Supporting Information
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The limitations of an exclusively colloidal view of protein solution hydrodynamics and rheology

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Methods

Size-exclusion chromatography multi-angle light scattering (SEC-MALS): SEC-MALS was performed using a TSK gel G3000 SW 7.8 mm×30 cm, 5µm (TOSOH Bioscience) column connected to an Agilent 1100 series equipped with a Diode Array Detector (DAD, Agilent 1100 series) and a refractive index detector (RID, Agilent 1100 series). Agilent HPLC system is connected to a Multi Angle Light Scattering (MALS) detector (DAWN EOS, Wyatt Technology, Santa Barbara, CA) equipped with a laser source 690 nm and 18 detectors at angles between 22.5 ^{o} and 147 ^{o}. All detectors were normalized based on a 90 ^{o} angle using a 2 mg/mL BSA standard (Pierce standard) and calibrated with Toluene. DAD, RID and MALS signals were imported and processed in the ASTRA V software (Wyatt Technology, Santa Barbara, CA). Mass average molar masses of all species were determined using the Zimm equation:

$$Kc/R_\theta = 1/M_w\,(1 + q^2R_g^2/3) + 2B_{22}c + \ldots..$$

$$K = 4\pi^2 n_0^2\,(dn/dc)^2 / N_A\lambda^4$$

n_o: refractive index,
dn/dc: refractive index increment (0.185 mL/g),
c: *BSA* concentration ($B_{22}c$ term is negligible as the samples are dilute),
B_{22}: second virial coefficient,
N_A: Avogadro's number,
λ: wavelength of the incident light,
R_g: radius of gyration,
q: scattering wave-vector.

Figures and Tables

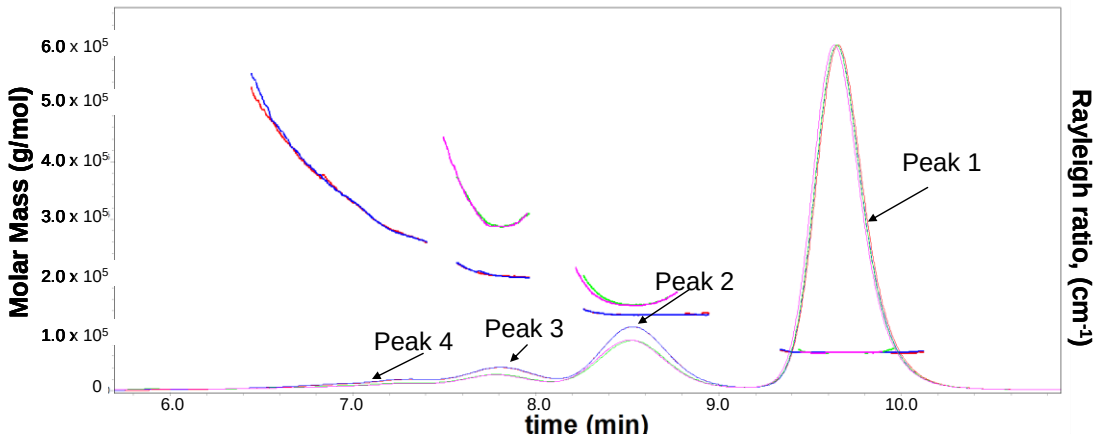


FIGURE S1: Size-exclusion chromatography multi-angle light scattering (SEC MALS) measurements for BSA at 10 mg/mL and pH = 4.0 (red), 5.0 (blue), 6.0 (light blue) and 7.4 (orange). The elution peak at 9.5 minutes from the SEC chromatograms in Fig. 1 in the text as the corresponding molar mass M_w = 67 kDa approximately equals BSA monomer M_w [1]. Molar masses corresponding to peaks 1 through 4 are shown in Table S1 below.

Table S1: Molar Masses of BSA fractions obtained from SEC MALS

pH	**Peak 1** (kDa)	**Peak 2** (kDa)	**Peak 3** (kDa)	**Peak 4** (kDa)
4.0	67	134	204	328 (528-250)
5.0	67	133	204	331 (510-250)
6.0	67	162	305 (380-280)	-
7.4	67	157	316 (420-300)	-

TABLE S1: Molar masses corresponding to eluted peaks 1 through 4 in Fig. S1.

Table S2: Size-exclusion chromatography data for Bovine Serum Albumin

pH	Monomer (%)	Clusters (%)
3.0	70	30
4.0	76	24
5.0	70	30
6.0	75	25
7.4	75	25

TABLE S2: SEC data summary for Fig. 2 in the text: percentage of monomer and higher-order soluble clusters in a 10 mg/mL BSA solution from single injections (250 μg)

Table S3: Prior studies of Bovine Serum Albumin solution rheology

Authors	**Concentration/Volume Fraction Range**	**pH range**
Present work	2 mg/mL - 400 mg/mL	3.0-7.4
Sharma A. Jaishankar, Y.C. Wang, G.H. McKinley (2)	10 mg/mL - 250 mg/mL	7.4
Brownsey, G.J., T.R. Noel, R. Parker, S.G. Ring (3)	200 mg/mL - 500 mg/mL	5.4
Tanford, C. and J. G. Buzzell. (4)	40 mg/mL	4.3-10.5
Tanford, C, J.G. Buzzell, D.G. Rands, and S.A. Swanson (5)	1-15 mg/mL	2.0-10.5
Saluja, A. and D.S. Kalonia (6)	10 mg/mL - 200 mg/mL	2.0-9.0
Gaigalas, A.K., V. Reipa, J.B. Hubbard, J. Edwards, and J. Douglas (7)	Volume Fraction: 0-0.2	4.0-6.0
Monkos, K. (8)	17.6 mg/mL - 363.4 mg/mL	5.2
Sun, S.F. (9)	~1 mg/mL	2.0-11.7
Lee, J. and Tripathi, A. (10)	20 mg/mL - 80 mg/mL	8.0
Heinen, M., F. Zanini, F. Roosen-Runge, D. Fedunova, and F. Zhang (11)	Up to 500 mg/mL	7.0 (unbuffered)

Giordano, R., G. Maisano, F. Mallamace, N. Micali, and F. Wanderlingh (12)	0.1-1 mg/mL	5.2-11.8
Jachimska, B., M. Wasilewska, and Z. Adamczyk. (13)	Volume Fraction: 0.001-0.008	3.0-9.5
Curvale, R. (14)	1.25-20 mg/mL	2.4-10.0
Yadav, S., S.J. Shire, and D.S. Kalonia (15)	25-300 mg/mL	4-7
Oates, K.M.N., W.E. Krause, R.L. Jones, and R.H. Colby (16)	11-44 mg/mL	5-7.4
Bloomfield, V. (17)	1.6 mg/mL	3.6
Bowen, W.R. and P.M. Williams (18)	0-20 mg/mL	6.0, 8.0, 10.0
Chikazumi, N. and Ohta, T. (19)	1 mg/mL	2.0
Ikeda, S. and Nishinari, K. (20)	10 mg/mL	7.0

TABLE S3: Comparison of experimental design space of this work and previous BSA solution rheology investigations. The volume fraction range is shown instead of protein concentration for some references, as the authors did not define protein volume fraction (7, 13), except for ref. (11).

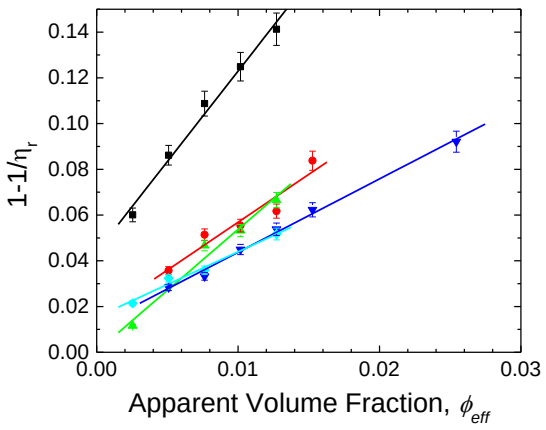


FIGURE S2: Intrinsic viscosity determination for BSA solutions at pH = 3.0 (squares), 4.0 (circles), 5.0 (triangles), 6.0 (inverted triangles), and 7.4 (diamonds) using volume fraction defined in Eq. 3 in text. The uncertainty in viscosity (one standard deviation) was determined from five different measurements with fresh sample loadings. In some cases, the uncertainties are smaller than the symbol sizes.

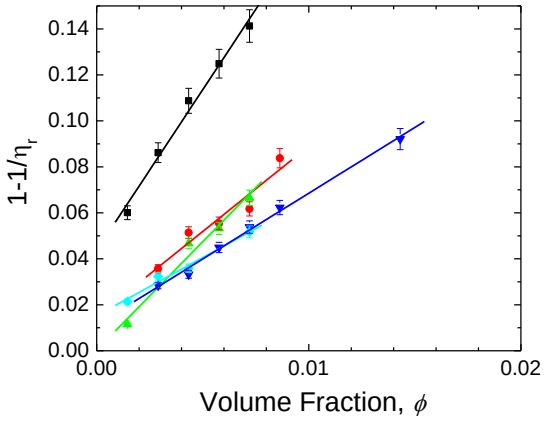


FIGURE S3: Intrinsic viscosity determination for BSA solutions at pH = 3.0 (squares), 4.0 (circles), 5.0 (triangles), 6.0 (inverted triangles), and 7.4 (diamonds) using volume fraction defined in Eq. (4) in text, which accounts for protein surface hydration. The same uncertainty in viscosity (standard deviation) applies here as in Fig. S2. In some cases, the uncertainties are smaller than the symbol sizes.

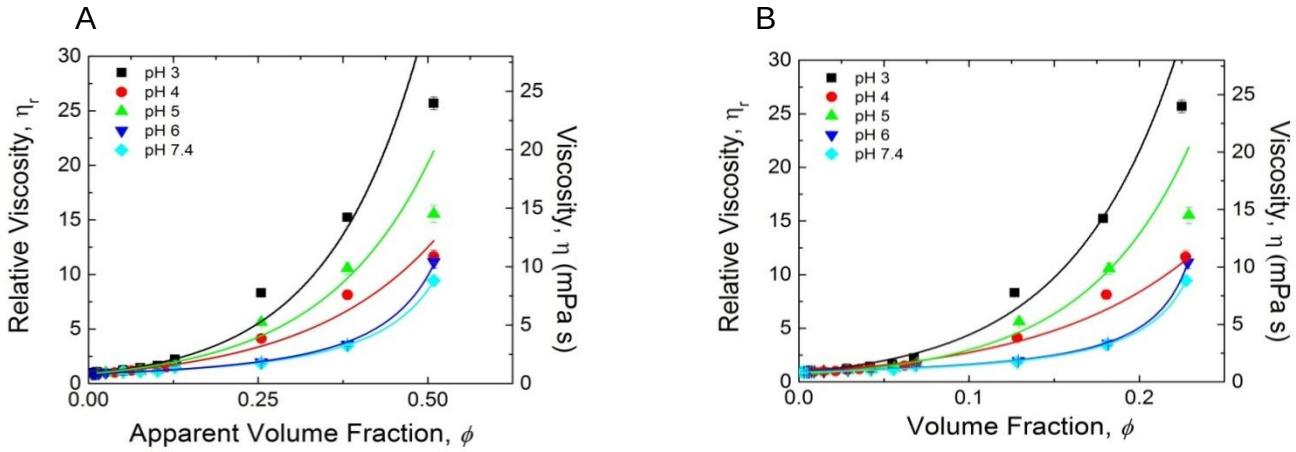
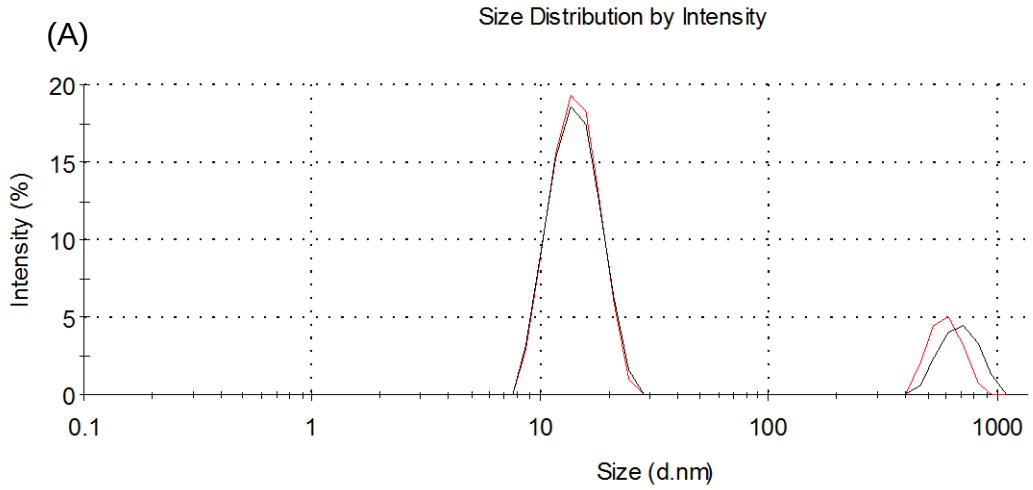
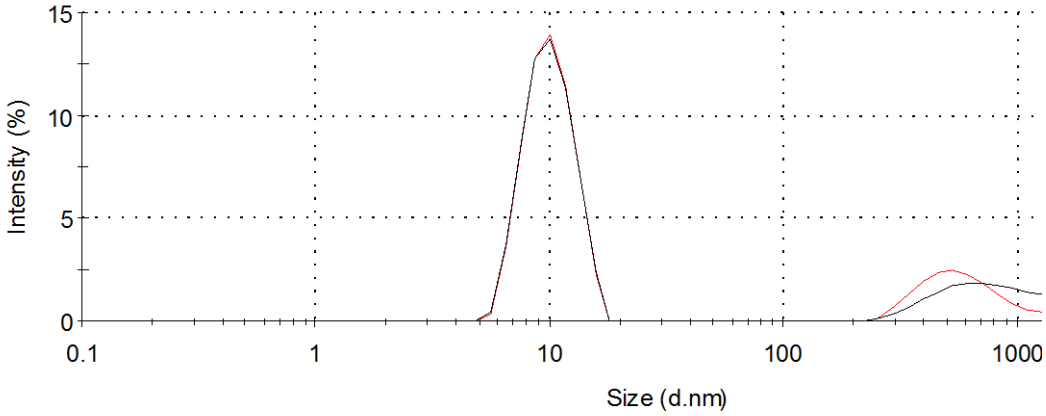


FIGURE S4: (A) Relative viscosity η_R vs. volume fraction, ϕ (calculated using Eq. 3) for BSA concentration, c_{BSA}, 2 mg/mL $\leq$ c_{BSA} $\leq$ 12 mg/mL at pH = 3.0 (squares), 4.0 (circles), 5.0 (triangles), 6.0 (inverted triangles) and 7.4 (diamonds). (B) Same data and symbol keys as in Fig. S4(A). Curves are non-linear regression fits to the Krieger-Dougherty model (Eq. 5) with *all* parameters freely floating with ϕ defined by Eq. 3 in (A) and Eq. 4 in (B), respectively.

(A)


Size Distribution by Intensity
Intensity (%)
20
15
10
5
0
0.1
1
10
100
1000
Size (d.nm)

(B)


Intensity (%)
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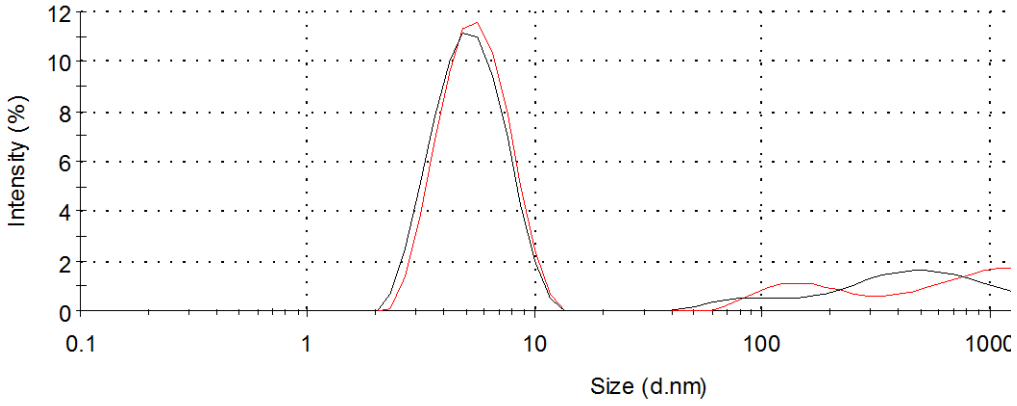
10
5
0
0.1
1
10
100
1000
Size (d.nm)

(C)


Intensity (%)
12
10
8
6
4
2
0
0.1
1
10
100
1000
Size (d.nm)

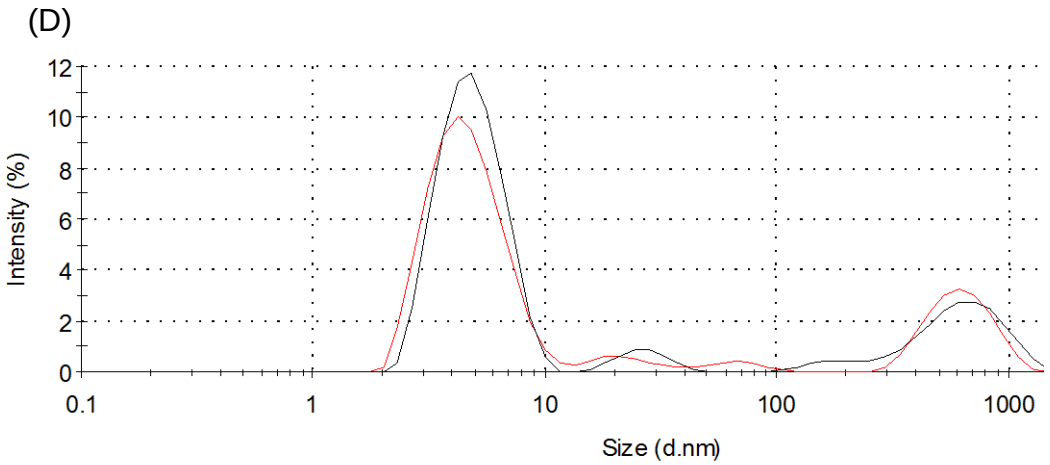


FIGURE S5: (A) Dynamic Light Scattering (DLS) data measured on a Zetasizer Nano for freshly reconstituted BSA solutions at two different time points (day 1: black, day 2: red) at 500 mg/mL and (A) pH 3, (B) pH 4, (C) pH 5, and (D) pH 7.4. Data are unreliable beyond 1 μm.

Supporting References

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