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DETECTING CONFORMATIONAL DIFFERENCES BETWEEN RNA 3D STRUCTURES

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Abstract

A method is described for detecting local conformational differences between two 3D structures of the same RNA molecule. These could be distinct 3D structures of the same molecule from the same organism, or homologous molecules from different organisms. In this approach, we detect the variability that exists among the relative translation and rotation operations that are needed to superimpose local neighborhoods after an initial rigid-body global superposition. Each translation and rotation is represented by a three dimensional vector. Thus, we investigate the variability within sets of multivariate data. We demonstrate that the method is able to identify both small- and large-scale conformational changes. Matlab/Octave programs to read RNA 3D structure files and compare structures have been developed and are freely accessible at:

<https://github.com/BGSU-RNA/ConformationalChange>.

Keywords

multivariate; outliers; RNA

1. Introduction

Over 3100 RNA 3D structures [9] have been deposited with the protein data bank (PDB) [3] and its partner, the nucleic acid data bank (NDB) [4]. Most of the 3D structures deposited have been determined with atomic or near-atomic precision by x-ray crystallography, cryo-electron microscopy, or nuclear magnetic resonance (NMR). RNA structures vary in size from hundreds to hundreds of thousands of atoms.

Not all of the crystal structures in the PDB represent distinct molecules. Of the 3100 RNA 3D structures deposited, about 1400 represent distinct RNA molecules [9]. The remaining RNA structures represent the same molecules bound to distinct ligands, i.e., drugs, ions, protein co-factors, or other RNAs, and in distinct functional states. For example, in some cases, small molecules like antibiotics can be diffused into existing crystals to bind to specific sites, and then the same crystal can be subjected to x-ray crystallography again [1]. As a concrete example, there are over 250 3D structures of the *Thermus thermophilus* (*T.th.*) small ribosomal subunit alone, cf.http://rna.bgsu.edu/rna3dhub/nrlist/view/NR_4.0_81883.33.

RNA molecules, like proteins, are dynamic entities which change shape as they bind to different interaction partners and cycle through functional states. Riboswitches change shape in response to their binding of their target. The ribosome, a large RNA-protein “molecular machine” that carries out template-directed protein synthesis, changes shape in dramatic ways. Each 3D structure of the intact ribosome catches the two subunits of the ribosome in different relative orientations as it binds each successive tRNA and adds amino acids to the growing protein chain [18]. Also of interest is the coordinated rotation of the head domain of the small (30S) subunit of the ribosome, which is required for movement of mRNA and tRNAs [8].

Moreover, RNA 3D structures are subject to a variety of smaller influences. Even in crystallized form, each copy of the RNA molecule can be in slightly different geometric conformations. Some parts of the molecule might be loose enough to move about quite a bit, which will make it more difficult to determine the locations of the atoms in these parts. In x-ray crystallography, one measures the intensity of x-rays diffracted from crystals of the molecules of interest as a function of scattering angle and infers the density of electrons at spatial points throughout a single copy of the molecule by 3D Fourier transformation. This is inherently imprecise due to variability in crystal quality. Then a crystallographer attempts to fit atoms and RNA nucleotides to the electron density. To the extent that parts of the molecule are mobile, the electron density may be too diffuse to fit uniquely. Similar considerations apply to cryo-electron microscopy and NMR.

Thus, RNA 3D structures are subject to two different kinds of variability: variability due to differences in interaction partners and experimental conditions, and variability due to thermal fluctuations, experimental measurement, and fitting. We would like to distinguish the variability between RNA 3D structures that is significant and biologically meaningful from that due to experimental or modeling errors. For example, research groups may want to analyze which regions of two crystal structures deviate from one another to determine whether errors have been made during the modeling process. Or it may be beneficial to learn what conformational changes take place within the 3D structure of a molecule when an antibiotic or other ligand is bound.

Fortunately, we have multiple structures for many RNA structures, especially ribosomal RNAs, and so are able to characterize routine variability by comparing such 3D structures. We note that when we compare two different crystal structures of the same molecule from the same organism, we know the exact correspondences between nucleotides of the two structures; thus, no alignment of the structures is required. When comparing homologous molecules from different organisms, we can make a structural alignment using R3D align [11].

The classical tool for comparing two different models of a molecule is the average root-mean-square deviations (RMSD) after optimal rigid-body superposition of the two structures. However, while global RMSDs do capture differences in the general shapes of the molecules, they do not provide any information regarding the variability that exists between the structures at a finer grained, local level of individual nucleotides or nucleotide neighborhoods. In [10], two metrics suitable to RNA comparisons are described - the

deformation index and deformation profile. The deformation index considers both base-base-stacking and base-base-pairing interactions while the deformation profile compares the structures at the nucleotide level for both intradomain and interdomain interactions. However, these do not indicate the nature or the extent of the deformation.

The goal of the present paper is to characterize and visualize the variability in local neighborhood locations and orientations in RNA 3D structures. Regions of variability will indicate conformational differences between the two structures. The method we describe in this paper finds and displays local conformational differences with clear intuitive interpretation and provides output suitable for further analysis and comparison of multiple structures of the same molecule.

2. Method

2.1. Using translations and rotations to detect conformational changes

The key idea of this paper is to analyze the variability that exists in the optimal superpositions of local neighborhoods, in comparison with the optimal global superposition of the structures. This will allow us to identify regions that are translated or rotated differently, relative to the rest of the structure.

An optimal rigid-body superposition of nucleotides $a_1, \dots, a_n$ of structure A onto nucleotides $b_1, \dots, b_n$ of structure B is computed as follows: let $\alpha_1, \dots, \alpha_n$ and $\beta_1, \dots, \beta_n$ be the geometric centers of the bases of $a_1, \dots, a_n$ and $b_1, \dots, b_n$ respectively. The optimal superposition then consists of a translation vector t and a rotation matrix R that minimize the squared error, ε , where

$$\varepsilon = \min_R \min_t \|\beta_i - R(\alpha_i - t)\|^2.$$

The optimal global rotation matrix R^* and translation vector T^* are obtained using the technique described by Berthold et al. [5]. These are used to superimpose the two structures globally.

Then for each nucleotide i in the first structure, its five-nucleotide local neighborhood is found (the nucleotide itself along with the four nearest nucleotides according to Euclidean distance), and then the translation vector t_i and rotation matrix R_i of the optimal superposition onto the corresponding five nucleotides in the second structure are found. Thus, t_i and R_i are found relative to T^* and R^* .

We note that while a rotation is typically represented as a 3×3 matrix, it can be expressed as a 3-dimensional vector defined by the unit vector corresponding to the axis of the rotation and multiplied by the angle of rotation in degrees. For our purposes, we will represent each R_i in this vector form.

2.2. Illustration of using translations to detect conformational changes

As an example of differences primarily due to thermal fluctuations, experimental measurement, and fitting, we compare the crystal structures found in PDB files 2UUB and 2UUC. Both files contain crystal structures of *Thermus thermophilus* 16S ribosomal RNA bound to an mRNA with a codon in the A-site and the antibiotic paromomycin. 2UUB is bound with a GUU-codon and 2UUC is bound with a GUA-codon [15]. Overall, we expect them to be very similar since they are both crystallizations of the same molecule bound to the same antibiotic. Also, the crystal structures were determined by the same research group and were deposited in the PDB just one week apart.

First, the translation vectors are analyzed. Figure 1 displays a 2D view of a scatterplot of the relative translation vectors, calculated as described above. It can be seen that the three-dimensional data points are roughly centered at zero. The more a point deviates from the mean, the greater the conformational difference between the corresponding neighborhoods of the nucleotides represented by that point. Of interest is the variability of the points about the mean as that provides a measure of the routine translational differences between nominally identical molecules. Most relative translation vectors have length 0.5 Ångstroms or less.

However, in the lower left of Figure 1, nucleotide 1257 appears to be an extreme data point as it is set apart from the rest of the data points. Figure 2 shows a portion of the global superposition including nucleotide 1257 from 2UUB and its local neighborhood (in red) together with corresponding nucleotides from 2UUC, which have the same nucleotide numbers. Nucleotides 1257 from the two structures do not superimpose well which makes it clear why the translation t_{1257} is farther removed from the origin than most other translation vectors. Its norm is approximately 1.4 Ångstroms.

The relative rotation vectors can be analyzed in a similar way as the translation vectors. The rotation vectors provide additional information by indicating a different type of conformational change. For example, the 5-nucleotide local neighborhood around base U1257 in 2UUB, shown in Figure 2, requires roughly a 26.2 degree rotation relative to the global, rigid body rotation that superposes the two structures. A scatterplot of rotation vectors is provided in the Github repository associated with this article in a folder called *figures*, under the name 2UUB_2UUC_Rotation_Comparison in PDF format and in Matlab/Octave figure format, so that the user can open and rotate the scatterplot. The direct URL for the figures folder is: <https://github.com/BGSU-RNA/ConformationalChange/tree/master/Figures>.

We are also able to detect larger regions of conformational difference by discovering clusters of data points that are scattered in the same direction from the mean. For example nucleotides 1026-1039 have relatively extreme values. A superposition of these nucleotides is provided in the Github figures folder under the name 2UUB_2UUC_Superposition_1026-1039.

2.3. Detecting outliers in multivariate data

We have seen above that extreme data points can be found by simply observing the scatterplot. However, a less subjective, more formal statistical process may be desired. In this section, we provide a description of the statistical methods that can be employed in order to measure variability and detect extreme values within multivariate data of this type.

We are essentially dealing with the problem of identifying outliers and other extreme data points within a sample of three-dimensional data. Detecting outliers is more difficult when dealing with multivariate data than with univariate data because multivariate data cannot be ordered in the same way that a univariate sample can so that extreme values simply show up on either end of a single continuum. With multivariate data, an extreme observation vector may have a large error in one of its components or smaller errors in several components. However, if the multivariate data is two-dimensional or three-dimensional, we may still perceive an observation to be particularly extreme when the data are displayed as a scatterplot as in Figures 1, 3, and 4.

Despite no obvious ordering of the data being present, it is necessary to adopt some notion of ordering in order to determine extremeness. The most common method of ordering multivariate data is to reduce each multivariate x to a scalar quantity $D(x)$ (often some type of distance measure), thereby creating a univariate data set from which extreme values can be detected [2].

We will represent each observation vector x_i (to represent both t_i and R_i) by its standardized distance from the mean. We denote $D(x_i)$ as D_i . Then we have

$$D_i^2 = \left(x_i - \bar{x} \right)' S^{-1} \left(x_i - \bar{x} \right),$$

where S is the sample variance-covariance matrix:

$$S = \frac{1}{n} \sum_{i=1}^n \left(x_i - \bar{x} \right) \left(x_i - \bar{x} \right)'.$$

The higher the value of D_i^2 , the greater the distance of the point from the mean. Thus the task of finding conformational changes among structures reduces to finding nucleotides whose corresponding data points have larger values for D_i^2 .

When the data is normally distributed, a formal outlier test exists that is based on $D_{(n)}^2$. This is because the Wilks' statistic [16] can be expressed in terms of $D_{(n)}^2$, where

$$D_{(n)}^2 = \max_i D_i^2.$$

The Wilks' statistic is

$$w = \max_i \frac{|(n-2) S_{-i}|}{|(n-1) S|},$$

where S_{-i} is the sample variance-covariance matrix of the data with the i th observation deleted. The Wilks' statistic can also be written as

$$w = 1 - \frac{n D_{(n)}^2}{(n-1)^2}.$$

Thus a test for an outlier can be based on $D_{(n)}^2$. Tables are available containing the upper 5% and 1% critical values of $D_{(n)}^2$ [12]. However, in our case no normality assumption is made since we are not particularly interested in the formal declaration of outliers. We are more interested in which points are more extreme relative to the others, for which an ordering of the D_i^2 values is sufficient.

In addition to discovering local regions of conformational change, we are also interested in determining the overall similarity of two structures. We know that the more similar two structures are, the closer the data points should be centered about the mean. Thus, if we study the overall variability of the data points, we can learn more about the overall similarity of the two structures. While this information is contained within the sample variance-covariance matrix S , a single numerical value for the overall multivariate scatter is often desired. The total sample variance, which is the trace of S , can serve this purpose. Since we are working with three dimensional data, we have:

$$\text{total sample variance} = s_{11} + s_{22} + s_{33}.$$

The square root of this quantity has appropriate units, Ångstroms or degrees and gives an indication of the typical relative translation or rotation.

3. Results

3.1. Analyzing total variability

First, we want to understand the variability in local conformations between two RNA 3D crystal structures of the same molecule from the same organism and produced by the same research group.

As our first example, we use two crystal structures of the *T.th.* 16S rRNA that were produced by the same research group. We use the crystal structure found in PDB file 1FJG [6] and the structure found in PDB file 1J5E [17].

As in Section 2.2, we can produce scatterplots of the translation and rotation vectors and use D_i^2 values to find regions of conformational change. The scatterplots of the translation and rotation vectors are included in the Github repository with names beginning with

1FJG_1J5E. Here we focus on the overall variability of the data points since that provides an indication of how similar the two structures are. The total sample variance for the translation vectors is found to be 0.2296, and the total sample variance for the rotation vectors is 10.6139. Taking square roots, the typical length of the relative translation vector is 0.4791 Ångstroms, and the typical rotation angle is 3.2579 degrees. For 2UUB and 2UUC, we obtain similar numbers, 0.2864 Ångstroms and 4.2705 degrees.

We next compare two crystal structures of the same molecule from the same organism, but where the crystal structures have been determined independently by different research groups. We expect there to be greater variability in the data points than in this case since there is additional variability in the modeling processes. We use the 1J5E crystal structure again, but compare to 2ZM6 which was solved more recently by an independent research lab [7]. As expected, the variances of the translation and rotation vectors are larger in the case when the structures were crystallized by different groups than by the same group. The square roots of total sample variance for the translation and rotation data are 0.9454 Ångstroms and 6.1262 degrees, respectively. These values are much larger than in the example described in the previous section. The scatterplots of the translation and rotation vectors are included in the Github repository with names starting with 1J5E_2ZM6.

3.2. Head rotation in the small ribosomal subunit

A dramatic example of conformational change occurs between two *T.th.* small ribosomal subunit structures, 1FJG [6] and 4QS0 [19]. In the second structure, the head of the small subunit has been crystallized in a rotated state in the act of translocation of the mRNA [8]. The fact that two large regions of the structure have different local rotation matrices compared to the global rotation is apparent in Figure 3.

Furthermore, the relative translation graph shows a spread of points, as the nucleotides in the head that are most distant from the hinges move the most, as is clearly shown in Figure 4. Figures 3 and 4 are provided in the Github repository with names starting with 1FJG_4QS0.

3.3. Comparing homologous molecules

Our examples thus far have compared two different crystal structures of the same molecule from the same organism. In this section, we analyze two homologous 16S rRNAs from different organisms. When we compare two molecules from different organisms, we do not know a priori which nucleotides correspond, so an alignment of the two structures is first required. Structural alignments are obtained using R3D align [11].

We analyze the conformational differences between the *T.th.* 1J5E [17] and *E.coli* 2AVY files [14]. A structural alignment was obtained using R3D align. Because 1J5E and 2AVY are crystal structures of different molecules, we expect there to be greater variability in the translation and rotation vectors than in the previous cases where the crystal structures are of the same molecule. The scatterplots for the translation and rotation vectors, colored by distance to the center of the molecule, are provided in the Github repository with names starting with 1J5E_2AVY. There are clear clusters of local neighborhoods that vary all in the same way. Most of these are at some distance from the center of the molecule, where more flexibility is allowed.

It is evident from the scatterplots that there is indeed more variability in this case. This is confirmed by analyzing the square roots of total sample variances which are computed to be 3.0869 Ångstroms and 30.4948 degrees. These values are much higher than in the other cases where the structures were from the same organism.

4. Software and Availability

The software consists of new programs that extend the FR3D and R3D align packages [11, 13]. We describe them briefly here; additional documentation within the programs and on Github explains how they work and how they can be easily modified.

CompareStructures.m

This program loads the RNA 3D structures, calculates the global superposition, superimposes local neighborhoods, and calculates the relative rotation and translation vectors. It optionally displays scatterplots as shown in the paper, returns a data structure containing all data, and writes the data to an excel spreadsheet. Points can optionally be colored by distance from the center of the structure, position in the sequence, or local geometric discrepancy between local neighborhoods (to bring out neighborhoods that are locally deformed).

Interact.m

This program creates the two-dimensional “Rotation-Translation” scatterplot shown in Figure 5. The user may click a point in the graph to visualize the local neighborhood, and then enlarge or shrink the local neighborhood. Nearby amino acids, if any, are also shown, in case they cause a conformational change.

Example_1FJG_4QS0.m

This script contains the commands to evaluate structures 1FJG and 4QS0, display the relative rotation and translation vectors, and interact with Figure 5. Other scripts are provided to replicate the other examples described within this paper, including Example_2UUB_2UUC.m, Example_1FJG_1J5E.m, Example_1J5E_2ZM6.m, and Example_1J5E_2AVY.m.

The software is available through Github at this URL: <https://github.com/BGSU-RNA/ConformationalChange>. As explained in the Readme file on Github, one also needs to install the FR3D program suite and, if desired, the R3D align programs. Support for reading mmCIF files is included in the current release of FR3D. The software has been extensively tested in the Matlab and Octave environments. The Readme file gives a number of pointers for working with Octave.

5. Conclusion

The procedure described in this paper, implemented in the Matlab programming language, is useful for quickly determining and visualizing local conformational differences between two 3D structures of RNA. These structures may represent the same molecule from the same organism or homologous molecules from different organisms. Comparing molecules from

different organisms first requires a 3D alignment, which can be determined by programs such as R3D align [11]. As a whole, this suite of programs provides valuable tools for researchers to quickly gain further insight into the function and structure of RNA 3D molecules.

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Figure 1.

View of 3D scatterplot of translation vectors for files 2UUB and 2UUC. Units are Ångstroms ($1\text{\AA} = 10^{-10}\text{ m}$). Points are colored according to the corresponding nucleotide's distance from the center of the structure. The colors range from blues to reds according to the color bar shown on the right. Extreme data points are labeled using the method outlined in Section 2.3, using a cut-off value of 25 for D_i^2 .

Figure 2.
Portion of the global superposition showing the neighborhoods of nucleotide 1257 in 2UUB (red) and 2UUC (gold). The neighborhood includes nucleotide 1257 along with the nearest four nucleotides in 3D space, which are nucleotides 1256, 1258, 1259, and 1260.

Figure 3.

Relative rotation vectors between chain A of 1FJG and chain A of 4QS0, colored by position in the sequence (from 1 to 1507). Nucleotides in the head occur between positions 910 to 1380, and these are colored yellow to orange in the figure. Their relative rotation vectors clearly stand apart from the others, on either side of the vector (0, 0, 0) representing the global rotation.

Figure 4.

Relative translation vectors between chain A of 1FJG and chain A of 4QS0, colored by position in the sequence (from 1 to 1507). Nucleotides in the head occur between positions 910 to 1380, and these are colored yellow to orange in the figure. Their relative translation vectors clearly stand apart from the others, on either side of the vector (0, 0, 0) representing the global translation.

Figure 5. Scatterplot of rotation angle versus norm of translation vector, for the example of 1FJG and 4QS0 discussed above. Points are colored by position in the sequence.