

# Modeling RNA loops using sequence homology and geometric constraints

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## ABSTRACT

**Summary:** RNA loop regions are essential structural elements of RNA molecules influencing both their structural and functional properties. We developed RLoop, a web application for homology-based modeling of RNA loops utilizing template structures extracted from the PDB. RLoop allows the insertion and replacement of loop structures of a desired sequence into an existing RNA structure. Furthermore, a comprehensive database of loops in RNA structures can be accessed through the web interface.

**Availability and Implementation:** The application was implemented in Python, MySQL and Apache. A web interface to the database and loop modeling application is freely available at <http://rloop.mpimp-golm.mpg.de>

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## 1 INTRODUCTION

Loop structures (i.e. regions without secondary structure) are not only essential structural elements accounting for the flexibility and structural diversity of RNA molecules. They are also important functional elements. For example, they frequently represent the site at which ligands are bound to the RNA molecule. The lack of canonical base pair formation in these regions makes them accessible for intra- (e.g. tertiary structure contact formation) and intermolecular contacts (e.g. binding of small molecules or other biomolecules). The disparity between the number of available RNA sequences on the one side and experimentally verified 3D structures on the other calls for an application of computational structure modeling to bridge this gap. Recently, we reported results from a comprehensive survey of RNA loop structures and established the basis for the application of homology modeling to RNA loops (Schudoma *et al.*, 2010). Sequence-similar loops were found to fold into very similar structures. As a critical sequence identity threshold beyond which this relationship no longer holds, 25% sequence identity was determined. Here, we present a web-based application for RNA loop structure modeling utilizing available structures extracted from the Protein Data Bank (PDB; Berman *et al.*, 2000). In contrast to the existing RNA 3D structure modeling applications [MC-Sym/MC-Fold (Parisien and Major, 2008), NAST/C2S (Jonikas *et al.*, 2009) and FARNA (Das

and Baker, 2007)] and RNA 3D structure databases [RNAjunction (Bindewald *et al.*, 2008), FRABASE (Popenda *et al.*, 2008) and SCOR (Tamura *et al.*, 2004)], our approach is specifically tailored to the application to RNA loop structures.

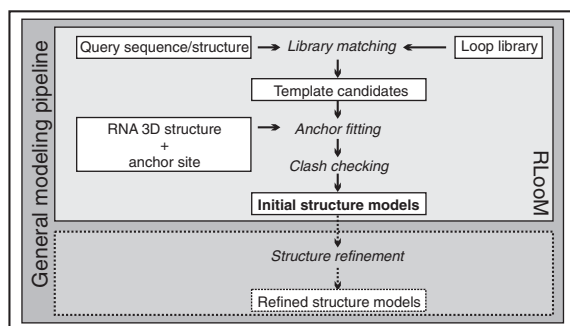
## 2 TEMPLATE LIBRARY GENERATION

We extracted loop structures [hairpins, internal loops, multiloops and (single-stranded) segments] from 1371 RNA structures in the PDB (December 2008) using secondary structure information obtained from MC-Annotate base pair annotations (Gendron *et al.*, 2001). Segments comprise bulges as well as the individual strands of internal loops and multiloops. The extraction followed these extraction rules: (i) Stems are regions of two or more continuous canonical base pairs (including GU/UG wobble pairs). (ii) Single base pairs and stacked base pairs inducing pseudoknots are not considered to be part of the secondary structure. (iii) Segments and hairpins include one additional *anchor* residue on either side. For hairpin loops, this corresponds to the closing base pair of their preceding stem. These anchors allow for the matching and insertion of a loop into a specified target site. (iv) Internal loops are composed of 1–2 (with the former case representing bulges) segments. Multiloops contain a number of individual strands less or equal to their number of branching stems. We clustered the raw loop datasets by sequence length, structural similarity [as given by the root mean square deviation (RMSD) of the superpositions of their reduced backbone atoms (P, O5', C5', C4', C3', O3')], and optionally, sequence identity. Using a range (0.5Å–3.0Å) of incremental (0.5Å) cutoffs, we generated eight clustered sets of template loop structures of different degrees of sequence and structural redundancy.

## 3 TEMPLATE LIBRARY CONTENT AND DATABASE USAGE

The database contains 13 085 hairpin loops, 46 361 single-stranded segments, 17 133 internal loops and 5756 multi-loops. For each loop, we provide information on intra-loop base pairs, sugar pucker, and base stackings as well as a visualization both as 3D structure model [using Jmol (<http://www.jmol.org/>)] and contact graph. Entries for structural clusters include the cluster representative structure, as well as a consensus sequence, and a list of the structures belonging to the cluster. Aside from browsing, the database can be queried by sequence (including wildcard searches or matchings with errors) or base pair patterns in MC-Search format.

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**Fig. 1.** RNA loop modeling workflow. White boxes highlight initial, intermediate and final data used in or created during the modeling process. Subroutines are given in italics.

## 4 LOOP MODELING

We adapted the protein loop modeling method of loops in proteins (Michalsky *et al.*, 2003) for RNA loops. The method finds the loop templates that agree with a given query sequence and fit best into a target site of a given RNA structure. The target site is specified by a number of nucleotide positions (anchors) that depends on the loop type [cf. Section 2 (3)]. Modeling queries are dispatched in a simple XML-like structured modeling script language – RLoOM Modeling Language (RLML, cf. RLoOM Online Help). The modeling workflow is depicted in Figure 1. The user submits an RNA 3D structure in PDB format (e.g. a crystal structure or a generated structural model) and specifies a target loop sequence and a target site (two or more anchors depending on loop type) for the loop to be fitted into. Additionally, a query sequence or structure [base pair pattern in MC-Search format (<http://major.irc.ca>)] for selecting suitable loop candidates is required. An initial candidate set is generated by matching the query sequence against the selected template library (cf. Section 2). Next, each candidate loop is inserted into the target site by computing the optimal superposition of the anchors of candidate loop and target site utilizing the algorithm by Kabsch (1978). The candidate loop is rotated according to the rotation matrix associated with the optimal superposition and translated into the target site. As a quality measure, we compute  $RMSD_a$ , the RMSD between the anchors after optimal superposition. Fitted candidate loops with  $RMSD_a > 5.0\text{\AA}$  are automatically rejected, since they likely require a substantial change in their backbone conformation to fit into the target site. The whole construct is then checked for steric clashes (defined as atomic distances of  $< 4.0\text{\AA}$ ) with any surrounding atom. The server returns the 10 best model structures (with minimum  $RMSD_a$ ) for download. These models should be subjected to an external refinement routine in order to correct geometric inconsistencies resulting from the loop fitting.

## 5 CONCLUSION

We reported RLoOM, a structure modeling web server for RNA loop modeling based on sequence homology and geometric

constraints dictated by the site of insertion via anchor nucleotides. The server uses loop templates extracted from experimentally verified structures in the PDB. A performed benchmark (Schudoma *et al.*, 2010) showed that homology modeling approach as implemented in RLoOM yielded more accurate structural predictions than an *ab initio*-based modeling server [the iFoldRNA webserver (Sharma *et al.*, 2008)] for target sequences with available sequence-similar template structures. For nine selected examples covering various loop sizes and types, our homology-based approach yielded significantly better results as judged by RMSD than obtained by using iFoldRNA [cf. Schudoma *et al.* (2010) Supplementary Table 1]. Evidently, for target loop sequences with no suitable structural example in the database, no prediction can be made. However, with the structural database steadily growing, this modeling gap is expected to shrink substantially over the coming years. RLoOM was designed to harness the wealth of structural information already available for applications in RNA modeling. RLoOM is expected to find many applications in RNA engineering and design.

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**Conflict of Interest:** none declared.

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