

RNA STRUCTURE ANALYSIS VIA THE RIGID BLOCK MODEL

by

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ABSTRACT OF THE DISSERTATION

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RNA structure is at the forefront of our understanding of the origin of life, the mechanisms of life regulation and control, and it plays a primordial role in some viruses. Our knowledge of the importance of RNA in cellular regulation is relatively new, (a bit more than a decade, Craig and Mello, Nature 1998) and along with the detailed structural elucidation of the transcription machine, the ribosome, has propelled interest in RNA understanding to a level which starts to closely resemble that given to proteins and DNA.

In these scheme of progressively understanding the landscape of functionality of such a complex polymer as RNA, one practical task left to the structural chemist is to understand the details of how structure relates to large scale polymer processes. With this in mind the fundamental problems which fuel the work described in this thesis are those of the conformations which RNA's assume in nature, and the aim to understand how RNA folds.

The RNA folding problem can be understood as a mechanical problem, therefore it's not foreign to use statistical mechanical methods combined with detailed knowledge of atomic level structure. Such methodology is mainly used in this work in a long term effort on understanding the intrinsic structural features of RNA, and how they might relate to it's folding.

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

As a molecule among molecules, each molecule is equally insignificant; as a world each one equally significant.

If I have been contemplating RNA, and then am told; but now all you know is RNA, my result does indeed sound trivial. For this represents the matter as if I had studied RNA as one among the many, many molecules in the world. But if I was contemplating RNA, 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.

Anonymous Chemist

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Chapter 1

Introduction

1.1 RNA

RNA plays a primordial role in life, and perhaps also in the early history of its origins [1]. In Biology RNA is a central player in the transcription and translation steps of what is known as its central dogma. In the last decade of the twentieth century it was found by Fire and Mello [2] that RNA also plays a role solely known to be the job of proteins, that is, RNA can regulate translation using non-coding RNA's (ncRNA's). Another fundamental discovery about RNA came in 2000 with the elucidation at atomic level detail of a non-coding RNA, the ribosome [2, 3, 4].

Since its very beginnings, structural understanding of RNA has proven to be a complex problem. It was not until 1956, three years after the famous Nature triad of papers by Crick, Wilkins, and Franklin [5], that Alex Rich and David Davies were able to produce dsRNA from poly-A and poly-U to produce a neatly diffracting X-ray pattern typical of a double-helical structure. It was not until 1965 that Robert Holley was able to obtain a complete sequence of tRNA and propose a secondary structure for it, and it was only until 1973, that the first complex, but small, tRNA structure, was solved at full atomic detail. Fifty seven years have passed since the description of the double helical structure of DNA, but still RNA faces more challenges with the possibility of finding a whole new ZOO of non-coding RNA structures [6], and the possibility of new engineered ones [7].

1.1.1 RNA folding

The first high resolution X-ray structure of RNA larger than a dinucleotide was that of yeast tRNA^{Phe} at 3 Å in 1974 [8, 9]. Thirty-six years later there are two orders of magnitude more RNA structural information [10], and new information from non-coding RNA's is expected [6]. This fact and the discovery of ribozymes [11, 12] has renewed interest in solving the RNA folding problem, that is, from primary

sequence, finding in an automatedⁱ way the native three-dimensional structure of RNA and its folding pathway. The RNA folding problem is usually seen as analogous to the protein folding problem, due both to the discovery of the enzymatic behavior of RNA [11, 12] and the complicated folding of large RNA molecules [16]. 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 [17]. 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 [18, 19, 20] of either protein or RNA.

1.2 Is RNA folding a hard or easy problem?

There are two trains of thought regarding the mechanism of RNA folding. One states that RNA folding is less complex than protein folding [21] 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 well known that secondary and tertiary interactions can be separated in the case of RNA by the absence or presence of Mg^{2+} [22] (see Figure 1.1), whereas secondary and tertiary elements are not as easily separable in proteins. The other point of view says

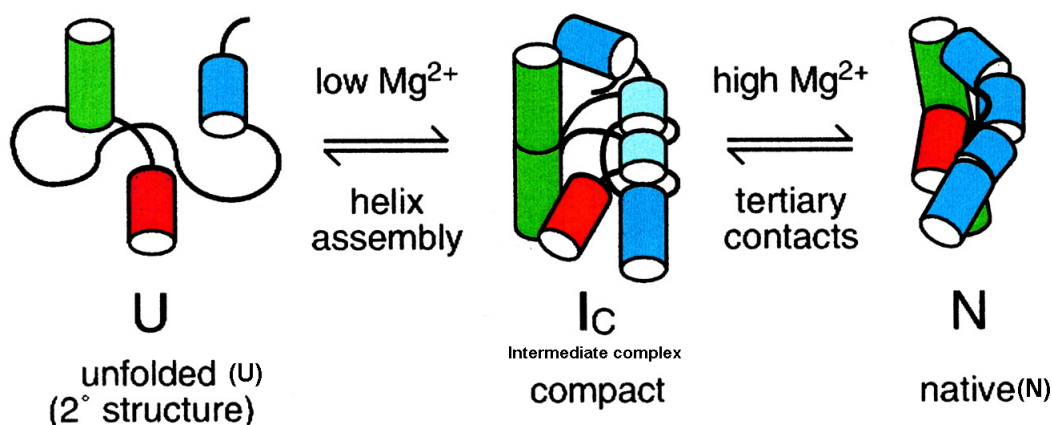


Figure 1.1: Separation of secondary and tertiary interaction in RNA [22]. Double helical secondary structure represented by individual cylinders and tertiary interactions by association of cylinders.

that RNA folding can be at least as complex as protein folding [23, 24] since there is no such thing as hydrophobic burial of regions of RNA as in the case of proteins. Instead, the electrostatic problem

ⁱThe 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 [13, 14] uses the Tinoco-Uhlenbeck dinucleotide postulate [15] to find total free energies.

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 and cations [25] 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 known has been investigated at low resolution. Professor Stephen Harvey and associates have simulated yeast tRNA^{Phe}, [26] and the assembly of the 30S subunit of the ribosome [27] at various levels of detail, initially using only one pseudoatom per helical region, and later one pseudoatom per nucleotide. Recently Major's group at Montreal has proposed a pipeline of two computer algorithms [28], one makes secondary structure predictions, and the other assembles 3D structures based on the best scoring secondary structures. 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 [29, 30, 31]. This type of work is often referred to as protein structural topology and there is no counterpart for RNA.

1.3 Experimental folding techniques

Traditionally RNA folding and unfolding have been followed calorimetrically and spectroscopically as a function of temperature and cation concentration [32, 33]. 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 [34]. 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 [35, 36, 37, 38]. 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 [39], one can deal with such cases and extract RNA folding free energies from single molecule experiments [40].

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 [41, 42, 43] 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 [44]. Tung and associates used this structure for an all-atom MD simulation of the movement of tRNA into a fluctuating ribosome [45]. This type of simulation might be useful in a reverse-folding approach to the RNA folding problem. To the best of our knowledge, such calculations haven't as yet been done for RNA.

1.4.1 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 [46, 47]. 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 [44, 48]. 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 [49] seem to contradict their older publications [48, 50], 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) [51]. 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 [32]. In fact, the stacking free energies for some sequencesⁱⁱ 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 [52, 53] 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 [32] or the folding of RNA. Poisson-Boltzmann theory offers a more detailed picture of the behavior of charged macroions in solution [54].

The local conformational space of RNA has been studied using a large set of available RNA structures from the Nucleic Acid Database (NDB) [55]. The torsion angles of the nucleotide steps have been clustered in the parameter space using different techniques [56, 57]. The root-mean-square deviations (RMSD) of the distances between closely spaced atoms in the phosphates, sugars, and bases, have also been clustered [58]. 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. Recently, the RNA Ontology Consortium (ROC) has proposed a consensus set of RNA dinucleotide conformers integrating the work of various groups [59].

1.4.2 RNA secondary structure algorithms and the lack of tertiary ones

From secondary structure prediction algorithms like Zuker's *mfold* program [60], Hofacker's Vienna RNA package [14], or Mathews Dynaling [61], 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 making possible a "relation of microscopic conformations to macroscopic properties" [62]. So far this type of model has not been generalized to take into account tertiary structural features, *i.e.*, interhelical interactions of RNA. In the last two to three years a boom in prediction of small (≈ 200 nucleotides) RNA 3D structures has started. Basically three types of approaches are being followed. One is that of using a coarse grained

ⁱⁱUnpaired terminal nucleotides UC/A UU/A at 1M NaCl.

model assigning a potential function to it, followed by a minimization procedure, and then a molecular mechanics (MM) all atom refinement [63, 64, 65]. Another starts from predicted secondary structures and assumes their helical regions adopt the A-form conformation, then mechanically thrusts residues as rigid bodies in the remaining non-helical regions, and finally carry out an MM optimization [66]. Finally, a pipeline between secondary structure prediction, and tertiary structure assembly is proposed. This pipeline uses as bridging concept between 2D and 3D structure, the graph theoretical definition of a minimum cycle basis, which for the case of nucleic acids is renamed by Major's group as Nucleic Cyclic Motifs (NCM) [28].

1.4.3 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 [67] (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.



Figure 1.2: Ribbon-coil schematic illustrating the fold and intermolecular units of a dimer of prealbumin, or transthyretin, taken from Richardson *et al.* [68]

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.

In the case of proteins the SCOP (Structural Classification of Proteins) database [69], classifies proteins, among other classifications, according to recurrent arrangements of secondary structure, that is, folds. The SCOR (Structural Classification of RNA) database [70, 71], aims to provide a similar classification to that obtained for proteins, but using RNA motifs instead. This classification focuses on the local folding of small pieces of RNA and cannot describe the overall fold.

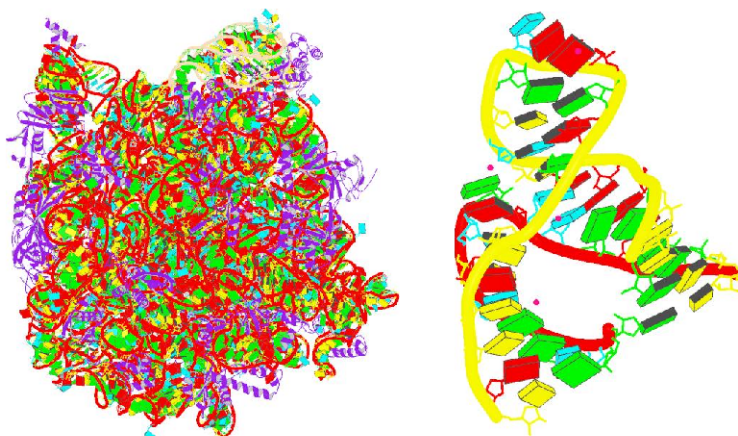


Figure 1.3: *Haloharcula marismortui*'s large ribosomal subunit (left) and hammerhead ribozyme (right). The figures were taken directly from the NDB web pages, and show a ribbon representation of the phosphate backbone, and a block representation for the nucleotide bases. From the figures it's clear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

1.4.4 RNA motifs

First, a word of caution must be given to the reader. The term “*RNA motif*” alone is used in the literature to describe three different levels of RNA organization, that is, RNA **sequence** motifs, RNA **secondary structure** motifs, or RNA **3D structure** motifs. We start by making such distinction as it is not always clearly mentioned in RNA literature, generating a great deal of confusion and bibliographical search frustration for the beginner. The kind of RNA motifs we are dealing with in this thesis are those of the third kind, that is, RNA **3D structure** motifs which we'll address from now on simply as RNA motifs. Yet another source of confusion in understanding RNA motifs is the lack of a unique definition. Three popular and somewhat recent definitions are:

- RNA motifs are “*Conserved structural subunits that make up the secondary structures of RNAs.*”[72]
- RNA motifs are “*Ordered stacked arrays of non-Watson-Crick base pairs that form distinct folds on the phosphodiester backbones of RNA strands.*”[73]
- “*An RNA Motif is a discrete sequence or combination of base juxtapositions found in naturally occurring RNA's in unexpectedly high abundance.*”[74]

From our point of view RNA motifs are to be understood as peculiar sets of geometrical (in the rigid block sense) arrangements in three dimensional space.

Even though there is no unique definition, we can think of three practical tasks regarding RNA motifs. That is, given an RNA 3D structure automatically identify [75, 76, 77], describe [78, 79, 80, 81, 82], and

find new [83, 84, 77, 85, 76] motifs.

1.5 Overview

Keeping always in mind the greater scope of the RNA folding problem, this thesis addresses various issues of RNA structural understanding using RNA crystallographic data from the Protein Data Bank (PDB). Such data has been analyzed statistically along with the use of a very rigorous rigid body formalism. In Chapter 2 the consensus clustering technique is used to classify RNA base-step parameters of non-ARNA conformations, and the resulting groups are localized and understood in the context of rRNA. Chapter 3 reconsiders previous work carried out by Dr. Yurong Xin at the Olson's lab, on classification of RNA base-pairs by resorting again to clustering analysis techniques, and database mining of the WWW available Base Pair Structures (BPS) database. In Chapter 4 we explore, using statistical analysis, the data available on RNA helical regions, and use it to compute the persistence length of double stranded RNA's and compare it to experimental results. In Chapter 5 we provide a new python software, pyRNAmotifs which interfaces with 3DNA to do a rigorous search of existing and perhaps new RNA motifs, and finally in Chapter 6 we propose the measurement and classification of RNA structures using a new graph theoretical index named folding index, based on a helical region "view" of RNA's, which is clearly concordant with the emerging necessity of new metrics beyond RMSD for structural understanding.

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Chapter 2

RNA Base Steps

The problem of classification of the space of conformations of RNA is not new, see for example, Olson 1972 [1], Saenger 1984 [2], and Gautheret 1993 [3]. This problem had only been addressed by a few researchers before the turn of the twenty first century, but starting in the year 2000 a vast amount of RNA structural information has become available with the elucidation of the structure of the 30S small ribosomal subunit of *Thermus thermophilus*, a bacterial ribosome [4, 5], and the 50S large ribosomal subunit of *Haloarcula marismortui*, an archaeal ribosome [6].

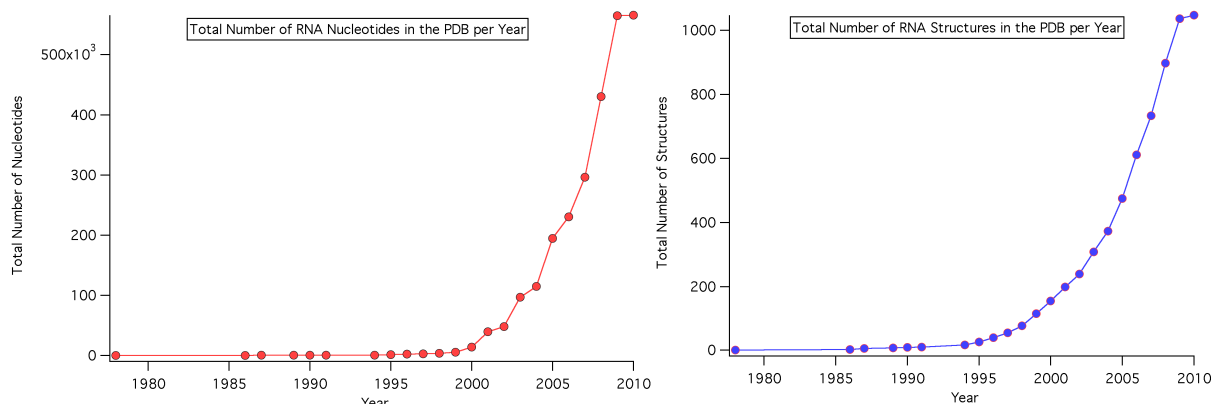


Figure 2.1: **Right:** Total number of RNA bases added to the PDB database between 2000 and 2010. **Left:** Total number of RNA structures solved yearly by X-Ray crystallography between 2000 and 2010.

Between 1978 and 2000 a total of 116 RNA structures with resolution greater than 3.5Å, and comprising around 5500 nucleotide bases are found in the Protein Data Bank (PDB), and between 2000 and today a total of 931 RNA structures comprising around 570000 nucleotide bases are found. That is, the increase in information due to the solution of large RNA structures is two orders of magnitude as pointed out by Noller [7] in 2005. Looking at the growth of RNA structural information from 2000 until today it's important to point out that, although the total number of RNA structures deposited in the PDB shows exponential growth (see left panel in Figure 2.1), the total number of RNA bases does not show such a well defined trend (see right panel in Figure 2.1). This is due to the size preponderance of ribosomal structures. That is, in 2005 nineteen ribosomal structures were deposited in the PDB, whereas in

2006 only four were deposited. So, even though interest in RNA seems to be growing since ribosomal structures have become available in 2000, and two Nobel prizes were awarded for work in RNA in 2006, along with the exciting possibilities of deciphering even larger RNA virus structures, still the growth of the RNA structural field is far from that of proteins if weighed by the growth of RNA structural information in the past seven years. This fact is due to the smaller sizes and structural diversity of RNA molecules crystallized to this date, which, as can be seen in Figure 2.2, is restricted to “compact” nucleotide ranges. A representative example of these characteristic ranges can be seen in Table 1.1 for structures larger than 300 bases.

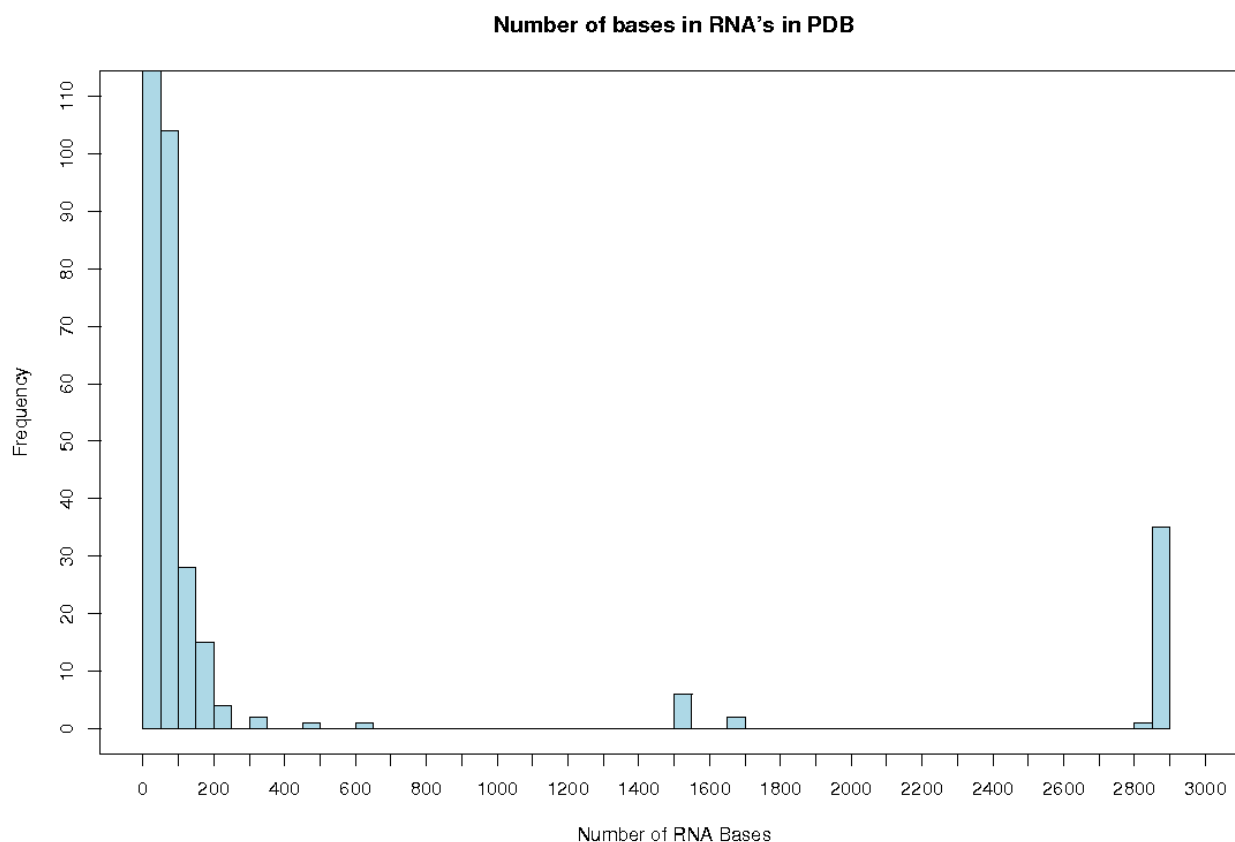


Figure 2.2: Frequency of nucleotide bases in RNA molecules found in the PDB classified by the size of RNA molecules. We define the size as the total number of nucleotide bases present per molecule.

The analysis of RNA conformational information contained in RNA structural data can be divided into three main perspectives: an atom based perspective; a bond based perspective; and a third, as yet unexplored to our knowledge, rigid-body based perspective. In the atom based perspective, either direct comparison of backbone atom positions is made [8], or a comparison of distances between a reduced set of atoms taken from the nucleotide backbone, sugar, and base [9]. The bond based

perspective is divided into three main categories; the first considers the consecutive covalent bonds in the RNA backbone and the glycosidic bond between the sugar and base, that is, six backbone torsion angles and one glycosidic torsion angle [8, 10, 11, 12, 13]; or alternatively the pseudo-bonds between consecutive P and C4' atoms and the resulting pseudo-torsion angles η and θ [1, 14, 15, 16]. The third category considers the networks of horizontal hydrogen bonding patterns coming from a definition of interacting edge boundaries in the nucleotide bases [17, 18, 19]. In this chapter we study the rigid body based perspective using clustering analysis.

| PDBID | Structure Name | Phylogenetic Group | Number of bases | Year |
|-------|--|--------------------|-----------------|------|
| 1l8v | Mutant of P4-P6 Domain of Group I Intron | Eukaryote | 314 | 2002 |
| 1fg0 | Central Loop in Domain V of 23S rRNA | Archaea | 499 | 2000 |
| 2nz4 | GlmS Ribozyme | Eukaryote | 604 | 2006 |
| 1xmq | 30S rRNA | Bacteria | 1522 | 2004 |
| 1ffk | 50S rRNA Subunit | Archaea | 2828 | 2000 |

Table 2.1: Some large RNA structures (>300 bases) elucidated in the last 7 years.

2.1 Consensus Clustering of Single Stranded Base Step Parameters

To our knowledge there has been no classification of rigid-body base-step parameters for RNA structures deposited at the PDB. It is important to note here that in crystal structures, RNA bases are determined more accurately than backbone torsion angles, as has been shown by Richardson and collaborators from analysis of van der Waals steric clashes. This can be seen more clearly in Figure 2.3, reproduced from Richardson's work [10], where the red and orange dots in the backbone atoms region denote steric clashes and the green and yellow dots in the base atoms region denote very good agreement with expected van der Waals distances.

2.1.1 Combining Fourier Averaging Results and Clustering Analysis

Using the coordinates files of 20 rRNA structures provided by Schneider et al.[12] we have used standard clustering analysis (CA) techniques to classify a set of non-ARNA base-steps using, rather than the torsion angles space, the base-step parameters space, that is, three translational parameters (Shift D_x , Slide D_y , Rise D_z), and three rotational parameters (Tilt τ , Roll ρ , Twist ω), which are described by the hexaparametric vector ν :

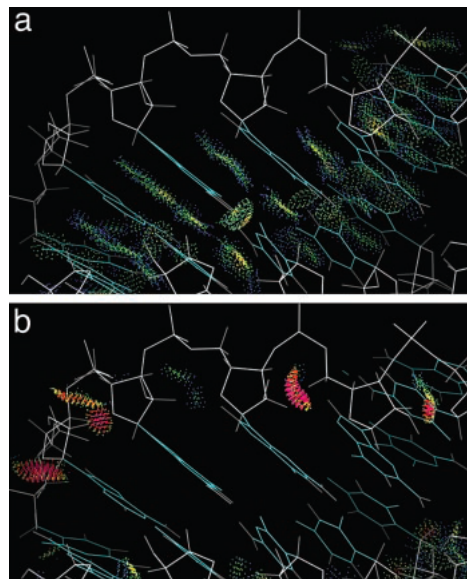


Figure 2.3: Figure taken from Richardson et al. [10] where the blue and green dots in a) mean very accurate van der Waals distances, and in b) the red and orange dots mean steric clashes, that is, distances outside the acceptable van der Waals range.

$$\nu = (D_x, D_y, D_z, \tau, \rho, \omega) \quad (2.1)$$

The results illustrated in Figures 2.4 and 2.5 were obtained by performing clustering analysis and consensus clustering on 20 structures provided by Schneider et al. [12]. These twenty structures were obtained by Schneider applying a Fourier averaging technique, and lexicographical clustering, to torsion angles of 23S rRNA. The methodology we used follows that used by others to recover the periodic table classification from multidimensional property vectors for elements [20, 21]. Table 2.2 shows the residue numbers of bases from 23S rRNA which belong to the main categories of Figure 2.5. To decide which residues of 23S rRNA belonged to the non-Atype clusters, a root mean squared deviation (RMSD) of 15 or less was required between step parameter vectors of 23S rRNA and the mean parameter vectors for the four non-Atype groups identified.

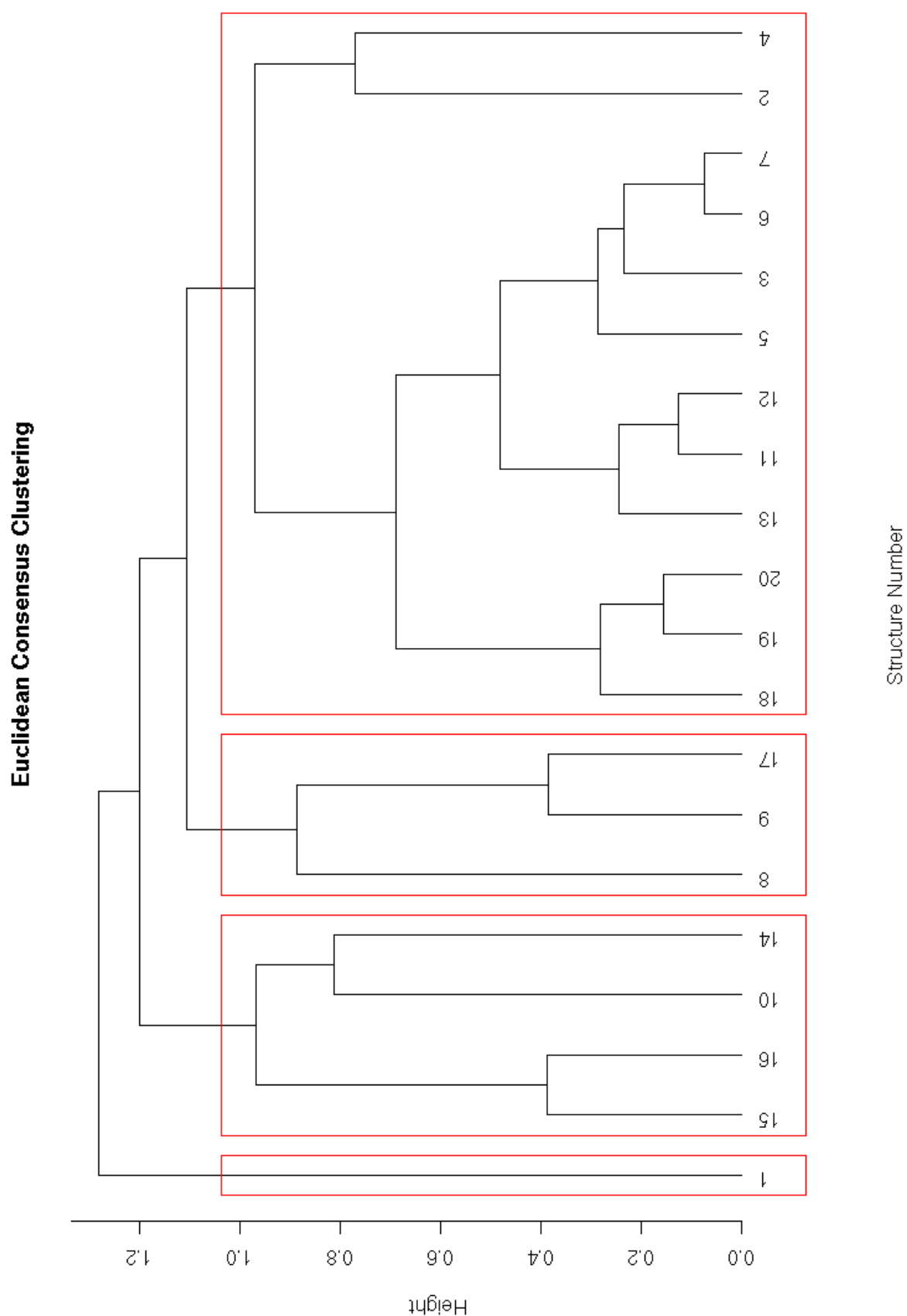


Figure 2.4: Dendrogram showing the results of consensus clustering of 20 non-Atype rRNA dinucleotides according to their hexadimensional base-step parameter vectors.

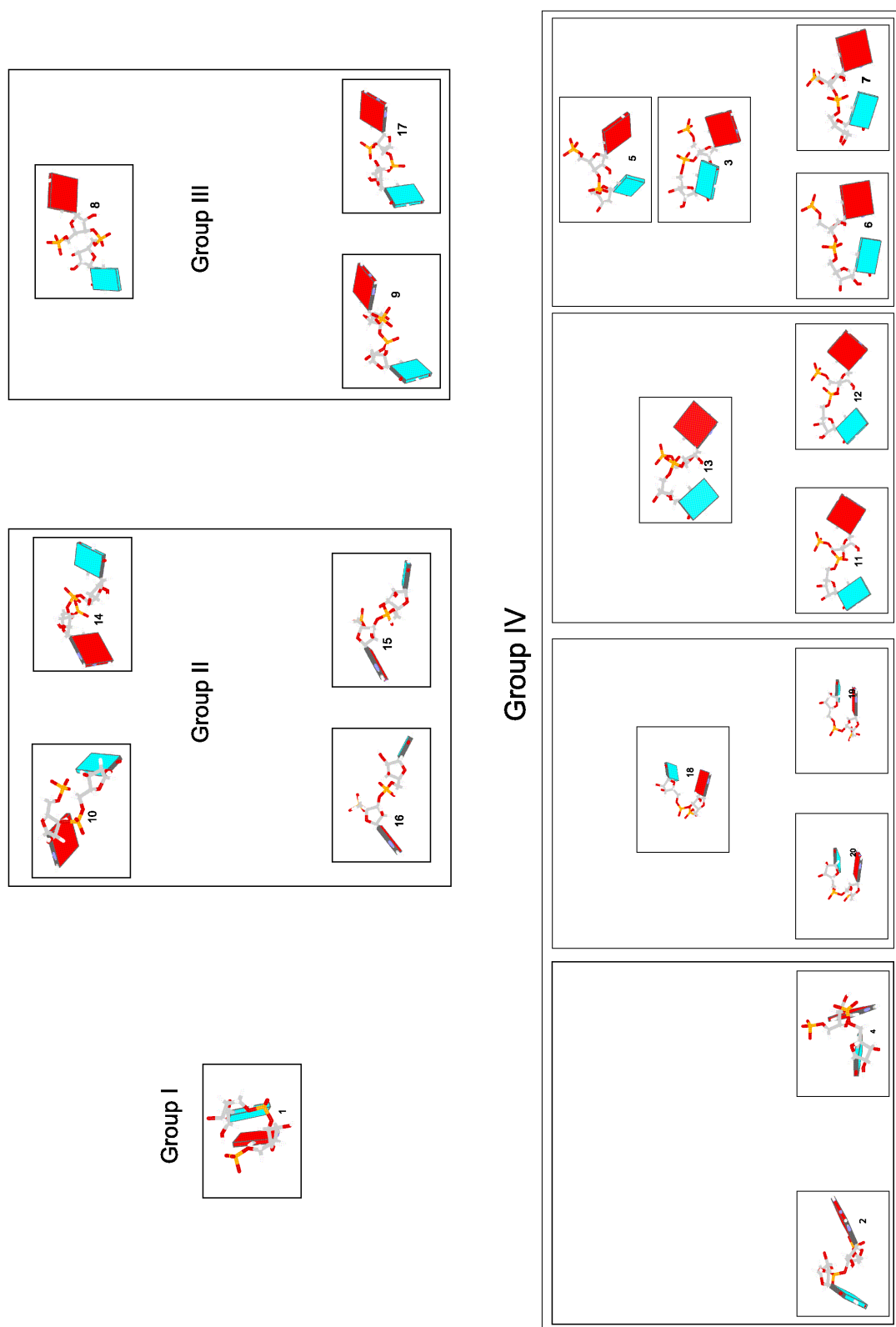


Figure 2.5: rRNA dinucleotide structures organized by clusters obtained from consensus clustering of their hexadimensional base-step parameter vectors.

| Total Number of Nucleotides | RMSD Limit | Group | Base-steps | Base-step Residue Number | Overlaps |
|-----------------------------|------------|-------|------------|---|---|
| 2754 | < 15 | I | 3 | 892, 2006, 2390 | |
| | | II | 5 | 459, 1279, 1653, 1919, 2302 | |
| | | III | 1 | 2109 | |
| | | IV | 35 | 79, 112, 128, 190, 213, 269, 358, 434, 488, 564, 706, 720, 775, 867, 966, 1292, 1503, 1543, 1614, 1766, 1874, 1908, 1971, 2017, 2257, 2427, 2516, 2540, 2755, 2782, 2810, 2826, 2874, 2882, 2913 | |
| | | IVa | 1 | 882 | |
| | | IVb | 807 | | |
| | | IVc | 9 | 306, 789, 854, 880, 1107, 1192, 1493, 1818, 2005 | |
| | | IVd | 35 | 175, 213, 246, 264, 304, 358, 464, 518, 531, 534, 588, 795, 938, 1214, 1231, 1316, 1340, 1370, 1605, 1745, 1766, 1971, 1976, 2010, 2017, 2291, 2320, 2428, 2469, 2481, 2516, 2532, 2755, 2826, 2882 | Only IVd with IV (213, 358, 1766, 1971, 2017, 2516, 2755, 2826, 2882) |

Table 2.2: Residue numbers for base-steps with RMSD values less than 15 between the reference base-step vectors from the four groups of non-A-type RNA dinucleotide conformations and all base-step vectors found in the 23S strand of *Haloarcula marismortui* large ribosomal subunit.

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Chapter 3

RNA Base-Pairing

The RNA base-pairs are reviewed again.

3.1 Canonical and Noncanonical Base-pairs, Methods Paper

3.2 Clustering of Yurong's Classification

Chapter 4

RNA Base Pair Steps

- 4.1 Analysis (Albany Poster) and Django Webserver**
- 4.2 Persistence Length vs. Hagerman**
- 4.3 AMBER: Persistence Length of Base-Pair Step Patterns**

Chapter 5

RNA Motifs

5.1 GNRA tetraloop

In order to compare our work to that of others on RNA structural motif localization and discovery, we ask the following questions:

1. Can the geometric rigid-block description of base-pairing and base-stacking solve the problem of defining RNA structural motifs?
2. Can we use quantities derived from the 3DNA software package to make an automatic search for a known motif, for example, the GNRA tetraloop motif, and perhaps find unknown motifs?

In the ROC meeting of May, 2009 a reduced dataset of RNA structures found at:

http://docs.google.com/Doc?id=dhrmkfmrn_13ftpbjcgq

was made available to participants with the purpose of allowing them to search for RNA motifs, which would later be compared between groups. We have modestly, and as of yet unsuccessfully, started to aim at solving question number two. Initially we are trying to identify all instances of the well known GNRA tetraloop motif in the 23S subunit of ribosomal RNA of *Thermus Thermophilus*, PDB-ID:1ffk using results from 3DNA and 3DNA-Parser, and using an automated process which could be later reproduced for any desired dataset. Our hope is that these baby steps will allow us to tackle the whole ROC dataset.

5.1.1 3DNA-Parser

We started by using Dr. Yurong Xin's 3DNA-Parser hoping that the description of the enclosing base pair in the loop, that is, the sheared G·A, would have a characteristic signature. We found that such is not the case. We know from Major et al. [4] that there should be at least 21 GNRA tetraloops in the 23S subunit of rRNA. We used the G2696 N2697 R2698 A2699 tetraloop as a seed (as can be seen in Figure 1.1) and found out that according to Dr. Xin's helical classification the enclosing G is classified

as S_{hq} and A is classified as H_e . We then searched all such instances for G-A base-pairs and we found

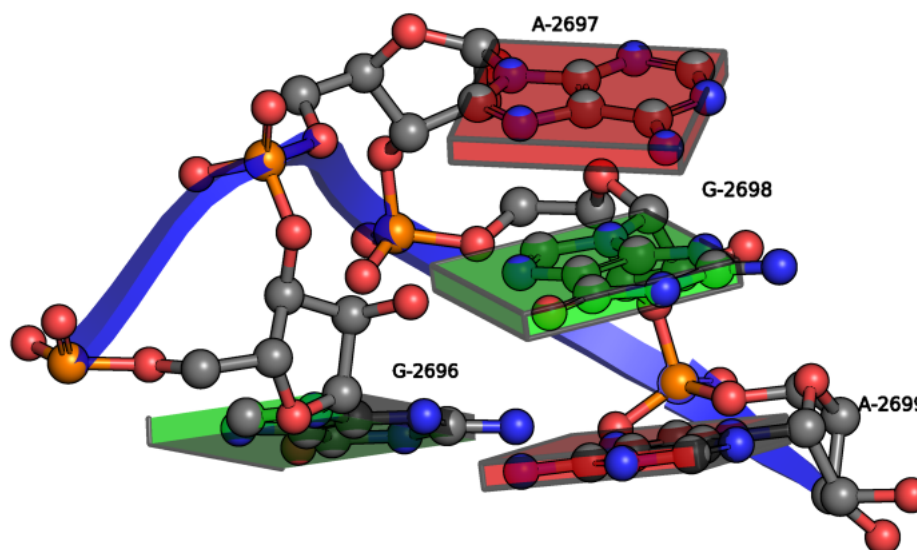


Figure 5.1: GNRA Tetraloop from *Thermus Thermophilus* 23S Ribosomal RNA PDB-ID:1ffk.

seven hits, but none were in fact GNRA tetraloops.

5.1.2 Overlap Scores

We clustered the overlap values imposing a cutoff of values of [1-8]. There are many values which are exactly zero (33%), so, without the cutoff the zero values "overshadow" the data. For this case we obtained a "good" dendrogram as seen in Figure 1.2.

The next step in this analysis will be to find the structures which correspond to this clusters and superimpose and align them using Kabsh's algorithm to be able to determine their RMSD's.

Many people start their RNA Motif identification and classification algorithms by splitting RNA structures into what is helical and what is not, and then finding interactions between these two groups. We believe that we could do a similar exercise with 3DNA by using the scalar product of helical axis vectors and once helical and non-helical regions are found we might be able to use 3DNA Parser to look for characteristic interactions.

5.2 Triplets on RNA (comparison to Laing et al.)

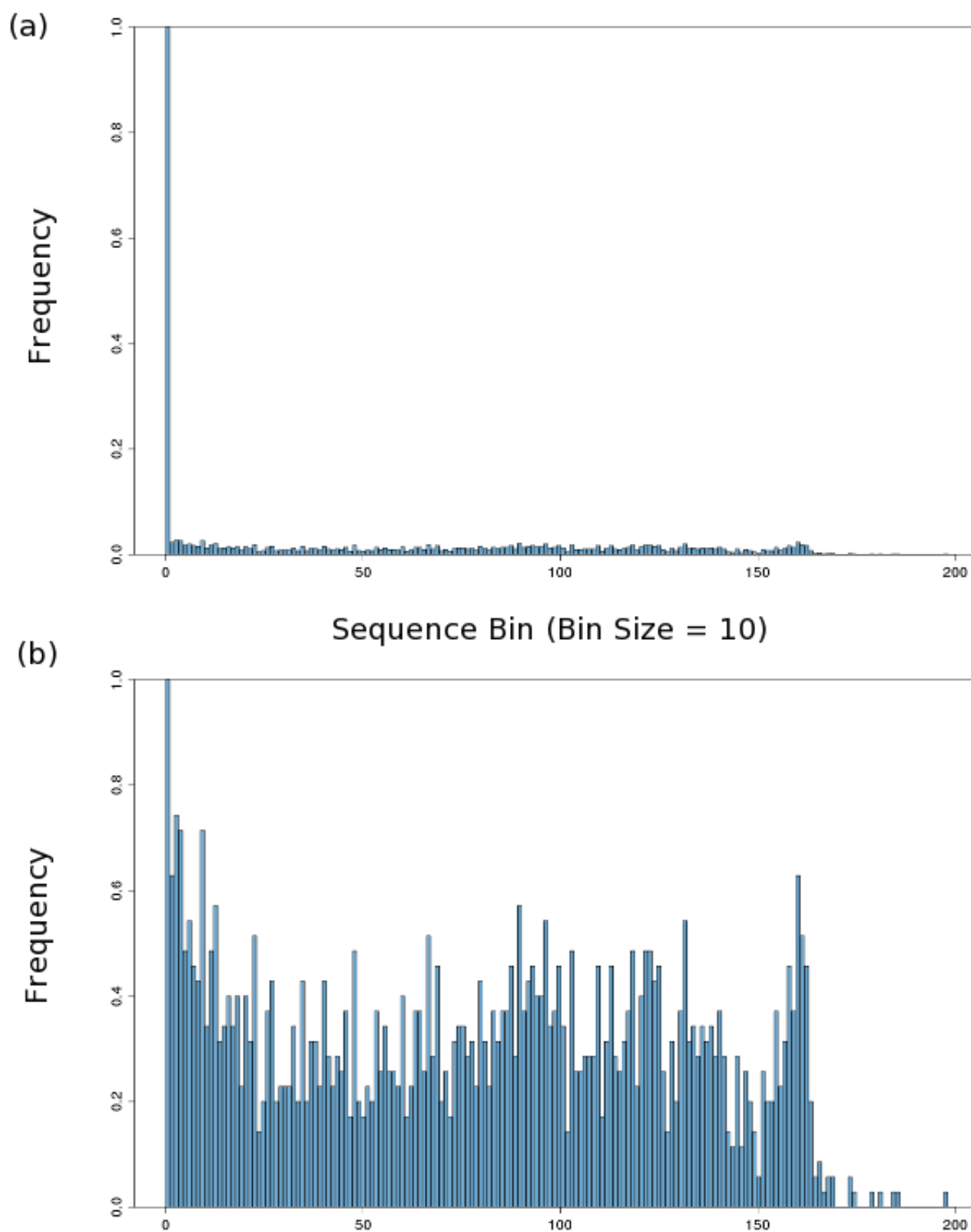


Figure 5.2: Normalized histograms showing the distribution of overlap values in the 23S subunit or *Thermus Thermophilus* rRNA, PDB-ID:1jjk. In histogram (a) all values are included, but in histogram (b) only values greater than zero are included. Notice the high preponderance of zero values, exactly 897 out of a total of 2705.

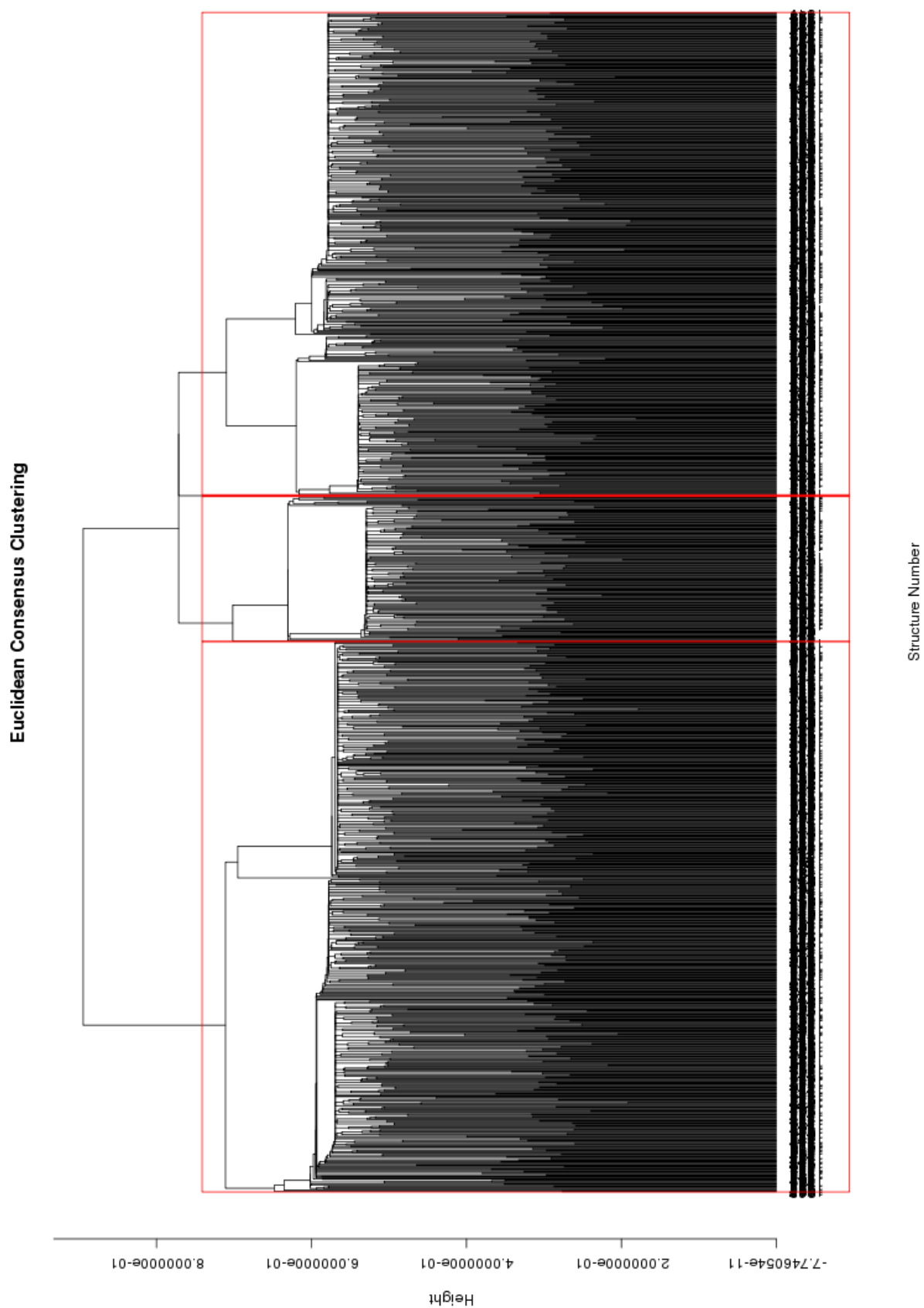


Figure 5.3: Dendrogram for consensus clustering of overlap scores in the ribosome. Zero values filtered out and remaining data normalized.

References

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Chapter 6

RNA Helical Regions and Graph Theory

Chapter on RNA Helical Region Recognition and description using graph theoretical descriptors.

Appendix A

Clustering Analysis (CA)

A.1 Hierarchical methods

The hierarchical clustering methods used were:

1. *Single linkage clustering*, where the minimum distance between elements of each cluster is taken as clustering criteria.

$$D(X, Y) = \min\{d(x_i, y_j) : x_i \in X, y_j \in Y\} \quad (\text{A.1})$$

where X and Y are vectors, and $d(x_i, y_j)$ is the distance between cluster elements.

2. *Complete linkage clustering*, where the maximum distance between cluster elements is the clustering criteria.

$$D(X, Y) = \max\{d(x_i, y_j) : x_i \in X, y_j \in Y\} \quad (\text{A.2})$$

3. *Average linkage clustering*, the mean distance between elements of each cluster is taken as clustering criteria.

$$D(X, Y) = \frac{1}{N_x * N_y} \sum_{i=1}^{N_x} \sum_{j=1}^{N_y} d(x_i, y_j) \quad (\text{A.3})$$

where N_x and N_y are the number of elements in respective clusters.

| Structure | Property I | Property II |
|-----------|------------|-------------|
| 1 | 1.00 | 5.00 |
| 2 | -2.00 | 6.00 |
| 3 | 2.00 | -2.00 |
| 4 | -2.00 | -3.00 |
| 5 | 3.00 | -4.00 |

Table A.1: Example of structures, considered as bidimensional vectors, to be clustered using the average linkage method and the Manhattan distance.

4. *Centroid linkage clustering*, uses the distance between cluster centroids, as clustering criteria.

$$D(X, Y) = d(\bar{x}, \bar{y}) \quad (\text{A.4})$$

$$\bar{x} = \frac{1}{N_x} \sum_{i=1}^{N_x} x_i \quad (\text{A.5})$$

$$\bar{y} = \frac{1}{N_y} \sum_{i=1}^{N_y} y_i \quad (\text{A.6})$$

$$(\text{A.7})$$

5. *Ward's Method*, uses the error sum of squares (ESS).

$$D(X, Y) = ESS(XY) - [ESS(X) + ESS(Y)] \quad (\text{A.8})$$

$$ESS(X) = \sum_{i=1}^{N_x} \left| x_i - \frac{1}{N_x} \sum_{j=1}^{N_x} x_j \right|^2 \quad (\text{A.9})$$

As an example lets think of a case where we have five structures. Each one of them is descibed by a bidimensional vector as illustrated in Table A.1.

The first step is to choose a distance definition. We chose Manhattan and the distance values between structures can be displayed in a lower triangular matrix as seen in equation A.10

$$d(X, Y) = \begin{vmatrix} & 1 & 2 & 3 & 4 \\ 1 & & & & \\ 2 & 4 & & & \\ 3 & 8 & 12 & & \\ 4 & 11 & 9 & 5 & \\ 5 & 11 & 15 & 3 & 6 \end{vmatrix} \quad (\text{A.10})$$

Let's calculate explicitly the Manhattan distance between structures 2 and 3,

$$d(2, 3) = |-2.00 - 6.00| + |2.00 - -2.00| = 12 \quad (\text{A.11})$$

Now that we have calculated the distances we need a clustering method, in this case, we will use the average linkage clustering method. The first step is to group whatever structures are closer, that is, structures 3 and 5 ($d(3, 5) = 3$). Now we find the mean distance between the elements of this cluster and the remaining unclustered structures, that is, structures 1, 2 and 4, we obtain the following mean distances

$$D(\{3, 5\}, 1) = \frac{1}{2 * 1} * (8 + 11) = 4.5 \quad (\text{A.12})$$

$$D(\{3, 5\}, 2) = \frac{1}{2 * 1} * (12 + 15) = 13.5 \quad (\text{A.13})$$

$$D(\{3, 5\}, 4) = \frac{1}{2 * 1} * (5 + 6) = 5.5 \quad (\text{A.14})$$

Since the distances between {3, 5} and all remaining unclustered vectors is higher than the distance between vectors 1 and 2 ($d(1, 2) = 4$) then {1, 2} are grouped. The following value, in hierarchical increasing order is 4.5 between {3, 5} and 1 (see equation A.12), but since 1 and 2 are already grouped we can't group {3, 5} with 1. The next value, following the lower to higher hierarchy, is 5 ($d(3, 4) = 5$), but we have already grouped 3 with 5, so we have to keep advancing in the hierarchy. The next value is 5.5, which corresponds to grouping {3, 5} with 4, so we cluster them. The only remaining possibility for grouping is, group {1, 2} and {4, 3, 5}, so we do it as illustrated in Figure A.1.

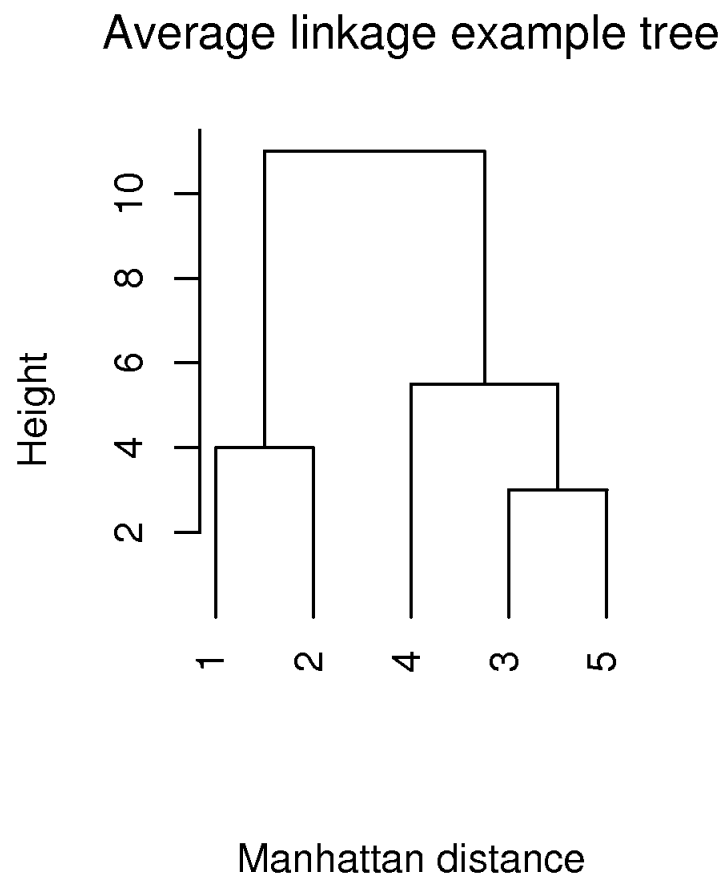


Figure A.1: Clustering tree for 5 bidimensional vectors using the Manhattan distance definition and the average linkage clustering method.

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