How to recognize native-like structure in a set of 3D RNA predictions

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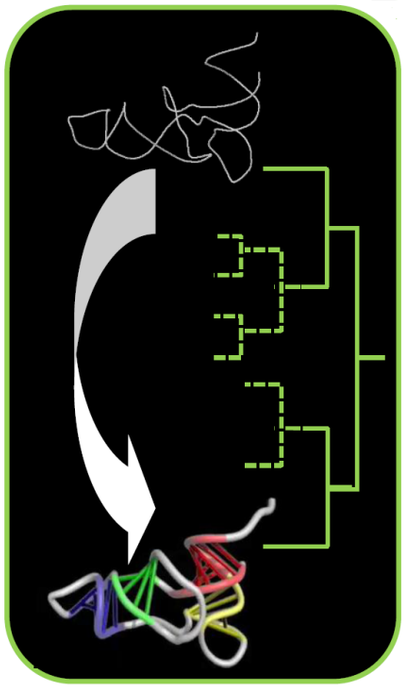
3D RNA structure prediction methods

ModeRNA

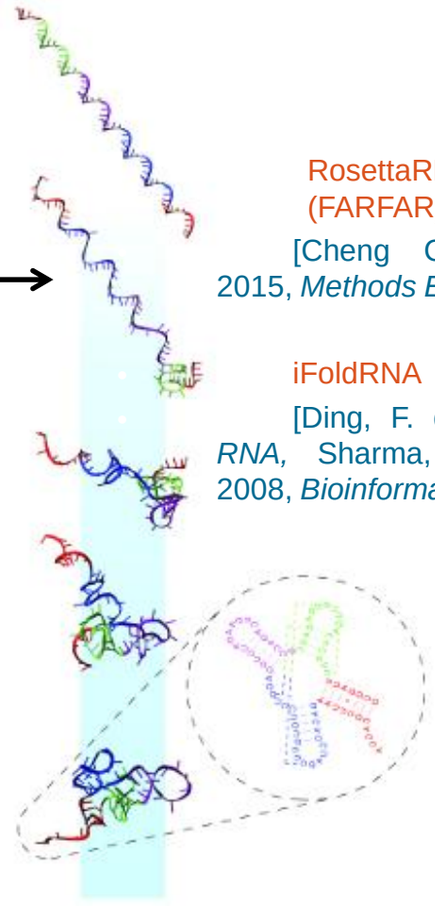
[Rother, K. et al., 2011, *Nucleic Acids Res., Bioinformatics*]



1. Homologous
2. *De novo* (*ab initio*)
3. Coarse-grained
4. Fragment-assembly
5. Machine learning

RosettaRNA (FARFAR)

[Cheng C.Y. et al., 2015, *Methods Enzymol.*]

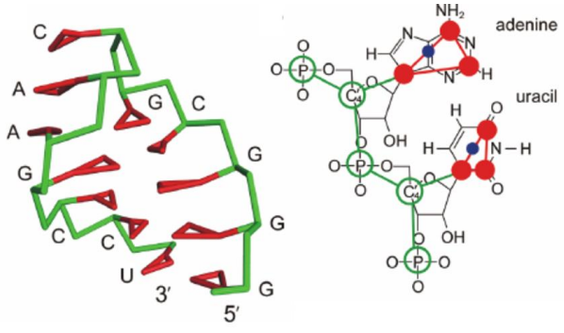


iFoldRNA

[Ding, F. et al., 2008, *RNA,* Sharma, S. et al., 2008, *Bioinformatics*]

SimRNA

[Boniecki, M.J. et al., 2016, *Nucleic Acids Res.*]

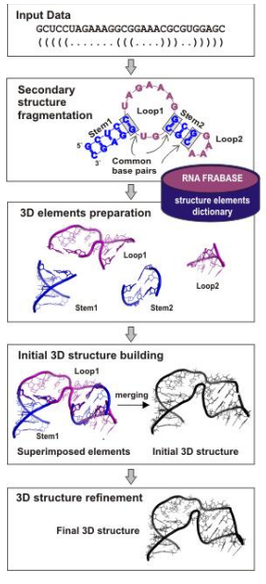


RNAComposer

[Popenda, M. et al., 2012, *Nucleic Acids Res.*]

Vfold

[Xu, X. et al., 2014, *PLoS One.*]



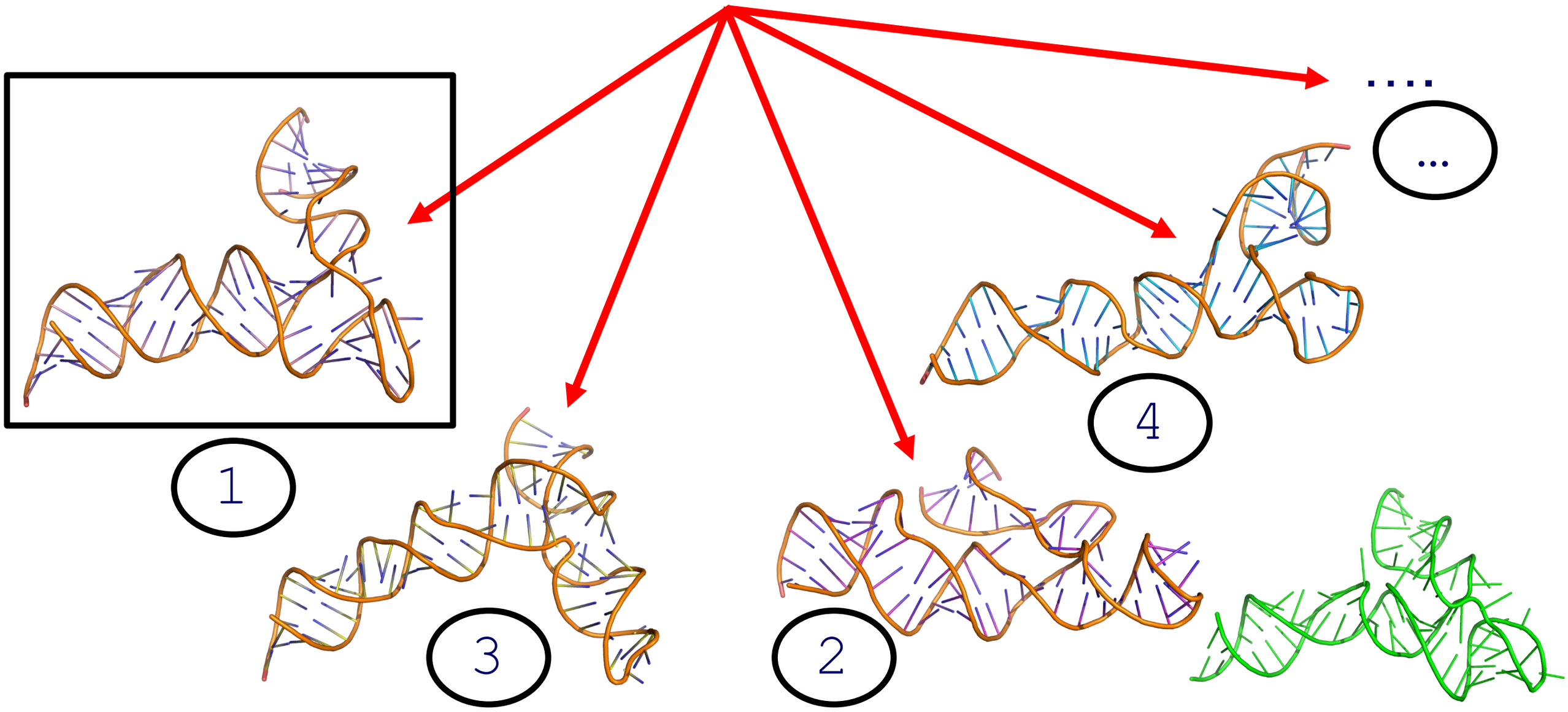
AlphaFold3 [Abramson, J. et al., 2024, *Nature*], Boltz-1 [Wohlwend, J. et al., 2024, *bioRxiv*], NuFold [Kagaya, Y. et al., 2025, *Nature Communications*], RhoFold+ [Shen, T. et al., 2024, *Nature Methods*], RoseTTAFoldNA [Baek, M. et al., 2024, *Nature*].

What is the problem?

Sequence (5′ to 3′):

CUCUGGAGAGAACCGUUUAAUCGGUCGCCGAAGGAGCAAGCU

CUGCGCAUAUGCAGAGUGAAACUCUCAGGCAAAAGGACAGAG



Why is it important?

- No single, reference-free approach to reliably assess the quality of 3D RNA structures is known so far (e.g., RASP, ARES, etc.).
- Experimenters often fail to select the single most promising model from the many available tertiary predictions.
- Even the authors of computational methods for predicting 3D RNA structures often cannot clearly identify the best prediction produced by their tool.
- The potential to efficiently filter unreliable 3D conformations is also crucial during global/local optimization in computational modeling.

What is the promising solution?

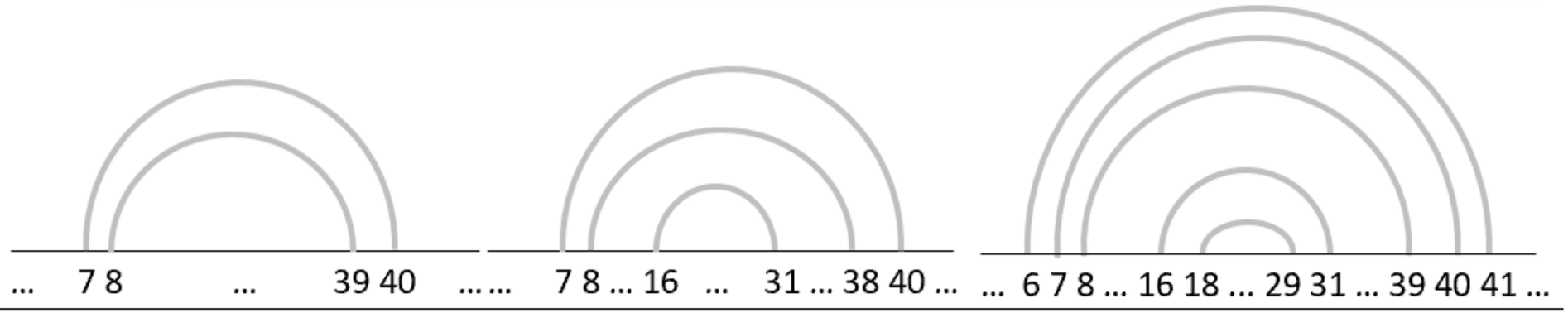
- Consensus-based methods assume that the structural region, which is conservative across most 3D models predicted by various approaches, can be classified as potentially appropriate.

- Inspiration comes from the evaluation results of the CASP9 model-quality predictions round:
  - "*all top-performing methods use consensus approaches to generate quality estimates*" [1].

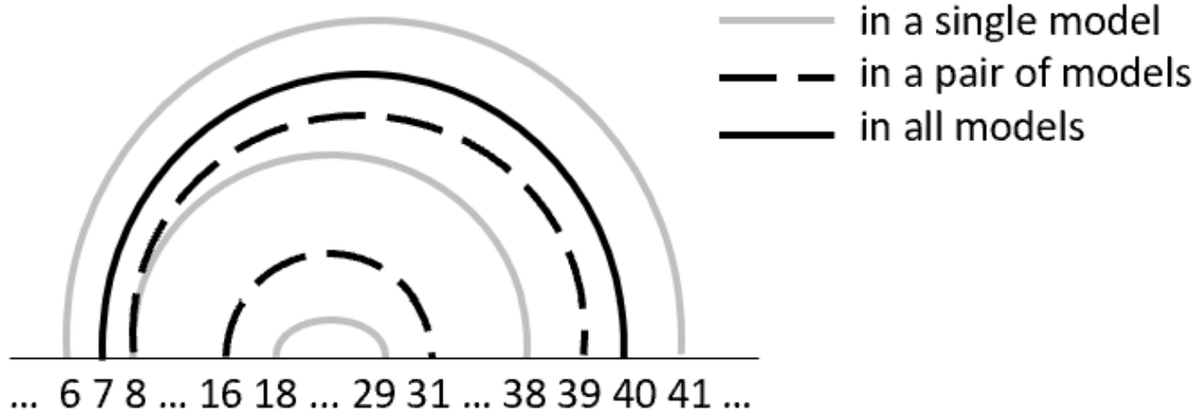
[1] Kryshtafovych, A., Fidelis, K., & Tramontano, A. (2011). Evaluation of model quality predictions in CASP9. *Proteins: Structure, Function, and Bioinformatics*, 79(S10), 91-106.

Our solution

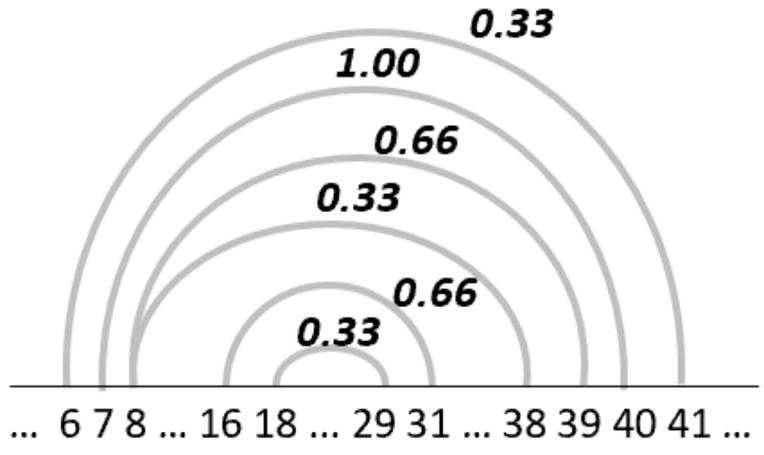
Step 1: Identify base pairs



Step 2: Construct a multiset of base pairs found in all models

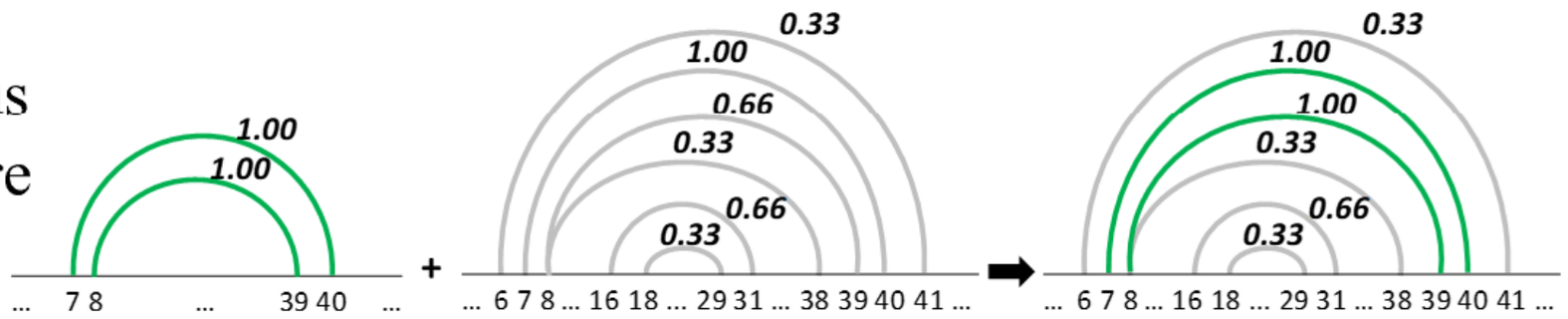


Step 3: Compute base pair confidence coefficient (*bpcc*) for each base pair

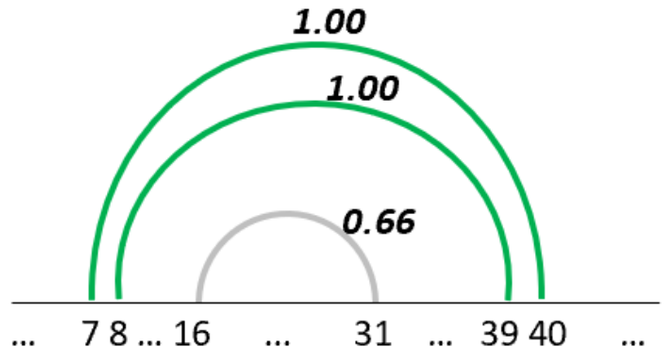


Our solution (2)

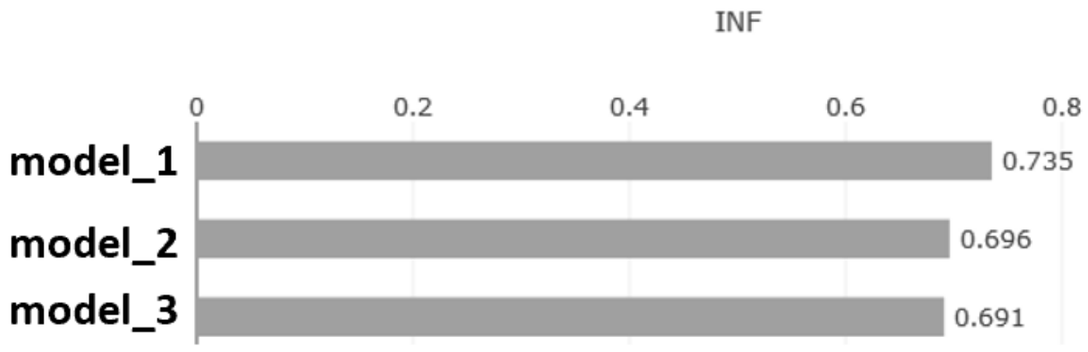
Step 4 (optional): Preserve consensus secondary structure from *Rfam*



Step 5: Construct reference secondary structure (base pairs with *bpcc* > *threshold*)



Step 6: Create INF-based ranking of 3D models compared to the reference structure



Extensions

- *Consensus build based on all interactions (instead of only high-confidence ones above the threshold).*
  - We provide *Conditionally weighted consensus* where 3D models are ranked based on how often each interaction appears across the input set.
- *Elimination of 3D models of low quality*.
  - Individual 3D models undergo *MolProbity* evaluation [1] and receive ratings of "good", "caution", or "warning" across four key metrics: clashscore, backbone conformation, bonds, and angles.
  - "Strict filter" accepts 3D models that received a score of "good" in clashscore, bonds, and angles criteria.
  - "Clashscore filter" accepts 3D models that received a "good" score in clashscore only.

[1] *MolProbity* validation cut-offs: http://molprobity.biochem.duke.edu/help/validation_options/summary_table_guide.html.

Base pairs annotation

- There are several SOTA tools to extract nucleotide interactions from given 3D coordinates.
- They differ in the rules used to identify base pairing and the input/output formats provided.

- *RNAtive* integrates and provides users with the following tools:
  - *Barnaba* (https://github.com/srnas/barnaba),
  - *BPNet* (https://github.com/computational-biology/bpnet),
  - *FR3D* (https://github.com/BGSU-RNA/fr3d-python/tree/latest),
  - *MC-Annotate* (https://github.com/major-lab/MC-Annotate),
  - *RNApolis Annotator* (https://github.com/tzok/rnapolis-py),
  - *RNAView* (https://github.com/rcsb/RNAView).

How to compute *INF* [1] and *F1* scores?

- S(R) – a set of bp interactions in the reference structure.
- S(M) – a set of bp interactions in the model.

- TP (True-Positive) – base pairs correctly predicted: TP = S(R) ∩ S(M).
- FP (False-Positive) – base pairs predicted incorrectly: FP = S(M) \ S(R).
- FN (False-Negative) – unpredicted base pairs: FN = S(R) \ S(M).

- $PPV = \frac{|TP|}{|TP|+|FP|}$ (precision), $TPR = \frac{|TP|}{|TP|+|FN|}$ (recall)
- $INF = \sqrt{PPV * TPR}$, $F1 = 2 * \frac{PPV * TPR}{PPV+TPR}$.

[1] Parisien, M., Cruz, J. A., Westhof, E., & Major, F. (2009). New metrics for comparing and assessing discrepancies between RNA 3D structures and models. *RNA*, 15(10), 1875-1885.

3D RNA structure quality assessment methods

Method	Reference	Description
Knowledge-based approaches		
RASP	Capriotti *et al.* (2011)	An all-atom statistical potential to rank models.
3dRNAscore	Wang *et al.* (2015)	Distance- and dihedral-dependent energies to evaluate models.
DFIRE	Zhang *et al.* (2020)	All-atom distance-dependent energy function based on a reference state (distance-scaled finite ideal-gas reference state).
BRiQ	Xiong *et al.* (2021)	Statistical energy function with a nucleobase-centric sampling algorithm, capturing full-orientation dependence of base-base, oxygen-base, and oxygen--oxygen interactions with the RNA backbone. Quantum-mechanical calculations ensure proper weights to minimize the possible effects of indirect interaction.
rsRNASP	Tan *et al.* (2022)	All-atom distance-dependent statistical potential based on residue separation.
cgRNASP, cgRNASP-C, cgRNASP-CN, cgRNASP-PC	Song *et al.* (2022); Tan *et al.* (2023)	Direct successor of rsRNASP, uses both long- and short-range interactions by residue separation.
Machine learning-based approaches		
RNA3DCNN_MD, RNA3DCNN_MDMC	Li *et al.* (2018)	Uses a Convolutional Neural Network to analyze every nucleotide individually with a 3D grid representation without manual structure extraction.
ARES	Townshend *et al.* (2021)	A deep neural network with an architecture formulated for direct 3D structure input that predicts the model's RMSD using the spatial position of atoms and their chemical element types.
PAMNet	Zhang *et al.* (2023)	The Physics-Aware Multiplex Graph Neural Network that induces a physics-informed bias that allows for modeling of local and non-local interactions of molecules based on different geometric information.
lociPARSE	Tarafder and Bhattacharya (2024)	A Locality-aware invariant point attention architecture that estimates the lDDT scores to assess the accuracy of each nucleotide and the atomic environment that surrounds it in a superposition-free manner.

Datasets and measures

- In the benchmark, we used two RNA 3D structure datasets:
  - *CASP15* [1] - diverse predictions submitted by independent groups modeling structures from a given sequence - 12 targets (1,672 models) – provided measures: ***RMSD***, ***lDDT***, ***TM-score***, *GDT-TS*,
  - *randstr decoy set* [2] - uniform decoys generated computationally by perturbing native structures - 85 targets (42,585 models) - provided measures: *RMSD-C3'*, *GDT-C3'*, *RMSD-ALL*, and *GDT-ALL*.

- We calculated INF_{WC}, $\boldsymbol{INF_{NWC}}$, and INF_{ALL} scores using *FR3D* [3] for base pair annotation.
  - We removed structural quality metrics whose rankings were highly correlated (*Spearman's ρ correlation*, *Fisher's z-transformation*, hierarchical clustering using the *Unweighted Pair Group Method with Arithmetic mean*, *dissimilarity threshold of 0.25*).

[1] Kryshtafovych, A., Antczak, M., Szachniuk, M., Zok, T., Kretsch, R. C., Rangan, R., ... & Moult, J. (2023). New prediction categories in CASP15. *Proteins: Structure, Function, and Bioinformatics*, 91(12), 1550-1557.
[2] Capriotti, E., Norambuena, T., Marti-Renom, M. A., & Melo, F. (2011). All-atom knowledge-based potential for RNA structure prediction and assessment. *Bioinformatics*, 27(8), 1086-1093.
[3] Sarver, M., Zirbel, C. L., Stombaugh, J., Mokdad, A., & Leontis, N. B. (2008). FR3D: finding local and composite recurrent structural motifs in RNA 3D structures. *Journal of mathematical biology*, 56(1), 215-252.

Ranking similarity measures

- To quantify the concordance between tool- and reference- based rankings, we selected four distinct ranking similarity measures:
  - *Spearman's* ρ, a standard measure of rank correlation,
  - *Kendall's* τ, which counts the number of concordant and discordant pairs between two rankings,
  - *Enrichment Score* (ES) [1], a top-k overlap metric commonly used in structural bioinformatics,
  - *Rank-Biased Overlap* (RBO) [2], which compares entire rankings while giving greater weight to agreement on top-ranked items.
- To synthesize them into a single robust score, we applied *Principal Component Analysis* (PCA) and extracted *the first principal component* (PC1).

[1] Tsai, J., Bonneau, R., Morozov, A. V., Kuhlman, B., Rohl, C. A., & Baker, D. (2003). An improved protein decoy set for testing energy functions for protein structure prediction. *Proteins: Structure, Function, and Bioinformatics: Structure, Function, and Bioinformatics*, 53(1), 76-87.
[2] Webber, W., Moffat, A., & Zobel, J. (2010). A similarity measure for indefinite rankings. *ACM Transactions on Information Systems* (TOIS), 28(4), 1-38.

Results (INF_{NWC} ranking performance)

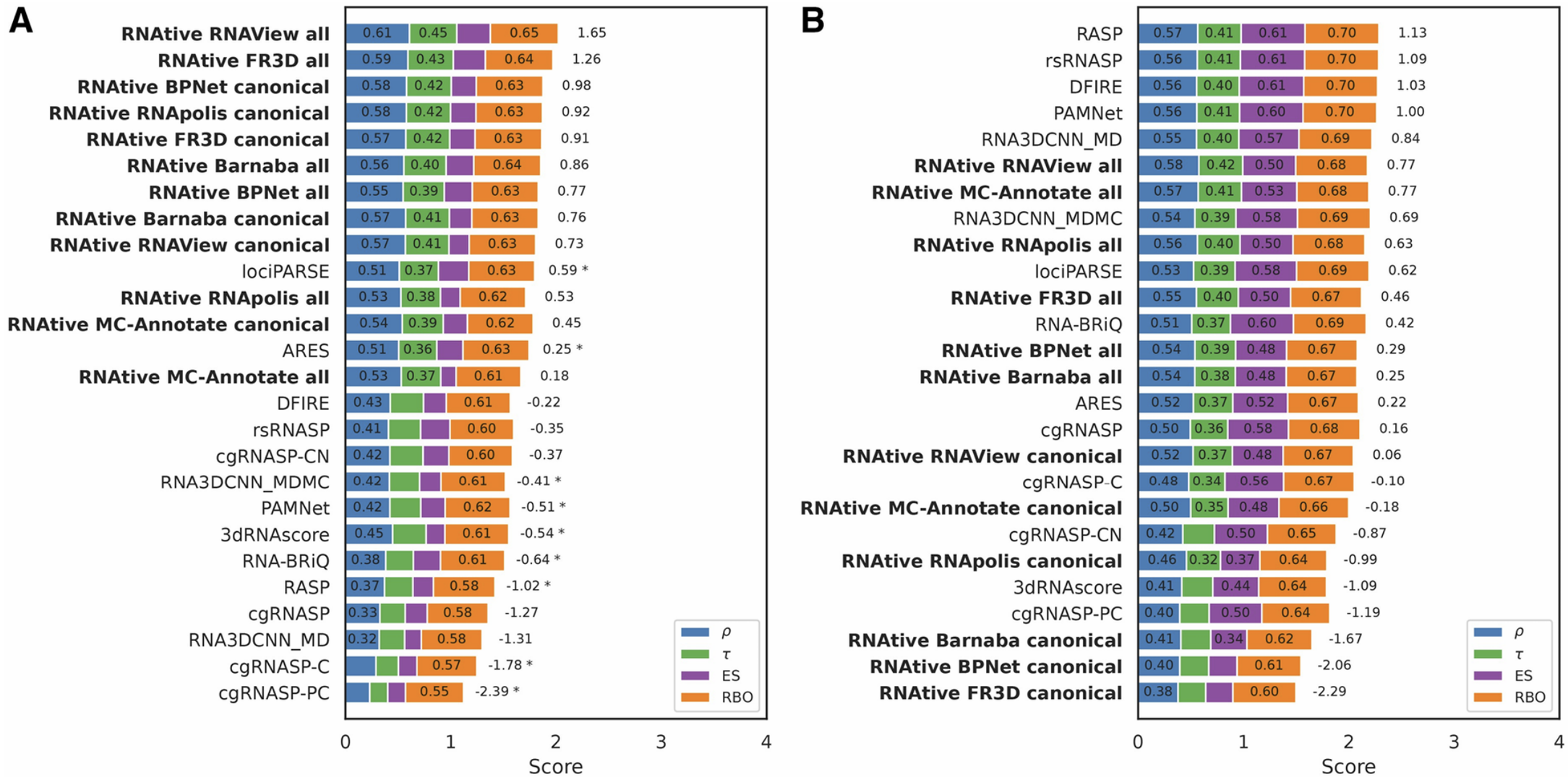
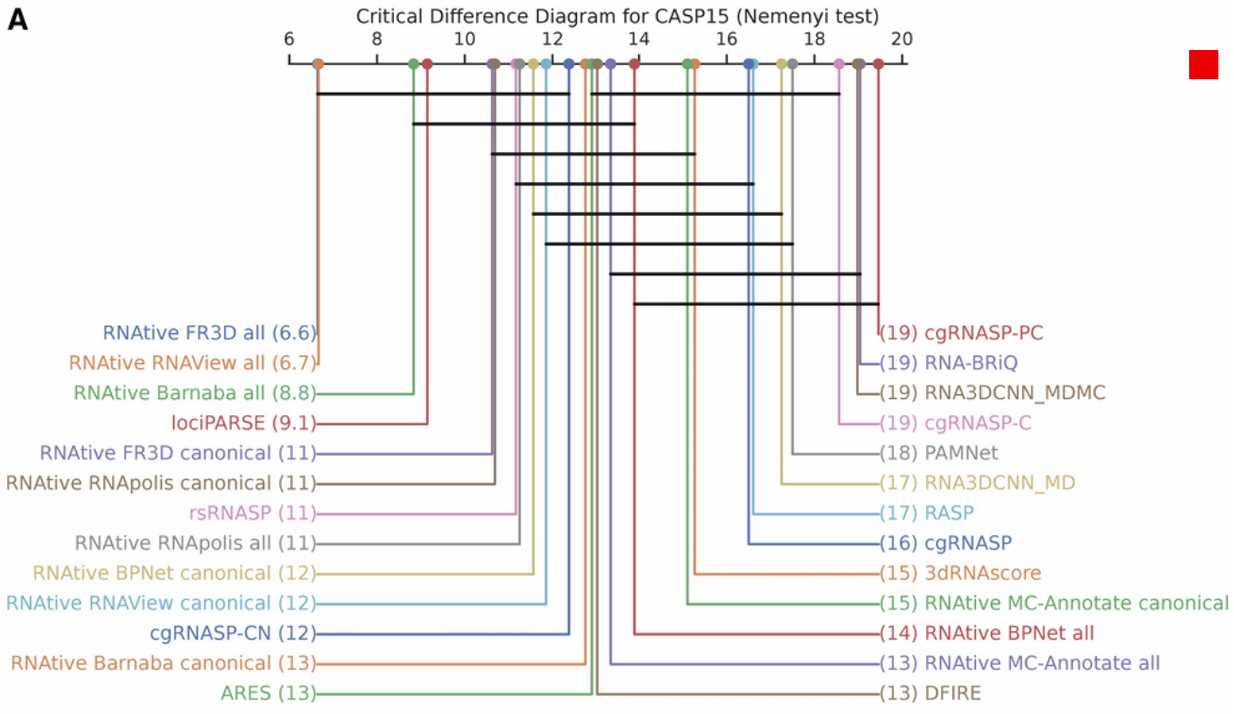


Figure 3. Ranking performance of scoring methods for the (A) CASP15 and (B) randstr decoy datasets. Each plot evaluates how the scoring methods rank RNA 3D models relative to the INF_{nWC} structural quality metric. Each bar reports four ranking similarity measures, averaged across all targets: Spearman's ρ, Kendall's τ, the normalized ES, and RBO. Methods are ordered by their score on the PC1 from the PCA of these four measures, providing a unified performance assessment. The PC1 score is labeled on each bar; its magnitude determines the ranking, though the absolute value is not directly interpretable. An asterisk (*) indicates that for a given method, $\geq 20\%$ of the underlying correlation coefficients (ρ or τ) were not statistically significant. Results for RNAtive, tested under different parameter settings, are shown in bold.

Results (Critical Difference diagrams)



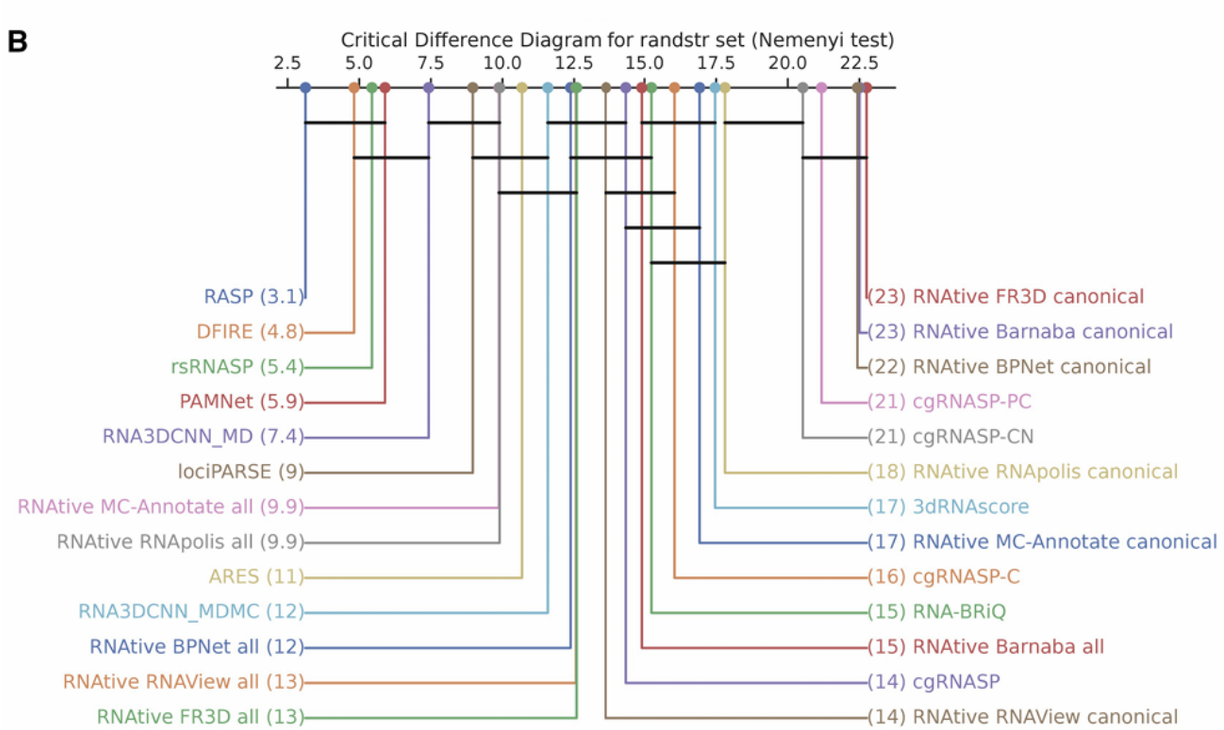


Figure 4. CD diagram for tool performance on the (A) CASP15 and (B) randstr decoys. The diagram shows the average rank of each tool across all evaluation tasks. Tools are ranked from best (left) to worst (right). Horizontal bars connect groups of tools whose performance is not statistically different according to a post-hoc Nemenyi test ($p < 0.05$).

- Global statistical comparison:
  - *Friedman's test* to confirm significant performance differences among the tools.
  - *Nemenyi post-hoc test* to perform all pairwise comparisons and identify tools differed significantly.
  - *Critical Difference (CD) diagrams* visually cluster tools whose performances are not statistically distinguishable.

RNAtive – input form

RNAtive is a consensus-based RNA structure analysis system designed to process multiple structural models sharing the same sequence and to identify reliable base pairs and stacking interactions. It supports model validation, improves structural predictions, and facilitates studies of RNA structure evolution. The tool accepts a minimum of two RNA 3D structure models in PDB or mmCIF format (with a total file size limit of 100 MB), and analyzes them using state-of-the-art base-pair annotation tools. Comparing annotations across all input models, it **generates a consensus structure** that highlights recurrent interactions, which are more likely to reflect stable, native-like folds. It then **evaluates and ranks the input models** based on their consistency with the derived consensus.

Example datasets: RNA-Puzzles models | U2 snRNA decoys | miRNA mir-663

NOTE: All uploaded RNA 3D structures must share the **exact same nucleotide sequence**.

RNA 3D models: Upload

- 1a9nR_M1.pdb
- 1a9nR_M2.pdb
- 1a9nR_M3.pdb
- 1a9nR_M4.pdb
- 1a9nR_M5.pdb
- 1a9nR_M6.pdb
- 1a9nR_M7.pdb
- 1a9nR_M8.pdb
- 1a9nR_M9.pdb

Reset

Model quality filter: No filter (keep all models)

2D structure constraints:

```
>strand_R
CCUGGUAUUGCAGUACCUCCAGGU
(((((.............)))))).
```

Base pair analyzer: RNApolis Annotator

Conditionally weighted consensus:

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Structural Bioinformatics

RNAtive to recognize native-like structure in a set of RNA 3D models

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Abstract

Motivation: Most widely used methods for evaluating RNA 3D structure models require experimental reference structures, which restricts their use for novel RNAs. They also often overlook recurrent structural features shared across multiple predictions of the same sequence. Although consensus approaches have proven effective in RNA sequence analysis and evolutionary studies, no existing tool applies these principles to evaluate ensembles of 3D models. This gap hampers the identification of native-like folds in computational predictions, particularly as AI-driven methods become increasingly prevalent.

Results: This paper presents RNAtive, the first computational tool to apply consensus-derived secondary structures for reference-free evaluation of RNA 3D models. RNAtive aggregates recurrent base-pairing and stacking interactions across ensembles of predicted 3D structures to construct a consensus secondary structure. It introduces a novel conditionally weighted consensus mode that treats interaction networks as fuzzy sets and uniquely allows integration of user-defined 2D structural constraints, enabling evaluation guided by experimental data. Input RNA models are ranked using two adapted binary-classification-based scores. Benchmarking against CASP15 competition data shows that models consistent with the consensus exhibit native-like structural features. The RNAtive web server offers an intuitive platform for comparing and prioritizing RNA 3D predictions, providing a scalable solution to address the variability inherent in deep learning and fragment-assembly methods. By bridging consensus principles with 3D structural analysis, RNAtive advances the exploration of RNA conformational landscapes and has potential applications in fields like therapeutic RNA design.

Availability: RNAtive is a freely accessible web server with a modern, user-friendly interface, available for scientific, educational, and commercial use at https://rnative.cs.put.poznan.pl/.

1 Introduction

RNA molecules exhibit remarkable structural versatility, adopting diverse three-dimensional (3D) conformations influenced by environmental factors and molecular interactions (Haque *et al.* 2018; Schroeder *et al.* 2004; Vicens and Kieft 2022; Leamy *et al.* 2016; Pai and Luca 2019). While a single sequence can yield multiple stable states, as in riboswitches and aptamers, evolutionary pressure preserves a functional structural core (Bevilacqua and Tolbert 2022). These conserved core motifs—such as catalytic sites or ligand-binding pockets—remain invariant across diverse conformations, while peripheral regions may vary. Identifying these recurrent motifs is central to the concept of consensus, which enables the extraction of biologically meaningful patterns from structural diversity (Haque *et al.* 2018; Leamy *et al.* 2016).

In bioinformatics, consensus approaches are robust strategies for extracting signals from heterogeneous data. They are used to identify functional motifs in sequence alignments, infer conserved base-pairing patterns from multiple sequence alignments to improve RNA secondary structure prediction, and define invariant cores from ensembles of experimental 3D models (Hofacker 2007; Bernhart and Hofacker 2009; Hamada 2015; Fukunaga and Hamada 2022; Pietrosanto *et al.* 2016; Zhao and Wang 2008).

Consensus principles, however, remain underutilized in the assessment of computational RNA 3D structure predictions. Current evaluation strategies predominantly rely on reference-dependent metrics, such as Root Mean Square Deviation (RMSD) or Interaction Network Fidelity (INF), that require an experimental structure, limiting their applicability to novel RNAs (Parisien *et al.* 2009; Zok *et al.* 2014; Wiedemann *et al.* 2017; Magnus *et al.* 2020; Mackowiak *et al.* 2024). Moreover, they fail to capture recurrent structural features across predicted conformations—a critical shortcoming given the high variability of models from emerging deep-learning methods. While other approaches can flag outliers or predict model quality, they do not systematically extract consensus signals from ensembles of predicted structures (Davis *et al.* 2007; Luwanski *et al.* 2022; Townshend *et al.* 2021).

To address this gap, we introduce RNAtive, a web-based tool that establishes a new paradigm for evaluating ensembles of RNA 3D models. Unlike traditional methods that score models in isolation, RNAtive exploits the synergistic information within the entire set of models to identify the most plausible structures when no reference is available. Beyond this conceptual shift, RNAtive incorporates several algorithmic innovations. The conditionally weighted consensus mode introduces a novel, threshold-free approach that treats

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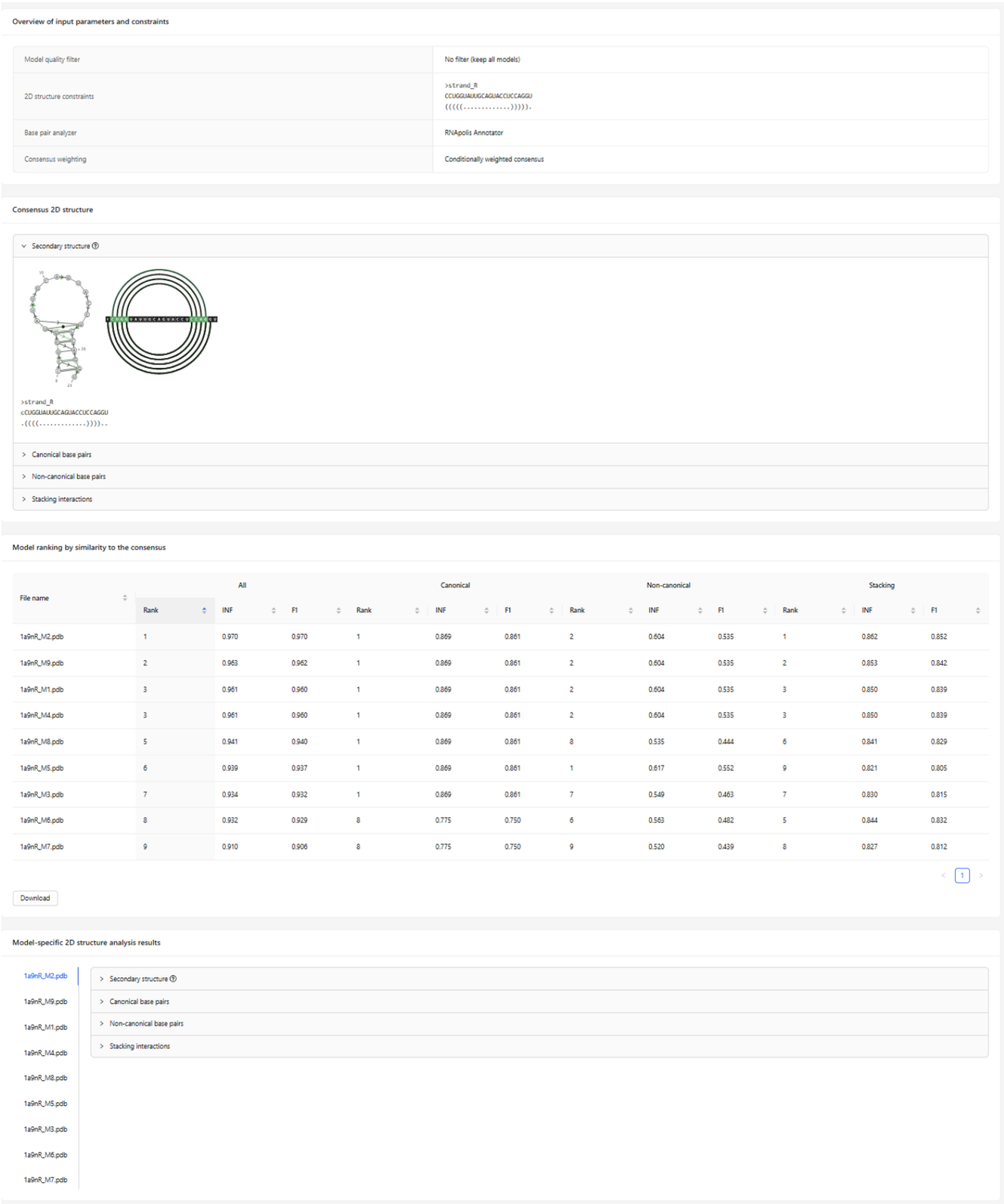
RNAtive – results view



Overview of input parameters and constraints

Model quality filter	No filter (keep all models)
2D structure constraints	>strand_R CCUGGUAUUGCAGUACCUCCAGGU (((((.............))))).
Base pair analyzer	RNApolis Annotator
Consensus weighting	Conditionally weighted consensus

Consensus 2D structure

Secondary structure

>strand_R
cCUGGUAUUGCAGUACCUCCAGGU
.(((((.............)))))..

Canonical base pairs
Non-canonical base pairs
Stacking interactions

Model ranking by similarity to the consensus

File name	All			Canonical			Non-canonical			Stacking		
	Rank	INF	F1	Rank	INF	F1	Rank	INF	F1	Rank	INF	F1
1a9nR_M2.pdb	1	0.970	0.970	1	0.869	0.861	2	0.604	0.535	1	0.862	0.852
1a9nR_M9.pdb	2	0.963	0.962	1	0.869	0.861	2	0.604	0.535	2	0.853	0.842
1a9nR_M1.pdb	3	0.961	0.960	1	0.869	0.861	2	0.604	0.535	3	0.850	0.839
1a9nR_M4.pdb	3	0.961	0.960	1	0.869	0.861	2	0.604	0.535	3	0.850	0.839
1a9nR_M8.pdb	5	0.941	0.940	1	0.869	0.861	8	0.535	0.444	6	0.841	0.829
1a9nR_M5.pdb	6	0.939	0.937	1	0.869	0.861	1	0.617	0.552	9	0.821	0.805
1a9nR_M3.pdb	7	0.934	0.932	1	0.869	0.861	7	0.549	0.463	7	0.830	0.815
1a9nR_M6.pdb	8	0.932	0.929	8	0.775	0.750	6	0.563	0.482	5	0.844	0.832
1a9nR_M7.pdb	9	0.910	0.906	8	0.775	0.750	9	0.520	0.439	8	0.827	0.812

Download

Model-specific 2D structure analysis results

1a9nR_M2.pdb
1a9nR_M9.pdb
1a9nR_M1.pdb
1a9nR_M4.pdb
1a9nR_M8.pdb
1a9nR_M5.pdb
1a9nR_M3.pdb
1a9nR_M6.pdb
1a9nR_M7.pdb

Secondary structure
Canonical base pairs
Non-canonical base pairs
Stacking interactions

- Overview of input parameters and constraints.
- Consensus 2D structure (canonical, non-canonical, and stacking interactions).
- Model ranking by similarity to the consensus (INF, F1; canonical, non-canonical, all, and stacking interactions).
- Model-specific 2D structure analysis results.

Limitations

- The reliability of our method depends significantly on the quality and diversity of the 3D models provided as input.

- *From an empty vessel, not even Solomon can pour.*

Future work

- *3D models at the input must be full-atom structures.*
  - We will consider coarse-grained representations of 3D structures.

- *There is no possibility to increase importance of the selected base pairs flexibly.*
  - We will provide custom user-defined weight values for selected base pairs independently.

Q&A?

Tomasz ZOK

Jan PIELESIAK

Marta SZACHNIUK

Thank you for your attention!

WWW: `http://www.cs.put.poznan.pl/mantczak`

Contact: `Maciej.Antczak@cs.put.poznan.pl`

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