Molecular characterization of the RNA-protein complex directing -2/-1 programmed ribosomal frameshifting during arterivirus replicase expression

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Supporting Information

Table S1. Primers used for nsp1β and PCBP2 mutations for EMSAs

Oligo Name	Sequence (5'to 3')
nsp1β Y131A Forward	CCAACGCAGGCTTCAAGTCCG
nsp1β Y131A Reverse	AGCGCCTTACCATGCACGCCCC
nsp1β R135A Forward	CAAGTCCGCGGCATGCGTGC
nsp1β R135A Reverse	AGCGCGCGTTGGAGGTACTTAC
PCBP2 R40A Forward	GGAGAGTGGTGCACGTATCAAC
PCBP2 R40A Reverse	TCCGCCATCTTCTTAACTGATTC
PCBP2 R57A Forward	TATCACTTTGGCTGGACCCAC
PCBP2 R57A Reverse	ATCGCCTCAGGACAATTCCCTTC
PCBP2 N325D Forward	CAAAATTGCGGATCCAGTGGAAGG
PCBP2 N325D Reverse	ATCTGCGCCCCAGACATC

Table S2. Primers used for frameshifting assays

Oligo Name	Sequence (5' to 3')	Purpose
EUnsp1b-G129A-fw	GGGCGTGCATGCTAAGTACCTCCAACGCAG	Site directed mutagenesis G129A
EUnsp1b-G129A-rev	TGGAGGTACTTAGCATGCACGCCCCACTTGG	Site directed mutagenesis G129A
EUnsp1b-K130A-fw	GGCGTGCATGGTGCUTACCTCCAACGCAG	Site directed mutagenesis K130A
EUnsp1b-K130A-rev	TGCGTTGGAGGTAAGCACCATGCACGCC	Site directed mutagenesis K130A
EUnsp1b-Y131A-fw	TGCATGGTAAGGCCCTCCAACGCAGGCTTC	Site directed mutagenesis Y131A
EUnsp1b-Y131A-rev	TGCGTTGGAGGGCCTTACCATGCACGCCCC	Site directed mutagenesis Y131A
EUnsp1b-L132A-fw	GCATGGTAAGTACGCCCAACGCAGGCTTCAAGTC	Site directed mutagenesis L132A
EUnsp1b-L132A-rev	GAAGCCTGCGTTGGGCGTACTTACCATGCACG	Site directed mutagenesis L132A
EUnsp1b-Q133A-fw	TAAGTACCTCGCACGCAGGCTTCAAGTCCG	Site directed mutagenesis Q133A
EUnsp1b-Q133A-rev	GAAGCCTGCGTGCGAGGTACTTACCATGCAC	Site directed mutagenesis Q133A

EUnsp1b-L136A-fw	CCAACGCAGGGCTCAAGTCCGCGGCATGC	Site directed mutagenesis L136A
EUnsp1b-L136A-rev	CATGCCGCGGACTTGAGCCCTGCGTTGGAG	Site directed mutagenesis L136A
EUnsp1b-Q137A-fw	TCCAACGCAGGCTTGCAGTCCGCGGCATGC	Site directed mutagenesis Q137A
EUnsp1b-Q137A-rev	CCGCGGACTGCAAGCCTGCGTTGGAGGTAC	Site directed mutagenesis Q137A
EUnsp1b-V138A-fw	GCAGGCTTCAAGCCCGCGGCATGCGTGCTG	Site directed mutagenesis V138A
EUnsp1b-V138A-rev	GCATGCCGCGGGCTTGAAGCCTGCGTTGGAG	Site directed mutagenesis V138A
EUnsp1b-R139A-fw	CTTCAAGTCGCTGGCATGCGTGCTGTGGTC	Site directed mutagenesis R139A
EUnsp1b-R139A-rev	CACAGCACGCATGCCAGCGACTTGAAGCC	Site directed mutagenesis R139A
EUnsp1b-G140A-fw	TTCAAGTCCGCGCCATGCGTGCTGTGGTCG	Site directed mutagenesis G140A
EUnsp1b-G140A-rev	CAGCACGCATGGCGCGGACTTGAAGCCTGC	Site directed mutagenesis G140A

EUnsp1b-M141A-fw	AGTCCGCGGCGCTCGTGCTGTGGTCGATCC	Site directed mutagenesis M141A
EUnsp1b-M141A-rev	CCACAGCACGAGCGCCGCGGACTTGAAGCC	Site directed mutagenesis M141A
EUnsp1b-R142A-fw	CCGCGGCATGGCTGCTGTGGTCGATCCTG	Site directed mutagenesis R142A
EUnsp1b-R142A-rev	CGACCACAGCAGCCATGCCGCGGACTTGAAG	Site directed mutagenesis R142A
EUnsp1b-fw	ACGAGAATTCCCATGGATGTCTGACGTTTACAGGTGGAAG	Amplify EUnsp1b and introduce upstream EcoRI and NcoI sites
3xFLAG-EUnsp1b-fw	GCCGAATTCCCATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGTCTGACGTTTACAGGTGGAAG	Amplify EUnsp1b and introduce upstream 3xFLAG tag and NcoI site
EUnsp1b-rev	ATCACCTGCAGGGCGGCCGCCTCGAGTCAGCCATACCACTTATGTG	Amplify EUnsp1b and introduce downstream XhoI, NotI and SbfI restriction sites
pL-seq	GATCTGATCTGGGGCCTC	Sequencing pL vector

Part 2

Biophysical characterization

Parameters	
Solvent **1x PBS (pH 7.4 @ 25°C), 100 mM KCl, 5% glycerol**	2.027 mM sodium dihdyrogen phosphate 7.973 mM disodium hydrogen phosphate 138 mM sodium chloride 102.7 mM potassium chloride ~684 mM glycerol
Solvent density[1] ρ (g/cm^3)	1.0354 ± 0.0005
Buffer viscosity[1] η (P)	0.01235 ± 0.0002
Temperature T (°C)	20.0
his$_6$-PCBP2 partial specific volume[1] $\bar{\nu}$ (cm^3/g)	0.73318
his$_6$-PCBP2 formula mass[2] M_w (kDa)	39749.32
his$_6$- PCBP2 absorption coefficient[2] ε_{280} (M^{-1} cm^{-1})	14815
PRRSV nsp1β partial specific volume[1] $\bar{\nu}$ (cm^3/g)	0.72777
PRRSV nsp1β formula mass[2] M_w (kDa)	23786.87
PRRSV nsp1β absorption coefficient[2] ε_{280} (M^{-1} cm^{-1})	46200
34nt ssRNA partial specific volume $\bar{\nu}$ (cm^3/g)	dependent on cation concentration expected range 0.50 - 0.65
34nt ssRNA formula mass[3] M_w (kDa)	10688.3
34nt ssRNA absorption coefficient[3] ε_{260} (M^{-1} cm^{-1})	323000

[1]Sednterp, [2]ProtParam, [3]IDTDNA

Table S3

Section 1:
Characterization of 34 nt ssRNA by Sedimentation Velocity

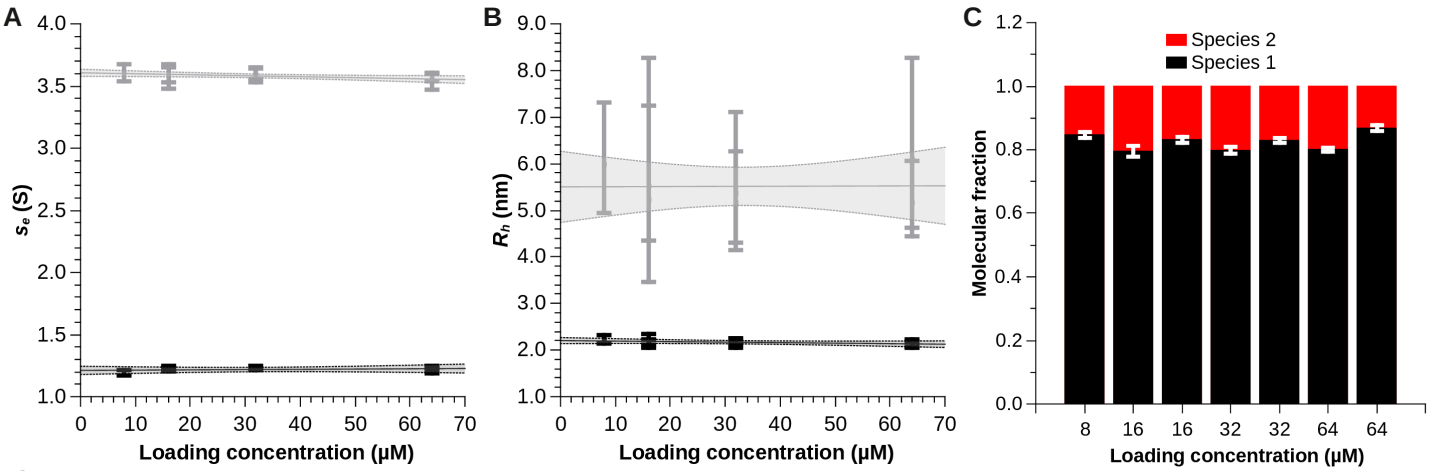


Fig. S1

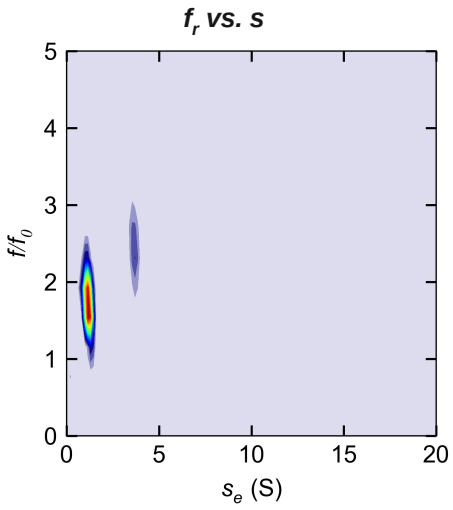
Results

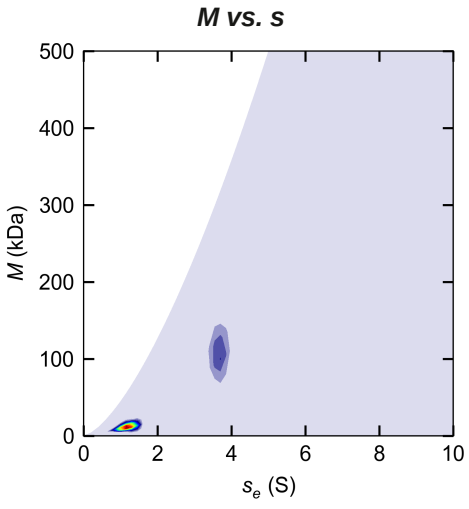
Species 1	
Sedimentation coefficient s^0_e	1.21 ± 0.04 S
Diffusion coefficient D^0_e	7.9 ± 0.3 ·10⁻⁷ cm²/s
Partial specific volume that yields correct mass (10.7 kDa)	0.62763 [0.60703 - 0.64704] cm³/g
Sedimentation coefficient $s^0_{20°C,\,w}$	1.59 [1.54 - 1.65] S
Hydrodynamic radius R_h^0	2.20 [2.12 - 2.29] nm
Species 2	
Sedimentation coefficient s^0_e	3.61 ± 0.03 S
Diffusion coefficient D^0_e	3.2 ± 0.5 ·10⁻⁷ cm²/s
Sedimentation coefficient $s^0_{20°C,\,w}$	4.74 [4.65 - 4.83] S
Hydrodynamic radius R_h^0	5.49 [4.84 - 6.35] nm
Molecular mass if RNA; would contain 7-8 strands	79.3 [68.2 - 90.5] kDa

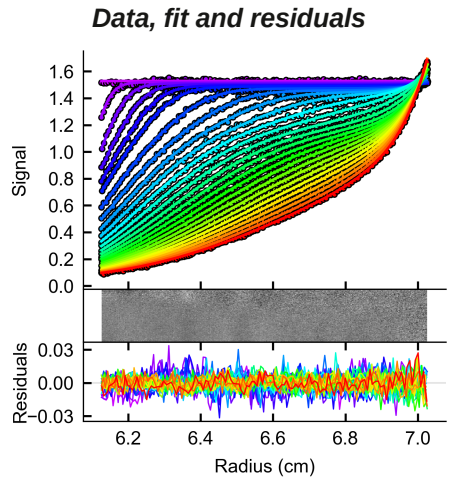
Uncertainties are given as 95% confidence intervals

Table S4

8 µM ssRNA monitored by absorbance optics
30000 rpm, λ = 278 nm



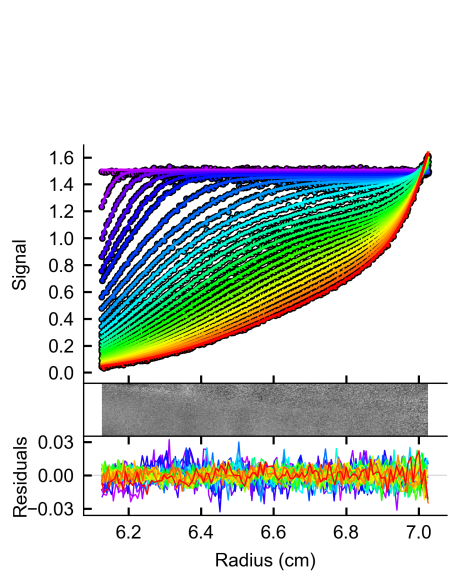




Reduced χ^2 [r.m.s.d]: 0.546565 [0.007393]

Species Analysis (30000 rpm, λ = 278 nm, exp. # 1)

Parameter	Value	95% confidence interval
Species 1		
Sedimentation coefficient s_e (S)	1.191056	1.1725 - 1.2098
Diffusion coefficient D_e (·10⁻⁷ cm²/s)	7.826990	7.5122 - 8.1480
Species 2		
Sedimentation coefficient s_e (S)	3.605013	3.5373 - 3.6741
Diffusion coefficient D_e (·10⁻⁷ cm²/s)	2.905149	2.3804 - 3.5159
Fraction (signal)	0.153196	0.1439 - 0.1631
Common		
Total concentration (signal)	1.508362	1.4841 - 1.5328
Meniscus position (cm)	6.081005	6.0774 - 6.0844
Bottom position (cm)	7.237708	7.2307 - 7.2447
Reduced χ2 of fit [rmsd]	0.5652639	
R.m.s.d. of fit	0.007518403	

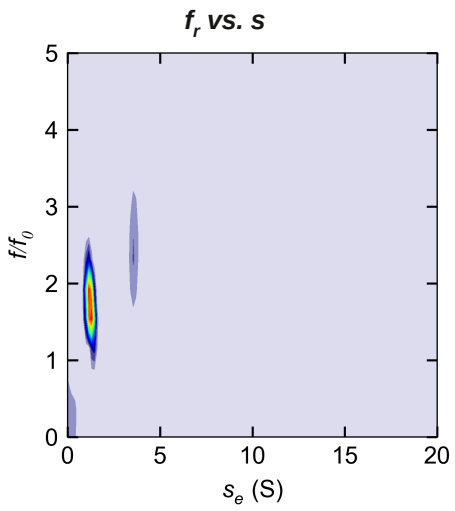


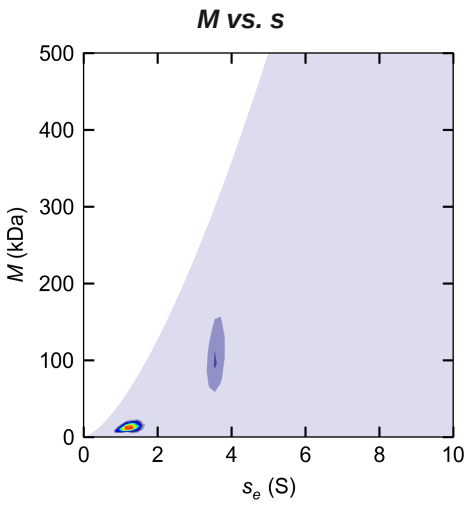
Reduced χ^2 [r.m.s.d]: 0.565264 [0.007518]

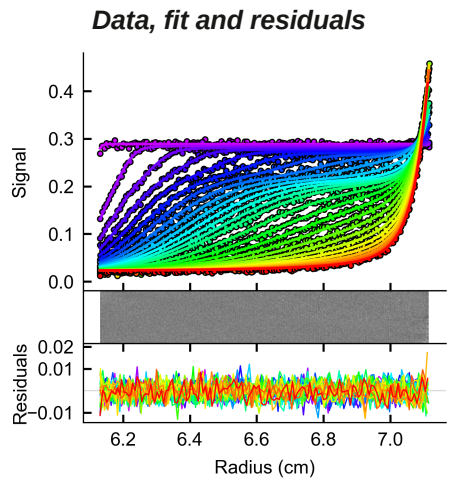
Fig. S2A

16 µM ssRNA monitored by absorbance optics

42000 rpm, λ = 294 nm

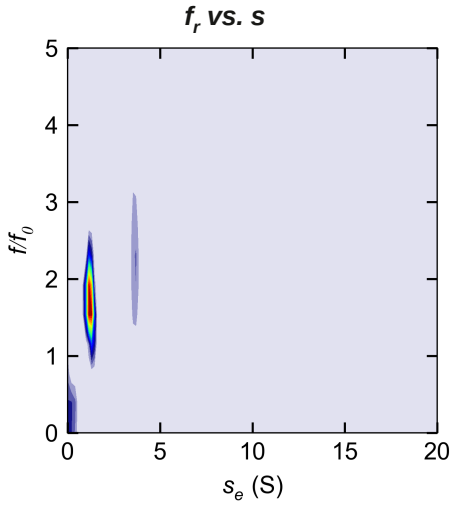


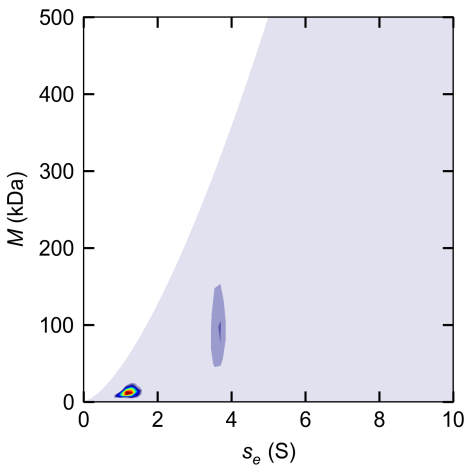


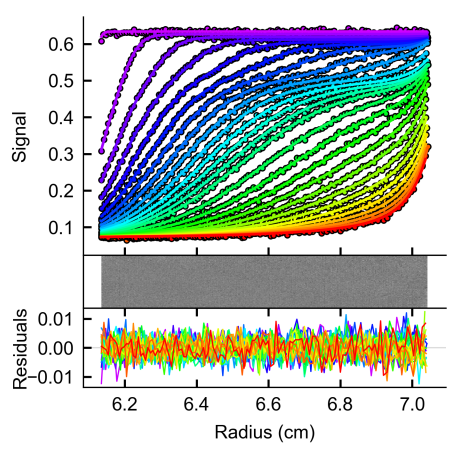


Reduced χ^2 [r.m.s.d]: 0.120617 [0.003473]

42000 rpm, λ = 291 nm



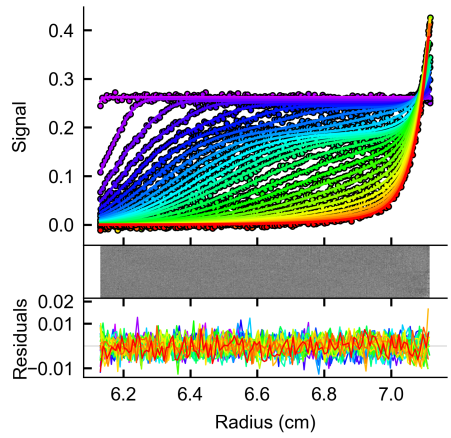




Reduced χ^2 [r.m.s.d]: 0.112694 [0.003357]

Species Analysis (42000 rpm, λ = 294 nm, exp. # 1)

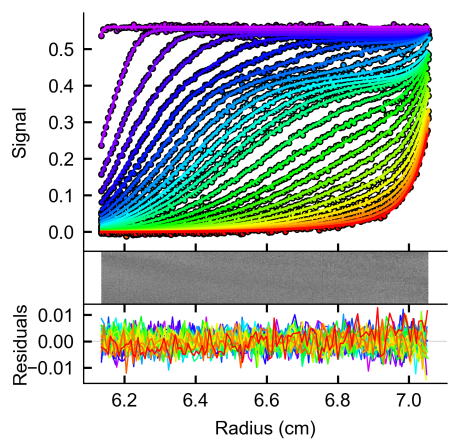
Parameter	Value	95% confidence interval
Species 1		
Sedimentation coefficient s_e (S)	1.229725	1.2078 - 1.2518
Diffusion coefficient D_e (·10⁻⁷ cm²/s)	7.837109	7.4467 - 8.2514
Species 2		
Sedimentation coefficient s_e (S)	3.579254	3.4808 - 3.6784
Diffusion coefficient D_e (·10⁻⁷ cm²/s)	3.316965	2.1005 - 5.0301
Fraction (signal)	0.204758	0.1884 - 0.2216
Common		
Total concentration (signal)	0.2623926	0.2545 - 0.2705
Meniscus position (cm)	6.113078	6.1073 - 6.1188
Bottom position (cm)	7.227485	7.2230 - 7.2323
Reduced χ2 of fit [rmsd]	0.1228484	
R.m.s.d. of fit	0.003504974	



Reduced χ^2 [r.m.s.d]: 0.122848 [0.003505]

Species Analysis (42000 rpm, λ = 291 nm, exp. # 2)

Parameter	Value	95% confidence interval
Species 1		
Sedimentation coefficient s_e (S)	1.215424	1.2044 - 1.2265
Diffusion coefficient D_e (·10⁻⁷ cm²/s)	8.173451	7.8048 - 8.4944
Species 2		
Sedimentation coefficient s_e (S)	3.590108	3.5299 - 3.6475
Diffusion coefficient D_e (·10⁻⁷ cm²/s)	3.140034	2.3970 - 4.0048
Fraction (signal)	0.168034	0.1588 - 0.1769
Common		
Total concentration (signal)	0.5605898	0.1588 - 0.1769
Meniscus position (cm)	6.117462	6.1147 - 6.1205
Bottom position (cm)	7.234521	7.2271 - 7.2411
Reduced χ2 of fit [rmsd]	0.1260963	
R.m.s.d. of fit	0.003551004	

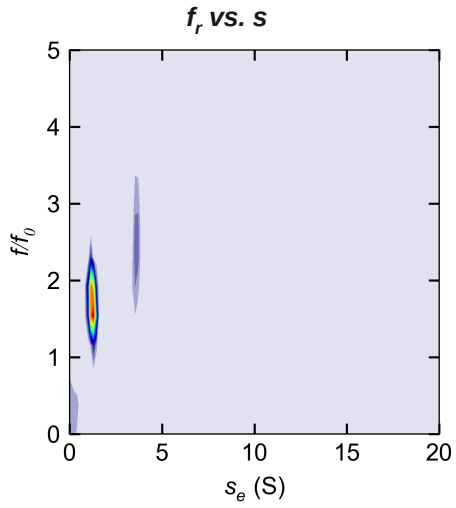


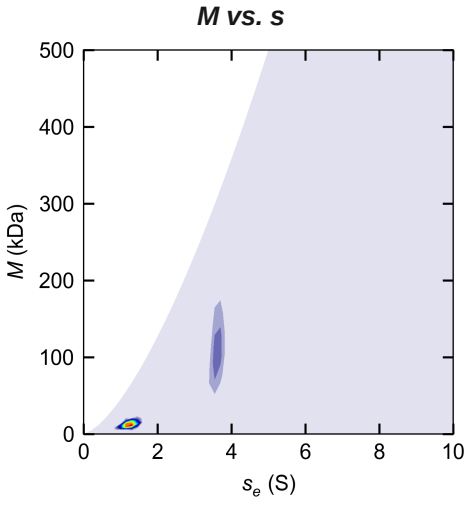
Reduced χ^2 [r.m.s.d]: 0.126096 [0.003551]

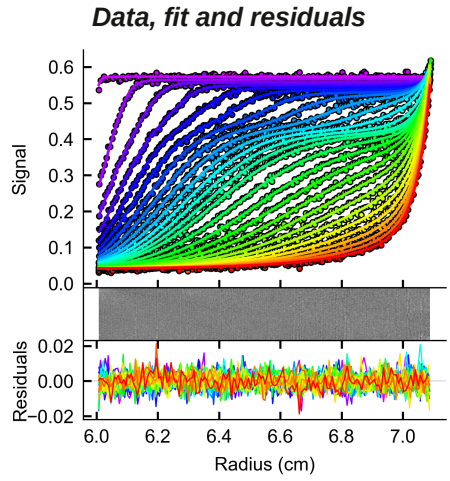
Fig. S2B

32 µM ssRNA monitored by absorbance optics

42000 rpm, λ = 294 nm

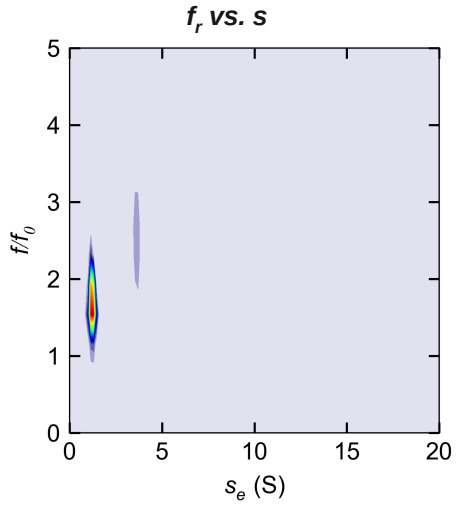


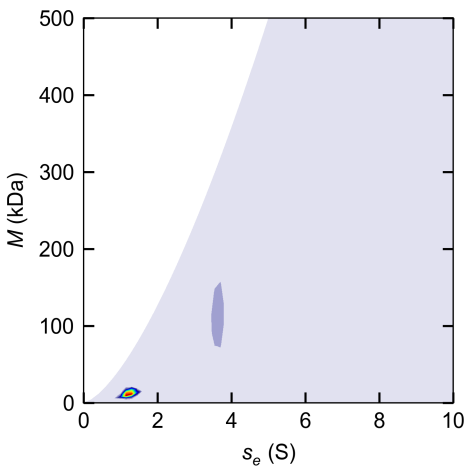


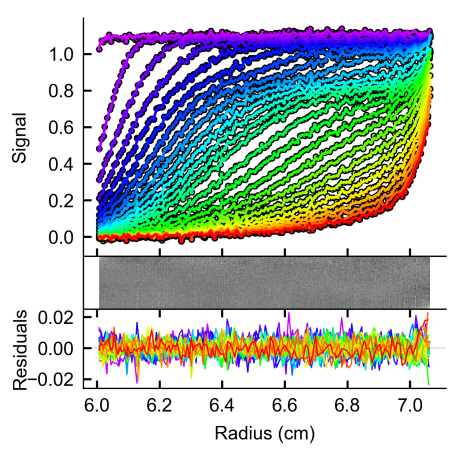


Reduced χ^2 [r.m.s.d]: 0.191756 [0.004379]

42000 rpm, λ = 291 nm



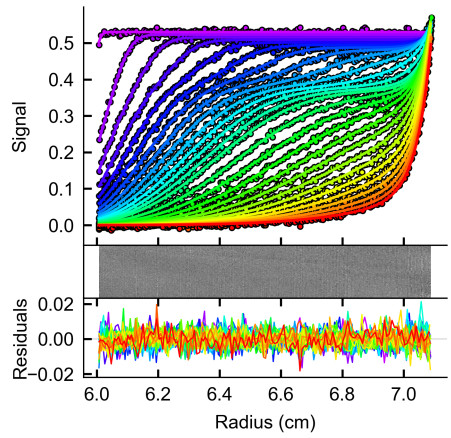




Reduced χ^2 [r.m.s.d]: 0.259183 [0.005091]

Species Analysis (42000 rpm, λ = 294 nm, exp. # 1)

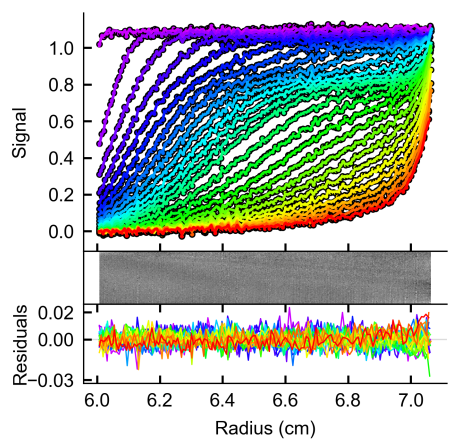
Parameter	Value	95% confidence interval
	Species 1	
Sedimentation coefficient s_e (S)	1.232830	1.2201 - 1.2468
Diffusion coefficient D_e (·10⁻⁷ cm²/s)	8.121437	7.7671 - 8.5080
	Species 2	
Sedimentation coefficient s_e (S)	3.585519	3.5308 - 3.6467
Diffusion coefficient D_e (·10⁻⁷ cm²/s)	3.231844	2.4451 - 4.1974
Fraction (signal)	0.202133	0.1919 - 0.2126
	Common	
Total concentration (signal)	0.5306498	0.5205 - 0.5417
Meniscus position (cm)	5.986057	5.9823 - 5.9896
Bottom position (cm)	7.236567	7.2309 - 7.2428
Reduced χ2 of fit [rmsd]	0.1983076	
R.m.s.d. of fit	0.004453174	



Reduced χ^2 [r.m.s.d]: 0.198308 [0.004453]

Species Analysis (42000 rpm, λ = 291 nm, exp. # 2)

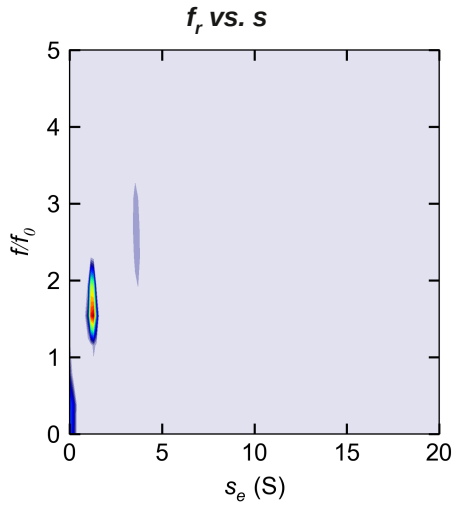
Parameter	Value	95% confidence interval
	Species 1	
Sedimentation coefficient s_e (S)	1.220184	1.2127 - 1.2280
Diffusion coefficient D_e (·10⁻⁷ cm²/s)	7.938753	7.7172 - 8.1994
	Species 2	
Sedimentation coefficient s_e (S)	3.596446	3.5560 - 3.6386
Diffusion coefficient D_e (·10⁻⁷ cm²/s)	3.366156	2.7780 - 4.0454
Fraction (signal)	0.170676	0.1640 - 0.1770
	Common	
Total concentration (signal)	1.088870	1.0756 - 1.1033
Meniscus position (cm)	5.989551	5.9874 - 5.9917
Bottom position (cm)	7.235867	7.2314 - 7.2412
Reduced χ2 of fit [rmsd]	0.2852372	
R.m.s.d. of fit	0.005340760	

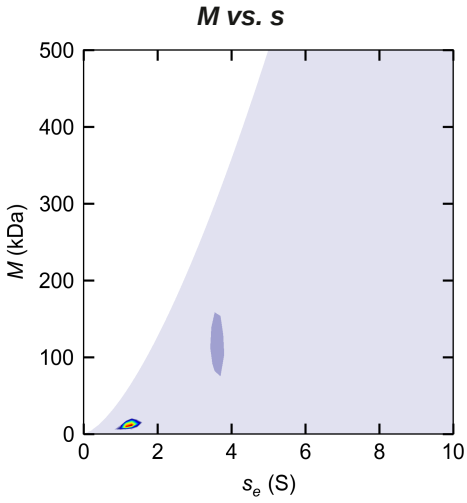


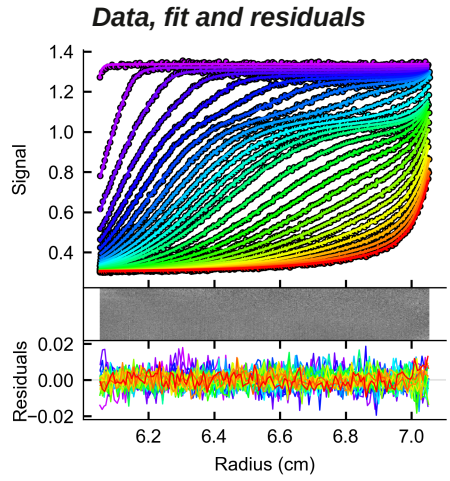
Reduced χ^2 [r.m.s.d]: 0.285237 [0.005341]

64 µM ssRNA monitored by absorbance optics

42000 rpm, λ = 294 nm

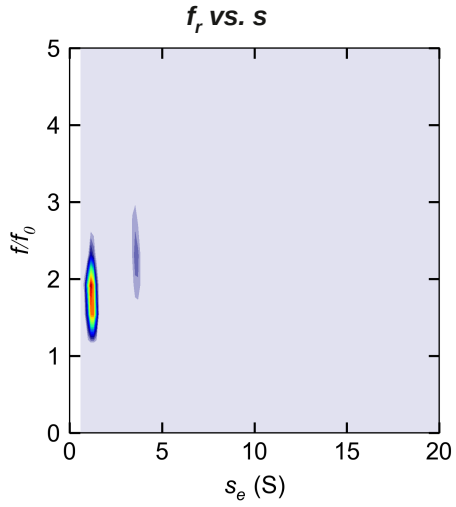


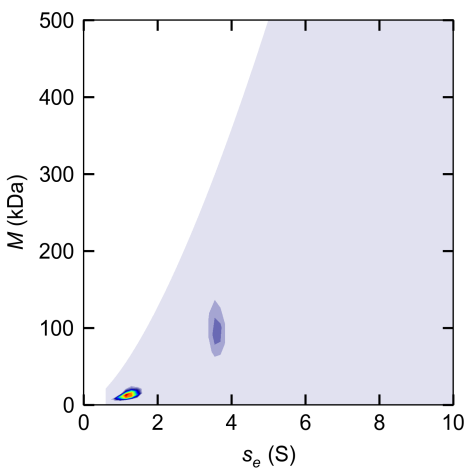


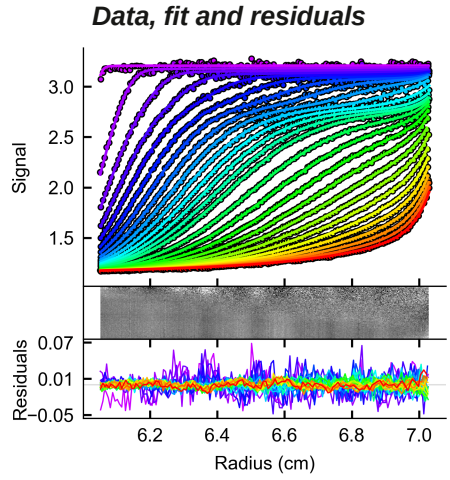


Reduced χ^2 [r.m.s.d]: 0.211508 [0.004599]

42000 rpm, λ = 291 nm



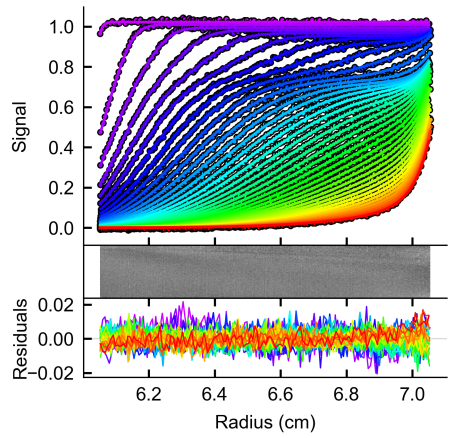




Reduced χ^2 [r.m.s.d]: 1.01949 [0.010097]

Species Analysis (42000 rpm, λ = 294 nm, exp. # 1)

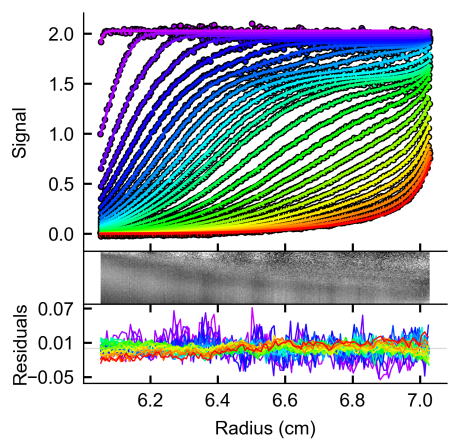
Parameter	Value	95% confidence interval
	Species 1	
Sedimentation coefficient s_e (S)	1.239898	1.2324 - 1.2474
Diffusion coefficient D_e (·10⁻⁷ cm²/s)	8.170592	7.9399 - 8.4006
	Species 2	
Sedimentation coefficient s_e (S)	3.572412	3.5362 - 3.6070
Diffusion coefficient D_e (·10⁻⁷ cm²/s)	3.355237	2.8711 - 3.9210
Fraction (signal)	0.198935	0.1930 - 0.2052
	Common	
Total concentration (signal)	1.024325	1.0124 - 1.0365
Meniscus position (cm)	6.025673	6.0235 - 6.0279
Bottom position (cm)	7.241323	7.2362 - 7.2464
Reduced χ2 of fit [rmsd]	0.2163596	
R.m.s.d. of fit	0.004651447	



Reduced χ^2 [r.m.s.d]: 0.21630 [0.004651]

Species Analysis (42000 rpm, λ 291 nm, exp. # 2)

Parameter	Value	95% confidence interval
	Species 1	
Sedimentation coefficient s_e (S)	1.201514	1.1918 - 1.2113
Diffusion coefficient D_e (·10⁻⁷ cm²/s)	8.107909	7.7838 - 8.4409
	Species 2	
Sedimentation coefficient s_e (S)	3.534074	3.4728 - 3.5943
Diffusion coefficient D_e (·10⁻⁷ cm²/s)	2.847448	2.1050 - 3.7689
Fraction (signal)	0.132244	0.1238 - 0.1408
	Common	
Total concentration (signal)	2.030704	1.9974 - 2.0654
Meniscus position (cm)	6.027494	6.0247 - 6.0302
Bottom position (cm)	7.245158	7.2363 - 7.2544
Reduced χ2 of fit [rmsd]	1.393352	
R.m.s.d. of fit	0.011804033	



Reduced χ^2 [r.m.s.d]: 1.393352 [0.011804]

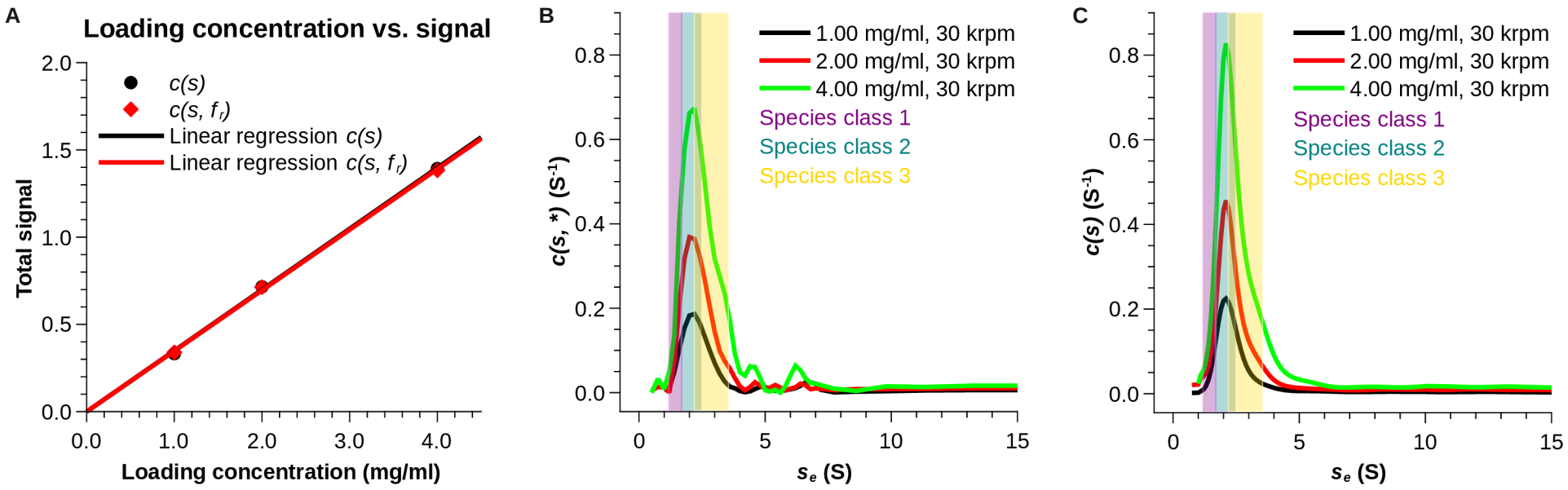
Fig. S2D

Section 2:

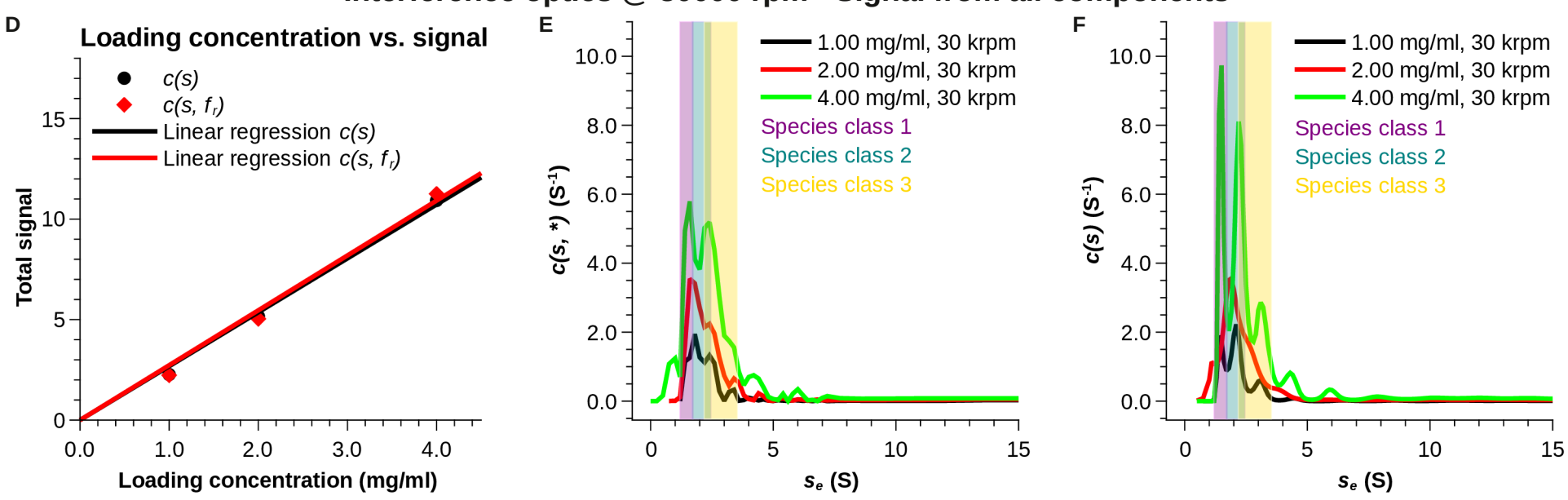
Characterization of Human PCBP2, PRRSV nsp1-β and PRRSV 34 nt ssRNA Complex by Sedimentation Velocity

Complex of human PCBP2, PRRSV nsp1-β and PRRSV 34 nt ssRNA

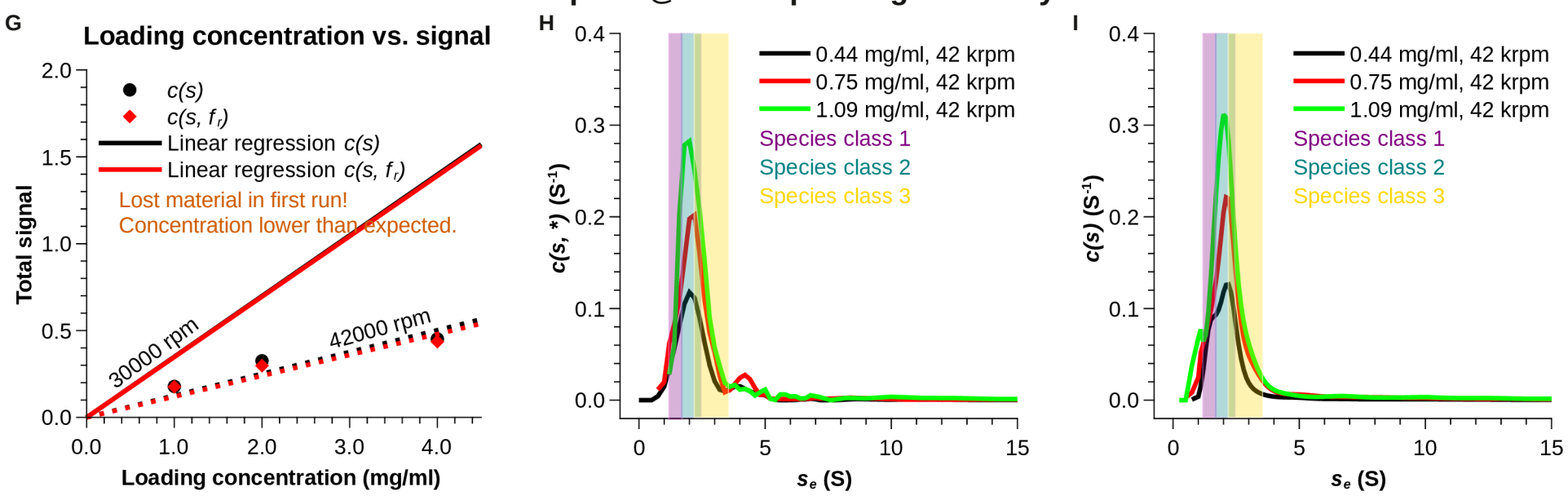
Absorbance optics @ 30000 rpm - Signal mainly from RNA



Interference optics @ 30000 rpm - Signal from all components



Absorbance optics @ 42000 rpm - Signal mainly from RNA



Interference optics @ 42000 rpm - Signal from all components

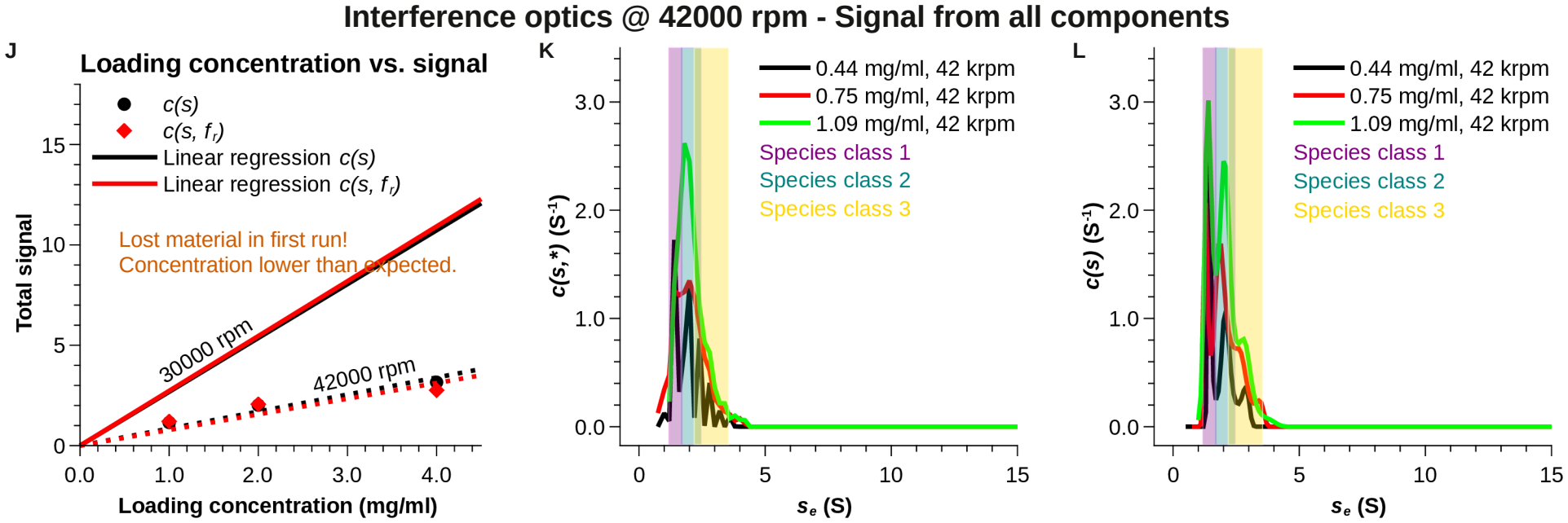


Fig. S3

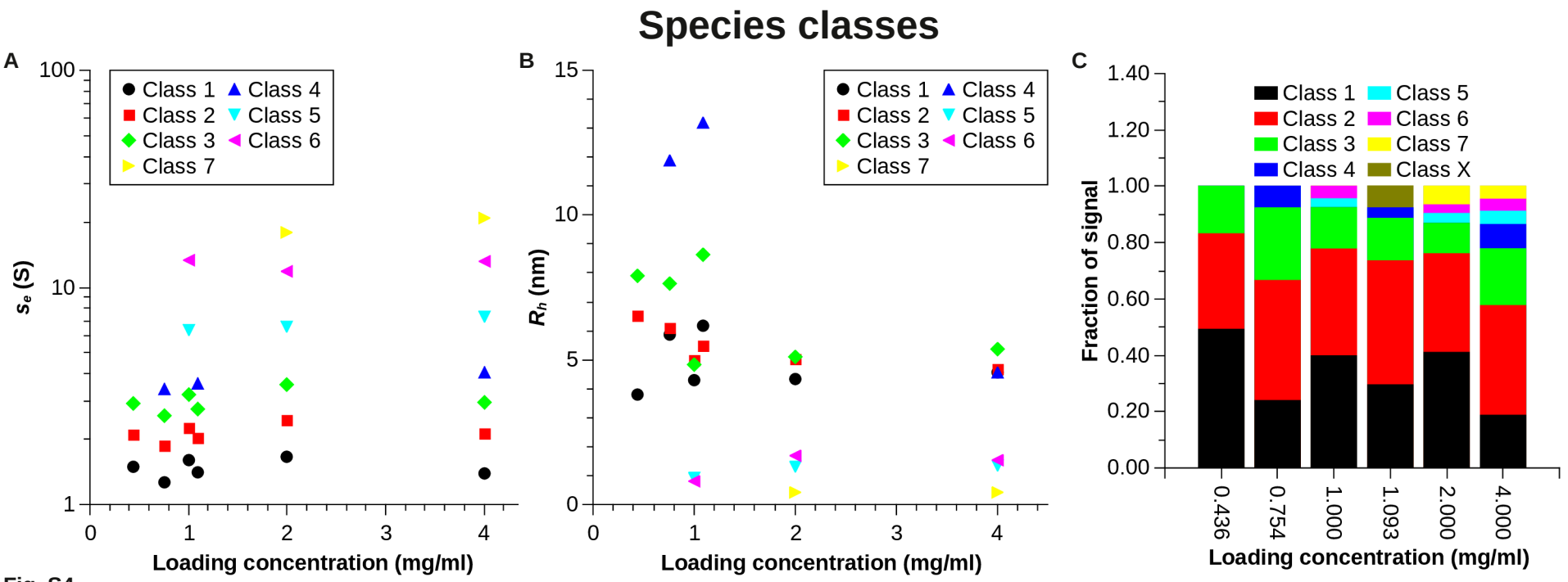


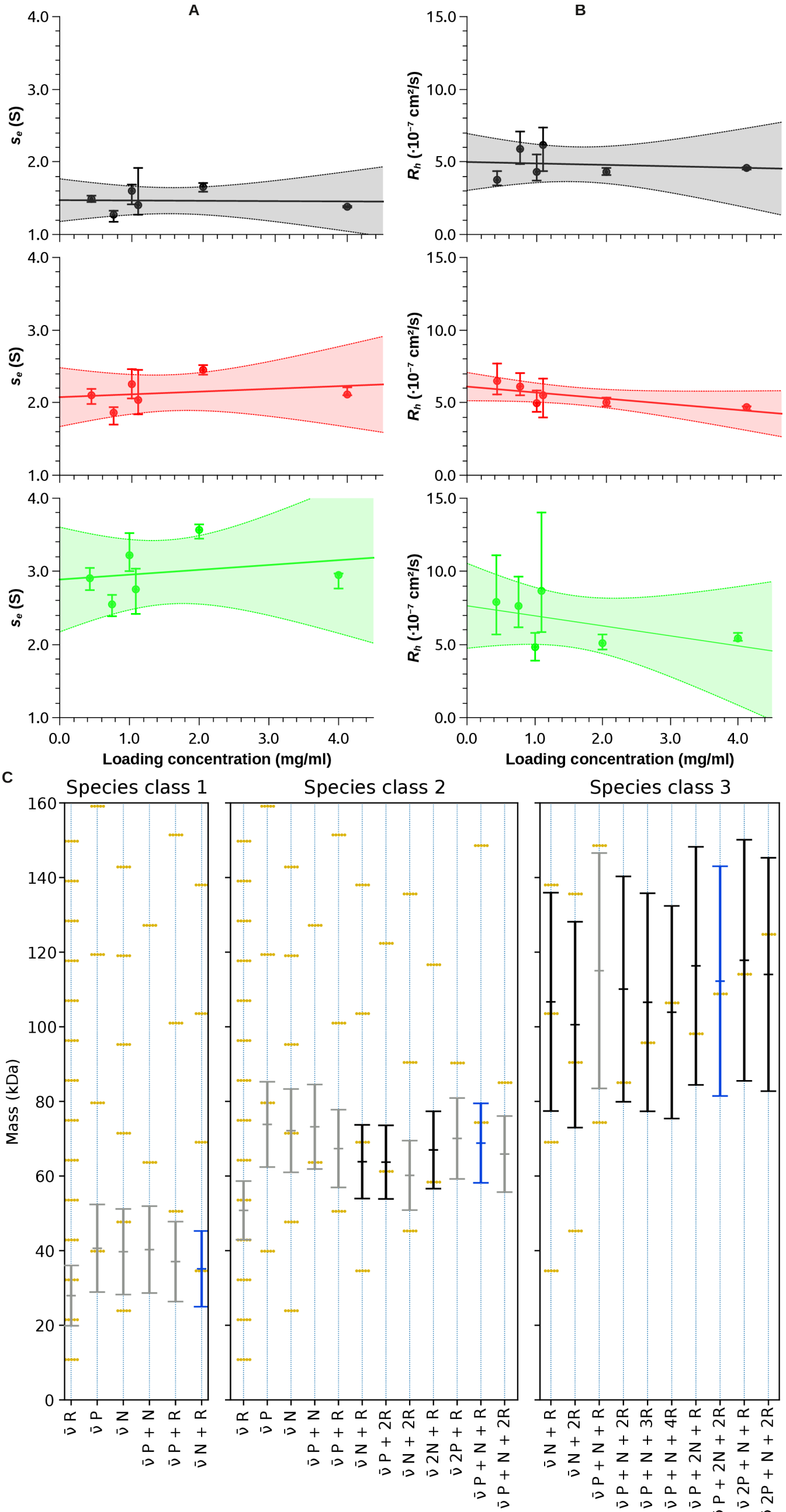
Fig. S4

Results

Species class 1	
Sedimentation coefficient $s^0_{20°C,\,w}$	2.02 [1.61 - 2.43] S
Diffusion coefficient $D^0_{20°C,\,w}$	4.52 [2.76 - 6.28] $\cdot 10^{-7}$ cm²/s
Hydrodynamic radius R_h^0	4.74 [3.41 - 7.74] nm
Species class 2	
Sedimentation coefficient $s^0_{20°C,\,w}$	2.84 [2.28 - 3.41] S
Diffusion coefficient $D^0_{20°C,\,w}$	3.51 [2.87 - 4.15] $\cdot 10^{-7}$ cm²/s
Hydrodynamic radius R_h^0	6.11 ± [5.17 - 7.46] nm
Species class 3	
Sedimentation coefficient $s^0_{20°C,\,w}$	3.96 [2.97 - 4.94] S
Diffusion coefficient $D^0_{20°C,\,w}$	2.92 [1.37 - 4.47] $\cdot 10^{-7}$ cm²/s
Hydrodynamic radius R_h^0	7.34 [4.79 - 15.61] nm

Table S5

Results



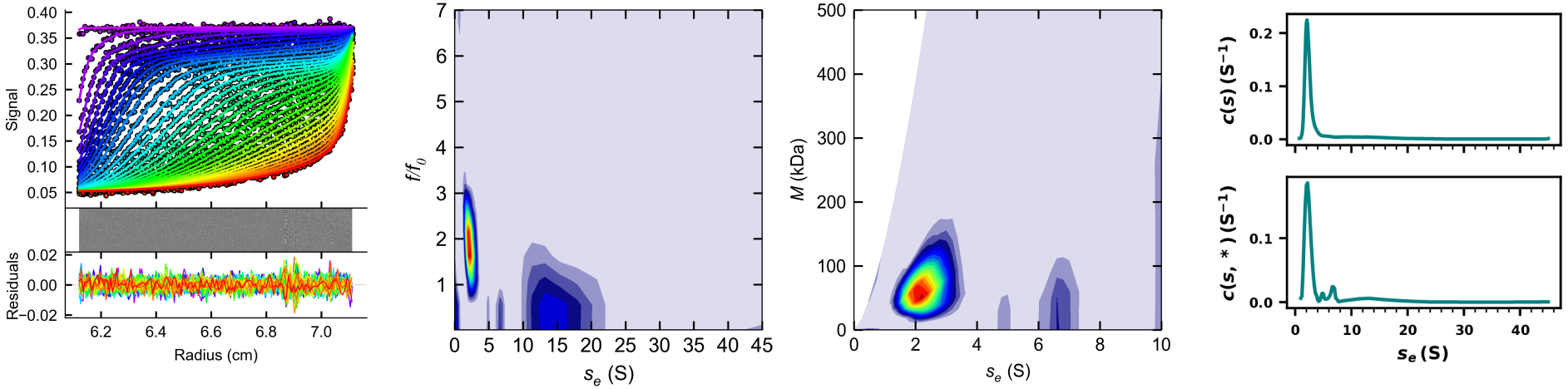
P: PCBP2, N: nsp1β, R: 34nt ssRNA

Complex of human PCBP2, PRRSV nsp1-β and PRRSV 34 nt ssRNA

Absorbance optics @ 30000 rpm - loading concentration 1.0 mg/ml

c(s, f_r) analysis

Data, fit and residuals ***f_r vs. s*** ***M vs. s*** ***1D distributions***

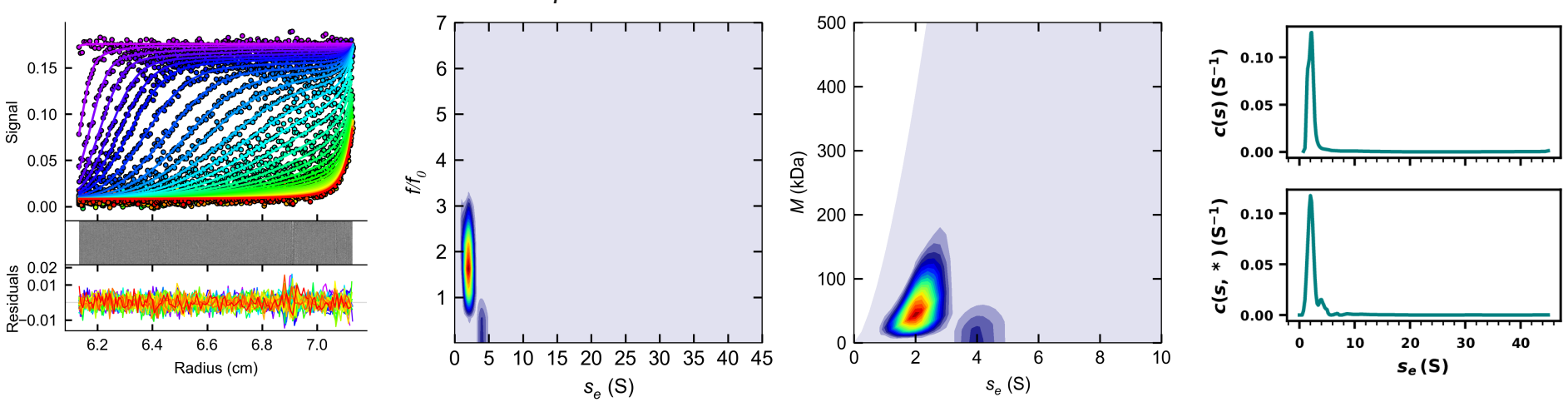


Reduced χ^2 [r.m.s.d]: [0.003905]

Absorbance optics @ 42000 rpm - actual concentration 0.44 mg/ml

c(s, f_r) analysis

Data, fit and residuals ***f_r vs. s*** ***M vs. s*** ***1D distributions***



Reduced χ^2 [r.m.s.d]: [0.003341]

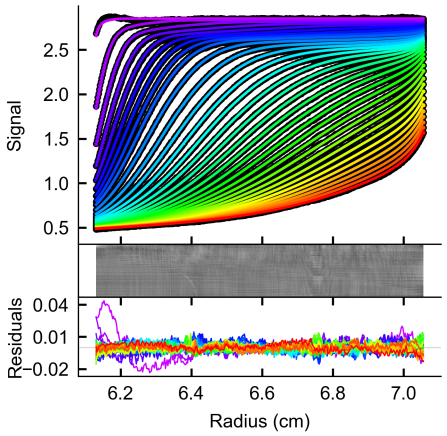
Fig. S6A

Complex of human PCBP2, PRRSV nsp1-β and PRRSV 34 nt ssRNA

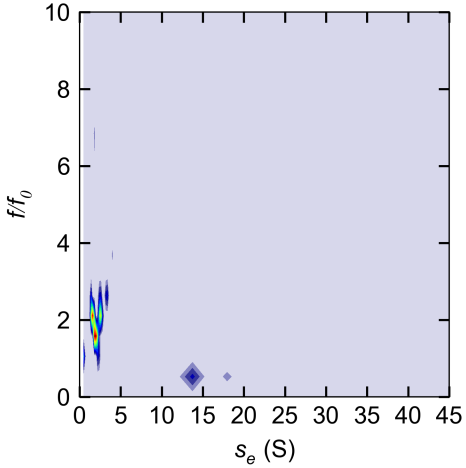
Interference optics @ 30000 rpm - loading concentration 1.0 mg/ml

c(s, f_r) analysis

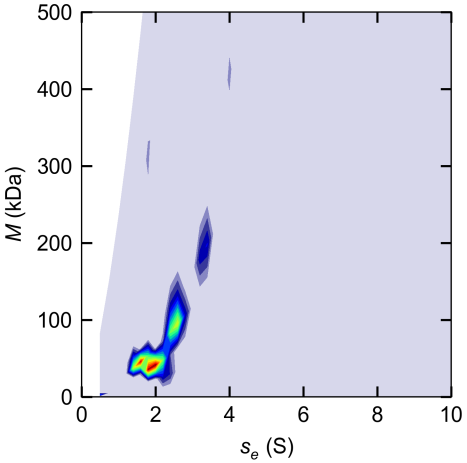
Data, fit and residuals



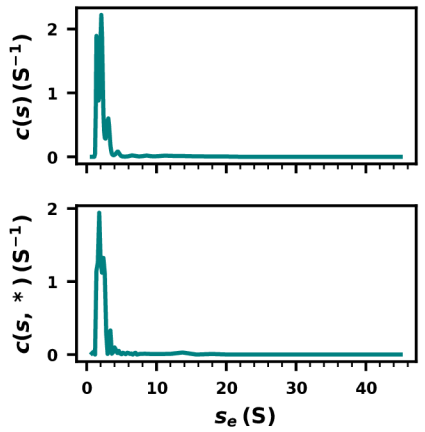
f_r vs. s



M vs. s



1D distributions

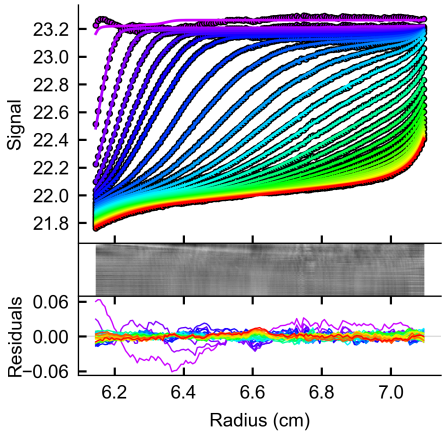


Reduced χ^2 [r.m.s.d]: [0.003815]

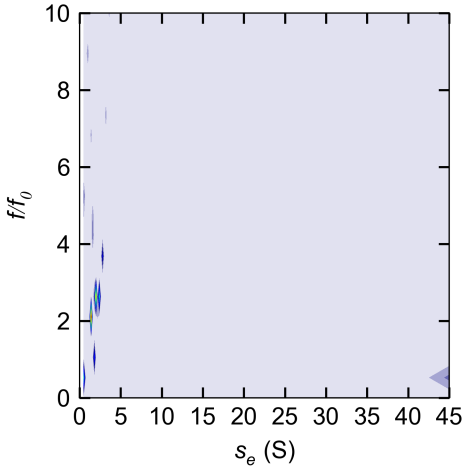
Interference optics @ 42000 rpm - actual concentration 0.44 mg/ml

c(s, f_r) analysis

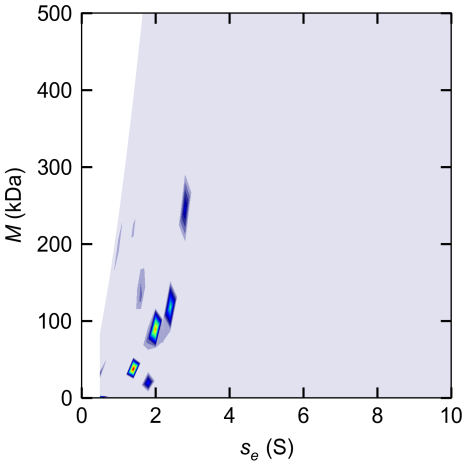
Data, fit and residuals



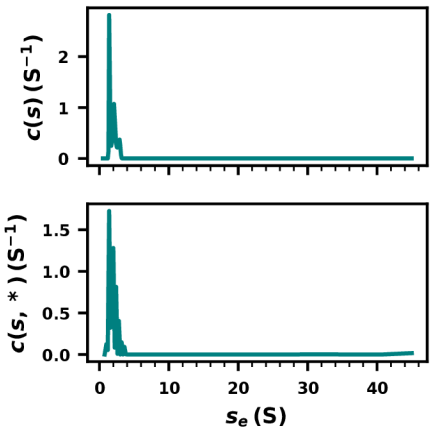
f_r vs. s



M vs. s



1D distributions



Reduced χ^2 [r.m.s.d]: [0.006460]

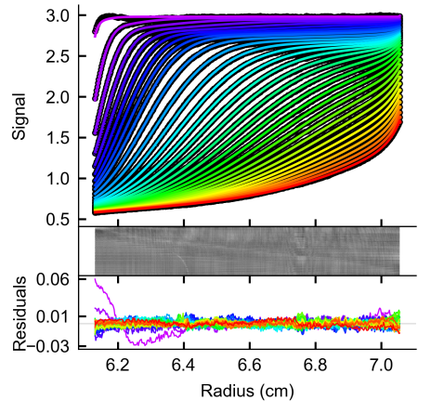
Fig. S6B

Complex of human PCBP2, PRRSV nsp1-β and PRRSV 34 nt ssRNA

Species Analysis (1.00 mg/ml, 30000 rpm, i. o.)

Parameter	Value	95% confidence interval
Species # 1 (class 1)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	2.185538	1.9320 - 2.3175
Molecular mass[1] *M* (Da)	39429.258626	31975.1983 - 43851.1943
Concentration (signal)	0.889672	
Species # 2 (class 2)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	3.086904	2.8131 - 3.3679
Molecular mass[1] *M* (Da)	64508.620238	56946.9871 - 72146.4835
Concentration (signal)	0.848381	
Species # 3 (class 3)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	4.413055	4.1166 - 4.8238
Molecular mass[1] *M* (Da)	89133.977232	75536.5637 - 117267.7025
Concentration (signal)	0.327668	
Species # 4 (class 5)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	8.680858	6.5740 - 11.0911
Molecular mass[1] *M* (Da)	33004.982556	27014.9568 - 41589.0286
Concentration (signal)	0.0689382	
Species # 5 (class 6)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	18.269621	17.2184 - 19.6377
Molecular mass[1] *M* (Da)	62045.164082	50792.5477 - 83330.0656
Concentration (signal)	0.101118	
Common		
Meniscus position (cm)	6.107007	6.1061 - 6.1081
Bottom position (cm)	7.216139	7.2101 - 7.2245
Buffer signal[2] frictional ratio f_r	2.382192	1.8745 - 3.3470
Reduced χ2 of fit [rmsd]	0.1871007	
R.m.s.d. of fit	0.004326	

[1]Substitute $\bar{\nu}$ = 0.73 cm^3/g
[2]Continuous *c(s)* from 0.0 - 0.8 S



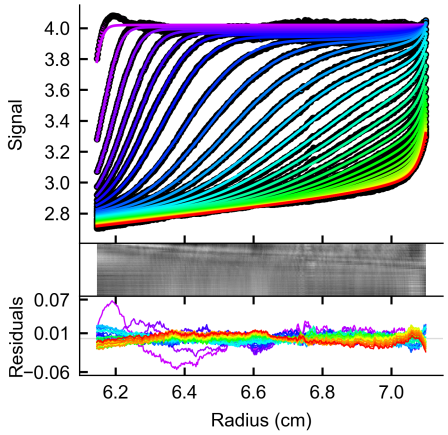
Reduced χ^2 [r.m.s.d]: 0.187101 [0.004326]

Complex of human PCBP2, PRRSV nsp1-β and PRRSV 34 nt ssRNA

Species Analysis (0.44 mg/ml, 42000 rpm, i. o.)

Parameter	Value	95% confidence interval
Species # 1 (class 1)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	2.045761	1.9890 - 2.0955
Molecular mass[1] *M* (Da)	32468.185230	28140.1889 - 35903.1468
Concentration (signal)	0.564412	
Species # 2 (class 2)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	2.874757	2.7103 - 2.9979
Molecular mass[1] *M* (Da)	78493.617851	67236.8428 - 93921.0463
Concentration (signal)	0.388871	
Species # 3 (class 3)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	3.976393	3.7610 - 4.1698
Molecular mass[1] *M* (Da)	131871.067920	94586.0501 - 201092.7657
Concentration (signal)	0.193644	
Common		
Meniscus position (cm)	6.107400	6.1056 - 6.1093
Bottom position (cm)	7.201615	7.1931 - 7.2121
Buffer signal[2] frictional ratio f_r	1.481410	1.2663 - 1.9532
Reduced χ2 of fit [rmsd]	0.5931672	
R.m.s.d. of fit	0.007702	

[1]Substitute $\bar{\nu} = 0.73$ cm^3/g
[2]Continuous *c(s)* from 0.0 - 0.8 S



Reduced χ^2 [r.m.s.d]: 0.593167 [0.007702]

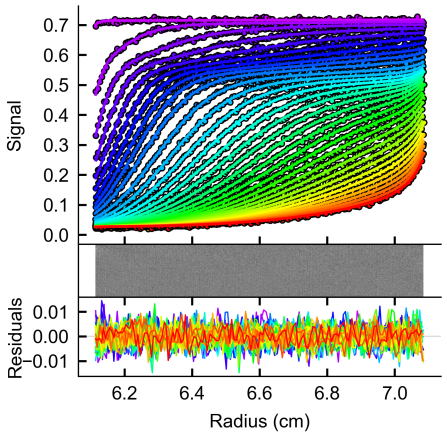
Fig. S6D

Complex of human PCBP2, PRRSV nsp1-β and PRRSV 34 nt ssRNA

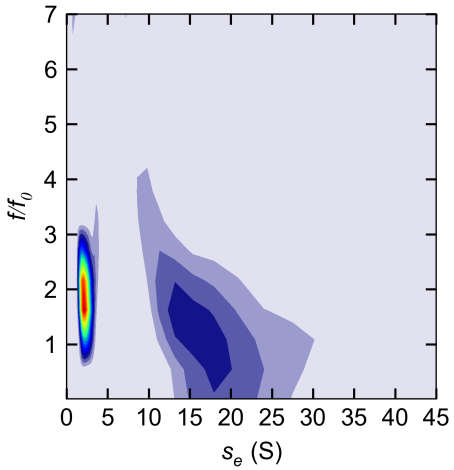
Absorbance optics @ 30000 rpm - loading concentration 2.0 mg/ml

c(s, f_r) analysis

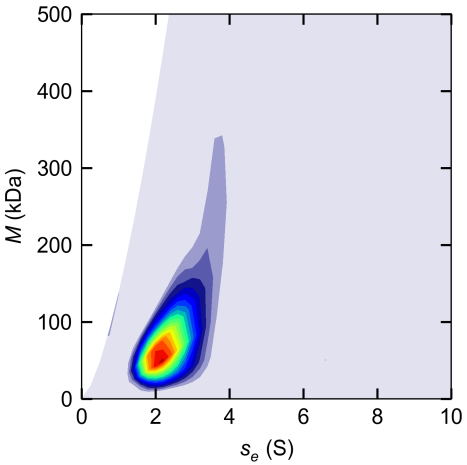
Data, fit and residuals



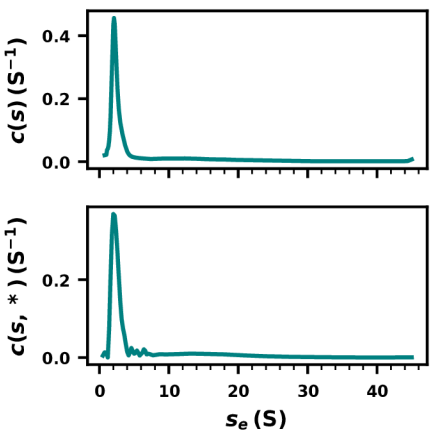
f_r vs. s



M vs. s



1D distributions

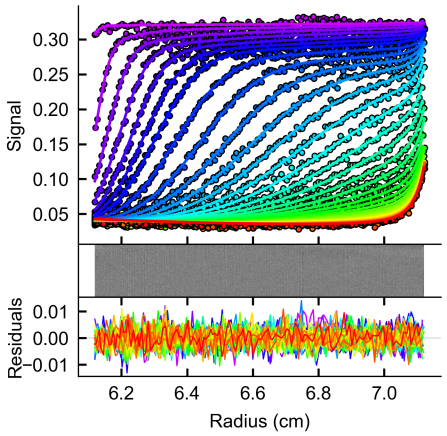


Reduced χ^2 [r.m.s.d]: [0.003730]

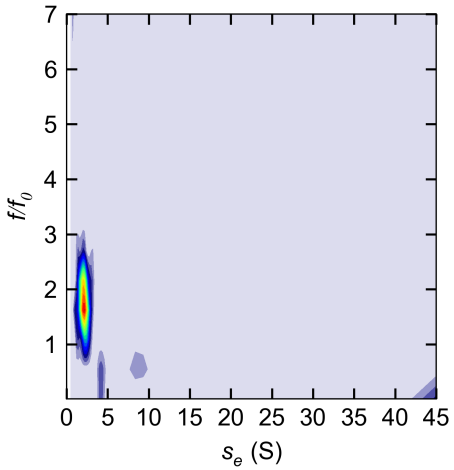
Absorbance optics @ 42000 rpm - actual concentration 0.75 mg/ml

c(s, f_r) analysis

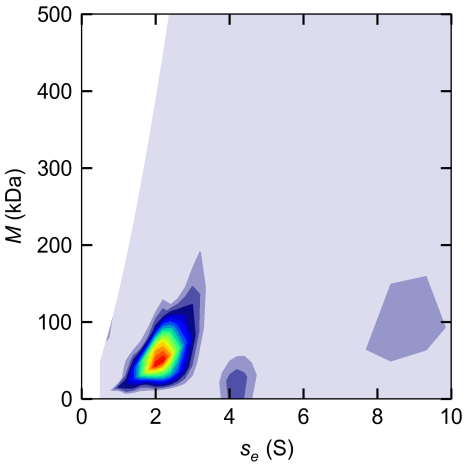
Data, fit and residuals



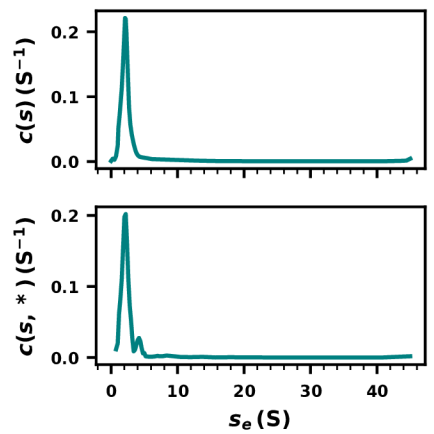
f_r vs. s



M vs. s



1D distributions



Reduced χ^2 [r.m.s.d]: [0.003382]

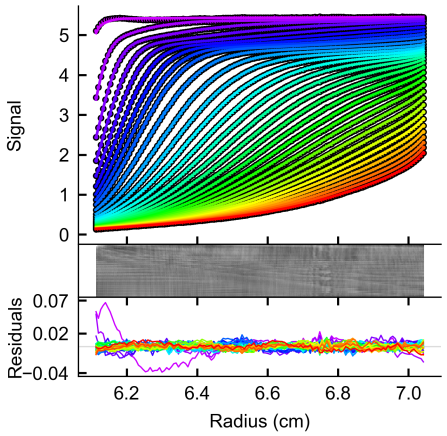
Fig. S6E

Complex of human PCBP2, PRRSV nsp1-β and PRRSV 34 nt ssRNA

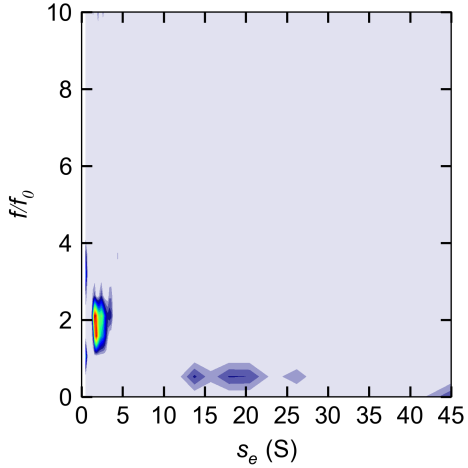
Interference optics @ 30000 rpm - loading concentration 2.0 mg/ml

c(s, f_r) analysis

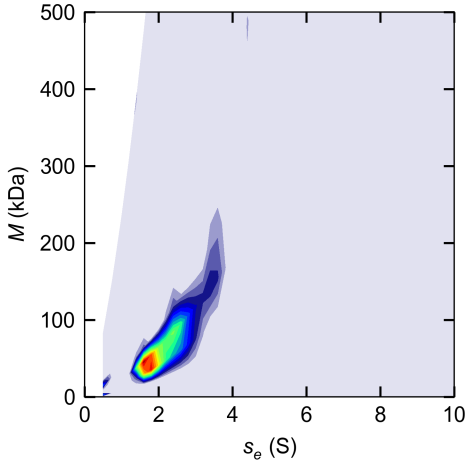
Data, fit and residuals



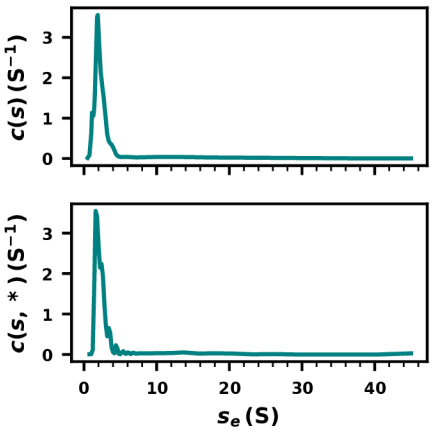
f_r vs. s



M vs. s



1D distributions

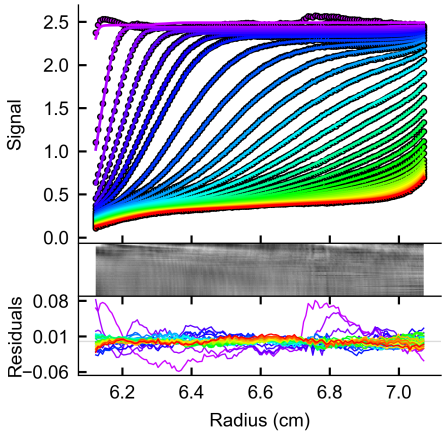


Reduced χ^2 [r.m.s.d]: [0.005117]

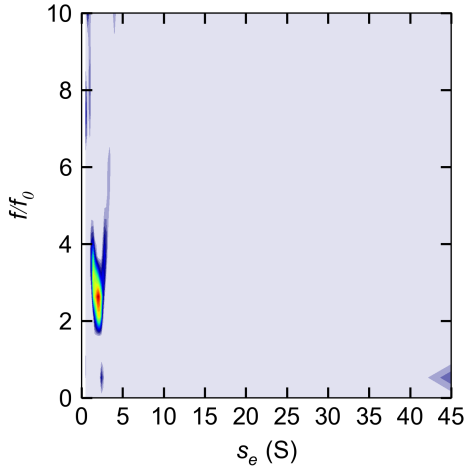
Interference optics @ 42000 rpm - actual concentration 0.75 mg/ml

c(s, f_r) analysis

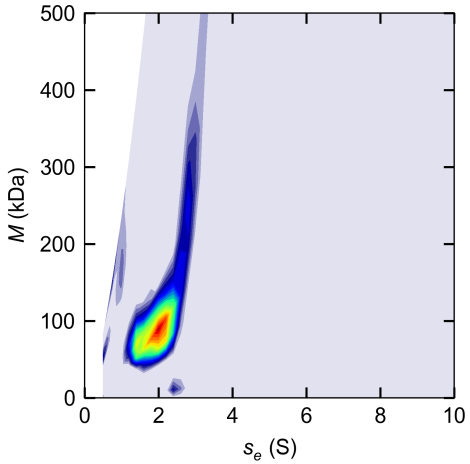
Data, fit and residuals



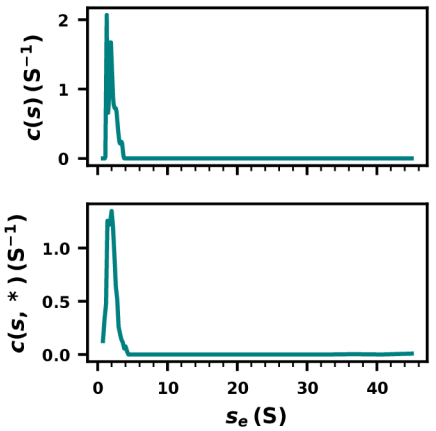
f_r vs. s



M vs. s



1D distributions



Reduced χ^2 [r.m.s.d]: [0.009482]

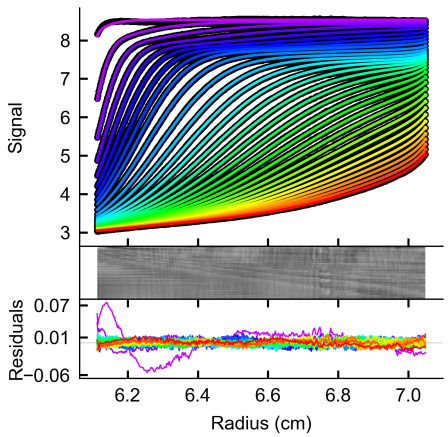
Fig. S6F

Complex of human PCBP2, PRRSV nsp1-β and PRRSV 34 nt ssRNA

Species Analysis (2.00 mg/ml, 30000 rpm, i. o.)

Parameter	Value	95% confidence interval
Species # 1 (class 1)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	2.259298	2.1789 - 2.3345
Molecular mass[1] *M* (Da)	40911.383621	39015.7993 - 42569.8757
Concentration (signal)	2.10665	
Species # 2 (class 2)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	3.362426	3.2647 - 3.4499
Molecular mass[1] *M* (Da)	70607.408753	66954.0626 - 74408.5887
Concentration (signal)	1.80079	
Species # 3 (class 3)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	4.879189	4.7237 - 4.9818
Molecular mass[1] *M* (Da)	104525.361816	94748.6103 - 115580.2252
Concentration (signal)	0.557564	
Species # 4 (class 5)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	9.007732	7.8572 - 10.1107
Molecular mass[1] *M* (Da)	49129.251609	38405.4058 - 69148.1903
Concentration (signal)	0.179173	
Species # 5 (class 6)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	16.172510	15.2395 - 17.1308
Molecular mass[1] *M* (Da)	114826.491328	86165.0080 - 173746.5190
Concentration (signal)	0.15973	
Species # 6 (class 7)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	24.574698	23.3895 - 25.9063
Molecular mass[1] *M* (Da)	42732.956296	38685.9354 - 49078.2030
Concentration (signal)	0.337733	
Common		
Meniscus position (cm)	6.078588	6.0778 - 6.0790
Bottom position (cm)	7.233989	7.2288 - 7.2399
Buffer signal[2] frictional ratio f_r	3.336644	2.8806 - 3.9916
Reduced χ2 of fit [rmsd]	0.3392895	
R.m.s.d. of fit	0.005825	

[1]Substitute $\bar{\nu} = 0.73$ cm^3/g
[2]Continuous *c(s)* from 0.0 - 0.8 S



Reduced χ^2 [r.m.s.d]: 0.339290 [0.005825]

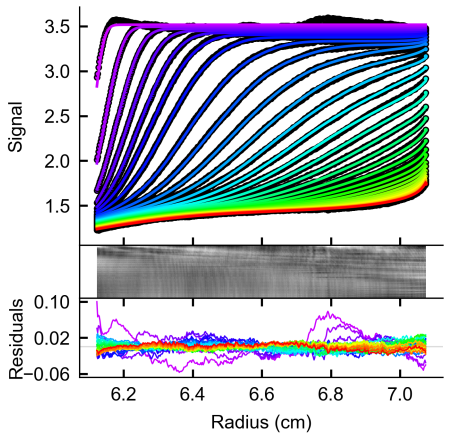
Fig. S6G

Complex of human PCBP2, PRRSV nsp1-β and PRRSV 34 nt ssRNA

Species Analysis (0.75 mg/ml, 42000 rpm, i. o.)

Parameter	Value	95% confidence interval
Species # 1 (class 1)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	1.737800	1.6152 - 1.8163
Molecular mass[1] *M* (Da)	42836.913056	36022.7457 - 53638.9211
Concentration (signal)	0.488908	
Species # 2 (class 2)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	2.545311	2.3295 - 2.6511
Molecular mass[1] *M* (Da)	65269.106310	58823.6621 - 72195.2814
Concentration (signal)	0.869682	
Species # 3 (class 3)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	3.497679	3.2748 - 3.6633
Molecular mass[1] *M* (Da)	112116.879536	90117.1719 - 141988.8079
Concentration (signal)	0.526914	
Species # 4 (class 4)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	4.655214	4.3692 - 4.8847
Molecular mass[1] *M* (Da)	232327.712281	148281.3925 - 426686.8809
Concentration (signal)	0.156663	
Common		
Meniscus position (cm)	6.093045	6.0911 - 6.0950
Bottom position (cm)	7.175154	7.1615 - 7.1904
Buffer signal[2] frictional ratio f_r	5.718135	4.3407 - 7.4711
Reduced χ2 of fit [rmsd]	0.9201238	
R.m.s.d. of fit	0.009592	

[1]Substitute $\bar{\nu} = 0.73$ cm^3/g
[2]Continuous *c(s)* from 0.0 - 0.8 S



Reduced χ^2 [r.m.s.d]: 0.920124 [0.009592]

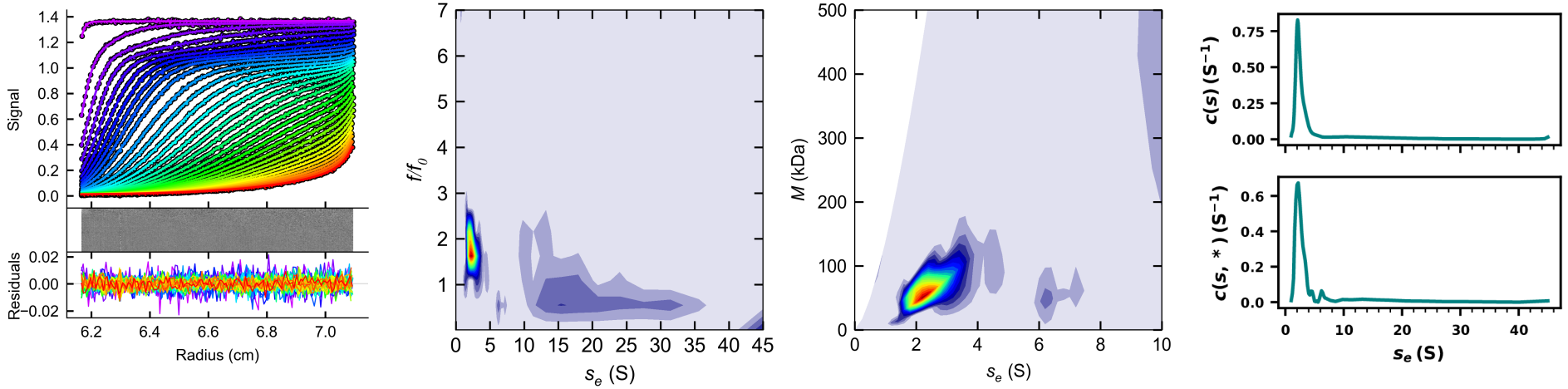
Fig. S6H

Complex of human PCBP2, PRRSV nsp1-β and PRRSV 34 nt ssRNA

Absorbance optics @ 30000 rpm - loading concentration 4.0 mg/ml

c(s, f_r) analysis

Data, fit and residuals ***f_r vs. s*** ***M vs. s*** ***1D distributions***

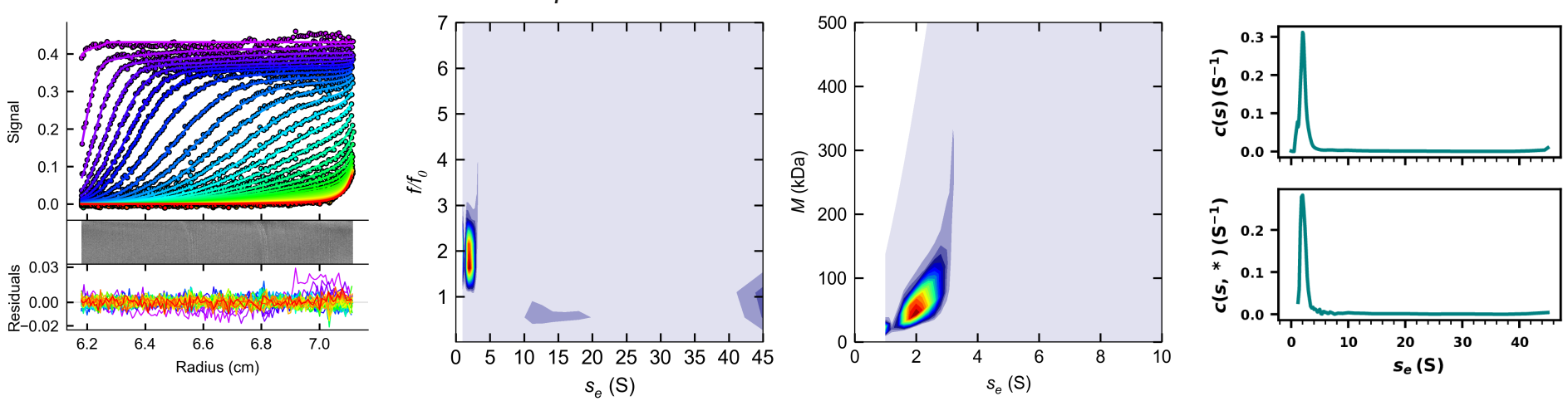


Reduced χ^2 [r.m.s.d]: [0.004508]

Absorbance optics @ 42000 rpm - actual concentration 1.09 mg/ml

c(s, f_r) analysis

Data, fit and residuals ***f_r vs. s*** ***M vs. s*** ***1D distributions***



Reduced χ^2 [r.m.s.d]: [0.004322]

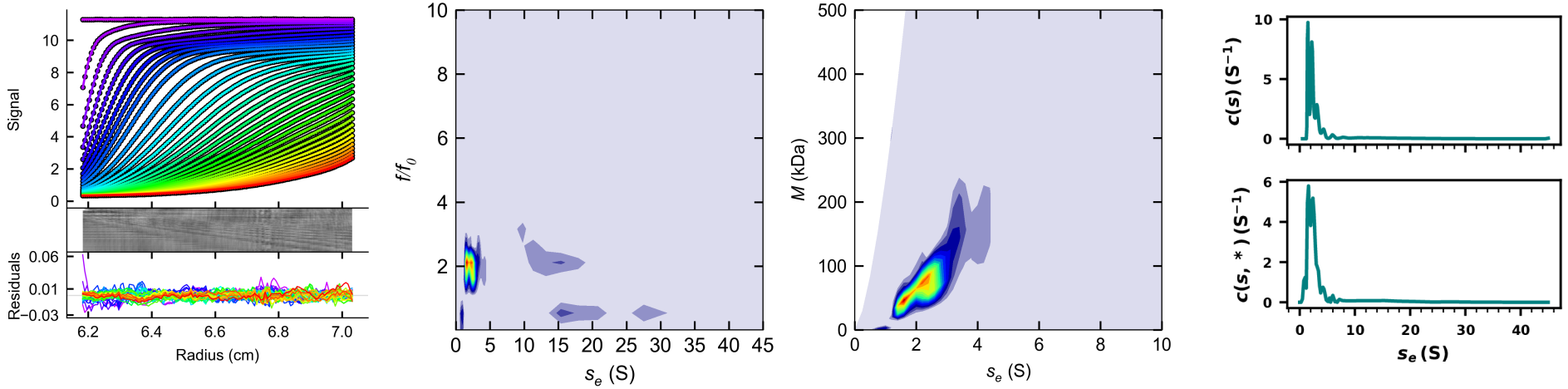
Fig. S6I

Complex of human PCBP2, PRRSV nsp1-β and PRRSV 34 nt ssRNA

Interference optics @ 30000 rpm - loading concentration 4.0 mg/ml

c(s, f_r) analysis

Data, fit and residuals ***f_r vs. s*** ***M vs. s*** ***1D distributions***

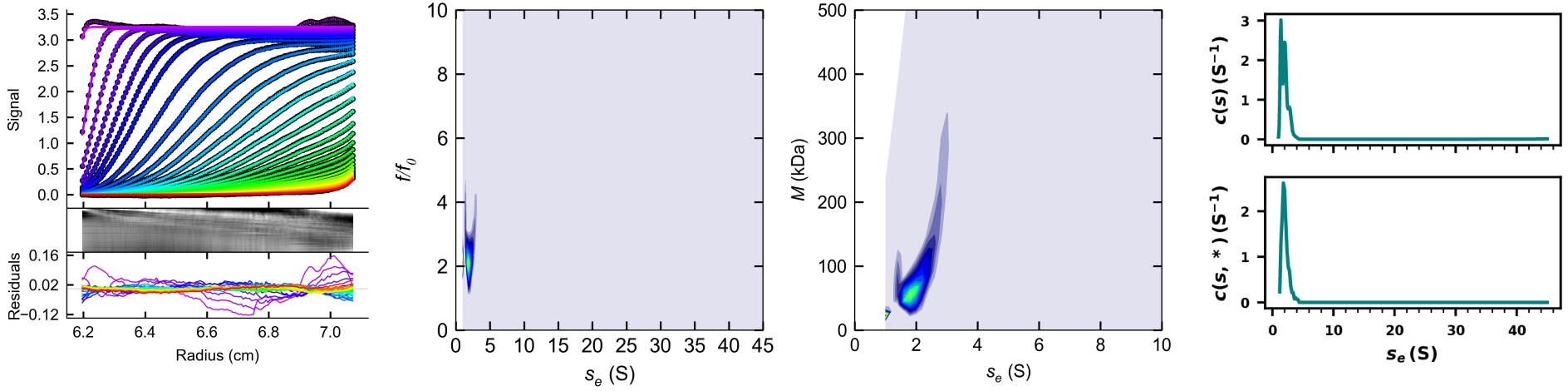


Reduced χ^2 [r.m.s.d]: [0.005735]

Interference optics @ 42000 rpm - actual concentration 1.09 mg/ml

c(s, f_r) analysis

Data, fit and residuals ***f_r vs. s*** ***M vs. s*** ***1D distributions***



Reduced χ^2 [r.m.s.d]: [0.019392]

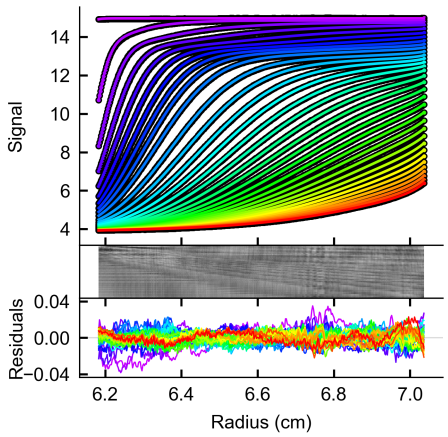
Fig. S6J

Complex of human PCBP2, PRRSV nsp1-β and PRRSV 34 nt ssRNA

Species Analysis (4.00 mg/ml, 30000 rpm, i. o.)

Parameter	Value	95% confidence interval
Species # 1 (class 1)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	1.893838	1.8767 - 1.9115
Molecular mass[1] *M* (Da)	36242.513823	35605.9184 - 36924.8539
Concentration (signal)	2.02274	
Species # 2 (class 2)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	2.895957	2.8717 - 3.0216
Molecular mass[1] *M* (Da)	56798.604800	56171.7866 - 57417.8843
Concentration (signal)	4.20148	
Species # 3 (class 3)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	4.032742	3.7914 - 4.0755
Molecular mass[1] *M* (Da)	91110.192293	88642.3598 - 93600.9839
Concentration (signal)	2.17643	
Species # 4 (class 4)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	5.558437	5.5059 - 5.7911
Molecular mass[1] *M* (Da)	106503.214501	97771.9841 - 113202.5506
Concentration (signal)	0.924506	
Species # 5 (class 5)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	9.980081	9.6854 - 10.2378
Molecular mass[1] *M* (Da)	55993.213184	50533.5330 - 65497.6998
Concentration (signal)	0.504232	
Species # 6 (class 6)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	18.163020	17.9089 - 18.4664
Molecular mass[1] *M* (Da)	116367.123008	102061.2320 - 138132.1828
Concentration (signal)	0.460625	
Species # 7 (class 7)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	28.605184	27.7262 - 30.0465
Molecular mass[1] *M* (Da)	50122.522087	49947.7361 - 50312.8130
Concentration (signal)	0.500927	
Common		
Meniscus position (cm)	6.154459	6.1540 - 6.1548
Bottom position (cm)	7.219491	7.2170 - 7.2245
Buffer signal[2] frictional ratio f_r	1.126869	0.9060 - 1.3521
Reduced χ2 of fit [rmsd]	0.5273897	
R.m.s.d. of fit	0.007262	

[1]Substitute $\bar{\nu} = 0.73$ cm^3/g
[2]Continuous *c(s)* from 0.0 - 0.8 S



Reduced χ^2 [r.m.s.d]: 0.527390 [0.007262]

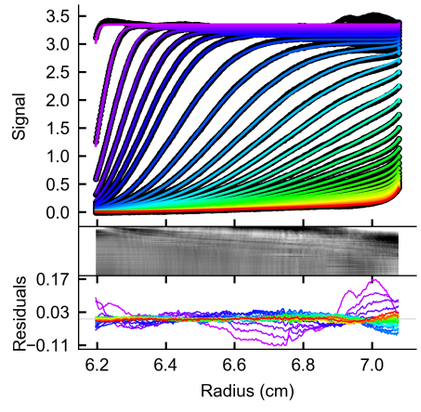
Fig. S6K

Complex of human PCBP2, PRRSV nsp1-β and PRRSV 34 nt ssRNA

Species Analysis (1.09 mg/ml, 42000 rpm, i. o.)

Parameter	Value	95% confidence interval
Species # 1 (class X)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	1.209722	0.8561 - 1.7601
Molecular mass[1] *M* (Da)	30400.731201	20063.1666 - >900000
Concentration (signal)	0.255537	
Species # 2 (class 1)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	1.922069	1.7474 - 2.6281
Molecular mass[1] *M* (Da)	49646.909726	42877.4221 - 61527.1873
Concentration (signal)	0.962814	
Species # 3 (class 2)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	2.787625	2.5211 - 3.3593
Molecular mass[1] *M* (Da)	64072.302750	54843.6506 - 109988.7832
Concentration (signal)	1.43393	
Species # 4 (class 3)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	3.776941	3.3105 - 4.1561
Molecular mass[1] *M* (Da)	136947.108751	87118.2414 - 219814.9212
Concentration (signal)	0.4912	
Species # 5 (class 4)		
Sedimentation coefficient $s_{20°C,\,w}$ (S)	4.936165	4.2947 - 5.5977
Molecular mass[1] *M* (Da)	273002.737766	108529.5424 - >900000
Concentration (signal)	0.119144	
Common		
Meniscus position (cm)	6.169227	6.1674 - 6.1710
Bottom position (cm)	7.181567	7.1682 - 7.2017
Buffer signal[2] frictional ratio f_r	4.608594	2.9507 - 28.0506
Reduced χ2 of fit [rmsd]	3.344057	
R.m.s.d. of fit	0.018287	

[1]Substitute $\bar{\nu}$ = 0.73 cm³/g
[2]Continuous *c(s)* from 0.0 - 0.8 S



Reduced χ^2 [r.m.s.d]: 3.344057 [0.018287]

Section 3:

Characterization of Human PCBP2, PRRSV nsp1-β and PRRSV 34 nt ssRNA Complex by SEC-SAXS

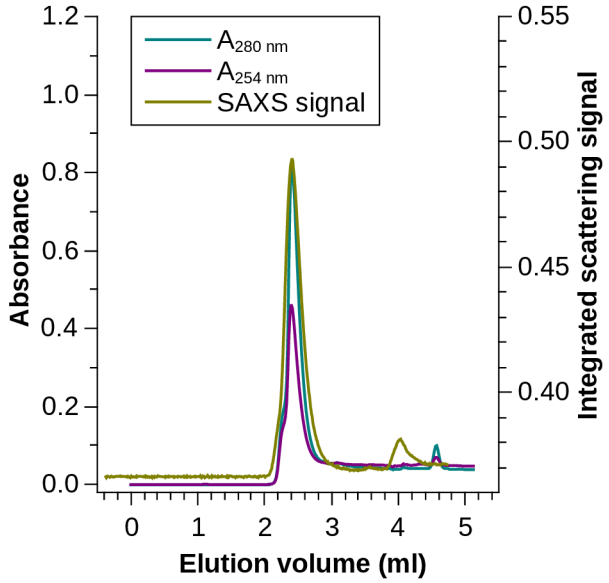

A280 nm
A254 nm
SAXS signal
Absorbance
Integrated scattering signal
Elution volume (ml)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.55
0.50
0.45
0.40
0
1
2
3
4
5

Fig. S7