



Spectral and Hydrodynamic Analysis of West Nile Virus RNA— Protein Interactions by Multiwavelength Sedimentation Velocity in the Analytical Ultracentrifuge

Zhang et al.

Presented by Liam Kerr

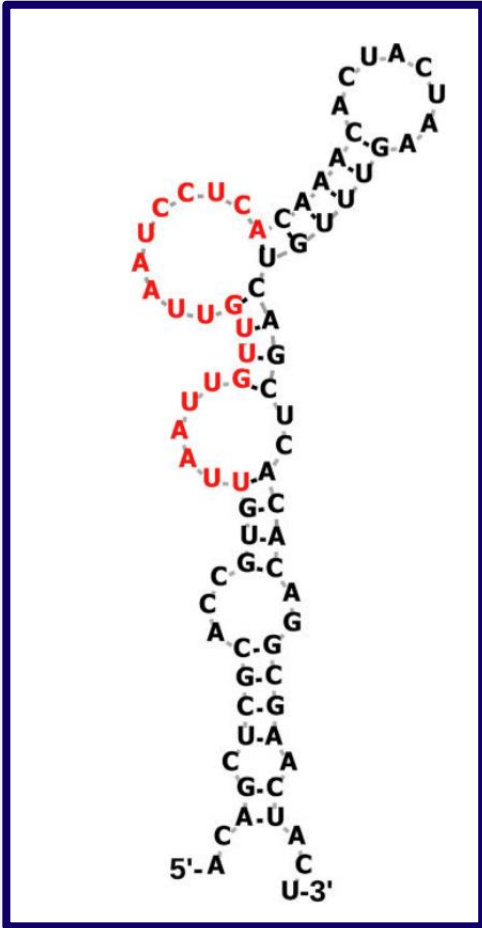
BCHM 4000 Spring 2024

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Overview

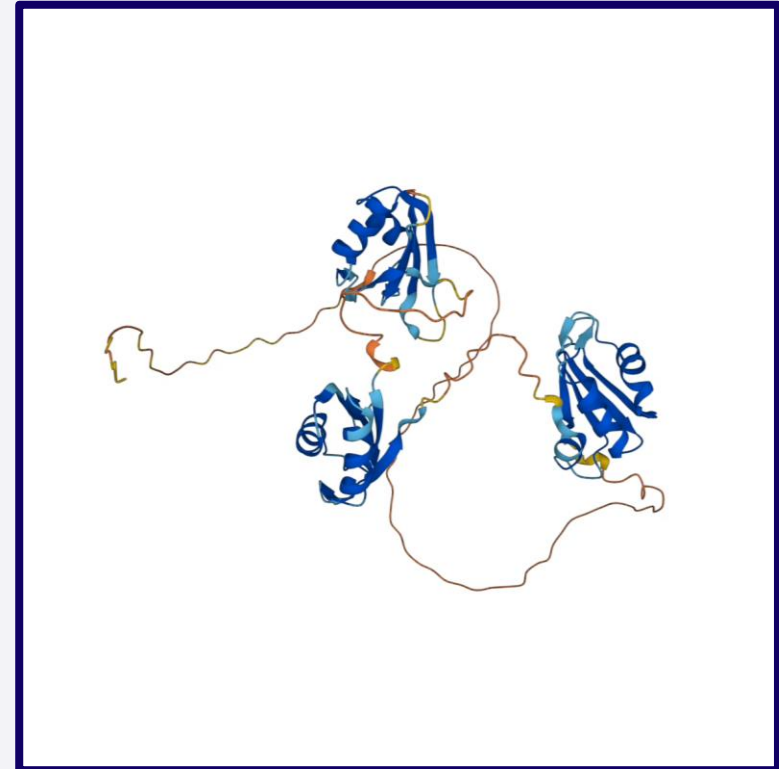
Background	Methods	Results/Conclusions
WNV and hTIAR	Preparation of WNV and hTIAR	Sedimentation determination of hTIAR:WNV Ratios
Characterization of RNA-protein interactions	Multi-Wavelength AUC Sedimentation Velocity analysis	Determination of Stoichiometry

West Nile Virus (WNV) and human T-cell restricted intracellular antigen-1-related protein (hTIAR)



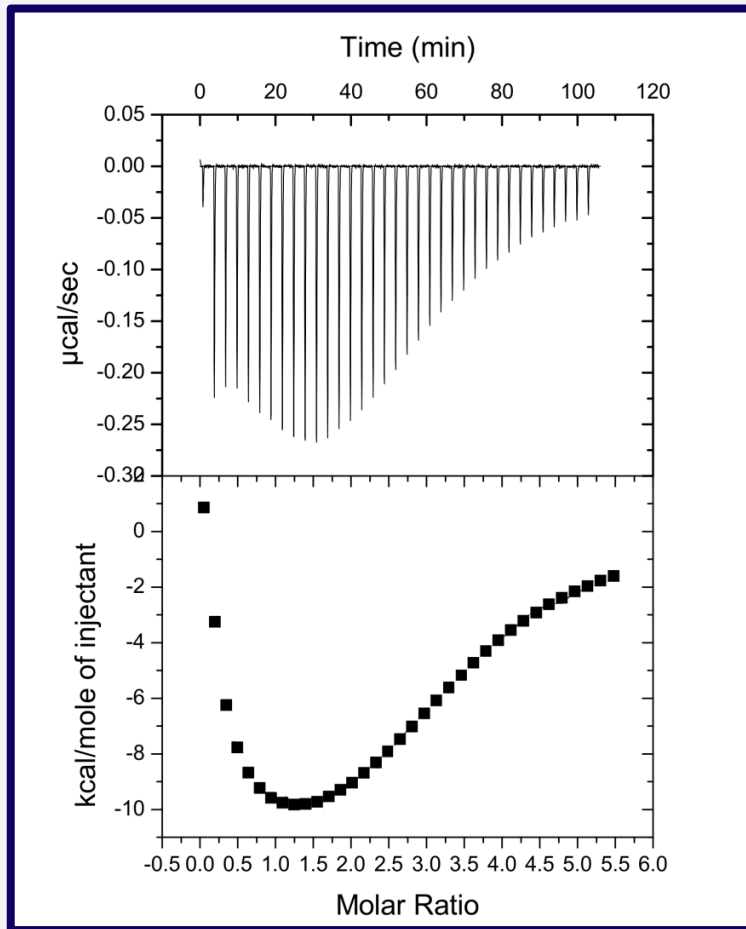
(Zhang et al., 2017)

- WNV is a single stranded RNA virus.
- Currently no therapeutic treatment for WNV.
- hTIAR is a multi-functional RNA binding protein.
- 3 RRM domains that bind to AU rich regions

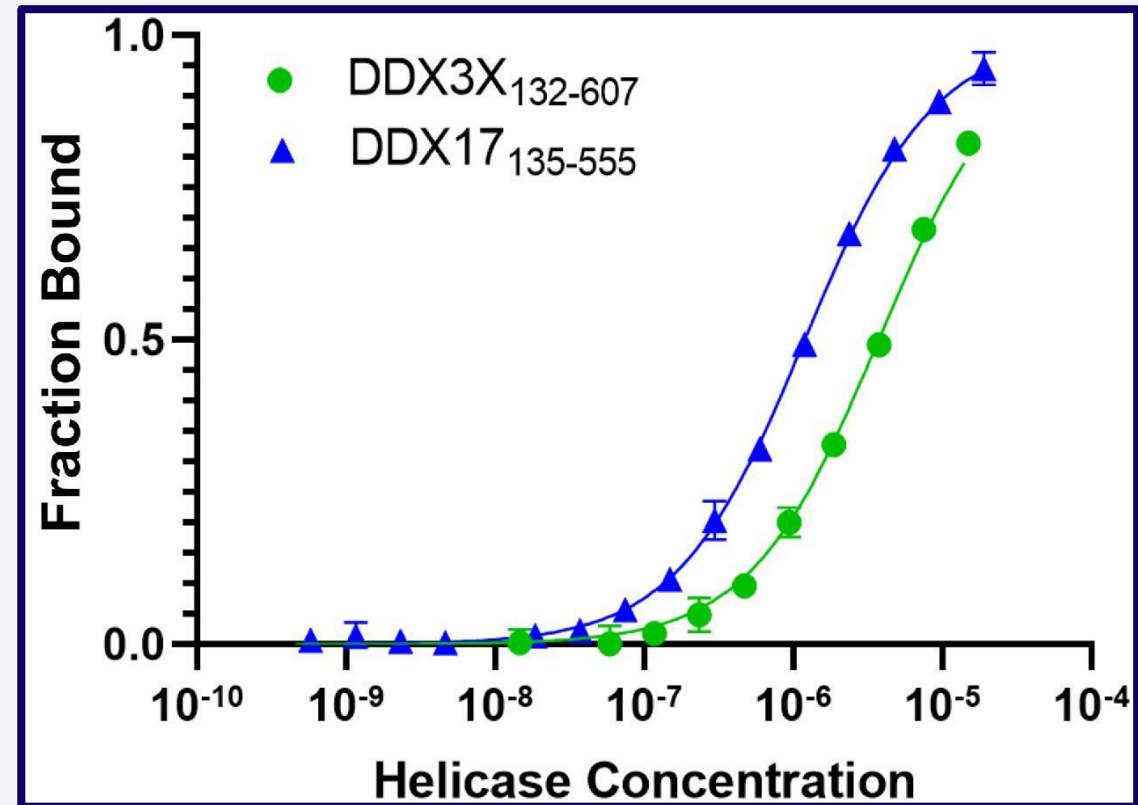


AF-P31483-F1 (Jumper et al., 2021)

Characterization of viral-RNA and host protein interactions

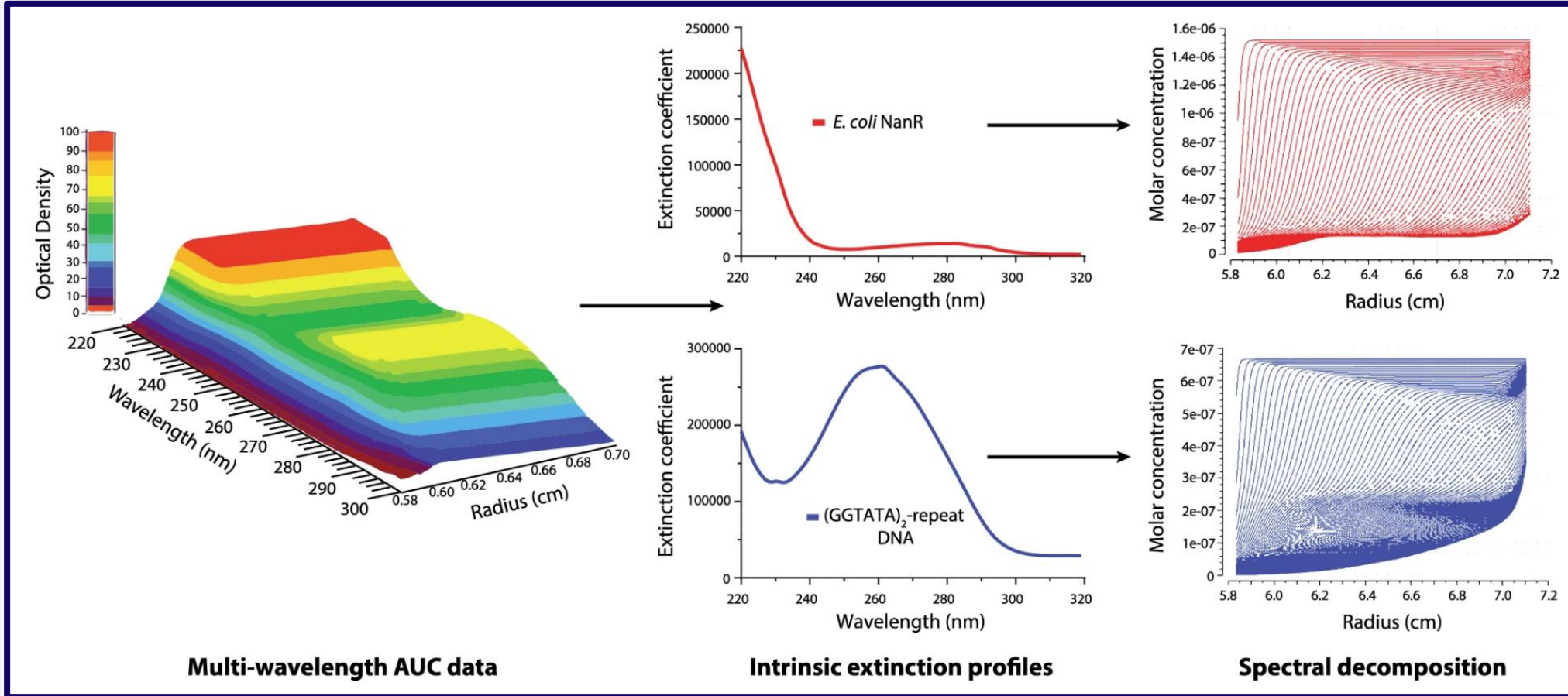


(Zhang et al., 2017)



(Gemmill et al., 2024)

Multi-Wavelength AUC Sedimentation Velocity analysis

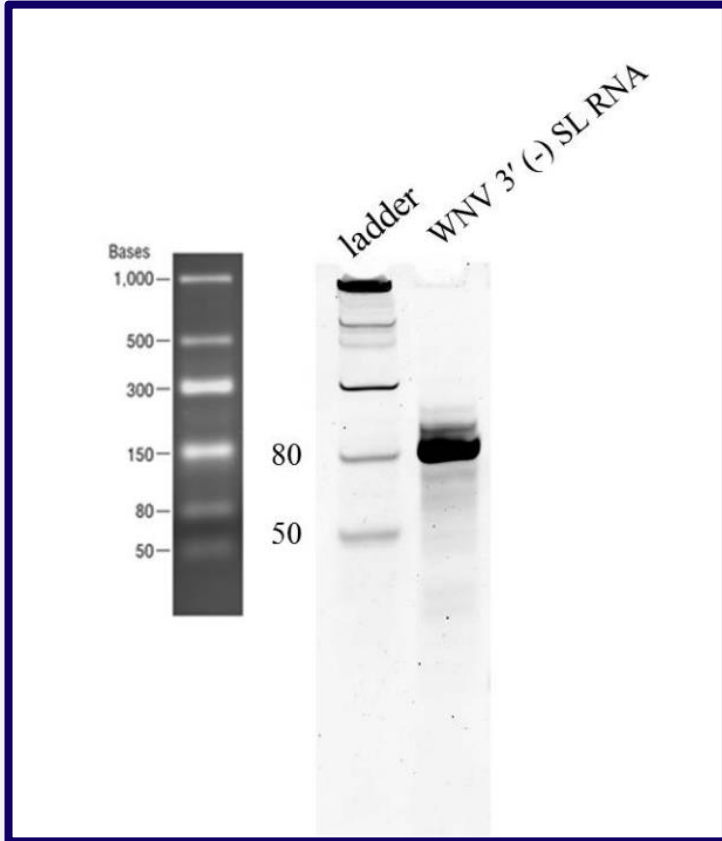


(Horne et al., 2020)

Overview

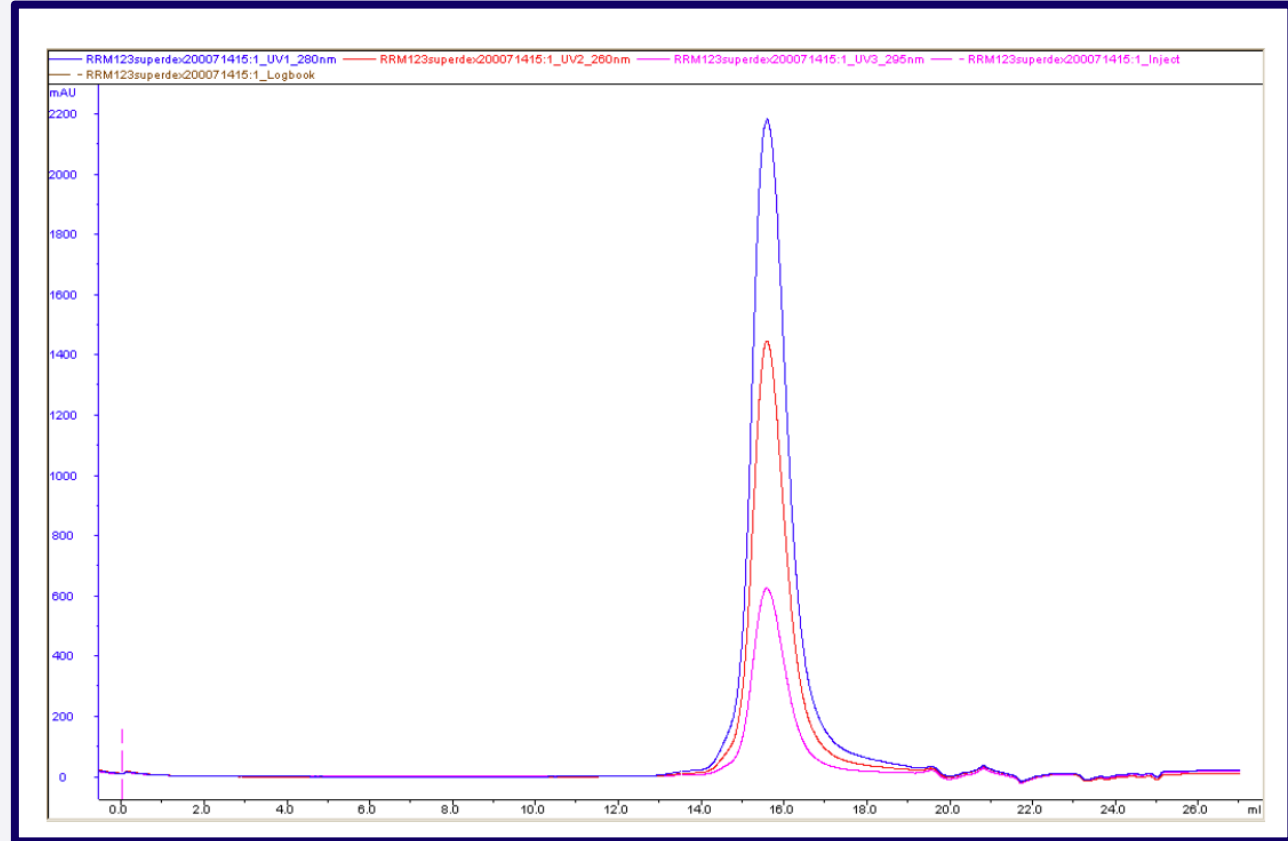
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Preparation of WNV stem loop RNA, and hTIAR



(Zhang et al., 2017)

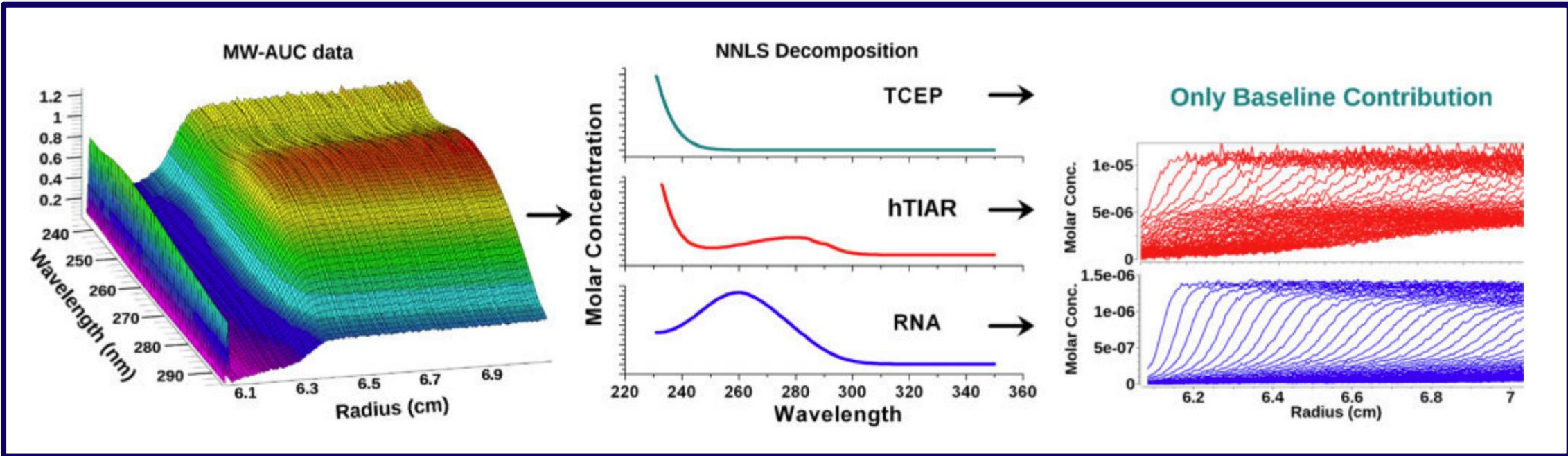
- RNA produced through transcription of amplified PCR product



(Zhang et al., 2017)

- hTIAR was purified through over expression followed by ammonium sulfate precipitation
- Chromatogram of S200 shows hTIAR purity

MWL-SV decomposition of hTIAR:WNV

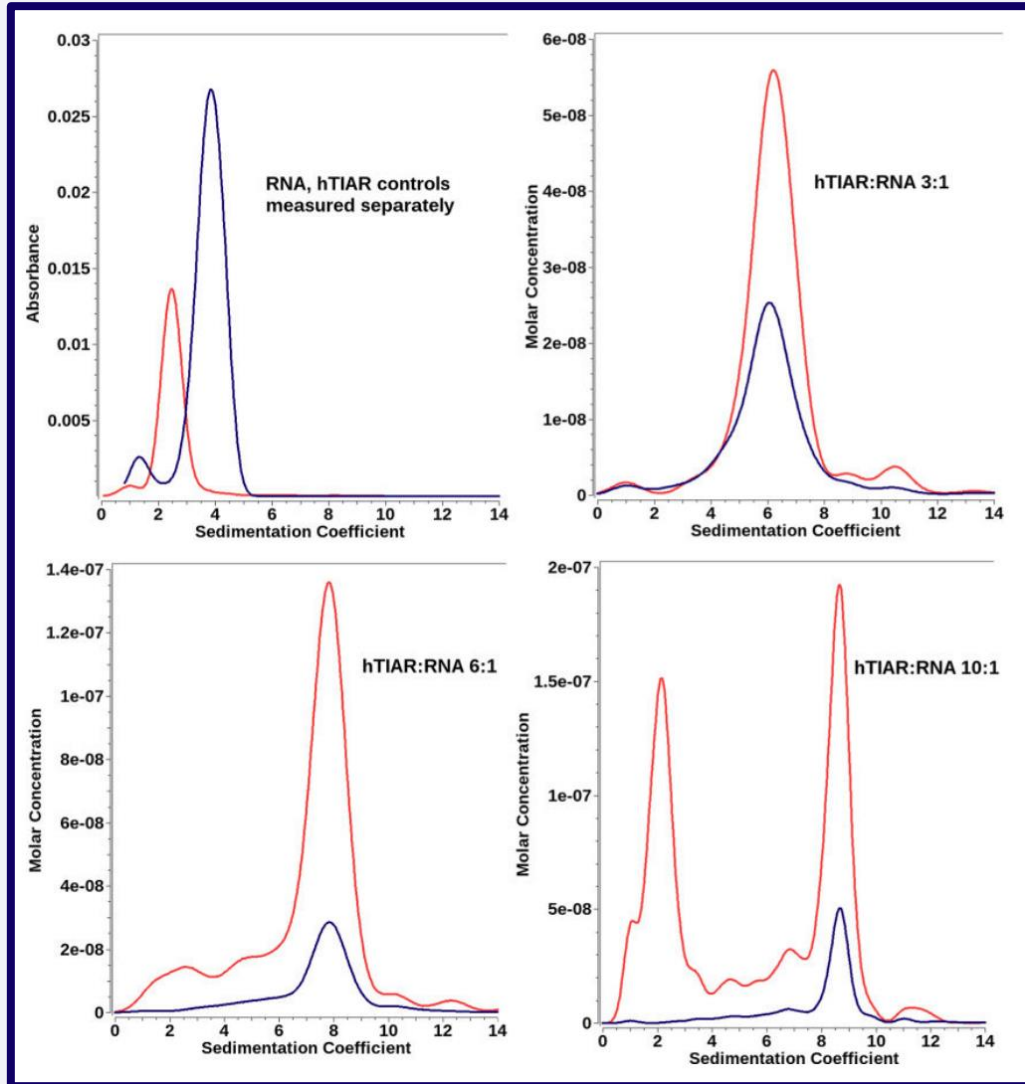


(Zhang et al., 2017)

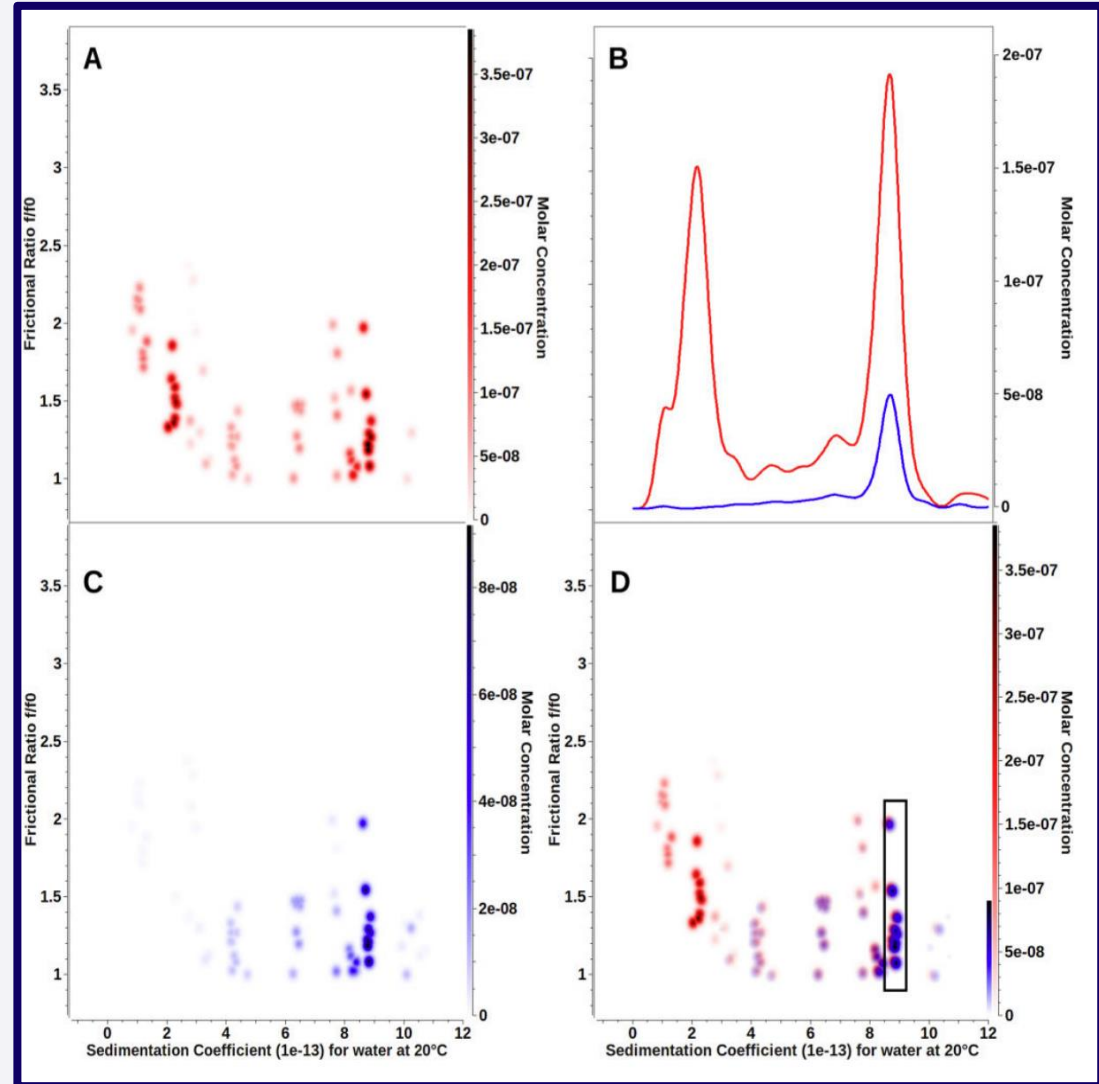
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Sedimentation determination of hTIAR:WNV interaction



(Zhang et al., 2017)



(Zhang et al., 2017)

Red: hTIAR
 Blue: WNV

Stoichiometry of hTIAR:WNV Interaction

Summary

	hTIAR	RNA	hTIAR:RNA		
			3:1	6:1	10:1
sedimentation coefficient (x 10 ⁻¹³ s)	2.45	3.87	6.43	7.69	8.78
measured molar ratio	n/a	n/a	1.63	4.25	4.3
predicted par. specific volume	0.728 mL/g	0.550 mL/g	0.672 mL/g	0.701 mL/g	0.701 mL/g
predicted molar mass	31.9 kDa	23.7 kDa	75.7 kDa	159.3 kDa	160.9 kDa
measured molar mass	31.9 kDa	22.9 kDa	N/D	N/D	152.7 kDa



Key Points

- MWL-AUC SV is a high-quality technique for identifying protein-RNA interactions
- MWL-AUC SV Allows for determination of stoichiometry of interactions as well as determining if higher order complex formation occurs



Questions?



Demeler, B. (n.d.). Measuring molecular interactions in solution using multi-wavelength analytical ultracentrifugation: combining spectral analysis with hydrodynamics. <https://doi.org/10.1042/bio04102014>

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