### RNA STRUCTURE ANALYSIS USING THE RIGID BLOCK MODEL

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#### **ABSTRACT OF THE DISSERTATION**

### **RNA Structure Analysis Using the Rigid Block Model**

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RNA structure is at the forefront of our understanding of the beginning of life, also the mechanisms of life regulation and control. The life regulation part is new, not ten years old. Primodial in understanding the cell. The practical purpuse for the chemist is to understand how RNA folds. It's mainly a mechanical problem, therefore it's not foreing to use statistical mechanics methods, combined with detailed knownledge of atomic level structure.

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As a thing among things, each thing is equally insignificant; as a world each one equally significant.

If I have been contemplating the stove, and then am told; but now all you know is the stove, my result does indeed sound trivial. For this represents the matter as if I had studied the stove as one among the many, many things in the world. But if I was contemplating the stove, it was my world, and everything else colorless by contrast with it ...

For it is equally possible to take the bare present image as the worthless momentary picture in the whole temporal world, and as the true world among shadows.

**Ludwig Wittgenstein** 

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### Chapter 1 Introduction

#### 1.1 RNA folding

#### 1.1.1 Interest in RNA folding

The first high resolution X-ray structure of RNA larger than a dinucleotide was that of yeast tRNA<sup>Phe</sup> at 3Å in 1974 [1, 2]. Thirty years later there are two orders of magnitude more RNA structural information [3]. This fact and the discovery of ribozymes [4, 5] has renewed interest in solving the problem, that is, from primary sequence, finding in an automated way the native three-dimensional structure of RNA and its folding pathway. The problem is usually seen as analogous to the protein folding problem, due both to the discovery of the enzymatic behavior of RNA [4, 5] and the complicated folding of large RNA molecules [9]. To take advantage of this analogy, a unified conceptual framework for describing RNA and protein folding, called the kinetic partitioning mechanism (KPM), has been developed by Thirumalai and Hyeon [10]. This and other methods are based on defining an adequate partition function for describing the correct conformational ensemble of folded, partially folded, and unfolded structures [11, 12, 13] of either protein or RNA.

### 1.1.2 Is RNA folding a hard or easy problem?

There are two trains of thought regarding RNA folding. One states that RNA folding is less complex than protein folding [14] because RNA is made up of a four letter alphabet of similar nucleotide units instead of a 20 letter alphabet of dissimilar amino acids. Therefore the number of possible sequential combinations is smaller. It is also known that secondary and tertiary interactions can be separated in the case of RNA by the absence or presence of Mg<sup>2+</sup> [15] (see Figure 1.1), whereas secondary and tertiary elements are not as easily separable in proteins. The other point of view says that RNA folding can be at least as complex as protein folding [16, 17] since there is no such thing as hydrophobic burial of regions of RNA as in the case of proteins. Instead, the electrostatic problem of having a complex charged backbone must be dealt with in the case of RNA. For instance, the interactions of the RNA polyanionic backbone with water

<sup>&</sup>lt;sup>i</sup>The term automated is used here to mean a theoretical model of tertiary folding, which could use experimental measures of secondary structure association in the same way that the traditional secondary structure folding model [6, 7] uses the Tinoco-Uhlenbeck dinucleotide postulate [8] to find total free energies.

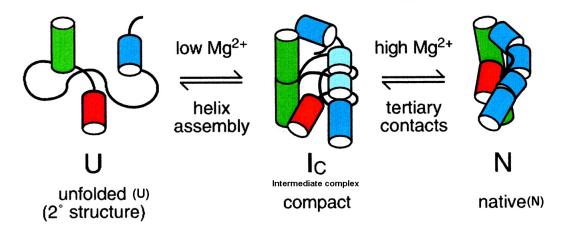


Figure 1.1: Separation of secondary and tertiary interaction in RNA [15]. Double helical secondary structure represented by individual cylinders and tertiary interactions by association of cylinders.

and cations [18] are not easily simulated with explicit solvent models as can be done for proteins. The aforementioned interactions of RNA need to be modeled implicitly, and must aim to describe long dynamic processes of the order of seconds to minutes, in contrast to the typical time scales of tens of microseconds associated with protein folding. Although secondary and tertiary structure can be separated experimentally, there have been few theoretical efforts to account for the folding of RNA from a random sequence of nucleotides into secondary structures and tertiary structures. What little is know has been investigated at low resolution. Professor Stephen Harvey and associates have simulated yeast tRNA<sup>Phe</sup> [19, 20] at various levels of detail, initially using only one pseudoatom per helical region, and later one pseudoatom per nucleotide. By contrast, in the case of proteins many groups have simulated the transition from secondary to tertiary structure, including some calculations which account for the strong coupling of secondary and tertiary structure [21, 22, 23]. This type of work is often referred to as protein structural topology and there is no counterpart for RNA.

#### 1.1.3 Experimental folding techniques

Traditionally RNA folding and unfolding have been followed calorimetrically and spectroscopically as a function of temperature and cation concentration [24]. While this approach works well for studying two-state folders, *i.e.*, structures which populate only two states (native and melted), in general RNA's are not two-state folders. RNA seems to go through a rugged free energy landscape of conformations in the process of folding [25]. The experimental solution to this problem is offered by single molecule techniques like fluorescence resonance energy transfer (FRET) and mechanical micromanipulation, in which the ends of RNA are attached to micron sized beads which are then pulled apart and monitored with a laser light trap [26, 27, 28, 29]. In the case of single molecule force-induced unfolding, state transitions often occur under non-equilibrium conditions, thereby making it difficult to extract equilibrium information from the data.

Recently Bustamante, Tinoco, and associates have shown that using the Crooks fluctuation theorem [30], one can deal with such cases and extract RNA folding free energies from single molecule experiments [31].

#### 1.1.4 RNA simulations

Network and molecular mechanics-molecular dynamics (MM-MD) methods provide useful information relevant to the RNA folding-unfolding problem, especially for describing fluctuations away from the native conformation. Gaussian network models [32, 33, 34] which treat RNA at less than atomic detail have been used to describe the motions of large RNA structures like the ribosome. Examples of the predicted normal modes of motion of the ribosome can be seen at: http://ribosome.bb.iastate.edu/70SnK mode. Using MM, Sanbonmatsu and coworkers obtained a static atomic model of the 70S ribosome structure through homology modeling [35]. Tung and associates used this structure for an all-atom MD simulation of the movement of tRNA into a fluctuating ribosome [36]. This type of simulation might be useful in a reverse-folding approach to the RNA folding problem. To the best of my knowledge, such calculations haven't as yet been done for RNA.

#### 1.1.5 Local nucleotide interactions

The molecular interactions which rule RNA structures at the nucleic acid base level. i.e., local level, are hydrogen bonding and stacking interactions. The former are related to base pairing and the latter, in most cases, to nucleotide steps. These interactions can be explored theoretically at various levels. At the highest level are ab-initio quantum mechanical calculations which are still too expensive for systems as large as hundreds of atoms. Such calculations, nevertheless, can tell a great deal about local electronic behavior. For example, Hobza and collaborators have found that the stacking interaction of free nucleotide bases is determined by dispersion attraction, short-range exchange repulsion, and electrostatic interaction. No specific  $\pi - \pi$  interactions are found from electron correlated ab-initio calculations [37, 38]. This is why force field methods have been so successful in the study of nucleic acids, since the empirical potentials used in such studies mimic well the quantum mechanically obtained energy profiles [35, 39]. A currently debated ab-initio finding is whether small fluctuations in the configurations of neighboring base pairs (dimers) are iso-energetic or not. Recent calculations of Sponer and Hobza [40] seem to contradict their older publications [39, 41], in which the stacking energies were reported to be relatively insensitive to dimer conformation. The new results use the so-called "coupled cluster singles doubles with triple electron excitations" CCSD(T) method, to account for electron correlation. Using this electron correlation energy correction, the stacking energy differences between dimer conformations turn out to be considerably higher than previously reported.

Single and double strand stacking free energies can be obtained calorimetrically. The most popular method used for obtaining such quantities is differential scanning calorimetry (DSC) [42]. These measurements show favorable dinucleotide stacking

free energies as large as -3.6 kcal/mol for double strand stacking. Experimentally, the magnitudes of these interactions are found to be sequence dependent [24]. In fact, the stacking free energies for some sequences<sup>ii</sup> are found to be negligible. Thus there may be no accountable stacking interaction at all for some sequences.

Besides taking into account the effects of stacking and hydrogen bonding, it is important to think at the same time about the polyelectrolyte nature of the RNA backbone. Manning's counterion condensation theory [43, 44] provides a simple and quantitative picture of the interactions of the double helical nucleic acid polyanion with its counterions, although it does not take into account the discrete nature of charge [24] or the folding of RNA. Poisson-Boltzmann theory offers a more detailed picture of the behavior of charged macroions in solution [45].

The local conformational space of RNA has been studied using a large set of available RNA structures from the Nucleic Acid Database (NDB) [46]. The torsion angles of the nucleotide steps have been clustered in the parameter space using different techniques [47, 48]. The root-mean-square deviations (RMSD) of the distances between closely spaced atoms in the phosphates, sugars, and bases, have also been clustered [49]. The latter studies are aimed at finding the common nucleotide base steps and base-pair building blocks which are given the name of RNA doublets.

### 1.1.6 RNA secondary structure algorithms and the lack of tertiary ones

From secondary structure prediction algorithms like Zuker's *mfold* program [50], or Hofacker's Vienna RNA package [7], one obtains a large ensemble of secondary structure graphs. These graphs can be analyzed with graph theory to produce a partition function describing a full arrangement of contacts for the total number of possible secondary structures [51]. So far this type of model has not been generalized to take into account tertiary structural features, *i.e.*, interhelical interactions of RNA.

#### 1.1.7 RNA overall fold

Whereas in the case of proteins one can describe the overall fold from the arrangement of secondary structure motifs, *i.e.*, using the helix-ribbon-coil images developed by Jane Richardson [52] (see Figure 1.2), there is still no comparable description of the overall fold of RNA. A ribbon representation of the sugar phosphate backbone helps to understand the folding of small RNA's, but in the case of the ribosome this type of representation is not sufficient, see Figure 1.3.

One can envision that a thorough investigation of the parameter space of translational and rotational degrees of freedom of the helical regions of RNA could give clues as to how we might see an overall fold in RNA structures.

<sup>&</sup>quot;Unpaired terminal nucleotides UC/A UU/A at 1M NaCl.

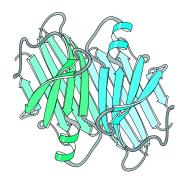


Figure 1.2: Ribbon-coil schematic illustraring the fold and intermolecular units of a dimer of prealbumin, or transthyretin, taken from Richardson *et al.* [53]

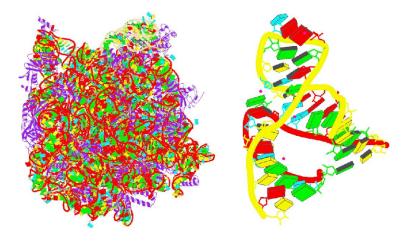


Figure 1.3: *Haloharcula marismortui's* large ribosomal subunit (left) and hammerhead ribozyme (right). The figures were taken directly from the NDB web pages, and show a ribbon representation of the phosphate backbone, and a block representation for the nucleotide bases. From the figures it's clear that, whereas the ribozyme fold can be clearly understood with this representation, the ribosome fold cannot.

In the case of proteins the SCOP (Structural Classification of Proteins) database [54], classifies proteins, among other classifications, according to recurrent arrangements of secondary structure, that is, folds. The SCOR (Structural Classification of RNA) database [55, 56], aims to provide a similar classification to that obtained for proteins, but using RNA motifs<sup>iii</sup> instead. This classification focuses on the local folding of small pieces of RNA and cannot describe the overall fold.

Structure, interactions, and reactivity are the conceptual pillars upon which chemistry stands. The aim of this proposal is to try to understand how these concepts relate to the RNA folding problem, by providing a new model for the three dimensional description of RNA.

<sup>&</sup>lt;sup>iii</sup>Leontis and Westhof [57] define RNA motifs as: "Directed and ordered arrays of non-WC (Watson-Crick) base-pairs forming distinctive foldings of the phosphodiester backbones of the interacting RNA strands"

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## **Chapter 2 RNA Base Steps**

This chapter deals with how starting from a backbone based view of RNA, we can make an interpretation at the step level using the block model.

## Chapter 3 RNA Base-Pairing

The RNA base-pair is reviewed again.

- 3.1 Canonical and Non. Methods Paper
- 3.2 Clustering of Yurong's classification

# Chapter 4 RNA Base Pair Steps

- 4.1 Analysis and Django Webserver
- 4.2 Persistence Length
- 4.3 AMBER: Persistence Length of Base-Pair Step Patterns

## **Chapter 5 RNA Motifs**

This is the introductory chapter.

## **Chapter 6 Introduction**

This is the introductory chapter.