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Supplemental Information

Solution Structure of *C. elegans* UNC-6: A Nematode Paralogue of the Axon Guidance Protein Netrin-1

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Solution structure and hydrodynamics of *C. elegans* UNC-6: a nematode paralogue of the vertebrate key axon guidance protein Netrin-1

Supporting Material

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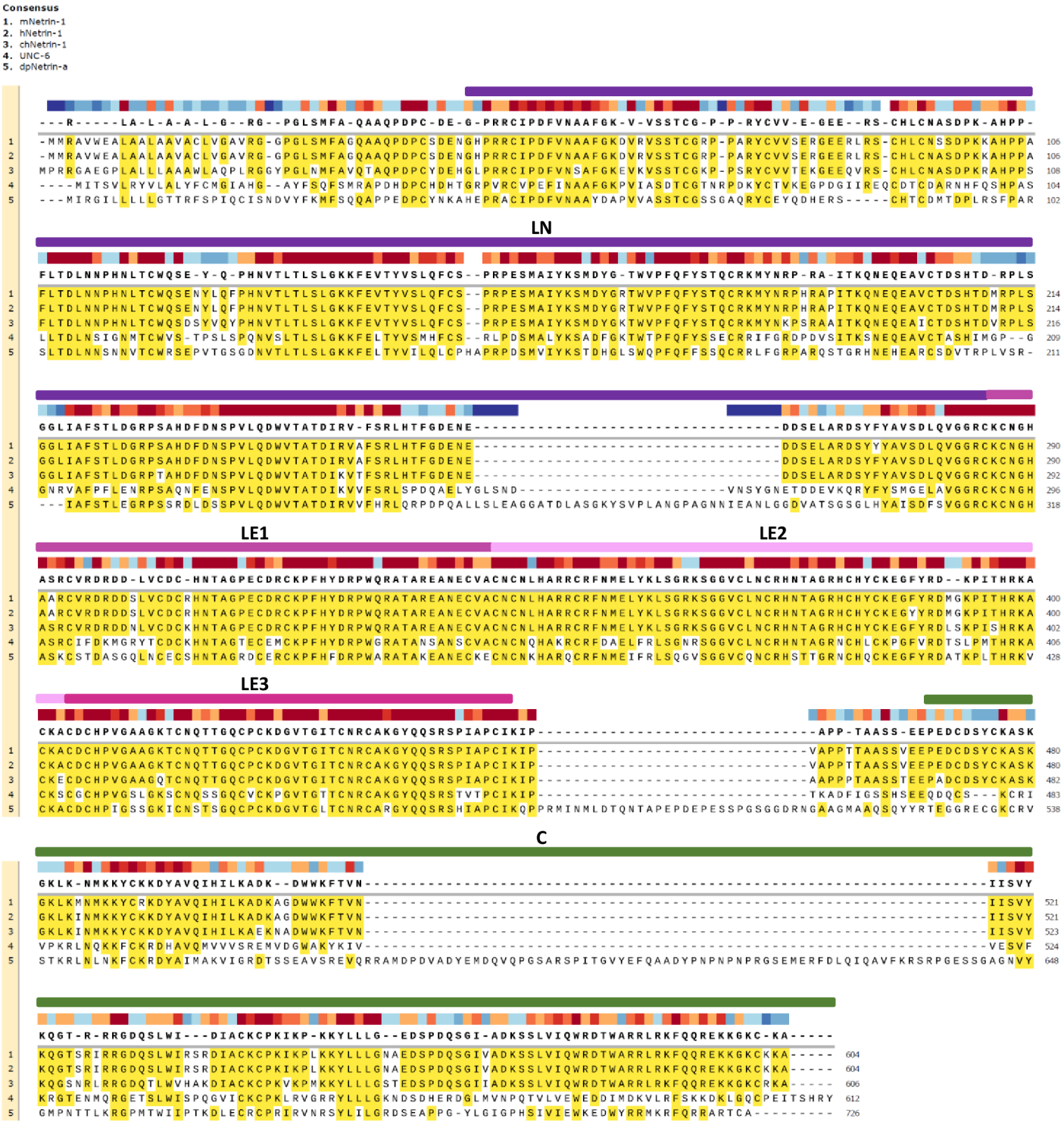


Figure S1: Sequence alignment of the amino acid sequences of various netrins performed using Clustal W. The domains (LN, LE and C) are outlined above the protein sequence with a heat map depicting the conservation of the amino acids (red is highly conserved; blue is not conserved). This alignment reveals that the LN and LE domains have multiple regions that are highly conserved whereas the C-domain is not well conserved.

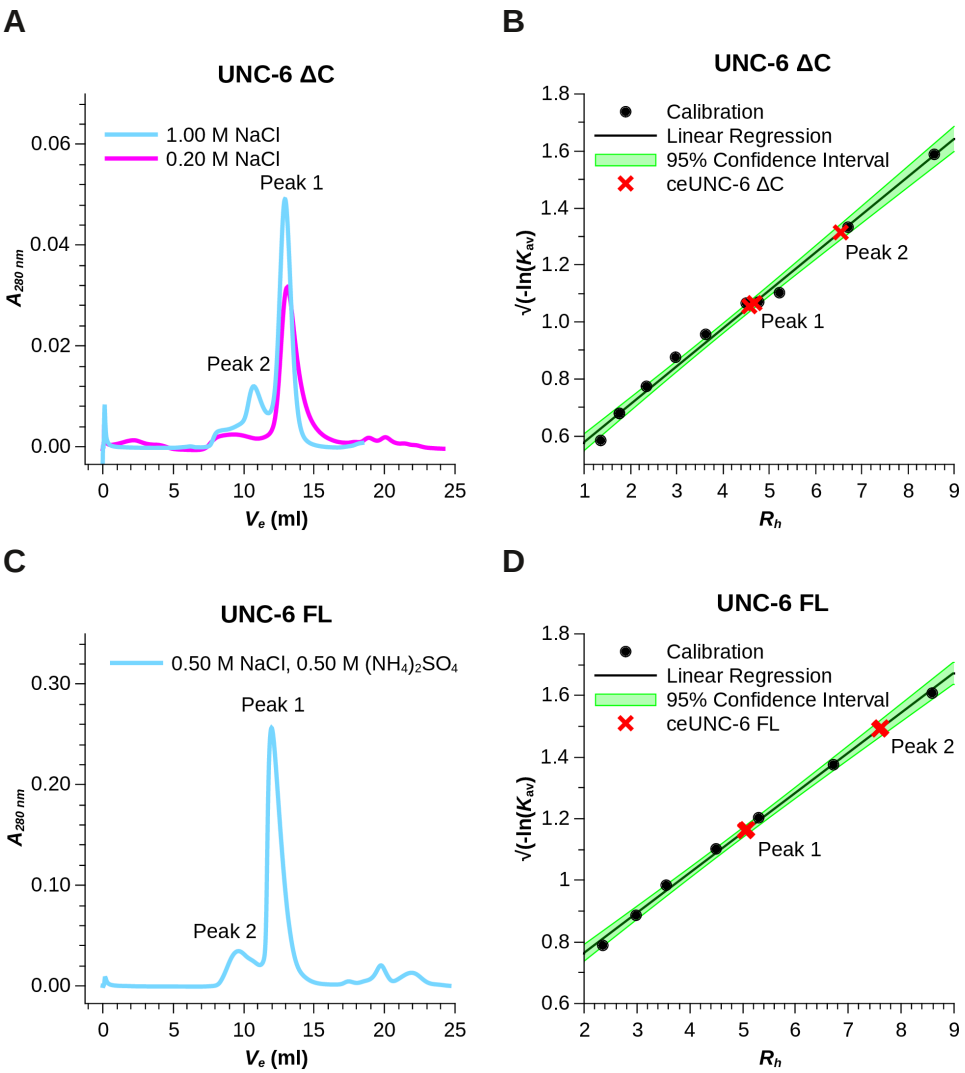


Figure S2: (A) UV trace of *C. elegans* UNC-6 ΔC eluting from a Superdex 200 10/30 GL column in 0.05 M tris, pH 7.5, 1.00 M NaCl (cyan) and 0.20 M NaCl (magenta). In high-ionic strength the protein elutes with two peaks (major peak 1 at 12.94 ml and minor peak 2 at 10.71 ml), but in low-ionic strength only as a single peak (at 13.09 ml). **(B)** The elution peaks correspond to hydrodynamic radii R_h of 4.7 ± 0.2 nm (peak 1 in high-ionic strength), 4.6 ± 0.2 nm (peak 1 in low-ionic strength) and 6.5 ± 0.3 nm (peak 2 in high-ionic strength) as derived from our calibration. **(C)** UV trace of *C. elegans* UNC-6 FL eluting from the Superdex 200 *increase* 10/300 GL (GE Healthcare) column. The protein elutes as a major peak at 12.00 ml (peak 1) and a minor peak at 9.63 ml (peak 2). **(D)** According to our column calibration, the hydrodynamic radii R_h of the eluting species are 5.1 ± 0.2 nm (peak 1) and 7.6 ± 0.3 nm (peak 2). The values are based on the elution volumes (V_e) obtained from four runs.

The Superdex 200 10/30 GL column was calibrated (1) with aprotinin from bovine lung (1.35 nm), cytochrome C from equine heart (1.77 nm), carbonic anhydrase from bovine erythrocytes (2.35 nm), ovalbumin from chicken egg (2.98 nm), conalbumin from chicken egg (3.64 nm), alcohol deydrogenase from *Saccharomyces cerevisiae* (4.50 nm), aldolase from rabbit muscle (4.77 nm), catalase from bovine liver (5.22 nm), ferritin from horse spleen (6.71 nm) and thyroglobulin from bovine thyroid (8.58 nm). The Superdex 200 *increase* 10/300 GL was calibrated (1) with carbonic anhydrase from bovine erythrocytes (2.35 nm), ovalbumin from chicken egg (2.98 nm), albumin from bovine serum (3.56 nm), alcohol deydrogenase (4.50 nm) from *Saccharomyces cerevisiae*, β-amylase from sweet potato (5.30 nm), ferritin from horse spleen (6.71 nm) and thyroglobulin from bovine thyroid (8.58 nm).

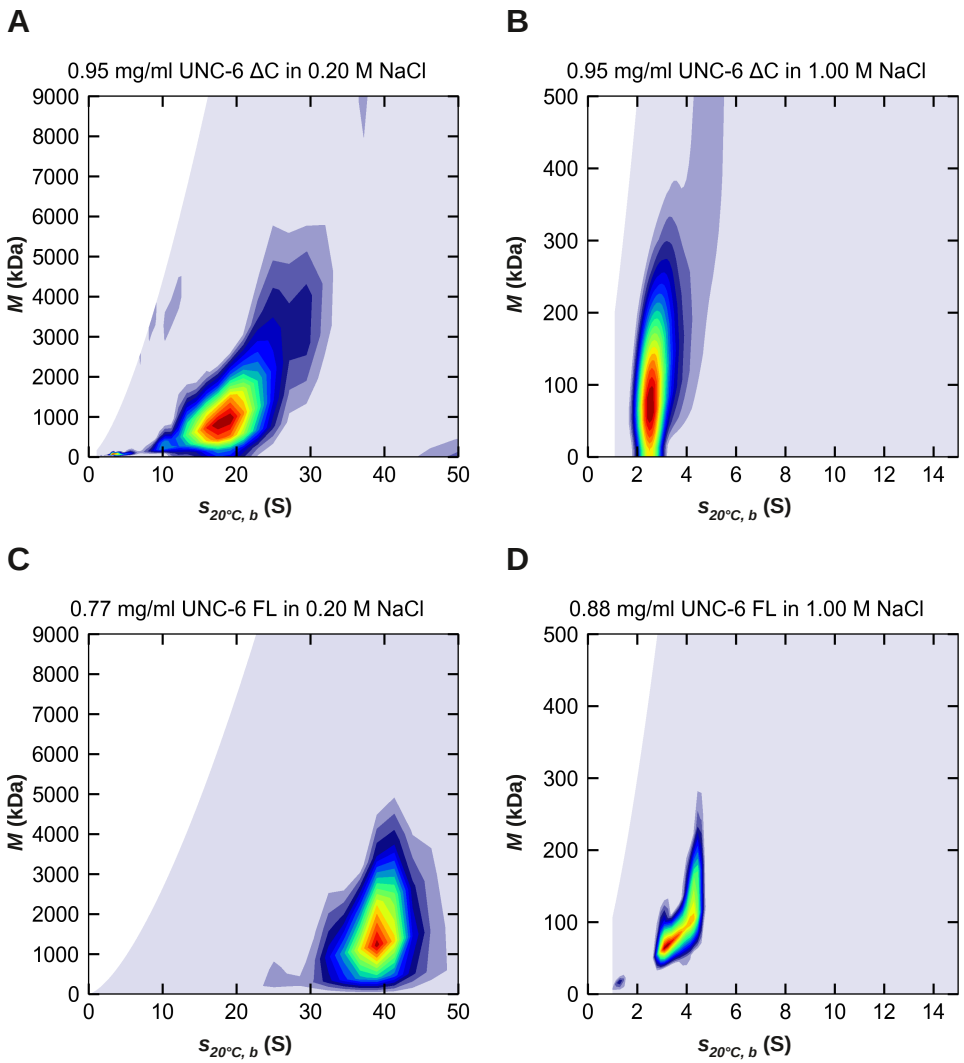


Figure S3: 2-dimensional *c(s, M)* distributions (2) obtained from the absorbance optics of **(A)** *C. elegans* UNC-6 ΔC in low-ionic strength buffer, **(B)** *C. elegans* UNC-6 ΔC in high-ionic strength solvent, **(C)** *C. elegans* UNC-6 FL in low-ionic strength buffer and **(D)** *C. elegans* UNC-6 FL in high-ionic strength solvent at concentrations close to 1 mg/ml. In low-ionic strength, the protein forms assemblies of high molecular mass in the range of 1 - 2 MDa. At high-ionic strength, the formation of these assemblies is suppressed and the distributions suggest populations of monomers, dimers and multimers in the case of the ΔC truncation and a monomer/dimer equilibrium (plus a small amount of a larger species) in the case of the full-length version. Interestingly, in low salt, the assemblies of the full-length protein have sedimentation coefficents twice as large as the truncated protein, however their masses are similar. This suggests that the shapes of the assemblies differ between the two protein versions.

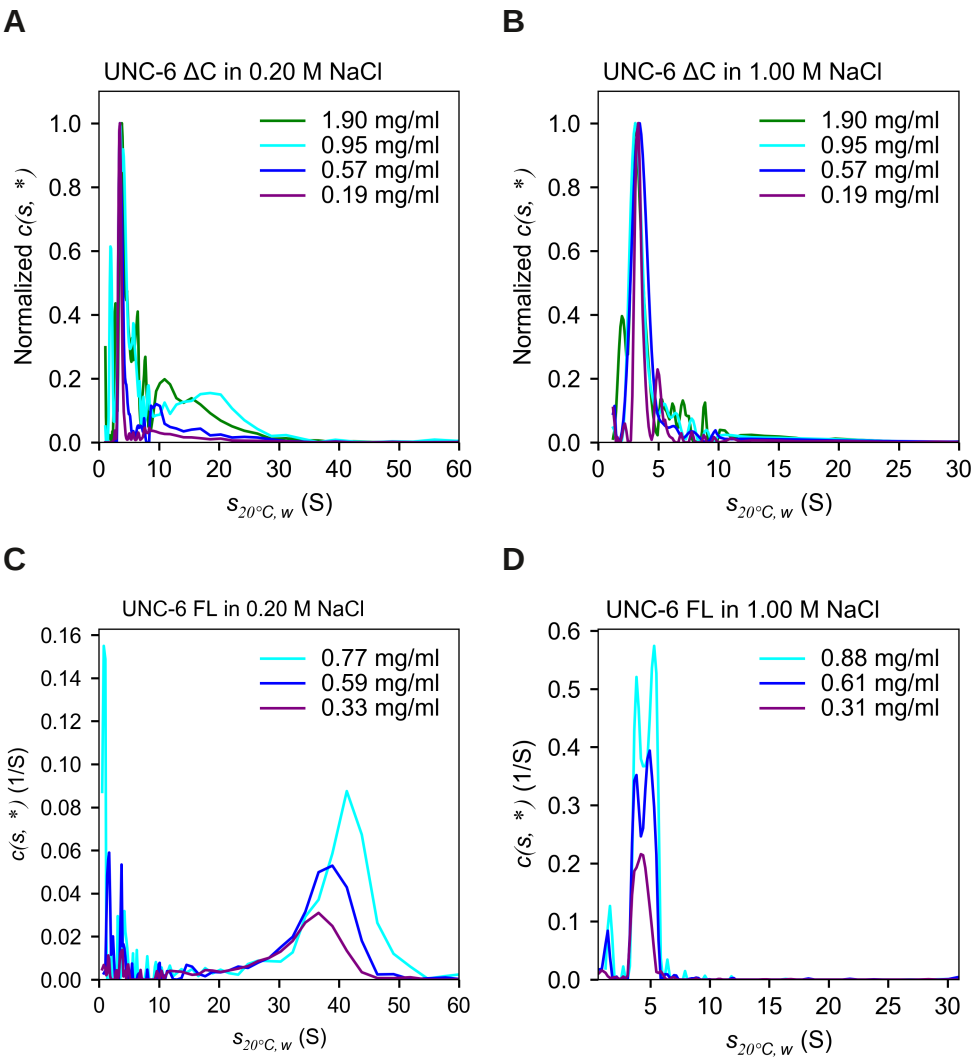


Figure S4: *c(s, *)* distributions (2) at different loading concentrations obtained from data recorded by the absorbance optics. **(A)** *C. elegans* UNC-6 ΔC in 0.05 M tris, pH 7.5, 0.20 M NaCl. For clarity, the distributions were normalized such that the maximum value is equal to 1.0. **(B)** *C. elegans* UNC-6 ΔC in 0.05 M tris, pH 7.5, 1.00 M NaCl. The distributions were normalized such that the maximum value is equal to 1.0. **(C)** *C. elegans* UNC-6 FL in 0.05 M tris, pH 7.5, 0.20 M NaCl. The distributions are shown at original scale. **(D)** *C. elegans* UNC-6 FL in 0.05 M tris, pH 7.5, 1.00 M NaCl. The distributions are shown at original scale.

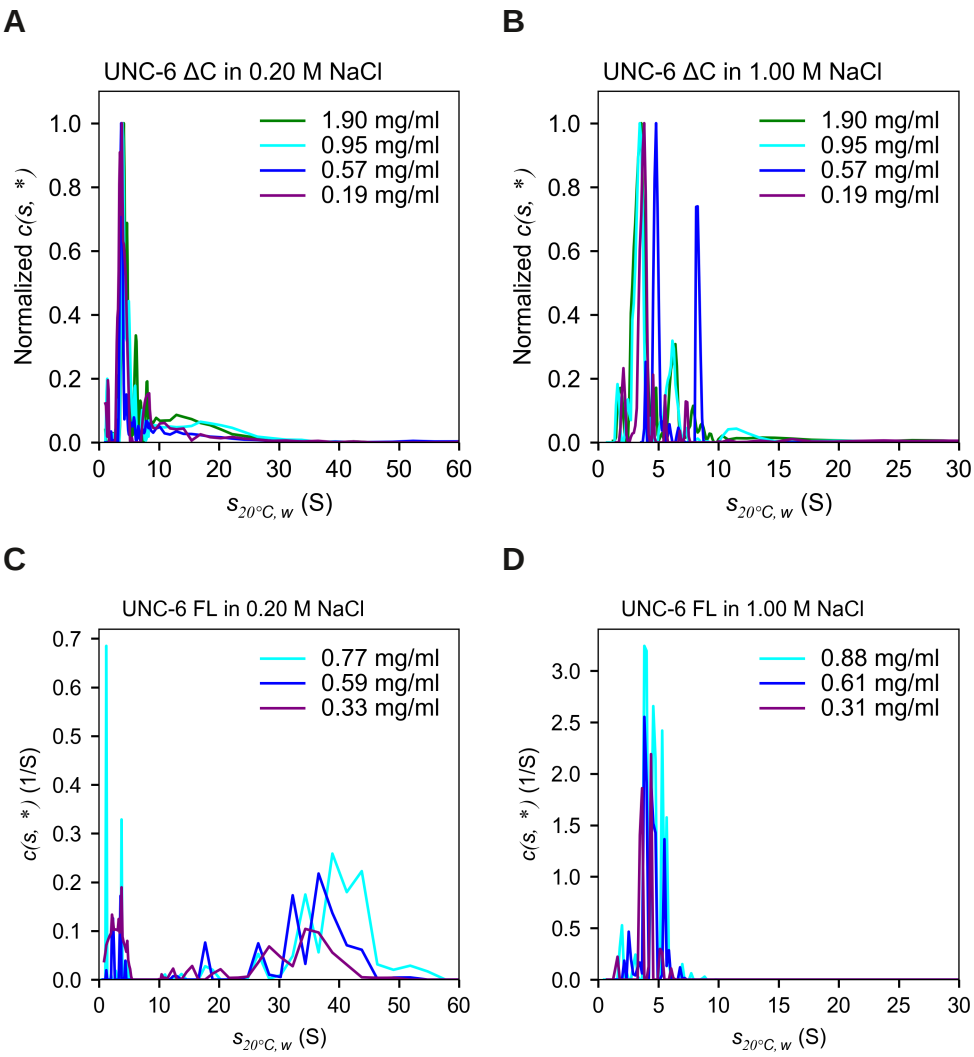


Figure S5: *c(s, *)* distributions (2) at different loading concentrations obtained from data recorded by the interference optics. **(A)** *C. elegans* UNC-6 ΔC in 0.05 M tris, pH 7.5, 0.20 M NaCl. For clarity, the distributions were normalized such that the maximum value is equal to 1.0. **(B)** *C. elegans* UNC-6 ΔC in 0.05 M tris, pH 7.5, 1.00 M NaCl. The distributions were normalized such that the maximum value is equal to 1.0. **(C)** *C. elegans* UNC-6 FL in 0.05 M tris, pH 7.5, 0.20 M NaCl. The distributions are shown at original scale. **(D)** *C. elegans* UNC-6 FL in 0.05 M tris, pH 7.5, 1.00 M NaCl. The distributions are shown at original scale.

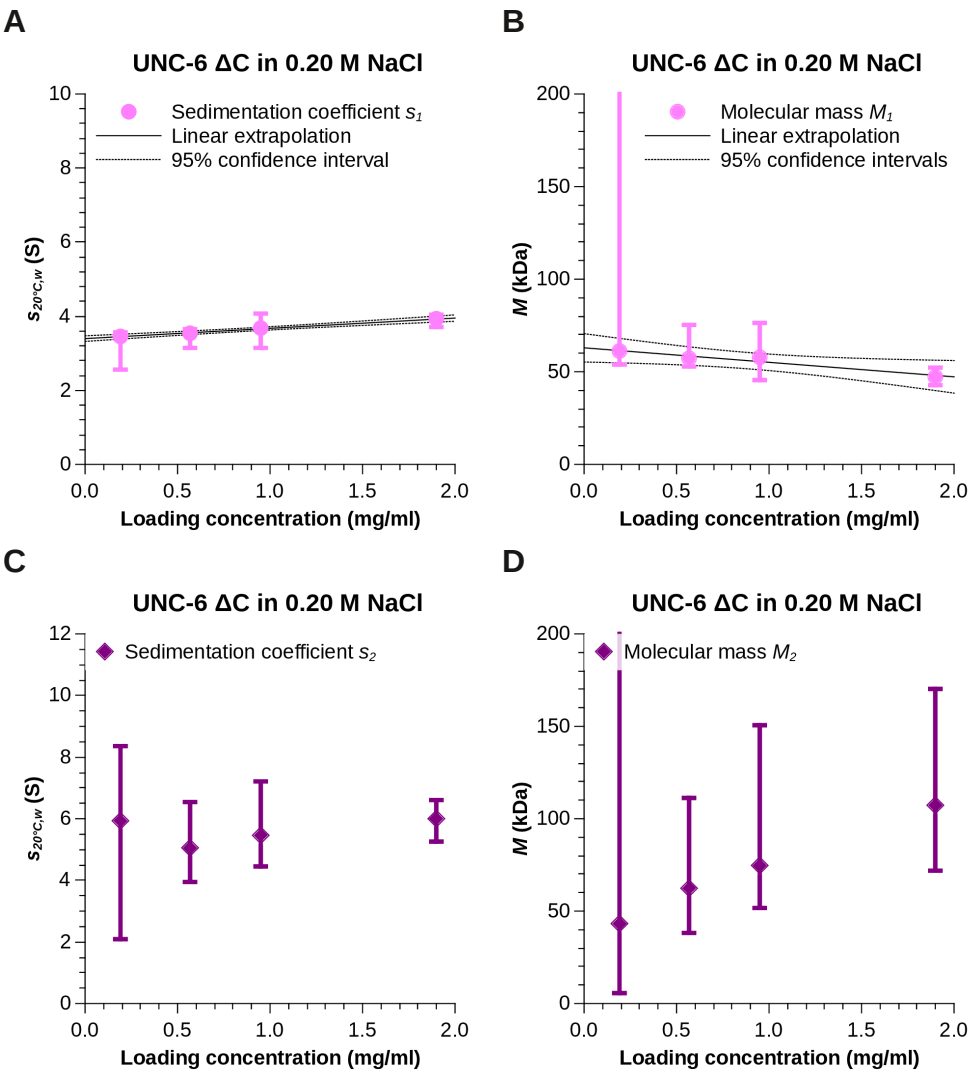


Figure S6: Sedimentation velocity data at different loading concentrations of *C. elegans* UNC-6 ΔC in 0.05 M tris, pH 7.5, 0.20 M NaCl with each loading concentration fitted independently to a hybrid continuous *c(s)* distribution model (3) with two discrete species. **(A and B):** Sedimentation coefficients **(A)** and molecular masses **(B)** of the stationary species which represents the monomeric *C. elegans* UNC-6 ΔC. The parameters were extrapolated to infinite dilution (black line) with the 95.4% confidence intervals of the extrapolation indicated by dotted lines. **(C and D):** The second species is an effective particle representing a time-average of interacting particles and therefore its apparent sedimentation coefficient **(C)** and molecular mass **(D)** increase with loading concentration. The vertical error bars represent 95.4 % confidence intervals of each fitted parameter. The data were measured using the absorbance optics.

Tag-free *C. elegans* UNC-6 ΔC in 0.20 M NaCl

Absorbance optics

Species analysis - extrapolation to infinite dilution

A

	Species 1	Species 2
Sedimentation coefficient $s_{20°C,w}$ (S)	3.4 ± 0.1	Effective particle*
Molecular mass *M* (kDa)	63 ± 8	Effective particle*
Diffusion coefficient *D* (10^{-7} cm^2/s)	4.6	Effective particle*
Hydrodynamic radius R_h (nm)	4.5	Effective particle*
Rel. molecular fraction *f*	0.644 - 0.860	0.14 - 0.356

95% confidence intervals, *extrapolation meaningless

B

Parameters	
Buffer density[1] ρ (g/cm^3)	1.007900
Buffer viscosity[1] η (P)	0.010357
Temperature T (°C)	20.0
Glycoprotein[2] partial specific volume[1] $\bar{\nu}$ (cm^3/g)	0.70662
Glycoprotein[2] formula mass[1] M_w (kDa)	58.65

[1]Sednterp, [2]calculation includes 5 N-linked core pentasaccharides

Table S1: (A): Experimental hydrodynamic parameters of monomeric *C. elegans* UNC-6 ΔC (species 1) in 0.05 M tris, pH 7.5, 0.20 M NaCl, as obtained from extrapolating the fitted parameters from the hybrid continuous *c(s)* distribution model (3) with two discrete species at each loading concentration to infinite dilution (see Figure S6). **(B)** Buffer and protein properties were calculated by the program SEDNTERP (6).

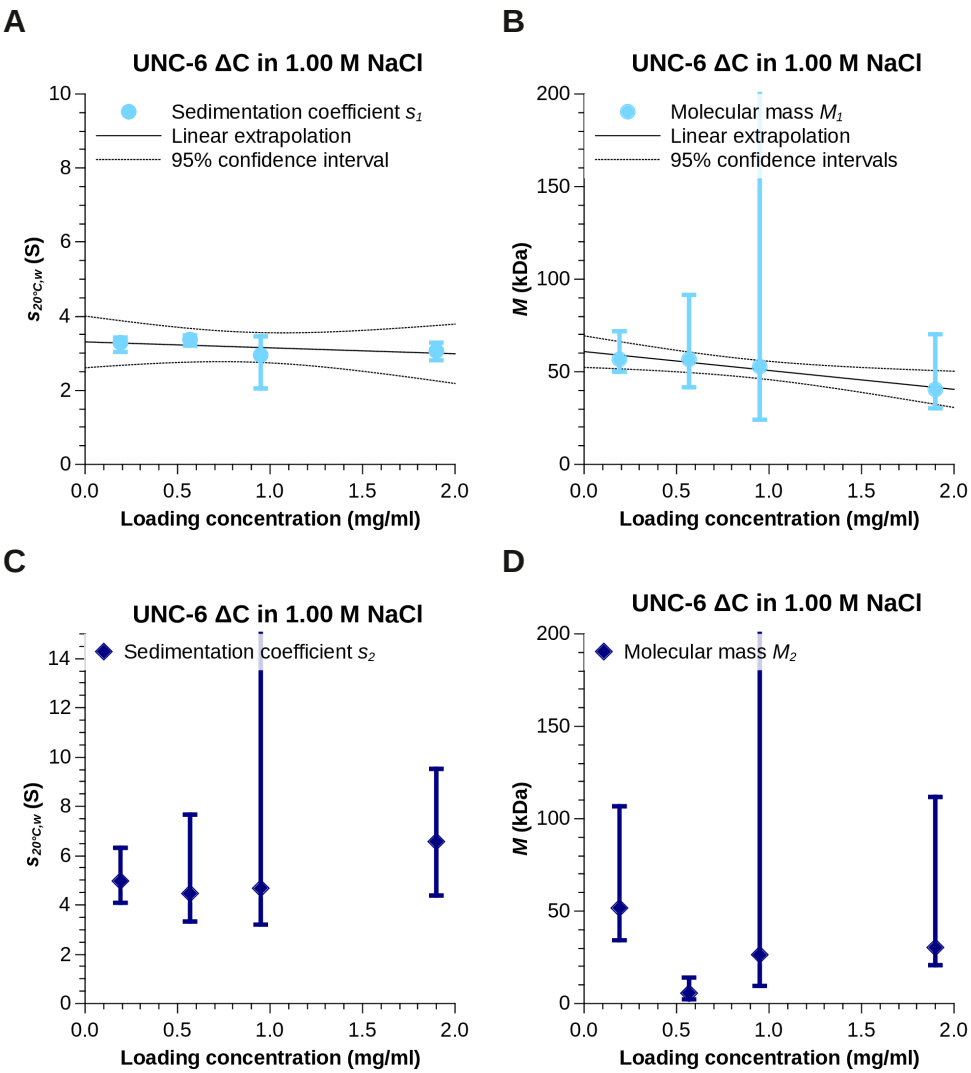


Figure S7: Sedimentation velocity data at different loading concentrations of *C. elegans* UNC-6 ΔC in 0.05 M tris, pH 7.5, 1.00 M NaCl with each loading concentration fitted independently to a non-interacting species model (4, 5) with two discrete species. **(A and B):** Sedimentation coefficients **(A)** and molecular masses **(B)** of the stationary species which represents the monomeric *C. elegans* UNC-6 ΔC. The parameters were extrapolated to infinite dilution (black line) with the 95.4% confidence intervals of the extrapolation indicated by dotted lines. **(C and D):** The second species is an effective particle representing a time-average of interacting particles and therefore its apparent sedimentation coefficient **(C)** and molecular mass **(D)** are expected to increase with loading concentration. The vertical error bars represent 95.4 % confidence intervals of each fitted parameter. The data were measured using the absorbance optics.

Tag-free *C. elegans* UNC-6 ΔC in 1.00 M NaCl

Absorbance optics

Species analysis - extrapolation to infinite dilution

A

	Species 1	Species 2
Sedimentation coefficient $s_{20°C,w}$ (S)	3.3 ± 0.7	Effective particle*
Molecular mass *M* (kDa)	61 ± 9	Effective particle*
Diffusion coefficient *D* (10^{-7} cm^2/s)	4 ± 2	Effective particle*
Hydrodynamic radius R_h (nm)	4.44 [3.14 - 7.61]	Effective particle*
Molecular fraction *f*	0.377 - 0.789	0.211 - 0.623

95% confidence intervals, *extrapolation meaningless

B

Parameters	
Buffer density[1] ρ (g/cm^3)	1.040120
Buffer viscosity[1] η (P)	0.011131
Temperature T (°C)	20.0
Glycoprotein[2] partial specific volume[1] $\bar{\nu}$ (cm^3/g)	0.70662
Glycoprotein[2] formula mass[1] M_w (kDa)	58.65

[1]Sednterp, [2]calculation includes 5 N-linked core pentasaccharides

Table S2: (A): Experimental hydrodynamic parameters of monomeric *C. elegans* UNC-6 ΔC (species 1) in 0.05 M tris, pH 7.5, 1.00 M NaCl, as obtained from extrapolating the fitted parameters from the non-interacting species model (4, 5) with two discrete species at each loading concentration to infinite dilution (see Figure S7). **(B)** Buffer and protein properties were calculated by the program SEDNTERP (6).

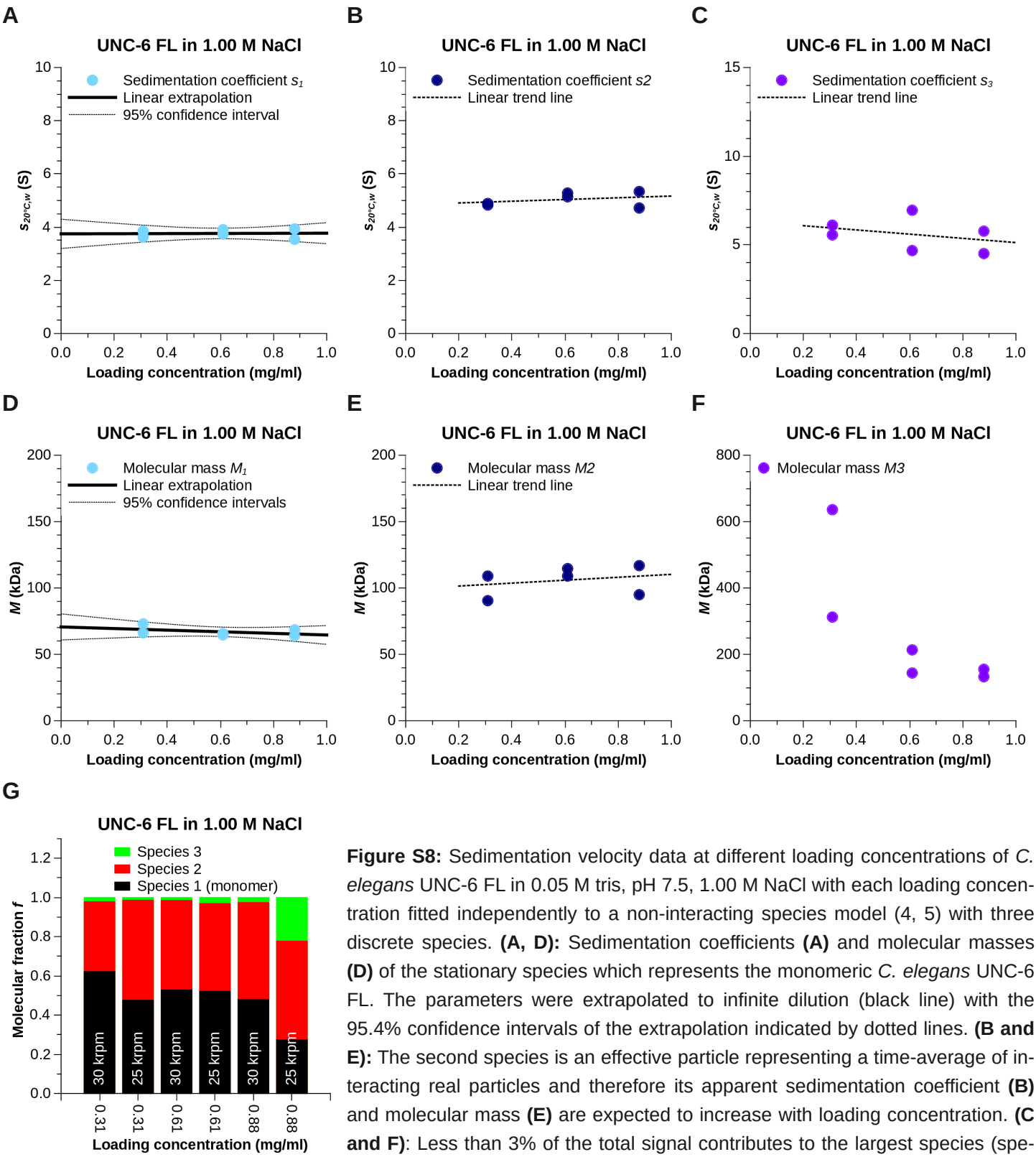


Figure S8: Sedimentation velocity data at different loading concentrations of *C. elegans* UNC-6 FL in 0.05 M tris, pH 7.5, 1.00 M NaCl with each loading concentration fitted independently to a non-interacting species model (4, 5) with three discrete species. **(A, D):** Sedimentation coefficients **(A)** and molecular masses **(D)** of the stationary species which represents the monomeric *C. elegans* UNC-6 FL. The parameters were extrapolated to infinite dilution (black line) with the 95.4% confidence intervals of the extrapolation indicated by dotted lines. **(B and E):** The second species is an effective particle representing a time-average of interacting real particles and therefore its apparent sedimentation coefficient **(B)** and molecular mass **(E)** are expected to increase with loading concentration. **(C and F)**: Less than 3% of the total signal contributes to the largest species (species 3), and therefore its mass and sedimentation coefficient are not well defined. Due to the insignificant contribution of species 3 to the signal, the parameters of species 2 can be averaged to yield underestimated values for the UNC-6 FL dimer (Table S3). The data were measured using the absorbance optics at rotor speeds of 30000 and 25000 rpm.

2x STREPII-tagged *C. elegans* UNC-6 FL in 1.00 NaCl

Absorbance optics

Species analysis

A

	Species‡ 1	Species 2 (effective particle*)
Sedimentation coefficient $s_{20°C,w}$ (S)	3.8 ± 0.6	5.0 ± 0.3
Molecular mass *M* (kDa)	70 ± 10	106 ± 12
Diffusion coefficient *D* (10^{-7} cm^2/s)		
Hydrodynamic radius R_h (nm)		
Molecular fraction *f*	0.27 - 0.63	0.35 - 0.52

95% confidence intervals,‡extrapolated to infinite dilution, *averaged values

B

Parameters	
Buffer density[1] ρ (g/cm^3)	1.040120
Buffer viscosity[1] η (P)	0.011131
Temperature T (°C)	20.0
Glycoprotein[2] partial specific volume[1] $\bar{\nu}$ (cm^3/g)	0.71101
Glycoprotein[2] formula mass[1] M_w (kDa)	76.49

[1]Sednterp, [2]calculation includes 6 N-linked core pentasaccharides

Table S3: (A): Experimental hydrodynamic parameters of *C. elegans* UNC-6 FL in 0.05 M tris, pH 7.5, 1.00 M NaCl obtained from fitting them to the data of each loading concentration to the non-interacting species model (4, 5) with three discrete species (see Figure S8). Species 1 is stationary and represents monomers. The parameters were extrapolated to infinite dilution. Species 2 is an effective particle containing contributions from interacting monomers and dimers with increasing weight from dimers at higher concentrations. Since we only measured a limited concentration range, we averaged the values. They underestimate the true value of the dimer. The parameters of species 3 are not well defined and are not shown. **(B)** Buffer and protein properties were calculated by the program SEDNTERP (6).

Figures S9:

Individual sedimentation velocity datasets of *C. elegans* UNC-6 ΔC

in 0.05 M tris, pH 7.5, 0.20 M NaCl

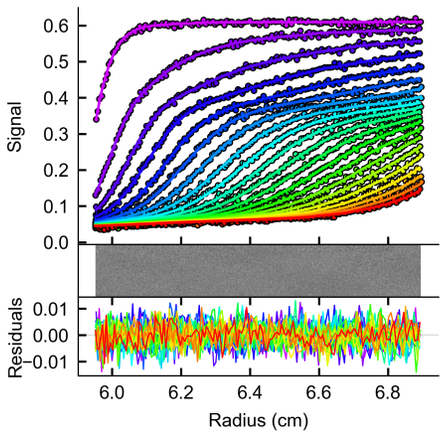
Figures were prepared using the computer software GUSSI (7) and Matplotlib (8).

0.19 mg/ml tag-free *C. elegans* UNC-6 ΔC in 0.20 M NaCl

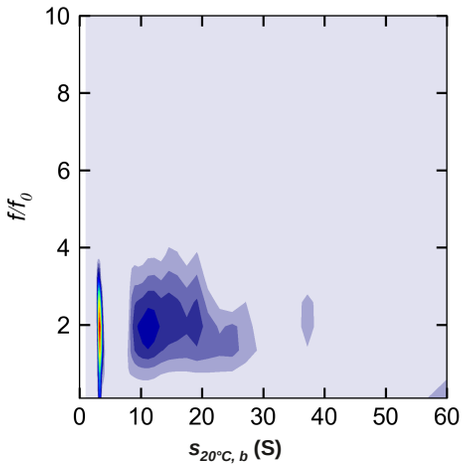
c(s, f_r) analysis

Absorbance optics @ 30000 rpm

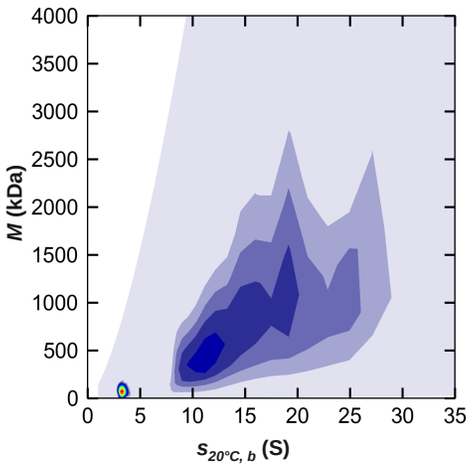
Data, fit and residuals



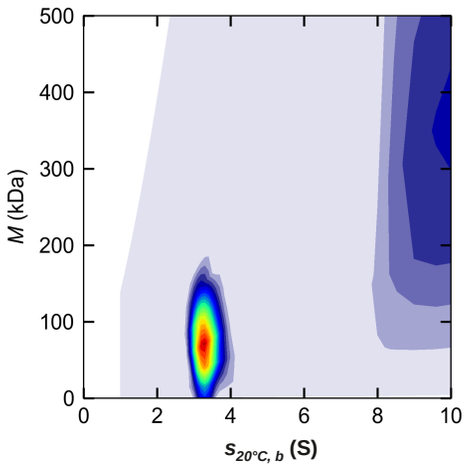
f_r vs. s



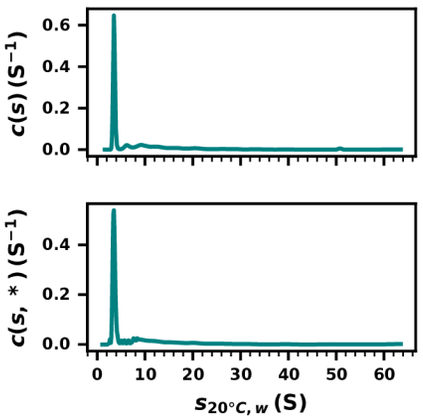
M vs. s



M vs. s



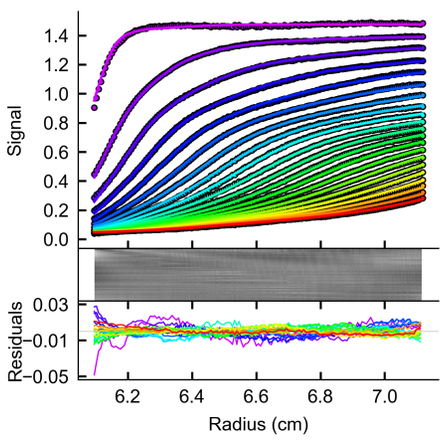
1D distributions



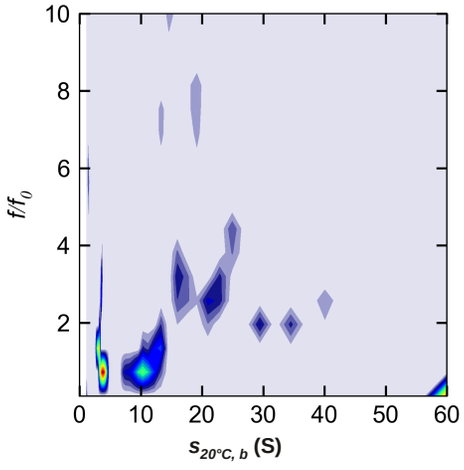
Reduced χ^2 [r.m.s.d]: 0.1629737 [0.004037]

Interference optics @ 30000 rpm

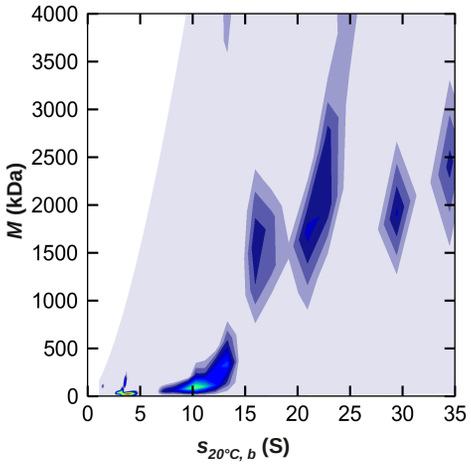
Data, fit and residuals



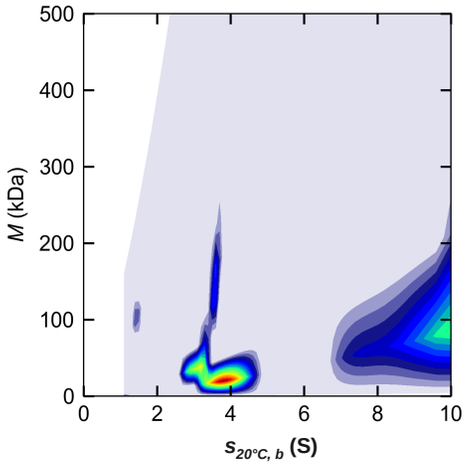
f_r vs. s



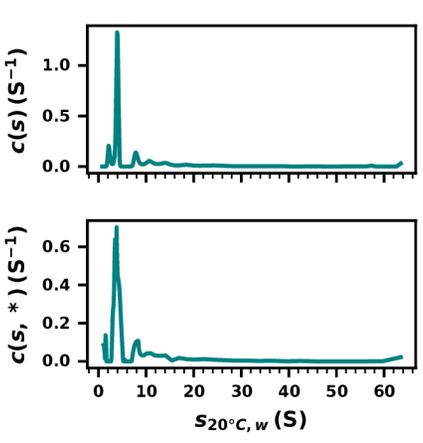
M vs. s



M vs. s



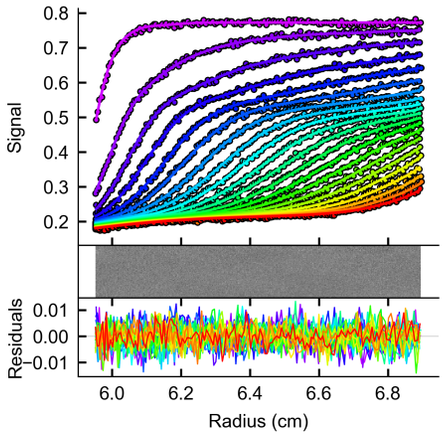
1D distributions



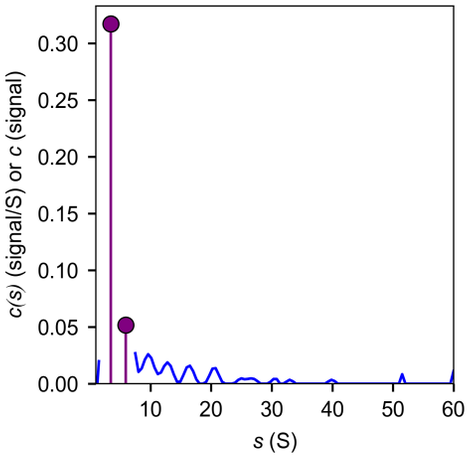
Reduced χ^2 [r.m.s.d]: 0.2090318 [0.004572]

Hybrid model with continuous distribution and two discrete species (absorbance optics @ 30000 rpm)

Data, fit and residuals



Species and distribution



	Species 1		Species 2	
	Best fit	95% confidence intervals	Best fit	95% confidence intervals
Sedimentation coefficient $s_{20°C,w}$ (S)	3.46	2.55 - 3.57	5.94	2.07 - 8.38
Molecular mass *M* (kDa)	61.1	53.8 - >10^6	43.0	5.7 -279.0
Diffusion coefficient *D* (10^{-7} cm^2/s)				
Rel. molecular fraction *f*	0.859	n/a	0.141	n/a
Reduced *χ2* [r.m.s.d]	0.1629801 [0.004037079]			

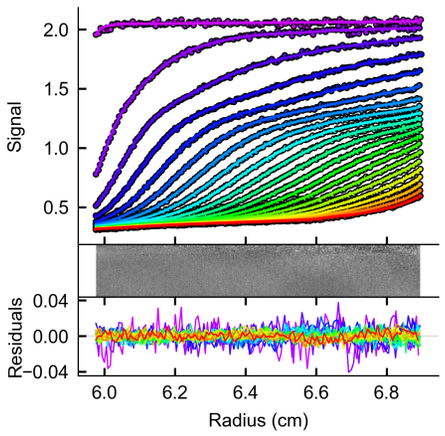
Figure S9A

0.57 mg/ml tag-free *C. elegans* UNC-6 ΔC in 0.20 M NaCl

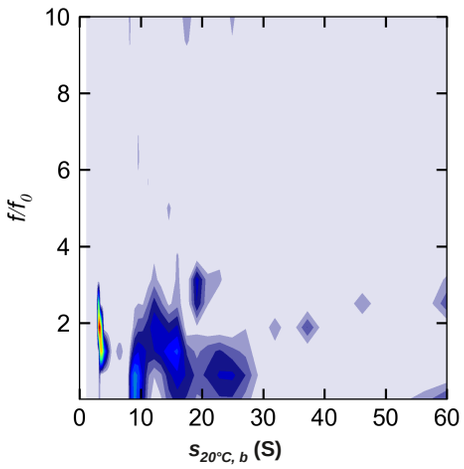
c(s, f_r) analysis

Absorbance optics @ 30000 rpm

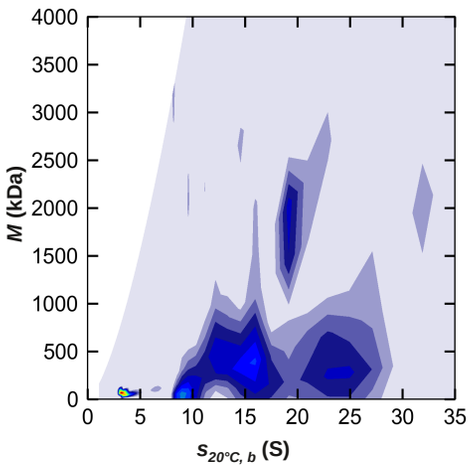
Data, fit and residuals



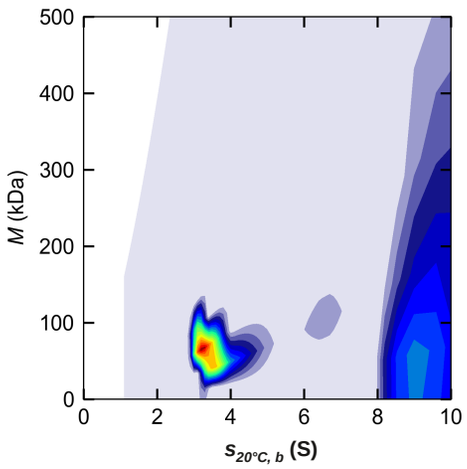
f_r vs. s



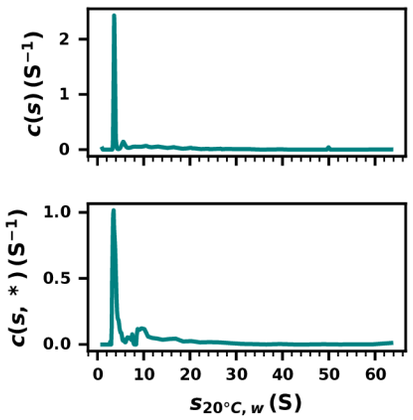
M vs. s



M vs. s



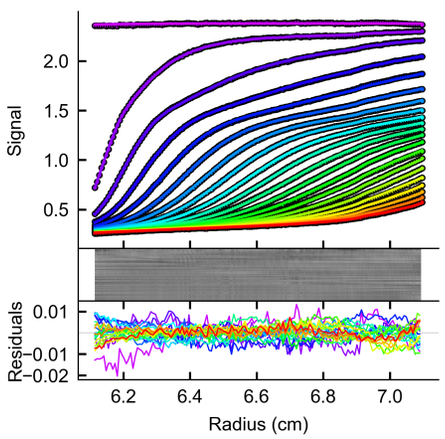
1D distributions



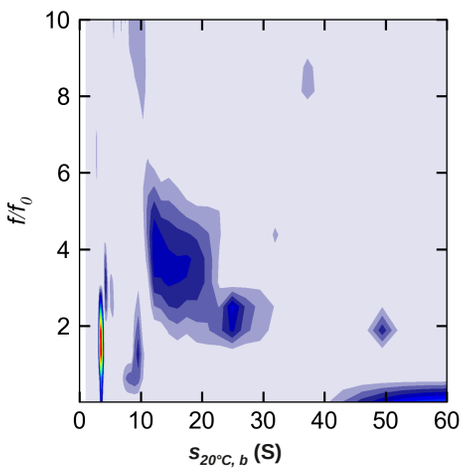
Reduced χ^2 [r.m.s.d]: 0.4094720 [0.006399]

Interference optics @ 30000 rpm

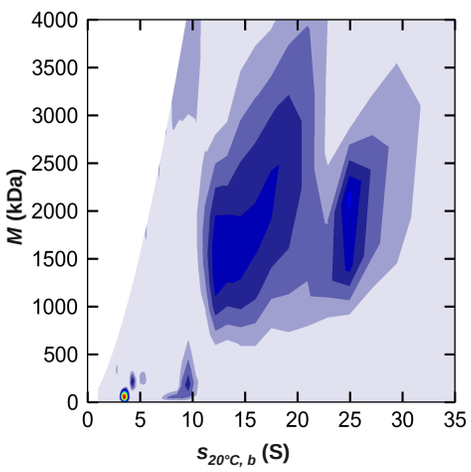
Data, fit and residuals



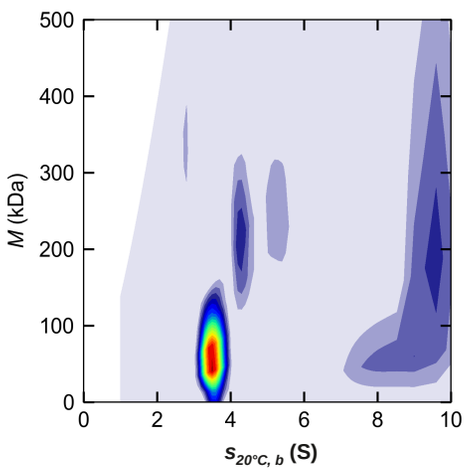
f_r vs. s



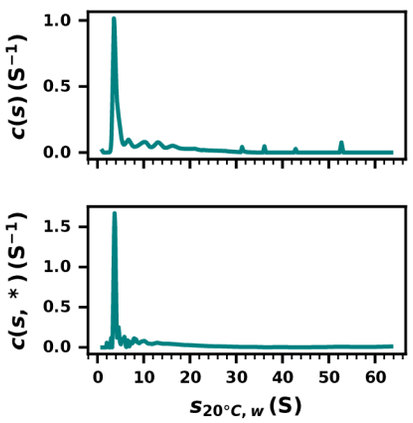
M vs. s



M vs. s



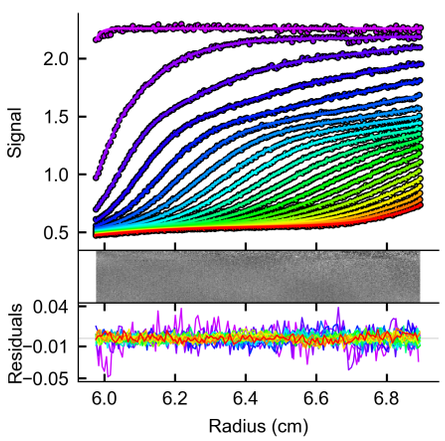
1D distributions



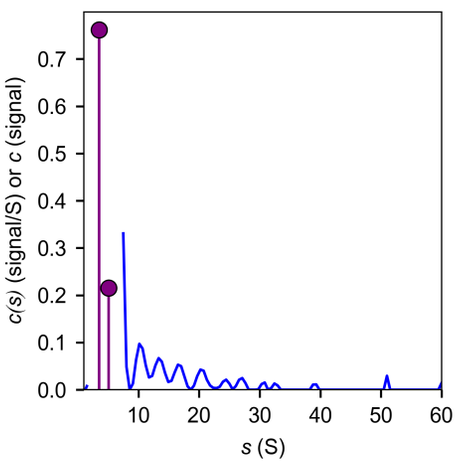
Reduced χ^2 [r.m.s.d]: 0.1605605 [0.004007]

Hybrid model with continuous distribution and two discrete species (absorbance optics @ 30000 rpm)

Data, fit and residuals



Species and distribution



	Species 1		Species 2	
	Best fit	95% confidence intervals	Best fit	95% confidence intervals
Sedimentation coefficient $s_{20°C,w}$ (S)	3.53	3.14 - 3.65	5.07	3.93 - 6.55
Molecular mass *M* (kDa)	57.2	52.5 - 75.4	62.5	38.4 - 111.3
Diffusion coefficient *D* (10^{-7} cm²/s)				
Rel. molecular fraction *f*	0.780	n/a	0.220	n/a
Reduced *χ2* [r.m.s.d]	0.4182122 [0.006466933]			

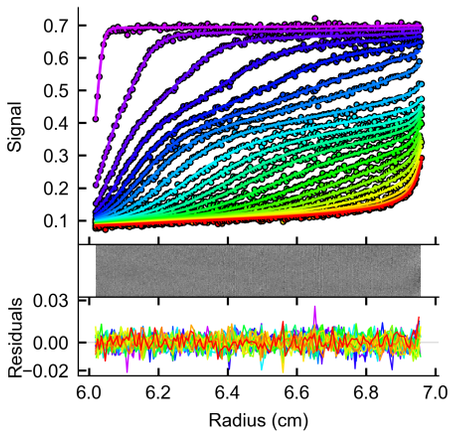
Figure S9B

0.95 mg/ml tag-free *C. elegans* UNC-6 ΔC in 0.20 M NaCl

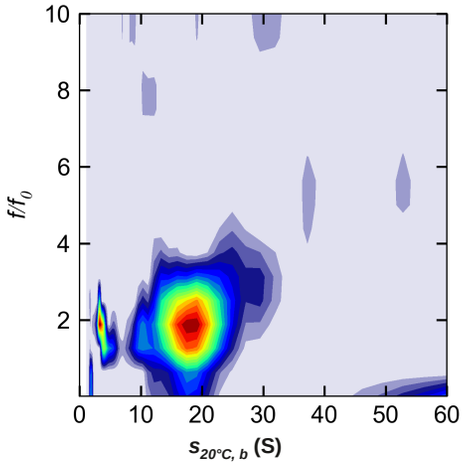
c(s, f_r) analysis

Absorbance optics @ 30000 rpm

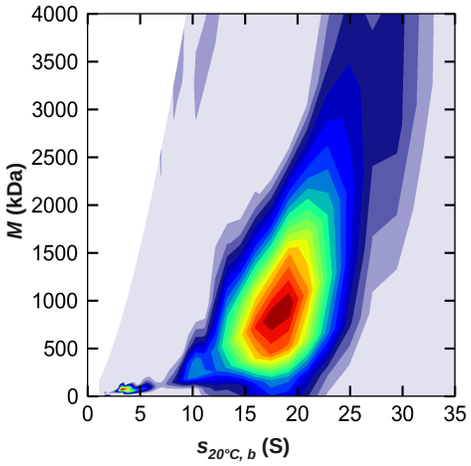
Data, fit and residuals



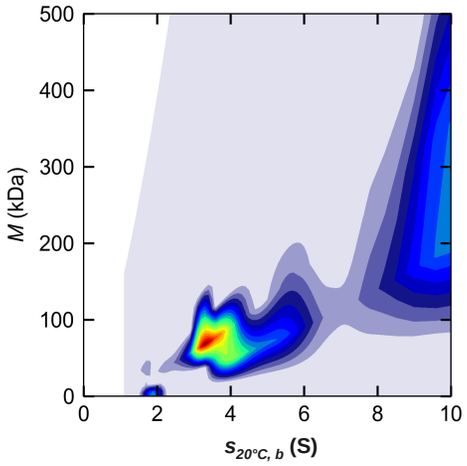
f_r vs. s



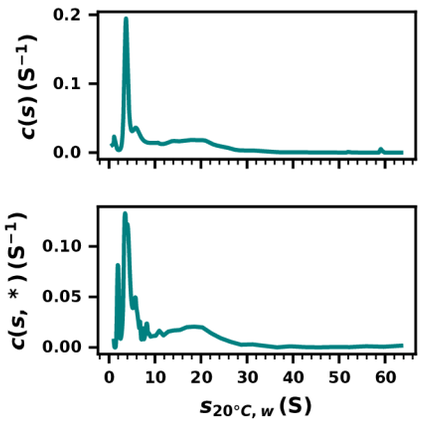
M vs. s



M vs. s



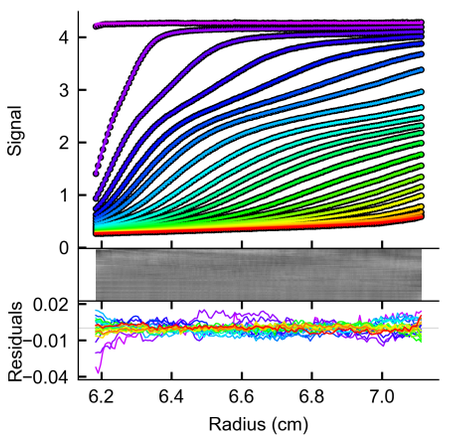
1D distributions



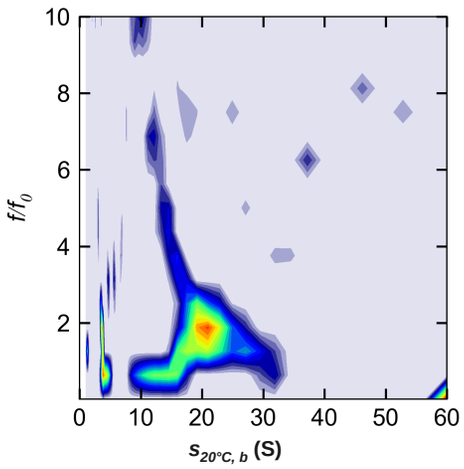
Reduced χ^2 [r.m.s.d]: 0.2461152 [0.004961]

Interference optics @ 30000 rpm

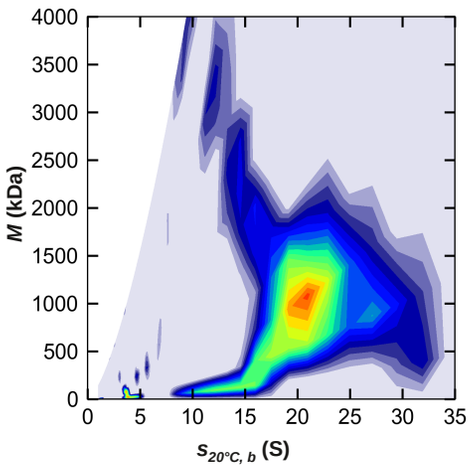
Data, fit and residuals



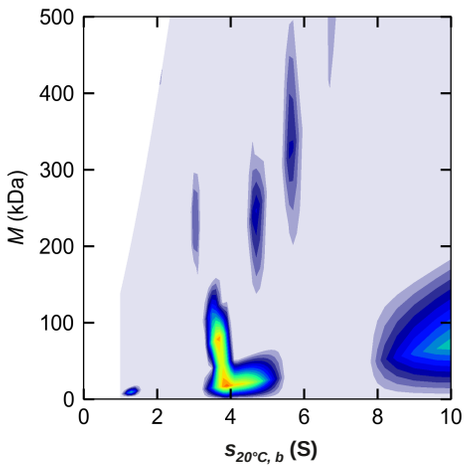
f_r vs. s



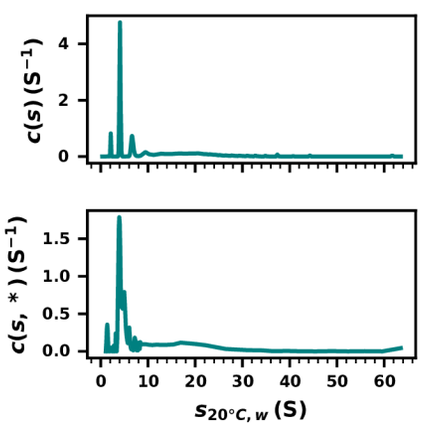
M vs. s



M vs. s



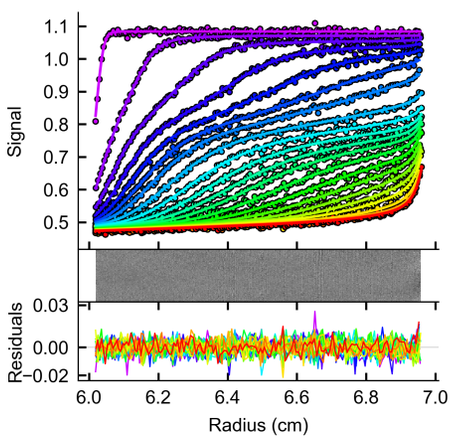
1D distributions



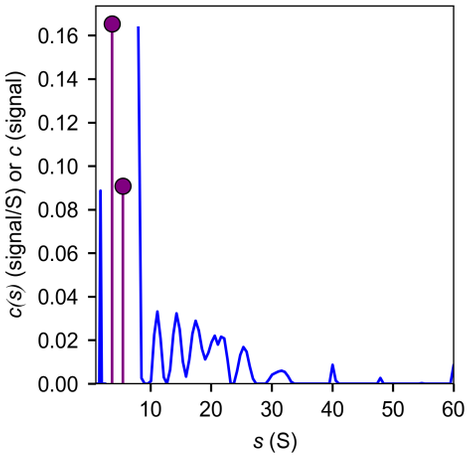
Reduced χ^2 [r.m.s.d]: 0.1690032 [0.004111]

Hybrid model with continuous distribution and two discrete species (absorbance optics @ 30000 rpm)

Data, fit and residuals



Species and distribution



	Species 1		Species 2	
	Best fit	95% confidence intervals	Best fit	95% confidence intervals
Sedimentation coefficient $s_{20°C,w}$ (S)	3.67	3.14 - 4.09	5.46	4.46 - 7.22
Molecular mass *M* (kDa)	57.9	45.7 - 76.7	74.6	51.6 - 150.3
Diffusion coefficient *D* (10^{-7} cm²/s)				
Rel. molecular fraction *f*	0.645	n/a	0.355	n/a
Reduced *χ2* [r.m.s.d]	0.2247424 [0.0047407]			

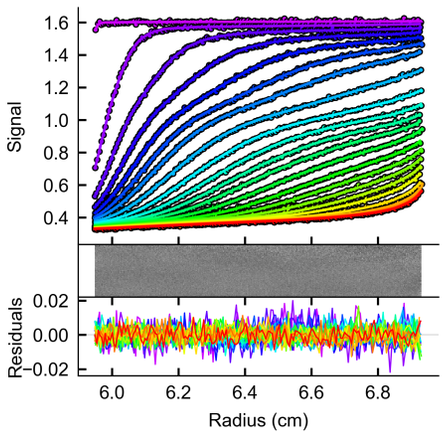
Figure S9C

1.90 mg/ml tag-free *C. elegans* UNC-6 ΔC in 0.20 M NaCl

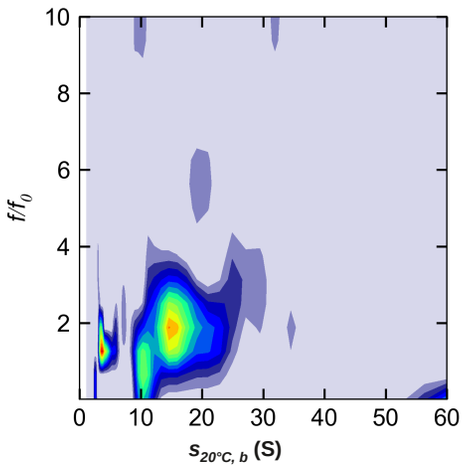
c(s, f_r) analysis

Absorbance optics @ 30000 rpm

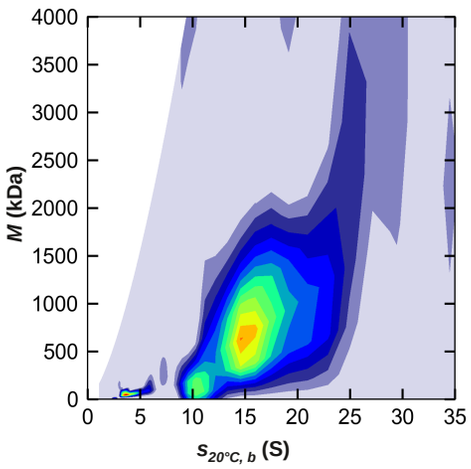
Data, fit and residuals



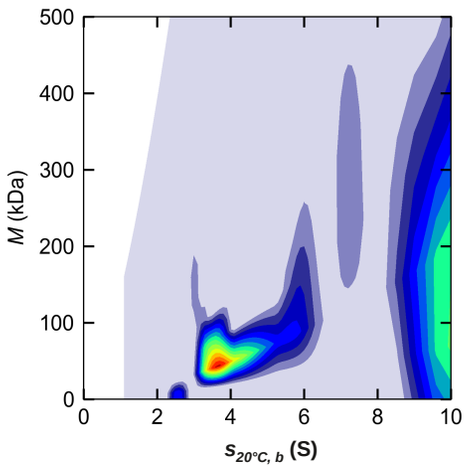
f_r vs. s



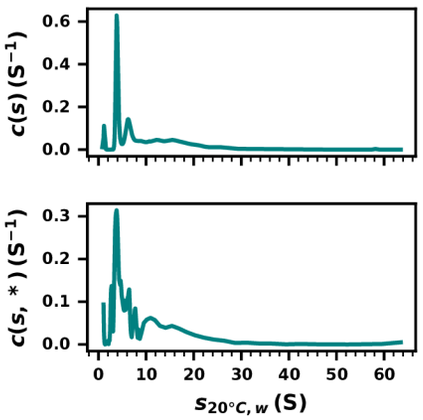
M vs. s



M vs. s



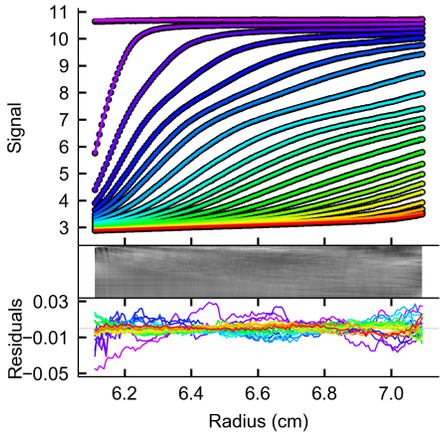
1D distributions



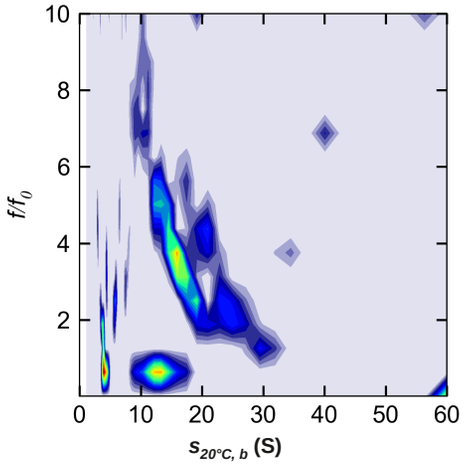
Reduced χ^2 [r.m.s.d]: 0.2155745 [0.004643]

Interference optics @ 30000 rpm

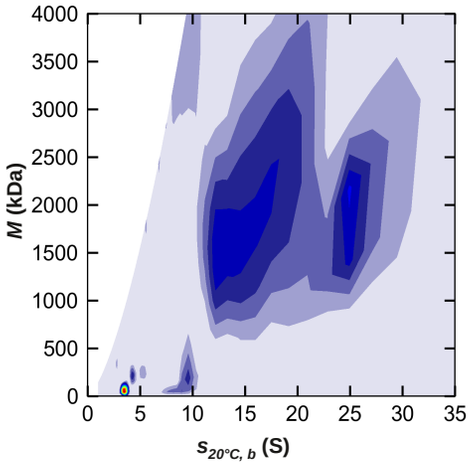
Data, fit and residuals



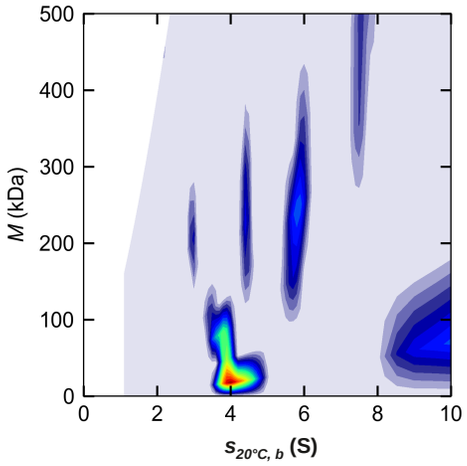
f_r vs. s



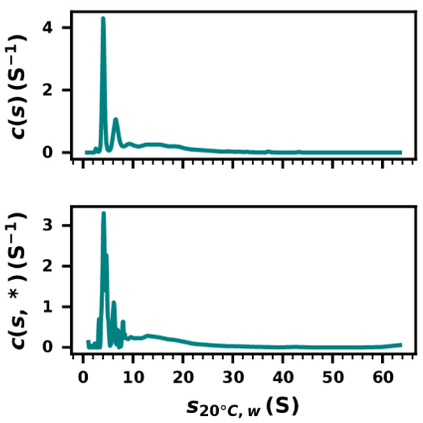
M vs. s



M vs. s



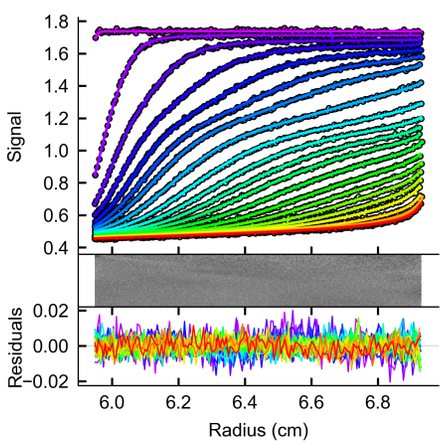
1D distributions



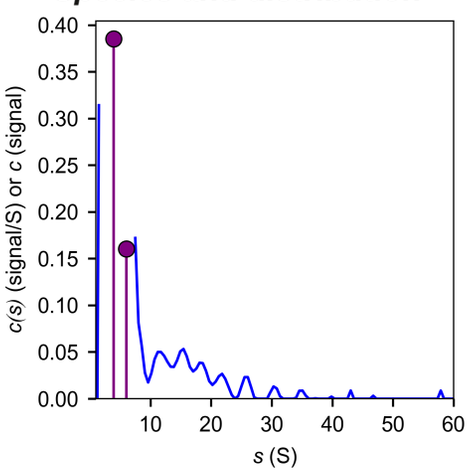
Reduced χ^2 [r.m.s.d]: 0.483025 [0.006950]

Hybrid model with continuous distribution and two discrete species (absorbance optics @ 30000 rpm)

Data, fit and residuals



Species and distribution



	Species 1		Species 2	
	Best fit	95% confidence intervals	Best fit	95% confidence intervals
Sedimentation coefficient $s_{20°C,w}$ (S)	3.92	3.70 - 4.06	6.01	5.25 - 6.61
Molecular mass *M* (kDa)	47.2	42.8 - 52.4	107.1	71.6 - 170.3
Diffusion coefficient *D* (10^{-7} cm²/s)				
Rel. molecular fraction *f*	0.706	n/a	0.294	n/a
Reduced *χ2* [r.m.s.d]	0.2267210 [0.004761523]			

Figure S9D

Figures S10:

Individual sedimentation velocity datasets of *C. elegans* UNC-6 ΔC

in 0.05 M tris, pH 7.5, 1.00 M NaCl

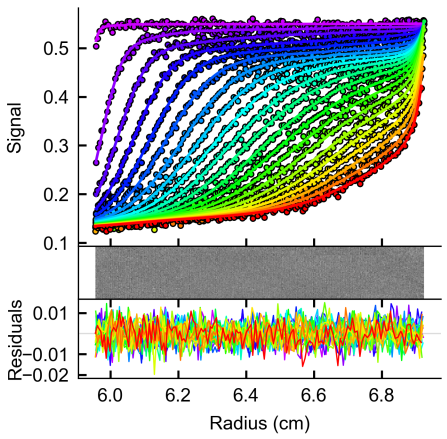
Figures were prepared using the computer software GUSSI (7) and Matplotlib (8).

0.19 mg/ml tag-free *C. elegans* UNC-6 ΔC in 1.00 M NaCl

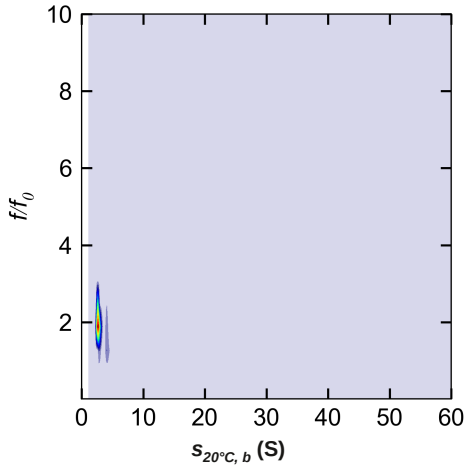
c(s, f_r) analysis

Absorbance optics @ 30000 rpm

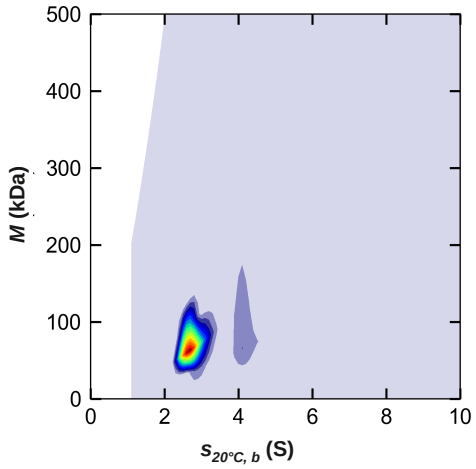
Data, fit and residuals



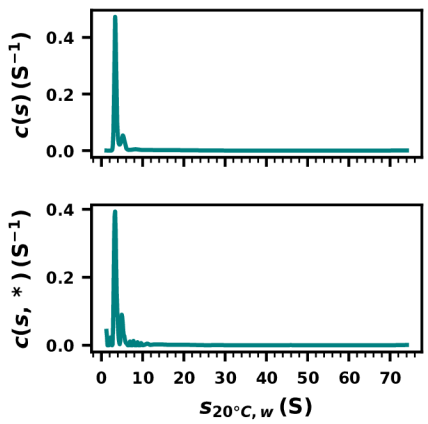
f_r vs. s



M vs. s



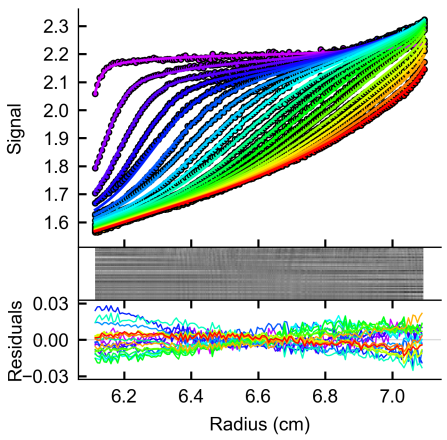
1D distributions



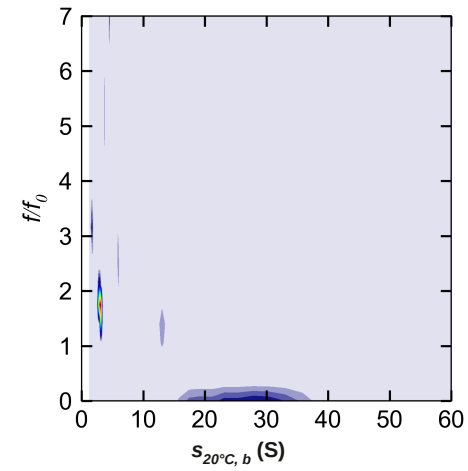
Reduced χ^2 [r.m.s.d]: 0.2324204 [0.004821]

Interference optics @ 30000 rpm

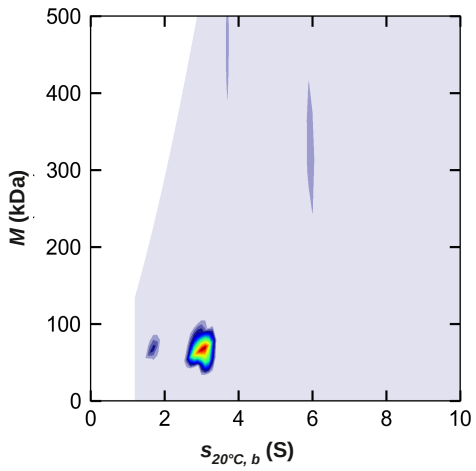
Data, fit and residuals



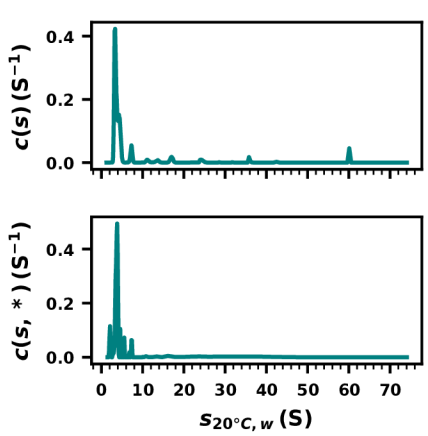
f_r vs. s



M vs. s



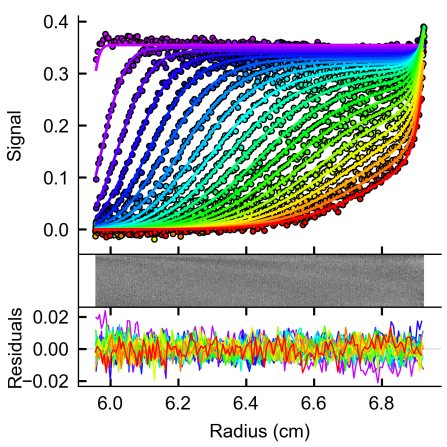
1D distributions



Reduced χ^2 [r.m.s.d]: 0.5041000 [0.007100]

Species analysis (absorbance optics @ 30000 rpm)

Data, fit and residuals



	Species 1		Species 2	
	Best fit	95% confidence intervals	Best fit	95% confidence intervals
Sedimentation coefficient $s_{20°C,w}$ (S)	3.28	3.02 - 3.43	4.96	4.07 - 6.34
Molecular mass *M* (kDa)	65.9	49.9 - 72.2	51.8	34.3 - 106.8
Diffusion coefficient *D* (10^{-7} cm²/s)	4.78	3.51 - 5.63	7.92	4.14 - 13.93
Molecular fraction *f*	0.785	0.462 - 0.904	0.215	0.096 - 0.538
Reduced *χ2* [r.m.s.d]	0.2765050 [0.005258374]			

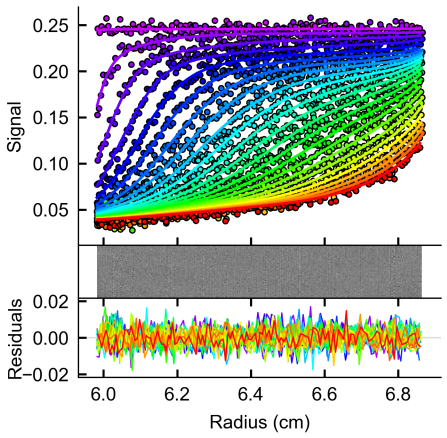
Figure S10A

0.57 mg/ml tag-free *C. elegans* UNC-6 ΔC in 1.00 M NaCl

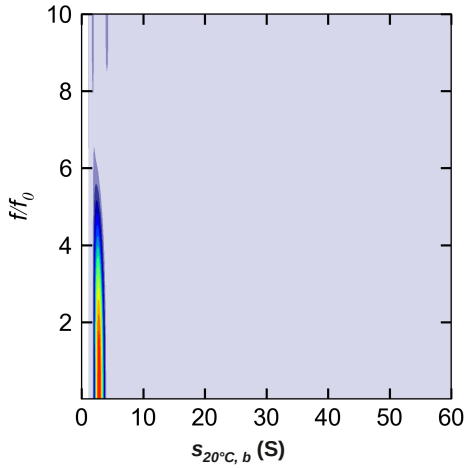
c(s, f_r) analysis

Absorbance optics @ 30000 rpm

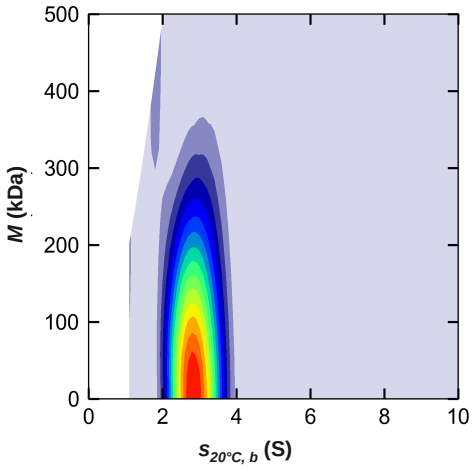
Data, fit and residuals



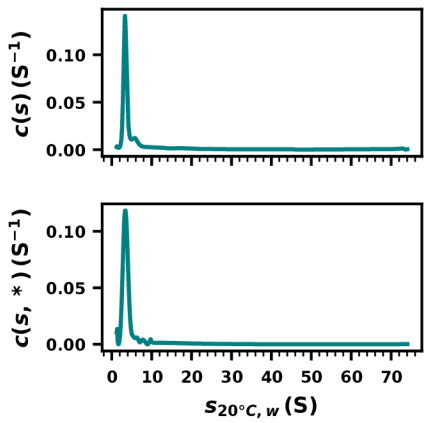
f_r vs. s



M vs. s



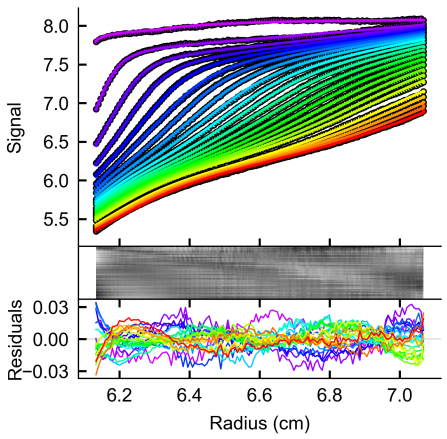
1D distributions



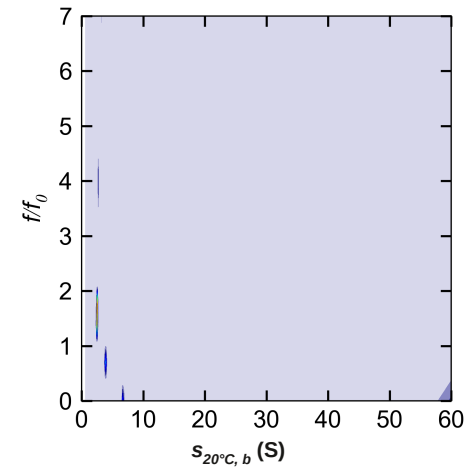
Reduced χ^2 [r.m.s.d]: 0.2216526 [0.004708]

Interference optics @ 30000 rpm

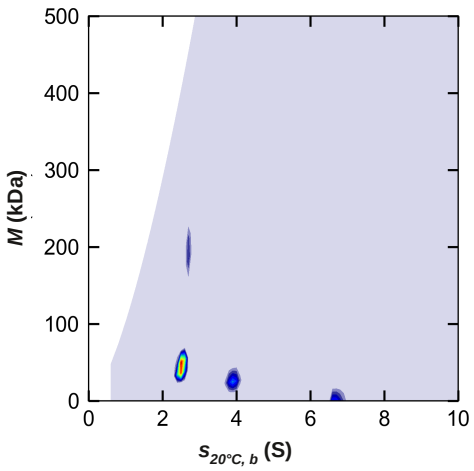
Data, fit and residuals



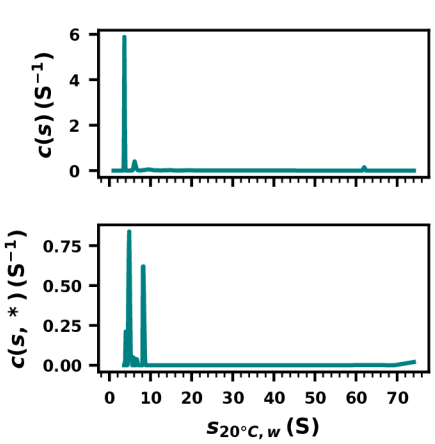
f_r vs. s



M vs. s



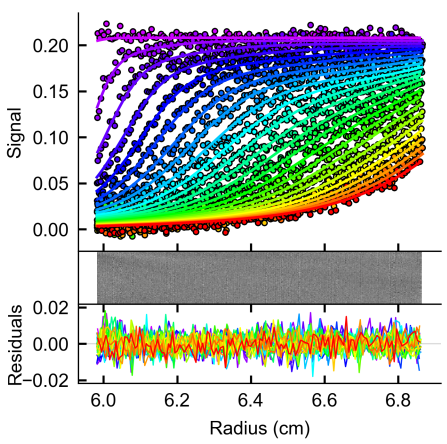
1D distributions



Reduced χ^2 [r.m.s.d]: 0.8691833 [0.009323]

Species analysis (absorbance optics @ 30000 rpm)

Data, fit and residuals



	Species 1		Species 2	
	Best fit	95% confidence intervals	Best fit	95% confidence intervals
Sedimentation coefficient $s_{20°C,w}$ (S)	3.36	3.21 - 3.49	4.45	3.34 - 7.69
Molecular mass *M* (kDa)	56.6	41.6 - 91.4	5.7	2.1 - 13.9
Diffusion coefficient *D* (10^{-7} cm²/s)	4.91	2.98 - 6.67	64.33	21.75 - 192.86
Molecular fraction *f*	0.575	0.376 - 0.822	0.425	0.178 - 0.624
Reduced *χ2* [r.m.s.d]	0.2306443 [0.004802544]			

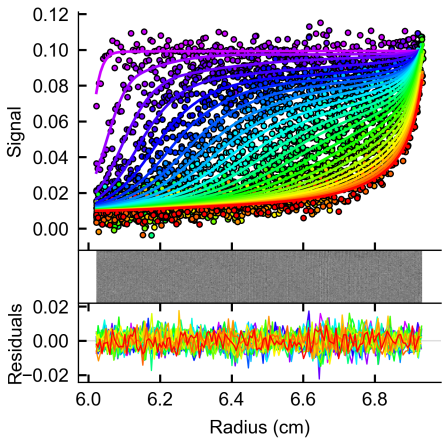
Figure S10B

0.95 mg/ml tag-free *C. elegans* UNC-6 ΔC in 1.00 M NaCl

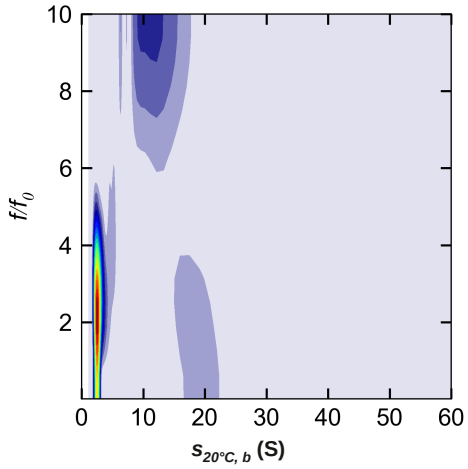
c(s, f_r) analysis

Absorbance optics @ 30000 rpm

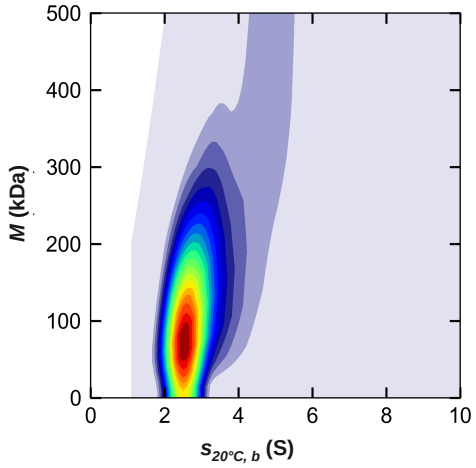
Data, fit and residuals



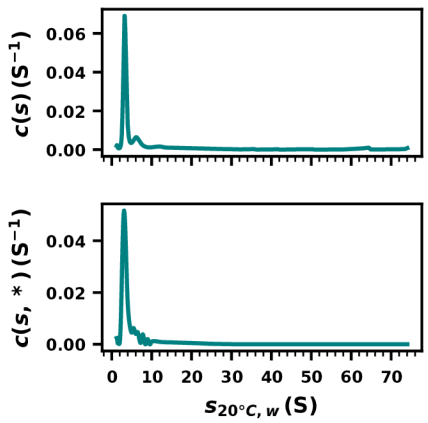
f_r vs. s



M vs. s



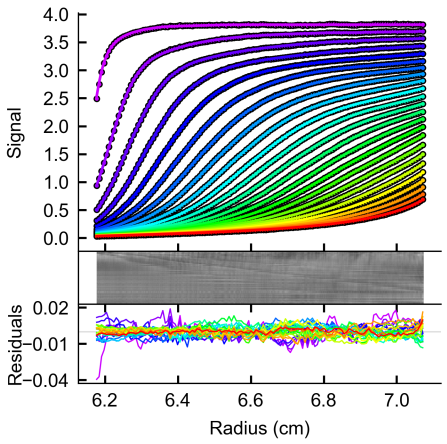
1D distributions



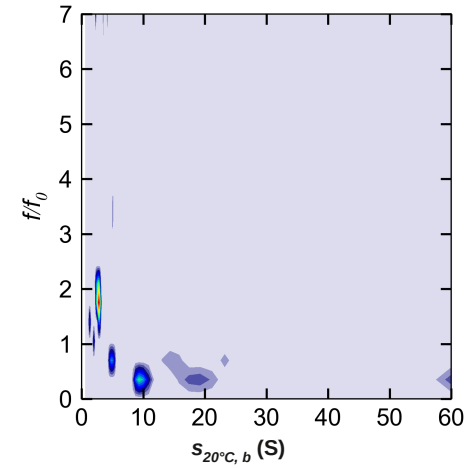
Reduced χ^2 [r.m.s.d]: 0.2281018 [0.004776]

Interference optics @ 30000 rpm

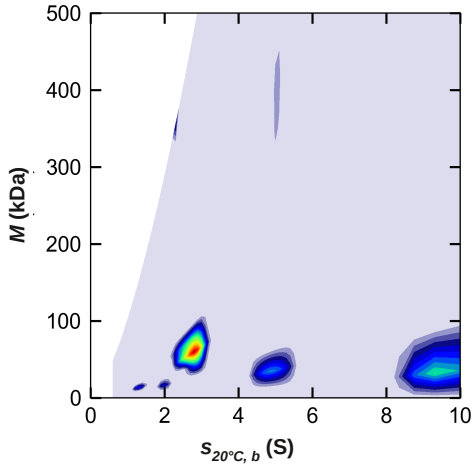
Data, fit and residuals



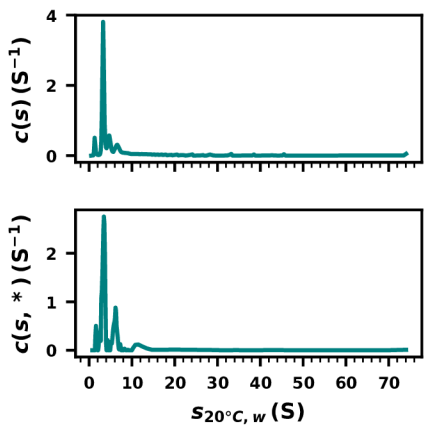
f_r vs. s



M vs. s



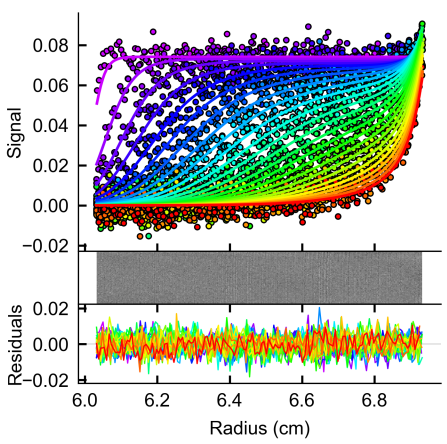
1D distributions



Reduced χ^2 [r.m.s.d]: 0.1921069 [0.004383]

Species analysis (absorbance optics @ 30000 rpm)

Data, fit and residuals



	Species 1		Species 2	
	Best fit	95% confidence intervals	Best fit	95% confidence intervals
Sedimentation coefficient $s_{20°C,w}$ (S)	2.94	2.04 - 3.46	4.67	3.20 - >20
Molecular mass *M* (kDa)	52.8	24.2 - >10^6	26.2	9.7 - >10^6
Diffusion coefficient *D* (10^{-7} cm²/s)	4.60	<0.01 - 11.27	14.74	6.71 - >500
Molecular fraction *f*	0.620	0.009 - 0.992	0.380	0.008 - 0.991
Reduced *χ2* [r.m.s.d]	0.2342305 [0.004839737]			

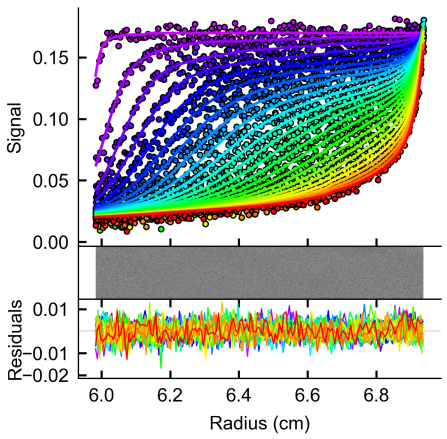
Figure S10C

1.90 mg/ml tag-free *C. elegans* UNC-6 ΔC in 1.00 M NaCl

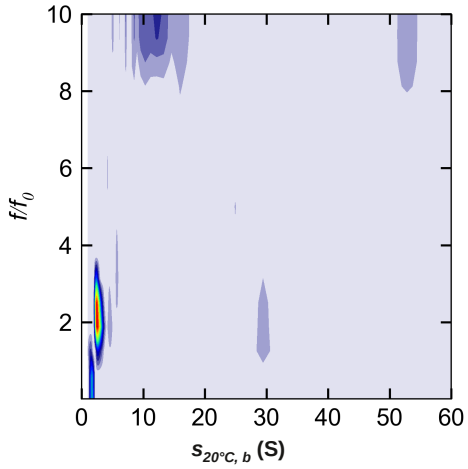
c(s, f_r) analysis

Absorbance optics @ 30000 rpm

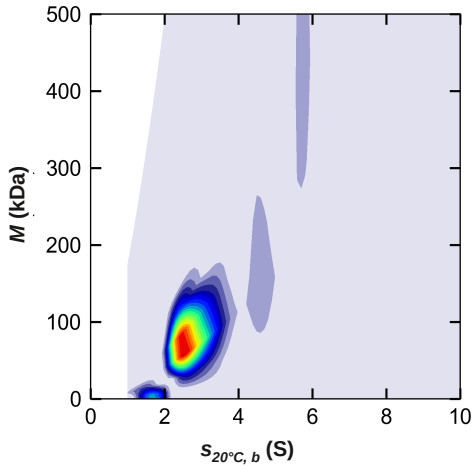
Data, fit and residuals



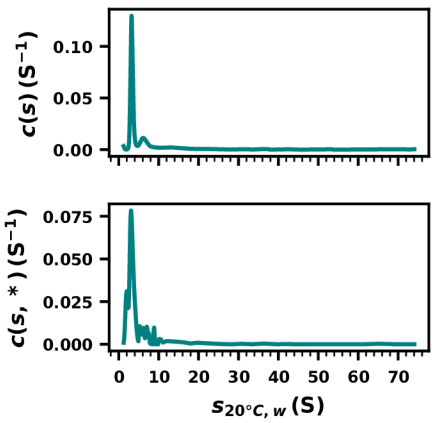
f_r vs. s



M vs. s



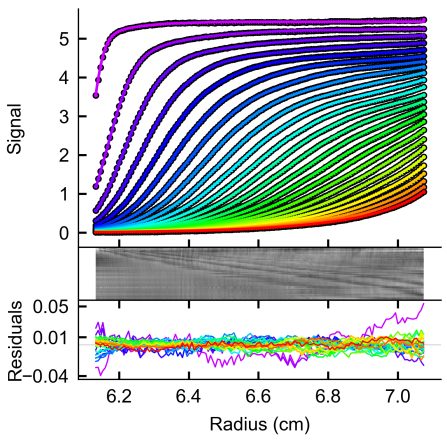
1D distributions



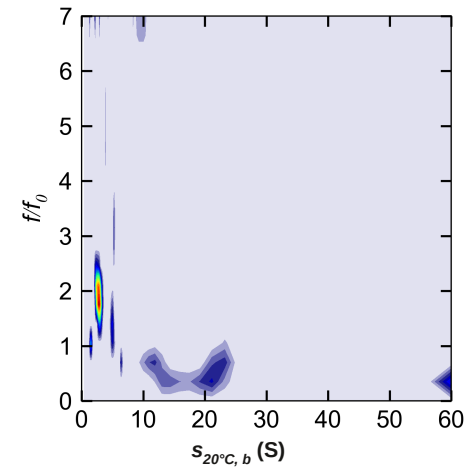
Reduced χ^2 [r.m.s.d]: 0.1394276 [0.003734]

Interference optics @ 30000 rpm

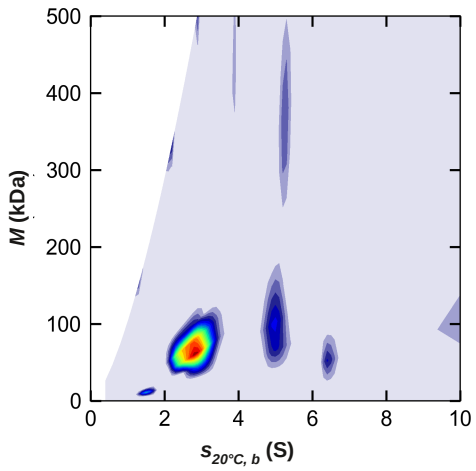
Data, fit and residuals



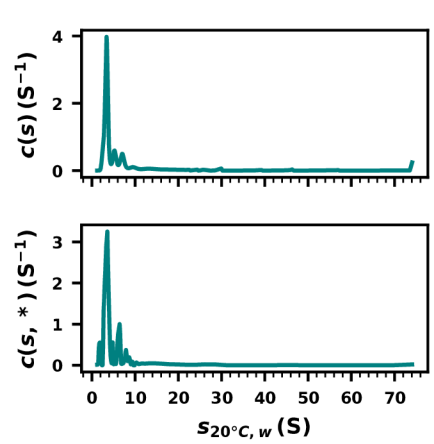
f_r vs. s



M vs. s



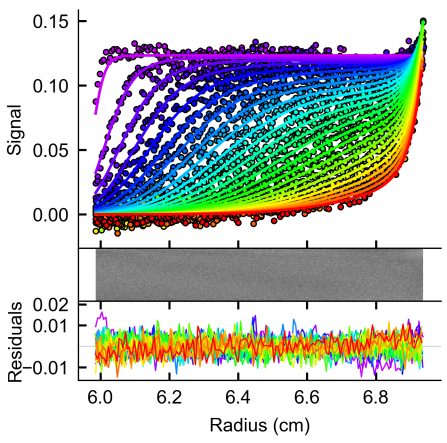
1D distributions



Reduced χ^2 [r.m.s.d]: 0.4769284 [0.006906]

Species analysis (absorbance optics @ 30000 rpm)

Data, fit and residuals



	Species 1		Species 2	
	Best fit	95% confidence intervals	Best fit	95% confidence intervals
Sedimentation coefficient $s_{20°C,w}$ (S)	3.06	2.80 - 3.28	6.59	4.39 - 9.54
Molecular mass *M* (kDa)	40 .5	30.1 - 70.2	30.5	20.7 - 112.0
Diffusion coefficient *D* (10^{-7} cm²/s)	6.26	3.36 - 8.79	17.89	5.27 - 30.06
Molecular fraction *f*	0.789	0.482 - 0.895	0.211	0.105 - 0.518
Reduced *χ2* [r.m.s.d]	0.1548478 [0.003935071]			

Figure S10D

Figures S11:

Individual sedimentation velocity datasets of *C. elegans* UNC-6 FL

in 0.05 M tris, pH 7.5, 0.20 M NaCl

2x STREPII-tagged *C. elegans* UNC-6 FL			
Property	**Value**	**Temperature**	**Source**
Partial specific volume $\bar{\nu}$	0.71101 cm³/g	20 °C	Sednterp
Molecular mass[1] M_w	76493.5 Da	20 °C	Sednterp
Hydration	0.386712 g/g	20 °C	Sednterp

[1]2xSTREPII-tagged *Caenorhabditis elegans* UNC-6 full-length with 6 common core pentasaccharides (12x β-D-N-Acetyl glucosamine (GlcNAc), 18x β-D-Mannose (Man), 6x glycosidic linkage)

0.05 M tris, pH 7.5, 0.20 M NaCl			
Density ρ	1.007900 g/cm³	20 °C	Sednterp
Viscosity η	0.010357 P	20 °C	Sednterp

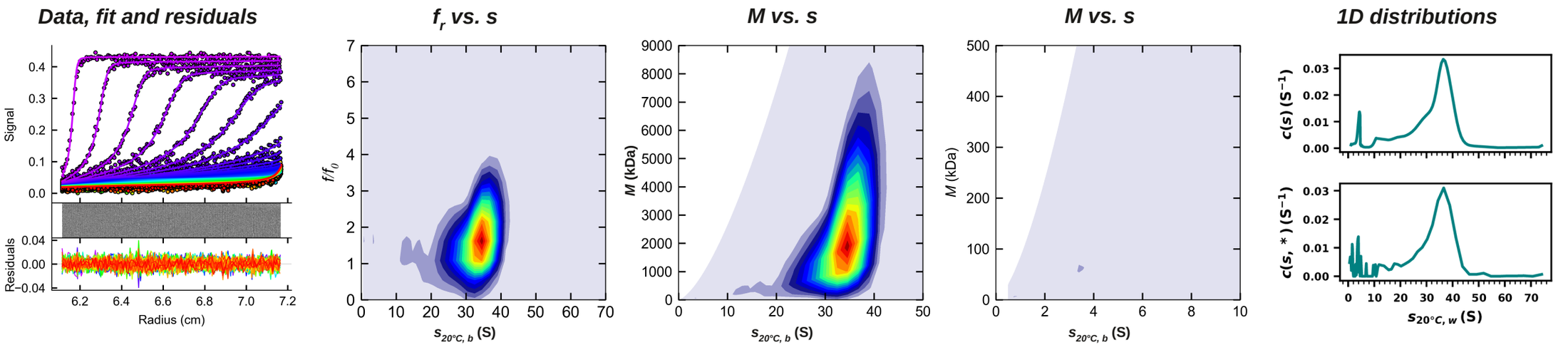
0.05 M tris, pH 7.5, 1.00 M NaCl			
Density ρ	1.040120 g/cm³	20 °C	Sednterp
Viscosity η	0.011131 P	20 °C	Sednterp

Figures were prepared using the computer software GUSSI (7) and Matplotlib (8).

0.33 mg/ml 2x STREPII-tagged *C. elegans* UNC-6 FL in 0.20 NaCl

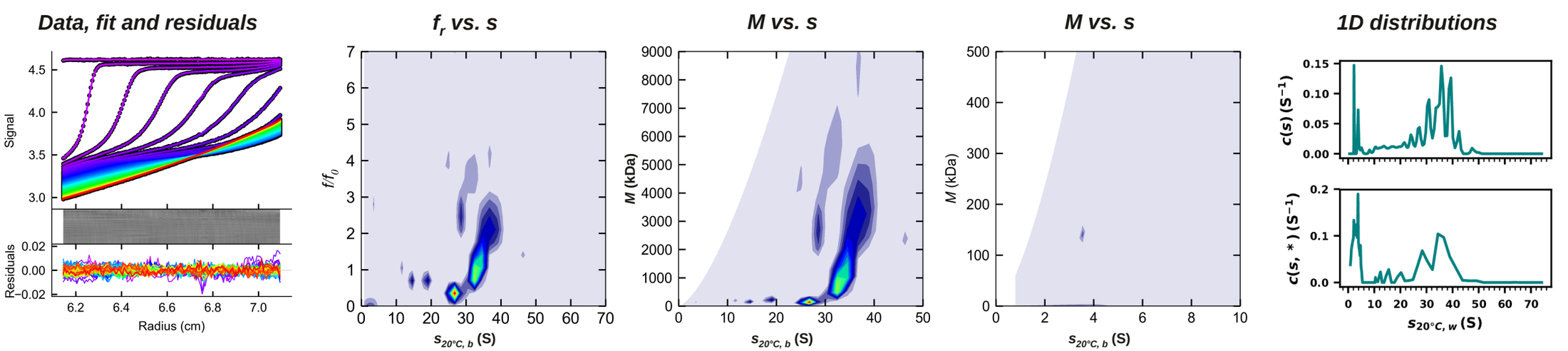
c(s, f_r) analysis

Absorbance optics @ 30000 rpm



Reduced χ^2 [r.m.s.d]: 0.4693620 [0.006851]

Interference optics @ 30000 rpm



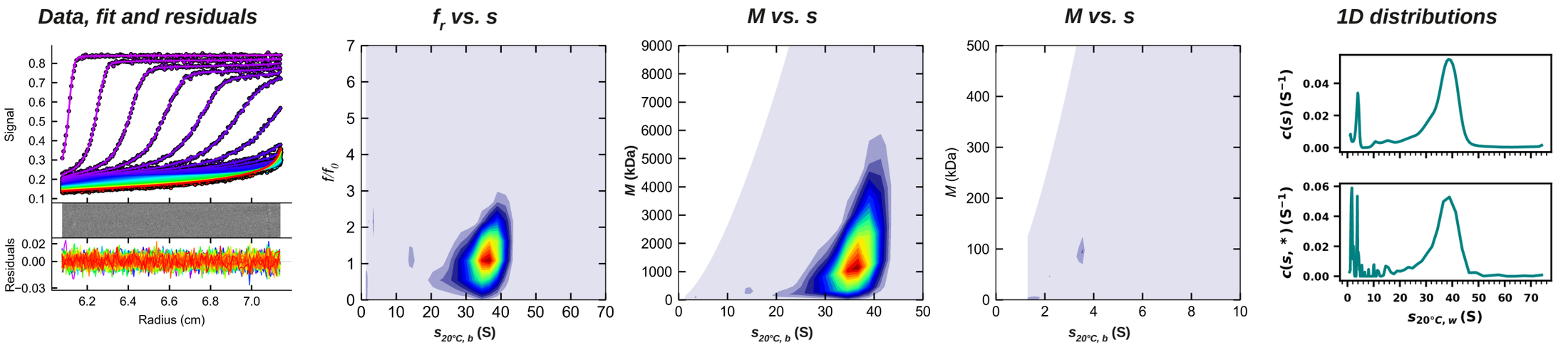
Reduced χ^2 [r.m.s.d]: 0.0730621 [0.002703]

Figure S11A

0.59 mg/ml 2x STREPII-tagged *C. elegans* UNC-6 FL in 0.20 NaCl

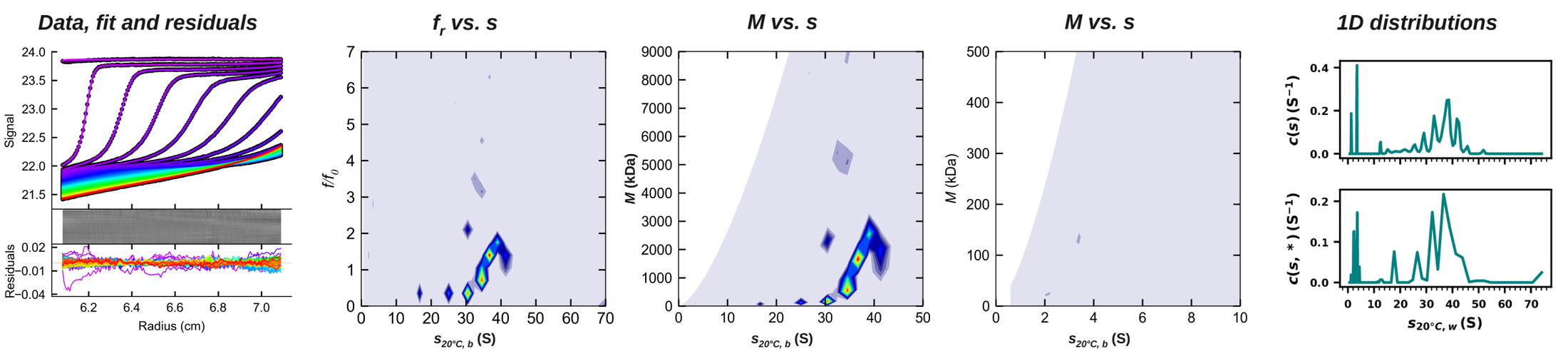
c(s, f_r) analysis

Absorbance optics @ 30000 rpm



Reduced χ^2 [r.m.s.d]: 0.2897669 [0.005383]

Interference optics @ 30000 rpm



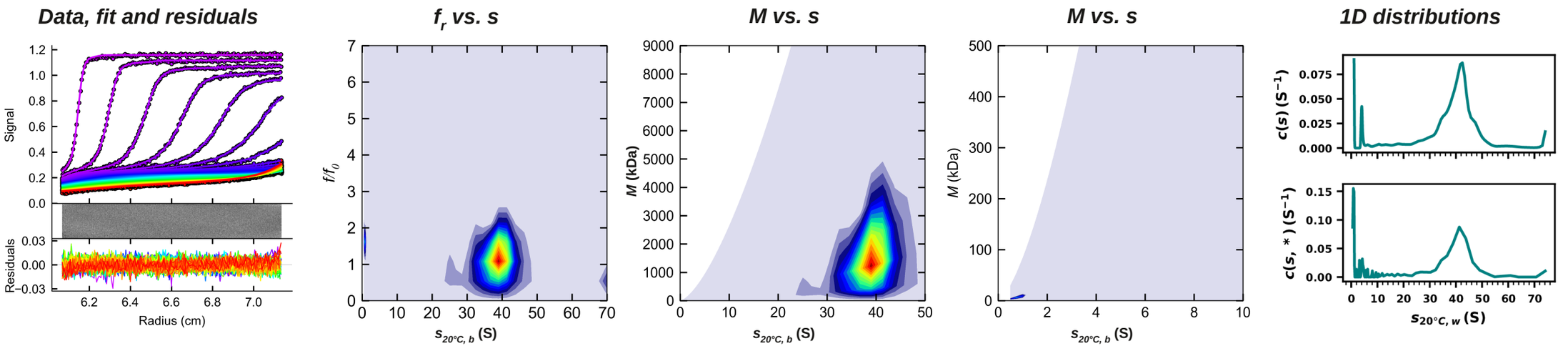
Reduced χ^2 [r.m.s.d]: 0.0964724 [0.003106]

Figure S11B

0.77 mg/ml 2x STREPII-tagged *C. elegans* UNC-6 FL in 0.20 NaCl

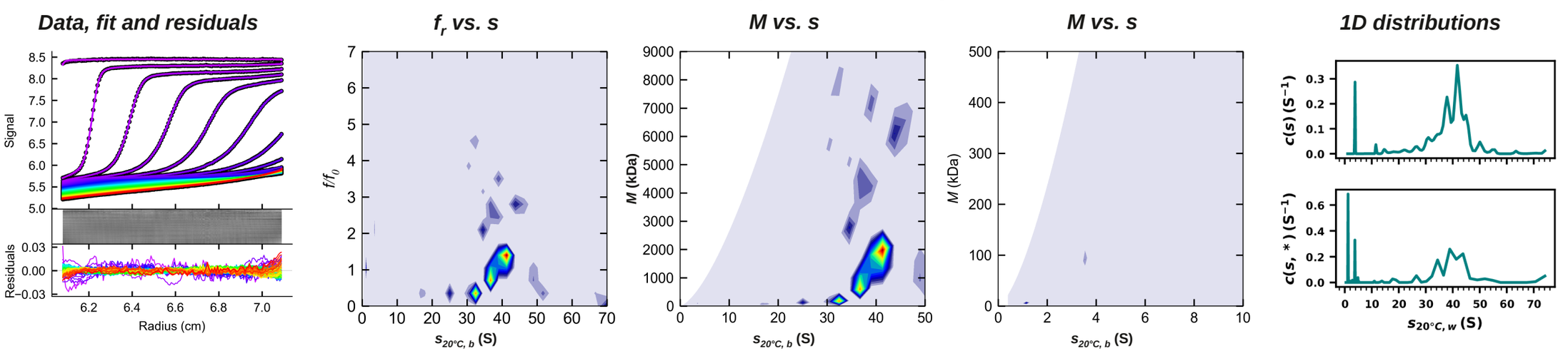
c(s, f_r) analysis

Absorbance optics @ 30000 rpm



Reduced χ^2 [r.m.s.d]: 0.3469210 [0.005890]

Interference optics @ 30000 rpm



Reduced χ^2 [r.m.s.d]: 0.1293841 [0.003597]

Figure S11C

Figures S12:

Individual sedimentation velocity datasets of *C. elegans* UNC-6 FL in 0.05 M tris, pH 7.5, 1.00 M NaCl

2x STREPII-tagged *C. elegans* UNC-6 FL			
Property	**Value**	**Temperature**	**Source**
Partial specific volume $\bar{\nu}$	0.71101 cm³/g	20 °C	Sednterp
Molecular mass[1] M_w	76493.5 Da	20 °C	Sednterp
Hydration	0.386712 g/g	20 °C	Sednterp

[1]2xSTREPII-tagged *Caenorhabditis elegans* UNC-6 full-length with 6 common core pentasaccharides (12x β-D-N-Acetyl glucosamine (GlcNAc), 18x β-D-Mannose (Man), 6x glycosidic linkage)

0.05 M tris, pH 7.5, 0.20 M NaCl			
Density ρ	1.007900 g/cm³	20 °C	Sednterp
Viscosity η	0.010357 P	20 °C	Sednterp

0.05 M tris, pH 7.5, 1.00 M NaCl			
Density ρ	1.040120 g/cm³	20 °C	Sednterp
Viscosity η	0.011131 P	20 °C	Sednterp

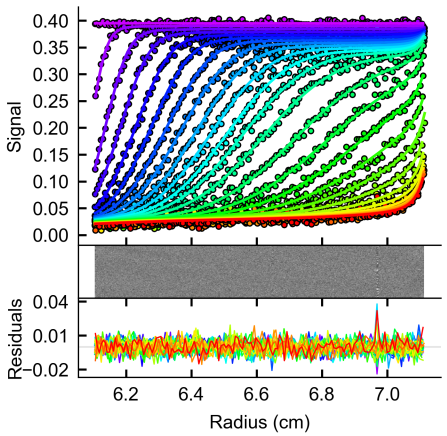
Figures were prepared using the computer software GUSSI (7) and Matplotlib (8).

0.31 mg/ml 2x STREPII-tagged *C. elegans* UNC-6 FL in 1.00 NaCl

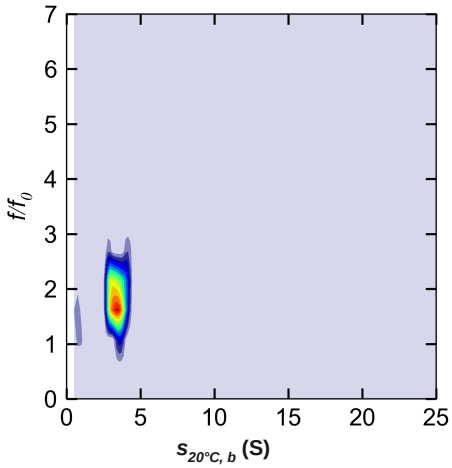
c(s, f_r) analysis

Absorbance optics @ 30000 rpm

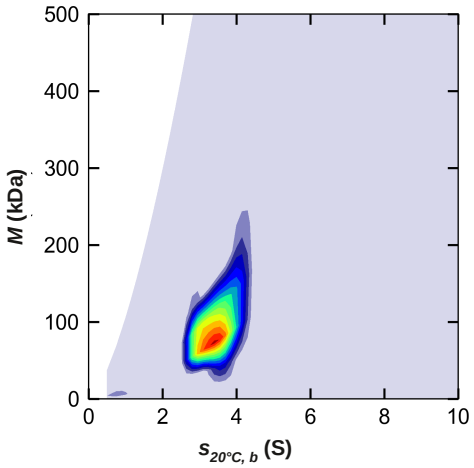
Data, fit and residuals



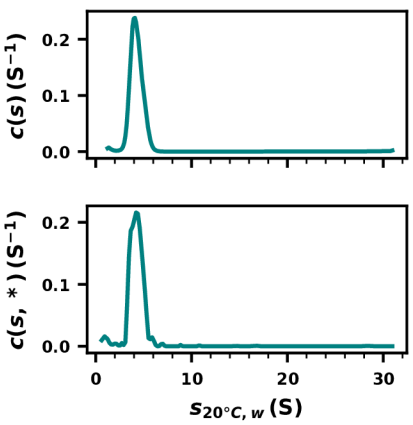
f_r vs. s



M vs. s



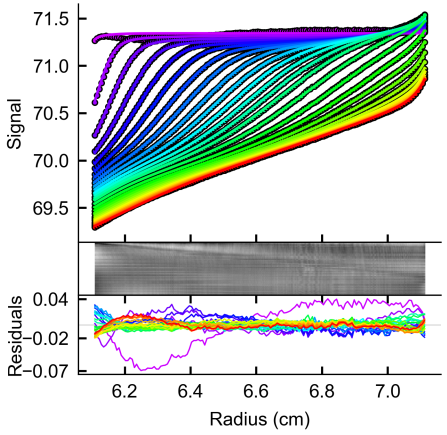
1D distributions



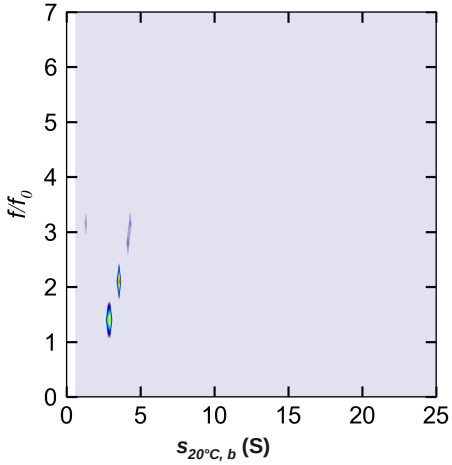
Reduced χ^2 [r.m.s.d]: 0.2775182 [0.005268]

Interference optics @ 30000 rpm

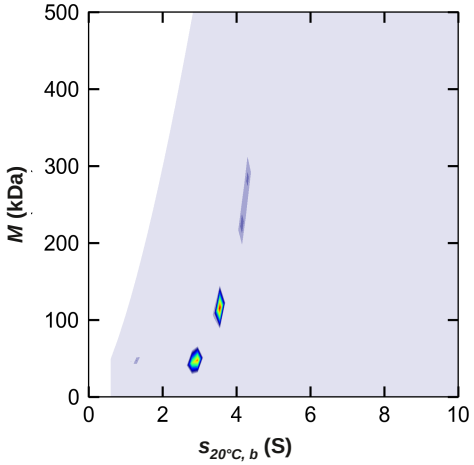
Data, fit and residuals



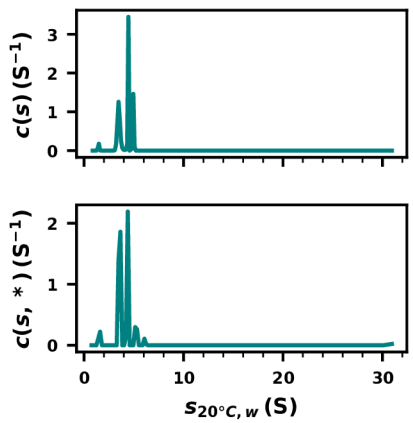
f_r vs. s



M vs. s



1D distributions



Reduced χ^2 [r.m.s.d]: 0.6760128 [0.008222]

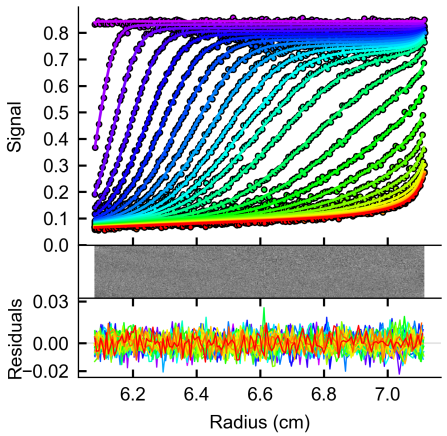
Figure S12A

0.61 mg/ml 2x STREPII-tagged *C. elegans* UNC-6 FL in 1.00 NaCl

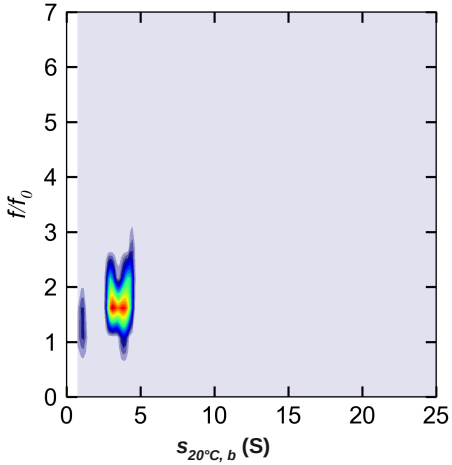
c(s, f_r) analysis

Absorbance optics @ 30000 rpm

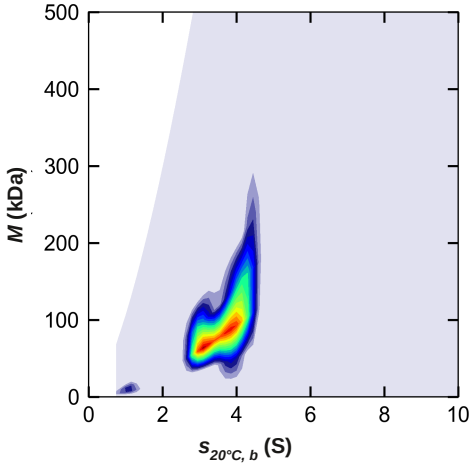
Data, fit and residuals



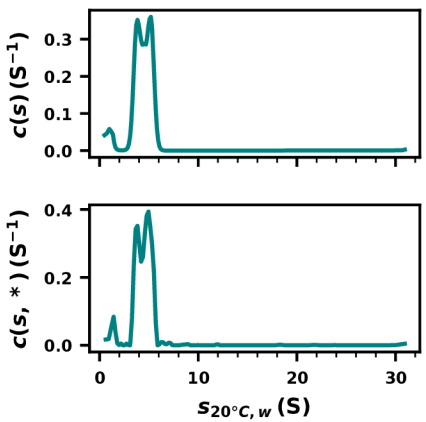
f_r vs. s



M vs. s



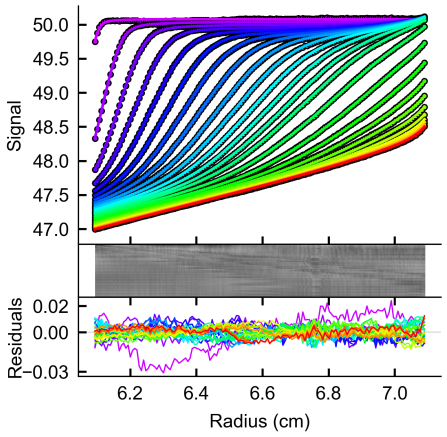
1D distributions



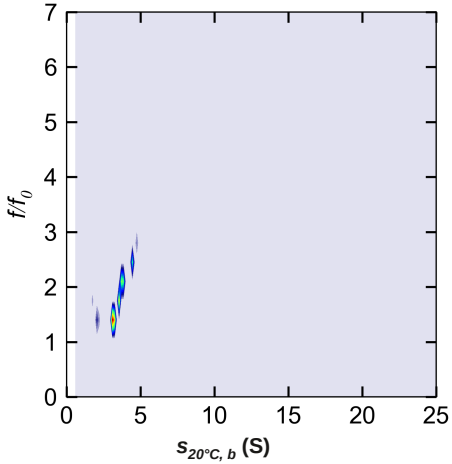
Reduced χ^2 [r.m.s.d]: 0.3343152 [0.005782]

Interference optics @ 30000 rpm

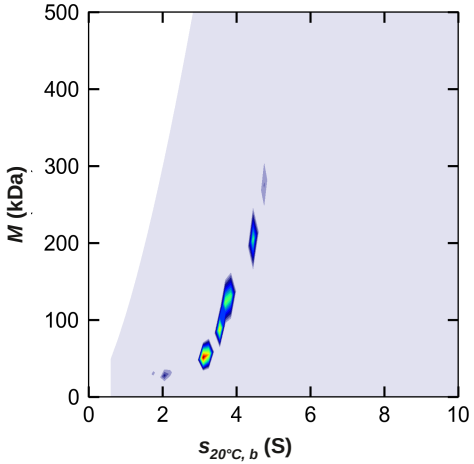
Data, fit and residuals



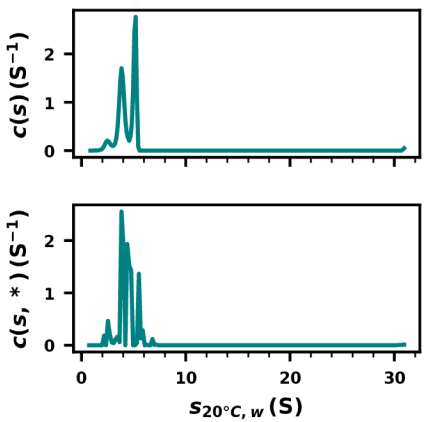
f_r vs. s



M vs. s



1D distributions



Reduced χ^2 [r.m.s.d]: 0.214369 [0.004630]

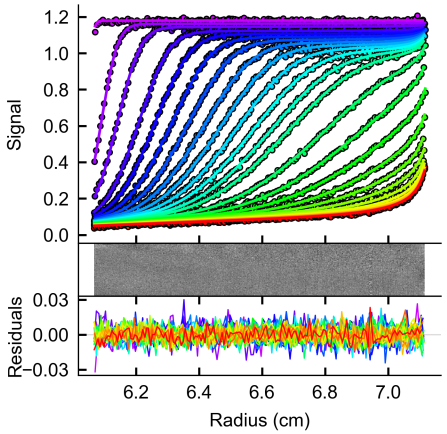
Figure S12B

0.88 mg/ml 2x STREPII-tagged *C. elegans* UNC-6 FL in 1.00 NaCl

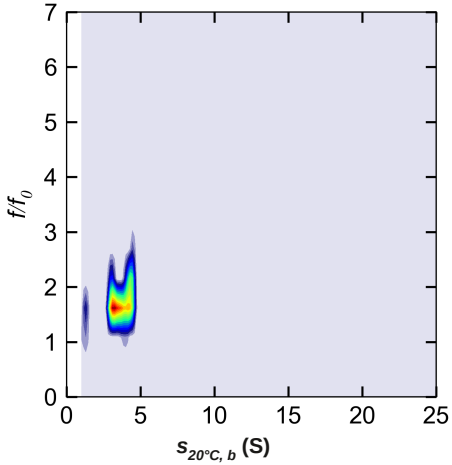
c(s, f_r) analysis

Absorbance optics @ 30000 rpm

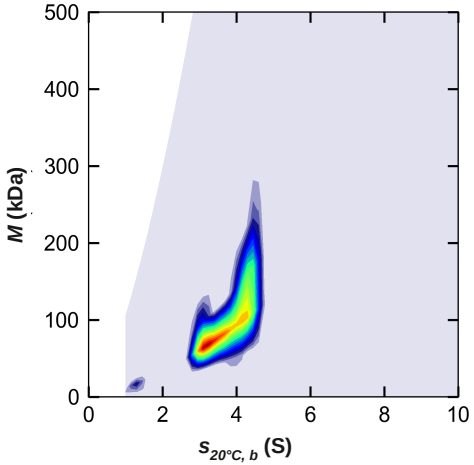
Data, fit and residuals



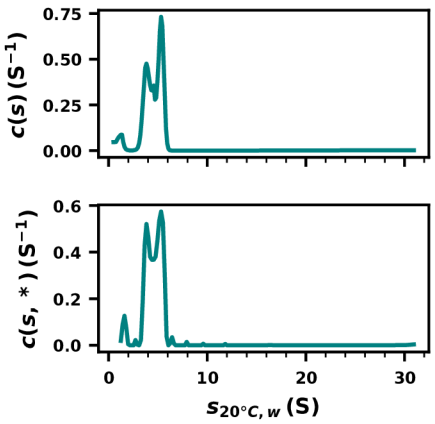
f_r vs. s



M vs. s



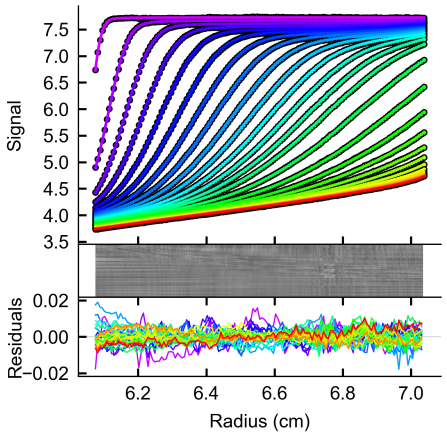
1D distributions



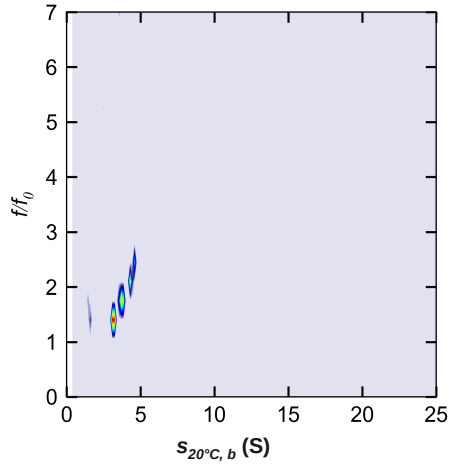
Reduced χ^2 [r.m.s.d]: 0.4215905 [0.006493]

Interference optics @ 30000 rpm

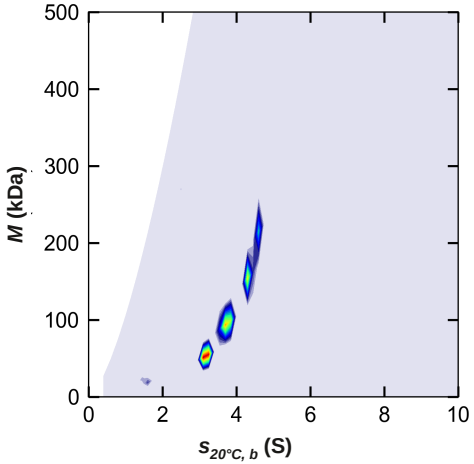
Data, fit and residuals



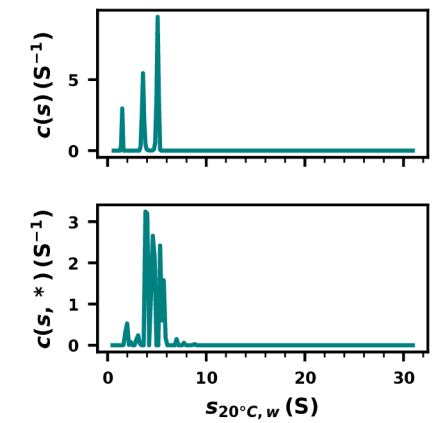
f_r vs. s



M vs. s



1D distributions



Reduced χ^2 [r.m.s.d]: 0.163216 [0.004040]

Figure S12C

Supporting References

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