



# Predicting RNA 2D and 3D structures with **SQUARNA** and **R3FOLD**

---

Eugene F. Baulin

Laboratory of RNA Algorithms

The International Institute of Molecular Mechanisms and Machines

Polish Academy of Sciences

Benasque, 5-17 July 2026

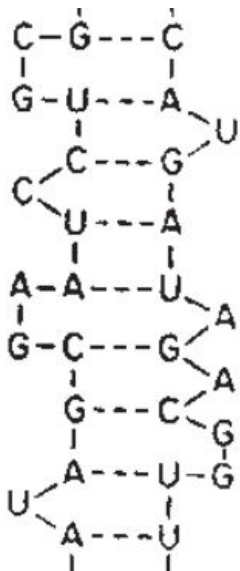
# Benasque 2022 & 2024



## 2D. Benasque 2024



### Maximum Weighted Matching

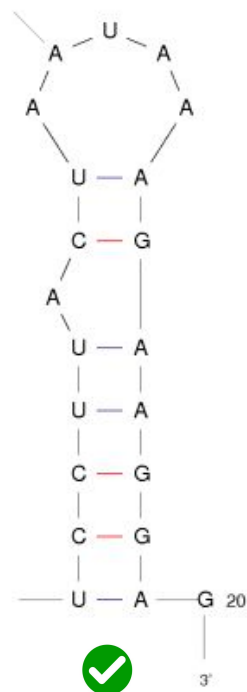


|   | C | G | A | A | G | U | C | C | U | U | U | A | C | G | U | A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| G | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 1 | 1 | 1 | 0 | 3 | 0 | 1 | 0 |
| A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 0 | 0 | 0 | 2 | 0 |
| A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 0 | 0 | 0 | 2 | 0 |
| G | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 3 | 0 | 1 | 0 |
| U | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 2 |
| C | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| C | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| U | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| U | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| U | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| U | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

base pair weights

|     | C | G | A | A | G | U | C | C | U | U | U | A | C | G | U | A |
|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C ( | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 |
| G ( | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 1 | 3 | 5 | 0 | 6 | 0 | 1 | 0 |
| A ( | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 5 | 5 | 0 | 0 | 0 | 2 | 0 |
| A ( | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5 | 3 | 0 | 0 | 0 | 2 | 0 |
| G ( | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 1 | 0 | 5 | 0 | 2 | 0 |
| U . | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 2 | 0 | 2 |
| C . | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| C . | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| U ) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| U ) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| U ) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| A . | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C ) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G ) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| U . | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A . | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

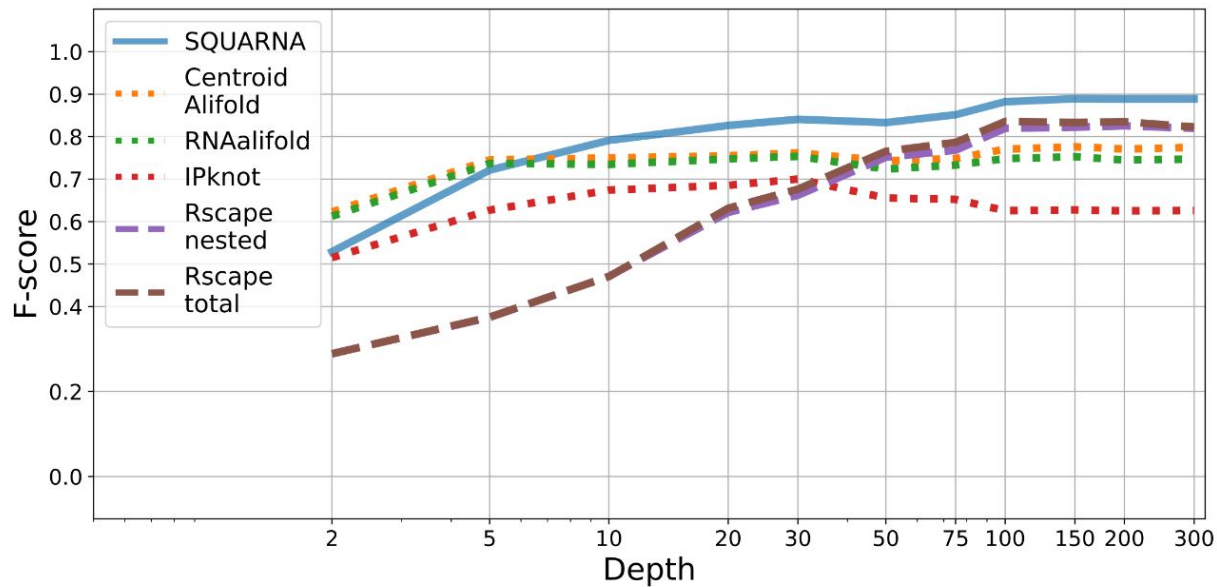
stem weights



## 2D. Benasque 2024

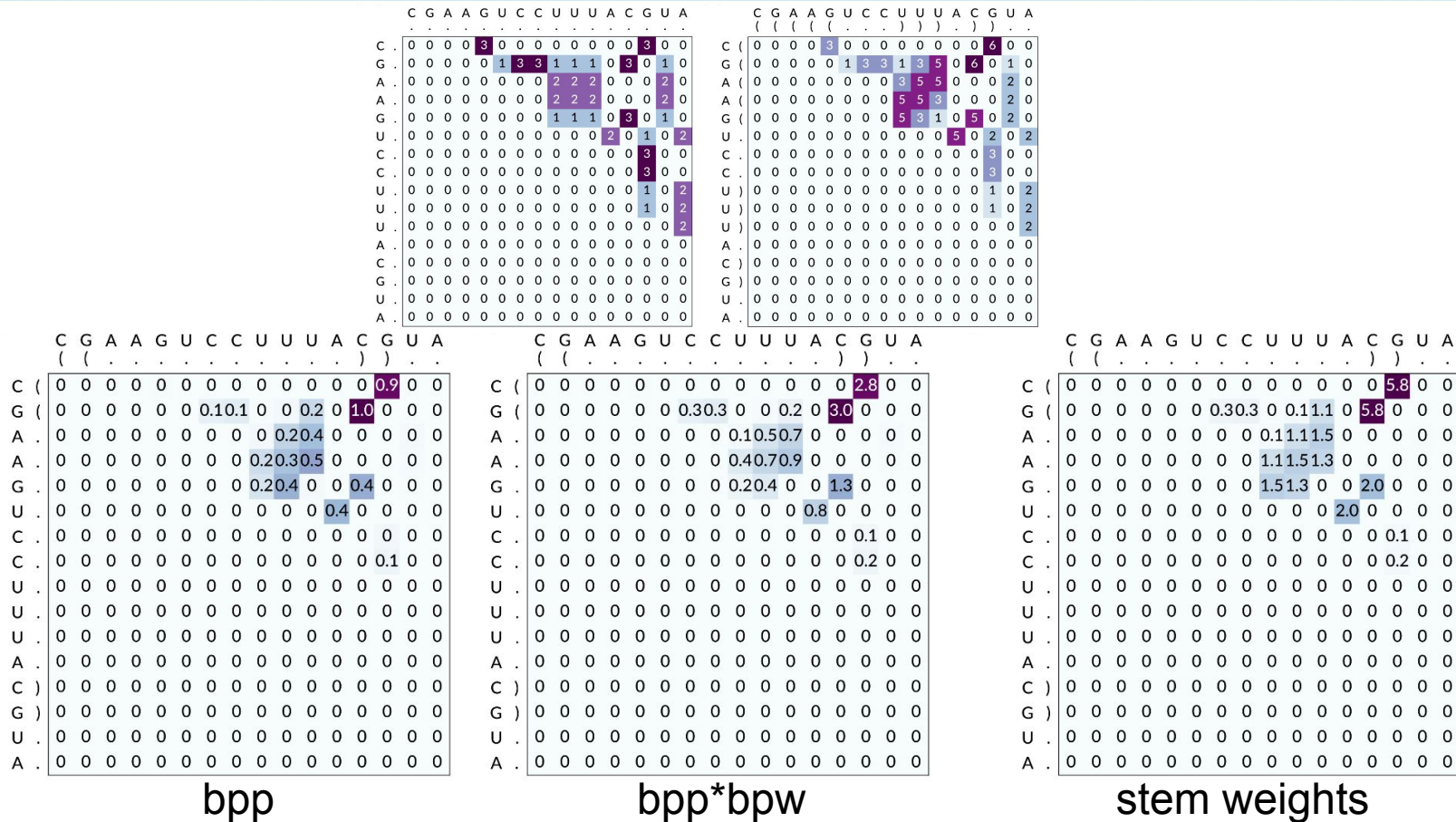


|         |   |   |   |   |   |   |   |   |    |    |    |   |    |    |    |   |
|---------|---|---|---|---|---|---|---|---|----|----|----|---|----|----|----|---|
|         | C | G | A | A | G | U | C | C | U  | U  | U  | A | C  | G  | U  | A |
|         | G | C | A | A | G | U | C | C | U  | U  | U  | A | G  | C  | U  | A |
|         | A | A | A | A | G | U | C | C | U  | U  | U  | - | U  | U  | U  | A |
|         | ( | ( | ( | ( | . | . | . | ) | )  | )  | )  | . | )  | .  | .  | . |
| C G A ( | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 5  | 5  | 7  | 0 | 8  | 21 | 10 | 0 |
| G C A ( | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 5  | 9  | 13 | 0 | 21 | 9  | 8  | 0 |
| A A A ( | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9  | 15 | 21 | 0 | 9  | 7  | 9  | 0 |
| A A A ( | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 21 | 15 | 0 | 7  | 5  | 11 | 0 |
| G G G ( | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 15 | 9  | 0 | 10 | 9  | 4  | 0 |
| U U U . | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 0  | 7 | 6  | 2  | 0  | 6 |
| C C C . | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 0  | 0 | 3  | 3  | 0  | 0 |
| C C C . | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 0  | 0 | 3  | 3  | 0  | 0 |
| U C U ) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 0  | 0 | 3  | 1  | 0  | 4 |
| U U U ) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 0  | 0 | 0  | 1  | 0  | 6 |
| U U U ) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 6 |
| A A - . | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0 |
| C G U ) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0 |
| G C U ) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0 |
| U U U . | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0 |
| A A A . | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0 |





## 2D. Single-sequence improvement - BPPs





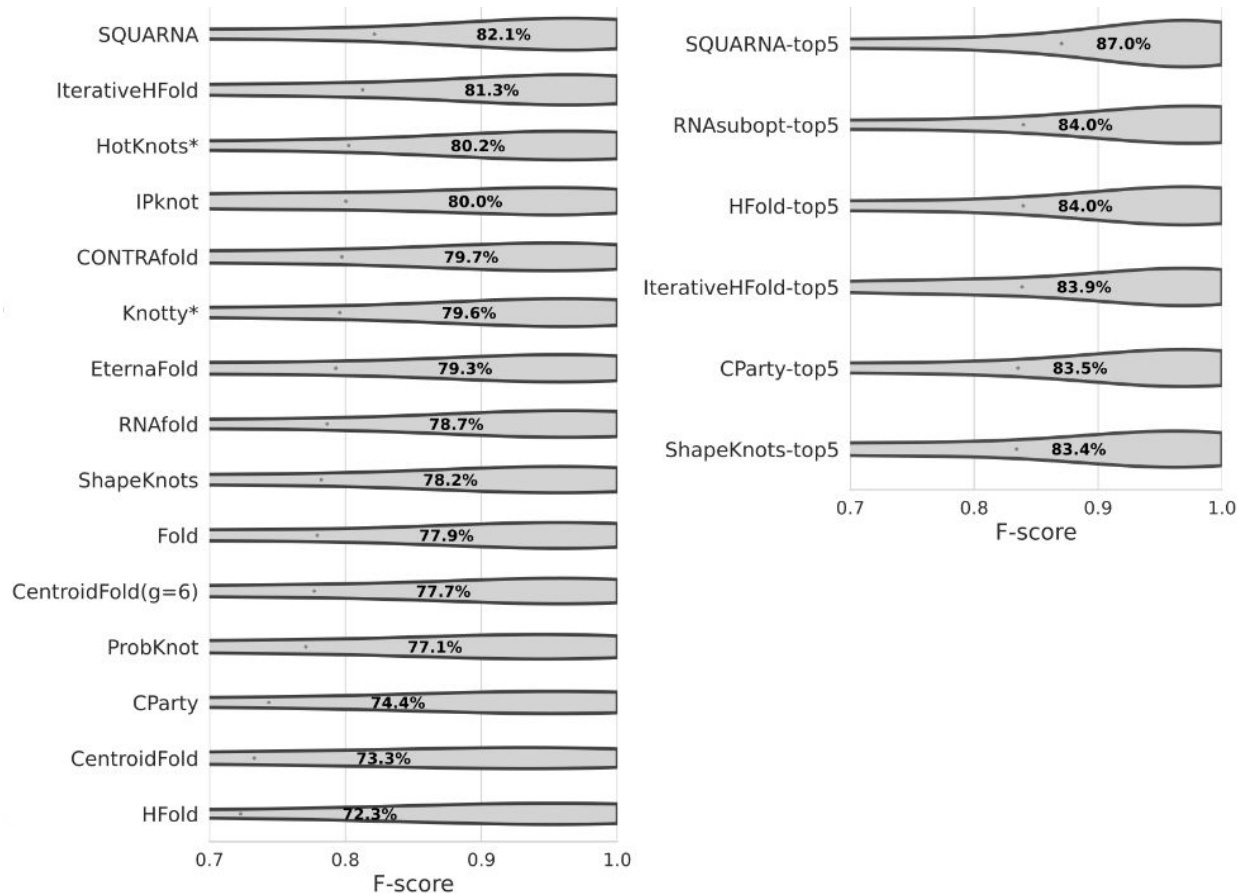
## 2D. How it worked

### “Train” dataset:

274 non-redundant RNA sequences from PDB

### Test dataset:

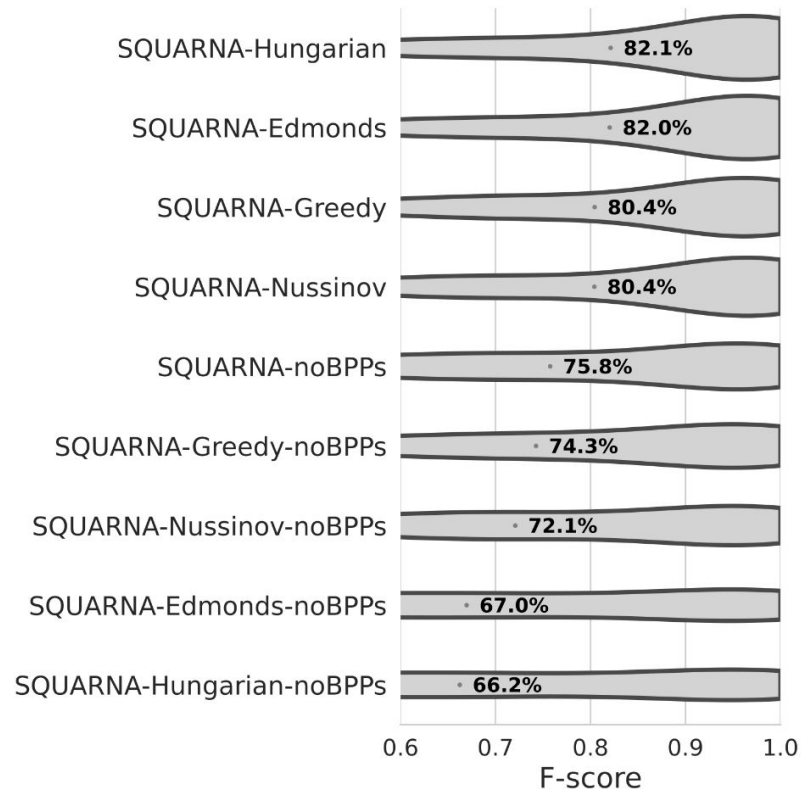
247 non-redundant RNA sequences from PDB, all < 80% similarity from train data



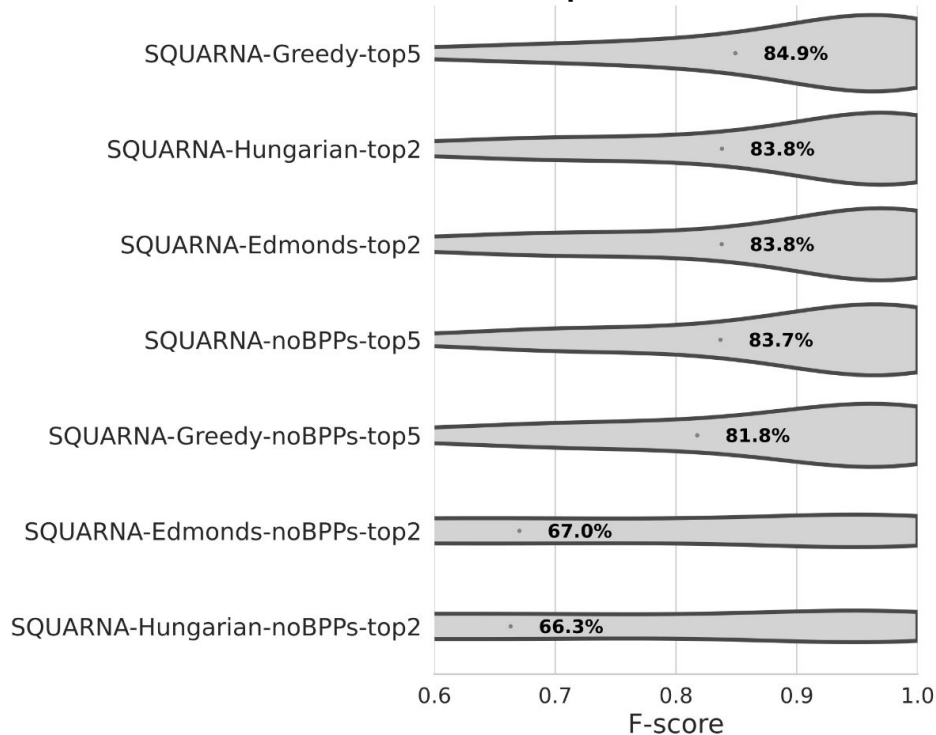
## 2D. Under the hood



### SQUARNA 82.1%



### SQUARNA-top5 87.0%



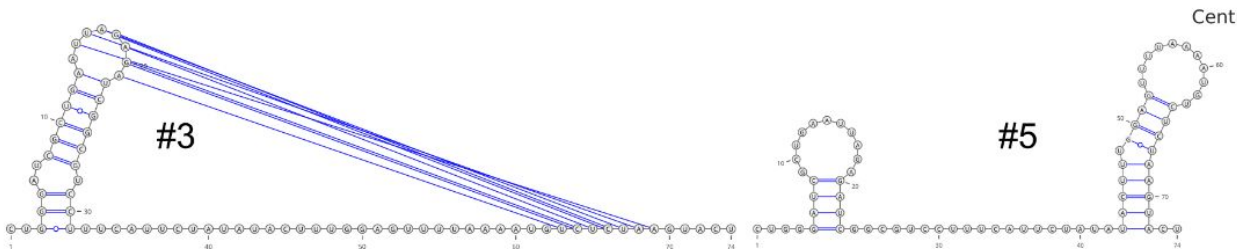
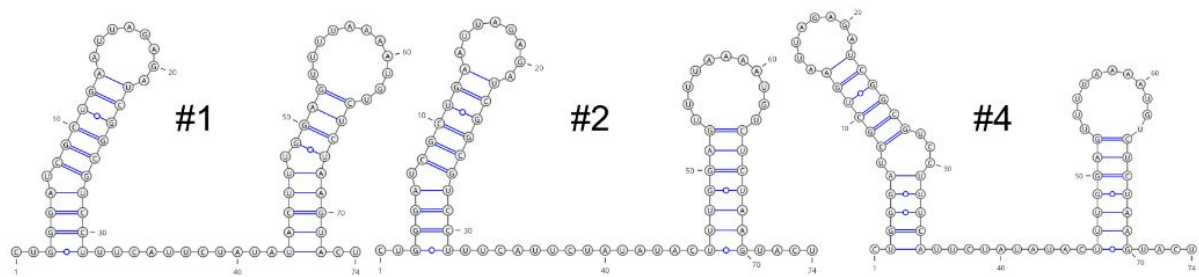
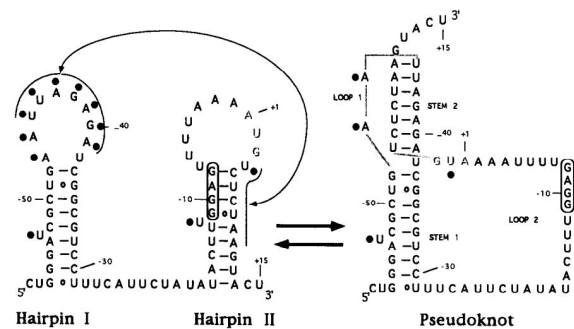


## 2D. Example: S15 leader sequence

CUGGAUCGCGUGAAUUAGAGAUCCGCGUCCUUUCAUUCUAUAUACUUUGGAGUUUUAAAAUGUCUCUAAGUACU

```

=====
..(((.(((.(((.....)))))))).(((.(((.(((.....)))))))). #1
..(((.(((.(((.....)))))))).(((.(((.(((.....)))))))). #2
..(((.(((.(((.([[[[[[[]]]]])).))).....]]]]]]].... #3
.(((.(((.(((.....)))))))).(((.(((.(((.....)))))))). #4
...(((.(((.....)))))).(((.(((.(((.....)))))))). #5
  
```



Bénard et al., 1996,  
10.1016/S0300-9084(96)80003-4

## 2D. Independent benchmark



Journal of Chemical Information and Modeling > Vol 66/Issue 7 > Article

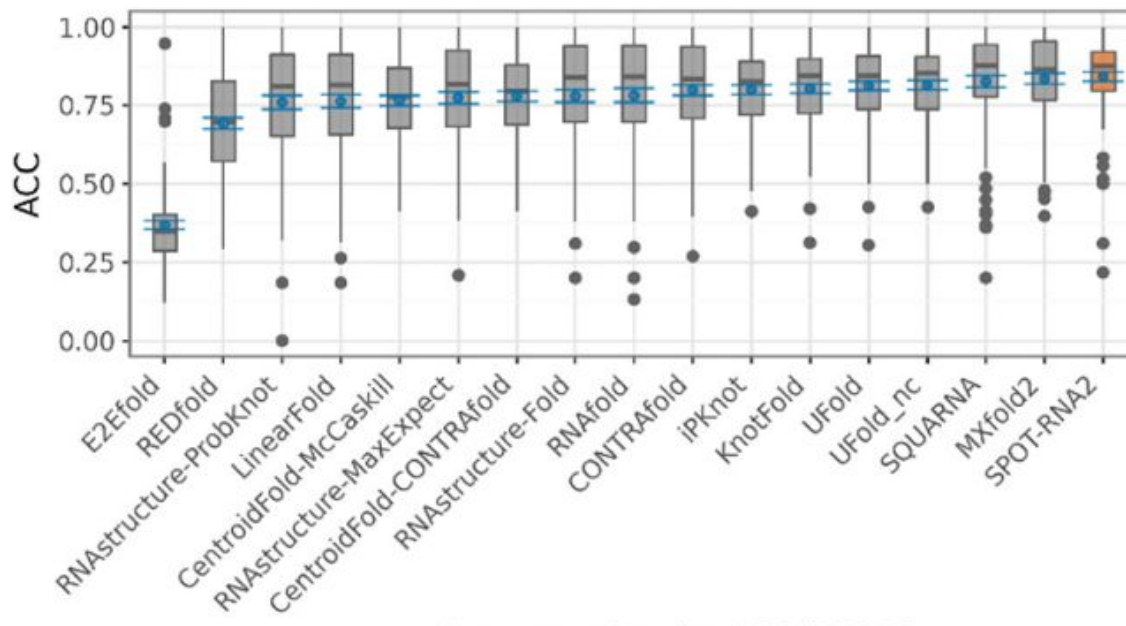
Open Access

Cite Share Jump to Expand

PHARMACEUTICAL MODELING | March 25, 2026

### Exploring Secondary Structure Predictions for RNA-Targeted Drug Discovery: Power and Challenges

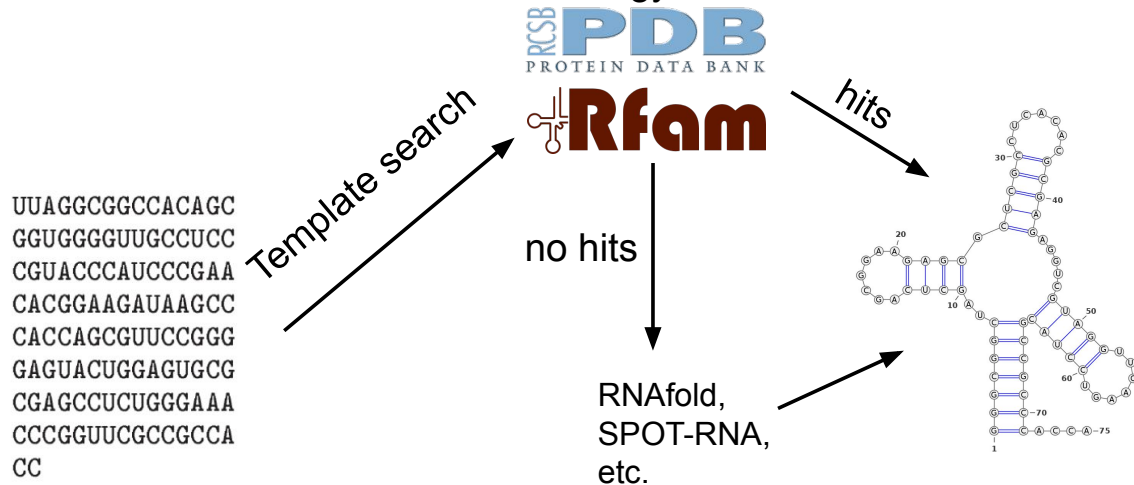
Zhengyue Zhang\*, Gaia Dolcetti, Christian Tyrchan, Leonardo De Maria, Giovanni Bussi, and Werngard Czechtizky



## 2D. And then we cheat :)



Common CASP/RNA-Puzzles strategy:



### SQUARNA + Infernal(cmscan)

```
~$ squarna s=GGGCGGCUAGCUCGCCUCACACGCGACAGGUCGUAGGUUCAAGUCCUACGCCGCCACCAGCGCGAAAGCGC rfam
```

```
Running Rfam search...: tRNA(1-57)
```

```
>inputseq
```

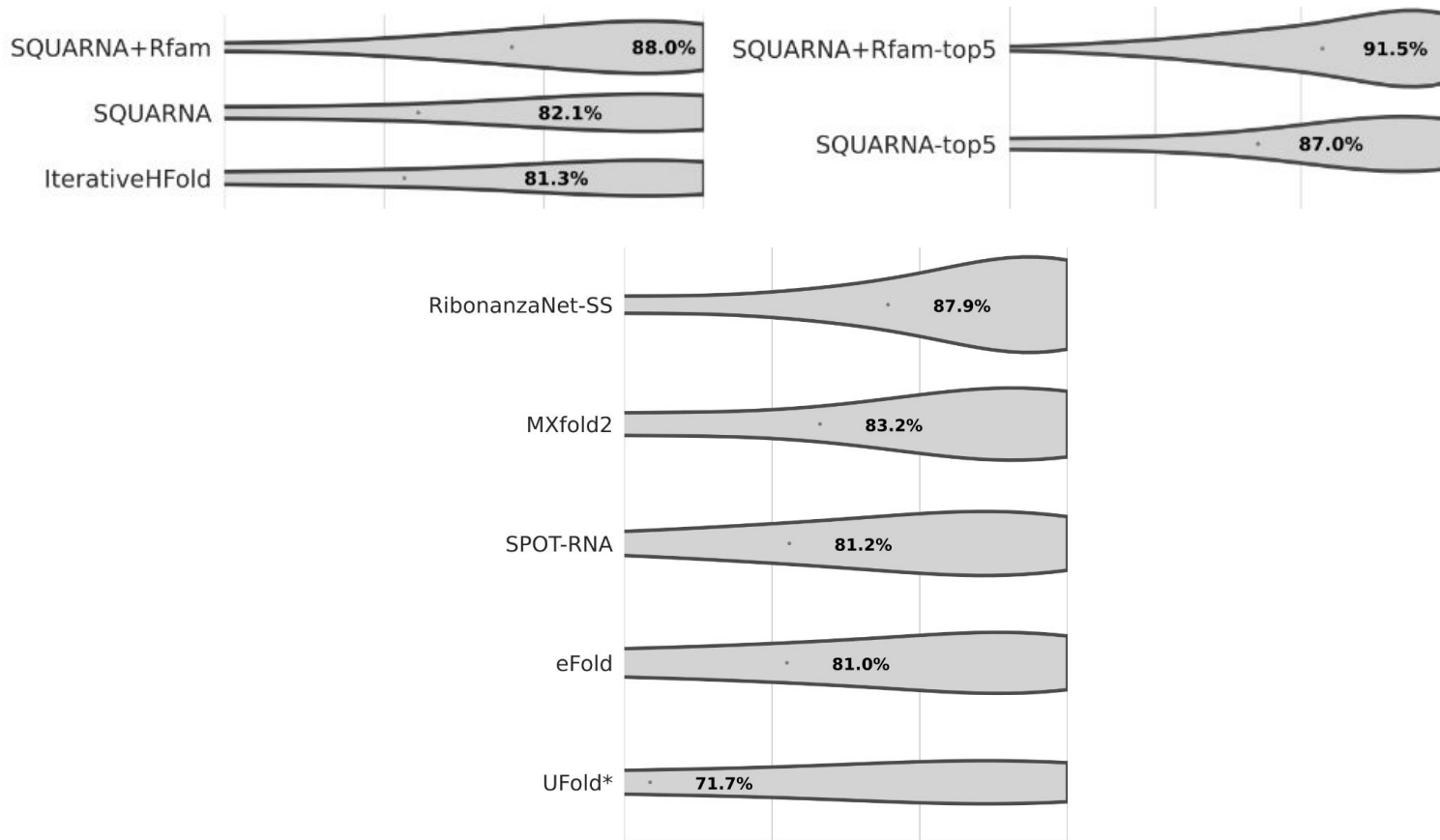
```
GGGCGGCUAGCUCGCCUCACACGCGACAGGUCGUAGGUUCAAGUCCUACGCCGCCACCAGCGCGAAAGCGC
```

```
(((((...(((.....)))))).....((((.....)))))).....restraints(tRNA(1-57))
```

```
=====
```

```
(((((...(((.....)))))).....((((.....)))))).....((((.....)))#1
```

## 2D. And then we cheat and beat AI :)





## G-quadruplex patterns

## Protein-binding motifs

# Web server

```
seq = "GCGCCAUGGGCCAUUGGGUGGGAUCUGGGAUGGCGC"
```

```
SQUARNA.Predict(inputseq = seq, g4 = True)
```

```
>inputseq
GCGCCAUUGGGCCAUAUGGGUGGGAUCUGGGAUGGCGC
.....+++.+++.+++.+++.+++.
=====
(((((((+++.+++.+++.+++.)))))))))
=====
(((((((+++.+++.+++.+++.+++.)))))))))

restraints(G4(+);
top_1_consensus
#1      103.254 206.508 0.5
```

```
seq = "GCGCCAUAAAUUGCACAAGGAGAAUGCAUGAAUGUACAUA AAAACUAACAAGAAACACAAAUGGCGC"
```

```
SQUARNA.Predict(inputseq = seq, rbp = True)
```

```
>inputseq
GCGCCAUAAAUUGCACAAGGAGAAUGCAUGAAUGUACAUA AAAACUAACAAGAAACACAAAUGGCGC
.....+++++.+++++.+++++.+++++++.+++++. (++++).
LIN28(19-23), RBF0X1/2(26-31), PUM(34-41), SF1/QKI(44-49), Fab(52-58))
```

((((( ((( . . . ++++++ . . . ++++++ . . . ++++++ . . . ++++++ . . . ++++++ . . . ++++++ . . . ) ) ) ) ) ) ) ) ) )

restraints(U:

top-1 consens

<https://larnal.imol.institute/>



## 3D. How to benchmark SQUARNA in CASP17?

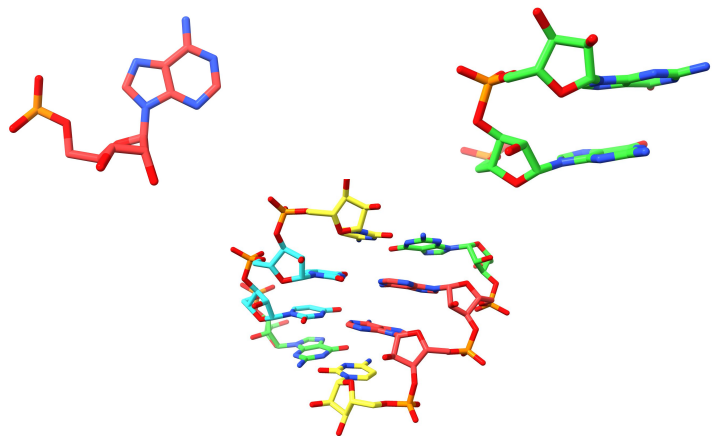
---



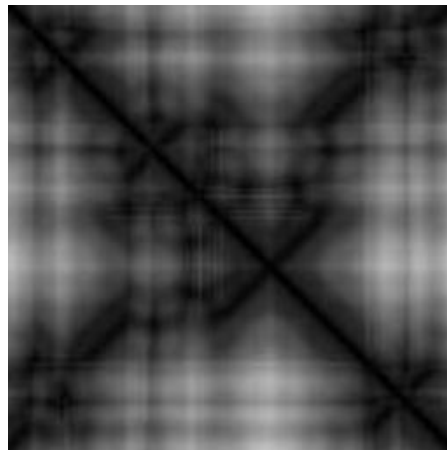


# 3D. “Dimensionality expansion”

2D -> pairwise distances -> 3D

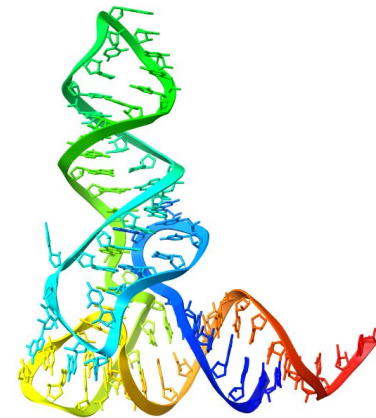


pairwise distances  $N \times N$



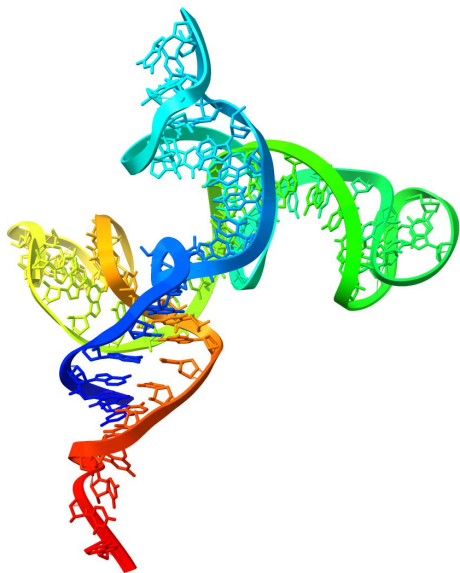
3D coordinates  $N \times 3$

MDS

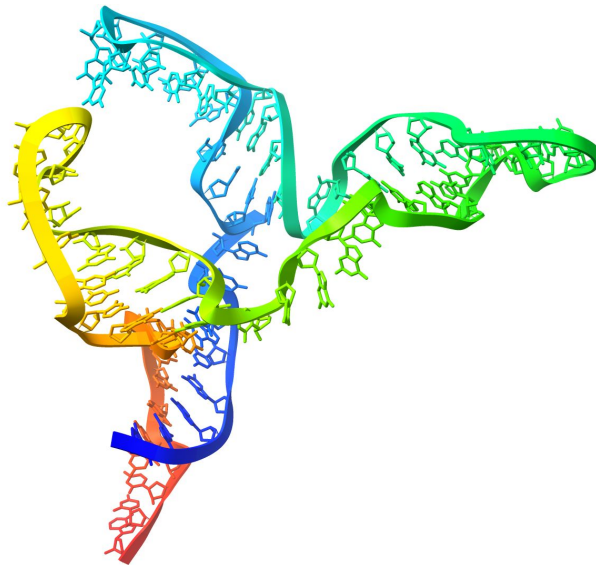


```
GGAGCGGUAGUUCAGUCGGUUAGAAUACCUGCCUGUCACGCAGGGGGUCGCGGGUUCGAGUCCCGUCCGUUCCGCCA
(((((((...((((.....))))).((((.....))))). ....((((.....)))))))))....
GGAGCGGUAGGUUCAGUCGGUUAGAAUACCUGCCUGUCACGCAGGGGGUCGCGGGUUCGAGUCCCGUCCGUUCCGCCA
(((((((...GUUC.....GAAU))))).((((.....))))). ....((((.....)))))))))....
GGAGCGGUAGUUCAGUCGGUUAGAAUACCUGCCUGUCACGCAGGGGGUCGCGGGUUCGAGUCCCGUCCGUUCCGCCA
(((((((...((((.....))))).((((.....))))).GG...((((.....)))))))))....
```

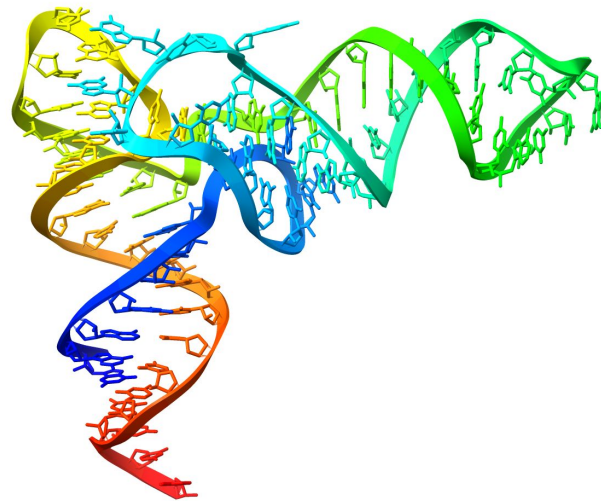
# 3D. How it worked: tRNA



MDS  
result

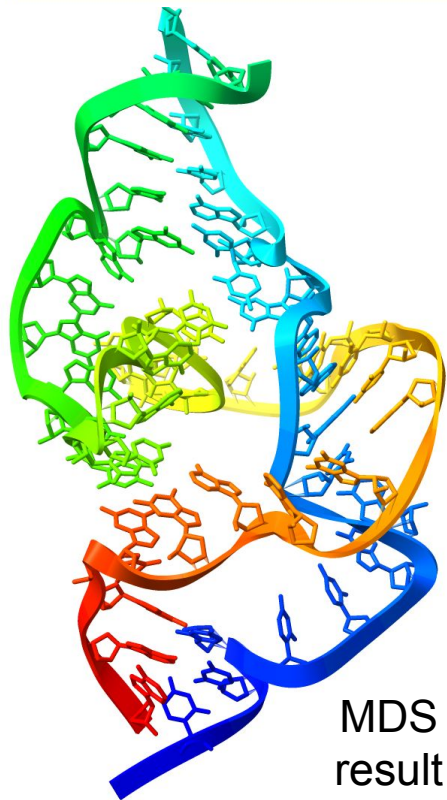


MDS  
result  
with the D-loop/T-loop  
lone pair

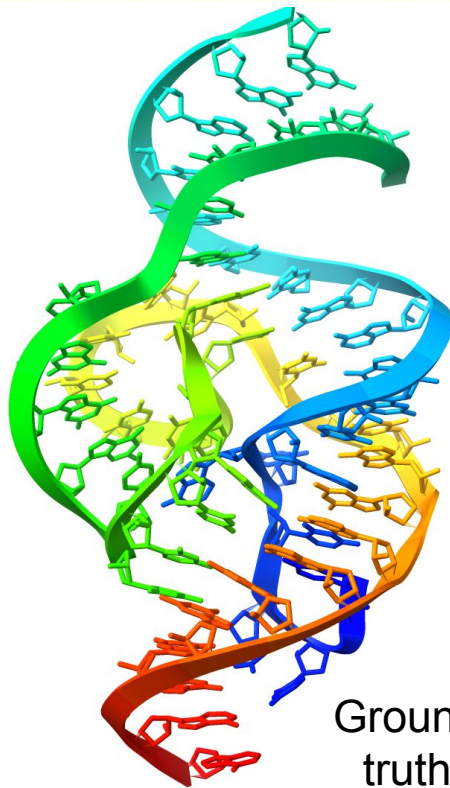


Ground  
truth

# 3D. How it worked: Twister ribozyme 4RGE



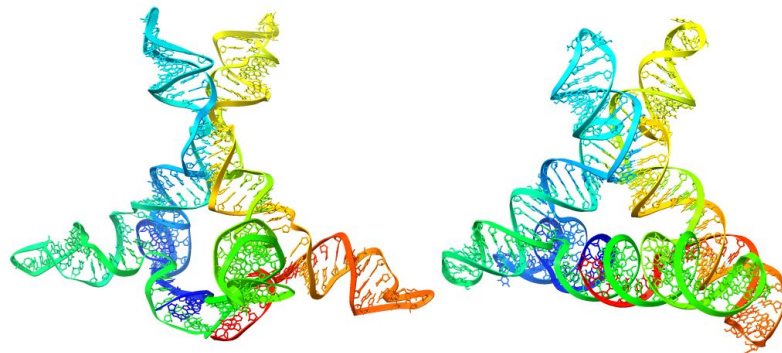
MDS  
result



Ground  
truth



They're the same picture.



Single-stranded Paranemic Crossover RNA Triangle  
8BTZ

UUUUUAAUGAAGCCACAGG&CCUGUGAGGGUCCUAAGCCCCUAAUUCAGAAGGGAAA  
.( (... (( {{ ((( (&))))).(((.[[ [..}]))).(...))..]]].)).



### 3D. R3FOLD, coming soon...

R3FOLD = SQUARNA + MDS + user restraints + three template search strategies

[LaRNAI Platform](#)   [About](#)   [Help](#)   [Standalone](#)

Contact  IMol  NATIONAL SCIENCE CENTRE  Baulin Lab

### Example 1

### Example 2

### Example 3

### Example 4

### Example 5

+ Reactivities

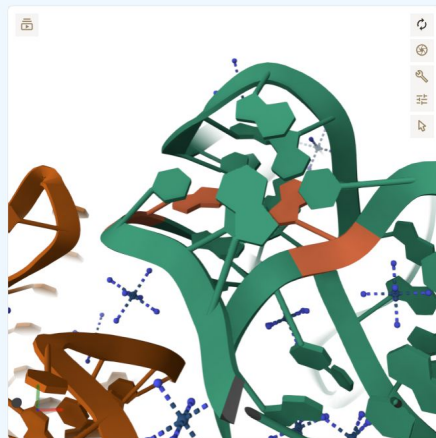
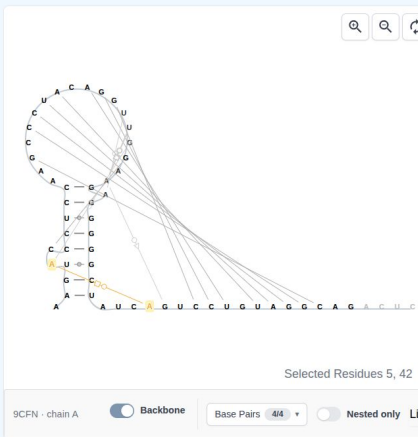
RNA sequence: ?

GGGGGCCACAGCAGAAGCGUUCACGUCGCAGCCCUGUCAGCCAUUGCACUCCGGCUGCGAAUUCUGCU

## Predict RNA 2D Structure (SQUARNA)

## Predict RNA 3D Structure (R3FOLD)

### Tertiary structure

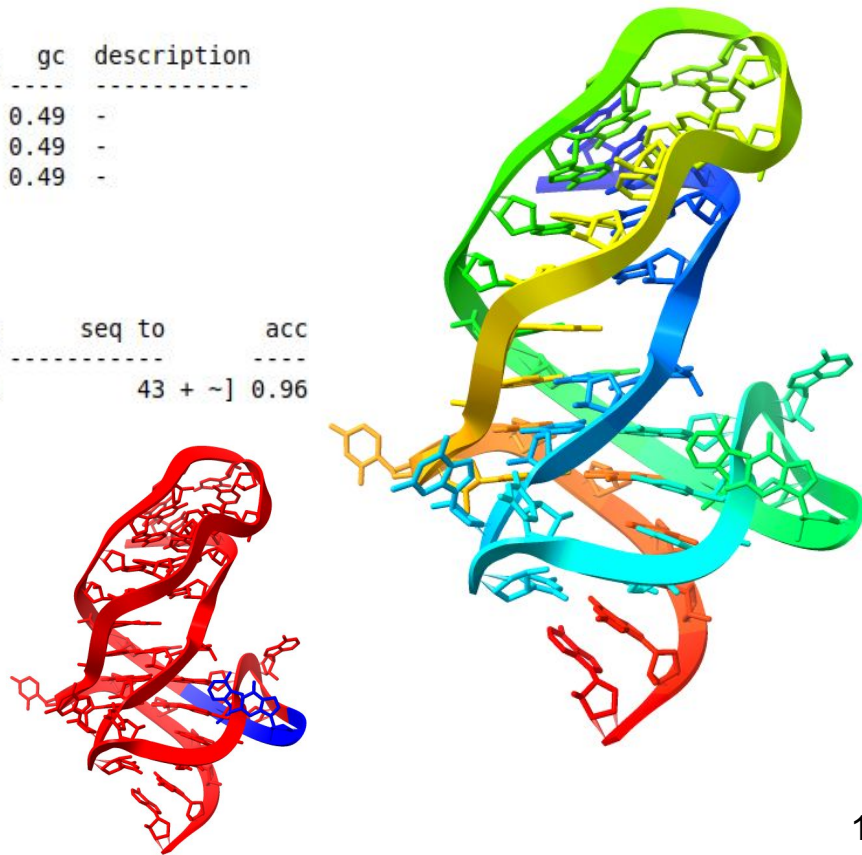


### Layered dot-bracket notation (Leontis–Westhof base pairs)

Greyed-out pairs are hidden in the current 2D view (via "Nested only" or the base-pair family filters).

[illegible]

with R2DT+Mol\* viz by  
Anton Petrov



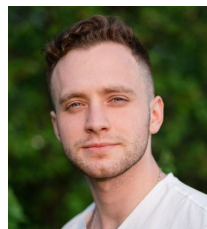


- SQUARNA is cool and all, try in Python/CLI with **pip install SQUARNA** or [larnal.imol.institute](https://larnal.imol.institute)
- MWM framework has P time algorithms, but we need better scoring matrices
- R3FOLD to be released in late 2026 / early 2027
  - 2D -> 3D transform for following simulations
  - automated template search
  - user-defined restraints
    - multiple full/partial templates
    - alignment-based / mapping-based (for permutations)

# Acknowledgments



Janusz Bujnicki



Davyd Bohdan



Grigory Nikolaev



Kunal Shewani

Maksim Serdakov



LaRNAI.imol.institute

 <https://github.com/febos/SQUARNA>

 <https://doi.org/10.64898/2026.06.30.735492>



ALTF-525-2022 fellowship  
to Eugene Baulin

## Thank you!



2017/25/B/NZ2/01294 to Janusz Bujnicki  
2024/53/B/NZ2/02718 to Eugene Baulin  
2024/55/D/NZ2/02049 to Eugene Baulin