

# Investigating RNA-RNA Interactions Through Computational and Biophysical Analysis

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## Investigating RNA–RNA interactions through computational and biophysical analysis

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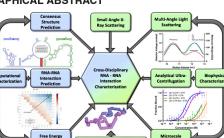
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### ABSTRACT

Numerous viruses utilize essential long-range RNA–RNA genome interactions, specifically flaviviruses. Using Japanese encephalitis virus (JEV) as a model system, we computationally predicted and then biophysically validated and characterized its long-range RNA–RNA genome interaction using the RNA computational assessment programs. We determine the primary RNA–RNA interacting site among JEV isolates and numerous related viruses. Following *in vitro* transcription of RNA, we provide, for the first time, characterization of an RNA–RNA interaction using size-exclusion chromatography coupled with multi-angle light scattering and analytical ultracentrifugation. Next, we demonstrate that the 5' and 3' terminal regions of JEV contain ~10 nm  $\text{M}_{\text{w}}$  containing minor RNA thermophoresis, and this affinity is significantly reduced when the conserved cyclization sequence is not present. Furthermore, we perform computational kinetic analyses validating the cyclization sequence as the primary driver of this RNA–RNA interaction. Finally, we examined the 3D structure of the interaction using small-angle X-ray scattering, revealing a flexible yet stable interaction. This pathway can be adapted and utilized to study various viral and human long-non-coding RNA–RNA

interactions and determine their binding affinities, a critical pharmacological property of designing potential therapeutics.

### GRAPHICAL ABSTRACT

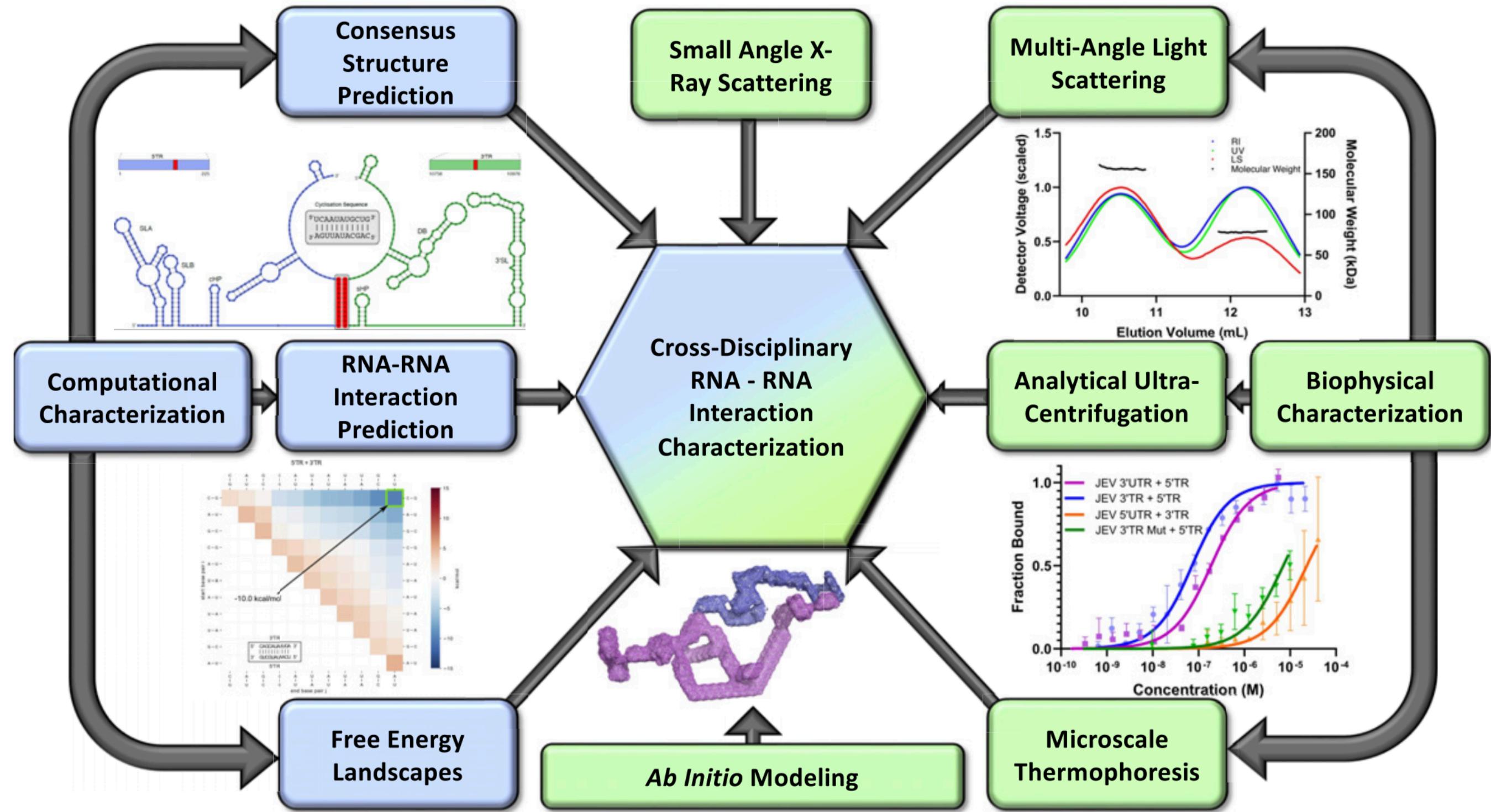


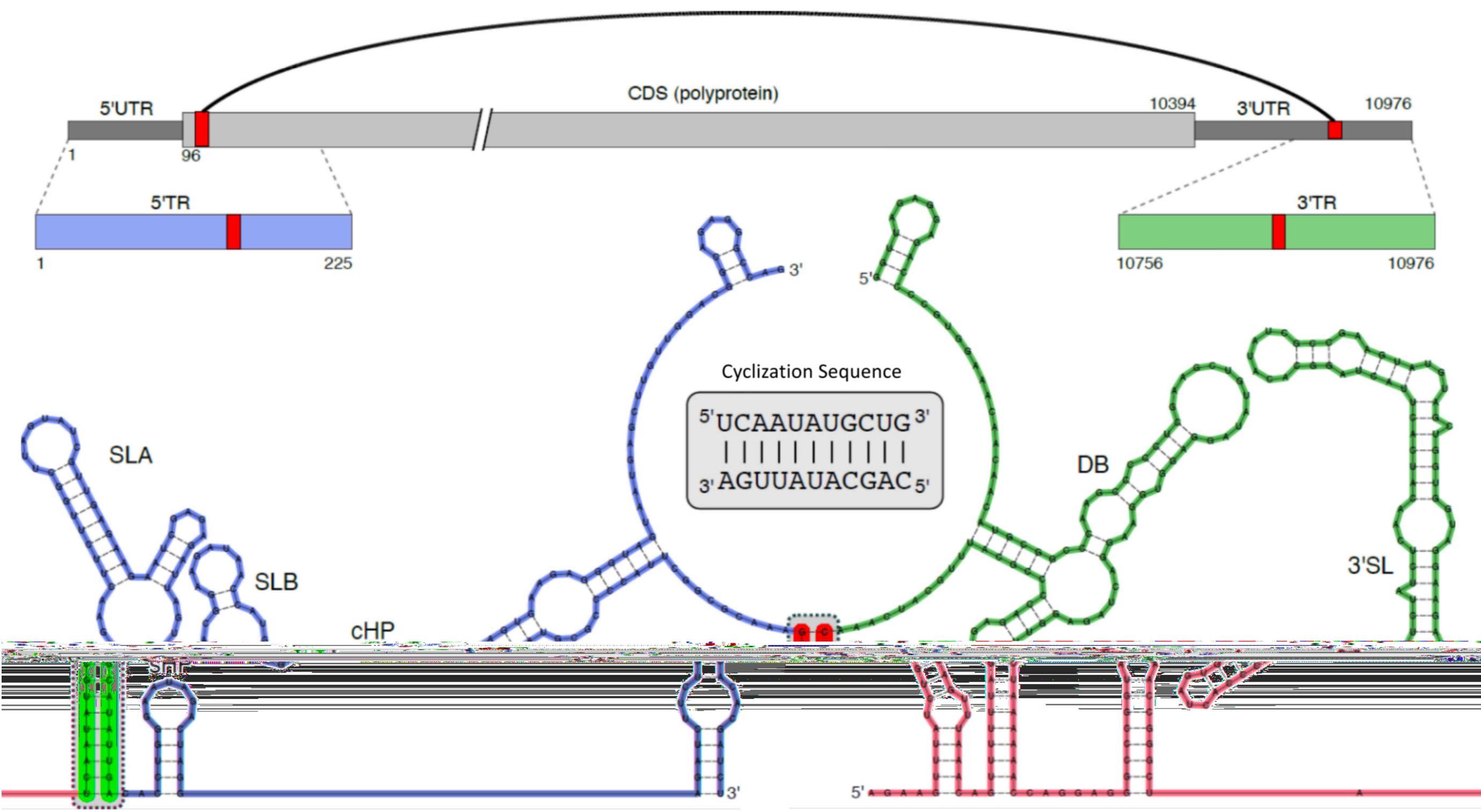
### INTRODUCTION

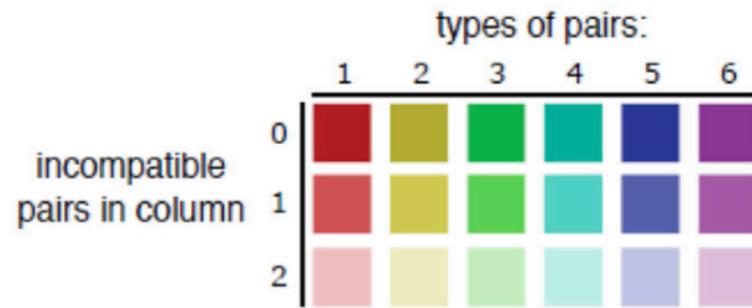
Computational and biophysical methods to obtain structure, properties and dynamics of protein–ligand interactions are well developed as a foundational component of many pharmaceutical discovery pipelines. As a result, nearly all currently approved drugs target one of ~700 disease-related proteins, despite an increasing number of diseases attributed to the >98% of the human genome,

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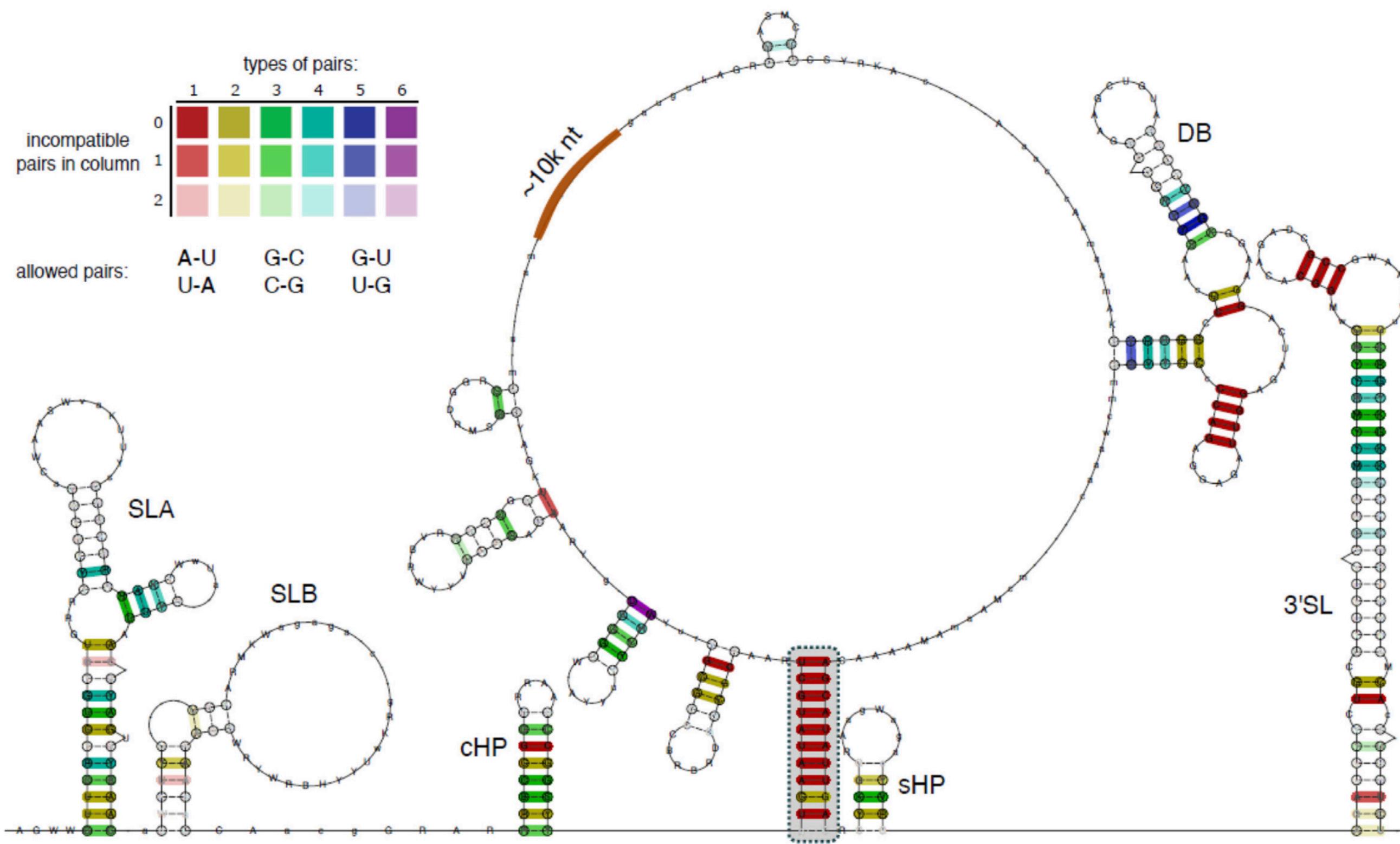


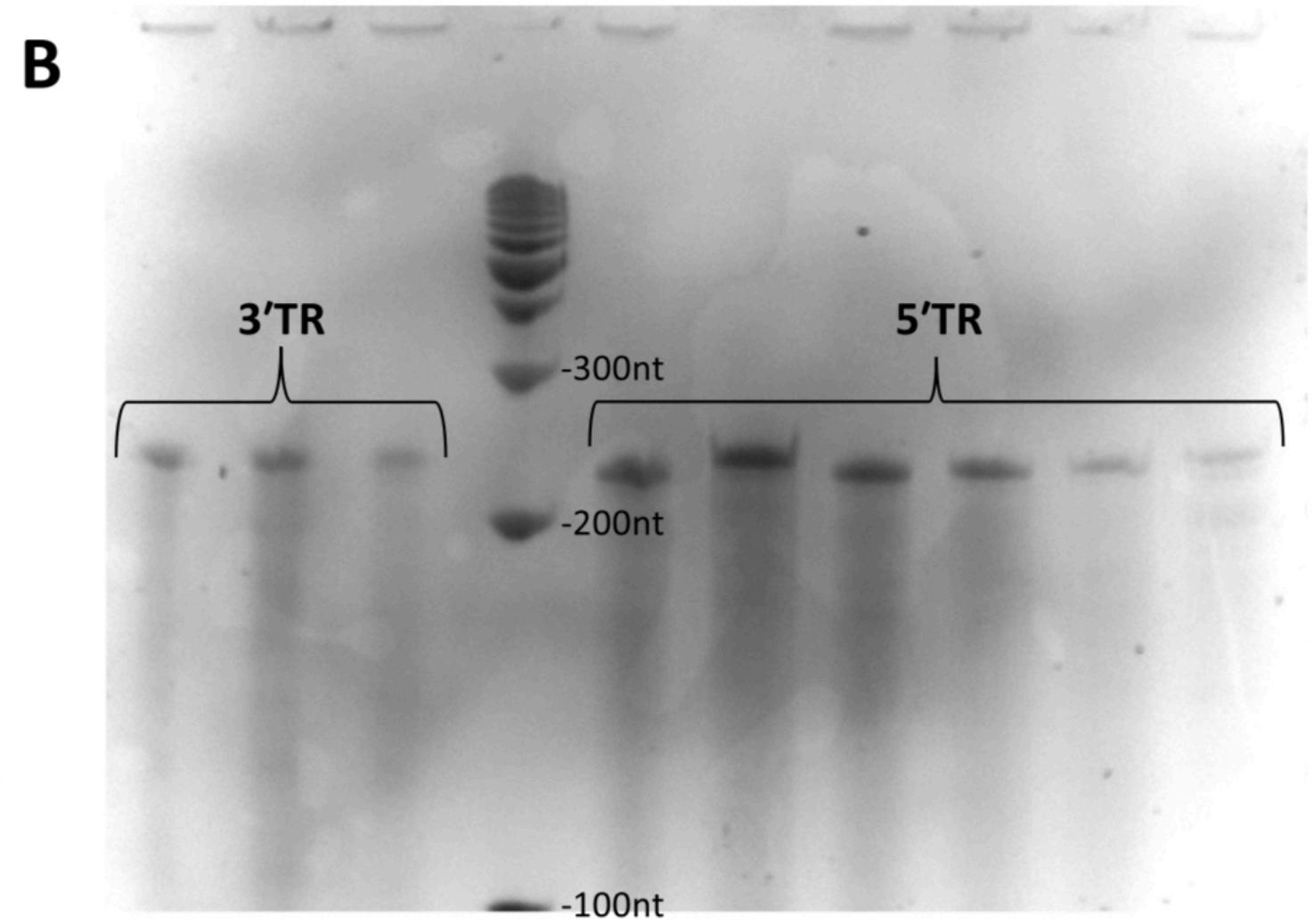
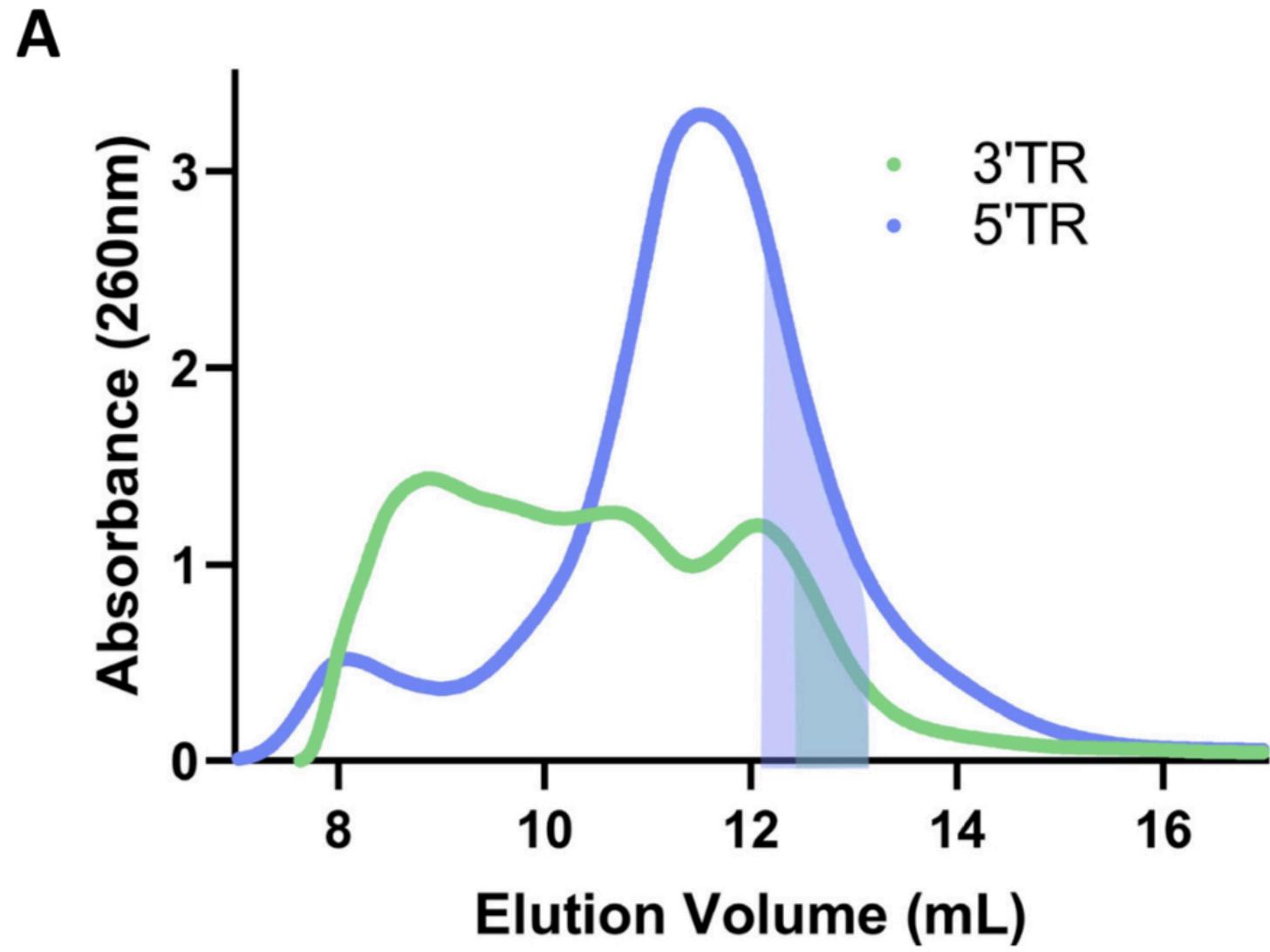


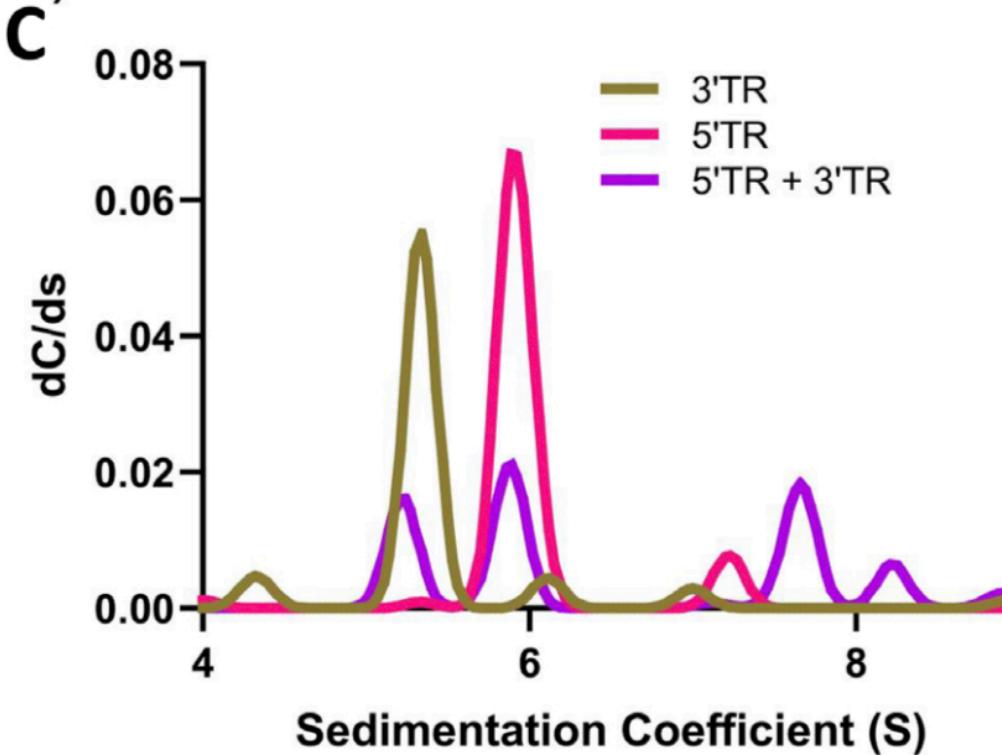
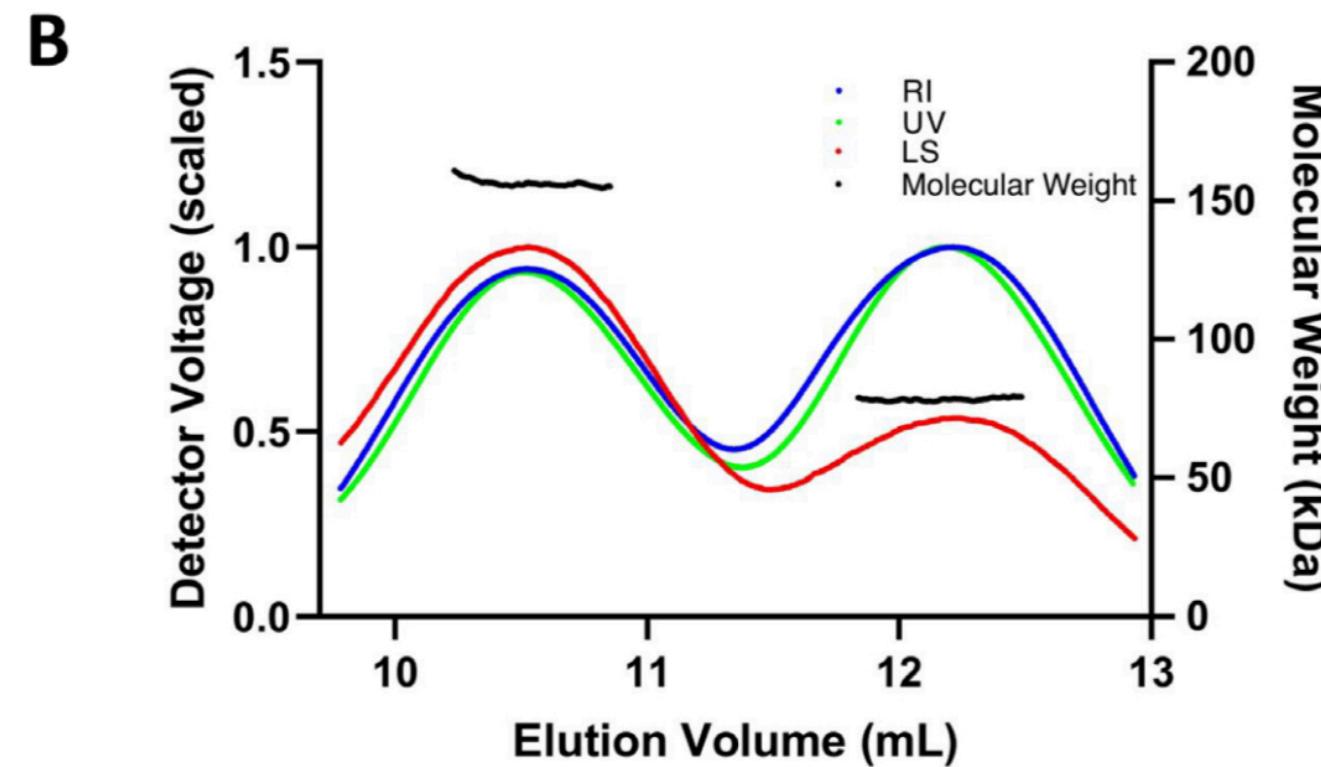
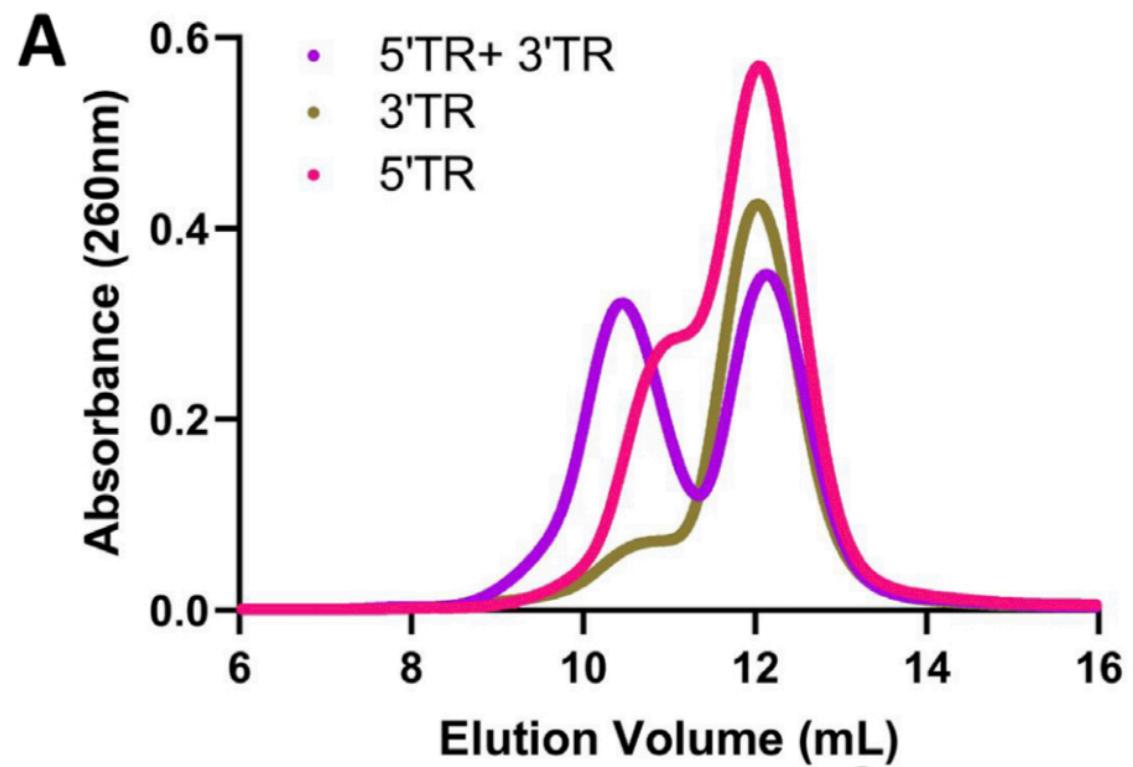


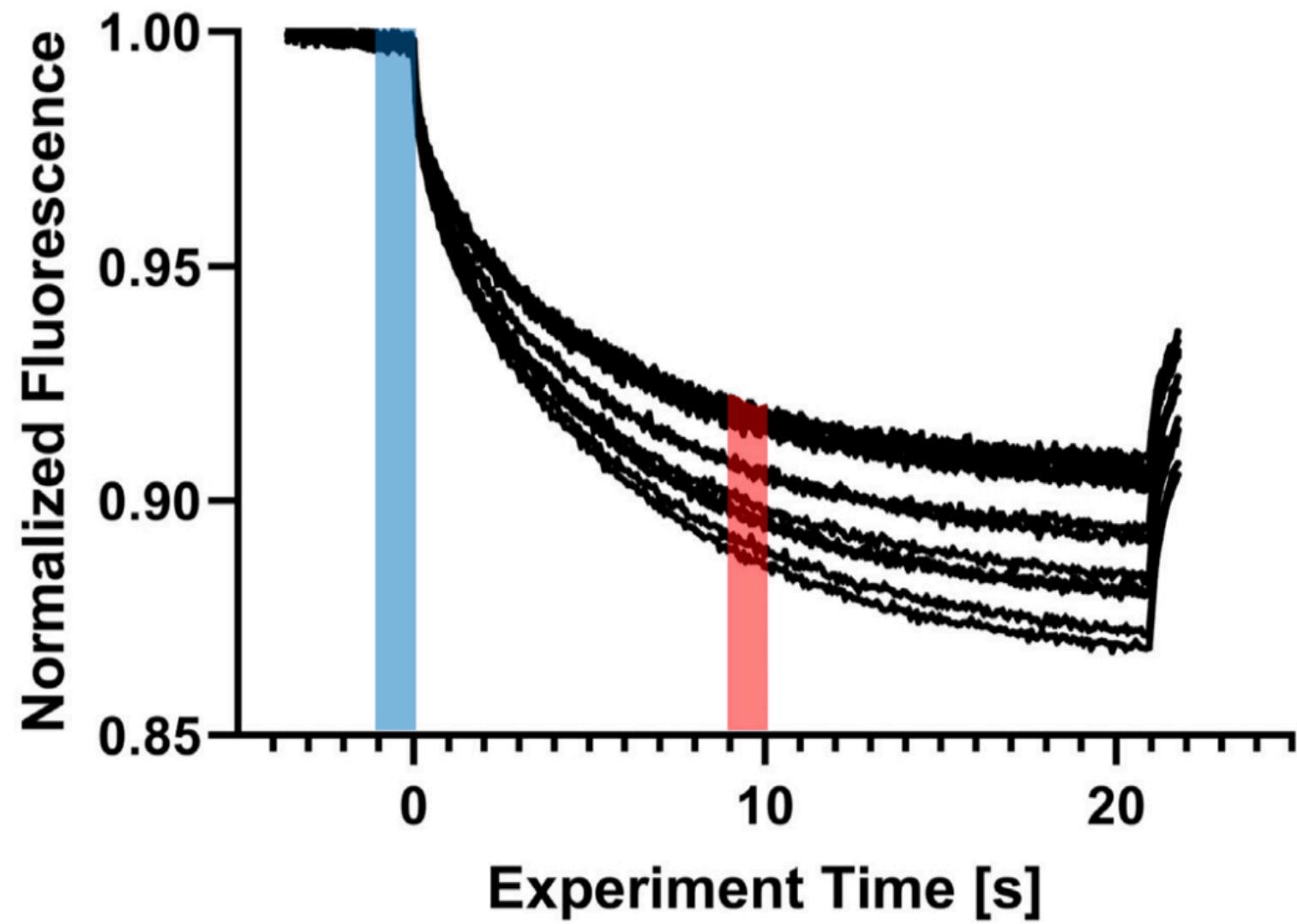
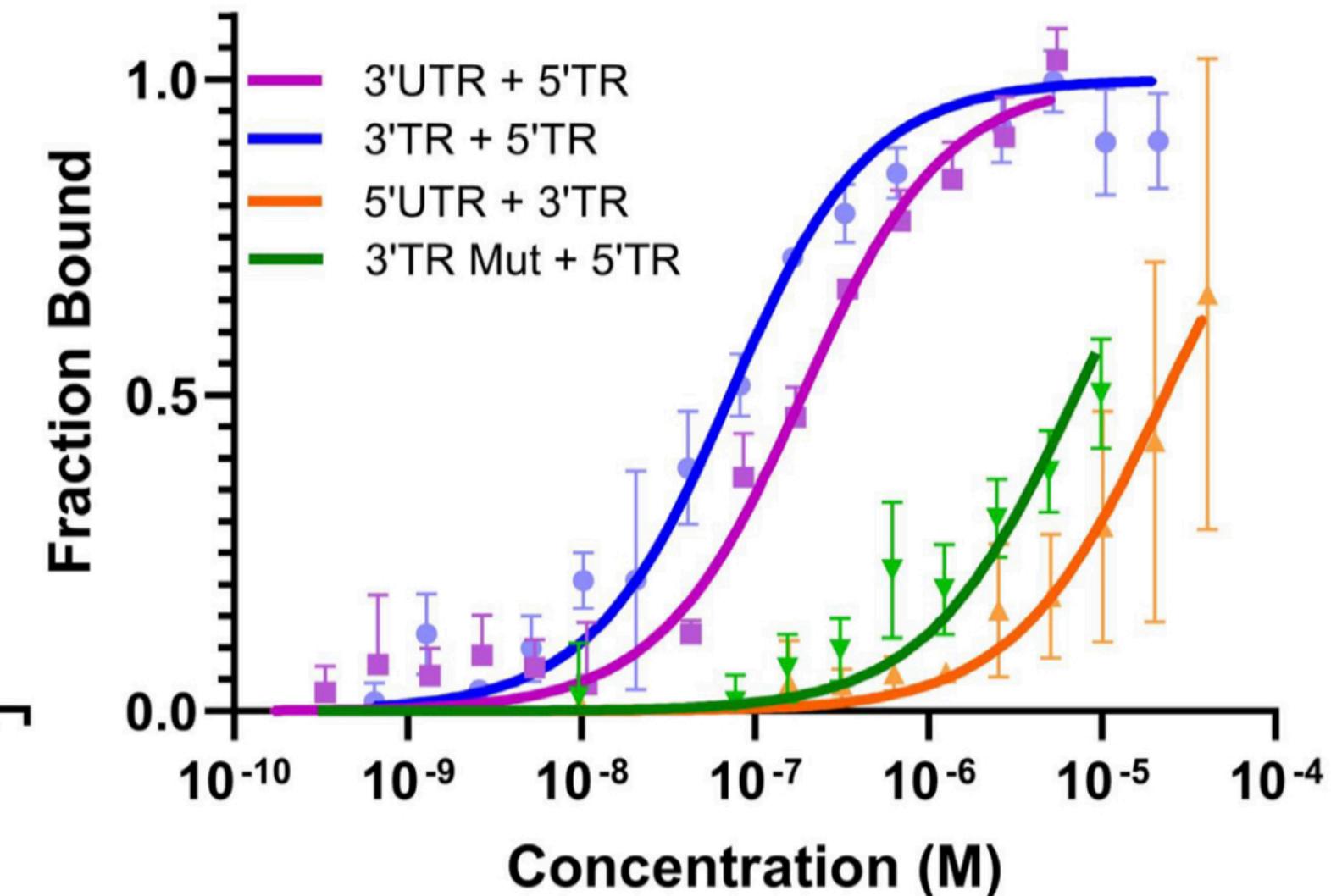
allowed pairs:

A-U	G-C	G-U
U-A	C-G	U-G







**A****B**

# 5' TR + 3' TR

