



RNApdbee 3.0:

A unified web server for comprehensive RNA secondary structure annotation from 3D coordinates

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M. Zurawski, M. Antczak, M. Szachniuk, T. Zok.

What is RNApdbee 3.0?

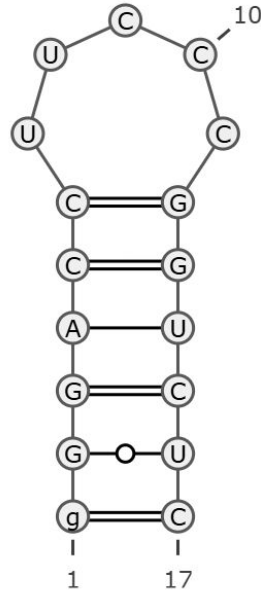


RNApdbee 3.0 is an online tool that allows users to annotate and visualise both 2D and 3D RNA/DNA structures using a plethora of state-of-the-art tools.

Introduction: primary, secondary and tertiary structure of RNA

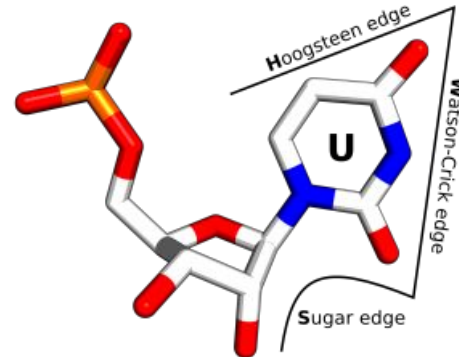
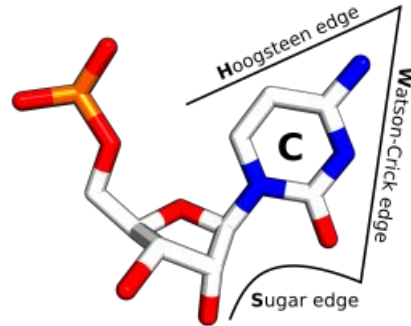
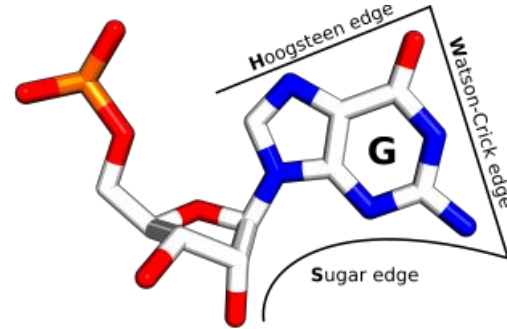
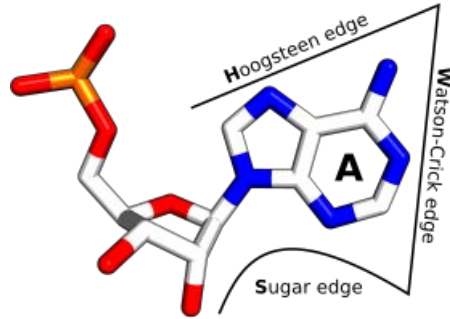


gGGACCUUCCCGGUCUC



pdb ID: 2LBK

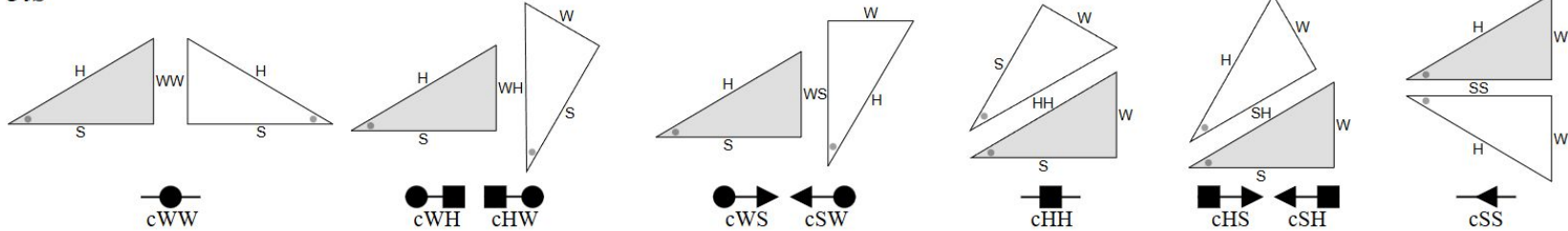
Introduction: interactions in RNA



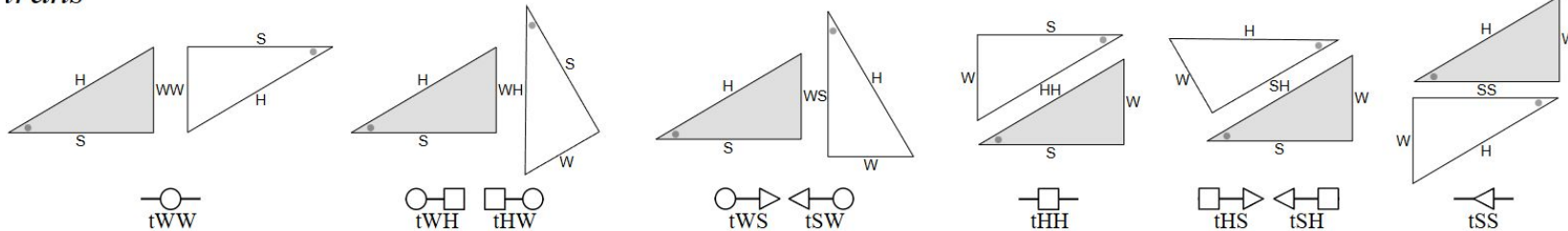
Introduction: interactions in RNA



cis



trans

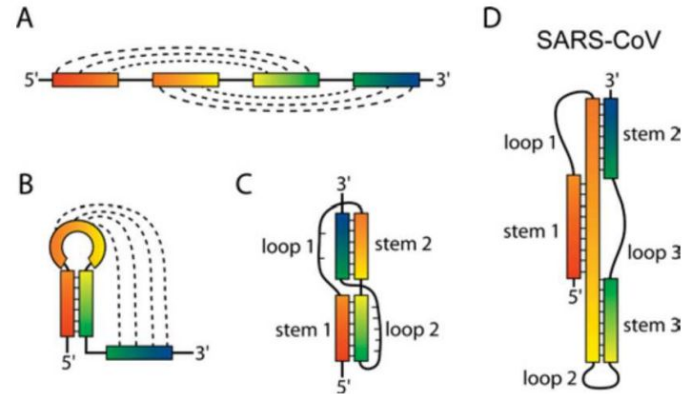


Introduction: base triples

<https://www.bgsu.edu/research/rna/databases/triples.html>

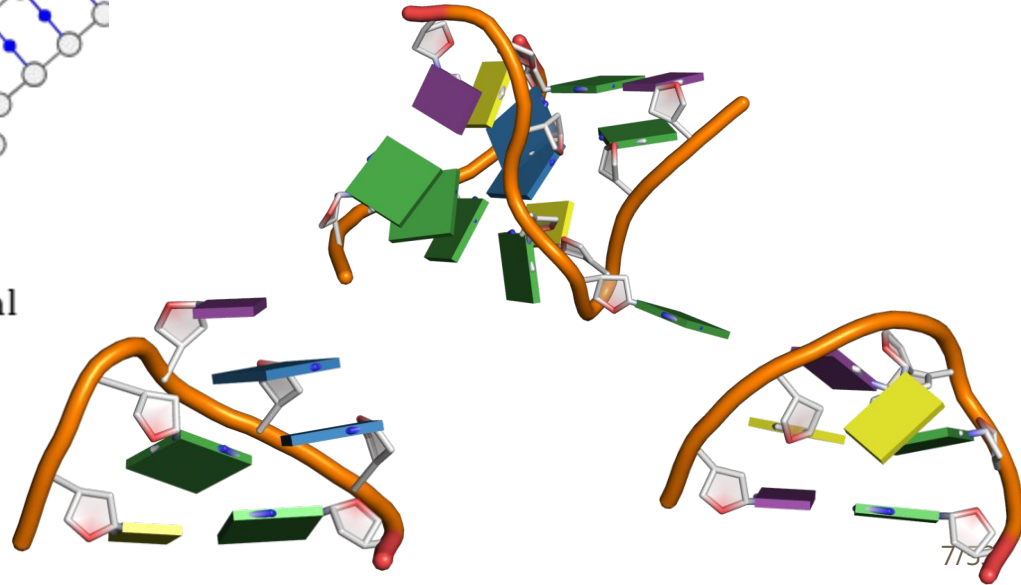
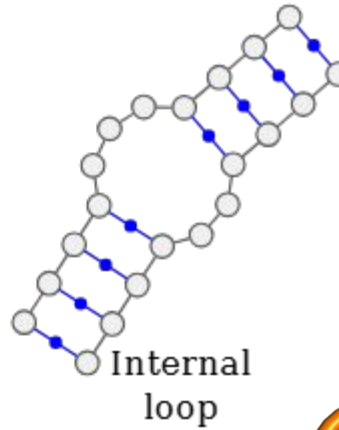
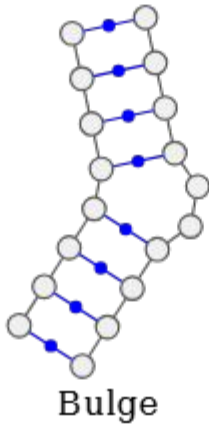
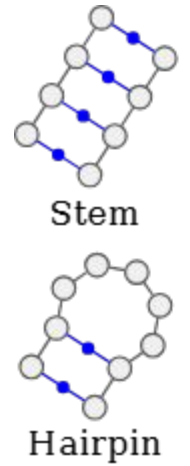


pseudoknots



Studnicka, G.M. et al. (1978). *Nucleic Acids Research*, 5(11), 4365–4387.

Introduction: motifs

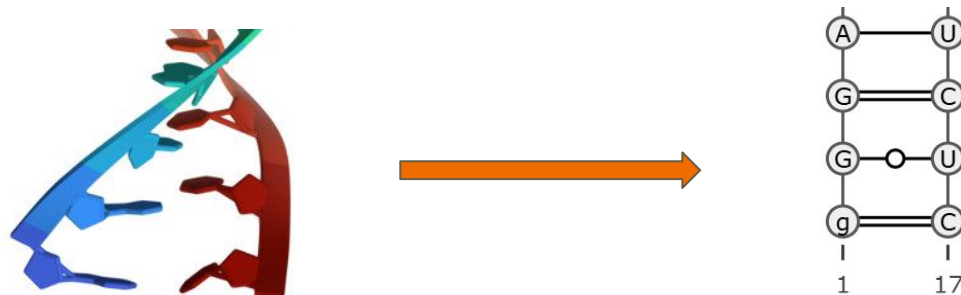


Introduction: what is annotation?



The PDB format provides just the spatial position of atoms within the molecule. The interactions need to be derived from it using an algorithm.

Annotation is the process of determining which nucleotides within a RNA structure interact (form a pair/triple)



RNAPdb 3.0 in short



In short - input

The user can provide either a 3D structure (.cif), or a 2D representation in .dbn, .ct or .bpseq

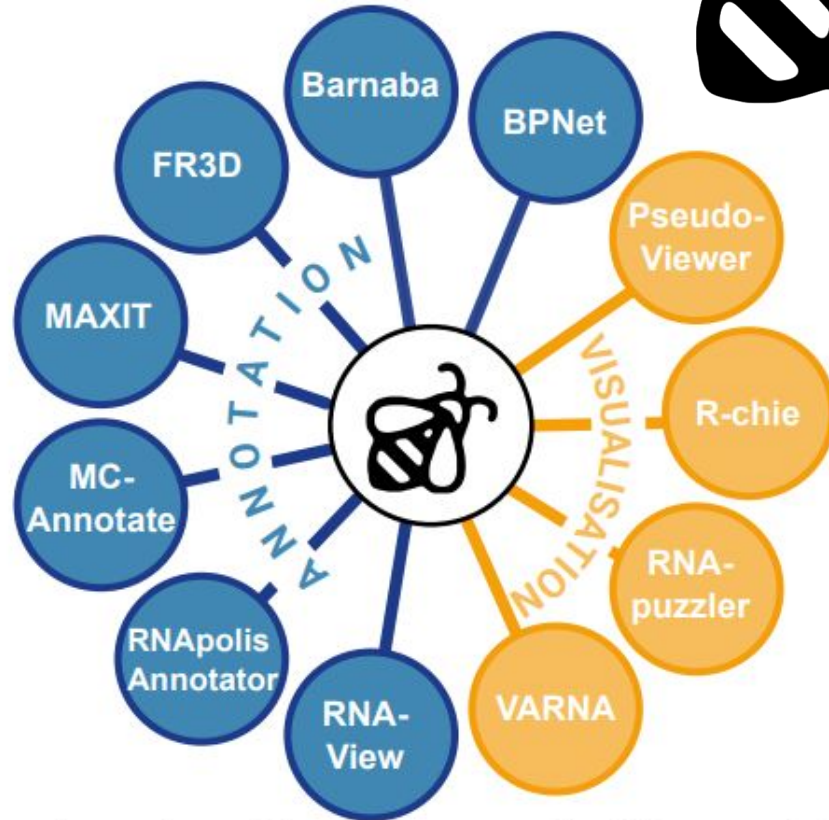


```
>strand_A
gCGGAUUUAgCUCAGuuGGGAGAGCgCCAGAcUgAAgA
(((((((.....[...])))).((((.....
ucUGGAGgUCcUGUGuuCGaUCCACAGAAUUCGCACCA
.))))......((((([...]).....))))))....
```

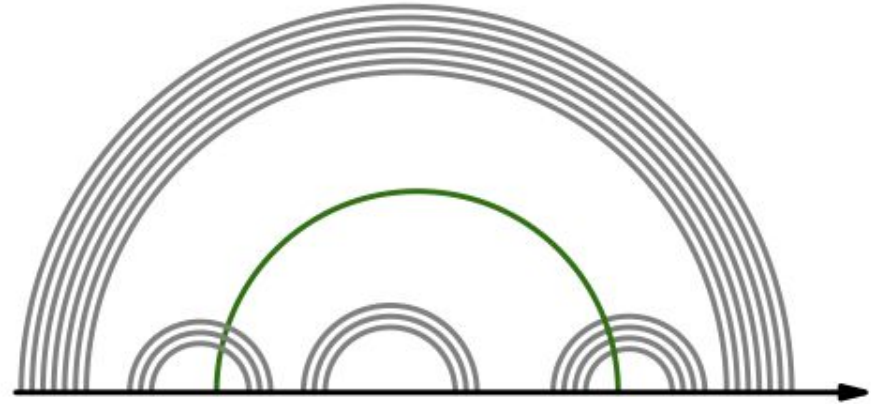
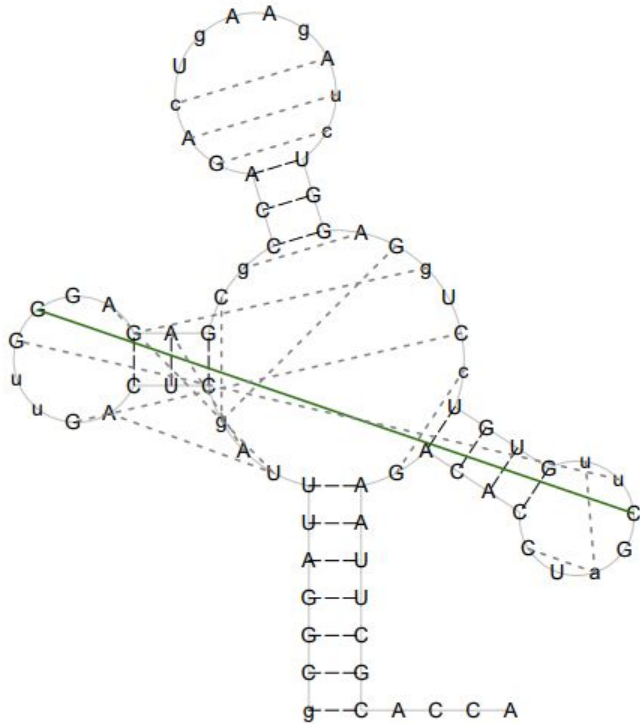
In short -annotation framework



- **base pair** annotation
- base **triples** detection
- **pseudoknot** identification and optimal encoding
- optional 2D representation of **non-canonical** basepairs
- **structural elements** detection
- **consensus construction**



In short - visualizations



Use scenarios



3D → (...)

Secondary structure of RNA derived from tertiary structure provided in PDB or PDBx/mmCIF file.

3D → multi 2D

Set of multiple secondary structures of RNA derived from tertiary structure provided in PDB or PDBx/mmCIF file, based on various results of intermediate processing steps.

2D → (...)

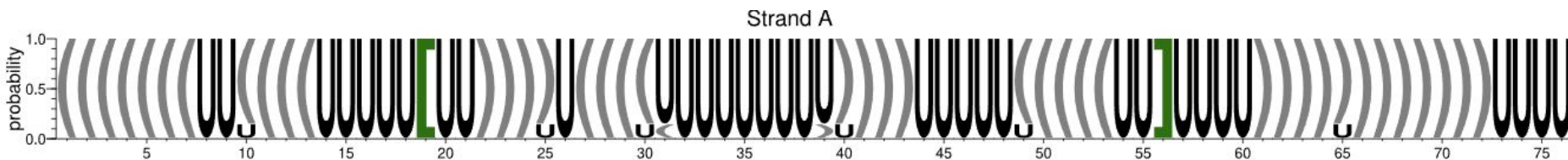
Secondary structure topology of RNA derived from base pair list provided in BPSEQ, CT or DBN.

Consensus 2D structure logo



The consensus 2D structure logo represents the combined annotations of all base pair annotators available in the RNAdbee 3.0.

The goal of this visualization is to notify the user about possible parts of the structure which are not well agreed upon by all the annotators.



How to navigate the website?



3D → (...)

2D → (...)

3D → multi 2D

Looking for (...) -> Image? See the [3.0 changes](#)

Tertiary to dot-bracket

Upload your tertiary RNA structure in PDBx/mmCIF file, choose one from example or provide valid Protein Data Bank ID. You can adjust your calculation with parameters shown below.

1 Upload RNA 3D structure

☒ From Protein Data Bank

7S3H



☐ From local file

Browse...

no file selected

☐ From PDBx/mmCIF example

2 For multimodel files, process

☒ First model only

☐ All models

3 Identify base pairs using

☒ RNAPolis Annotator

☐ FR3D

☐ BPNNet

☐ Barnaba

☐ RNAView

☐ MC-Annotate

☐ MAXIT

How to navigate the website?



Include non-canonical ones:

- ☒ In visualization only
- ☐ In text and visualization
- ☐ Do not include
- ☐ Remove isolated, canonical base pairs

4 Handle structural elements

- ☒ Including pseudoknots
- ☐ Ignoring pseudoknots

5 Visualize secondary structure using

- ☒ VARNA
- ☐ RNApuzzler
- ☐ PseudoViewer
- ☐ R-chie
- ☐ No image

Run

How to navigate the website?



Analyzed first model using RNApolis Annotator. Non-canonical base pairs annotated in visualization only. Structural elements handled using pseudoknots. Isolated, canonical base pairs were not removed.

Model 1

Dot Bracket

BPSEQ

CT

Structural elements

Messages

Canonical interactions

Non-canonical interactions

Interstrand interactions

Stacking interactions

Base-phosphate interactions

Base-ribose interactions

▼ show

▼ show

▼ show

▼ show

▼ show

▼ show

▼ show

▼ show

▼ show

▼ show

▼ show

▲ hide

Image

Generated by VARNA procedure. 

 See forna visualization



How to navigate the website?



CT

^ hide

```
162
1 G 0 2 0 1
2 A 1 3 0 2
3 C 2 4 0 3
4 G 3 5 0 4
5 C 4 6 0 5
6 A 5 7 0 6
7 T 6 8 0 7
8 a 7 9 53 8
9 a 8 10 52 9
10 a 9 11 51 10
11 g 10 12 50 11
```

Structural elements

^ hide

```
Stem N.8 N.30 aaagatgagacaatggcgattac ((((((((((((((((((((((( YYYYYYYYYYYYYYYYYY T.1 T.23 gtaatcgccattgtctcatcttt )))))))))))))))) YYYYYYYYYYYYYYYYYY
Stem R.69 R.72 ACUU ((( RYYY R.77 R.80 AAGU ))) RRRY
Stem R.82 R.87 GCACCG (((((( RYRYR R.91 R.96 CGGUGC ))))) YRRYRY
Loop N.30 T.1 cg () YY
Loop R.72 R.77 UGAAAA (....) YRRRRR
Loop R.87 R.91 GAGUC (...) RRRY
Single strand R.80 R.82 UGG ).( YRR
Single strand 5' N.8 N.8 a ( Y
Single strand 5' R.68 R.69 AA .( RR
Single strand 3' R.96 R.97 CU ). YY
```

How to navigate the website?



Base-ribose interactions

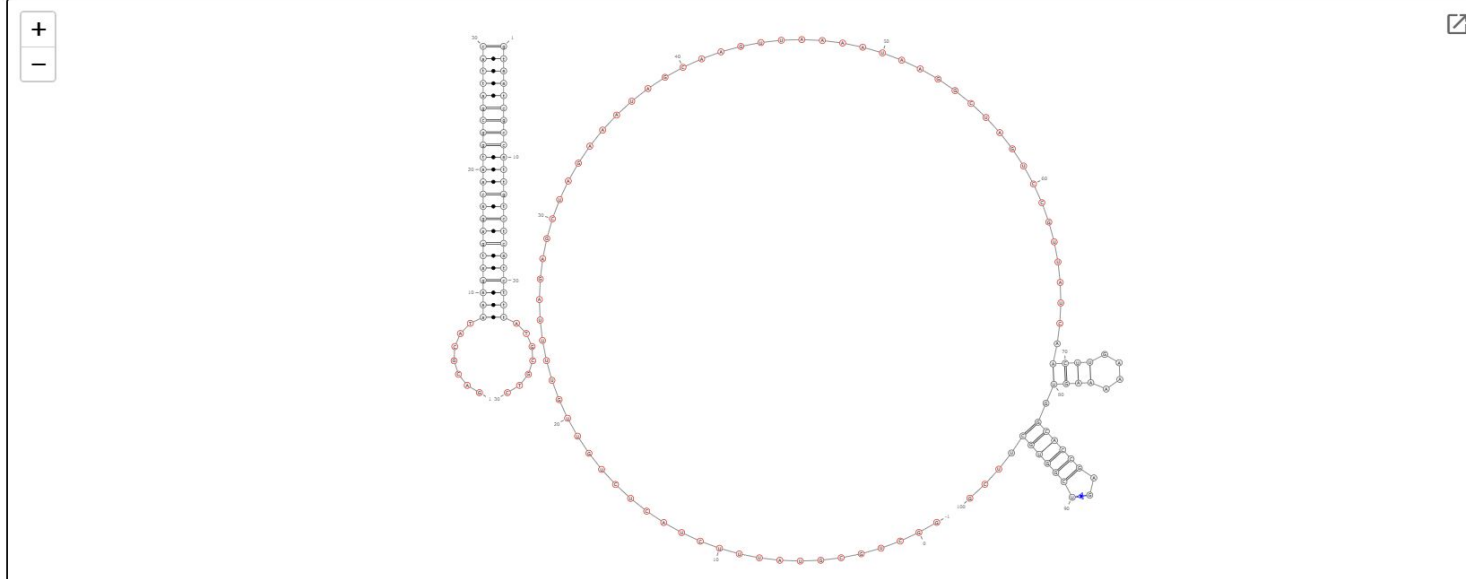
▼ show

Image

▲ hide

Generated by VARNA procedure. 

 See form visualization



How to navigate the website?



Tertiary to multi secondary

Upload your tertiary RNA structure in PDBx/mmCIF file, choose one from example or provide valid Protein Data Bank ID. You can adjust your calculation with parameters shown below.

1 Upload RNA 3D structure

☒ From Protein Data Bank

1EHZ



☐ From local file

Browse...

no file selected

☐ From PDBx/mmCIF example

2 During base pair identification

☐ Include non-canonical base pairs

☐ Remove isolated, canonical base pairs

3 Visualize secondary structure using

☒ VARNA

☐ RNApuzzler

☐ PseudoViewer

☐ R-chie

☐ No image

Run

How to navigate the website?



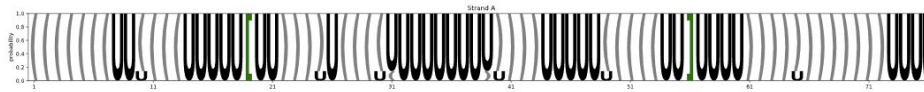
Non-canonical base pairs were not included. Isolated, canonical base pairs were not removed.

Consensus visualization

Generated by WebLogo procedure 



 hide



How to navigate the website?



Choose scenario:

3D → (...)

2D → (...)

3D → multi 2D

Looking for (...) -> Image? See the [3.0 changes](#)

Secondary to dot-bracket

Upload your secondary RNA structure in BPSEQ, CT or DBN file or choose one from example. You can adjust your calculation with parameters shown below.

1 Upload RNA base pair list

☐ From local file (BPSEQ/CT/DBN)

Browse...

no file selected

☒ From DBN

```
>strand_A
gCGGAUUUAgCUCAGuuGGGAGAGCgCCAGAcUgAAgAucUGGA6gUCcUGUGuuCGaUCCACAGAAUUCGCACCA
(((((((...(((.....[.])...))(((.....))).....((((([..].....))))))))))....
```

available examples

1

2

3

data uploaded from: 1EHZ.dbn

Reset

☐ From BPSEQ

☐ From CT

2 Handle structural elements

☒ Including pseudoknots

How to navigate the website?



2 Handle structural elements

- ☒ Including pseudoknots
- ☐ Ignoring pseudoknots
- ☐ Remove isolated, canonical base pairs

3 Visualize secondary structure using

- ☒ VARNA
- ☐ RNApuzzler
- ☐ PseudoViewer
- ☐ R-chie
- ☐ No image

Run

How to navigate the website?



Structural elements handled using pseudoknots. Isolated, canonical base pairs were not removed.

Dot Bracket

✓ show

BPSEQ

✓ show

CT

✓ show

Structural elements

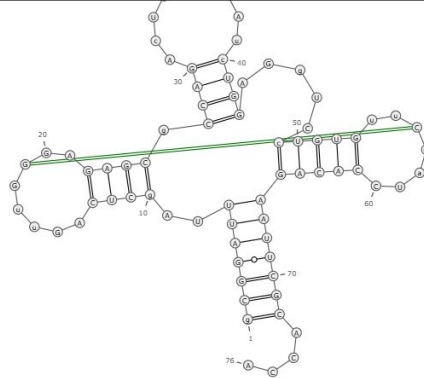
✓ show

Image

^ hide

Generated by VARNA procedure. 

 See forna visualization



How to navigate the website?



Download results

Reanalyze with different parameters

Reanalyze your structure with different parameters

Reanalysis means that you can perform another calculation with modified parameters. Input file will remain the same as in your first calculation. New calculation will be added to your results. That way you can easily compare one calculation to another.

1 Handle structural elements

- ☒ Including pseudoknots
- ☐ Ignoring pseudoknots
- ☐ Remove isolated, canonical base pairs

2 Visualize secondary structure using

- ☒ VARNA
- ☐ RNApuzzler
- ☐ PseudoViewer
- ☐ R-chie
- ☐ No image

Run

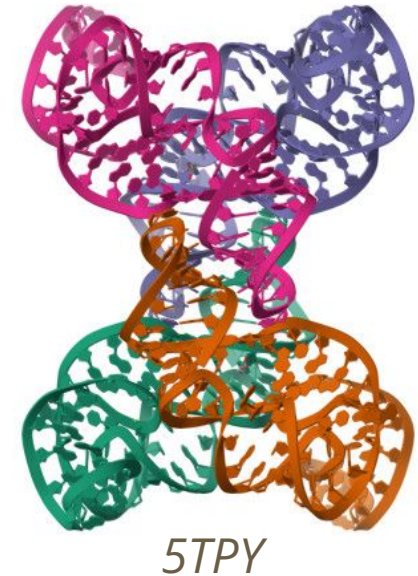
What can be achieved using RNApdbee 3.0?



Consensus ranking of Zika virus RNA models

An example of a robust structural comparison.

The problem: perform a comparison of 52 candidate models of the Zika virus exonuclease-resistant RNA from the 18th RNA-Puzzles challenge



Consensus ranking of Zika virus RNA models



All seven **RNApdbee's** annotators were used to process **each of the models** (Barnaba, BPNet, FR3D, MAXIT, MCAnnotate, RNApolis Annotator, and RNAView)

All of the resulting canonical and non-canonical base pairs were used to calculate **Interaction Network Fidelity (INF)** for each model relative to the target, creating **seven distinct rankings**.

$$PPV = \frac{|TP|}{|TP| + |FP|}$$

$$STY = \frac{|TP|}{|TP| + |FN|}$$

$$INF = \sqrt{PPV \cdot STY}$$

A bootstrapped statistical procedure was used to unify the rankings – using the **PC1** from Principal Component Analysis (**PCA**) a **composite score** is calculated.

Aforementioned **bootstrapping** was performed to the score with 10 000 iterations to assess statistical confidence, yielding a **median rank** and a **95% Confidence Interval** for each model.

Consensus ranking of Zika virus RNA models

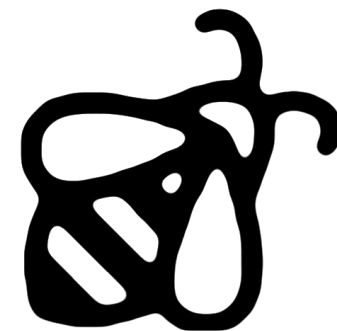


Our analysis identified a clear group of top-performing models.

Group	Model	Median rank	Rank 95% CI lower	Rank 95% CI upper
Das	1	1	1	2
Das	3	2	1	5
Das	2	3	2	4
Chen	1	4	2	5
Chen	2	5	3	6
Das	4	6	5	6
Das	5	7	7	7
Dokholyan	2	8	8	9
RNAComposer	2	9	8	13
Dokholyan	1	10	9	12
Dokholyan	3	12	9	21
3dRNA	3	13	10	20

8 models consistently appear in the top spots. Their narrow 95% CIs confirm their high rank, a finding that remained consistent across the interaction network identification tools used.

Consensus ranking of Zika virus RNA models



Chen	4	30	13	35
RW3D	3	30	24	34
Ding	5	31	24	34
3dRNA	5	32	26	35
Chen	5	33	25	35
SimRNA	2	35	35	36
RNAComposer	5	36	22	48
Ding	3	37	36	39
RNAComposer	3	38	36	42
Ding	1	39	37	42
Lee	2	39	37	41
LeeASmodel	2	41	37	42
Lee	5	42	39	44
LeeASmodel	5	43	41	44
Lee	4	44	42	45
LeeASmodel	4	45	44	46
Lee	1	46	43	47
LeeASmodel	1	47	45	47
SimRNA	3	48	47	48
SimRNA	1	49	49	50
Lee	3	50	49	51
LeeASmodel	3	51	50	51
Ding	4	52	52	52

A similar cluster of models with narrow 95% CI formed at the bottom of the ranking

Identification of key interactions in an RNA 3D structure

The goal of this example is to test how different annotating tools provide different outputs for the same molecule.



PDB ID 1X8W

Identification of key interactions in an RNA 3D structure

*The self-splicing group I intron from *Tetrahymena thermophila* is a very complex molecule, which poses a challenge to structural analysis even 40 years since its discovery.*

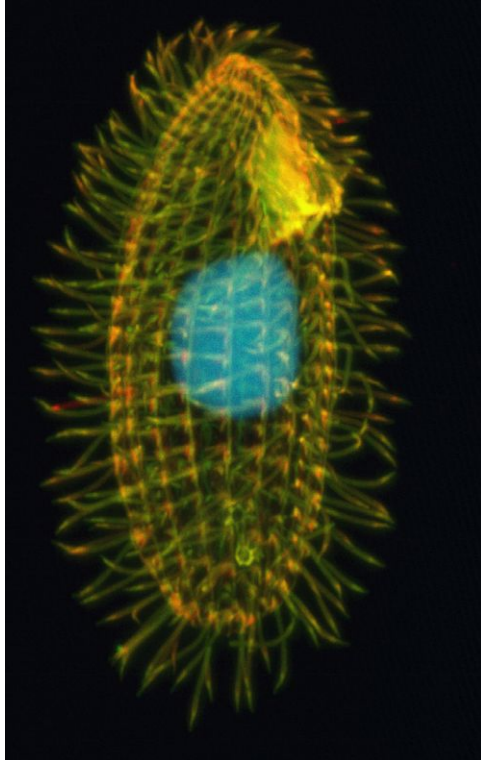
Its core contains multiple motifs and rare interactions



PDB ID 1X8W



Identification of key interactions in an RNA 3D structure



PDB ID 1X8W

Identification of key interactions in an RNA 3D structure

- guanosine binding site **triples**
G414:G264-C311, A263: C262-G312, A261:A265-U310, and A306: C266-G309.
- triple-helical scaffold featuring a **ribose-ribose** contact (A104-C217)
- base **triple** (A105:C216- G257)
- **base-ribose** contact (U106-U258)
- domain-connecting fragment comprising a **single hydrogen bond pair** (C217-C255)
- base **triple** (A218:C102-G272),
- **base phosphate contacts** between A256 and G272/ U273.
- interdomain interaction formed by **stacked** A324 and A125
- base **triple** A325:G119-U202



PDB ID 1X8W

Identification of key interactions in an RNA 3D structure

Base pair annotators integrated in RNAPdb all have different capabilities:

MAXIT detects only base pairing, Barnaba adds stacking, others, i.e., MC-Annotate, RNAView, BPNet, FR3D, and RNAPolis Annotator, also annotate base-phosphate and base-ribose contacts.



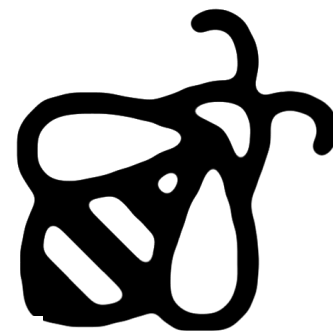
PDB ID 1X8W

Identification of key interactions in an RNA 3D structure



Crucial Interaction	Interaction Type	Component	Barnaba	BPNet	FR3D	MAXIT	MC-Annotate	RNApolis	Annotator	RNAView
G414:G264-C311	Base triple	cWW	A,B,C,D	A,B,C,D	A,B,C,D	A,B,C,D	A,B,C,D	A,B,C,D		A,B,C,D
		cHW	A,B,C,D	A,B,C,D	A,B,C,D	-	B,C,D	A,B,C,D		A,B,C,D
A263:C262-G312	Base triple	cWW	B,C,D	B,C,D	B,C,D	B,C,D	B,C,D	B,C,D		B,C,D
		cHS	A,B,C,D	-	-	A	-	A,D		-
		tHS	-	A,B,C,D	A,B,C,D	-	A,B,C,D	B,C		A,B,C,D
		BR	-	C	A,B,C,D	-	A,B,C	A,B,C,D		-
A261:A265-U310	Base triple	cWW	A,B,C,D	A,B,C,D	A,B,C,D	A,B,C,D	A,B,C,D	A,B,C,D		A,B,C,D
		tWH	A,B,C,D	C,D	A,B,C,D	-	A,D	B,C,D		A,B,C,D
		BPh	-	-	B	-	D	B		-
A306:C266-G309	Base triple	cWW	A,B,C,D	A,B,C,D	A,B,C,D	A,B,C,D	A,B,C,D	A,B,C,D		A,B,C,D
		tHH	-	-	A,D	-	-	-		B
A104-C217	Base-ribose	cSS	A,D	B,C,D	A,C,D	-	D	-		-
		cWS	-	-	-	-	-	D		-
		BR	-	B,D	-	-	A,B,D	A,D		-
A105:C216-G257	Base triple	cWW	A,B,C,D	A,B,C,D	A,B,C,D	A,B,C,D	A,B,C,D	A,B,C,D		A,B,C,D
		tSS	B	A,D	A,B,C,D	-	B	B,C,D		A,B,C,D
		tWS	-	B	-	-	-	-		-
		BR	-	B	C,D	-	B	B,C,D		-

Identification of key interactions in an RNA 3D structure

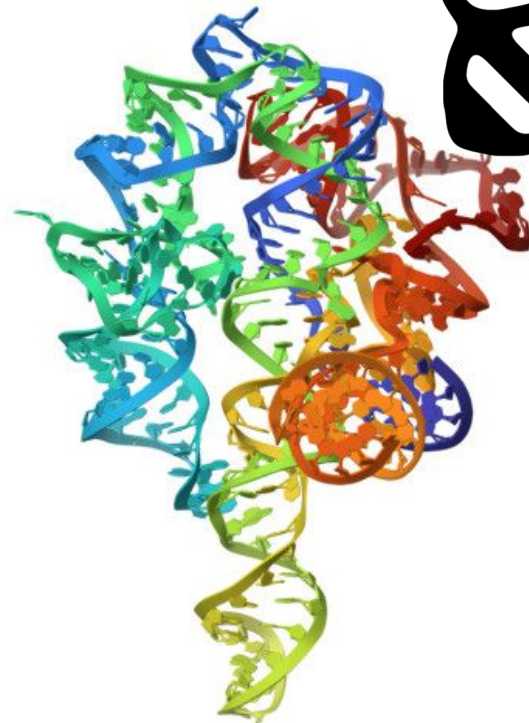


Crucial Interaction	Interaction Type	Component	Barnaba	BPNet	FR3D	MAXIT	MC-Annotate	RNApolis	Annotator	RNAView
U106-U258	Ribose-ribose	-	-	-	-	-	-	-	-	-
C217-C255	Base pair (1H)	tWH	B,C,D	D	C,D	-	-	C,D	B,C,D	
		tWW	-	-	-	B	-	-	-	-
A218:C102-G272	Base triple	cWW	A,B,D	A,B,D	A,B,D	A,B,D	A,B,D	A,B,D	A,B,D	
		cSS	A,B	A,B	A,B	-	B	A,B	-	-
A256-G272	Base-phosphate	BPh	-	A,B,C,D	-	-	-	C,D	-	-
		BR	-	-	A,B,D	-	-	B	-	-
A125-A324	Stacking	S	B,D	D	B,D	-	B,D	B,D	-	-
		BR	-	A	B	-	A	A,B	-	-
A325:G119-U202	Base triple	cWW	A,B,C	A,B,D	A,B,C,D	A,B,C,D	A,B,C	A,B,C,D	A,B,C,D	
		cSS	B	A	A,B	-	A	-	B	
		cSW	A	B	-	-	-	A,B	-	-
		S	-	-	D	-	-	-	-	-
		BR	-	A,B	-	-	A,B,D	A,B	A	

Identification of key interactions in an RNA 3D structure

Takeaways:

- **No tool is a true gold standard**
- **Human supervision is still required**
- MAXIT most conservative, shows only the most certain interactions
- FR3D and RNAPolis Annotator exhaustive annotations, possibly at the risk of including ambiguous contacts
- Barnaba, BPNet, and RNAView occupy a middle ground



The crystal structure of molecule's catalytic core (PDB ID 1X8W)

What does the version 3.0 improve over 2.0?



- **PDB and PDBx/mmCIF with improved interoperability**
- **DBN** added as a 2D format
- **New annotators and old one updated:** RNAPolis Annotator, FR3D (new, Python-based version), BPNet, Barnaba, RNAView (v2.0.0 with native PDBx/mmCIF support), MC-Annotate, MAXIT
- **MILP-based exact algorithm** for pseudoknot encoding
- **New visualization:** RNApuzzler, forna
- **Leaflet map-based visualization** adapted for extremely complex and large structures
- partial support of non-canonical interaction visualization with **RNApuzzler and PseudoViewer**
- **Base triples** are now detected and provided to the user
- structural elements now utilize the new **PDBx/mmCIF data structure**

Accessibility

RNApdbee 3.0 is publicly available at <https://rnapdbee.cs.put.poznan.pl/>

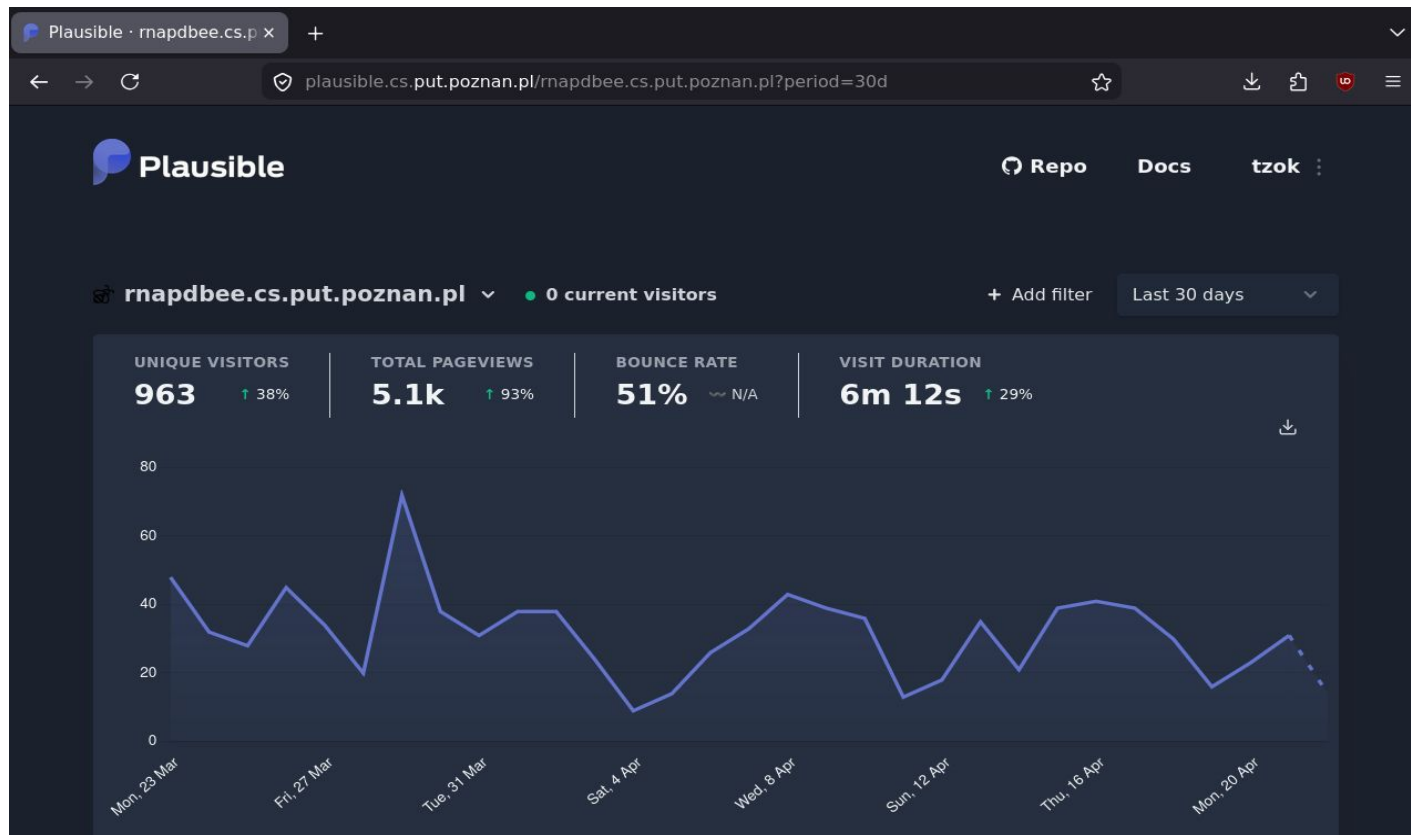
It can also be deployed by the user.

The system is **dockerized** and therefore does not require complex installation, but I would like to note, that a powerful machine is advised to run it.

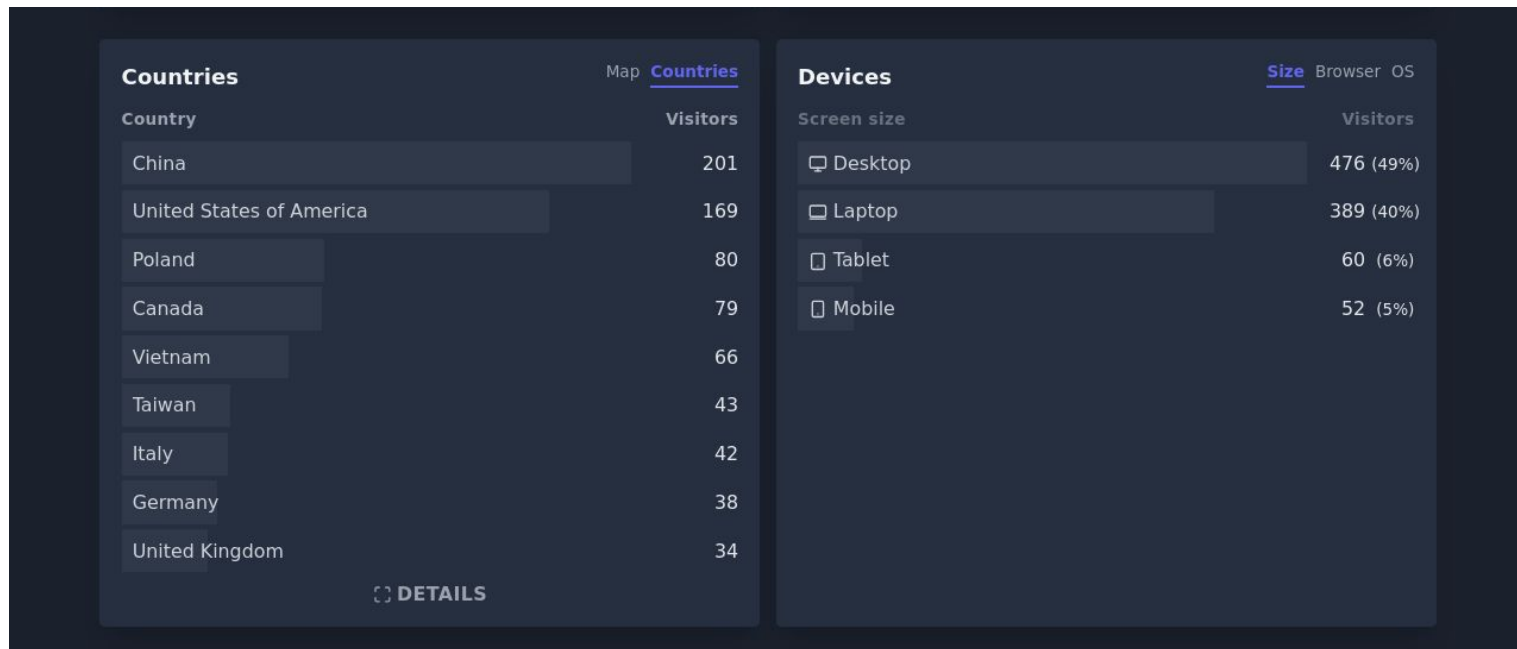
The web interface is written in Angular, while the backend is Java-based.



Is our tool useful at all tho?



Is our tool useful at all tho?



Is our tool useful at all tho?

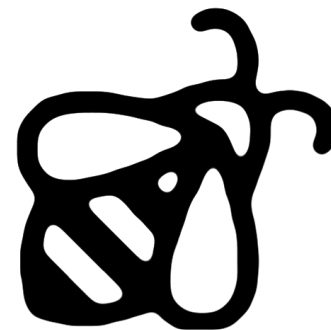


Top Sources		All	Medium	Source	Campaign
Source	Visitors				
 Google	228				
 Bing	49				
 DuckDuckGo	6				
 Yandex	6				
 sciencedirect.com	5				
 chatgpt.com	4				
 biorxiv.org	3				
 learning.hs-mittweida.de	3				
 doubao.com	2				
 DETAILS					

Is our tool useful at all tho?



Top Sources		All Medium Source Campaign
Source	Visitors	
 Google	228	
 Bing	49	
 DuckDuckGo	6	
 Yandex	6	
 sciencedirect.com	5	
 chatgpt.com	4	
 biorxiv.org	3	
 learning.hs-mittweida.de	3	
 doubao.com	2	
DETAILS		



Web Server

RNApdbee 3.0: A unified web server for comprehensive RNA secondary structure annotation from 3D coordinates ☆

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[Maciej Antczak](#)^{1,2}, [Marta Szachniuk](#)^{1,2}, [Tomasz Zok](#)¹  

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<https://doi.org/10.1016/j.jmb.2026.169795> ↗

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Thank you for your attention



NARODOWE CENTRUM NAUKI

NCN SONATA 2023/51/D/ST6/01207.
2024–2027

RNApdbee 3.0 in more detail



Input: scenario 3D -> (...)



Upload
RNA 3D structure
(mmCIF/PDB file
or PDB ID)

Specify inclusion of
noncanonical base pairs
in text/visualization

Select only the first
model or all models
for processing

Choose whether to
discard isolated
canonical base pairs

Choose whether to
generate 2D structure
visualization

Select tool for base pair
identification

Specify inclusion of
pseudoknots during
structural element
identification

Input: scenario 3D -> multi (...)



Upload
RNA 3D structure
(mmCIF/PDB file
or PDB ID)

Choose whether to
discard isolated
canonical base pairs

Choose whether to
include noncanonical
base pairs

Choose whether to
generate 2D structure
visualization

Input: scenario 2D -> (...)



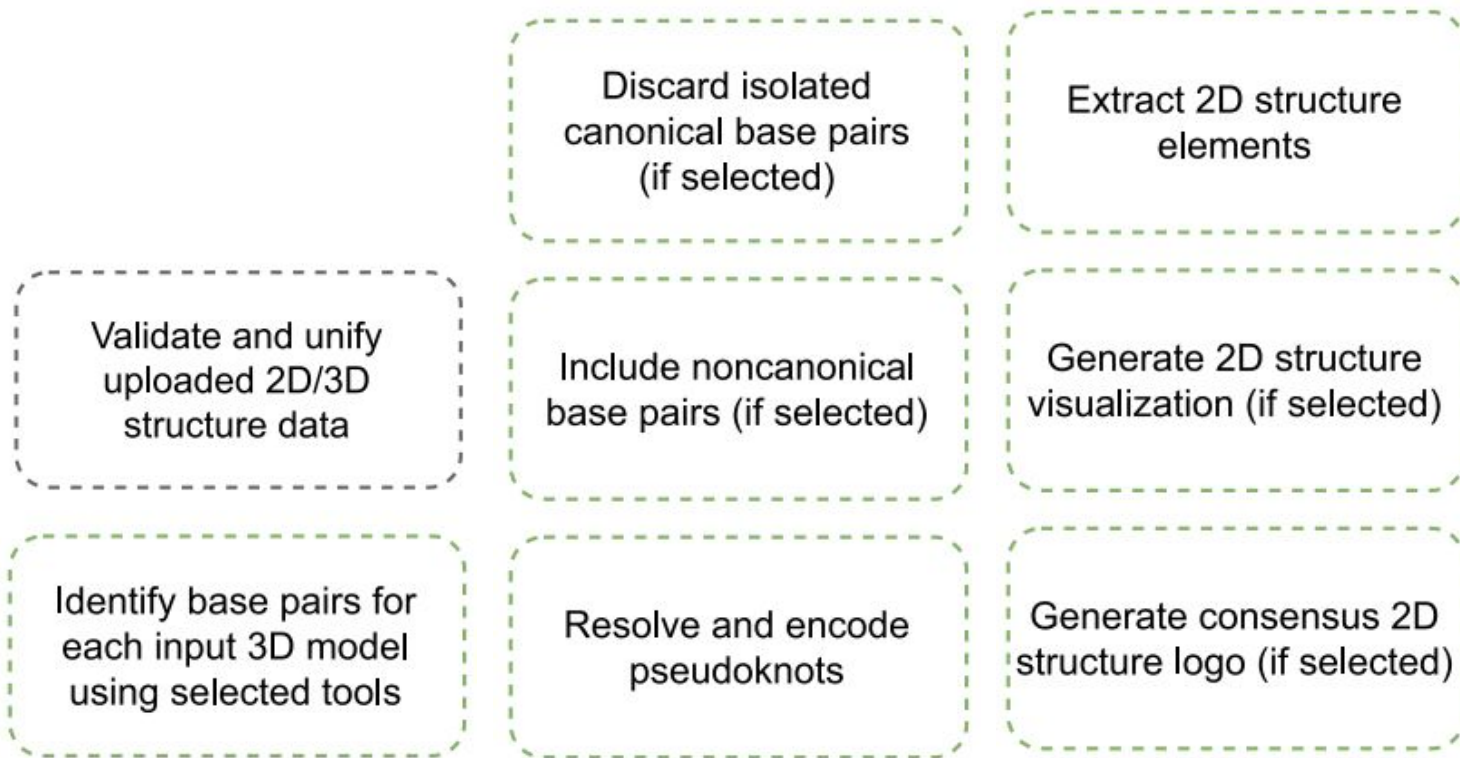
Upload
RNA 2D structure
(BPSEQ/CT/DBN file)

Choose whether to
discard isolated
canonical base pairs

Specify inclusion of
pseudoknots during
structural element
identification

Choose whether to
generate 2D structure
visualization

Processing...



Output



RNA 2D structure
in BPSEQ, CT, extended
DBN, and visualization
(if selected)

2D structure element
annotations

Consensus 2D
structure logo (optional)