Rapid high-resolution size distribution analysis for adeno-associated virus using high speed SV-AUC

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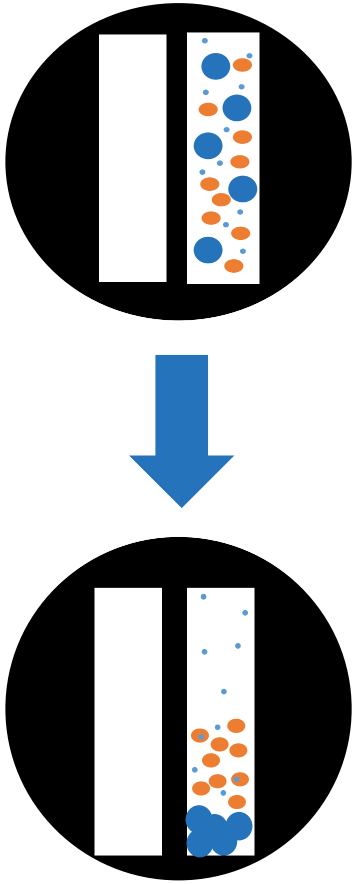
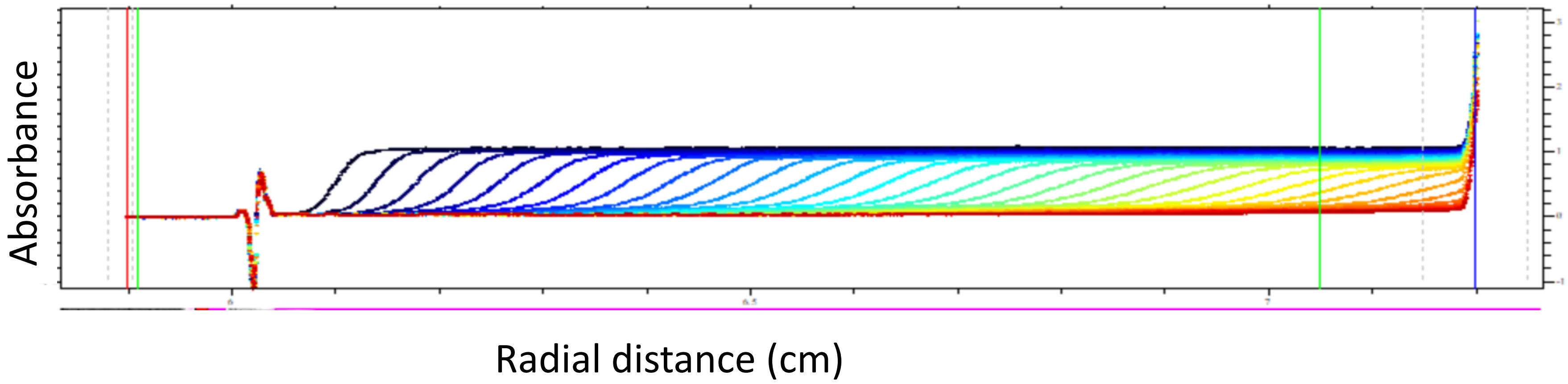
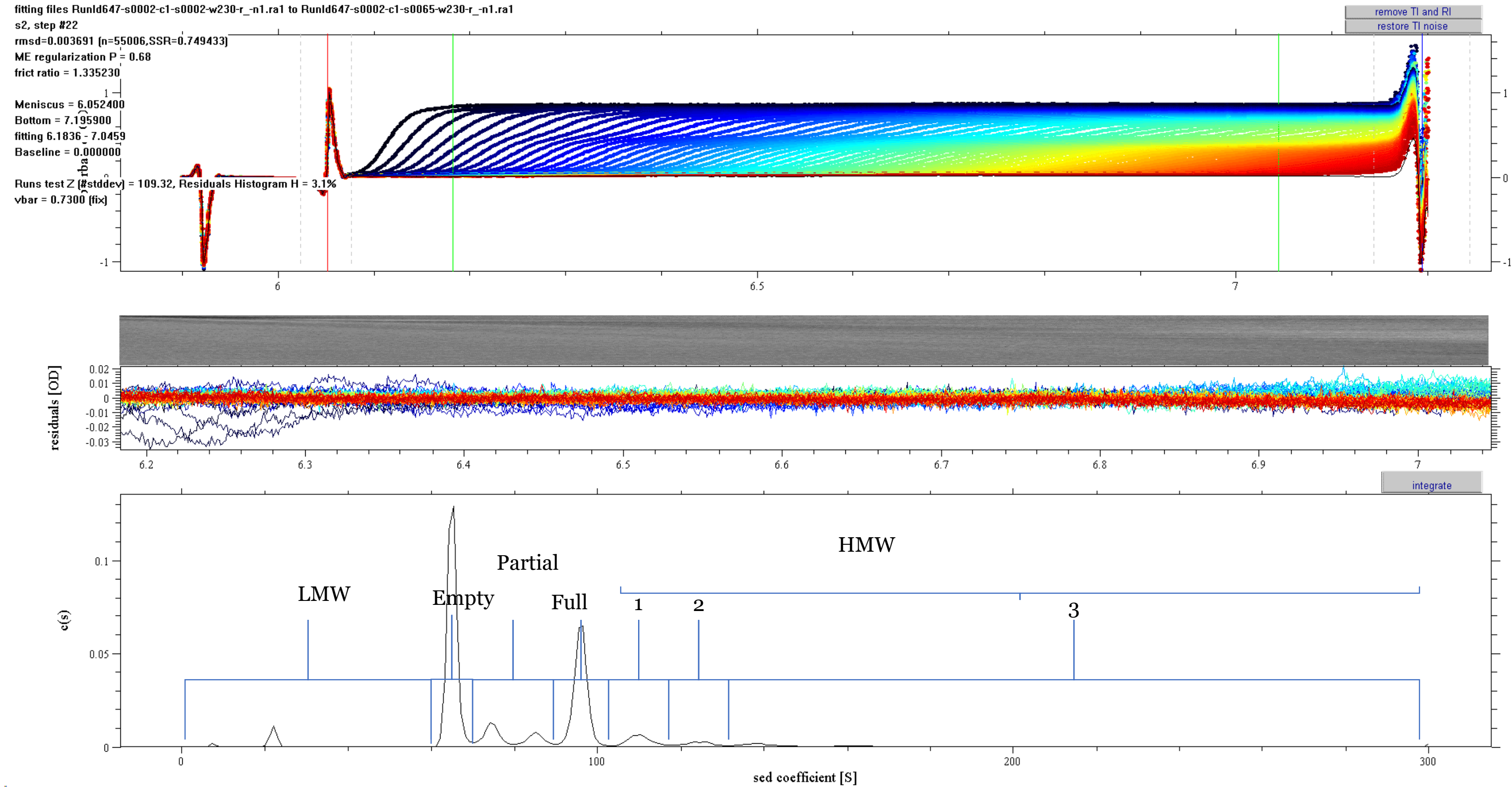
Analytical Ultracentrifugation

- Gold standard for size distribution quantitation
- Can be performed in formulation buffer
- Data is fit to the Lamm equation for quantitation





c(s) Distribution of AAV (15k run)



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fitting files RunId647-s0002-c1-s0002-w230-r_-n1.ra1 to RunId647-s0002-c1-s0065-w230-r_-n1.ra1
s2, step #22
rmsd=0.003691 (n=55006,SSR=0.749433)
ME regularization P = 0.68
frict ratio = 1.335230

Meniscus = 6.052400
Bottom = 7.195900
fitting 6.1836 - 7.0459
Baseline = 0.000000

Runs test Z (#stddev) = 109.32, Residuals Histogram H = 3.1%
vbar = 0.7300 (fix)
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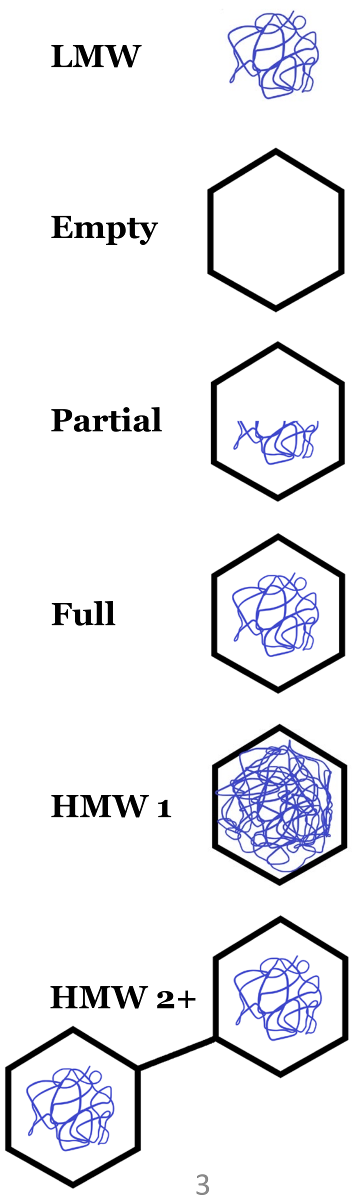


LMW

Empty

Partial

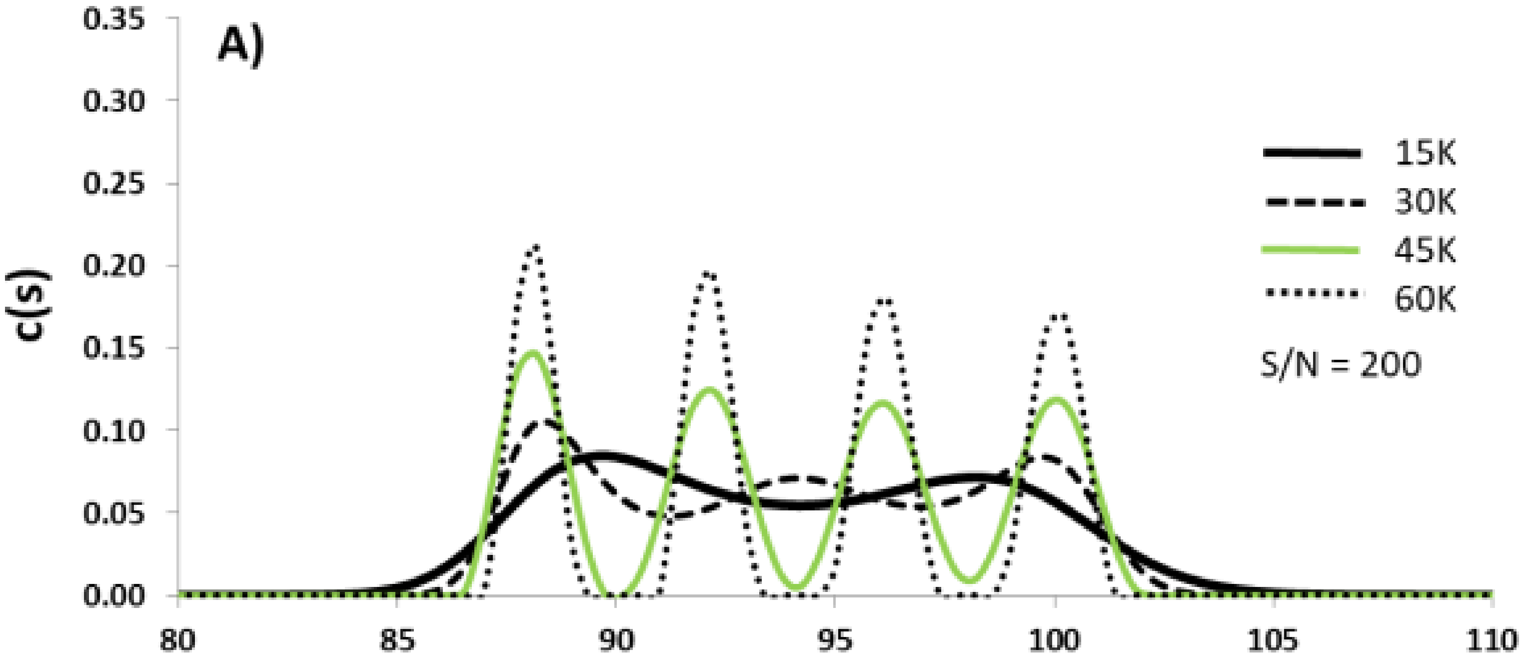
Full

HMW 1

HMW 2+

Simulations demonstrate higher speed improves resolution

- At a 45k run speed AAV sediment in less than 15 minutes



<u>Simulated data</u>

- Four discrete species
- 25% peak area each
- Four s difference between neighboring species

Comparison of absorbance and interference detection

Absorbance	Interference
• Signal linearity between (0.1 and 1.5 AU)	• Signal linearity over multiple orders of magnitude
• Scan 1 cell every 20 seconds	• Scan 7 cells in 5 seconds
• Chromophores absorb at specific wavelengths allowing for specific detection	• All molecules refract light
• Buffer matching not critical	• Meniscus and buffer matching is critical

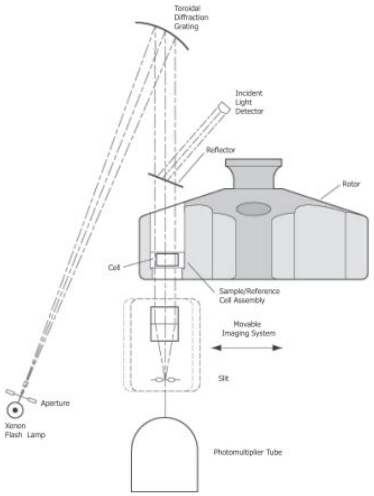


Figure 1.9 Schematics of the commercial absorbance optical system of the XL-A analytical ultracentrifuge from Beckman Coulter (courtesy of M. Rhyner, Beckman Coulter, Inc.).

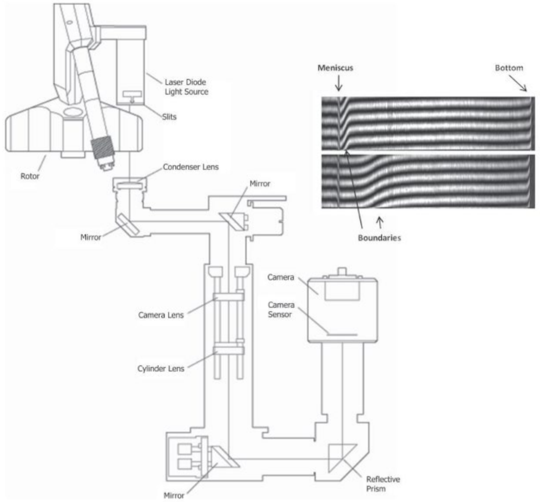


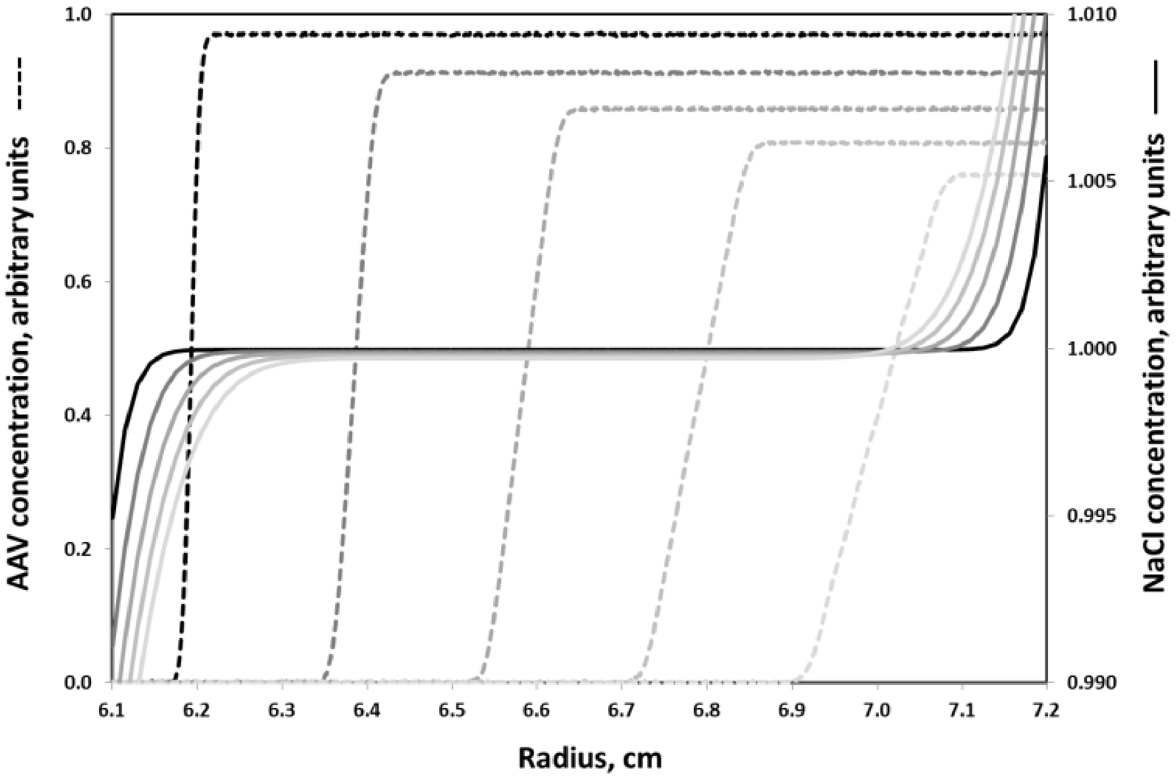
Figure 1.11 Schematics of the commercial Rayleigh interference optical system of the XL-I analytical ultracentrifuge from Beckman Coulter (courtesy of M. Rhyner, Beckman Coulter, Inc.). Included at top right are snapshots of the fringe picture at two different points in time.

Schuck P, Zhao H, Brautigam CA, Ghirlando R. Basic principles of analytical ultracentrifugation. CRC Press; 2016 Jan 5.

Interference detection buffer options

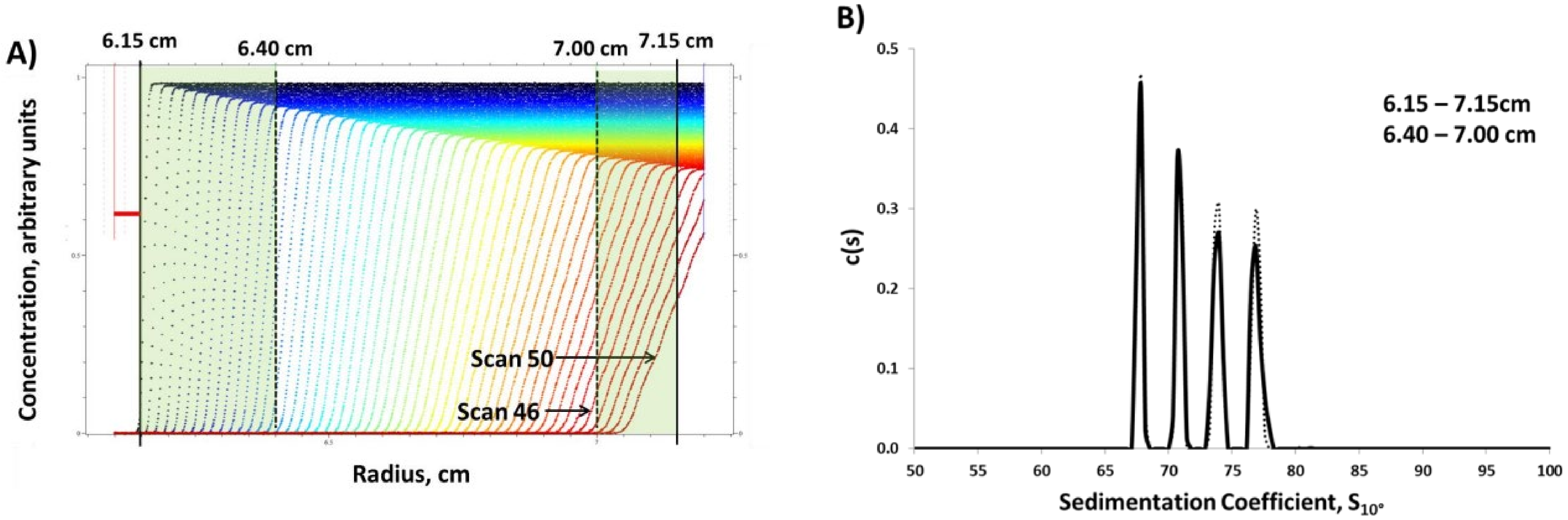
- Requires rigorous matching of buffer and sample through dialysis and the use of meniscus matching centerpieces
- Buffer mismatch can be modeled using SEDFIT but this is often difficult with a large number of buffer components
- Can also run water in the reference sector and model components in c(s) distribution
- Avoid doing any of these options by out running the buffer

AAV outruns buffer components



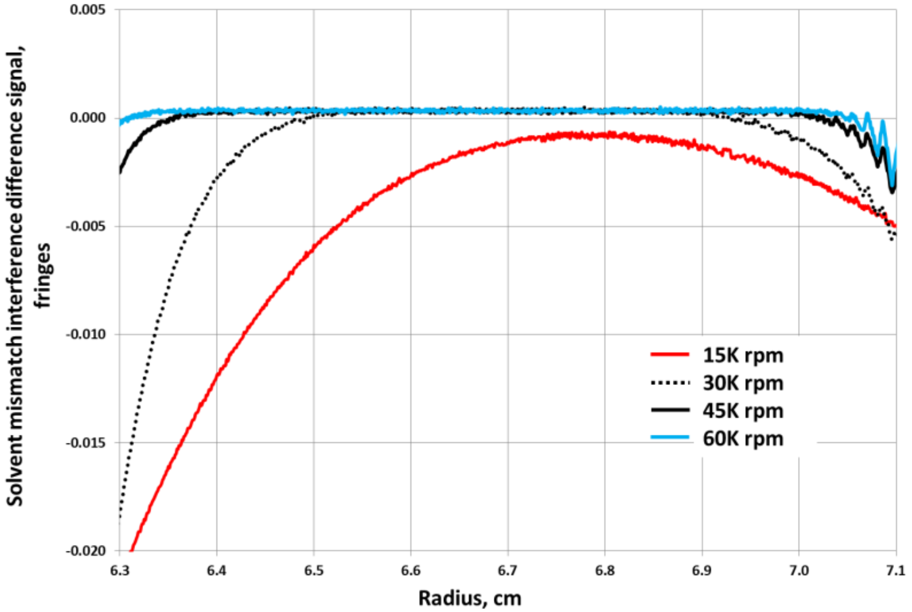
- By the time the AAV has sedimented the buffer components are only a small fraction of the way down the cell

Decreased radial fitting window does not impact the resulting c(s) distribution

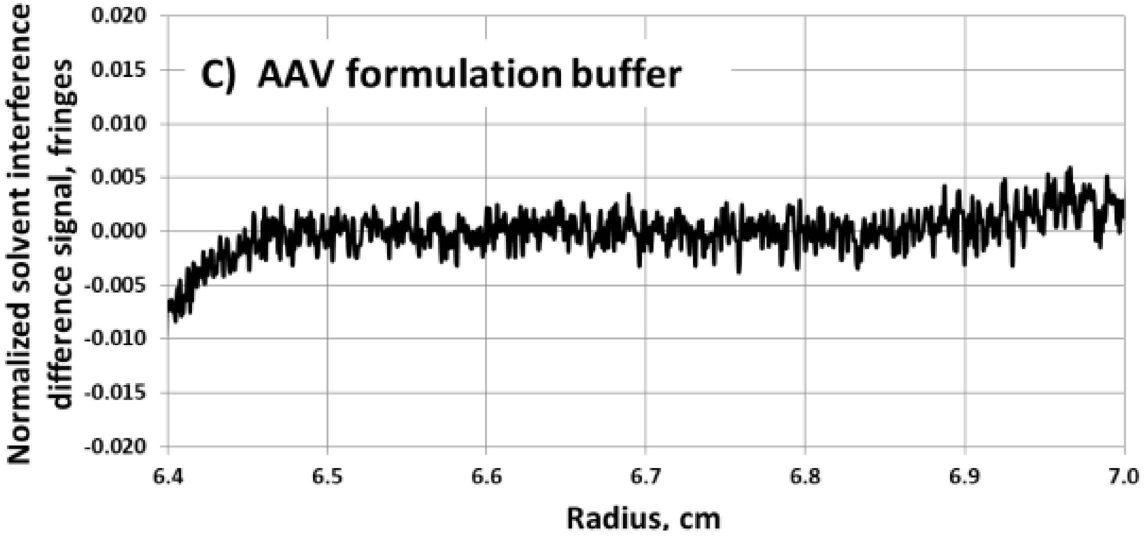


Buffer mismatch simulations agreement with experiment

Simulation

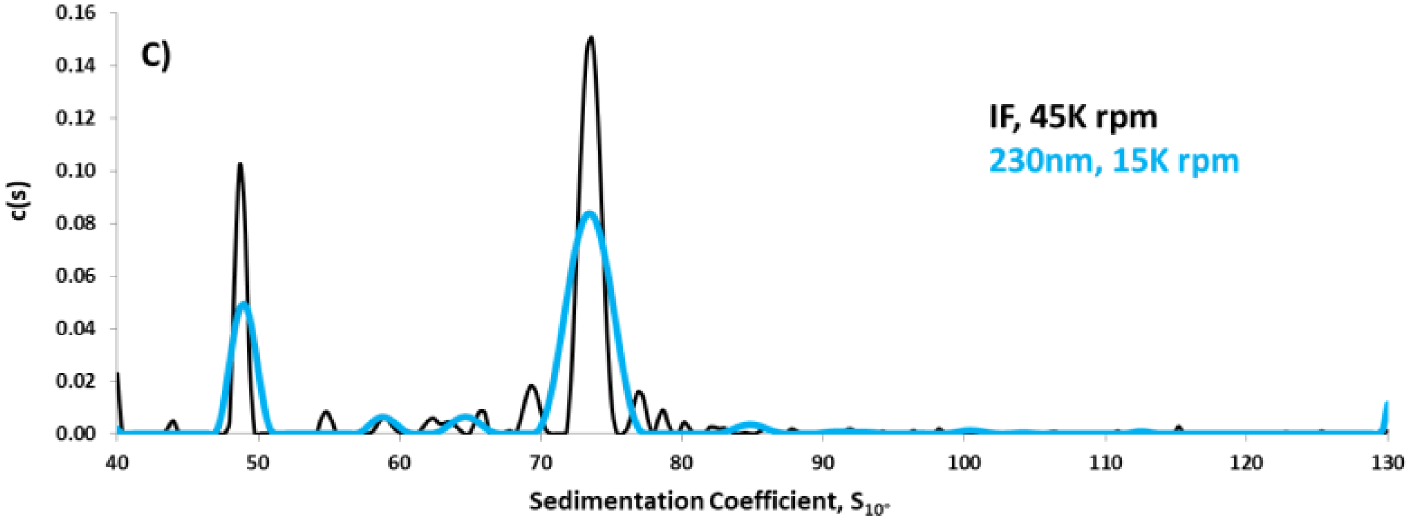


Experiment (45k rpm)



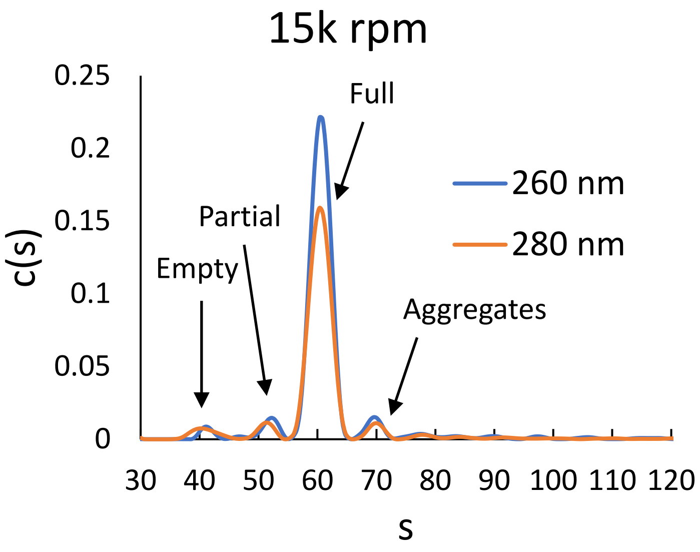
- Comparing the last scan of an AAV run with a 5 % compositional mismatch and 10 uL difference in loading volume

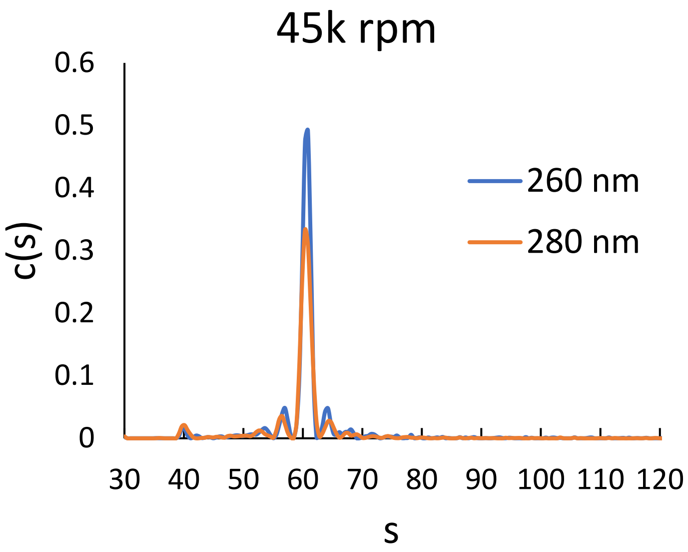
Comparison of empirical results at 45k and 15k rpm



	45k Interference (n = 4)	15k OD 230 nm (n = 2)	15k interference (n = 2)
%Empty	20.7	21.3	22.1
Empty s	49	48.8	48.7
% Partial	10.4	7	7.4
% Full	57.7	64.3	63.5
Full s	73.5	73	73.5
% HMW	10.4	7.6	6.7

Absorbance detection indicates ratio of protein to DNA content of each peak

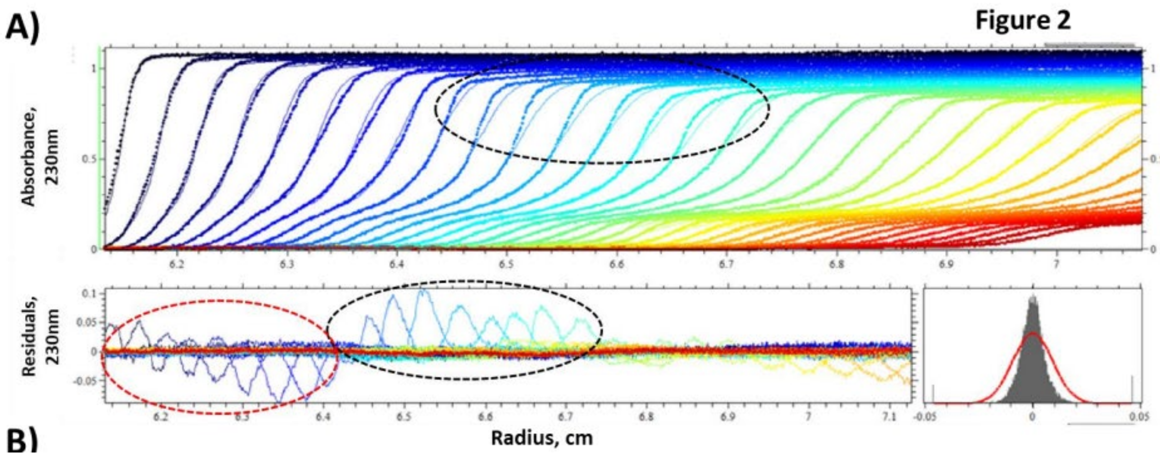


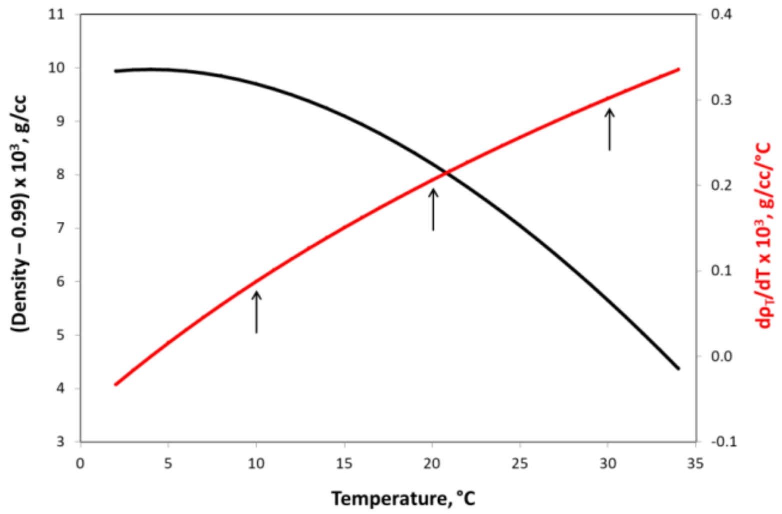


Attribute	15k (n = 3)	45k (n = 3)
Empty		
s	41.2 (0.5)	39.9 (0.06)
% (260 nm)	2.1 (0.1)	1.5 (0.1)
260/280	0.66 (0.01)	0.53 (0.04)
Partial		
% (260 nm)	4.7 (0.5)	11.5 (0.5)
260/280	1.19 (0.07)	1.12 (0.01)
Full		
s	60.5 (0.1)	60.6 (0.4)
% (260 nm)	82.9 (3.1)	73.9 (2.9)
260/280	1.28 (0.03)	1.29 (0.02)
Aggregate		
% (260 nm)	10.3 (3.2)	13.1 (2.6)
260/280	1.37 (0.08)	1.42 (0.04)

*Data in table presented as mean (std deviation)

Boundary sharpening from convection can be mitigated by running at low temperature





- At 5 C the change in density w.r.t temperature is 0 for water

Berkowitz SA, Laue T. Boundary convection during sedimentation velocity in the Optima analytical ultracentrifuge. Analytical Biochemistry. 2021 Oct 15;631:114306.

Conclusions

- Interference detection can be simplified avoiding the need for rigorous buffer and meniscus matching
- The resolution of the method impacts determination of drug product purity
- Running at higher speeds increases the resolution
    - This is true for all macromolecules with high enough mass (lentivirus, LNP, etc.)

Acknowledgements

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- Tom Laue
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Berkowitz S, Larson N, Bou-Assaf G, Laue T. Rapid high-resolution size distribution analysis for adeno-associated virus using high speed SV-AUC. bioRxiv. 2023:2023-05.