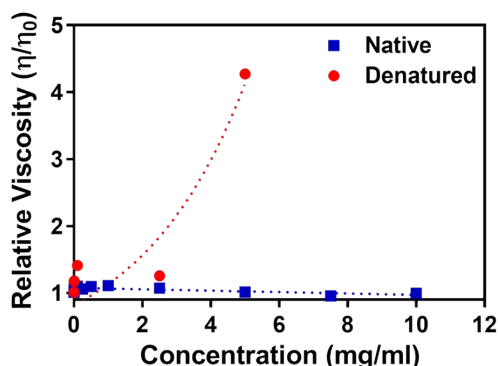


Fig. S2. Semi-Quantitative SDS-PAGE. SDS-PAGE gel analysis of non-specific adsorption of BSA on polystyrene particles. N represents native and D represents denatured. An integrated intensity comparison was performed on LI-COR software to compare the bands on the gel.

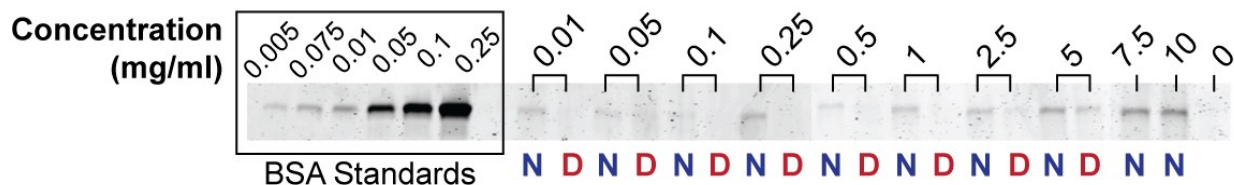


Table S3. Integrated Intensity Measurements. LI-COR Odyssey software is used to determine the integrated intensity and back calculate the amount of nonspecifically adsorbed BSA onto polystyrene surfaces post-washing. All calculations were based on a standard curve of integrated intensity bands on an SDS-PAGE of known BSA concentrations of 0.005, 0.0075, 0.01, 0.05, 0.1, and 0.25 mg/ml ($R^2 = 0.99$). All values are normalized by the integrated intensity signal measured from the background signal of the SDS-PAGE gel.

Concentration (mg/ml)	0.003 ± 0.000	0.036 ± 0.004	0.068 ± 0.007	0.127 ± 0.003	0.324 ± 0.003	0.613 ± 0.016
Native post-wash (mg/ml)	0.003 ± 0.001	0.012 ± 0.004	0.011 ± 0.010	0.010 ± 0.003	0.020 ± 0.016	0.014 ± 0.013
Denatured post wash (mg/ml)	0.003 ± 0.001	0.006 ± 0.001	0.008 ± 0.004	0.014 ± 0.008	0.024 ± 0.029	0.005 ± 0.004
Concentration (mg/ml)	1.184 ± 0.011	2.914 ± 0.035	5.622 ± 0.007	8.170 ± 0.019	10.451 ± 0.079	
Native post-wash (mg/ml)	0.012 ± 0.009	0.018 ± 0.014	0.024 ± 0.008	0.015 ± 0.008	0.011 ± 0.012	
Denatured post wash (mg/ml)	0.050 ± 0.083	0.142 ± 0.236	0.054 ± 0.070	N/A	N/A	

Fig. S3. SDS-PAGE of Proteins. SDS-PAGE gel verifying presence of insulin versus BSA in the solution.

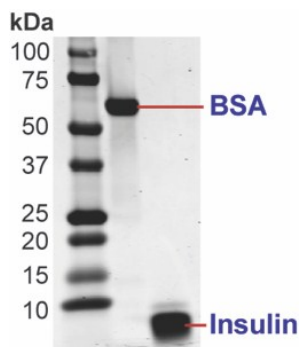


Fig. S4. Insulin Native PAGE. Native PAGE of native and denatured insulin solutions at a concentration of 0.25 mg/ml, where most denatured insulin remains at the gel's entrance (surrounded by a red box) and the native samples propagate into the gel (blue box). The first lane, reading left to right, contains only native insulin, the second lane contains only heat treated, denatured insulin, and the third, leftmost lane contains a mixture of 50% native and 50% degraded insulin.

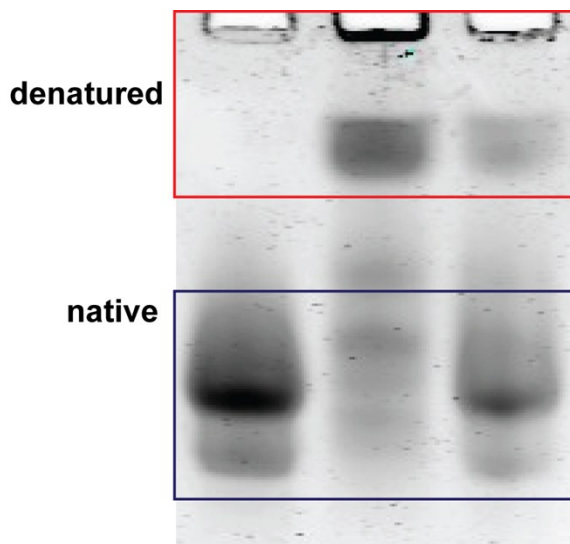


Table S4. Relative Viscosity of Insulin in PBS. Raw data values for the relative viscosity of native and denatured insulin suspended in 1X PBS pH 2.5 using PD (Figure 5A).

Concentration (mg/ml)	0.00 ± 0.00	0.61 ± 0.04	2.06 ± 0.07	3.1 ± 0.02	6.12 ± 0.08
Native	1.00 ± 0.03	1.01 ± 0.04	0.96 ± 0.03	1.01 ± 0.04	0.97 ± 0.02
Denatured	1.00 ± 0.01	1.03 ± 0.01	1.33 ± 0.06	1.48 ± 0.07	2.72 ± 0.06

Table S5. Relative Viscosity of Insulin in HEPES pH 2.5. Raw data values for the relative viscosity of native and denatured insulin suspended in 2.5 mM HEPES pH 2.5 using PD (Figure 5B).

Concentration (mg/ml)	0.00 ± 0.00	0.88 ± 0.06	2.08 ± 0.01	4.08 ± 0.11	5.80 ± 0.05	7.96 ± 0.03
Native	1.00 ± 0.03	0.99 ± 0.03	1.05 ± 0.04	0.97 ± 0.03	0.97 ± 0.02	1.09 ± 0.03
Denatured	1.00 ± 0.03	1.07 ± 0.05	1.00 ± 0.04	1.26 ± 0.08	1.95 ± 0.14	3.27 ± 0.26

Table S6. Relative Viscosity of Insulin in HEPES pH 8.2. Raw data values for the relative viscosity of native and denatured insulin suspended in 2.5 mM HEPES pH 8.2 using PD (Figure 5C).

Concentration (mg/ml)	0.02 ± 0.01	1.17 ± 0.04	2.74 ± 0.08	5.07 ± 0.01	7.48 ± 0.05	9.73 ± 0.04
Native	1.00 ± 0.03	1.01 ± 0.04	1.05 ± 0.04	1.01 ± 0.03	1.01 ± 0.02	1.09 ± 0.03
Denatured	1.00 ± 0.03	1.07 ± 0.05	1.00 ± 0.04	1.26 ± 0.08	1.95 ± 0.14	3.27 ± 0.26

Native	1.00 ± 0.02	1.02 ± 0.01	1.03 ± 0.02	1.01 ± 0.01	1.03 ± 0.02	1.05 ± 0.02
Denatured	1.00 ± 0.02	1.02 ± 0.02	1.01 ± 0.00	1.12 ± 0.01	1.21 ± 0.06	1.45 ± 0.03

Table S7. Relative Viscosity of Insulin Mixtures in PBS. Raw data values for the relative viscosity of native and denatured insulin combinations (denatured:native) suspended in 1X PBS pH 2.5 using PD (Figure 6B).

100:0	90:10	80:20	70:30	60:40	50:50	40:60	30:70	20:80	10:90	0:100
2.87	2.01	1.88	1.91	1.73	1.64	1.61	1.45	1.35	1.25	1.01
± 0.1	± 0.2	± 0.0	± 0.1	± 0.1	± 0.2	± 0.1	± 0.11	± 0.0	± 0.0	± 0.04
0	0	8	1	8	8	9		7	2	

Table S8. Relative Viscosity of Insulin Mixtures in HEPES. Raw data values for the relative viscosity of native and denatured (denatured:native) insulin combinations suspended in 2.5 mM HEPES pH 8.2 using PD (Figure 6C).

100:0	90:10	80:20	70:30	60:40	50:50	40:60	30:70	20:80	10:90	0:100
1.18	1.15	1.14	1.14	1.14	1.13	1.12	1.12	1.10	1.08	1.07
± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0		± 0.0	± 0.0	
5	2	4	4	3	2	4	± 0.04	3	2	± 0.01

Table S9. Relative Viscosity of IgG. Raw PD data values for the relative viscosity of IgG (Figure 7).

Concentration (mg/ml)	0	10	20	30	40	50
Native	1.00 ± 0.0	1.14 ± 0.0	1.20 ± 0.0	1.35 ± 0.0	1.52 ± 0.0	1.61 ± 0.0
	3	2	3	2	4	2