Supporting Information

Enhanced Sample Handling for Analytical Ultracentrifugation With 3D-Printed Centerpieces

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Supporting Materials and Methods

Analytical Ultracentrifugation

SV experiments were carried out in a ProteomeLab XL-A/I (Beckman Coulter, Indianapolis IN) following standard techniques[15] unless mentioned otherwise. Samples were filled using a pipettor with long gel loading tips, to achieve ≈12 mm solution column height. For example, 400 µL samples were loaded into standard 12 mm pathlength double-sector centerpieces. The rotor was temperature equilibrated at a nominal 20 °C while resting in the rotor chamber prior to start of centrifugation. After acceleration to 50,000 rpm, data acquisition was commenced using the Rayleigh interference optical system and/or the absorbance optical system.

Materials

Bovine serum albumin (BSA) (catalog # A7030; Sigma Aldrich, St. Louis, MO) samples were dissolved in 10 mM sodium phosphate, pH 7.4, 150 mM NaCl; hen egg lysozyme (catalog # L6876; Sigma Aldrich, St. Louis, MO) was dialyzed against 10 mM sodium acetate pH 4.6, 100 mM NaCl. BSA monomer was prepared by gel filtration (Superdex 200 16/60; GE Healthcare Bio-Sciences, Uppsala, Sweden) followed by concentration in an Amicon Ultra-4 Centrifugal Filter Unit with 10 kDa cutoff (Millipore Sigma, Burlington, MA).

Supporting Table 1

Summary of sedimentation coefficients and signal amplitudes of BSA monomer peak in replicate series

Values are reported as mean and standard deviation for of 3D Printed Centerpiece (3D1-3D3) and conventional Epon centerpieces (EC1 – ED3) in interference optics (top) and absorbance optics (bottom). For comparison, the data plotted in **Figure 2** are 3D2 and EC2.

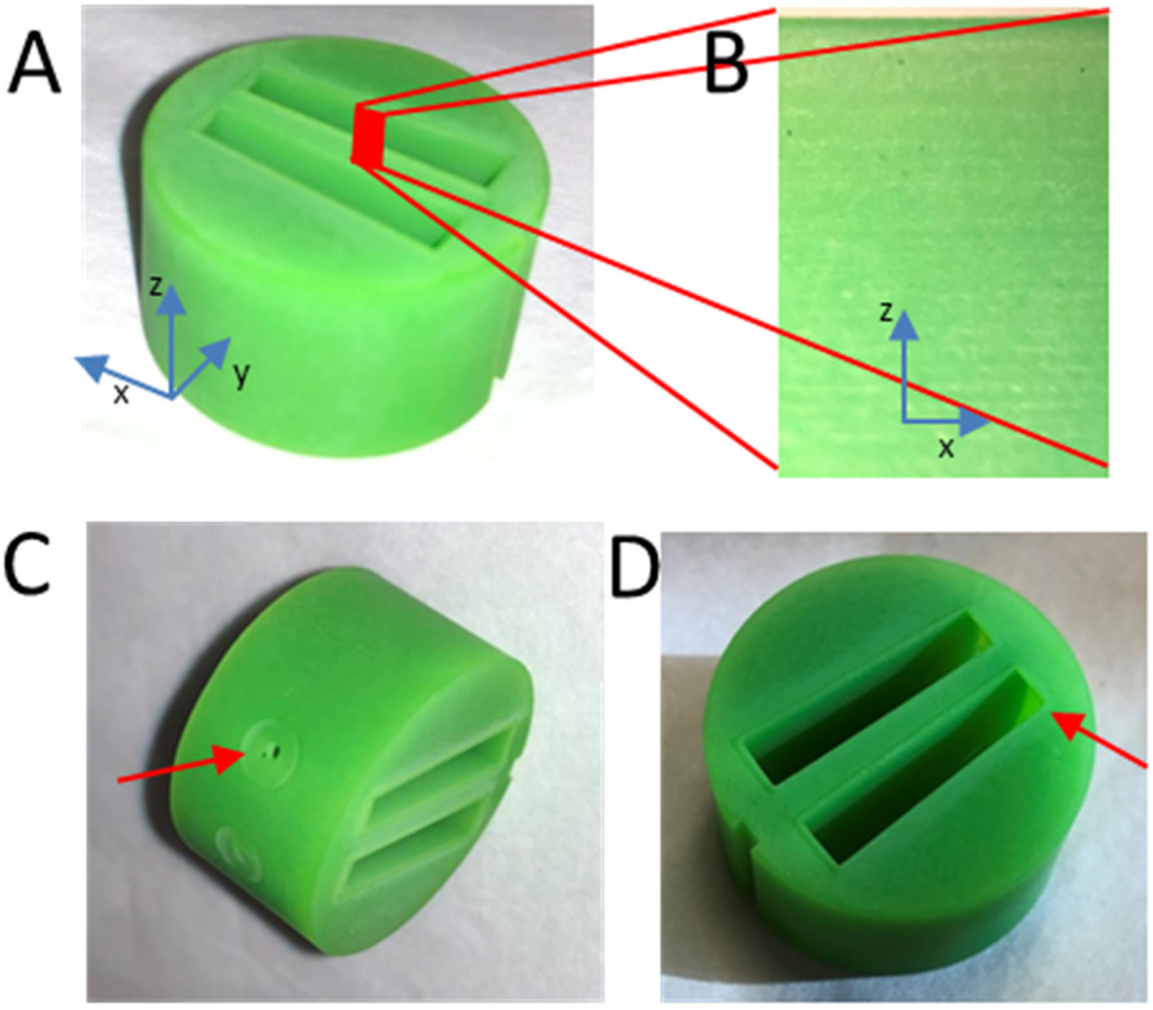
	IF					
	s-value (S)			signal of monomer peak (%)		
	mean	std	std/mean	mean	std	std/mean
3D1	4.301	0.024	0.6%	69.1%	0.7%	1.0%
3D2	4.313	0.022	0.5%	70.2%	1.0%	1.4%
3D3	4.299	0.024	0.6%	68.8%	1.4%	2.1%
EC1	4.309	0.019	0.4%	69.8%	0.9%	1.2%
EC2	4.312	0.014	0.3%	70.5%	0.9%	1.2%
EC3	4.310	0.020	0.5%	69.8%	0.8%	1.2%

	ABS					
	s-value (S)			signal of monomer peak (%)		
	mean	std	std/mean	mean	std	std/mean
3D1	4.334	0.019	0.4%	72.3%	1.1%	1.6%
3D2	4.328	0.016	0.4%	72.4%	0.8%	1.1%
3D3	4.329	0.016	0.4%	71.8%	0.8%	1.1%
EC1	4.341	0.020	0.5%	71.8%	0.7%	1.0%
EC2	4.345	0.014	0.3%	72.3%	0.9%	1.3%
EC3	4.340	0.020	0.4%	72.0%	0.8%	1.1%

Supporting Figures

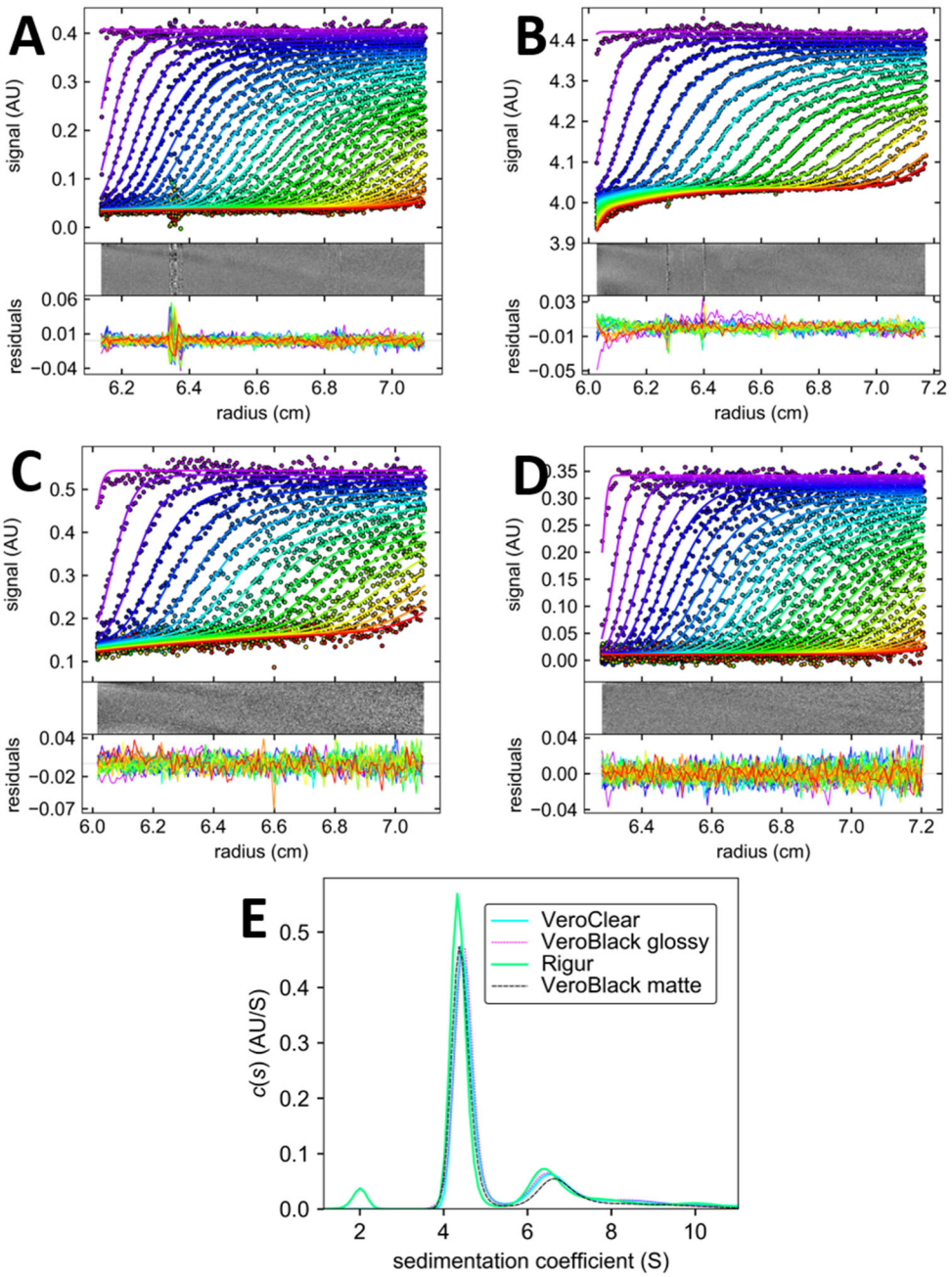
Supporting Figure S1: Pictures of 3D Printed Centerpiece by Stereolithography in Epoxy-Based Photopolymer (Microfine Green)

Photographs of 3D printed centerpiece fabricated by stereolithography in Microfine Green in the design of Figure 1. (A) Side view, with axis indicating the z-direction of printing, and the x-y plane normal to z. The red patch highlights an area that is shown in (B) after cutting the centerpiece open to observe the inner surface of the sector in a microscope. Periodic lines are visible in the z-direction corresponding to the different print layers. (C) View of the filling and venting holes. (D) View with side illumination from which the curved dome top can be discerned.



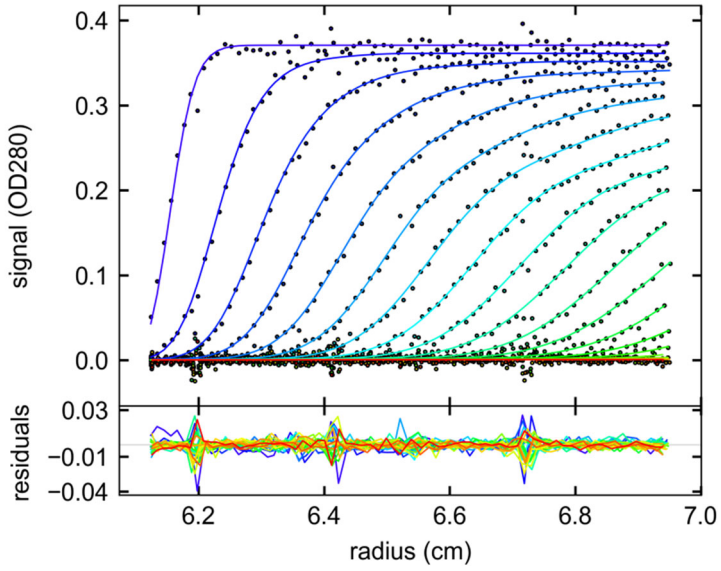
Supporting Figure S2: Sedimentation velocity data of 3D printed centerpieces in different materials

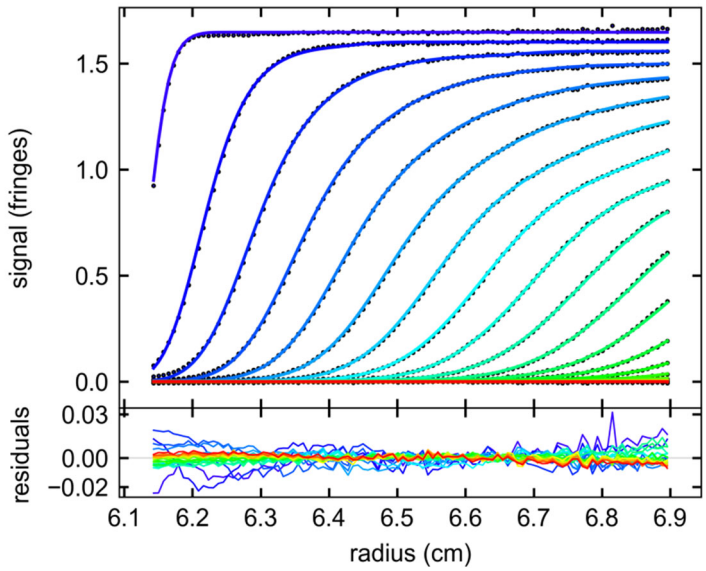
Comparison of SV data and uncorrected c(s) distribution analysis of a BSA sample sedimenting at 50,000 rpm, recorded at 280 nm, using 3D printed long-column centerpieces in acrylic VeroClear (A), glossy VeroBlackPlus (B), polypropylene-like Rigur (C), and acrylic VeroBlack matte (D).



Supporting Figure S3: Sedimentation velocity data of 3D printed centerpiece in run #11 of replicate series

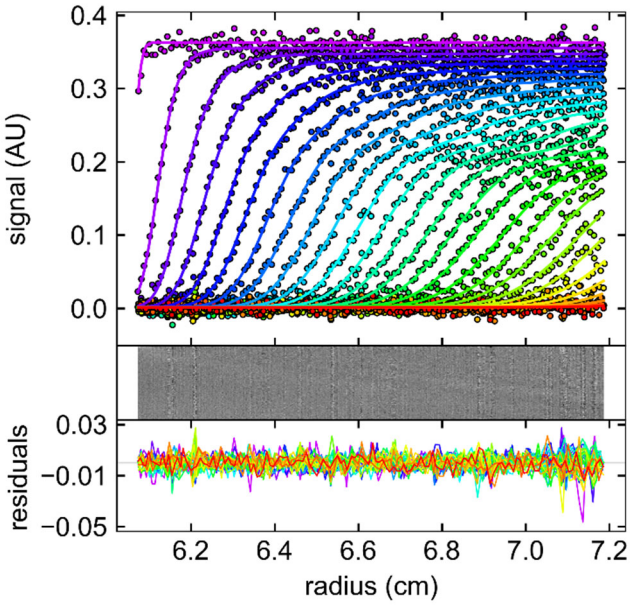
Shown are data from 3D printed centerpiece #2, in round 11 of the replicate series. Data show sedimentation of 0.5 mg/ml BSA at 50,000 rpm as acquired by absorbance (top) and Rayleigh interference optical systems (data points, showing every 2nd scan with every 5th data point of absorbance and 10th data point of interference data). Solid lines are the best-fit distributions from the standard *c*(*s*) analysis.

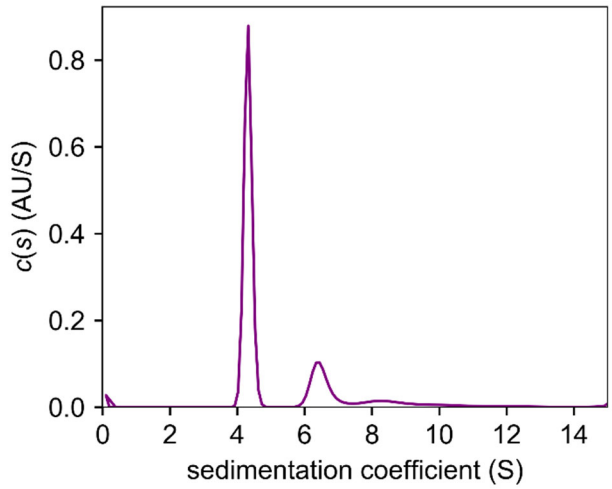




Supporting Figure S4: Mechanical stability of the long-column 3D printed centerpiece at 60,000 rpm

A 3D printed long-column centerpiece was filled with a sample of BSA in PBS, inserted in a 4-hole rotor and sedimented at 60,000 rpm. **Top:** Absorbance optical SV data recorded at 280 nm (data points) and best-fit *c*(*s*) distribution (lines). Appended in the lower panels are the residuals bitmap and overlay. **Bottom:** Uncorrected *c*(*s*) distribution from the analysis of the absorbance data.

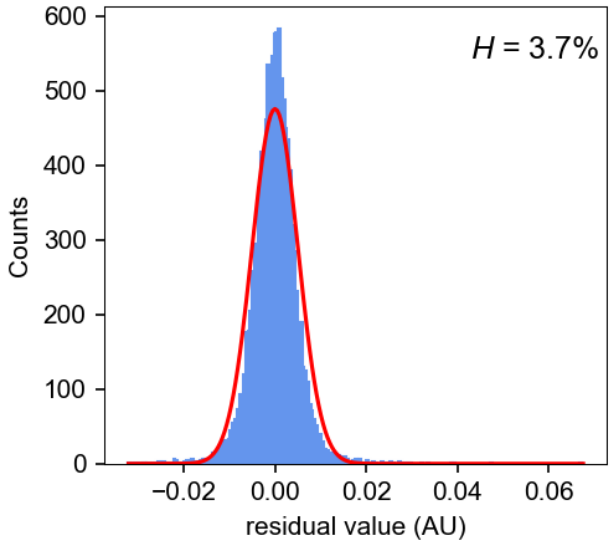




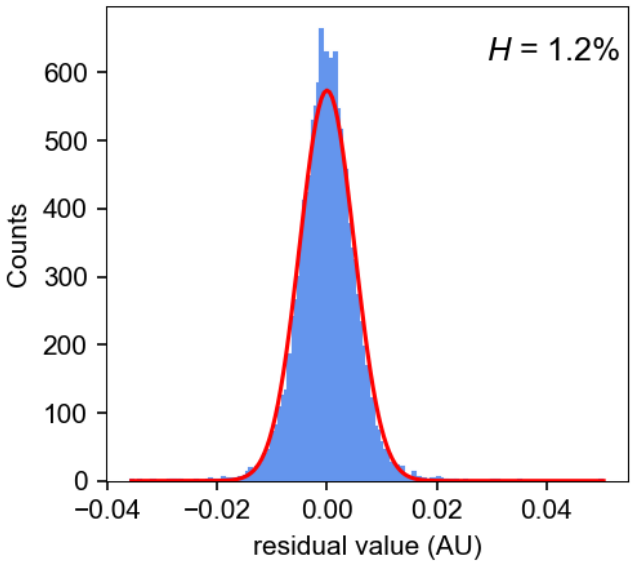
Supporting Figure S5: Residuals histograms of long-column 3D printed *vs* conventional centerpiece.

Residuals are from the single-species lysozyme fit shown in **Figure 3**, binned (blue) and fitted with a Gaussian (red) in GUSSI. The deviation from the bin heights and the Gaussian provides a measure for normalcy of the residuals, as quantified in the H-value introduced previously (Ma, J.; Zhao, H.; Schuck, P. A Histogram Approach to the Quality of Fit in Sedimentation Velocity Analyses. *Anal. Biochem.* **2015**, *483* (1), 1–3)

Conventional Epon centerpiece:

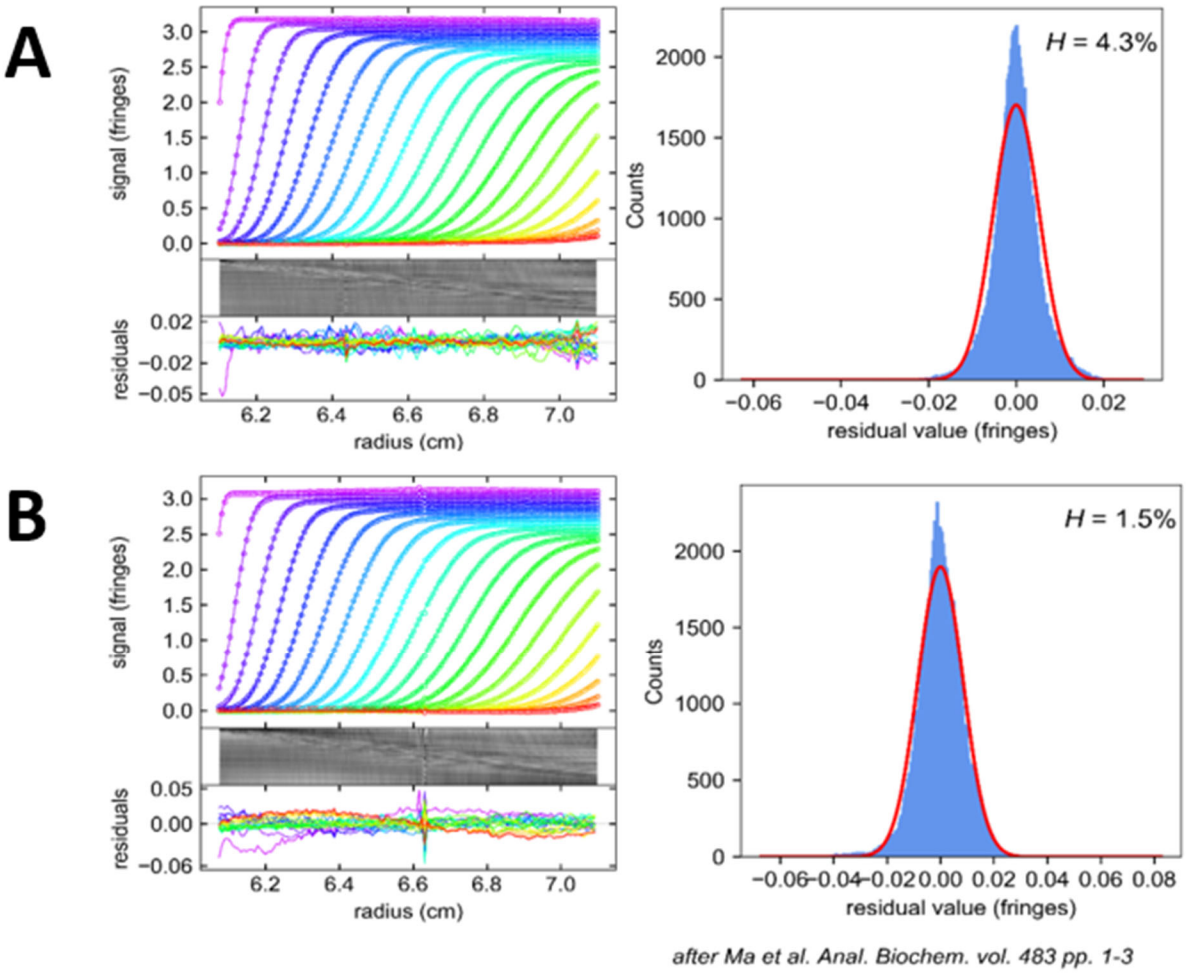


Long-column 3D printed centerpiece:

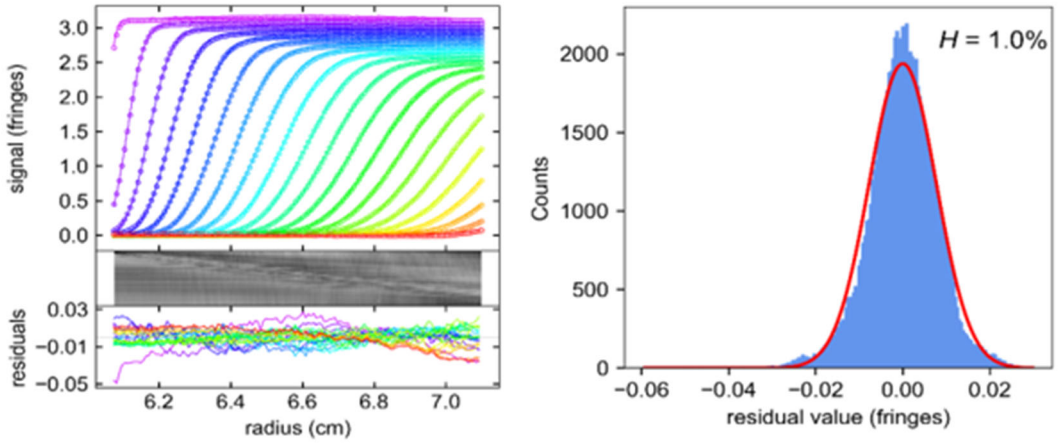


Supporting Figure S6: Comparison of BSA monomer SV data from narrow-sector and conventional centerpieces

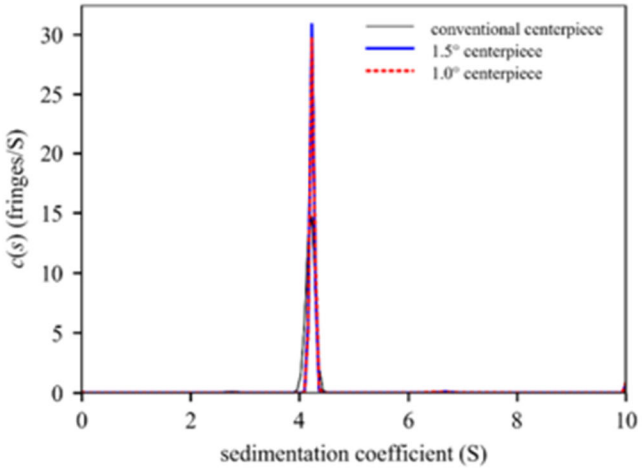
Fractionated BSA monomer was sedimented in conventional Epon centerpiece (A), and 3D printed narrow-sector centerpieces with 1.5° sector angles (B) and with 1.0° sector angles (C). Left panels show the SV data and fit with *c*(*s*) model, and right panels show the residuals histograms. (D) *c*(*s*) distributions of all centerpieces.



C


signal (fringes)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
residuals
0.03
−0.01
−0.05
6.2
6.4
6.6
6.8
7.0
radius (cm)
Counts
2000
1500
1000
500
0
H = 1.0%
−0.06
−0.04
−0.02
0.00
0.02
residual value (fringes)

D


conventional centerpiece
1.5° centerpiece
1.0° centerpiece
c(s) (fringes/S)
30
25
20
15
10
5
0
0
2
4
6
8
10
sedimentation coefficient (S)