

Combinatorial approaches to RNA folding

Part I: Basics

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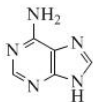
Math 4500, Spring 2022

What is RNA?

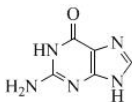
There are three major **macromolecules** that are essential to all forms of life:

- **RNA** (*Ribonucleic acid*)
 - **DNA** (*Deoxyribonucleic acid*)
 - **Proteins**
- } nucleic acids
- } biochemical compounds

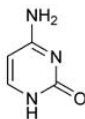
Nucleic acids are biological molecules built from strings of **nucleotides**.



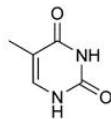
adenine



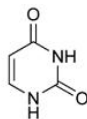
guanine



cytosine



thymine



uracil

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

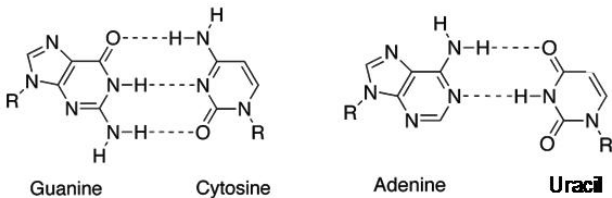
DNA strands consist of A, C, G, and T.

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

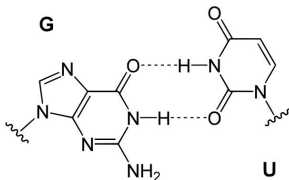
What is RNA?

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

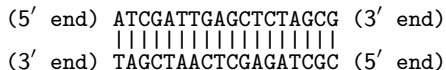
Other bonds are either chemically impossible (GT, AC), or thermodynamically unstable (purine–purine, pyrimidine–pyrimidine) and thus very rare.

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.”

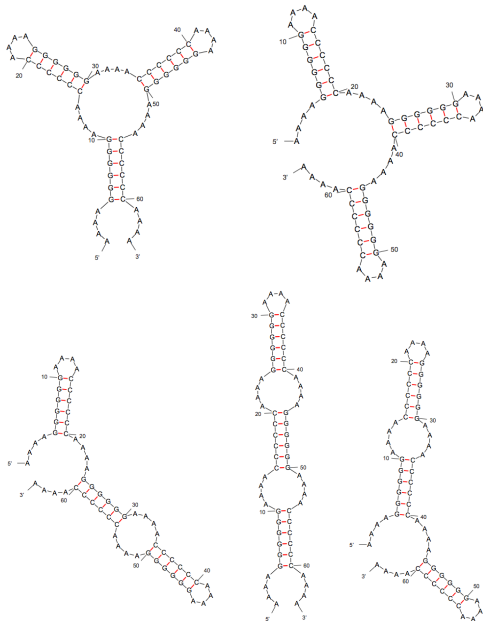
Single strands of DNA or RNA are written in the 5'-to-3' direction.

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



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

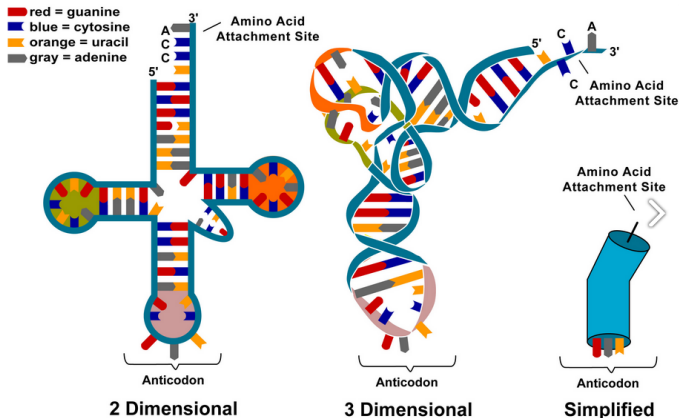
How does RNA fold? [image from C. Heitsch; Georgia Tech]



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.



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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 mathematicians, computer scientists, engineers, without too much biology knowledge.

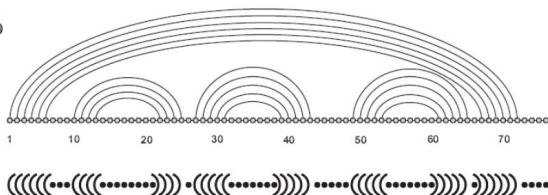
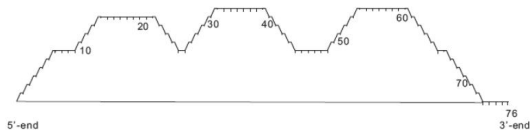
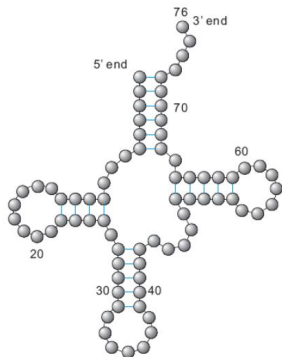
Before we proceed, we will need to establish a combinatorial framework for describing RNA strands.

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** $V = [n] = \{1, \dots, n\}$ and a collection of **edges**, or **arcs**, $E = \{(i, j) \mid i < j\} \subsetneq V \times V$.

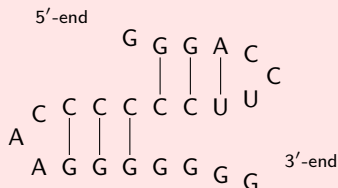
There are several natural combinatorial models we can associate with RNA strands:



Secondary structures

Exercise

Consider the following fold of the RNA sequence GGGACCUUCCCCCAAGGGGGGG:



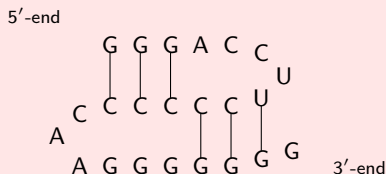
- (i) Draw the corresponding **arc diagram**.
- (ii) Write out this secondary structure in **point-bracket notation**.
- (iii) Draw the corresponding **Motzkin path**.

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**.

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 **Motzkin path**.

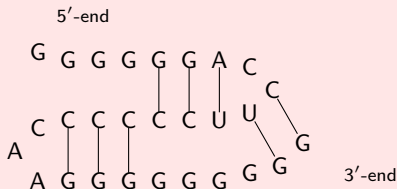
Which of these go wrong, now that there are crossing arcs?

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.

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:

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

and a maximal such σ is its *size*.

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

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

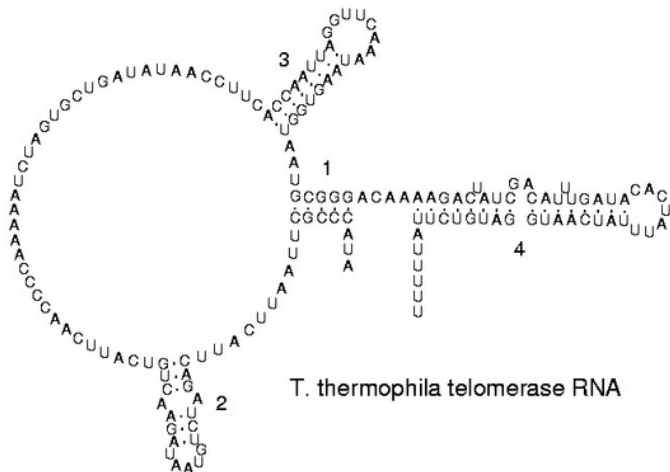
It is common to assume that $\sigma = 2$ and $\lambda = 3$ or $\lambda = 4$.

Mathematical questions

- How can we enumerate the number of structures with certain parameters? This may require *asymptotic 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.



T. thermophila telomerase RNA

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**.

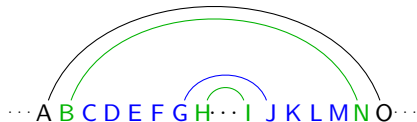
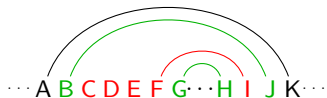
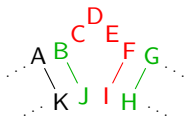
Loop decomposition

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 still a well-defined decomposition. (We won't go into details.)

