Combinatorial approaches to RNA folding
Part I: Basics

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What is RNA?

There are three major macromolecules that are essential to all forms of life:

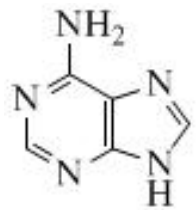
- RNA (*Ribonucleic acid*)
- DNA (*Deoxyribonucleic acid*)

} nucleic acids

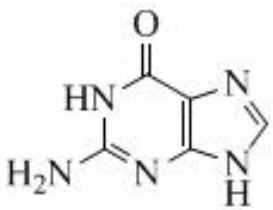
- Proteins

} biochemical compounds

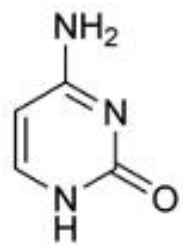
Nucleic acids are biological molecules built from strings of nucleotides.



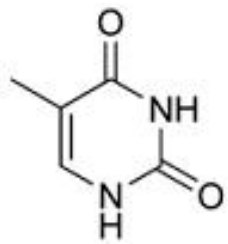
adenine



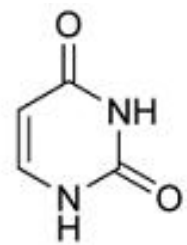
guanine



cytosine



thymine



uracil

A and G are *purines*. C, T, and U are *pyrimidines*.

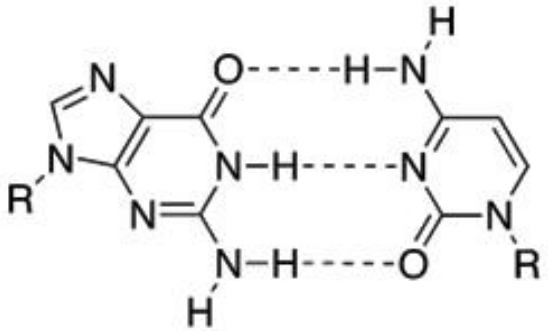
DNA strands consist of A, C, G, and T.

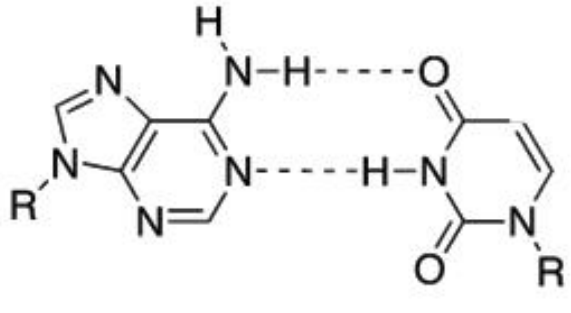
RNA strands consist of A, C, G, and U.

What is RNA?

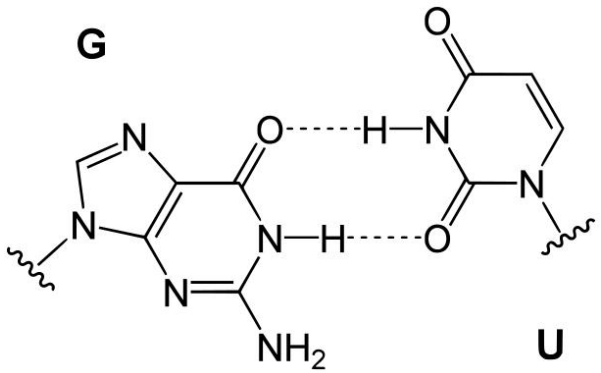
Combinatorially, an RNA strand is a length-n sequence (of bases, or nucleotides), over the alphabet $\{A, C, G, U\}$.

Bases can bond: A with U, and C with G. (*Watson–Crick* base pairs.)





Additionally, U can bond with G. (Called a *wobble-pair*).



Nucleic acid strands

Other bonds are either chemically impossible (GT, AC), or thermodynamically unstable (purine–purine, pyrimidine–pyrimidine) and thus very rare.

Nucleotides are strung together along a sugar-phosphate backbone, called a strand.

Strands of nucleic acid have directionality: a $5'$ end "five prime end" and a $3'$ end "three prime end."

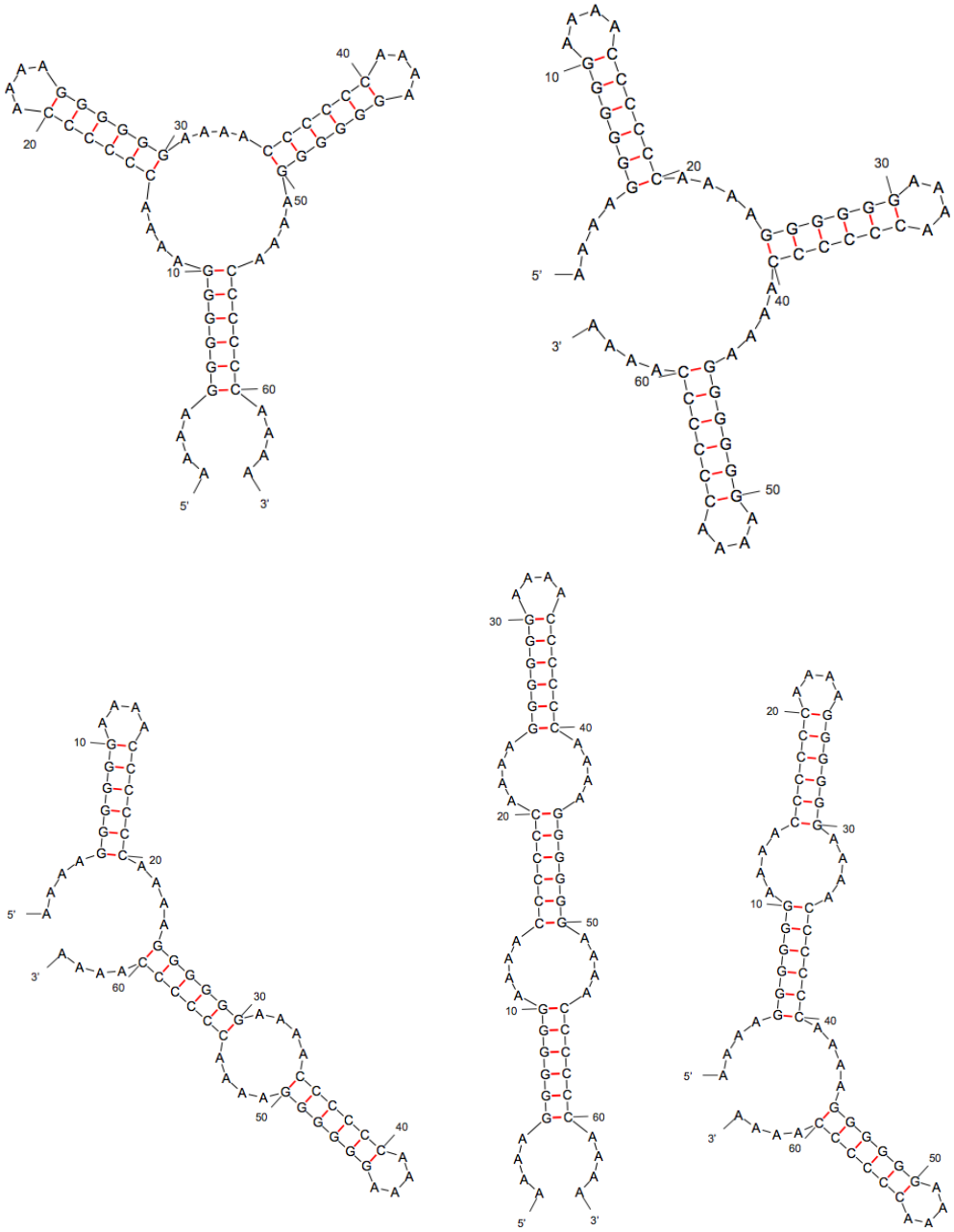
Single strands of DNA or RNA are written in the $5'$-to-$3'$ direction.

DNA consists of two strands that bond together, in opposite directions. One strand thus determines the other stand. For example:

```
(5′ end) ATCGATTGAGCTCTAGCG (3′ end)
         ||||||||||||||||||
(3′ end) TAGCTAACTCGAGATCGC (5′ end)
```

RNA consists of a single strand. It can fold and bond to itself. It is much less structurally constrainted than DNA!

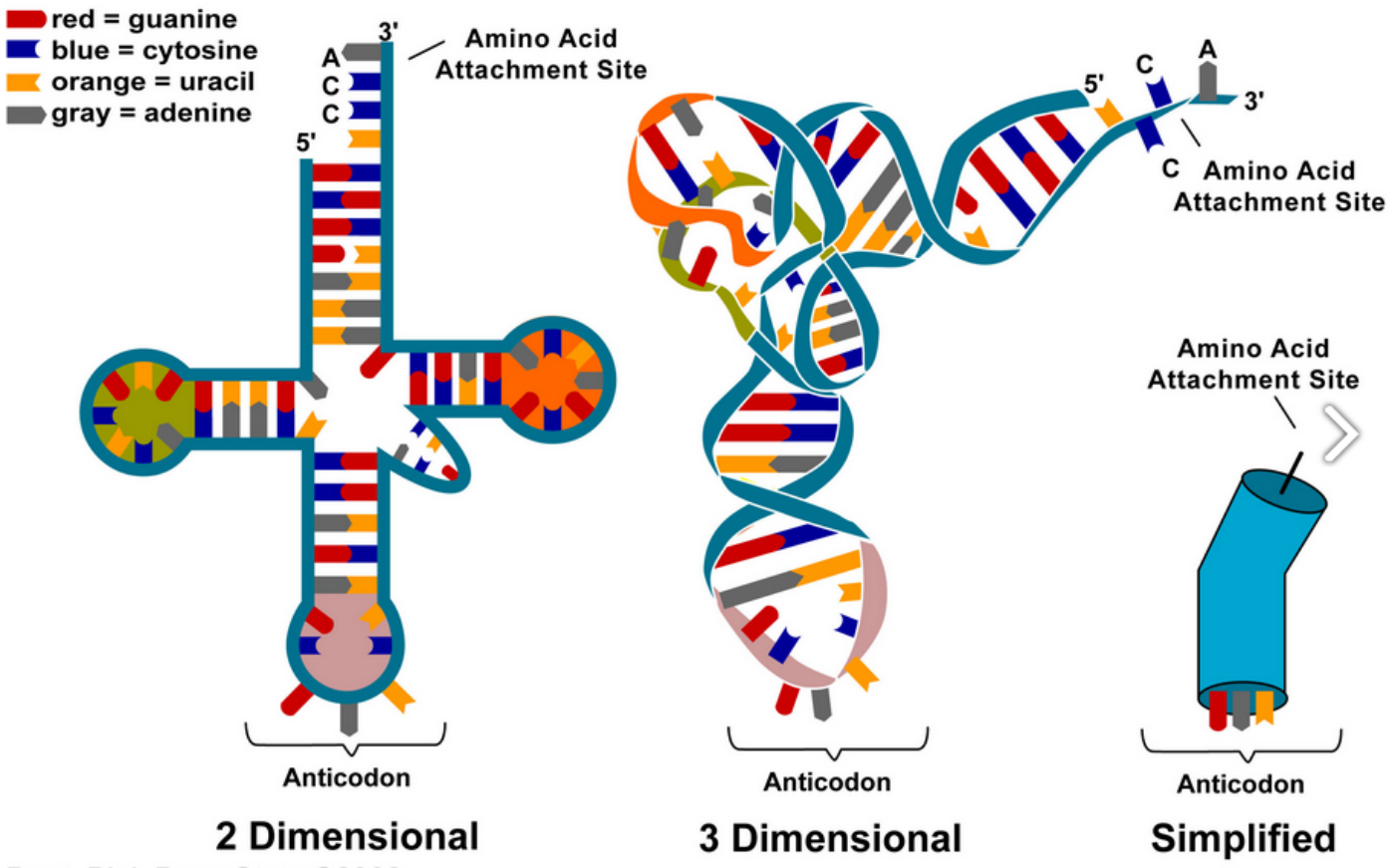
How does RNA fold? [image from C. Heitsch; Georgia Tech]



RNA folding

The physical structure of a folded RNA strand can be described on several levels.

- *Primary structure*: The raw sequence of nucleotides.
- *Secondary structure*: The bonding between nucleotides on a single strand.
- *Tertiary structure*: Embedding (e.g., twisting, knotting, etc.) of the strand in 3-dimensional space.



Central questions about RNA folding

Questions

1. Given an RNA strand, can we predict how it will fold?
2. How does the structure that an RNA strand (or protein) folds into affect its function? ("*structure-to-function problem*")

Question 2 above is more purely biological.

In contrast, Question 1 can be attacked by mathematicans, computer scientists, engineers, without too much biology knowledge.

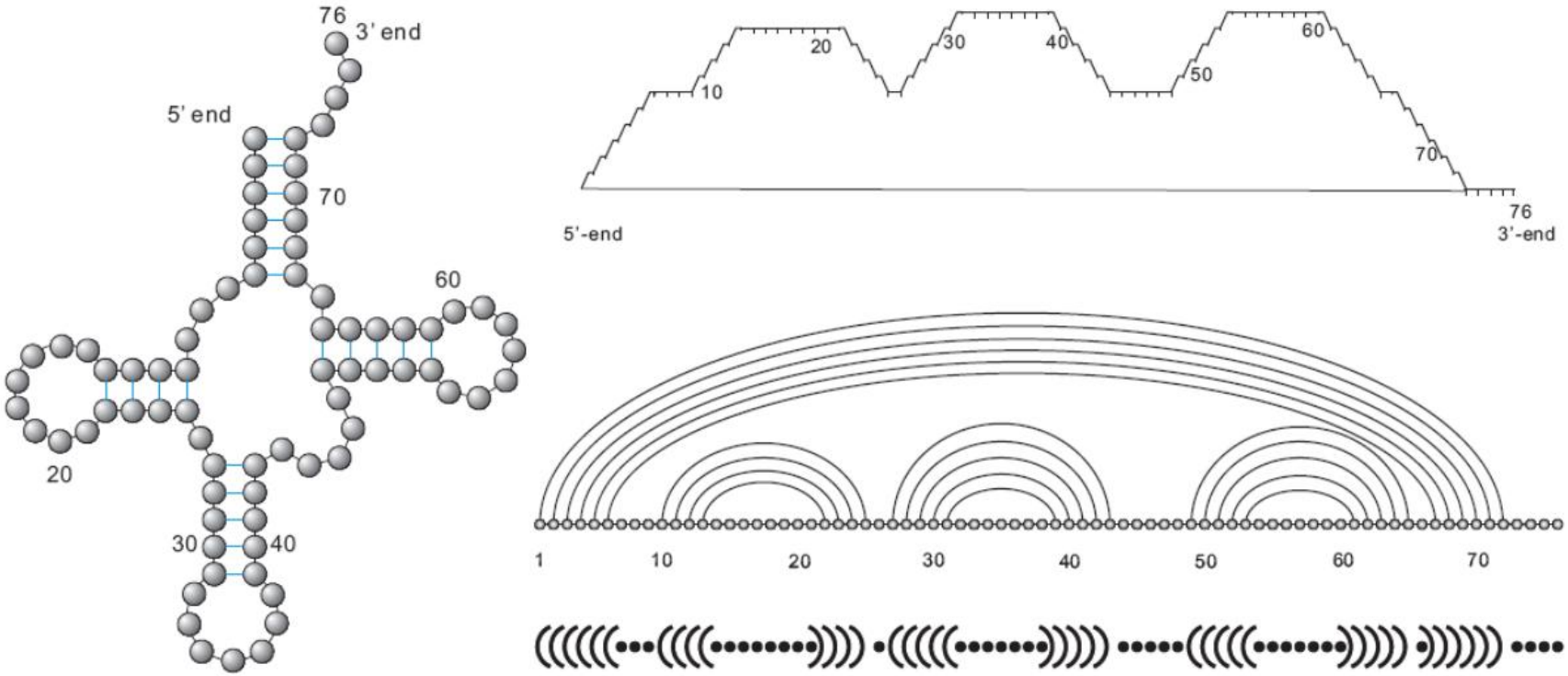
Before we proceed, we will need to establish a combinatorial framework for describing RNA strands.

Combinatorial models of RNA

To each base, we associate a vertex. We use an edge to denote a bond.

The arc diagram of an RNA folding consists of vertices $V = [n] = \{1, \ldots, n\}$ and a collection of edges, or arcs, $E = \{(i, j) \mid i < j\} \subsetneq V \times V$.

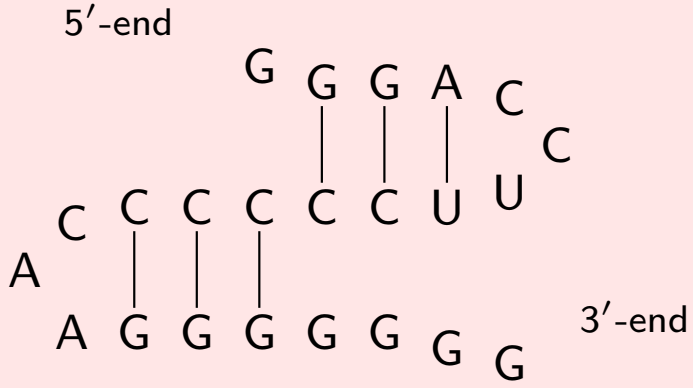
There are several natural combinatorial models we can associate with RNA strands:



Secondary structures

Exercise

Consider the following fold of the RNA sequence GGGACCUUCCCCCCAAGGGGGGG:



(i) Draw the corresponding arc diagram.

(ii) Write out this secondary structure in point-bracket notation.

(iii) Draw the corresponding Motskin path.

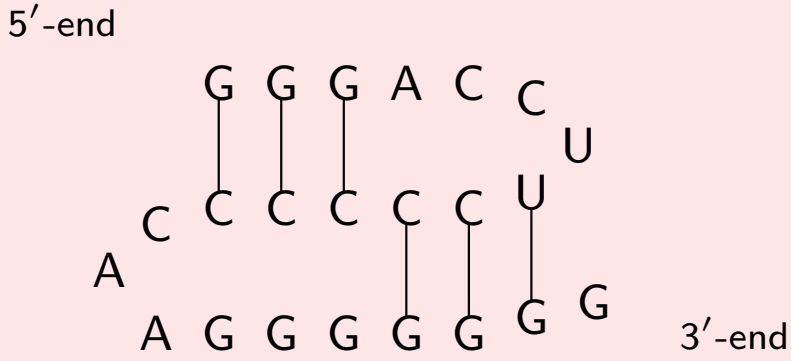
You should notice that your arc diagram has no crossings.

Formally, two arcs (i_1, j_1) and (i_2, j_2) (with $i_1 < i_2$) are *crossing* if $i_1 < i_2 < j_1 < j_2$. An arc diagram is non-crossing if it has no crossing arcs. Such an RNA structure is (unfortunately) called a secondary structure.

Pseudoknots

Exercise

Consider the following fold of the same RNA sequence:



(i) Draw the corresponding arc diagram.

(ii) Write out this secondary structure in point-bracket notation.

(iii) Draw the corresponding Motskin path.

Which of these go wrong, now that there are crossing arcs?

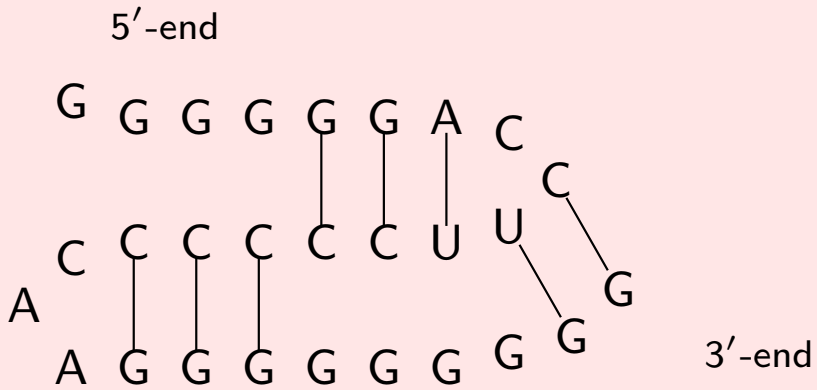
An RNA structure is a pseudoknot if its arc diagram has crossings.

An arc diagram is k-noncrossing if there is no set of k mutually crossing arcs.

Pseudoknots

Exercise

Consider the following fold of the same RNA sequence:



(i) Draw the corresponding arc diagram. What is the smallest k for which this is k-noncrossing .

(ii) What if the first G bonds with the C "directly below" it (vertex 17). Does this change the k from the previous part?

(iii) Draw a picture of a folded RNA strand (like the one above) that is 4-noncrossing but not 3-noncrossing.

Parameters

The length of an arc (i, j) is $|i - j|$. An arc of length k is called a k-arc.

A stack (or stem or helix) is a sequence of nested arcs:

$$(i, j), (i + 1, j - 1), \ldots, (i + (\sigma - 1), j - (\sigma - 1)),$$

and a maximal such σ is its *size*.

For thermodynnamical reasons, there are several key features of interest to us:

- The minimum loop size (i.e., arc-length), λ.
- The minimum stack size, σ.

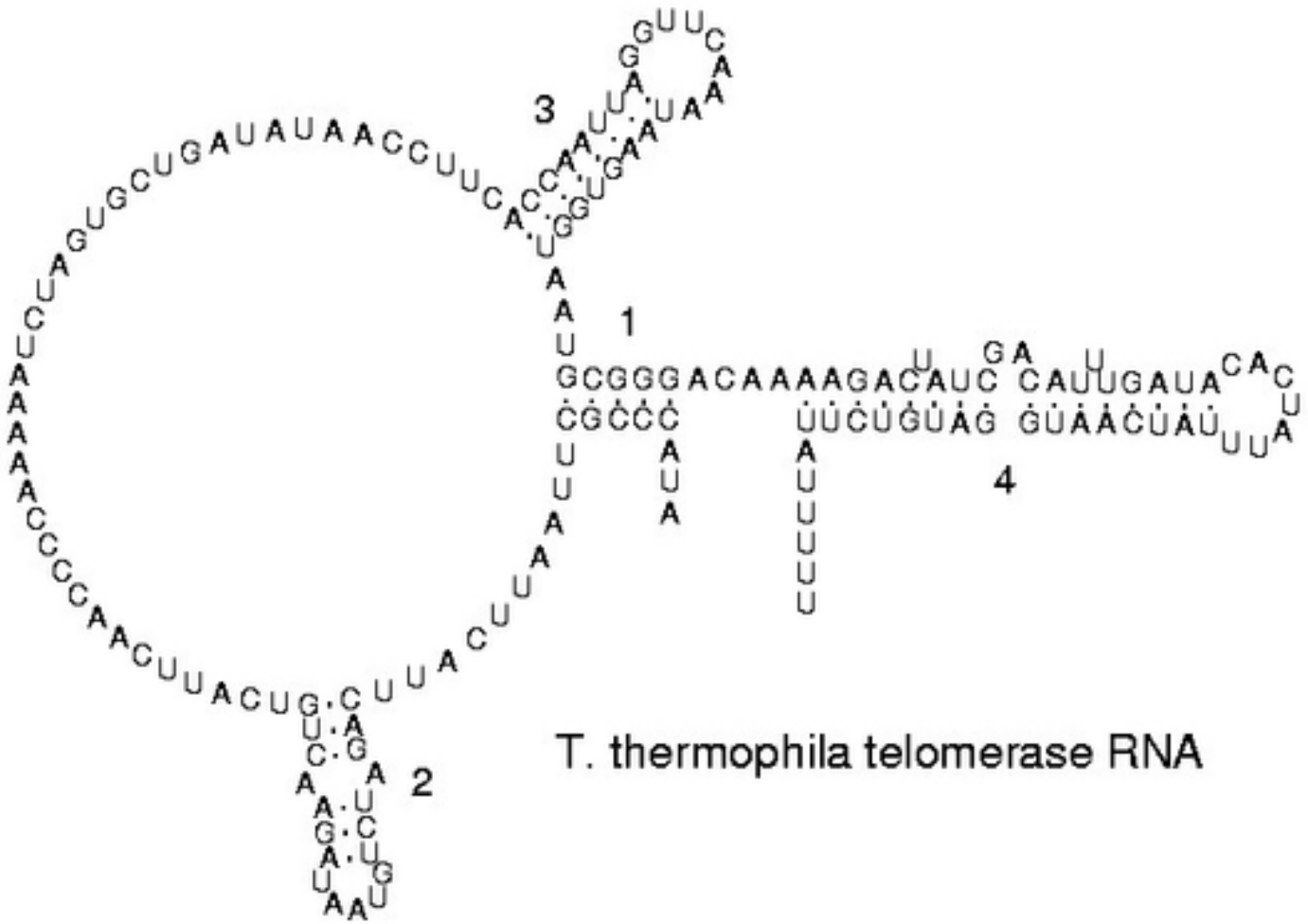
It is common to assume that $\sigma = 2$ and $\lambda = 3$ or $\lambda = 4$.

Mathematical questions

- How can we enumerate the number of structures with certain parameters? This may require *asympotic analysis*.
- How can we *uniformly generate* an RNA structure?
- What is the distribution of certain motifs (e.g., base-pairs, hairpin loops, etc.) in these structures?
- What is the *topology* of one of these structures?

Loop decomposition

Every secondary structure can be described by its loops, which come in different types.



Loop decomposition

Given a basepair (i, j) with $i < v < j$, say that v is accessible from (i, j) if there is no basepair (i', j') such that $i < i' < v < j' < j$.

Loosely speaking, v is accessible from (i, j) if it can *"look up and see the arc* (i, j)*."*

A basepair (v, w) is accessible from (i, j) if both v and w are accessible.

The k-loop closed by (i, j) is the set of $(k - 1)$ basepairs and the isolated bases that are accessible from (i, j).

We do NOT include either i or j in the k-loop closed by (i, j).

The size of a loop is the number of isolated bases in it

Loop types

0. The vertices not accessible from any arcs form the unique 0-loop, or null loop L_0.
1. A 1-loop is called a hairpin loop
2. There are three types of 2-loops: bulge loops, interior loops, and stacked pairs.
3. A k-loop for $k \geq 3$ is called a multiloop.

Loop decomposition

2-loops

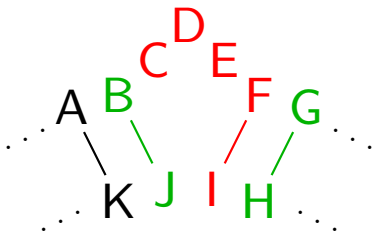
Suppose (i', j') is the unique accessible base pair from (i, j). Then the resulting 2-loop is:

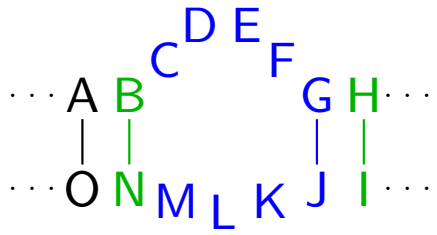
2a. a stacked pair if $i - i' = j' - j = 1$;

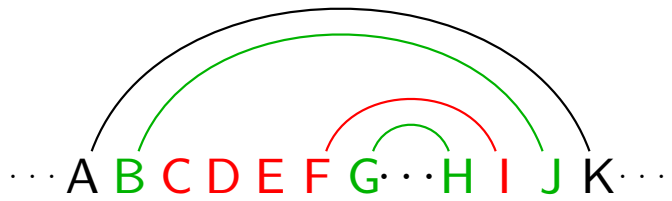
2b. a bulge loop if exactly one of $i - i'$ and $j' - j$ is > 1;

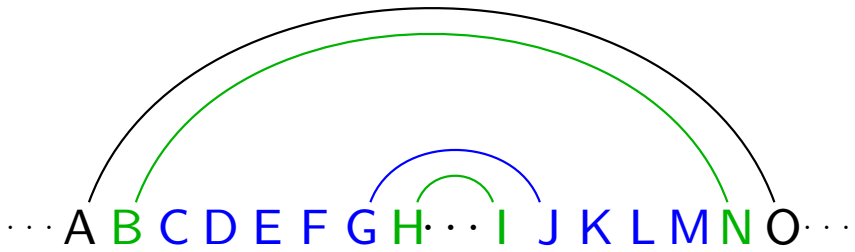
2c. an interior loop if both $i - i'$ and $j' - j$ are > 1;

Two 2-loops: a bulge loop (left) and an interior loop (right). Each secondary structure also contains two 2-loops that are stacked pairs.









Loop decomposition with pseudoknotting

Things get a little more complicated when the diagram contains a pseudoknot, but there is is still a well-defined decomposition. (We won't go into details.)