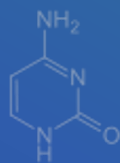


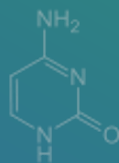
Cytosine



G

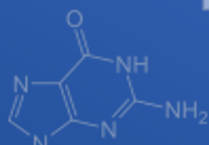
Nucleobases

Cytosine



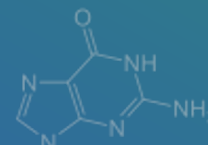
C

Guanine



G

Guanine



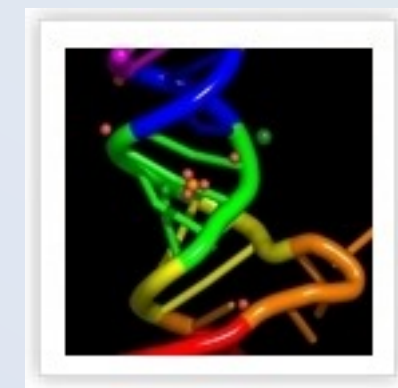
G

# RNA-Puzzles Website and Automation

Zhichao (Chichau) Miao  
Guangzhou Laboratory  
Guangzhou Medical University  
[www.rnacentre.org](http://www.rnacentre.org)

Computational Approaches to RNA  
Structure and Function, Benasque, Spain

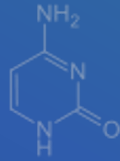
1 August 2024



# Outline

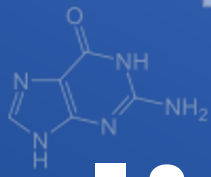
- Introduction to RNA-Puzzles
- Evaluation workflow
- Website
- Perspectives

Cytosine



G

Guanine



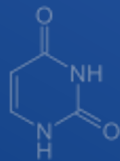
G

Adenine



A

Uracil



U

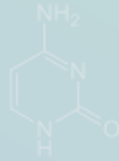
Nucleobases  
of RNA



RNA

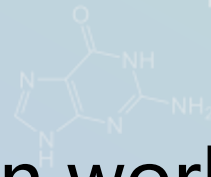
Ribonucleic acid

Cytosine



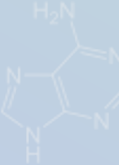
G

Guanine



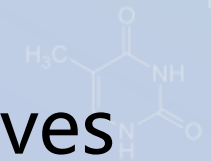
G

Adenine



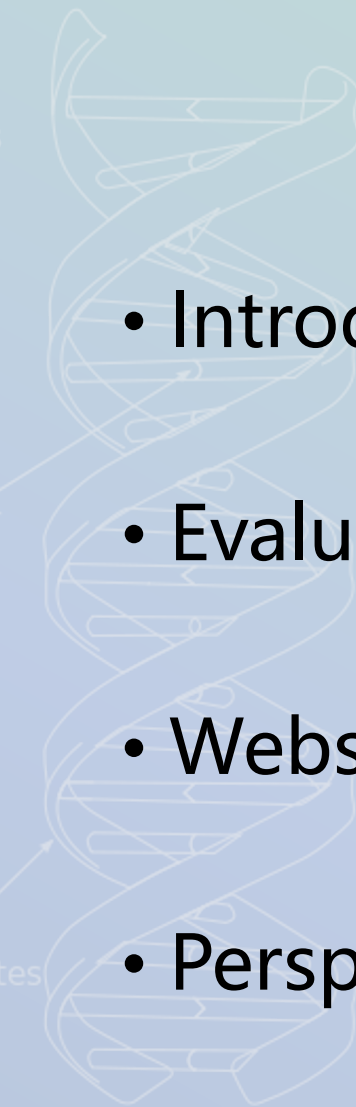
A

Thymine



T

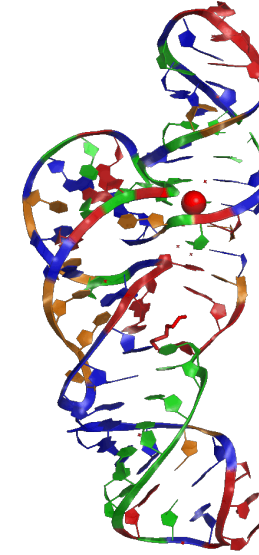
Nucleobases  
of DNA



DNA

Deoxyribonucleic acid

5'-GGGUCAGGCCGGCGAAAGUCG  
CCACAGUUUGGGGAAAGCUGU  
GCAGCCUGUAACCCCCCACGA-3'  
AAGUGGG



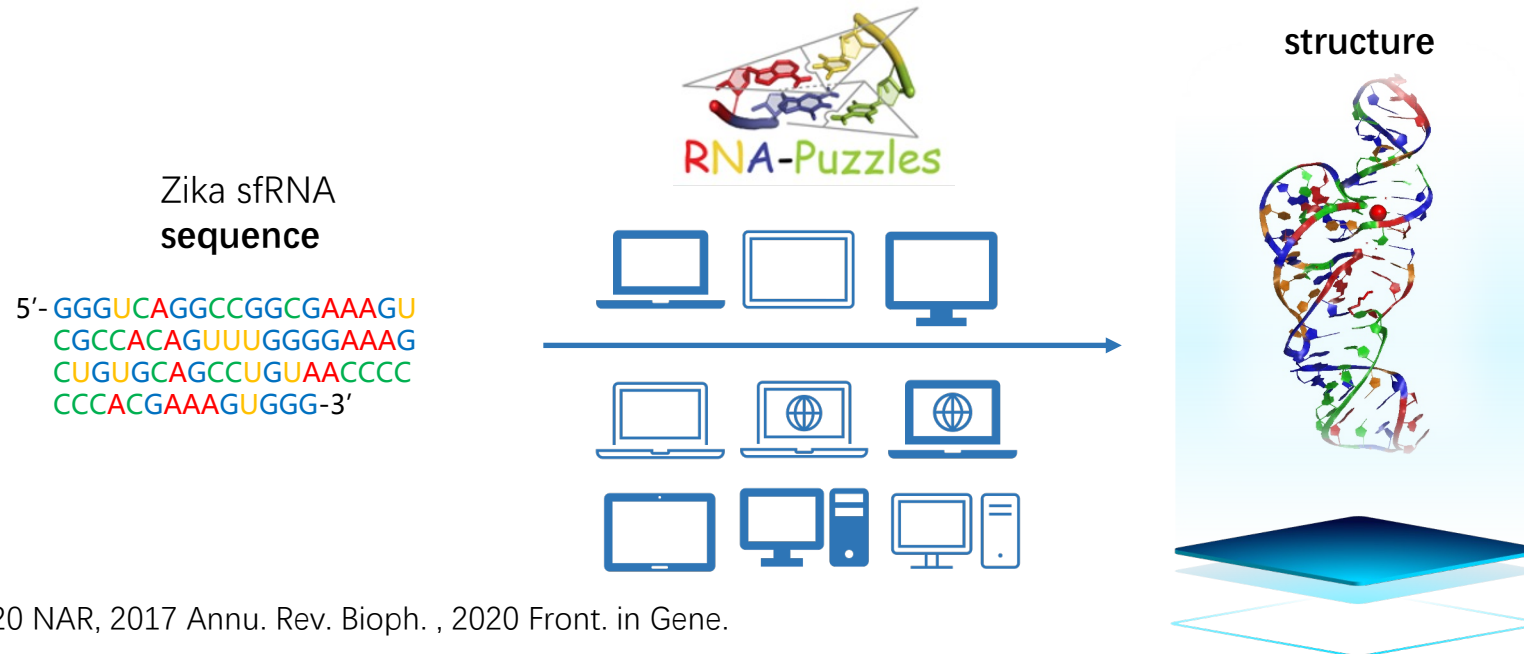
**One Sequence**  **One Unique Structure**  
(usually)

- This is true for both proteins and RNA molecules
- Similar structures may have low sequence identity!

# RNA-Puzzles



CASP	RNA-Puzzles
Protein Structure Prediction	RNA Structure Prediction
Community wide	Community wide
Since 1994	Since 2011
Journal: Proteins, IF 3.8	Journal: RNA, IF 4.3
Basically solved by AlphaFold	Break-through Expected in near future





To **determine the capabilities and limitations** of current methods of 3D RNA structure prediction based on sequence,



To find whether and how **progress** has been made,



To identify whether there are specific **bottlenecks** that hold back the field,



To promote the available methods and guide potential users in the choice of suitable tools for **real-world problems**,



To encourage the RNA structure prediction community in their efforts to improve the current tools and to make **automated prediction tools** available,



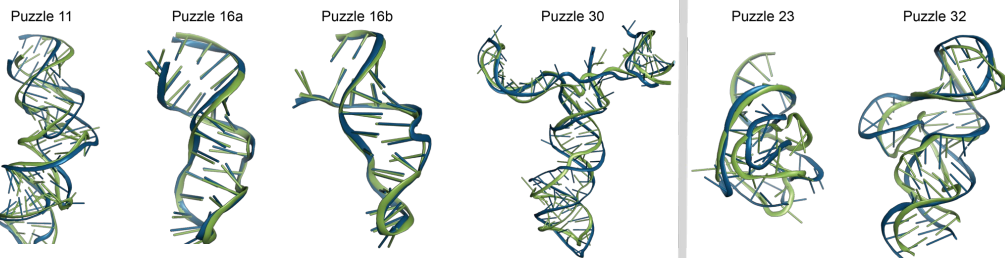
To explore the underlying **mechanism** of ligand-RNA binding and the conformational changes in riboswitches.

- Structure recruitment
- Preparation (3-7 days)
- Prediction
  - Automated web server: **48 hours**.
  - Human experts: **3-4 weeks**.
- Post-experiment prediction
  - 1D and 2D mutate-and-map data
- Results Submissions
  - **5 models** max for each prediction method
- Assessment
- Summary, Release

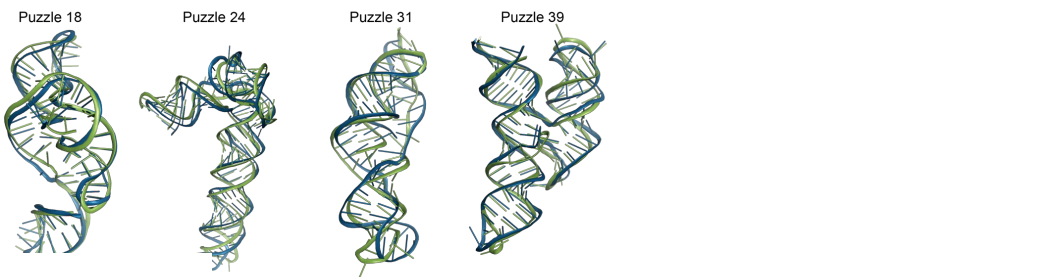
**We call for experimentalists to contribute structures!**

# Overview in Puzzle Round V: 23 Puzzles

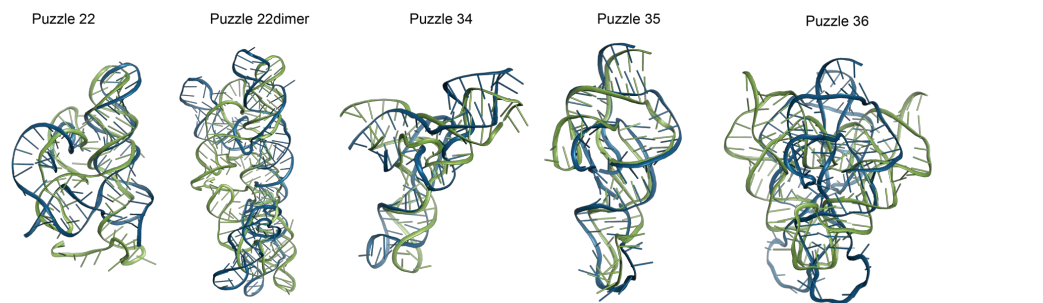
## RNA Elements



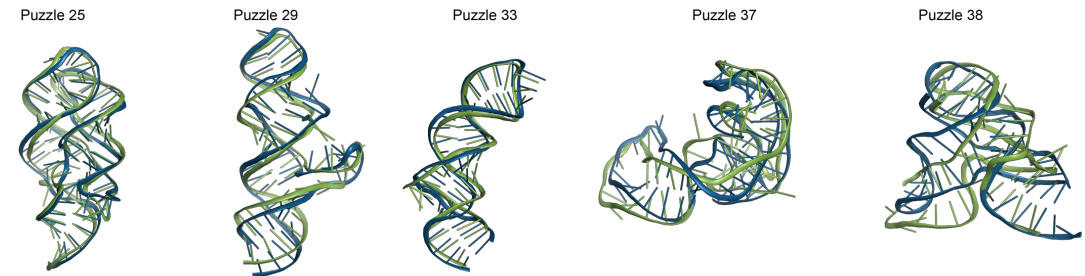
## Viral Elements



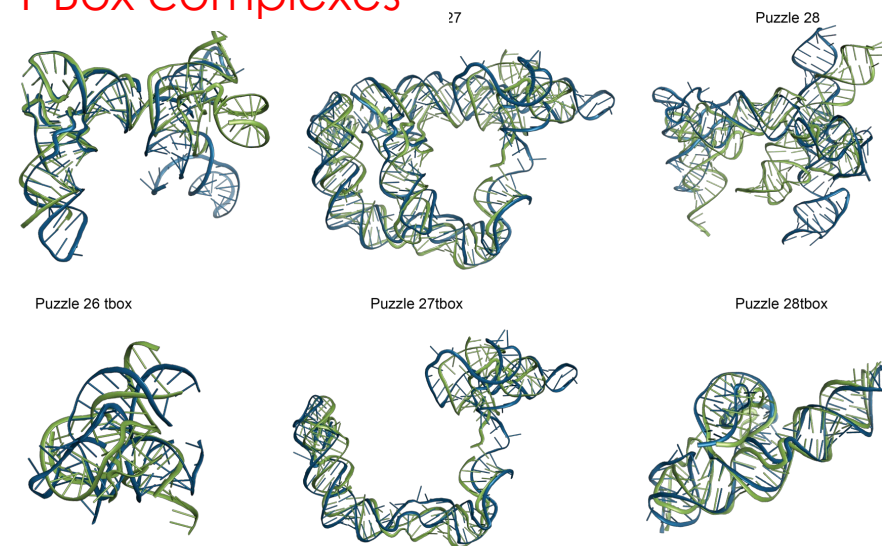
## Ribozymes



## Riboswitches



## T-Box complexes





rename

Sequence complete?  
Rename residue names  
Rename atom names  
Sort atoms  
Assign aligned region



format  
validation



evaluation

Target information  
Metric table  
Superimposition  
JSmol alignment  
Deformation profile



visualisation

PZ45\_RNAComposer\_3

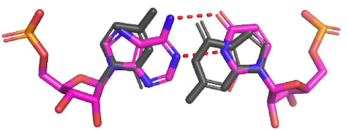
PZ#\_Method\_model#

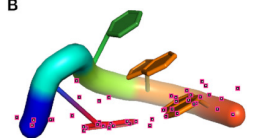
\* No space please

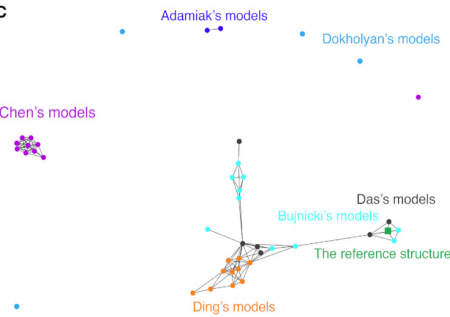
RMSD based on best Ref  
Interaction Network fidelity  
Deformation Index  
TM-score  
IDDT  
Clash score  
MCQ



# RNA-Puzzles Toolkit and rna-tools

**A**  C:G → A:U

**B**  PyMOL>clarna  
chains: A 4 7  
A 4 A 7 bp G A SH\_tran 0.72

**C**  Adamiak's models  
Dokholyan's models  
Chen's models  
Das's models  
Bujnicki's models  
The reference structure  
Ding's models

**D** Command-line interface:  

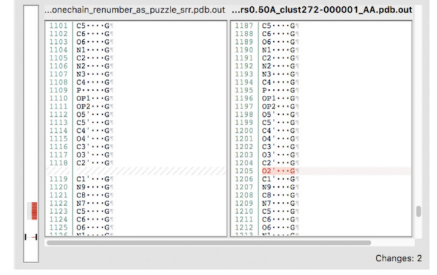
```
$ rna_pdb_toolsx.py --is-pdb input/I19V_A.pdb  
True  
$ rna_pdb_toolsx.py --is-pdb input/image.png  
False
```

  
or a Python script:  

```
>>> from rna_tools.lib import *  
>>> s = RNAStructure("input/I19V_A.pdb")  
>>> s.is_pdb()  
True
```

  
or a Jupyter Notebook:  

```
from rna_tools.tools.ma_alignment import ma_alignment  
alignment = ma_alignment.RNAAlignment(fetch="RF00167")  
alignment.plot()  
Richie: plot saved to richie.png
```

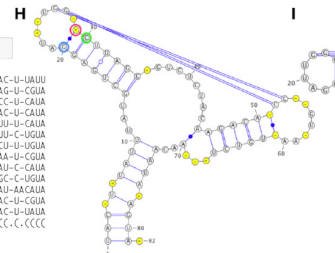
**E**  ..onechain\_renumber\_as\_puzzle\_srr.pdb.out ...rs0.60A\_clust272-000001\_AA.pdb.out

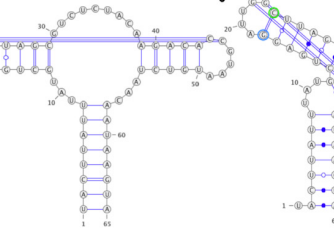
**F**

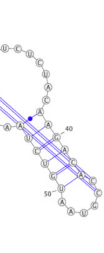
```
seq = Seq.Seq("GGGGCGGGCGCUCUACUCCUGCGCAAGCGGGGCGAGCCGUGCCGUGCAAGCCGGGACCCGCC")  
seq.predict_ss()  
(((((((.....)))))).....)))))).....)))))).....))))))  
seq.predict_ss(method="RNAsubopt")  
(((((((.....)))))).....)))))).....)))))).....)))))).....)))))) -34.40  
(((((((.....)))))).....)))))).....)))))).....)))))).....)))))) -34.40  
(((((((.....)))))).....)))))).....)))))).....)))))).....)))))) -33.90  
(((((((.....)))))).....)))))).....)))))).....)))))).....)))))) -34.90  
(((((((.....)))))).....)))))).....)))))).....)))))).....)))))) -33.90  
(((((((.....)))))).....)))))).....)))))).....)))))).....)))))) -33.90  
seq.predict_ss(method="pknot")  
(((((((.....)))))).....)))))).....)))))).....)))))).....))))))  
seq.predict_ss(method="centroid_fold")  
(((((((.....)))))).....)))))).....)))))).....)))))).....)))))) (g=1,th=0.5,ew=27.53)  
seq.predict_ss(method="context_fold")  
(((((((.....)))))).....)))))).....)))))).....)))))).....))))))
```

**G**

```
alignment.cols[1:10]  
# STOCKHOLM 1.0  
AE009948.1/1094322-1094400 AC-U-UAAU  
AL591975.1/251196-251218 AG-U-CGUA  
US1115.1/15606-15691 CC-U-CAUA  
AF270087.1/2594-2679 AC-U-CAUA  
BA000026.3/1103900-1104044 UU-U-CAUA  
CP000879.1/2164462-2164546 UU-C-UGUA  
CP001022.1/459677-459761 CU-U-UGUA  
AKY02019176.1/1629-1713 AA-U-CGUA  
ABD01000002.1/29705-29789 AU-C-CAUA  
CP000232.1/2146033-2146118 GC-C-UGUA  
AHL04000013.1/228668-228953 AU-AA-CAUA  
CP000903.1/3101736-3101822 AC-U-CGUA  
CP000721.1/2204691-2204778 AC-U-UAAU  
#=GC SS_cons  
(((((.....)))))).....)))))).....)))))).....))))))
```

**H** 

**I** 

**J** 

🏠 rna-tools

Search docs

USER DOCUMENTATION

Getting Started: I want to ...

RNA PDB Tools

RNA Tools

PyMOL4RNA

Selection

Workflows

Tips

RNA Puzzle Submission

Jupyter Notebooks & rna-tools

Emacs & rna-tools

Geekbook & rna-tools

Install

DEVELOPER DOCUMENTATION

Install & Configure

Git Quickref

Configuration

Documentation

Add a new tool to the package

Docs » Welcome to rna-tools' documentation!

## Welcome to rna-tools' document

rna-tools: a toolbox to analyze structures and simulations of

The code of the project can be found at GitHub <https://github>

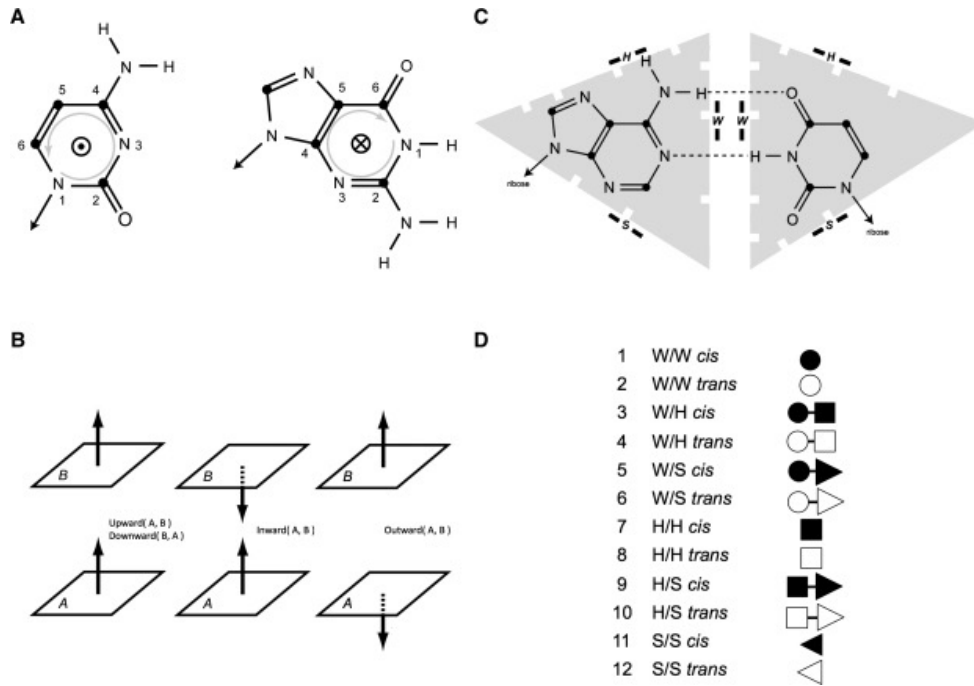
This documentation can be found at <http://rna-tools.rtfid.io>.

If something does not work for you, please make an issue, use [tools/issues](#).

## User Documentation

- Getting Started: I want to ...
  - fetch a structure from the PDB database
  - fetch a biological assembly
  - get sequences of a bunch of PDB files
  - get secondary structures of your PDB files
  - delete a part of of your structure
  - get numbering of your structure and rename chains
  - edit your structure (rename chain)
  - extract part of your structure
  - find missing atoms in my structure
  - mutate residues
  - add missing atoms

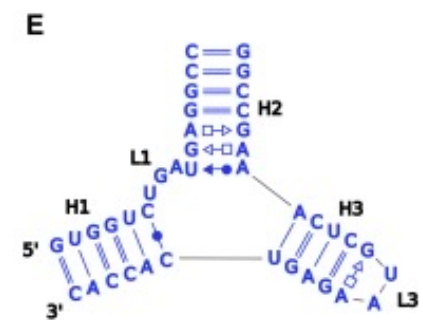
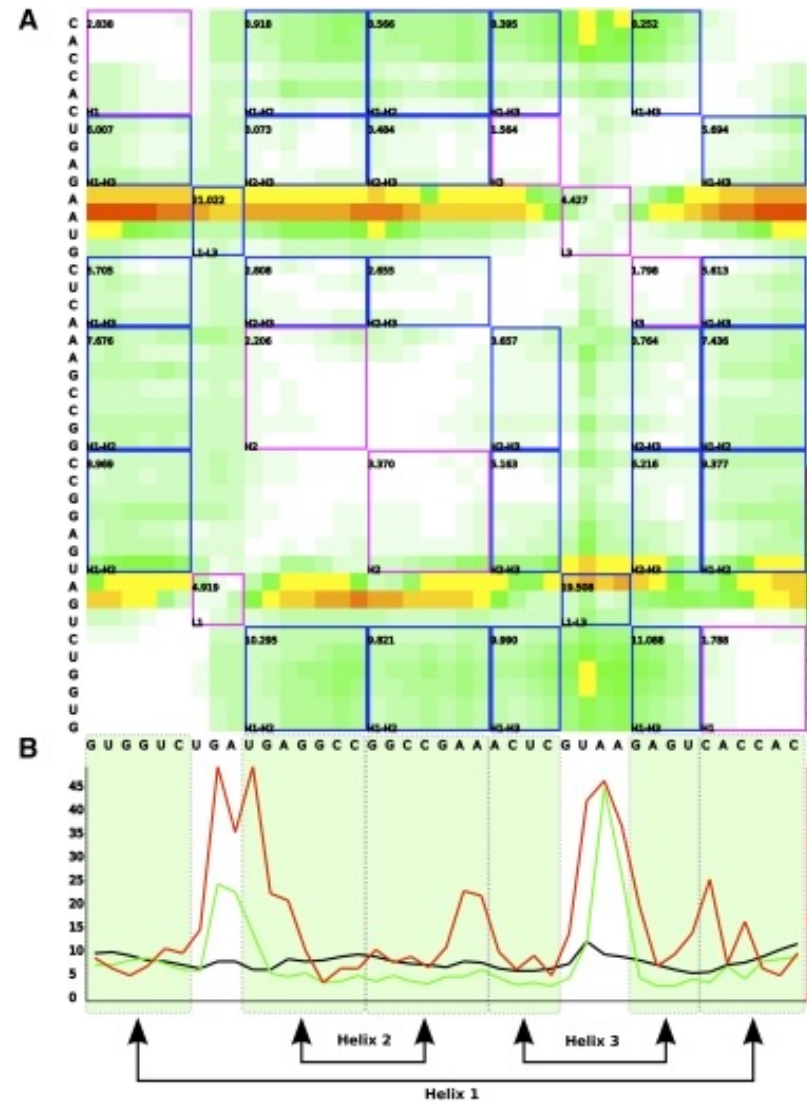
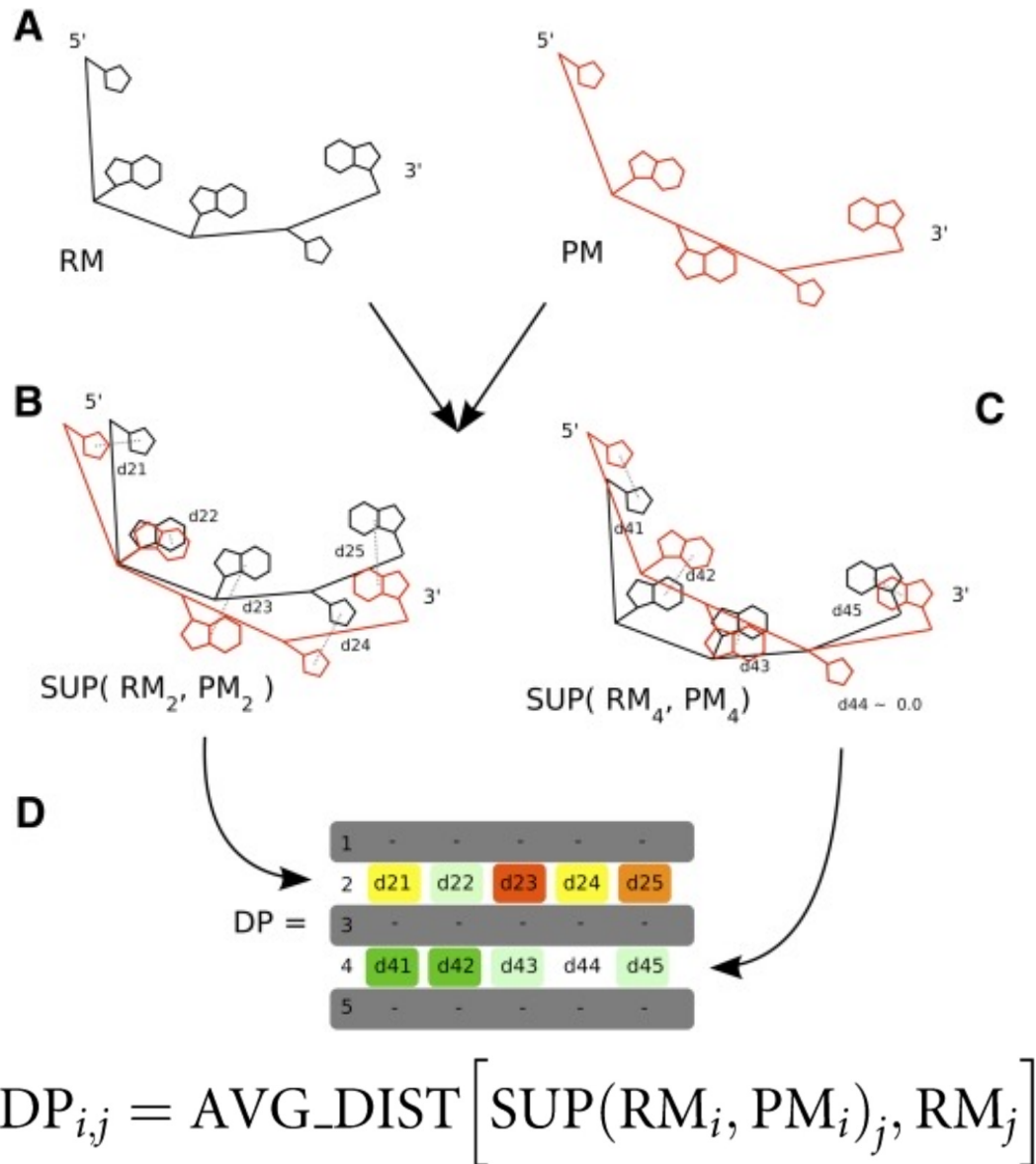
# Interaction Network Fidelity



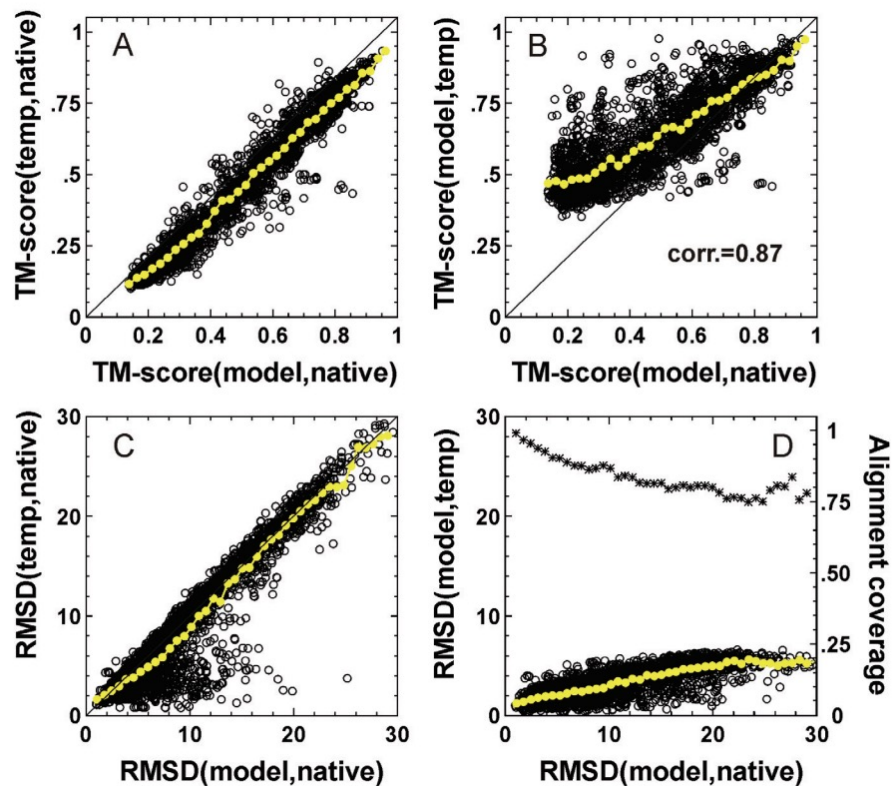
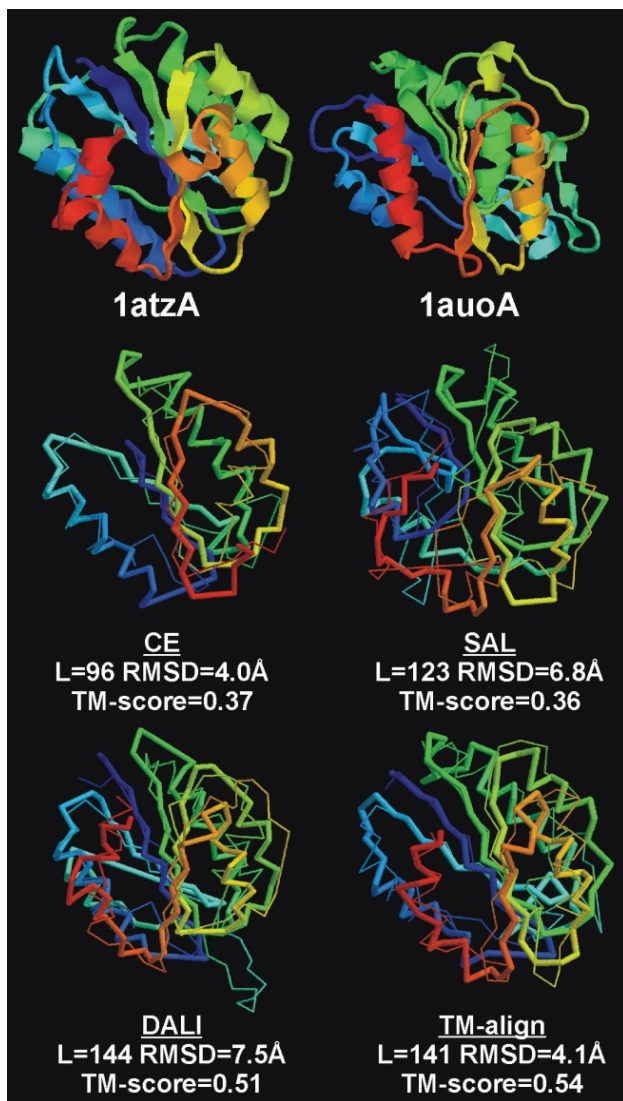
		Predicted Class			
		Positive	Negative		
Positive	Positive	True Positive (TP)	False Negative (FN) <b>Type II Error</b>	<b>Sensitivity</b> $\frac{TP}{(TP + FN)}$	
	Negative	False Positive (FP) <b>Type I Error</b>	True Negative (TN)		<b>Specificity</b> $\frac{TN}{(TN + FP)}$
		<b>Precision</b> $\frac{TP}{(TP + FP)}$	<b>Negative Predictive Value</b> $\frac{TN}{(TN + FN)}$	<b>Accuracy</b> $\frac{TP + TN}{(TP + TN + FP + FN)}$	

$$MCC = \sqrt{PPV \times STY},$$

# Deformation profile: local superimposition



# TM-score/LG-score

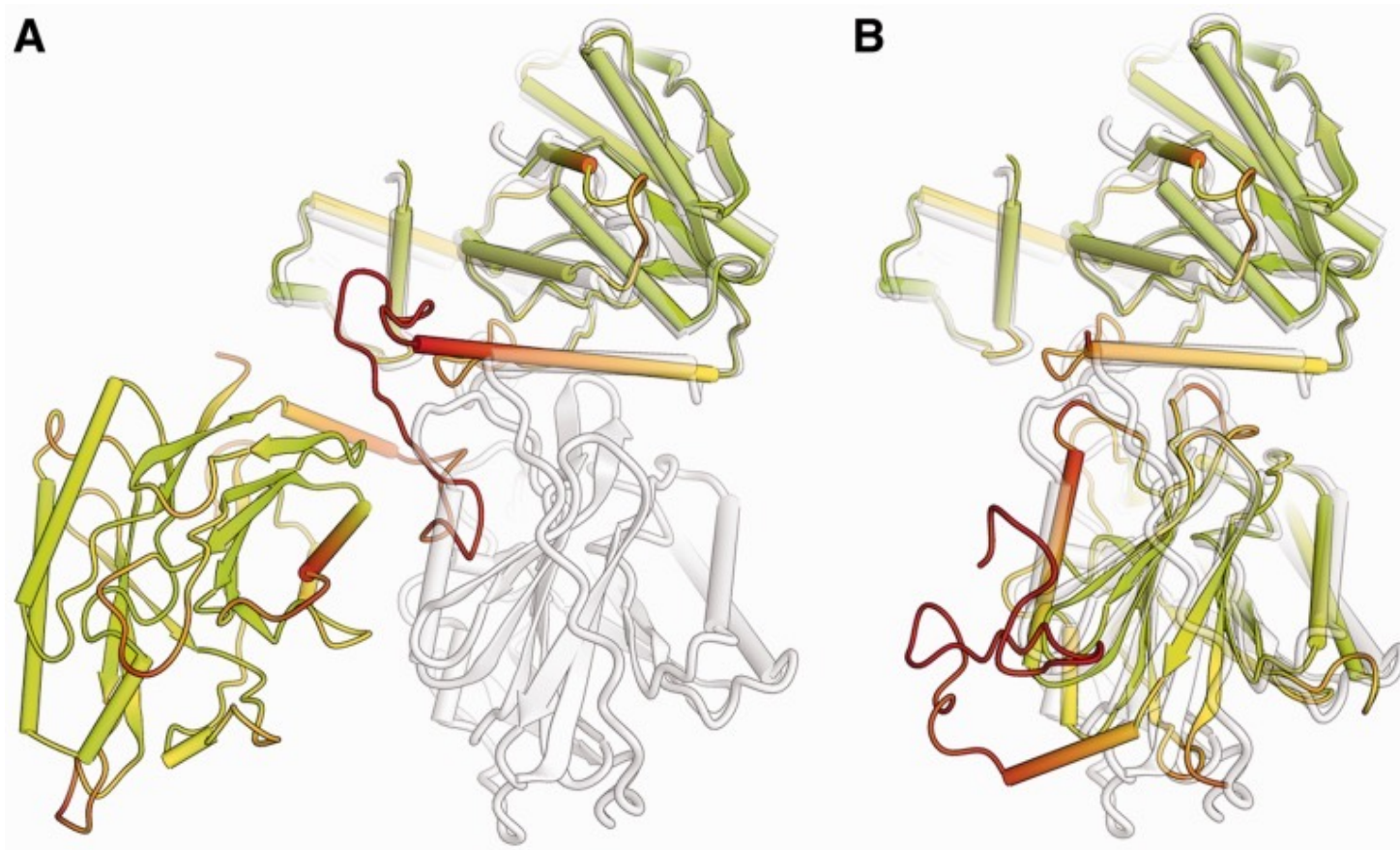


- Obtain the longest aligned fragment
- The score is normalized to [0, 1] for all length structures
- Consider sequential comparison
- Only 1 chain to compare

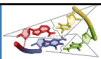
Levitt, M. and Gerstein, M. (1998) A unified statistical framework for sequence comparison and structure comparison. Proc. Natl Acad. Sci. USA, 95, 5913–5920.

Y. Zhang, J. Skolnick, TM-align: A protein structure alignment algorithm based on TM-score, Nucleic Acids Research, 33: 2302-2309 (2005)

# IDDT: local superimposition-free



RF00026: 6zym\_6\_1\_79  
TM-score: 0.515  
RMSD: 4.64 Å



## RNA-Puzzles

is a collective experiment for blind RNA structure prediction.

The sequence of a solved RNA structure is confidentially communicated to participating modelling groups a couple of weeks prior to publication. The results are assessed and presented in common publications involving structuralists and modellers.

### Aims of the project

- To determine the capabilities and limitations of current computational methods of 3D RNA structure prediction based on sequence;
- To assess progress made and to improve assessment protocols and methods;
- To identify whether there are specific bottlenecks that hold back progress;
- To promote the available methods and guide potential users in the choice of suitable tools;
- To encourage the RNA structure prediction community in their efforts to improve the current tools and to make automated prediction tools available;
- To explore the underlying mechanism of ligand-RNA binding and associated conformational changes.

### Basic Rules **(PLEASE READ CAREFULLY)**

- Confidential agreement required from new users: "I will use the provided sequence data for prediction purposes only. I will not distribute the sequences in any form outside my working group."
- It is strongly recommended to avoid assessment error that all the predictions be formatted in standard **PDB format**. We provide a python script [here](#) for generating standard PDB format files into which the coordinates need to be replaced by the predictors.
- Please name the submission file without space in the file name. The suggested names are: PZ#\_Method.pdb for web servers and PZ#\_GroupName.pdb for human predictors.
- RNA chains should be named in alphabetical order: A for the first chain, B for the second chain, and so on.
- Up to **FIVE** prediction models should be submitted as the final results for each puzzle from each prediction method. Each prediction group can submit results from several prediction methods (automatic or manual, etc.).
- Please put all prediction models together in one PDB file as in the NMR format (Separate models by lines: "MODEL n" and "ENDMDL").
- For submission, please either use the online [registration and submission](#) portal (see the open-puzzle tag: <http://www.rnapuzzles.org/open-puzzle/>) or send us an [email](#) (Please send an email to [e.westhof@ibmc-cnrs.unistra.fr](mailto:e.westhof@ibmc-cnrs.unistra.fr) and cc. [ibmc.cnrs@gmail.com](mailto:ibmc.cnrs@gmail.com)).
- For structure comparison metrics, please refer to RNA-Puzzles toolkit at <https://chichaumiau.github.io/rnapuzzlestoolkit>. Please find the reference at [Nucleic Acids Res. 2020 Jan 24;48\(2\):576-588](#). To use evaluation metrics in CASP15, please also refer to the repos: <https://github.com/DasLab/casp-rna> and [https://github.com/DasLab/CASP15\\_RNA\\_EM](https://github.com/DasLab/CASP15_RNA_EM). The reference can be found at [biorxiv](#).

## RNA-Puzzles

A collective blind evaluation of RNA three-dimensional structure prediction



### Recent Posts

[Submission Process](#)

[Research Spectrum](#)

[Recent Engagements](#)

[Participating Teams](#)

[Methodological Progress](#)

### GitHub Repos

[PuzzlesRoundV-reproducibility](#)

[RNA\\_assessment](#)

Basic RNA 3D structure comparison metrics in RNA-Puzzles, including INF, DI and RMSD. It also includes 'RNA\_normalizer' tool in `pdb_utils`.

[raw\\_dataset\\_and\\_for\\_assessment](#)

This repository includes all the submitted RNA structures in RNA-Puzzles, include the 'raw\_dataset' and the 'normalized\_dataset'

**Updated Rules**

# More complete information

RNA-Puzzles  **Results** Open Puzzles Groups Publications Resource  

ORDINAL

## Results

### Puzzle 39



**Sequence (5' to 3'):**  
GGUAAAACAGCCUGUGGGUGAAACACA CCCACAGGGCCCAUUGGGCGCUAGCAC  
UCUGGUAUCACGGUACCUUUGUGCGCC UGUUUUACC

**Puzzle:** B3 cloverleaf RRE

Crystal structure kindly provided by: [Deepak Koirala](#)

**Reference:** Crystal structure of a highly conserved enteroviral 5' cloverleaf RNA replication element. *Nat Commun* 14: 1955-1955. (2023)

**PDB id:** [8dp3](#)

**Puzzle Submission/Deadline:** 2023.01.18/02.08

[raw prediction](#) | [Assessment results](#)

### Puzzle 38



**Sequence (5' to 3'):**  
GCGGCGUUGCGUCCGAAAGUCUAAACAG ACACGGCCGCUUAAAAACAAAAGGAGA

**Puzzle:** NAD+ II riboswitch (another)

Crystal structure kindly provided by: [Lin Huang](#)

**Reference:** Crystal structures of the NAD+-II riboswitch reveal two distinct ligand-binding pockets. *Nucleic Acids Res* 51: 2904-2914 (2023)

**PDB id:** [8hb8](#)

**Puzzle Submission/Deadline:** 2012.11.30/12.21

[raw prediction](#) | [Assessment results](#)

## RNA-Puzzles

A collective blind evaluation of RNA three-dimensional structure prediction

### Recent Posts

- [Submission Process](#)
- [Research Spectrum](#)
- [Recent Engagements](#)
- [Participating Teams](#)
- [Methodological Progress](#)

### GitHub Repos

- [PuzzlesRoundV-reproducibility](#)
- [RNA\\_assessment](#)  
Basic RNA 3D structure comparison metrics in RNA-Puzzles, including INF, DI and RMSD. It also includes 'RNA\_normalizer' tool in pdb\_utils.
- [raw\\_dataset\\_and\\_for\\_assessment](#)  
This repository includes all the submitted RNA structures in RNA-Puzzles, include the 'raw\_dataset' and the 'normalized\_dataset'
- [toolkit-ansible-role](#)  
Ansible role for RNA-Puzzles Toolkit. Contains ansible-container config and an example playbook

Puzzle time

# New registration system

The screenshot shows the RNA-Puzzles website interface. The top navigation bar includes 'RNA-Puzzles', 'Results', 'Open Puzzles' (highlighted in orange), 'Groups', 'Publications', and 'Resource'. A search bar is located on the right. The main content area features a 'Log In' button and a 'Sign Up' link. Below these are input fields for 'Email' and 'Password', both with placeholder text 'Please enter email' and 'Please enter password' respectively. A 'Forgot password' link is positioned below the password field. A 'Log In' button is located at the bottom right of the form. The right sidebar contains a 'RNA-Puzzles' section with a description: 'A collective blind evaluation of RNA three-dimensional structure prediction'. It includes social media icons for GitHub, Email, Twitter, and LinkedIn. Below this are sections for 'Recent Posts' (listing Submission Process, Research Spectrum, Recent Engagements, Participating Teams, and Methodological Progress) and 'GitHub Repos' (listing PuzzlesRoundV-reproducibility and RNA\_assessment).

Automatic registration with email

Human identity confirmation  
(otherwise account deleted)

Email will be added to mail list

Sequence information available  
during puzzle open time

Sequence information sent to the  
mail list



# Group Information

RNA-Puzzles



Results

Open Puzzles

Groups

Publications

Resource

Search



ORDINAL

## Group

Prediction Group

Yang Group

Xiong Group

Perez Group

Dokholyan Group

Ding Group

Das Group

Szachniuk Group (Formerly Adamiak)

SoutheRNA Group

LCBio Group

Kollman Group

Boniecki Group

YagoubAli Group

## RNA-Puzzles

A collective blind evaluation of RNA  
three-dimensional structure prediction

RNA-Puzzles



Results

Open Puzzles

Groups

Publications

Resource

## Xiong Group

**Xiong** group is from School of Life Science, University of Science and Technology of China.  
(Click here to find more information about Xiong Group)

### Publications:

Chen K, Zhou Y, Wang S, Xiong P. RNA tertiary structure modeling with BRiQ potential in CASP15. *Proteins*. 2023 Dec;91(12):1771-1778. doi: 10.1002/prot.26574. Epub 2023 Aug 28. PMID: 37638558.

Posted by Chichau Miao ordinal group

« Perez Group

Yang Group »

### raw\_dataset\_and\_for\_assessment

This repository includes all the submitted RNA structures. The Puzzles, include the 'raw\_dataset' and the 'normalized\_dataset'

### toolkit-ansible-role

Ansible role for RNA-Puzzles Toolkit. Contains ansible-container config and an example playbook

### comparison\_metrics\_list

Prediction group information is provided

1. Link to the group/webserver
2. Related publications

## Publication

### Group highlights

#### RNA 3D Structure Comparison Using RNA-Puzzles Toolkit

Magnus, M., & Miao, Z.#

[RNA Structure Prediction. Springer US, 263-285 \(2023\).](#)

RNA-Puzzles toolkit is a computational resource derived from RNA-Puzzles, which includes (i) decoy sets generated by different RNA 3D structure prediction methods; (ii) 3D structure normalization, analysis, manipulation, and visualization tools; and (iii) 3D structure comparison metric tools.

#### Computational Pipeline for Reference-Free Comparative Analysis of RNA 3D Structures Applied to SARS-CoV-2 UTR Models.

Gumna J., Antczak M., Adamiak RW., Bujnicki JM., Chen S.-J., Ding F., Ghosh P., Li J., Mukherjee S., Nithin C., Pachulska-Wieczorek K., Ponce-Salvatierra A., Popenda M., Sarzynska J., Wirecki T., Zhang D., Zhang S., Zok T., Westhof E., Miao Z.#, Szachniuk M., & Rybarczyk A.#

[Int. J. Mol. Sci.23, 9630 \(2022\).](#)

A computational pipeline dedicated to reference-free high-throughput comparative analysis of 3D RNA structures. They show its application in the RNA-Puzzles challenge, in which five participating groups attempted to predict the three-dimensional structures of 5'- and 3'-untranslated regions (UTRs) of the SARS-CoV-2 genome.

#### RNA-Puzzles toolkit: a computational resource of RNA 3D structure benchmark datasets, structure manipulation, and evaluation tools.

Magnus, M., Antczak, M., Zok, T., Wiedemann, J., Lukasiak, P., Cao, Y., Bujnicki, J. M., Westhof, E., Szachniuk, M. & Miao, Z.#

[Nucleic Acids Res. 48, 576-588 \(2020\).](#)

A computational resource including (i) decoy sets generated by different RNA 3D structure prediction methods (raw, for-evaluation and standardized datasets), (ii) 3D structure normalization, analysis, manipulation, visualization tools (RNA\_format, RNA\_normalizer, rna-tools) and (iii) 3D structure comparison metric tools (RNAQUA, MCQ4Structures).

#### Evaluation of the stereochemical quality of predicted RNA 3D models in the RNA-Puzzles submissions.

Carrascoza, F., Antczak, M., Miao, Z.#, Westhof, E. & Szachniuk, M.#

[RNA \(2021\). doi:10.1261/rna.078685.121.](#)

The evaluation is based on standards of RNA stereochemistry that the Protein Data Bank requires from deposited experimental structures. Deviations from standard bond lengths and angles, planarity, or chirality are quantified. A reduction in the number of such deviations should help in the improvement of RNA 3D structure modeling approaches.

#### Advances in RNA 3D Structure Modeling Using Experimental Data.

Li, B., Cao, Y., Westhof, E. & Miao, Z.#

[Front. Genet. 11:574485, \(2020\).](#)

They summarize the experiments that can be integrated into RNA structure modeling as well as the computational methods based on these experimental data. We also demonstrate how computational modeling can help the experimental determination of RNA structure. We highlight the recent advances in computational modeling which can offer reliable structure models using high-throughput experimental data.

#### RNA Structure: Advances and Assessment of 3D Structure Prediction.

Miao, Z.# & Westhof, E.#

[Annu. Rev. Biophys. 46, 483-503 \(2017\).](#)

They hierarchically review the structural elements of RNA and how they contribute to RNA 3D structure. They focus our analysis on the non-WC base pairs and on RNA modules. Several computer programs have now been designed to predict RNA modules. They describe the RNA-Puzzles initiative, which is a community-wide, blind assessment of RNA 3D structure prediction programs to determine the capabilities and bottlenecks of current predictions.

## RNA-Puzzles

A collective blind evaluation of RNA three-dimensional structure prediction



### Recent Posts

Submission Process

Research Spectrum

Recent Engagements

Participating Teams

Methodological Progress

### GitHub Repos

PuzzlesRoundV-reproducibility

**RNA\_assessment**  
Basic RNA 3D structure comparison metrics in RNA-Puzzles, including INF, DI and RMSD. It also includes 'RNA\_normalizer' tool in pdb\_utils.

**raw\_dataset\_and\_for\_assessment**  
This repository includes all the submitted RNA structures in RNA-Puzzles, include the 'raw\_dataset' and the 'normalized\_dataset'

**toolkit-ansible-role**  
Ansible role for RNA-Puzzles Toolkit. Contains ansible-container config and an example playbook

**comparison\_metrics\_list**  
Assessment metrics tools to compare RNA 3D structures.

**Deformation\_Profile**  
RNA deformation profile, to account for structure comparison in 2D

@RNA-Puzzles on GitHub

### Categories

group (16)

news (7)

results (39)

table (66)

## Full List of publications

(# corresponding author)

Publications from the RNA-Puzzles community can be found on ([pubmed](#)).

### RNA 3D Structure Comparison Using RNA-Puzzles Toolkit

Magnus, M., & Miao, Z.#

[RNA Structure Prediction. Springer US, 263-285 \(2023\).](#)

### Computational Pipeline for Reference-Free Comparative Analysis of RNA 3D Structures Applied to SARS-CoV-2 UTR Models. International Journal of Molecular Sciences.

Gumna J., Antczak M., Adamiak RW., Bujnicki JM., Chen S.-J., Ding F., Ghosh P., Li J., Mukherjee S., Nithin C., Pachulska-Wieczorek K., Ponce-Salvatierra A., Popenda M., Sarzynska J., Wirecki T., Zhang D., Zhang S., Zok T., Westhof E., Miao Z.#, Szachniuk M., & Rybarczyk A.#

[Int. J. Mol. Sci.23, 9630 \(2022\).](#)

### RNA-Puzzles toolkit: a computational resource of RNA 3D structure benchmark datasets, structure manipulation, and evaluation tools.

Magnus, M., Antczak, M., Zok, T., Wiedemann, J., Lukasiak, P., Cao, Y., Bujnicki, J. M., Westhof, E., Szachniuk, M. & Miao, Z.#

[Nucleic Acids Res. 48, 576-588 \(2020\).](#)

### Evaluation of the stereochemical quality of predicted RNA 3D models in the RNA-Puzzles submissions.

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[RNA \(2021\). doi:10.1261/rna.078685.121.](#)

### Advances in RNA 3D Structure Modeling Using Experimental Data.

Li, B., Cao, Y., Westhof, E. & Miao, Z.#

[Front. Genet. 11:574485, \(2020\).](#)

### RNA Structure: Advances and Assessment of 3D Structure Prediction.

Miao, Z.# & Westhof, E.#

[Annu. Rev. Biophys. 46, 483-503 \(2017\).](#)

### RNA-Puzzles Round IV: 3D structure predictions of four ribozymes and two aptamers.

Miao, Z., Adamiak, R. W., Antczak, M., Boniecki, M. J., Bujnicki, J., Chen, S.-J., Cheng, C. Y., Cheng, Y., Chou, F.-C., Das, R., Dokholyan, N. V., Ding, F., Geniesse, C., Jiang, Y., Joshi, A., Krokhotin, A., Magnus, M., Mailhot, O., Major, F., Mann, T. H., Piątkowski, P., Pluta, R., Popenda, M., Sarzynska, J., Sun, L., Szachniuk, M., Tian, S., Wang, J., Wang, J., Watkins, A. M., Wiedemann, J., Xiao, Y., Xu, X., Yesselman, J. D., Zhang, D., Zhang, Y., Zhang, Z., Zhao, C., Zhao, P., Zhou, Y., Zok, T., Żyła, A., Ren, A., Batey, R. T., Golden, B. L., Huang, L., Lilley, D. M., Liu, Y., Patel, D. J. & Westhof, E.#

[RNA 26, 982-995 \(2020\).](#)

### RNA-Puzzles Round III: 3D RNA structure prediction of five riboswitches and one ribozyme.

Miao Z, Adamiak RW, Antczak M, Batey RT, Becka AJ, Biesiada M, Boniecki MJ, Bujnicki JM, Chen SJ, Cheng CY, Chou FC, Ferré-D'Amaré AR, Das R, Dawson WK, Ding F, Dokholyan NV, Dunin-Horkawicz S, Geniesse C, Kappel K, Kladwang W, Krokhotin A, Łach GE, Major F, Mann TH, Magnus M, Pachulska-Wieczorek K, Patel DJ, Piccirilli JA, Popenda M, Purzycka KJ, Ren A, Rice GM, Santalucia J Jr, Sarzynska J, Szachniuk M, Tandon A, Trausch JJ, Tian S, Wang J, Weeks KM, Williams B 2nd, Xiao Y, Xu X, Zhang D, Zok T, Westhof E.

[RNA. 2017 May;23\(5\):655-672.](#)

### RNA-Puzzles Round II: assessment of RNA structure prediction programs applied to three large RNA structures.

Miao Z, Adamiak RW, Blanchet MF, Boniecki M, Bujnicki JM, Chen SJ, Cheng C, Chojnowski G, Chou FC, Cordero P, Cruz JA, Ferré-D'Amaré AR, Das R, Ding F, Dokholyan NV, Dunin-Horkawicz S, Kladwang W, Krokhotin A, Lach G, Magnus M, Major F, Mann TH, Masquida B, Matelska D, Meyer M, Peselis A, Popenda M, Purzycka KJ, Serganov A, Stasiewicz J, Szachniuk M, Tandon A, Tian S, Wang J, Xiao Y, Xu X, Zhang J, Zhao P, Zok T, Westhof E.

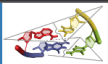
[RNA. 2015 Jun;21\(6\):1066-84.](#)

### RNA-Puzzles: a CASP-like evaluation of RNA three-dimensional structure prediction.

Cruz JA, Blanchet MF, Boniecki M, Bujnicki JM, Chen SJ, Cao S, Das R, Ding F, Dokholyan NV, Flores SC, Huang L, Lavender CA, Lisi V, Major F, Mikołajczak K, Patel DJ, Phillips A, Putoń T, Santalucia J, Sijenyi F, Hermann T, Rother K, Rother M, Serganov A, Skorupski M, Soltysinski T, Sripathidevong P, Tuszyńska I, Weeks KM, Waldsich C, Wildauer M, Leontis NB, Westhof E.

[RNA. 2012 Apr;18\(4\):610-25.](#)

RNA-Puzzles



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## Open\_puzzle

Log In

Sign Up

✉ Email:

Please enter email

🔑 Password:

Please enter password

[Forgot password](#)

Log In

<http://www.rnapuzzles.org/open-puzzle/>

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Log In

Sign Up

User:

Please enter user

Email:

Please enter email

Country:

Please enter country

Institute/company:

Please enter the name of your school or institute

Lab:

Please enter the laboratory name

Lab webpage:

Please enter Lab webpage

Method:

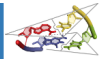
Please enter the prediction method

Password:

Please enter password

The password should be at least 8 characters, with uppercase letters, lowercase letters, special characters and at least one digit

For identification



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## Open\_puzzle

Institute/company:

Please enter the name of your school or institute

Lab:

Please enter the laboratory name

Lab webpage:

Please enter Lab webpage

Method:

Please enter the prediction method

Password:

Please enter password

The password should be at least 8 characters, with uppercase letters, lowercase letters, special characters and at least one digit.

Confirm Password:

Please confirm your password

Agree to the confidential agreement.

**I will use the provided sequence data for prediction purposes only. I will not distribute the sequences in any form outside my working group.**

Register



Agree to the confidential agreement.

**I will use the provided sequence data for prediction purposes only. I will not distribute the sequences in any form outside my working group.**

Register

Protect the structural biologists who provide solution structures for us

Hello [redacted],

Thanks for your registration with RNA-Puzzles.

Please click the link below to confirm your registration.

!!Please note: click the confirmation link means you have agreed to our term: **I will use the provided sequence data for prediction purposes only. I will not distribute the sequences in any form outside my working group.**

[https://openpuzzle.bio-it.tech/puzzle/v1/activate?  
token=\[redacted\]](https://openpuzzle.bio-it.tech/puzzle/v1/activate?token=[redacted])

Click here to activate

Regards,

RNA-Puzzles group

Hello [redacted],

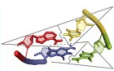
Welcome! Your registration with RNA-Puzzles is completed.

Regards,

RNA-Puzzles group

Then you will get an email from RNA-Puzzles to activate your account.

RNA-Puzzles



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## Open\_puzzle

### FASTA

Edit user

Logout

Puzzle name	Sequence	Open time	Close time
PZ42	<a href="#">PZ42.fa</a>	2023-08-09 20:00:00	2023-09-20 20:00:00
PZ41	<a href="#">PZ41.fa</a>	2023-08-09 20:00:00	2023-09-20 20:00:00

Showing 1 to 2 of 2 rows

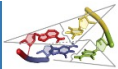
### PDB

Submit Your Prediction

[View the submission format of RNA-Puzzles.](#)

User	Lab	Puzzle name	Method	File name	Upload name	Submit date
No matching records found						

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Edit user information

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## Open\_puzzle

### FASTA

Puzzle name	Sequence	Open time	Close time
PZ42	<a href="#">PZ42.fa</a>	2023-08-09 20:00:00	2023-09-20 20:00:00
PZ41	<a href="#">PZ41.fa</a>	2023-08-09 20:00:00	2023-09-20 20:00:00

Showing 1 to 2 of 2 rows

### PDB

Submit Your Prediction

[View the submission format of RNA-Puzzles.](#)

User	Lab	Puzzle name	Method	File name	Upload name	Submit date
No matching records found						

Edit user

Logout

Edit user



User:

Bowen Xiao

Country:

China

Institute/company:

Guangzhou National Laboratory

Lab:

Miao Lab

Lab webpage:

<https://www.rnacentre.org/>

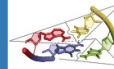
Method:

Human Prediction

Close

Submit





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## Open\_puzzle

Click to download open puzzles sequences file.  
(Only open puzzles will display at here)

### FASTA

Edit user

Logout

Puzzle name	Sequence	Open time	Close time
PZ42	<a href="#">PZ42.fa</a>	2023-08-09 20:00:00	2023-09-20 20:00:00
PZ41	<a href="#">PZ41.fa</a>	2023-08-09 20:00:00	2023-09-20 20:00:00

Showing 1 to 2 of 2 rows

### PDB

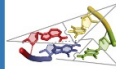
Submit Your Prediction

[View the submission format of RNA-Puzzles.](#)

User	Lab	Puzzle name	Method	File name	Upload name	Submit date
No matching records found						

# Submit your prediction

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## Open\_puzzle

FASTA

Edit user

Logout

Puzzle name	Sequence	Open time	Close time
PZ42	<a href="#">PZ42.fa</a>	2023-08-09 20:00:00	2023-09-20 20:00:00
PZ41	<a href="#">PZ41.fa</a>	2023-08-09 20:00:00	2023-09-20 20:00:00

Showing 1 to 2 of 2 rows

PDB

Submit Your Prediction

[View the submission format of RNA-Puzzles.](#)

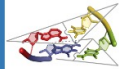
User	Lab	Puzzle name	Method	File name	Upload name	Submit date
No matching records found						

Submit your prediction at here

It will check your submission format automatically.

Your submission will list at here

RNA-Puzzles



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## Open\_puzzle

### FASTA

Edit user

Logout

Puzzle name	Sequence	Open time	Close time
PZ42	<a href="#">PZ42.fa</a>	2023-08-09 20:00:00	2023-09-20 20:00:00
PZ41	<a href="#">PZ41.fa</a>	2023-08-09 20:00:00	2023-09-20 20:00:00

Showing 1 to 2 of 2 rows

### PDB

Submit Your Prediction

[View the submission format of RNA-Puzzles.](#)

User	Lab	Puzzle name	Method	File name	Upload name	Submit date
No matching records found						

## RNA-Puzzles format

The submission format of RNA-Puzzles

[View on GitHub](#)

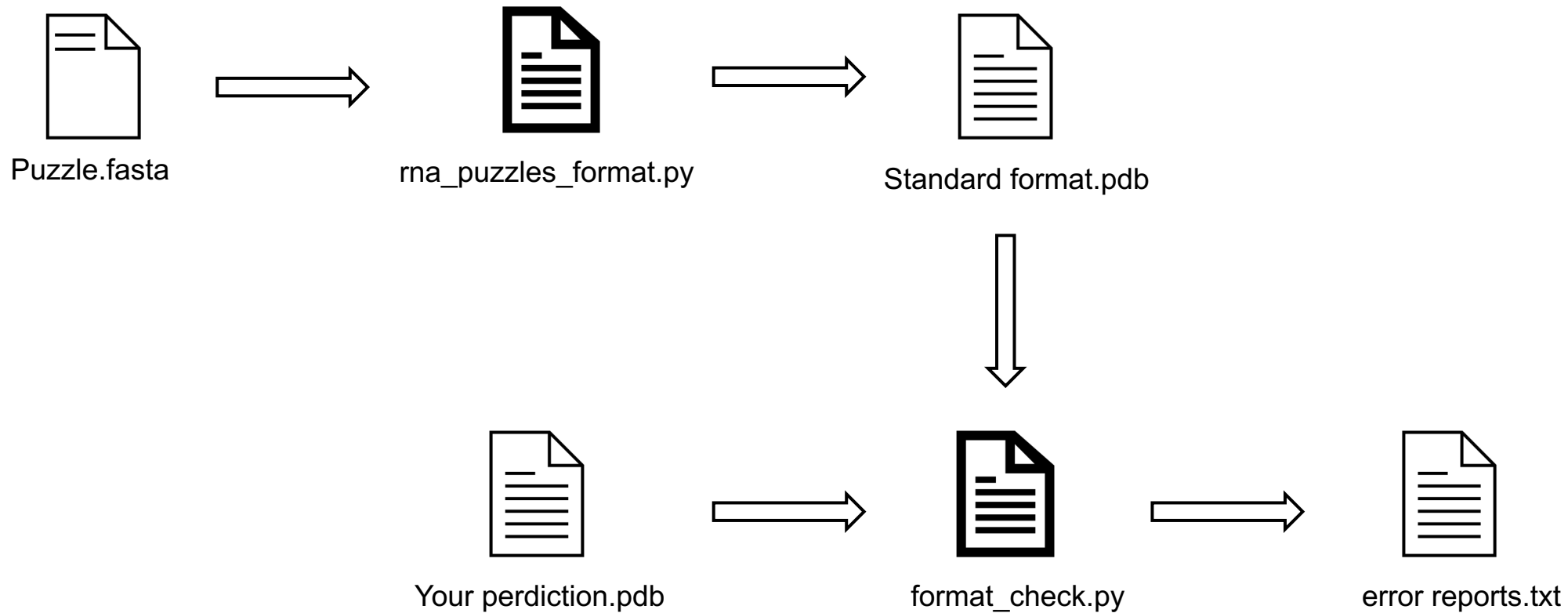
[Download .zip](#)

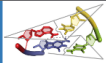
[Download .tar.gz](#)

Download the scripts

### RNA-Puzzles

RNA-Puzzles a collective and blind experiment in three-dimensional (3D) RNA structure prediction. It aims at: To determine the capabilities and limitations of current methods of 3D RNA structure prediction based on sequence; To find whether and how progress has been made, as well as what has yet to be done to achieve better solutions; To identify whether there are specific bottlenecks that hold back the field; To promote the available methods and guide potential users in the choice of suitable tools for real-world problems; To encourage the RNA structure prediction community in their efforts to improve the current tools and to make automated prediction tools available; To explore the underlying mechanism of ligand-RNA binding and the conformational changes in riboswitches.





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## Results

### Puzzle 39



**Sequence (5' to 3'):**

GGUAAAACAGCCUGUGGGUGAAAACACA CCCACAGGGCCCAUUGGGCGCUAGCAC  
UCUGGUAUCACGGUACCUUUGUGCGCC UGUUUUACC

**Puzzle:** B3 cloverleaf RRE

Crystal structure kindly provided by: [Deepak Koirala](#)

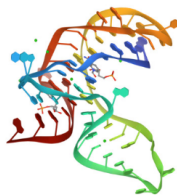
**Reference:** Crystal structure of a highly conserved enteroviral 5' cloverleaf RNA replication element. *Nat Commun* 14: 1955-1955. (2023)

**PDB id:** [8dp3](#)

[raw prediction](#) | [Assessment results](#)

Click here to get the assessment results.

### Puzzle 38



**Sequence (5' to 3'):**

GCGGCGUUGCGUCCGAAAGUCUAAACAG ACACGGCCGCUUAAAAACAAAAGGAGA

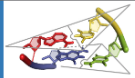
**Puzzle:** NAD+ II riboswitch (another)

Crystal structure kindly provided by: [Lin Huang](#)

**Reference:** Crystal structures of the NAD+-II riboswitch reveal two distinct ligand-binding pockets. *Nucleic Acids Res* 51: 2904-2914 (2023)

**PDB id:** [8hb8](#)

[raw prediction](#) | [Assessment results](#)



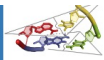
## Puzzle PZ39 Table

Please click the tags to sort data.

Lab	Num	RMSD	DI all	INF all	INF wc	INF nwc	INF stacking	Clash Score	P-value	mcq	TM-score	best sol.	Detail
GeneSilico4	1	3.383	3.656	0.925	1.000	-	0.894	0.350	0.00e+00	26.71	0.6360	1	<a href="#">-&gt;</a>
Chen	1	3.959	4.343	0.912	1.000	0.000	0.878	0.350	0.00e+00	17.34	0.5530	1	<a href="#">-&gt;</a>
GeneSilico3	1	5.447	6.093	0.894	1.000	0.000	0.851	0.350	0.00e+00	20.69	0.4340	1	<a href="#">-&gt;</a>
Szachniuk	1	5.605	6.169	0.909	0.987	0.000	0.887	7.380	0.00e+00	20.03	0.4620	1	<a href="#">-&gt;</a>
Ding	1	7.452	8.838	0.843	0.960	0.000	0.795	8.780	0.00e+00	19.36	0.4430	1	<a href="#">-&gt;</a>
DasSWM	1	8.023	8.986	0.893	0.987	0.000	0.874	1.760	0.00e+00	17.07	0.4040	1	<a href="#">-&gt;</a>
GeneSilico2	1	8.768	9.920	0.884	1.000	0.000	0.847	0.350	0.00e+00	20.09	0.4030	1	<a href="#">-&gt;</a>
Xiong	1	15.634	17.821	0.877	1.000	0.000	0.832	8.800	1.43e-11	13.76	0.2840	1	<a href="#">-&gt;</a>
Perez	1	17.120	21.588	0.793	0.973	0.000	0.735	0.350	2.79e-09	22.20	0.2660	1	<a href="#">-&gt;</a>
Dfold	1	18.073	28.267	0.639	0.466	0.000	0.738	126.890	5.81e-08	21.64	0.2840	1	<a href="#">-&gt;</a>
Yang	1	18.762	27.648	0.679	0.743	0.000	0.685	52.340	4.41e-07	35.00	0.4300	1	<a href="#">-&gt;</a>
DasEMinfo	1	19.329	23.740	0.814	0.961	0.000	0.753	8.080	2.10e-06	21.33	0.2930	1	<a href="#">-&gt;</a>
Dokholyan	1	19.975	23.444	0.852	0.960	0.000	0.816	101.090	1.11e-05	21.15	0.2960	1	<a href="#">-&gt;</a>
SimRNA	1	20.392	23.897	0.853	0.973	0.000	0.816	87.400	3.03e-05	23.32	0.3970	1	<a href="#">-&gt;</a>
GeneSilico1	1	20.433	22.902	0.892	0.987	0.000	0.856	0.000	3.33e-05	21.27	0.3930	1	<a href="#">-&gt;</a>

Click here to get the assessment result detail for each submission.

RNA-Puzzles



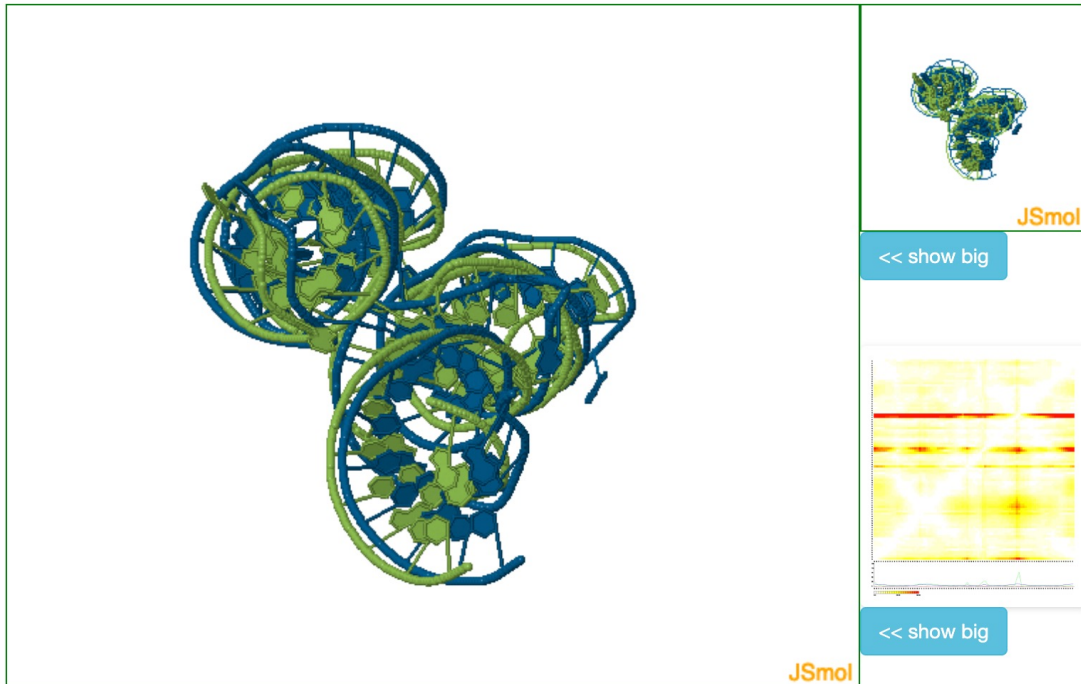
Results Open Puzzles Groups Publications Resource

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## Show

Puzzle **PZ39** Model 1 from **GeneSilico4** lab

Model Coordinates



GREEN: X-Ray structure; BLUE: Proposed model

RNA-Puzzles



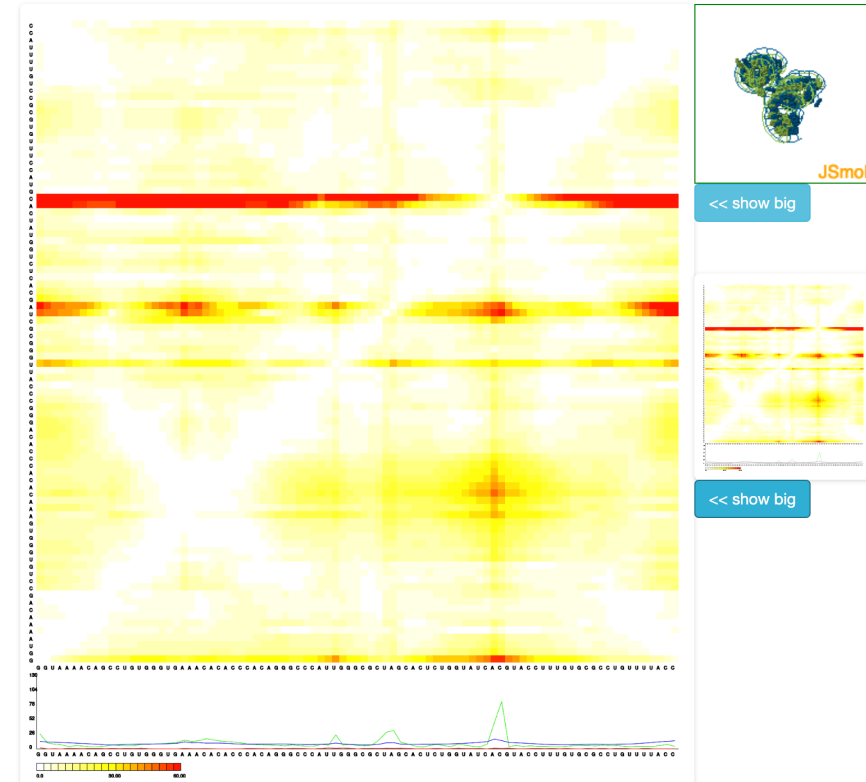
Results Open Puzzles Groups Publications Resource

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## Show

Puzzle **PZ39** Model 1 from **GeneSilico4** lab

Deformation Profile Matrix



Click figure to zoom in.



- It is important to model using both human- and web-based approaches for each Puzzle.
- We are eager to implement new metrics (run automatically within the framework of RNA Puzzles pipeline).



Eric Westhof

## Crystallographers:

- Adrian Ferre-D'Amare, Jinwei Zhang (NIH)
- Dinshaw Patel(MSKCC, New-York)
- Thomas Hermann (UCSD)
- David M J Lilley (Dundee)
- Jeffrey S. Kieft (UC denver)
- Barbara L. Golden (Purdue)
- Robert T. Batey (ucolorado boulder)
- Joseph A Piccirilli (uchicago)
- Alexander Serganov (NYU, New-York)
- Anne-Catherine Dock-Bregeon (ENSP, Paris)
- Benoît Masquida (CNRS, Strasbourg)
- Amy Ren (Zhejiang University)
- Lin Huang
- Yijing Liu
- Kelly Nguyen

## Modelling:

- Ryszard W. Adamiak
- Janusz M. Bujnicki
- Shi-Jie Chen
- Rhiju Das
- Feng Ding
- Nikolay V. Dokholyan
- Jooyoung Lee
- Francois Major
- Kevin Weeks
- Yi Xiao
- Karissa Y. Sanbonmatsu
- Alberto Perez
- Markus Kollmann
- Peng Xiong
- Yaoqi Zhou

## Fundings

### R&D Programs of Guangzhou Laboratory

- GZNL2024A01002
- GZNL2023A01006
- SRPG22-003
- SRPG22-006
- SRPG22-007
- HWYQ23-003

### Natural Science Foundation of China

- 32270707

### National Key R&D Programs of China

- 2021YFF1200903
- 2023YFF1204701



Welcome new prediction groups!



## Openings:

### Computational Biology

- 2025 PhD students (3)
- Co-PI (2)
- Postdocs (3)
- RA (3)
- Interns

### Biochemistry

- Postdocs (2)

Please email:

[miao\\_zhichao@gzlab.ac.cn](mailto:miao_zhichao@gzlab.ac.cn)



Thanks!