Metrics on RNA secondary structure ensembles

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RNA secondary structure is defined as the set of base-pairing interactions between the constituent bases of a RNA sequence. The function of a RNA is largely determined by its secondary structure. Consequently, if two or more RNAs share a similar secondary structure it is likely that they share a similar function. There exist various metrics that allow one to measure similarity between two RNA secondary structures (see [4])

A typical assumption made in RNA structure studies is that a particular RNA sequence forms a single, well-defined structure, such RNAs are referred to as *stable* or *highly structured*. However, it is more accurate to think of RNA sequences as able to adopt a range of possible conformations with varying degrees of probability [2, 3, 1, 5]. RNAs that have a tendency to adopt a large number of distinct conformations are termed *flexible*. The untranslated regions (UTRs) of many mRNAs are generally considered to be so [2]. The probability distribution over all possible structural conformations a particular RNA sequence can form under a given set of conditions is referred to as its *ensemble*.

The goal of this project is define a set of metrics that allow one to quantify the spread of structural conformations within an ensemble (a measure of flexibility) and measure distances between structural ensembles (as opposed to just the distance between the most probable conformation in each of the two ensembles, as is currently the standard).

These metrics are expected to be applied to real-world data, to test whether associations exist between the measured properties of the RNA ensembles and different functional annotations. For example, ensembles corresponding to mutant sequences which are measured as "far" from the wild-type ensemble might be associated with disease more frequently than can be accounted for by chance.

Primary objective:

1. Let $\mathcal{E}_1$ and $\mathcal{E}_2$ denote structural ensembles corresponding to two sequences, $\mathcal{S}_1$ and $\mathcal{S}_2$, folded under conditions $\mathcal{C}_1$ and $\mathcal{C}_2$, respectively. Design two computationally efficient metrics for structural ensembles.The first metric, $\mathbb{V}(\mathcal{E}_1)$, should approximate the within-ensemble variance (partially a proxy of structural flexibility). The second metric, $d(\mathcal{E}_1, \mathcal{E}_2)$ should approximate distances between ensembles and must have the property that $d(\mathcal{E}_1, \mathcal{E}_2) \approx 0$, when $\mathcal{E}_1 = \mathcal{E}_2$. Note that stochastic sampling of secondary structures from ensembles will be used to compute the desired metrics, implying the metrics provide only approximate answers.

Possible tasks:

1. Introduction to computer representations of RNA secondary structure.

2. Understand the difference between thermodynamic and SCFG approaches to RNA secondary structure prediction.

3. Read [4] - describes various metrics for measuring distances between RNA secondary structures (not ensembles!). You should focus on understanding the mountain metric.

4. Read [2] - motivates why structural ensembles are useful to consider.

5. Before computing the desired statistics, i.e. $\mathbb{V}(\mathcal{E}_1)$ and $d(\mathcal{E}_1, \mathcal{E}_2)$, you need to be able to generate statistically representative samples (RNA secondary structures) from RNA ensembles. There are multiple approaches to sampling (we recommend using the first approach):

   (a) Use ViennaRNA's RNAsubopt to stochastically draw samples with probability proportional to their Boltzmann weights. Requires programmatically shelling to the RNAsubopt executable. Thermodynamic only. For example, the following command: `RNAsubopt.exe --stochBT=1000 --temp=37.0 < input_seq.fas > output_sample.txt`, will take as input a sequence, fold it at 37.0C and generate a sample of 1000 secondary structures from the folded ensemble.

   (b) Implement a MCMC algorithm to sample from either a thermodynamic or SCFG ensemble. Requires designing valid proposadl between RNA secondary structures. Will likely require implementing a parallel tempering algorithm due to the multi-modal nature of RNA ensembles. Has the disadvantage that it will be slow and samples are dependent (auto-correlated).

   (c) Sample using inside-outside probabilities. For SCFGs only, programmatically difficult, but will generate independent samples. Is the SCFG analogue of RNAsubopt's stochastic traceback.

6. Plot $\mathbb{V}(\mathcal{E}_i)$ against different values of temperature T_i for a single sequence. We expect ensemble variance to be positively correlated with temperature.

7. As a test case, compute distances using the ensemble distance metric between a wild-type RNA ensemble and single-nucleotide mutant disease-associated RNA ensembles. Test whether ensemble distance from the wild-type ensemble correlates with disease-association, one way to do this is to randomly generating mutant sequences that all differ from the wild-type by exactly one nucleotide.

8. Benchmark $\mathbb{V}(\mathcal{E}_1)$ against known stable RNA structures and known flexible RNA structures. Flexible structures are expected to have higher $\mathbb{V}(\mathcal{E}_1)$.

9. Perform a PCA analysis in order to visualise secondary structure ensembles in 2D, as in [2].

   (a) Generate samples from the wild-type $\mathcal{E}_w$ and mutant ensembles: $\mathcal{E}_1, \ldots, \mathcal{E}_m$.

   (b) Compute mountain vectors [4] for each sample in each ensemble.

   (c) Perform a seperate PCA analysis for each of the ensembles.

10. A comparison of ensembles generated via the two different approaches (thermodynamic vs. SCFGs). Relies on being able to sample from both thermodynamic and SCFG ensembles, as described in 6).

Long-term objectives:

1. Perform a large scale analysis of human RNAs, and test whether flexible structures (as measured using $\mathbb{V}(\mathcal{E}_1)$) are associated with certain functional annotations.

2. Develop a robust and computationally efficient method for pairwise alignment of RNA structural ensembles. This would be particularly valuable, as alignment becomes necessary when comparing two structural ensembles corresponding to two RNA sequences of differing length. This would eventually be expected to be applied on a genome-wide scale (see [2]).

References

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