Variable Field Analytical Ultracentrifugation: II. Gravitational Sweep Sedimentation Velocity

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Supplementary Material

Table S1: Rotor speed schedules in Figure 1. Listed are the times after start of centrifugation at which a rotor speed change was initiated to the new target speed indicated. The time at which the new target was achieved is dependent on the rotor speed difference, and was based on an acceleration rate of 280 rpm/sec. In this form, the table adheres to the conventions in the speedsteps.txt file in SEDFIT.

time (sec)	**power-law (rpm)**	**constant field (rpm)**	**linear (rpm)**	**ad hoc (rpm)**
	red	blue	orange	green
0	3,000	18,970	3,000	5,000
2,700	4,185	26,833	3,923	10,000
5,400	5,838	32,863	4,846	20,000
8,100	8,143	37,947	5,769	28,000
10,800	11,359	42,426	6,692	35,000
13,500	15,846	46,476	7,615	40,000
16,200	22,104	50,200	8,538	45,000
18,900	30,834	53,666	9,462	49,000
21,600	43,012	56,921	10,385	53,000
24,300	60,000	60,000	11,308	57,000
27,000			12,231	60,000
29,700			13,154	
32,400			14,077	
35,100			15,000	

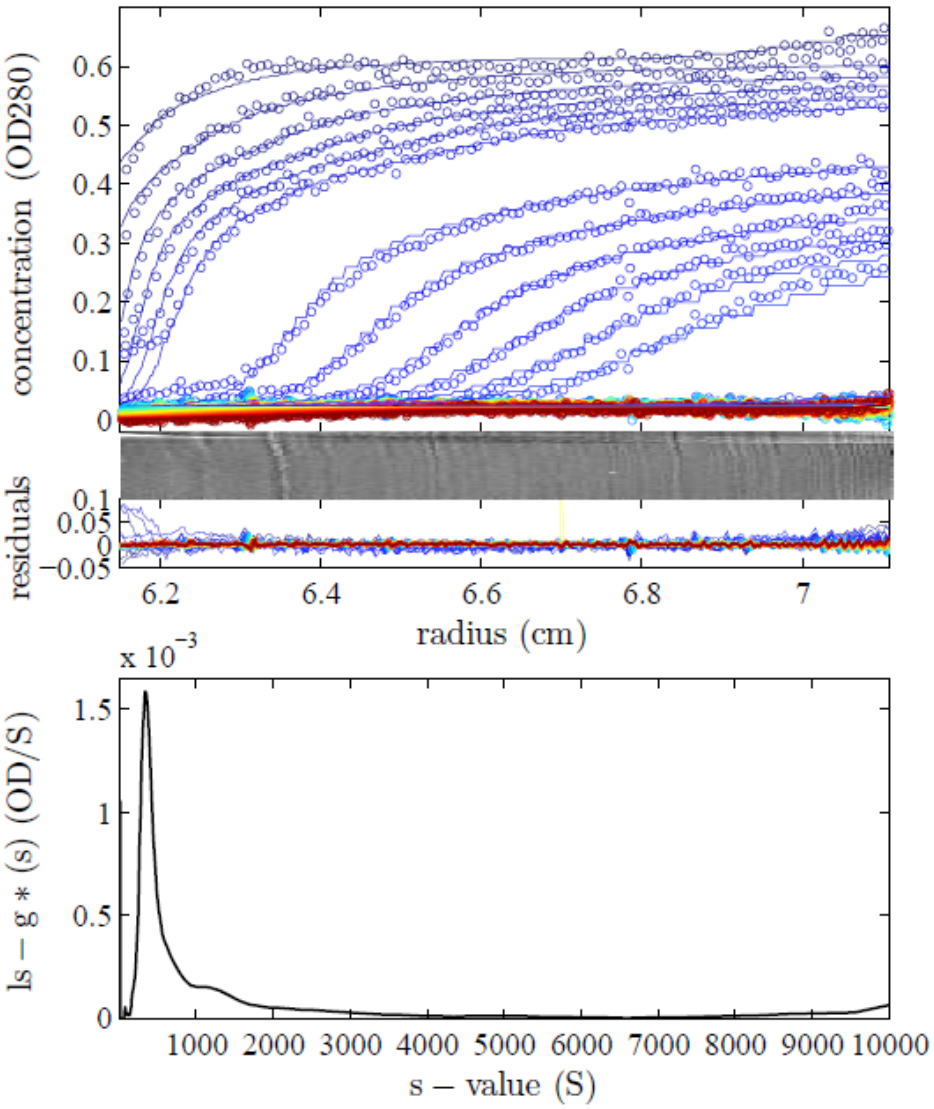

Figure S1: Sedimentation coefficient distribution ls-g*(s) of a preparation of DNA coated Au nanoparticles diluted in TRIS buffered saline. Top: Absorbance data at 280 nm (circles, only every 3rd data point shown) were acquired in a gravitational sweep experiment using the same 'extended constant field increase' rotor speed schedule shown in green in Figure 1, identical to the centrifugal field sequence used in the protein mixture experiments shown in Figure 3. The analysis of the data was carried out with a ls-g*(s) model for a broad distribution of non-diffusing particles, superimposed with a single diffusing species with apparent s-value of 1.02 S and 12 kDa (based on a partial specific volume scale of 0.73 ml/g in normal solvent conditions at 20 °C). The best-fit model is shown as solid lines. Middle: residuals in overlay and bitmap format, leading to an rmsd of 0.0066 OD.

const. speed 50 krpm (black)

const. field increase (blue)

ad hoc extended const. field increase (green)

power-law (red)

linear increase 3 – 18 krpm (orange)

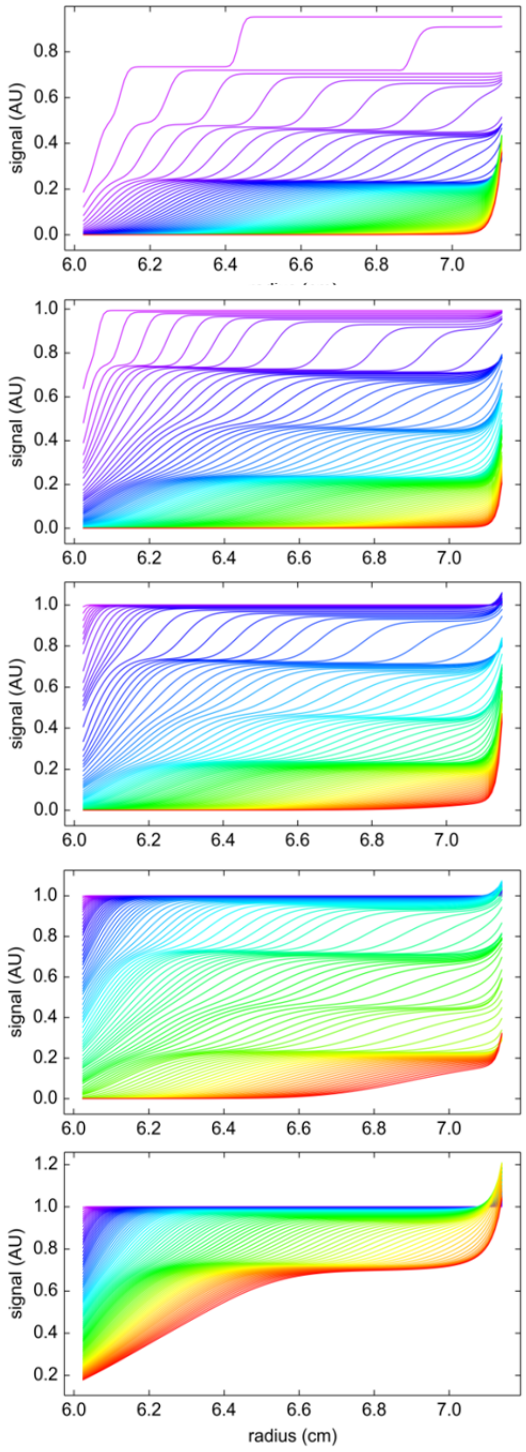

Figure S2: Simulated sedimentation profiles for an equal mixture of small to large protein species similar to Fig. 3 (2.5 S, 25 kDa; 10 S, 200 kDa; 20 S, 550 kDa; 70 S, 3.5 Mda) at different rotor speed profiles from Fig. 1. 100 scans are simulated in 10 min intervals, and plotted in the same color temperature indicating time.

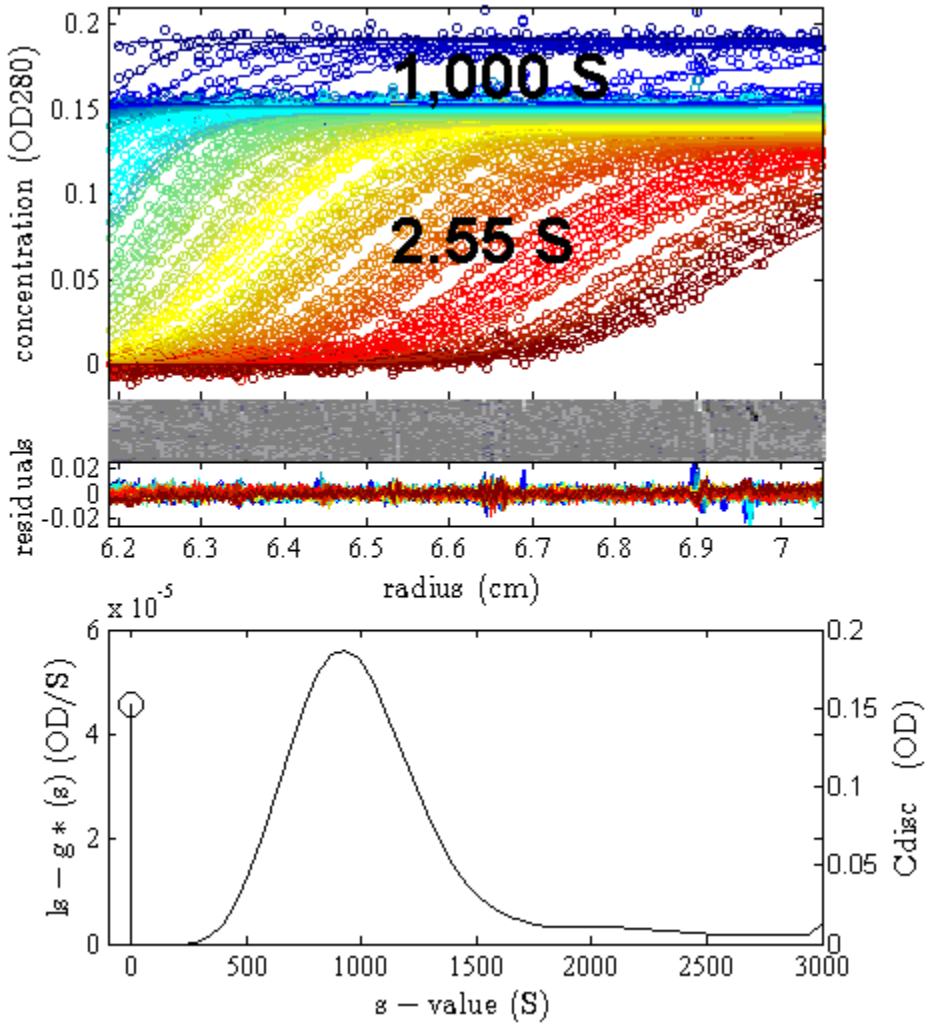

Figure S3: Sedimentation coefficient distribution ls-g*(s) of an aged preparation of streptavidin-coated 15 nm Au nanoparticles diluted in TRIS buffered saline. Top: Absorbance data at 280 nm (circles, only every 3rd data point shown) were acquired in a gravitational sweep experiment using the same 'extended constant field increase' rotor speed schedule shown in green in Figure 1, identical to the centrifugal field sequence used in the protein mixture experiments shown in Figure 3. The analysis of the data was carried out with a ls-g*(s) model for a broad distribution of non-diffusing particles, combined with an extra single diffusing species with best-fit apparent s-value of 2.6 S and 40 kDa (based on a partial specific volume scale of 0.73 ml/g in normal solvent conditions at 20 °C). The best-fit model is shown as solid lines. Middle: residuals in overlay and bitmap format, leading to an rmsd of 0.0034 OD. Bottom: ls-g*(s) distribution (solid line, left axis) with extra discrete species (stem, right axis).