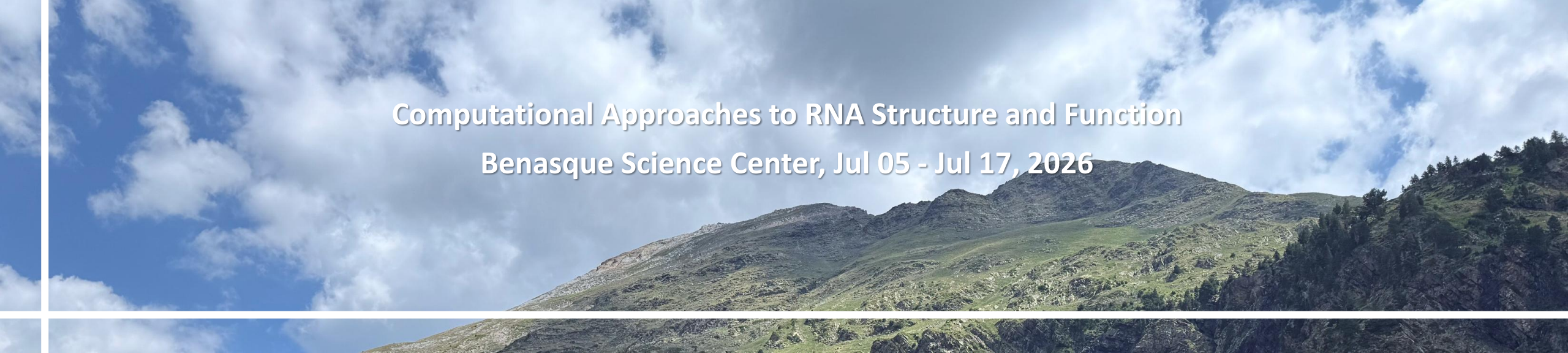




Computational Approaches to RNA Structure and Function
Benasque Science Center, Jul 05 - Jul 17, 2026

Modeling the 3D structure of a riboswitch: a case study

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Riboswitch structure targeted in CASP15 (R1117, 30 nt)

Information for predicting groups

Molecule: **PreQ1 class I type III riboswitch**

Organism: K. pneumoniae

Residues: 30

Method: X-RAY

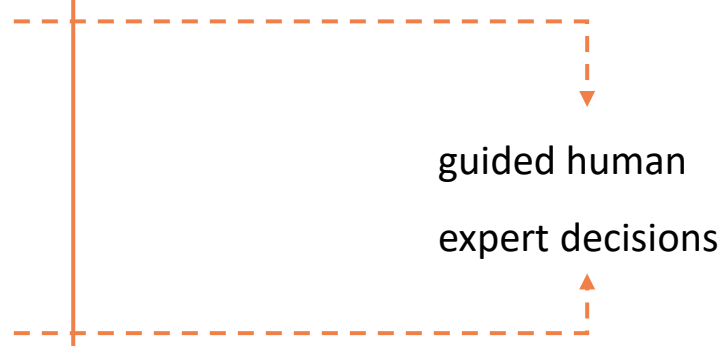
Additional information: **Structure contains a ligand (PRF)**

Sequence:

>R1117 0

UUGGGUUCCCUCACCCCAAUCAUAAAAAGG

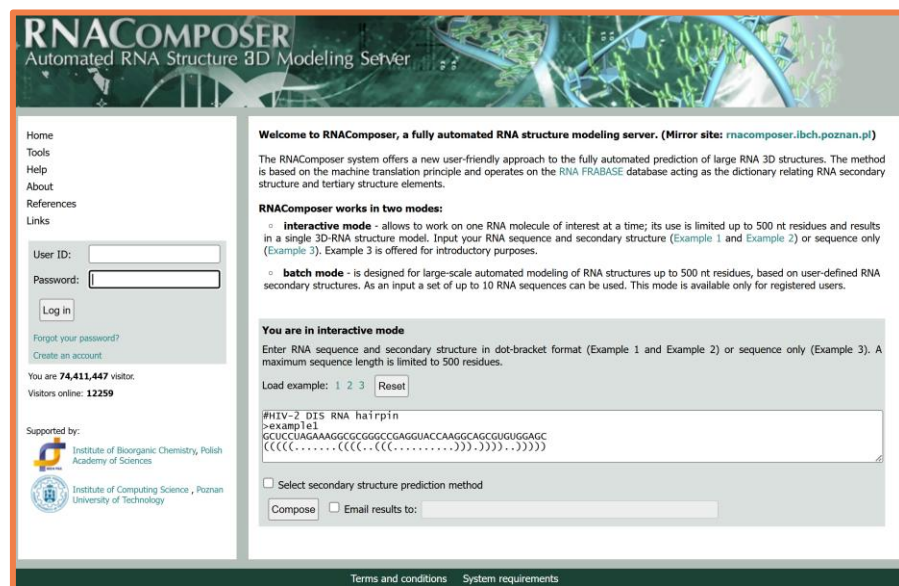
guided human
expert decisions



RNApolis group approach

Web server prediction (RNAComposer)

<https://rnacomposer.cs.put.poznan.pl/>



Popenda *et al.* *Nucleic Acids Res* 2012, 40(14):e112
Antczak *et al.* *Acta Biochim Pol* 2016, 63(4):737-744
Sarzynska *et al.* *Proteins* 2023, 91(12):1790–1799

Human expert modeling

1. Collecting prior knowledge

2. Prediction from sequence

3. Prediction from consensus 2D

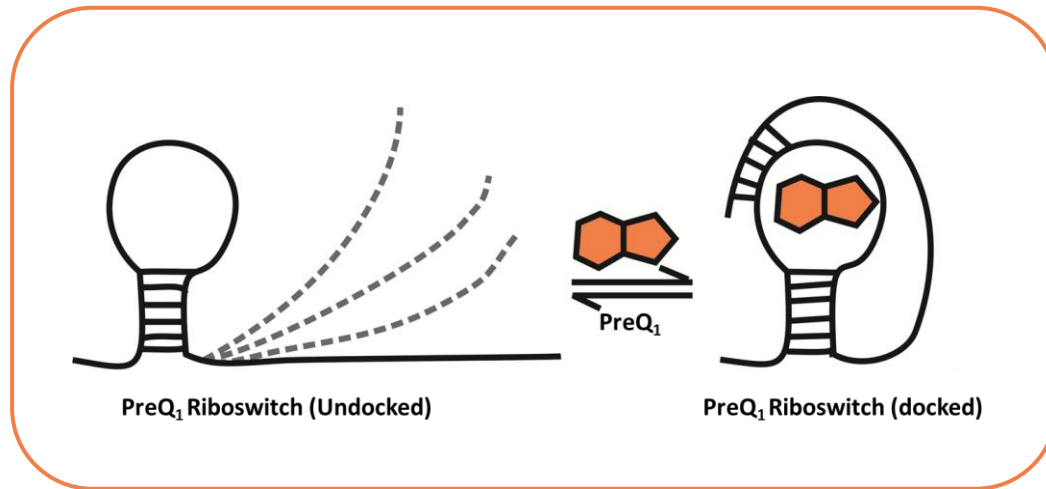
4. Homology modeling

5. Critical analysis & model selection

Collecting available structural and biological information

The class I prequeuosine riboswitch (preQ₁)

- regulates genes involved in the synthesis and transport of preQ₁ (an intermediate in queuosine biosynthesis);
- contains one of the smallest naturally occurring riboswitch aptamer domains.



- In the ligand-bound state, the preQ₁ aptamer adopts an **H-type pseudoknot** structure.

Collecting available structural and biological information

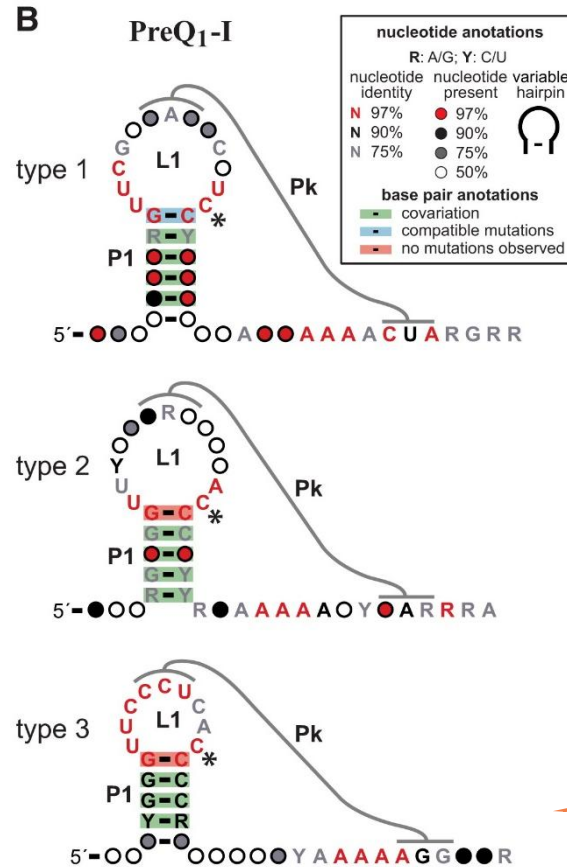
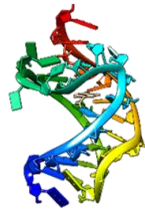
type 1

1KIV
7REX



type 2

2L1V (NMR),
3FU2,
3Q50, 3Q51,
6VUI, 6VUH,
3GCA,
6E1S, 6E1T, 6E1U,
6E1V, 6E1W,
7E9E, 7E9I



(B) Consensus sequences and secondary structure models of the three types of preQ1-I riboswitches.

McCown et al. Chemistry and
Biology 21,880-889,2014

type 3 (target)

No representative structure in PDB.

Collecting available structural and biological information

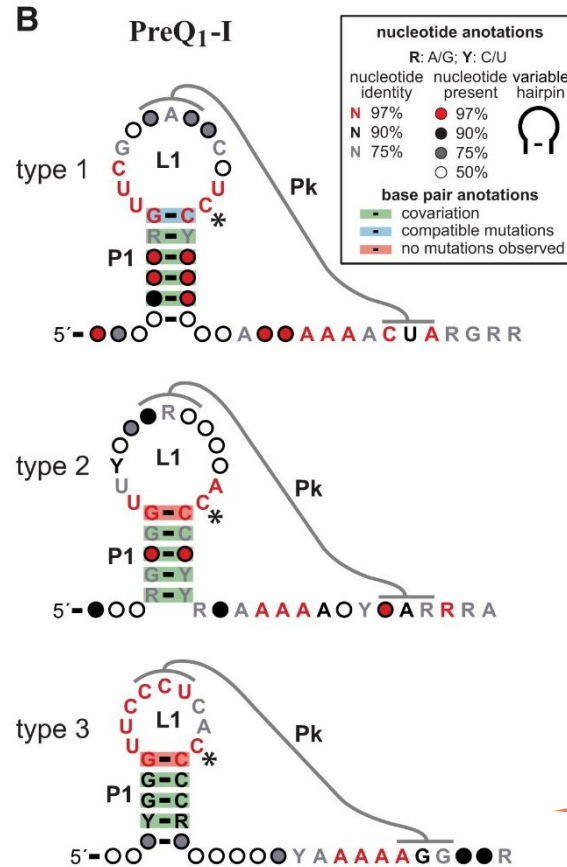
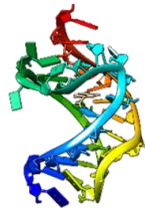
type 1

1KIV
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3FU2,
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6E1S, 6E1T, 6E1U,
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(B) Consensus sequences and secondary structure models of the three types of preQ1-I riboswitches.

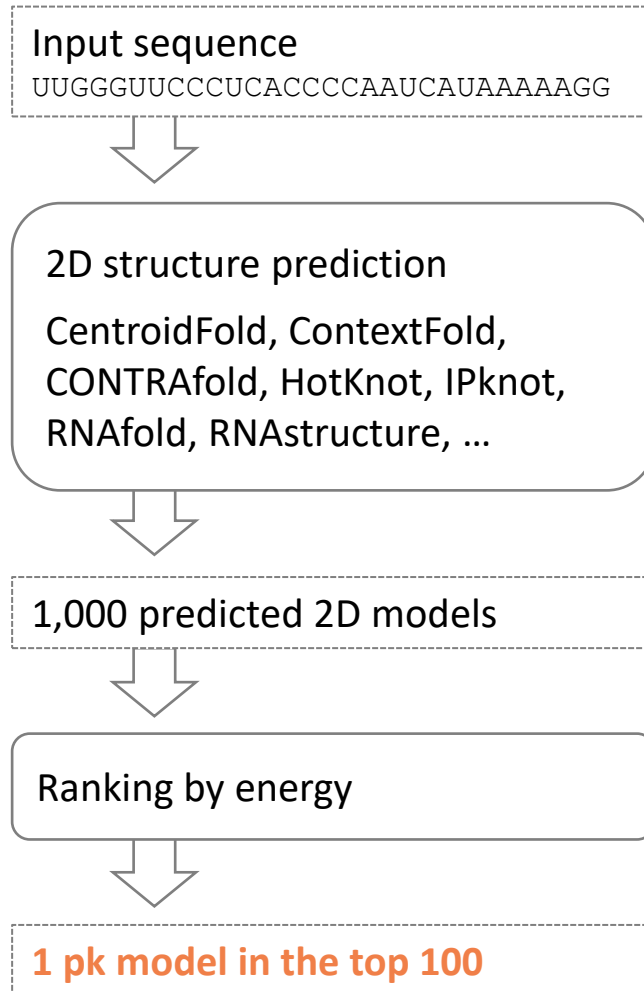
McCown et al. Chemistry and
Biology 21,880-889,2014

EASY

type 3 (target)

No representative structure in PDB.

Web server: sequence-based prediction

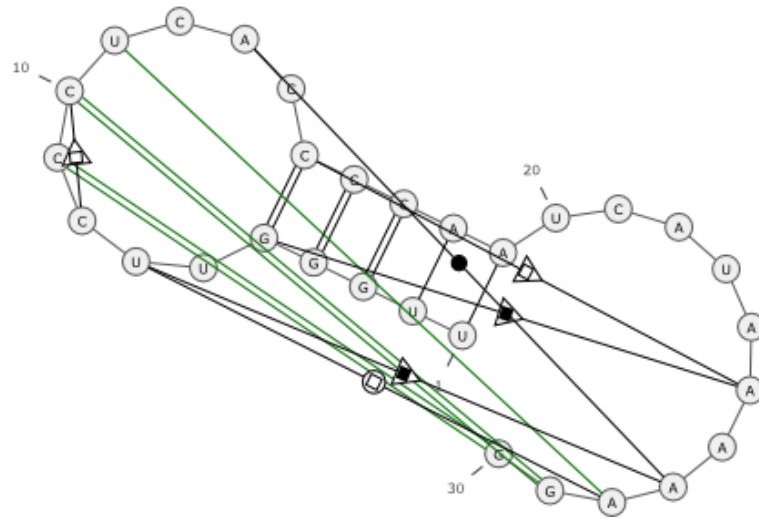


Model No.	Radius of Gyration	Total Energy (by Xplor)	Clash Score	2D Structure Prediction Tool	Secondary structure derived from 3D model (RNApdbee + canonical bps only)
1	13,042	-581,426	20,9	ContextFold	. ((((. (.....) .))) . (.....) .
2	13,51	-577,158	22,99	ContextFold	. ((((. (.....) .))) . (.....) .
3	15,389	-563,741	20,9	ContextFold	. ((((. (.....) .))) . (.....) .
4	14,992	-563,214	16,72	ContextFold	(((((. (.....) .))) . (.....) .
5	14,616	-563,085	15,67	ContextFold	. ((((. (.....) .))) . (.....) .
6	15,028	-558,735	12,54	ContextFold	. ((((. (.....) .))) . (.....) .
7	15,164	-557,393	16,72	ContextFold	. ((((. (.....) .))) . (.....) .
8	14,655	-554,107	12,54	ContextFold	. ((((. (.....) .))) . (.....) .
9	16,957	-552,254	19,85	ContextFold	. ((((. (.....) .))) . (.....) .
10	15,017	-551,41	13,58	ContextFold	. ((((. (.....) .))) . (.....) .
11	14,175	-550,549	10,45	ContextFold	. ((((. (.....) .))) . (.....) .
12	15,081	-547,064	13,58	ContextFold	. ((((. (.....) .))) . (.....) .
13	15,355	-545,919	25,08	ContextFold	. ((((. (.....) .))) . (.....) .
14	15,1	-545,674	15,67	ContextFold	. ((((. (.....) .))) . (.....) .
15	15,393	-544,342	13,58	ContextFold	. ((((. (.....) .))) . (.....) .
16	15,379	-538,223	16,72	ContextFold	. ((((. (.....) .))) . (.....) .
17	14,81	-536,726	16,72	ContextFold	. ((((. (.....) .))) . (.....) .
18	16,043	-535,287	7,31	ContextFold	. ((((. (.....) .))) . (.....) .
19	15,229	-532,994	17,76	ContextFold	. ((((. (.....) .))) . (.....) .
20	15,035	-532,476	9,4	ContextFold	. ((((. (.....) .))) . (.....) .
21	13,365	-527,382	24,03	HotKnots	(((((... [[[...]]) . (.....) .
22	15,215	-526,421	18,81	ContextFold	. ((((. (.....) .))) . (.....) .
23	15,595	-525,492	22,99	ContextFold	. ((((. (.....) .))) . (.....) .
24	15,603	-525,226	18,81	ContextFold	. ((((. (.....) .))) . (.....) .
25	18,878	-524,321	17,76	IPknot	(((((. (.....) .))) . (.....) .
26	14,404	-523,785	20,9	ContextFold	. ((((. (.....) .))) . (.....) .

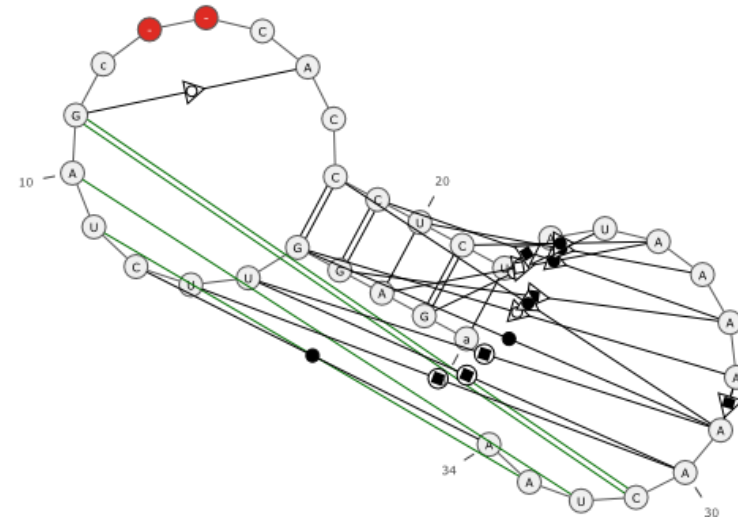
R1117

Web server: 2D structure-based prediction

RNAComposer web server model (type 3)



3FU2, X-ray, resolution: 2.85 Å (type 2)

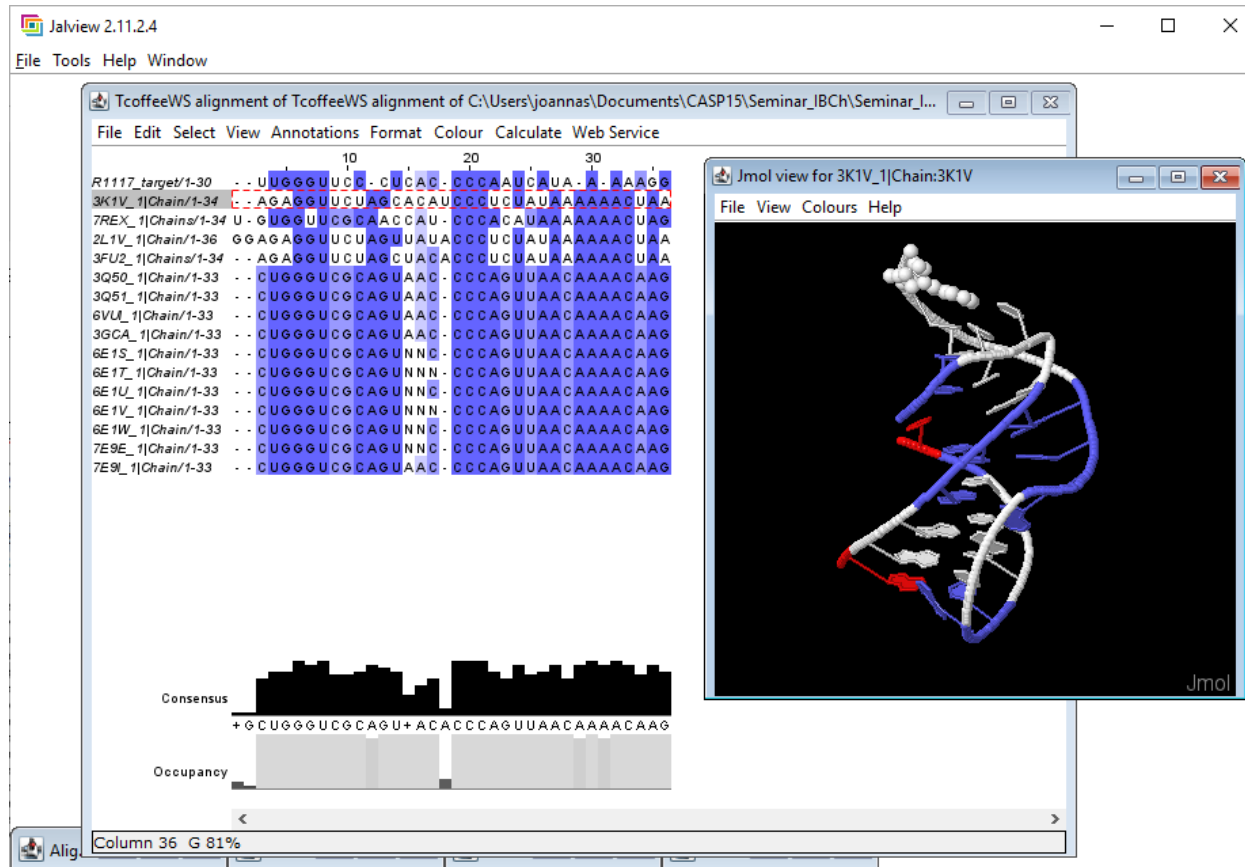


Predicted 3D models **do not reconstruct the noncanonical** tertiary interactions in the minor groove correctly.

Homology modeling by human experts

Template preparation

sequence alignment of available templates → superposition of the template 3D structures



Homology modeling by human experts

Template entered as a single structural block:

(((((...[[[...)))))...]]]
↓ ↓ ↓ ↓
[[[[[...{{{...}}]]]]...}}}



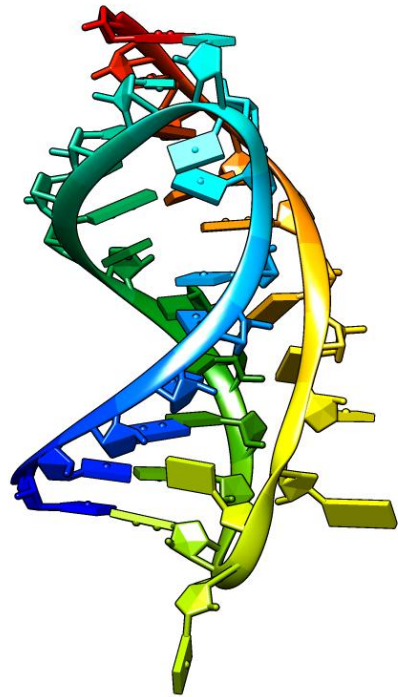
RNAComposer runs structure refinement:
two-step minimization (CYANA, XPLOR)



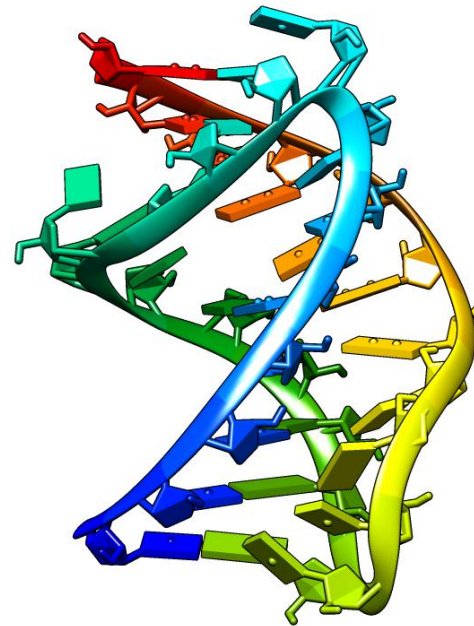
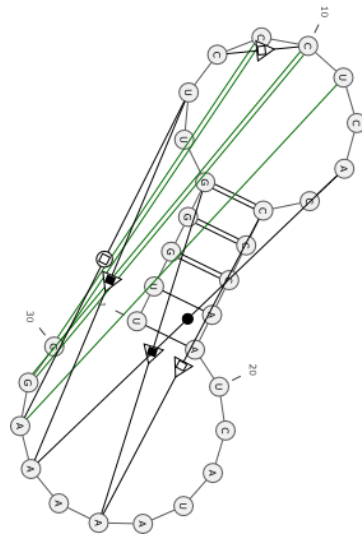
Final 3D model energy: -20 kcal/mol/residue

```
joannas@konwalia: ~/casp15/r1117
joannas@frabase2:~/casp15/r1117> more r1117_js1.dot
>R1117_js1
UUGGGUUCCCUACCCCAUCAUAAAAAGG
[[[[[...{{{...}}]]]]...}}}]
joannas@frabase2:~/casp15/r1117> more 3fu2C_tmp1.sbs
1;1
SS1
ATOM      1  P      A C  1      13.409  43.199  48.293  1.00 11.89      P
ATOM      2  OP1    A C  1      13.180  44.569  47.775  1.00 14.35      O
ATOM      3  OP2    A C  1      14.512  42.928  49.229  1.00 12.68      O
ATOM      4  O5'    A C  1      12.097  42.630  48.984  1.00 10.34      O
ATOM      5  C5'    A C  1      10.783  42.741  48.264  1.00 42.59      C
ATOM      6  C4'    A C  1       9.788  41.994  49.122  1.00 41.20      C
ATOM      7  O4'    A C  1      10.123  40.585  49.163  1.00 41.02      O
ATOM      8  C3'    A C  1       9.737  42.408  50.579  1.00 40.03      C
```

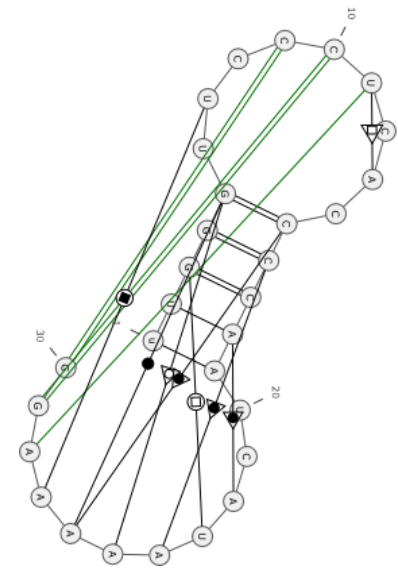
Web server vs human model



predicted from the
consensus 2D structure



modeled from a
homologous 3D template



estimated accuracy: 4 Å

Results of R1117 structure prediction

153 riboswitch models submitted by 34 participating groups

#	Model	Gr. Code	Gr. Name	vs structure							
				RMSD (glob.)	LDDT	TMscore	GDT_TS	INF_all	INF_stack	INF_wc	INF_nwc
1.	R1117TS287_5	287	Chen	2.82	0.709	0.416	85.34	0.85	0.86	1.00	0.68
2.	R1117TS287_2	287	Chen	2.25	0.736	0.387	84.48	0.84	0.86	1.00	0.60
3.	R1117TS128_1	128	GeneSilico	2.43	0.694	0.303	79.31	0.84	0.86	1.00	0.63
4.	R1117TS287_1	287	Chen	2.47	0.729	0.407	86.21	0.83	0.86	1.00	0.58
5.	R1117TS232_4	232	AIchemy_RNA2	2.64	0.728	0.378	82.76	0.83	0.86	1.00	0.60
6.	R1117TS232_2	232	AIchemv_RNA2	2.51	0.732	0.425	85.34	0.82	0.81	1.00	0.68
7.	R1117TS081_1	081	RNApolis	2.74	0.655	0.308	80.17	0.82	0.79	1.00	0.74
8.	R1117TS287_3	287	Chen	2.27	0.689	0.426	86.21	0.81	0.80	1.00	0.67
9.	R1117TS128_3	128	GeneSilico	2.57	0.689	0.301	78.45	0.81	0.91	0.93	0.47
10.	R1117TS128_4	128	GeneSilico	2.99	0.672	0.249	74.14	0.81	0.86	1.00	0.50
11.	R1117TS232_1	232	AIchemy_RNA2	2.27	0.747	0.401	85.34	0.80	0.76	1.00	0.76
12.	R1117TS232_5	232	AIchemy_RNA2	2.75	0.713	0.396	84.48	0.80	0.79	1.00	0.63
13.	R1117TS287_4	287	Chen	2.01	0.705	0.371	82.76	0.80	0.83	1.00	0.54
14.	R1117TS081_5	081	RNApolis	4.44	0.514	0.175	63.80	0.76	0.82	1.00	0.24
15.	R1117TS185_5	185	BAKER	3.73	0.567	0.191	68.11	0.75	0.85	0.93	0.00

Overall metric summary

RMSD: 2.01 – 27.68 Å

LDDT: 0.179 – 0.747

INF_{all}: 0.28 – 0.85

INF_{wc}: 0.00 – 1.00

INF_{nwc}: 0.00 – 0.76

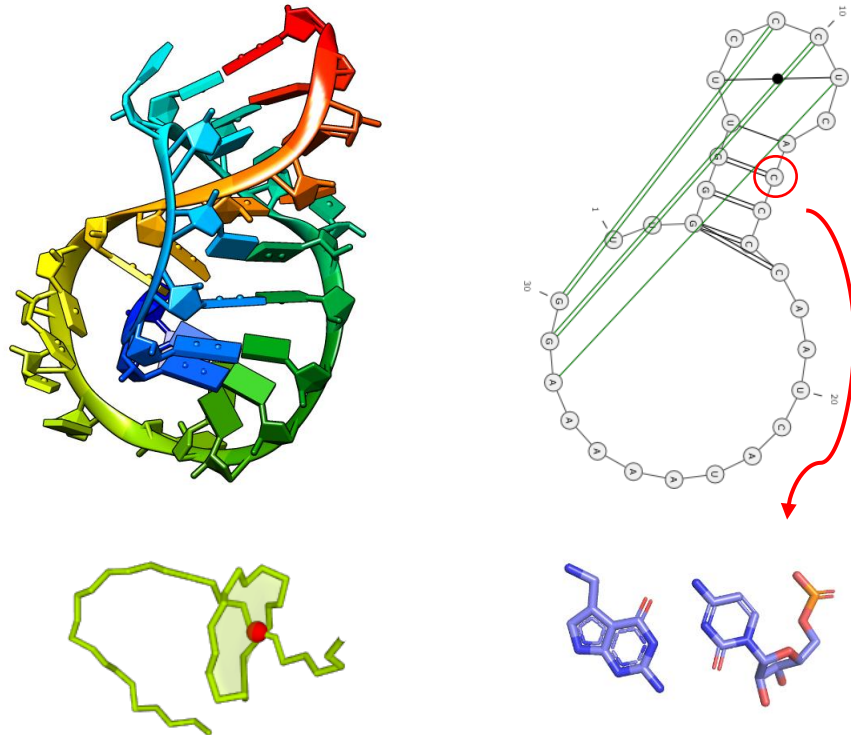
INF_{stack}: 0.37 – 0.91

Ranked 7th by INF_{all}

Ranked 13th by RMSD

Some models with lasso loops

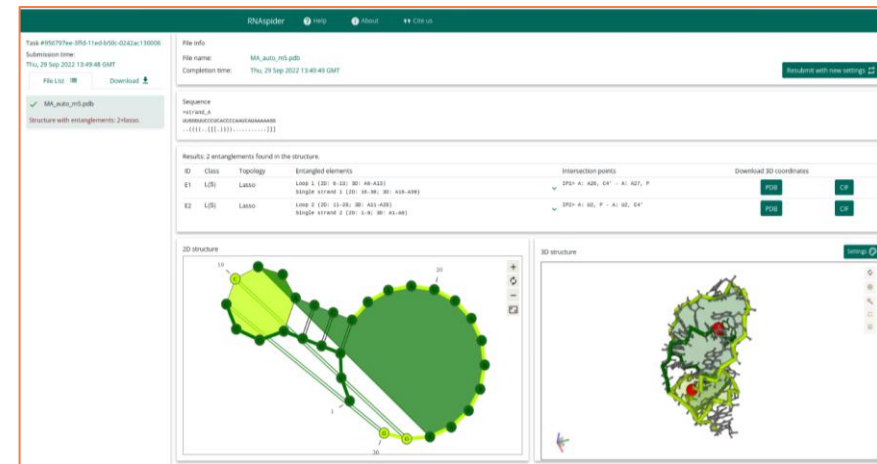
- Mispredicted 2D structure
- C14, which binds the ligand, is involved in HB.



- Lasso loop detected during topology validation.

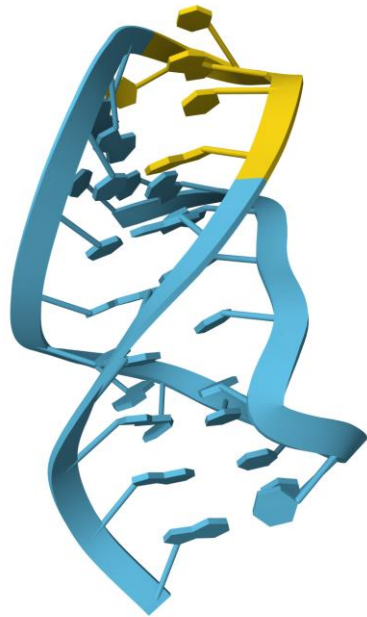


RNAspider: <https://rnaspider.cs.put.poznan.pl/>



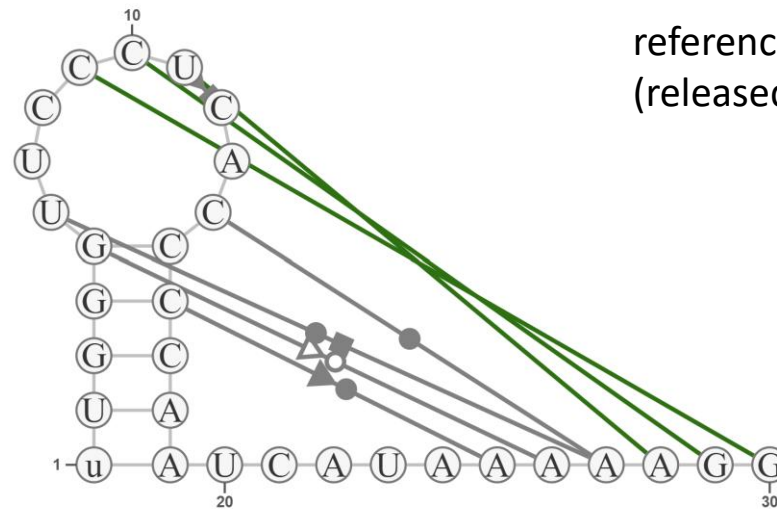
Popenda *et al. Nucleic Acids Res* 2021, 49(17):9625-9632
Luwanski *et al. Nucleic Acids Res* 2022, 50(W1):W663-W669

Out of curiosity I ran AlphaFold3



prediction obtained: 13.07.2026

reference structure: 8FZA
(released: 30.08.2023)



pLDDT Confidence Score

- Very high (pLDDT > 90)
- Confident (90 > pLDDT > 70)
- Low (70 > pLDDT > 50)
- Very low (pLDDT < 50)

RMSD:	3.14 Å	→	24 th in the RMSD ranking
LDDT:	0.641	→	14 th in the LDDT ranking
INF _{all} :	0.700	→	26 th in the INF _{all} ranking
TM-score:	0.263	→	36 th in the TM-score ranking

Acknowledgements

RNApolis team
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& RNA-Puzzles:

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Mariusz Popenda

Joanna Sarzynska

Tomasz Zok

Marta Szachniuk



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