Applying DMS-MaPseq for Evaluating RNA-Ligand Binding

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Background

- RNAs play critical and diverse roles in the cell ranging from protein translation and genetic regulation
- Dysfunction implicated in many diseases such as cancers and neurological disorders
- Structure of RNA presents significant challenges in conventional drug discovery
- Few RNAs have been drugged with ribosomal RNA being drugged extensively
- Most known chemical space of RNA-binding molecules lack drug-like characteristics
- Recent developments have found chemical space of RNA binders with drug-like properties
- Chemical space must be investigated to further development of RNA targeted therapeutics
- Current techniques to assess RNA-ligand interactions are either limited in their throughput or in the targets or molecules that can be evaluated

Purpose

- Further develop chemical probing as a technique to evaluate RNA-ligand interactions
- Advent of multiplex sequencing allows for evaluation of thousands of RNAs in single experiments
- Standardize this technique by using known set of RNA binding motifs with their known binders
- Provide basis for high-throughput assays for the discovery of novel RNA binding molecules

RNA Binding Molecules

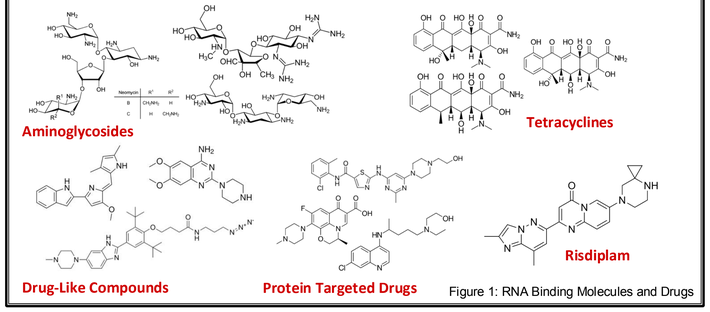

Figure 1: RNA Binding Molecules and Drugs

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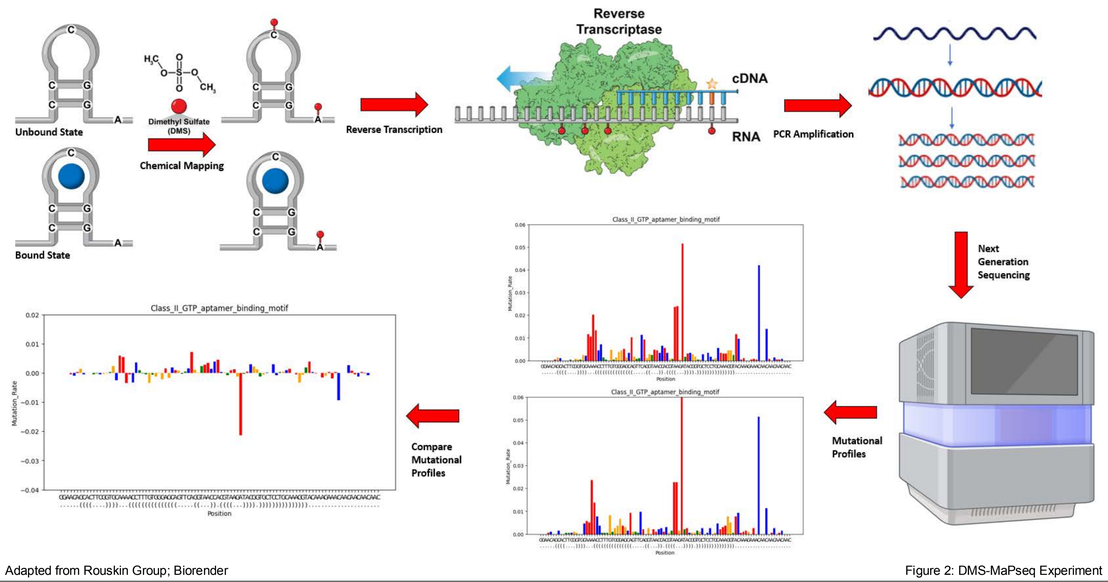
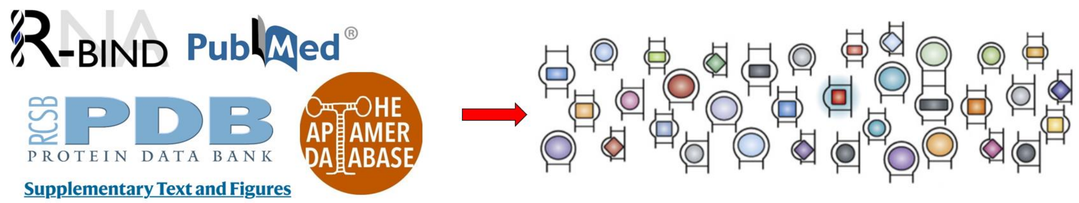
DMS-MaPseq Overview



Adapted from Rouskin Group; Biorender

Figure 2: DMS-MaPseq Experiment

Collection of Known Binding Motifs



Umuhire Juru and Hargrove 2020

Figure 3: Designing Libraries of Known Binding Motifs

ATP Aptamer Analysis

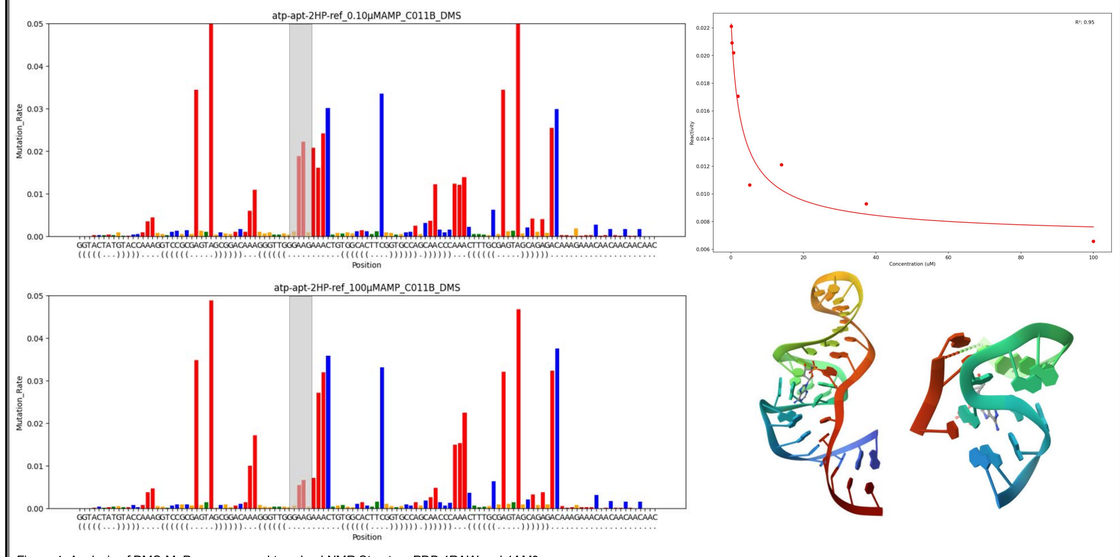


Figure 4: Analysis of DMS-MaPseq compared to solved NMR Structure PDB 1RAW and 1AM0

Initial Results

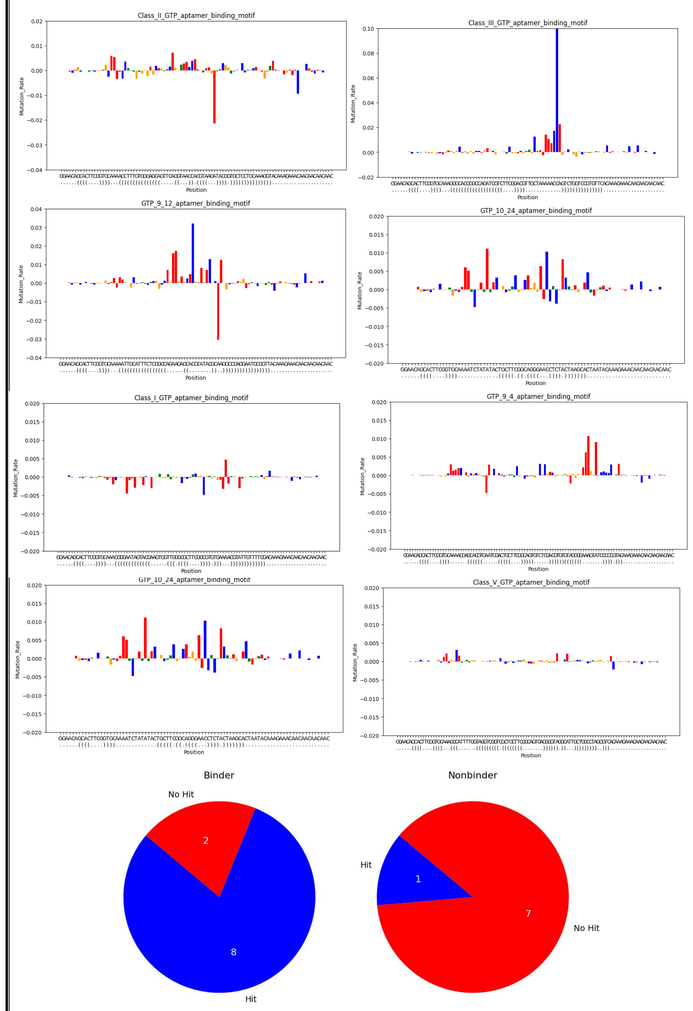


Figure 5. Analysis of GTP Aptamer Constructs in Library

Discussion

- Replicate experiments for higher resolution data
- Test other ligands on libraries
- Determine generalizability of technique
- Perform experiments at elevated pH to probe Uracil and Guanine
- Test other chemical probes such as SHAPE

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