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Applications Note

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Rclick: a web server for comparison of RNA 3D structures

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Abstract

Summary: RNA molecules play important roles in key biological processes in the cell and are becoming attractive for developing therapeutic applications. Since the function of RNA depends on its structure and dynamics, comparing and classifying the RNA 3D structures is of crucial importance to molecular biology. In this study, we have developed Rclick, a web server that is capable of superimposing RNA 3D structures by using clique matching and 3D least-squares fitting. Our server Rclick has been benchmarked and compared with other popular servers and methods for RNA structural alignments. In most cases, Rclick alignments were better in terms of structure overlap. Our server also recognizes conformational changes between structures. For this purpose, the server produces complementary alignments to maximize the extent of detectable similarity. Various examples showcase the utility of our web server for comparison of RNA, RNA–protein complexes and RNA-ligand structures.

Availability and implementation: The Rclick web server is freely accessible at http://mspc.bii.a-star.edu.sg/minhn/rclick.html

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

Comparing RNA 3D structures gives us unique insights into their evolution and function. While there are numerous methods for comparing and classifying protein 3D structures (Cuff *et al.*, 2009; Kolodny *et al.*, 2005; Murzin *et al.*, 1995; Stebbings and Mizuguchi, 2004), there have been very few such methods for RNA 3D structures (Rother *et al.*, 2011). Given the rapid pace with which new RNA structures are deposited in the PDB (Berman *et al.*, 2000), it is crucial to have tools to classify and categorize these structures and investigate them for similarities at different levels. Here, the Rclick web server is designed to provide a user-friendly interface to compare RNA 3D structures and produces accurate alignments when the structures are similar.

The performance of Rclick was compared to other popular servers and methods that align RNA 3D structures, including ARTS (Dror *et al.*, 2005, 2006), SARA (Capriotti and Marti-Renom, 2008, 2009), CLICK (Nguyen *et al.*, 2011), SETTER (Hoksza and Svozil, 2012; Cech, *et al.*, 2012), and R3D Align (Rahrig *et al.*, 2010, 2013) on different benchmark RNA datasets (Supplementary Figs. S4–S6 and Supplementary Tables S1–S4). The structure overlaps of Rclick in these comparisons were better. Figure 1 and Supplementary S1 and S2A and B in show the utilities of Rclick for comparison of large ribosomal subunit, RNA-protein complexes and RNA-ligand structures. While Rclick produced accurate alignments of RNA–protein interactions between two Ribonuclease III (PDB codes 2LUP and 1RC7, Fig. 1), when compared to other web servers such as SETTER (Cech, *et al.*, 2012) and ARTS (Dror *et al.*, 2006) which could not align correctly RNA-binding sites. Supplementary Figure S2A and B show two thiamine pyrophosphate (TPP) riboswitches (PDB codes 2CKY chain A and 2GDI chain X)

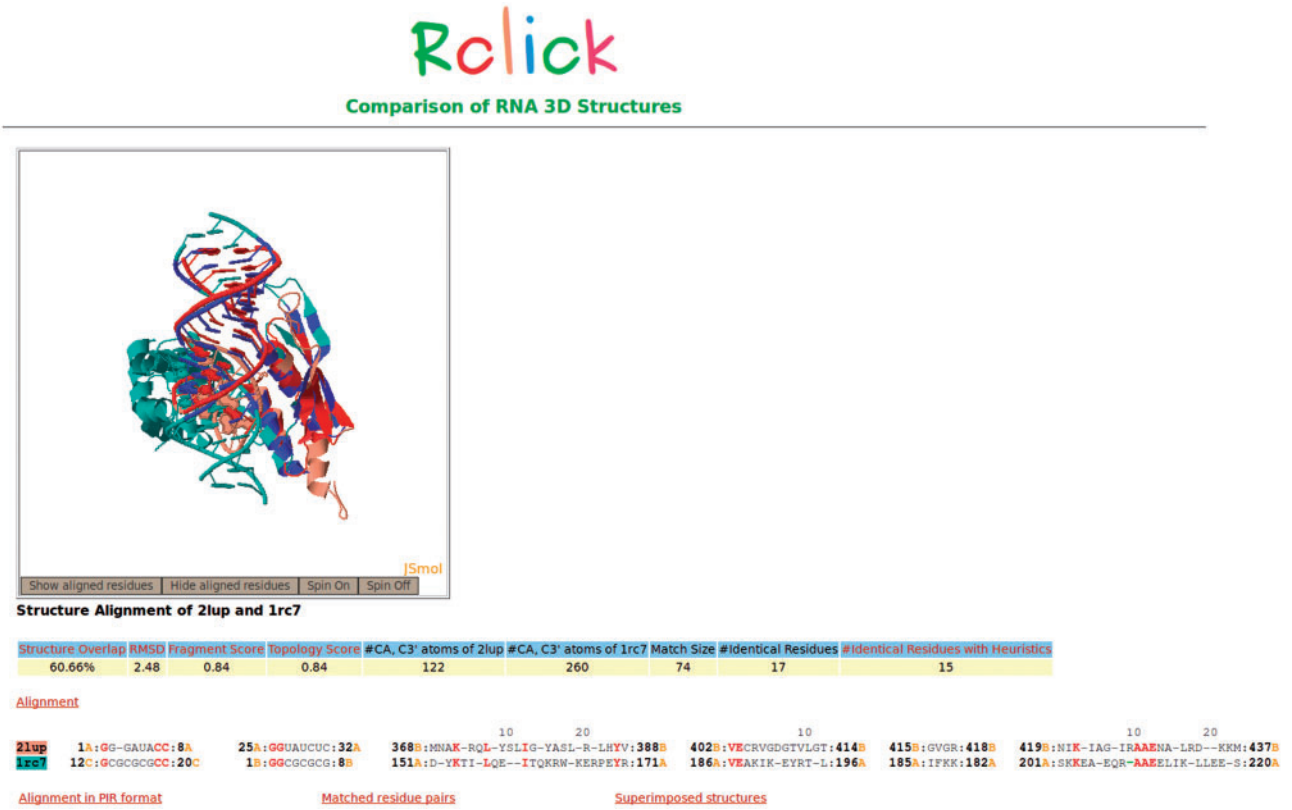

Fig. 1. Snapshots of the Rclick server showing the output of a structural alignment of two RNA that belong to Ribonuclease III family, PDB codes 2LUP and 1RC7. Rclick shows the accurate alignment of RNA–protein interactions between 2LUP (salmon) and 1RC7 (green). The superimposed residues of 2LUP and 1RC7 are shown in red and blue colors, respectively. The conserved residues are shown in bold and red lettering

aligned with one another, and the similarities of conformation of the bound TPP ligands in both RNA structures. These examples highlight the capabilities of our web server that produces accurate alignments of RNA structures and additionally aids in the general area of prediction of RNA–protein and RNA-ligand interactions. In addition, to the best of our knowledge, this is the first RNA structure comparison server that shows conformational changes in the RNA structures being compared (Supplementary Fig. S3A and B).

2 Program overview

Our server Rclick aligns RNA 3D structures by enhancing the CLICK algorithm that matches cliques of points (Nguyen and Madhusudhan, 2011; Nguyen *et al.*,2011). As in the original implementation, CLICK only optimally superimposes a pair of protein 3D structures independent of topology. In this study, cliques are optimal groupings of representative atoms (C3′ for RNA structures, and C3′ and C^{α} for RNA–protein complexes) within a certain spatial proximity. Any pairwise distance amongst clique members is less than the set threshold. A pair of RNA structures is structurally aligned by matching cliques based on the superimposition of their Cartesian coordinates with 3D least squares fitting. The objective here is to establish local structural similarities.

Clique matching identifies structurally equivalent residues in the two structures. Using these equivalences, a final 3D least squares fit is performed to superimpose the two RNA structures. The matching of cliques is not necessarily unique, i.e. it is possible to generate multiple structural alignments. The chosen alignment is the one that maximizes structure overlap. Here, structure overlap (also called equivalent positions) is defined as the percentage of the representative atoms in the structure A that are within 4 Å [root mean square deviation (RMSD) cutoff] of the corresponding atoms in the superimposed structure B. The user can set RMSD cutoff parameter within 3 and 6 Å in the Rclick server.

By optimizing representative atoms and parameters for RNA structures, the average structure overlap values of Rclick alignments are better than those of CLICK on the benchmark datasets (Supplementary Tables S1–S4).

3 Server description

3.1 Input

Input RNA structure can be submitted by specifying the 4-letter code for an existing structure deposited in the PDB or by uploading a structure in PDB format. In addition to the 4-letter code, the user can specify the identity of particular chains from the query structure. Fragments of structures are also acceptable as input, as long as there are three or more representative atoms. An explanation of the input structure is provided in the help pages.

3.2 Output

The structure alignment is shown in two lines, one line per structure. The number and chain identifier of the first aligned residue on both structures precedes the listing of the residue one letter codes. Each time the alignment fragments, the number and chain identifier of the last residue in the fragment are also listed. Accompanying each alignment is a 3D rendition of the structural superimposition using JSMol. The superimposed residues on both structures will be

displayed when the user clicks the button labelled 'Show aligned residues' in the JSMol viewer (Fig. 1).

Should there be conformational changes in the RNA structures being compared, Rclick first reports the largest alignment, in terms of the number of residues aligned. The method then seeks to compare the regions of the RNA structures that were not aligned first. The detection of further structural similarity results in additional output alignments, shown one below the other in the aforementioned format (Supplementary Figs. S3A and B).

The alignments are downloadable in PIR format and in Rclick format that shows one equivalent representative atom match per line. Also downloadable are the coordinates of the superimposed structures in PDB format.

Statistics relevant to the alignment including structure overlap, RMSD, fragment score, topology score, number of representative atoms in the two structures, length of the match and the number of identical residue matches are displayed in a table. Detailed help pages explain the significance of the different alignment measures.

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