Differential Scanning Fluorimetry: A High-Throughput Screening Method for Monitoring RNA Stability

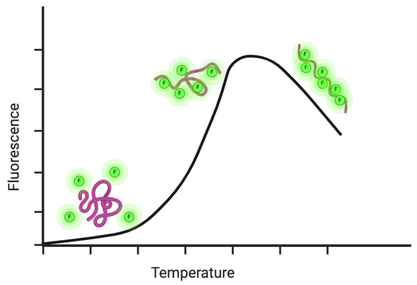
Chloé Dennis, Mentors: Victoria Ogunkunle, Nolan Blackford, Dr. Robert Silvers
Department of Chemistry and Biochemistry | Institute of Molecular Biophysics

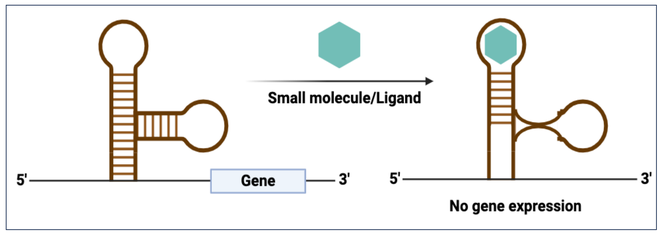
Abstract

Functional RNAs are characterized by 3-D structure and ligand binding. The riboswitches Neomycin and PreQ1 are well-characterized molecules with clear structured transitions induced by ligand binding. These molecules can serve as a model system for developing tools to monitor structural transitions in RNA folds. We have developed a system which uses intercalating dyes to monitor structural transitions by differential scanning fluorimetry. Within data we are able to identify clear structural shifts for the ligand bound and unbound states of both the Neomycin and PreQ1 riboswitches. We anticipate this approach will be widely applicable to other functional RNAs such as riboswitches. These results are pertinent in this field and others because functional RNAs are critical regulators of gene expression.

Background

- Differential scanning fluorimetry (DSF) is a method that can be utilized to quantify protein stability.
- Intercalating dyes bind to the hydrophobic part of the protein
- Unfolding is measured as a function of fluorescence intensity with temperature



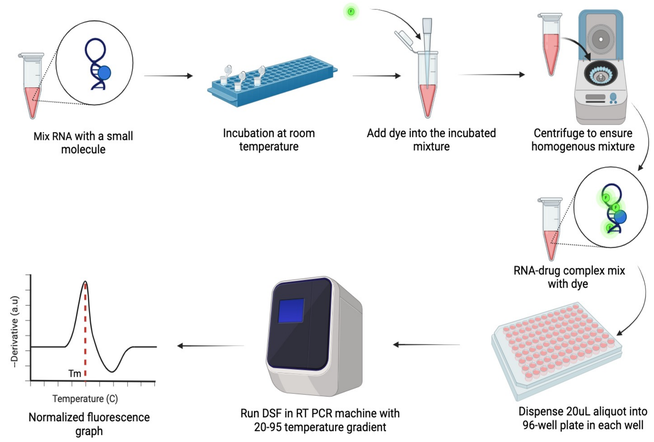


- Riboswitches regulate gene expression by binding to ligands.
- It consists of two domains
  - The aptamer domain: directly binds the ligand
  - The expression platform: regulates gene expression

Images made from BioRender.com

Methodology

- Begin by mixing specific working concentration RNA with a small molecule (drug) in a buffer solution and incubate at room temperature.
- Add 4000x RiboGreen (dye) into the tube and centrifuge to ensure a homogeneous mixture.
- Then dispense 20 uL aliquot into each well of a 96-well plate, seal with microplate films.
- Centrifuge the sample and then run in a real-time PCR machine with 20-95 ℃ temperature gradient.
- Finally, observe the fluorescence intensity.



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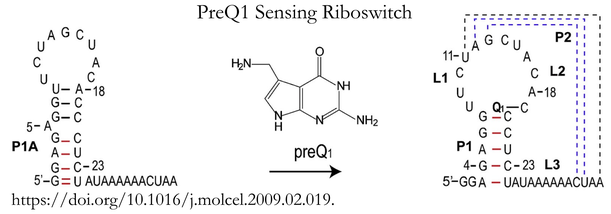
Results

PreQ1 Sensing Riboswitch



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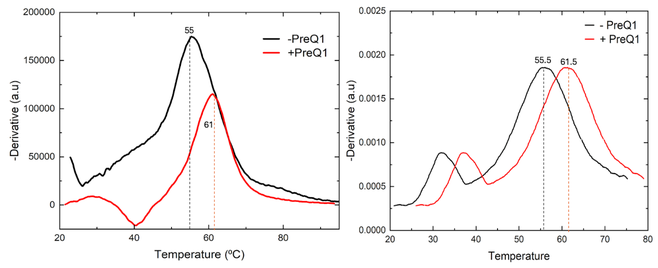
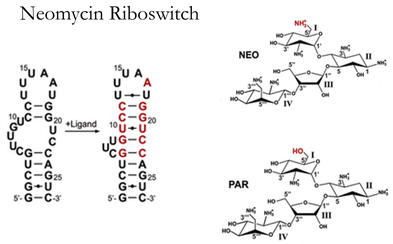
DSF (left) and UV-VIS spectroscopy of PreQ1 Riboswitch (right) produce concordant results when measuring RNA stability



Results

Neomycin Riboswitch



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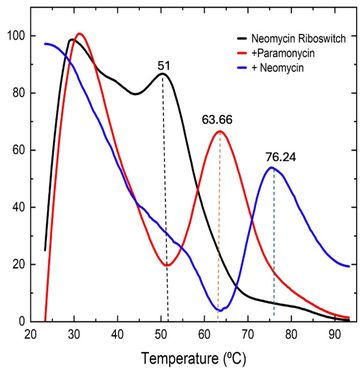
Ligand Concentration (uM)	Neomycin T_M (℃)	Paramomycin T_M (℃)
10	59.27	63.79
60	82.87	70.62
80	82.65	72.12
120	84.64	72.38

DSF of Neomycin Riboswitch with its ligands shows differing levels of stability depending on which ligand is bound



Conclusions

- PreQ1 binds to the PreQ1 Riboswitch and increases the melting temperature by approximately 6℃.
- Because of this increase in melting temperature, we can say that the presence of PreQ1 further stabilizes the PreQ1 Riboswitch.
- The Neomycin-Sensing Riboswitch binds both Paramomycin and Neomycin, discriminating between the difference in the functional groups of each ligand.
- Neomycin stabilizes the RNA more than the Paramomycin and this is observable by the variation in their melting temperatures.

Comparing the data resulting from the DSF and UV methods further validates the DSF results. Conclusively, we demonstrate DSF as a high-throughput screening method for determining RNA stability.

References

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