1 Subjects

- What is RNA 2^{nd} structure?
- Computing a pseudo-knot free RNA 2^{nd} structure.

2 Notes

2.1 RNA and second structure

Messenger RNA is often described as a linear, unstructured sequence, only interesting for the protein amino acid sequence that it encodes.

However, many non-coding RNA's exist which adopt sophisticated three-dimensional structures, and even catalyse biochemical reactions. RNA is typically produced as a single stranded molecule, which then folds to form a number of short base-paired stem, this is what we call the seconday structure of the RNA.

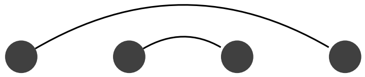
RNA is a polymer of four different nucleotide subunits, we abbreviate them A, C, G and U. In DNA, thymine T replaces uracil U. $G - C$ and $A - U$ form hydrogen bonded base pairs. $G - C$ form three hydrogen bonds and tend to be more stable than $A - U$ pairs which form only two. Some non-canonical pairs also forms, like the $G - U$ pair, and others which distort regular A-form RNA helices.

Base pairs are approximately coplanar and are almost always *stacked* onto other base pairs. Such contiguous stacked base pairs are called *stems.* In three dimensional space, the stems generally form a regula (A-form) double helix. We typically represent the RNA 2^{nd} structure in two-dimensional pictures.

Single stranded subsequences bounded by base pairs are called loops. A loop at the end of a stem is called a *hairpin loop.* Simple substructures which just consist of a stem and a loop is called *stem loops* or *hairpins.* Single stranded bases occuring within a stem are culled a *bulge* or *bulge loop* if the single stranded bases on only on side of the stem. it's called an *interior loop* if there is a bulge on both sides. If a loop connects three or more stems, then it is called a *multibranched loop.*

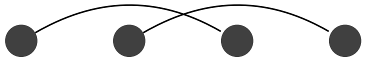
Base pairs almost always occur in a nested fashion in RNA secondary structure. Base pairs are nested if we can draw arcs over them, and none of the arcs intersect. Formally, if i, j is a base pair and i', j' is a base pair then $i < i' < j' < j$. If it happens that these arcs would cross, then they are called *pseudo-knots.*

Just to spell it out, this is nexted:



This is juxtaposed:

This is overlapping (pseudo-knot):



If there are no pseudo-knots, then we can represent it as a planar graph, and in general it is easier to find the compute the secondary structure with the least "free energy" without the pseudo-knots. Fortunately, there are very few pseudo-knots compared to the number of base pairs in nested secondary structure, so it is usually acceptable to sacrifice the information in pseudo-knots in return of efficient algorithms.

2.2 Predicting 2^{nd} RNA structure

Usually, when we want to predict the secondary stucture, we will try to minimize the amount of "free energy". The first example we will look at, bases the prediction on the primary structure (the simple sequence) only. For this we have Nussinov and Zuker's Mfold algorithm. Other methods use comparative structure prediction which is based on a prior alignment. As well as probabilistic methods.

2.2.1 Nussinov

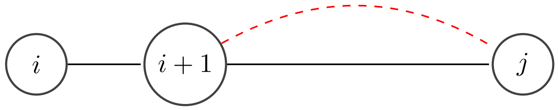
When we need to predict the secondary structure, there are many plausible secondary structures. An RNA of length 200 has over 10^{50} possible base-paired structures. Therefore, we need both a function that assigns the correct structure the highest score, and an algorithm for evaluating the scores.

Nussinov attempts to find the structure with the most base pairs, it is a dynamic programming approach, which calculate the best structure for small subsequences and work outwards. Let's first introduce som notations:

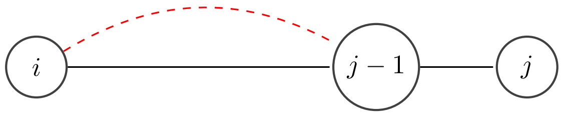
- seq the RNA sequence of $\{A, C, G, U\}$
- $seq[i, j]$ the RNA sequence from position i to j
- str the best 2^{nd} structure for seq of $\{(,), .\}$
- $str[i, j]$ the best 2^{nd} structure for $seq[i, j]$
- $score[i, j]$ the number of base pairs in $str[i, j]$

In the Nussinov algorithm we look at four cases:

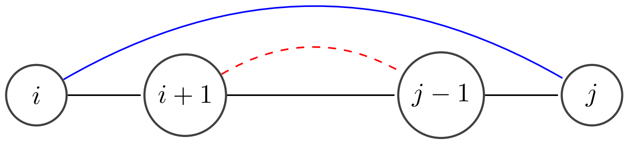
i being unpaired and $str[i + 1, j]$, that is we just prepend i to the rest of the structure:



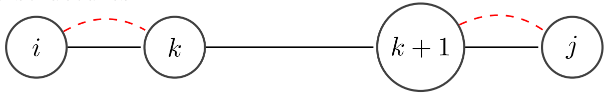
j being unpaired and $str[i, j-1]$, that is we just append j to the rest of the structure:



$seq[i] \cdot seq[j]$ and $str[i+1, j-1]$, that is we add the base-pair i, j to the rest of the structure:



$str[i, k]$ and $str[k+1, j]$ for some $i < k < j$, that is we just concatenate the two structures:



We then find the one of these cases which returns the highest score, which can be described formally as:

$$score[i,j] = \begin{cases} 0 \text{ if } j-1 < 2 \\ \max \begin{cases} score[i+1,j] \\ score[i,j-1] \\ score[i+1,j-1] + 1 if seq[i] \cdot seq[j] \\ max_{i<k<j-1}(score[i,k] + score[k+1,j]) \end{cases} \end{cases}$$

We have to save all the results in table of space $\mathcal{O}\left(n^2\right)$ and it will take $\mathcal{O}\left(n^3\right)$ to compute. We then simply start at the top-right corner of the table produced by the algorithm (the index corresponding to the first and last index) and traceback through the table. The path we trace back through the table is the optimal structure.

This can also be described as a stochastic CFG:

$$\begin{aligned} &S \to aS|cS|gS|uS && (i \text{ unpaired}) \\ &S \to Sa|Sc|Sg|Su && (j \text{ unparied}) \\ &S \to aSu|cSg|gSc|uSa && \text{(i,j pair)} \\ &S \to SS && \text{bifurcation} \end{aligned}$$