**Exercise SHAPE guided RNA folding**

The phenylalanine tRNA from yeast has the following sequence:

GCGGAUUUAGCUCAGUUGGGAGAGCGCCAGACUGAAGAUCUGGAGGUCCUGUGUUCGAUCCACAGAAUUCGCACCA

It is known that tRNAs in general fold into the a characteristic cloverleaf secondary structure with strong conservation of most of the basepairs in the structure. The crystal structure of the phenylalanine tRNA from yeast has been solved and confirms the cloverleaf structure for this RNA and the presence of the canonical tRNA base pairs.
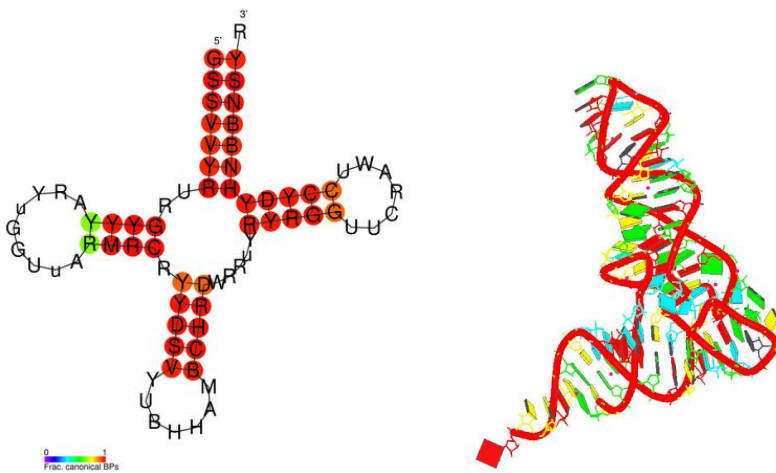

Folding exercise:

1. Folding of the yeast phenylalanine tRNA sequence

- Go to the RNAstructure server for folding of a single RNA. This server combines four separate prediction and analysis algorithms: calculating a partition function, predicting a maximum free energy (MFE) structure, finding structures with maximum expected accuracy, and pseudoknot prediction. This server takes a sequence, either RNA or DNA, and creates a highly probable, probability annotated group of secondary structures, starting with the lowest free energy structure and including others with varied probabilities of correctness. Other structures are included because the minimum free energy structure may not be the correct one.
- Paste in the sequence of the phenylalanine tRNA and predict the structure with the default settings. Browse the results of the folding. How many alternative structures are reported by RNAstructure? Does the predicted structures match the tRNA consensus structure shown above?

2. Folding of the yeast phenylalanine tRNA sequence using SHAPE constraints

SHAPE data for this RNA can be found in the RNA mapping database at Stanford. The data is averaged from 14 replicates over 7 independent experiments.:
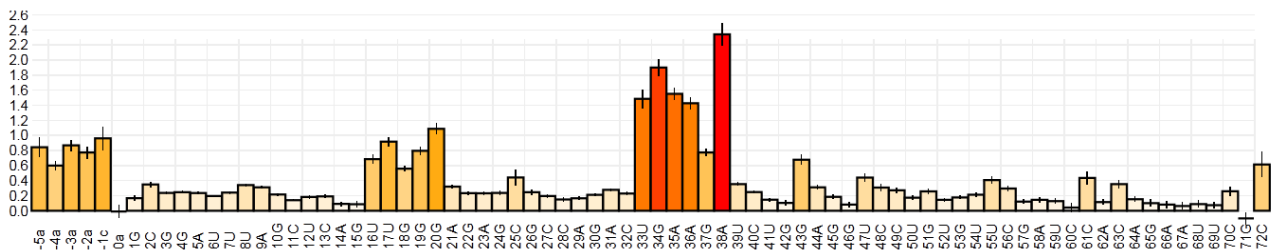
- Open a new tab and go back to the RNAstructure server for folding of a single RNA. Paste in the tRNA sequence and name it. Then download this file with the SHAPE values from the tRNA probing and upload it under Select SHAPE Constraints File. Again run the program with the default settings.
- Browse the results of the constrained folding and compare to the unconstrained. Does the constrained structure prediction resemble the canonical tRNA structure? Are the SHAPE reactive positions located in the loops? Take a closer look at the RNAstructure partition results and compare with the results from the unconstrained folding.

References:

RNAstrucure is developed by the Mathews Lab and is described in Reuter, J. S., & Mathews, D. H. (2010). RNAstructure: software for RNA secondary structure prediction and analysis. The software is also available for download.

RMDB is a repository of RNA structure probing by chemical or enzymatic mapping and is described in Cordero, P.; Lucks, J. B. & Das, R. (2011), The Stanford RNA Mapping Database for sharing and visualizing RNA structure mapping experiments