Towards Understanding Flaviviral Genome Cyclization

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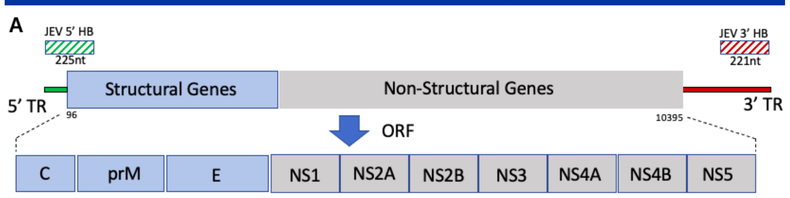
Introduction

Flaviviruses are a family of single stranded (+) sense RNA viruses which have been responsible for global concern such as the recent outbreaks of Zika and Dengue. Flaviviruses are highlighted by both the World Heath Organization and the Centers for Disease Control as global health threats([1]). These viruses have highly-conserved terminal regions (TRs). The 3' TR consists of approximately 700 untranslated nucleotides, varying virus to virus([2]).

JEV Terminal Regions



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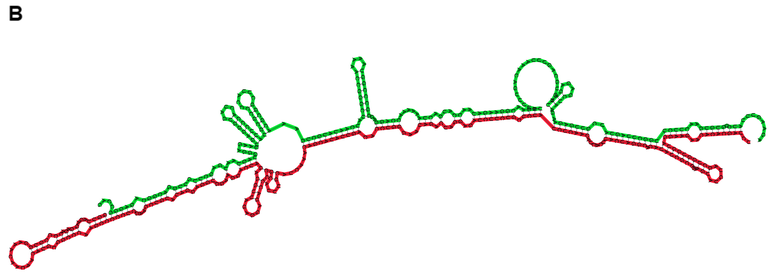

Figure 1. JEV genomic organization. (**A**) Schematic of the JEV Genome. Shaded regions indicate the portions of the TRs investigated. (**B**) Computationally predicted secondary structure of selected JEV 5' TR RNA (green) and 3' TR RNA (red) in complex.

Biophysical Approach to Viral Cyclization

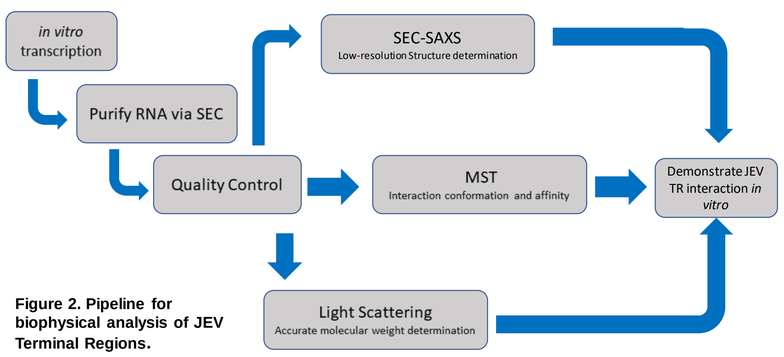

Figure 2. Pipeline for biophysical analysis of JEV Terminal Regions.

Bioinformatics Analysis of JEV TRs

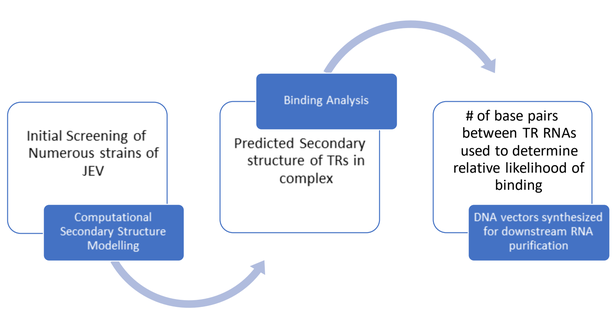

Figure 3. Computational Determination of theoretical binding likelihood from base pair interactions.

Purification & Quality Control

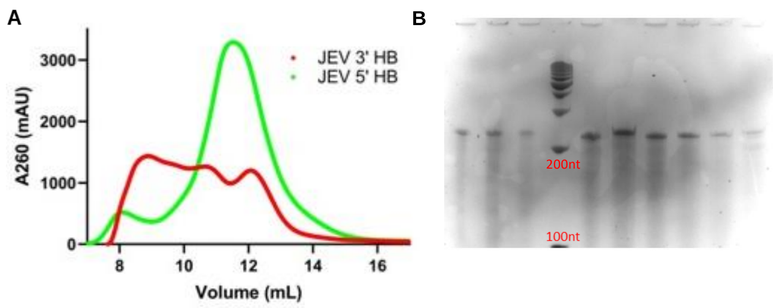

Figure 4. Purification and Quality analysis of *in vitro* transcribed JEV HB RNA. (A) Elution profile of JEV 3' HB (green) and 5' HB (red). **(B)** Urea-PAGE showing JEV 3' HB (left) and 5' HB (right) following SEC purification.

Multi-Angle Laser Light Scattering

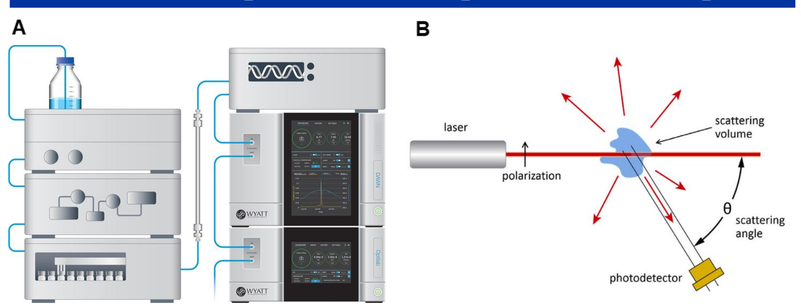

Figure 5. Size Exclusion Chromatography paired with DAWN light scattering detector and Wyatt Refractometer. (A) Visual representation of SEC-MALS equipment . **(B)** Depiction of laser light scattering and detection. A Series of 18 detectors ranging from 15° - 160° utilized to measure light scattering from biomolecule. Direct measurement of molecular weight derived from scattering.

Multi-Angle Laser Light Scattering (SEC-MALLS)

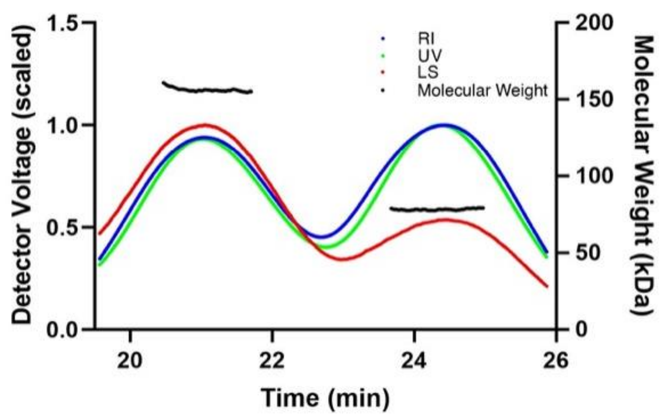

Figure 6. Molecular weight determination through SEC-MALS. Molecular weight shown as a function of time. Complex (~150kDa) elutes prior to monomers (~75kDa) and shows homogeneity across each respective peak as indicated by the horizontal molecular weight.

Microscale Thermophoresis

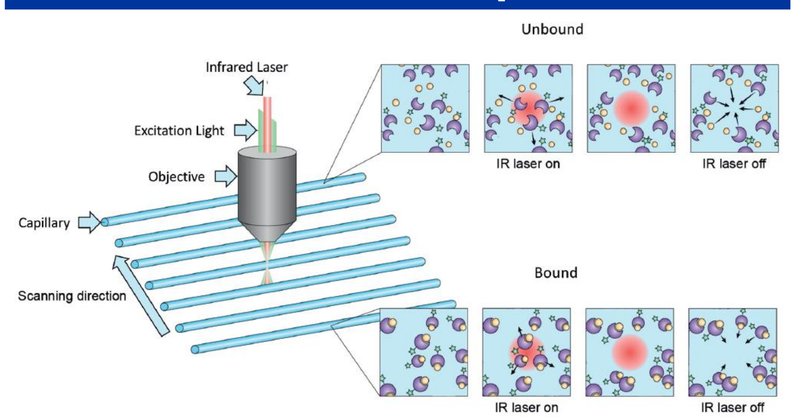

Figure 7. Microscale Thermophoretic theoretical approach. One of two of the interacting samples requires fluorescent labeling of one of the two interacting partners (target). The change in thermophoresis between an interacting pairs of molecules is compared and a dissociation constant can be calculated[6]

JEV TR RNA Interacts *in vitro*

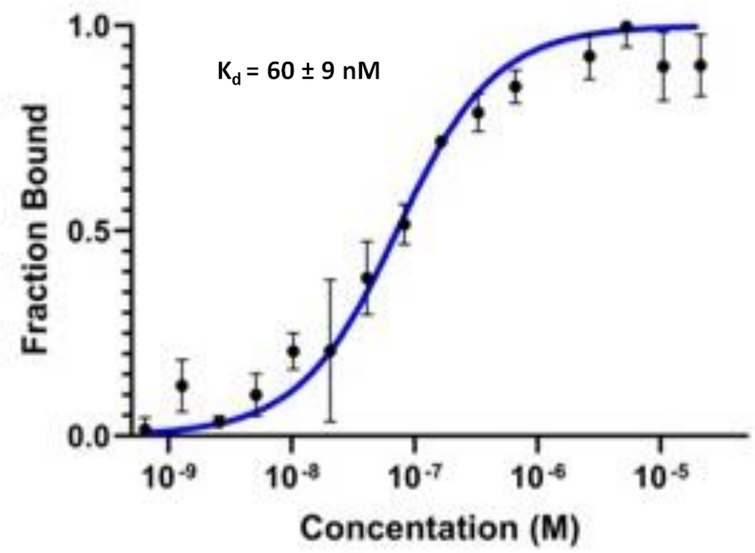

Figure 8. Microscale Thermophoresis showing an interaction between JEV 5' HB and JEV 3' HB. Binding curve generated from a 16 point serial dilution of JEV 3' HB while maintaining a constant concentration of fluorescently labelled JEV 5' HB.

Conclusions

- JEV terminal regions can be purified in sufficient quantity and homogeneity
- A variety of biophysical techniques demonstrate JEV TR interaction *in vitro*

Future Directions

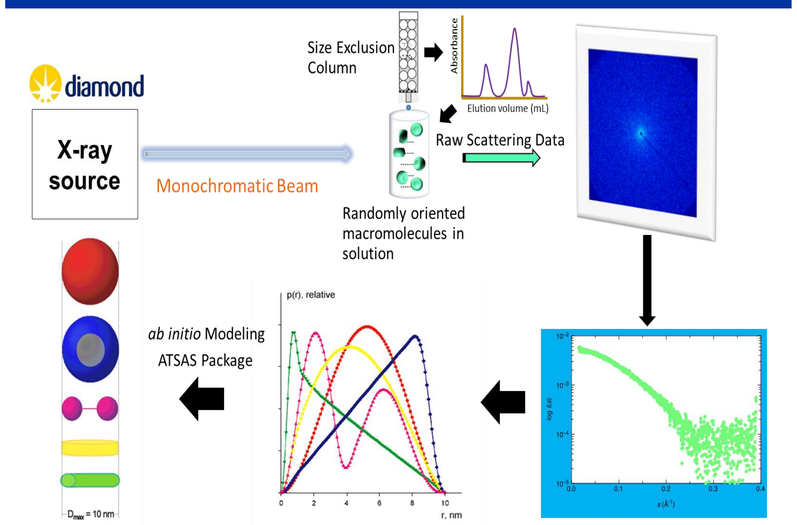

Figure 9. Pipeline of the SAXS workflow. Raw solution x-ray scattering data is buffer subtracted and radially averaged, and the inverse scattering data is transformed to real space data into form of the Paired Distribution graph (p(r) relative plot) that can be used to predict relative molecular shape[5]

References

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2. Wengler, G., (1981) *Virology* 113, 544–555.
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5. Mrozowich et al., 2018, *Journal of Visualized Experiments*
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