

Vfold Modeling of RNA Targets in CASP16

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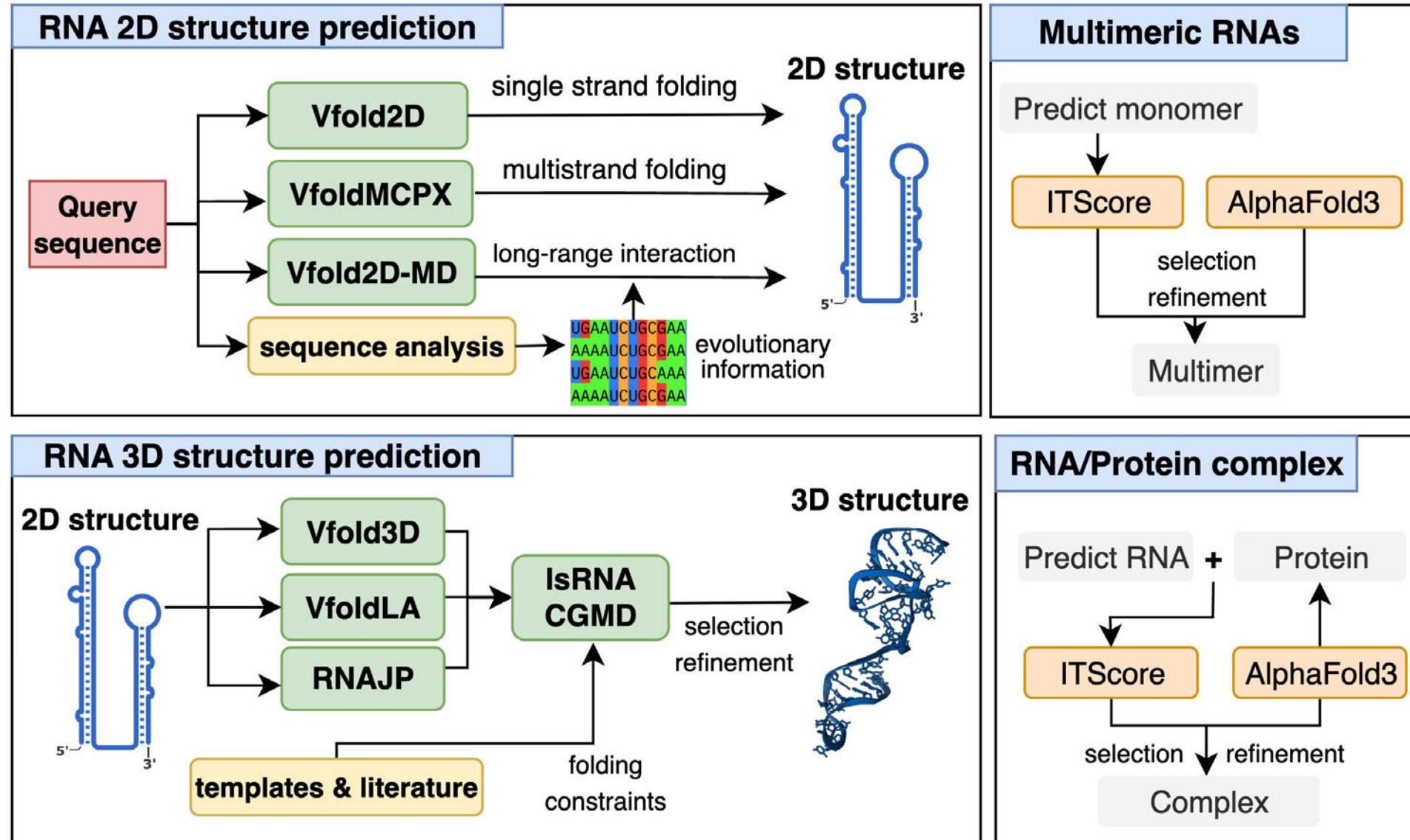
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AF3-assisted physics-based modeling

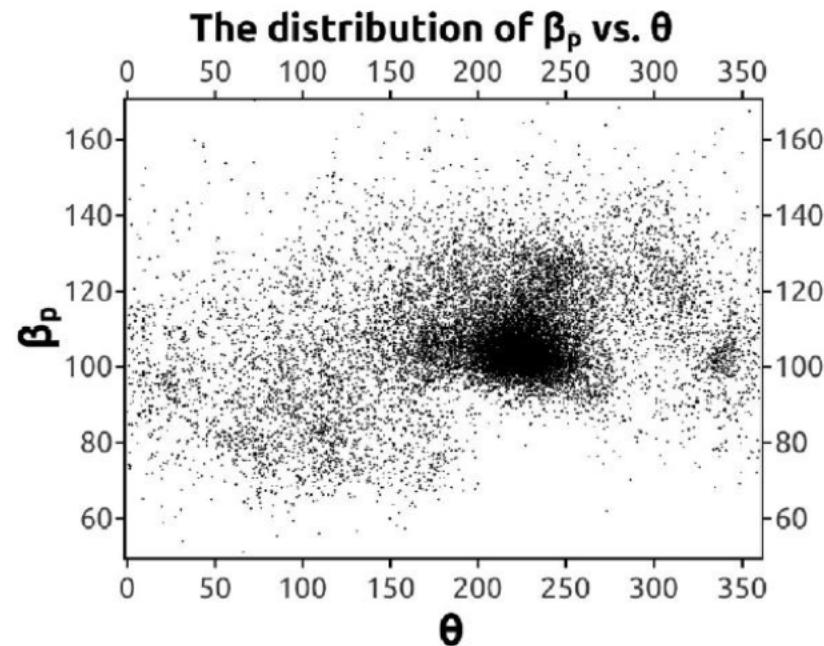
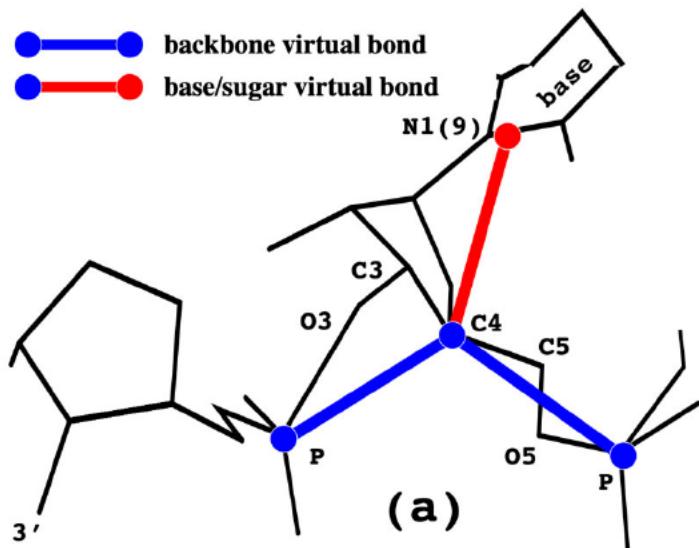


ITScore for docking: Zou lab

Vfold toolbox – Physics-based models

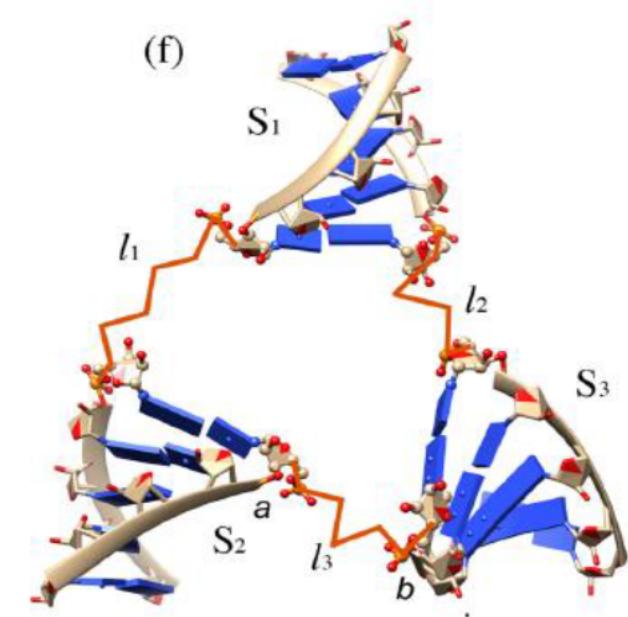
To predict 2D structure from the sequence

Free energy modeling: **Vfold2D** (monomer) **VfoldMCPX** (multimer)



A virtual bond (4 beads)
representation of RNA conformations

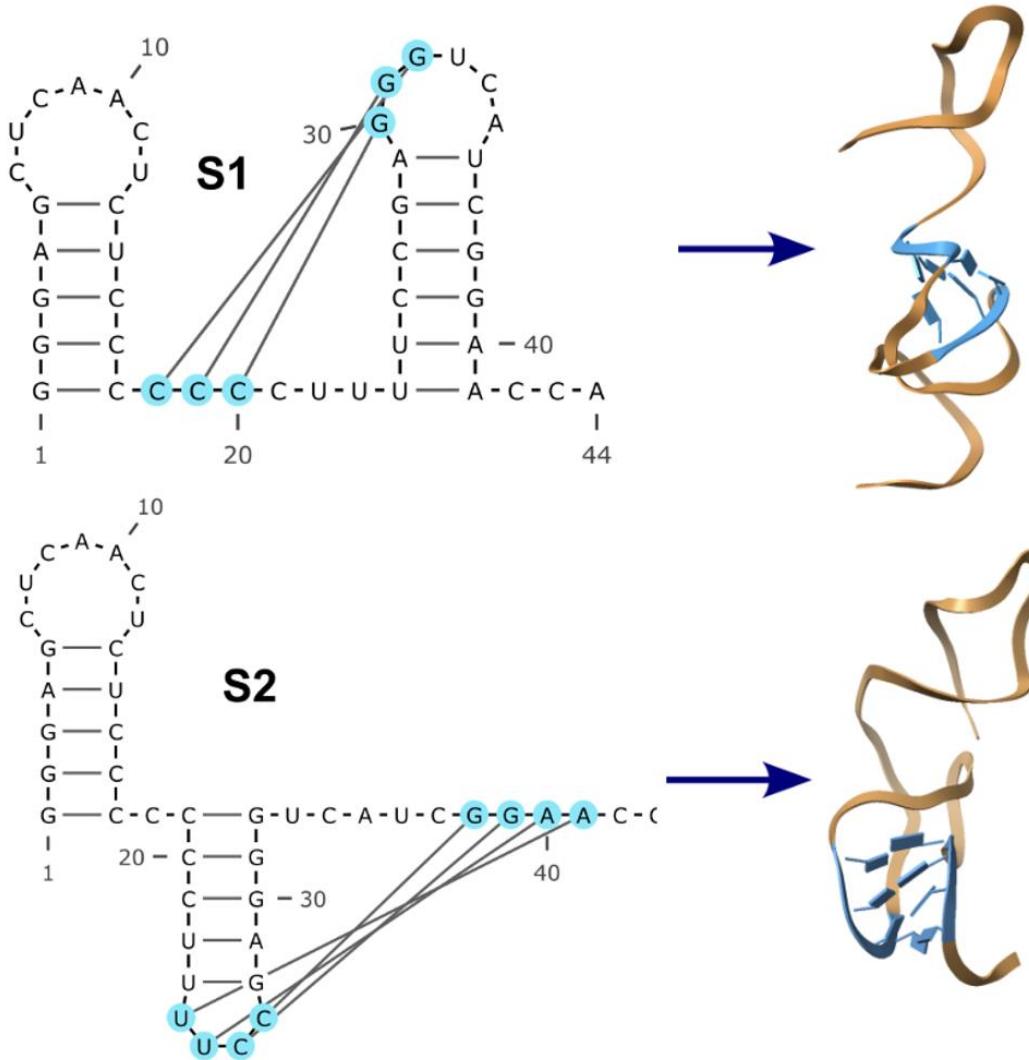
MC sampling guided by known structures



Free energy parameters for motifs

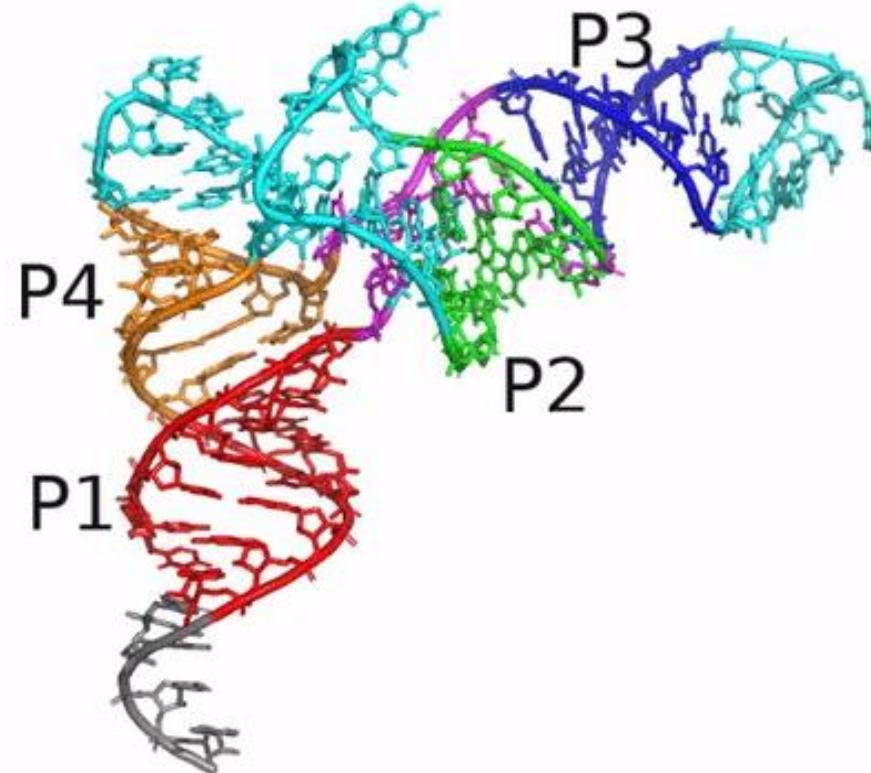
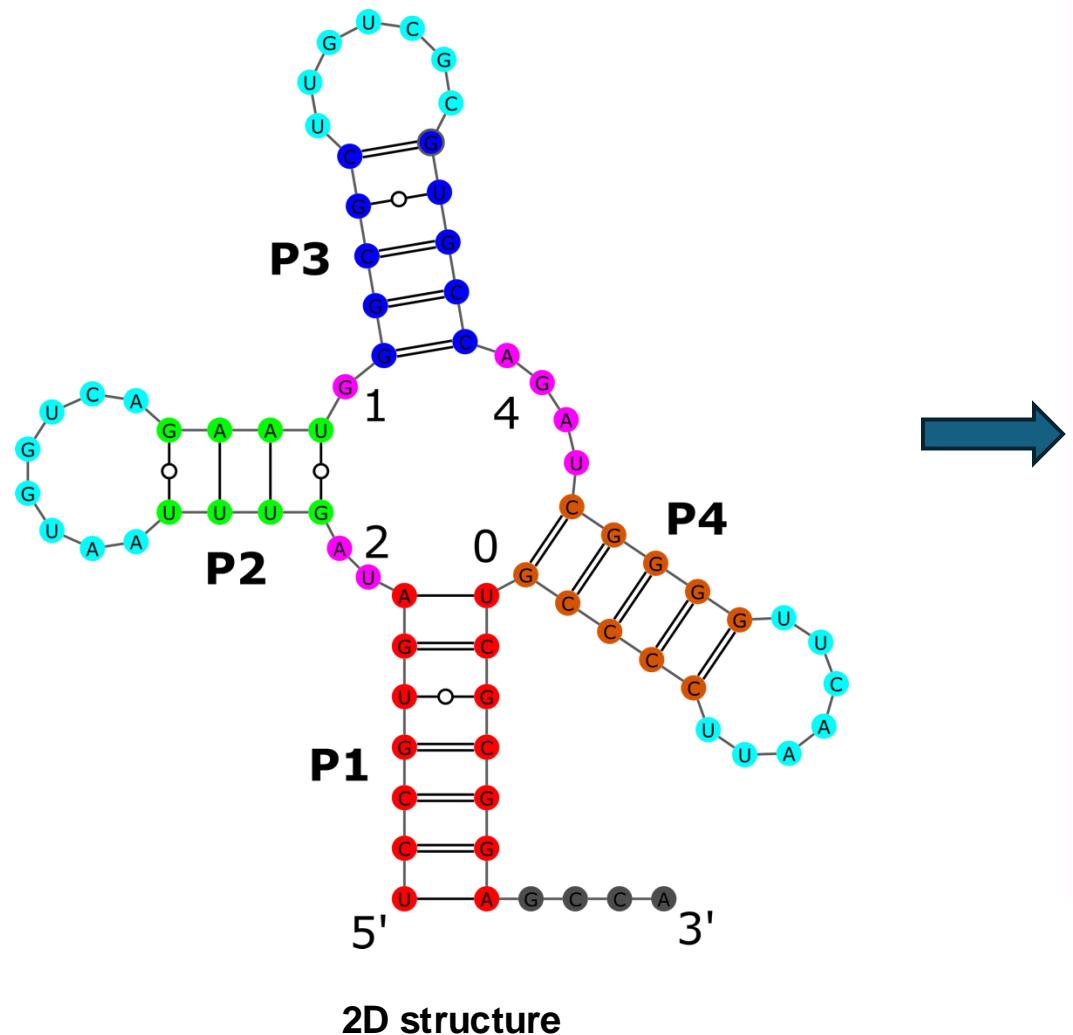
To predict long-range kissing interactions

Vfold2D-MD (unpublished)

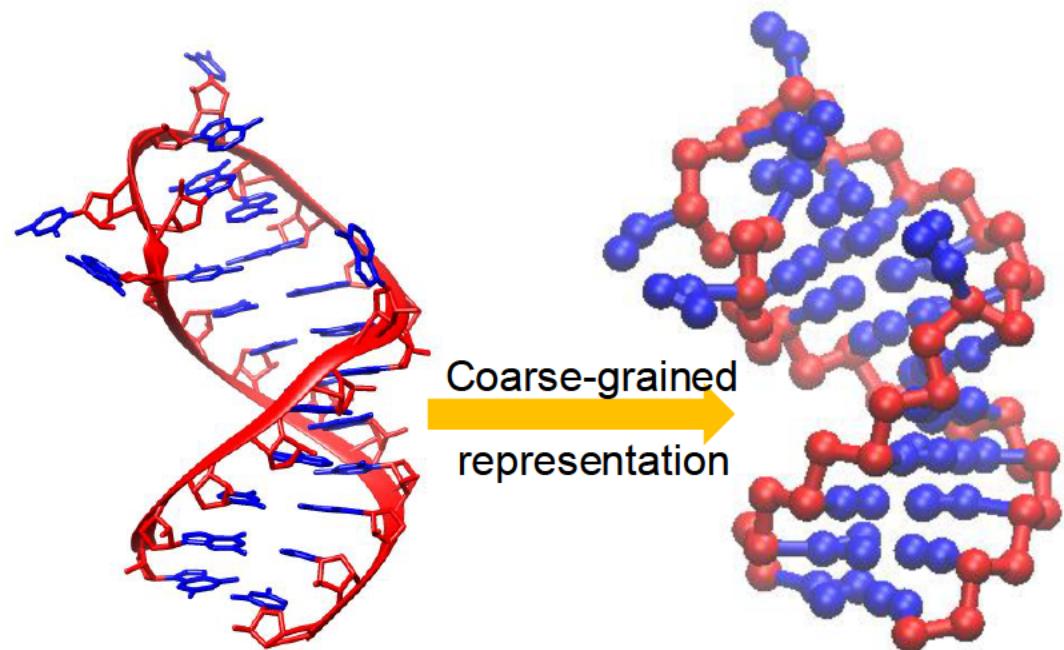


To generate (initial) 3D from 2D

Template-based modeling: Vfold3D & VfoldLA:



To predict the (final) 3D from (2D+initial 3D) MD simulation: IsRNA



Local connectivity

$$E_{total} = \boxed{\sum E_{bond}(b) + \sum E_{angle}(\theta) + \sum E_{torsion}(\phi)} \\ + \sum E_{bp}(b, \theta, \phi) + \sum E_{pair}(r) + \sum E_{LJ}(r)$$

Base pairing interactions Non-local pairwise interactions Excluded volume

A Bayesian FF derived from known structures

IsRNA: Coarse-grained molecular dynamics simulation

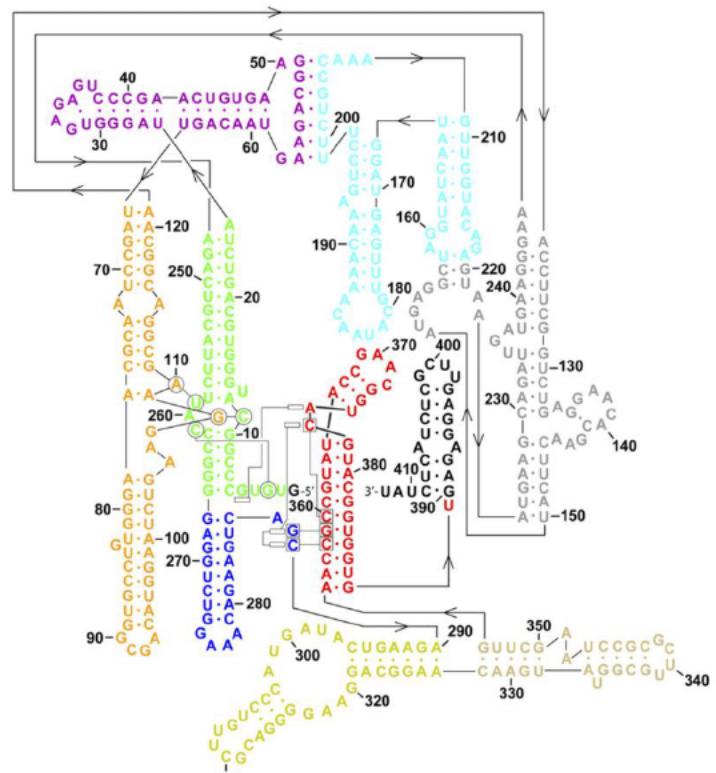
2D structure



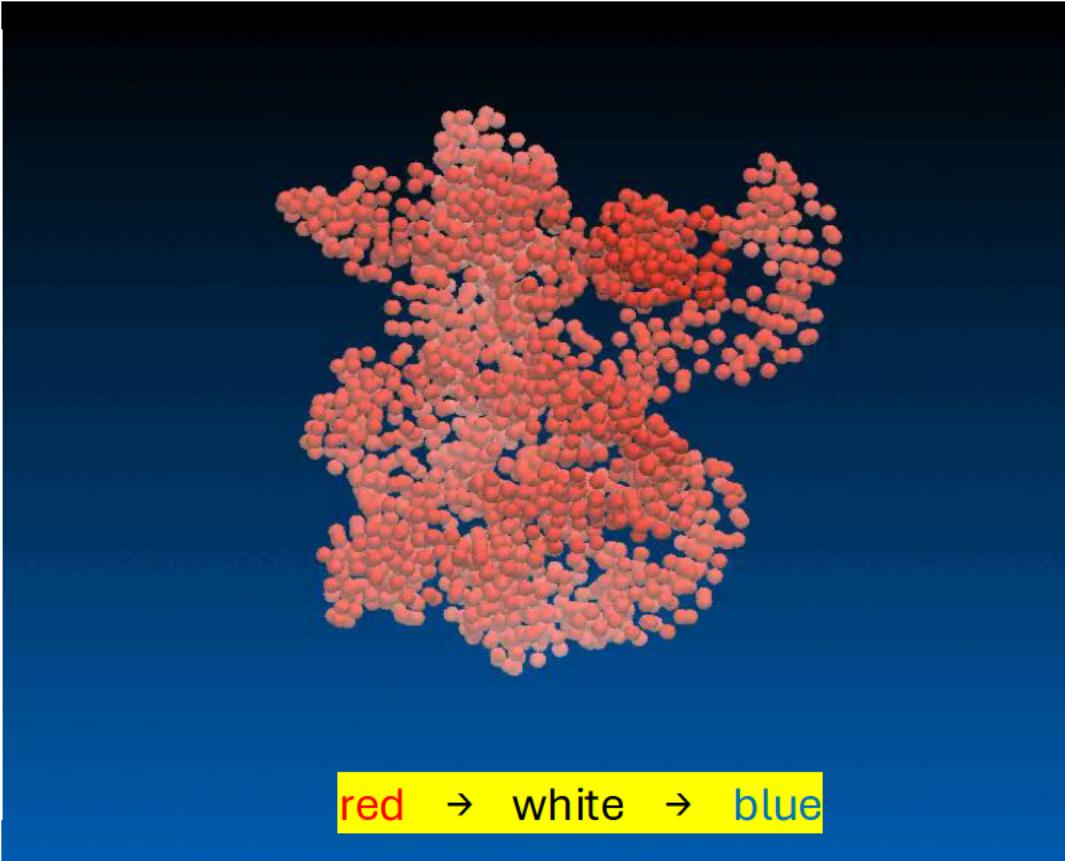
coarse-grained MD simulation



2D & 3D structures

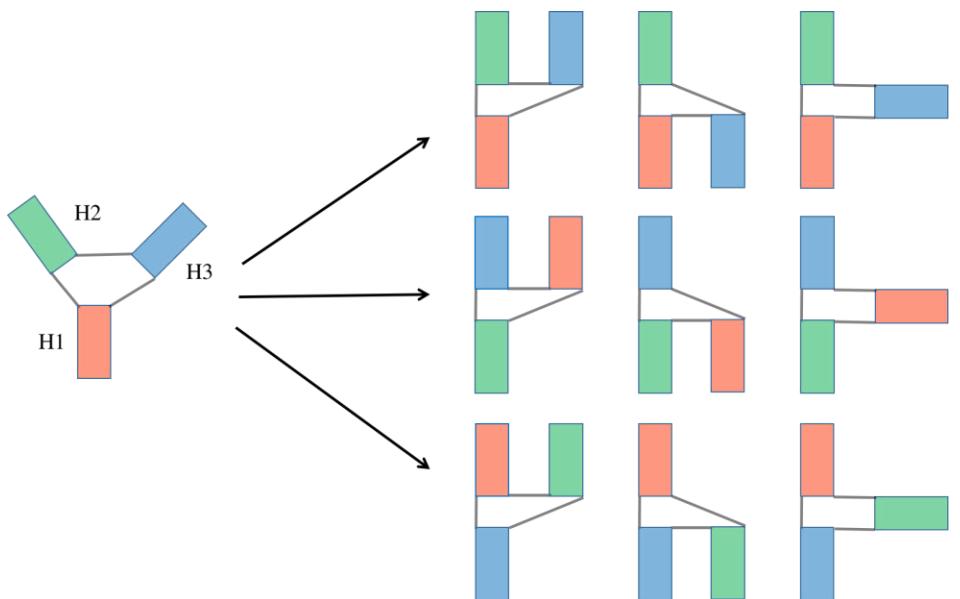


Group II intron (412 nts)



- Can also predict sub-populated alternative folds

To predict the (final) 3D from (2D+initial 3D) RNAJP: MC/MD hybrid simulation

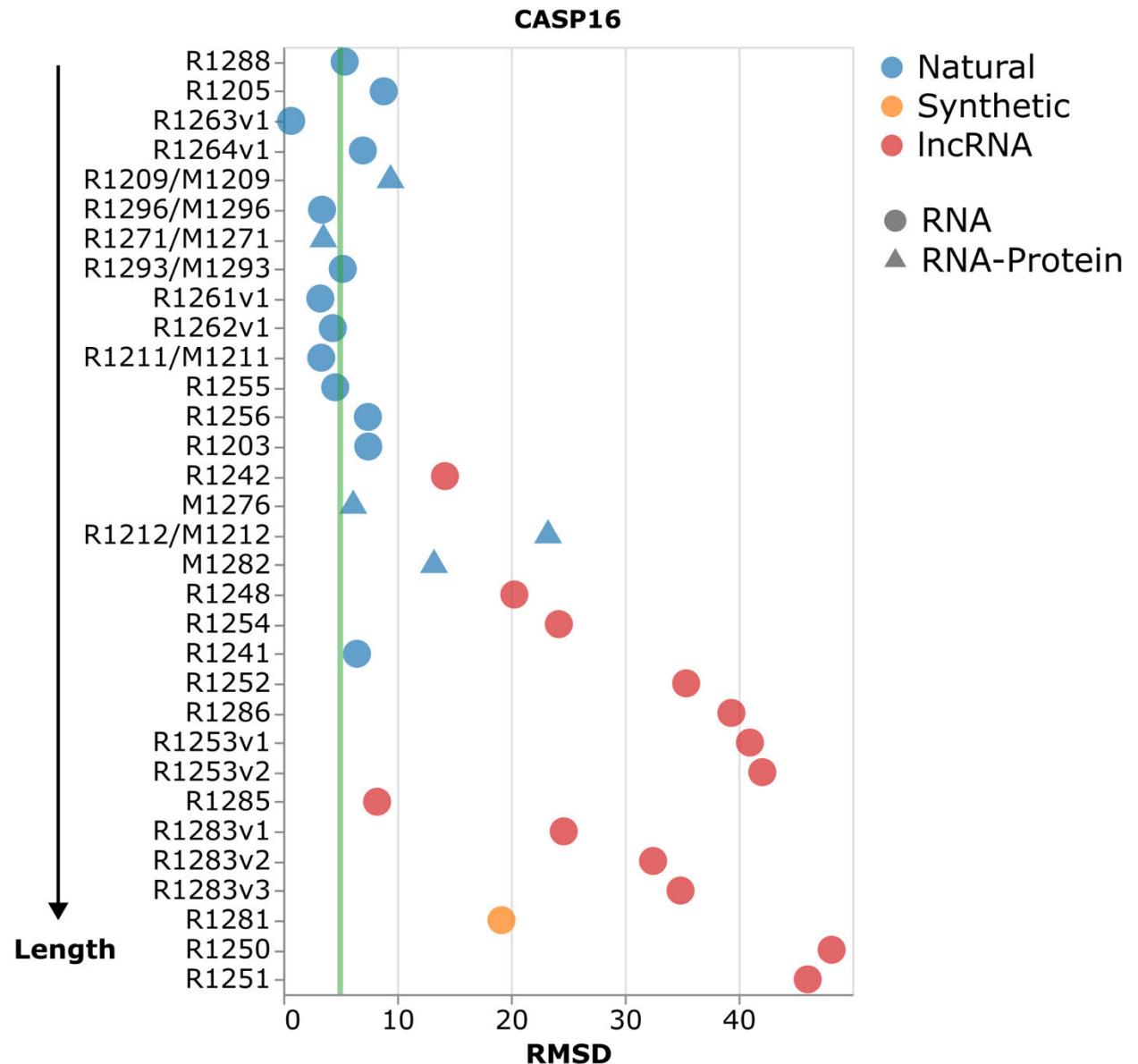


AlphaFold 3

- If AF3 models agree with the Vfold predictions
We are happy!
- If AF3 models are different from the Vfold predictions
We are also happy: AF3 model + 2D → MD simulation

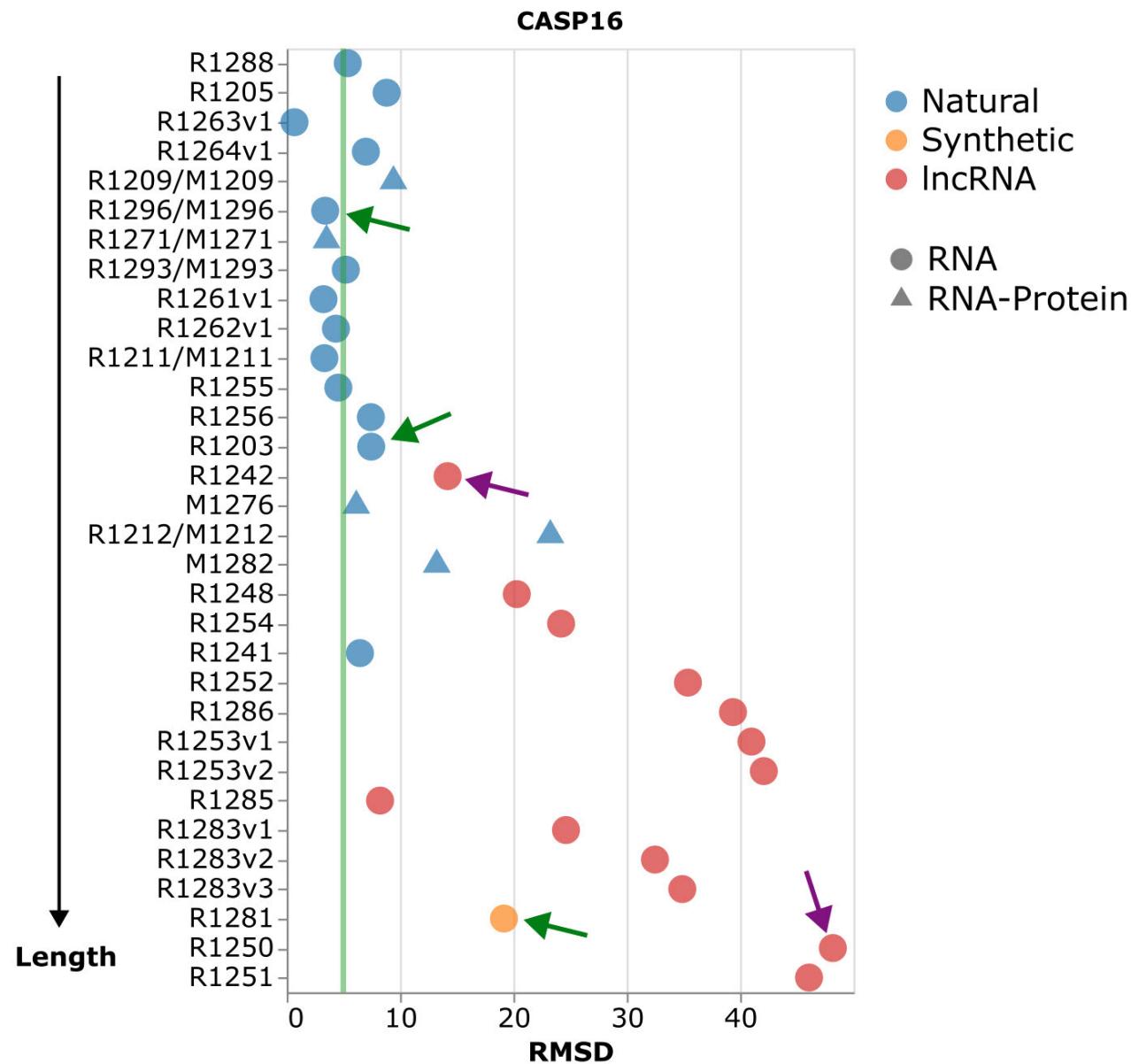
We are always happy with AF3!

Vfold performance in CASP16 – an overview



- **Strong performance** on **small RNAs and natural RNAs** due to the availability of (some) templates and/or the computationally efficient simulations capable of generating extensive structural ensembles for analysis.
- **Poor performance** on **lncRNAs**, as template information and additional structural information is not readily available, resulting in computationally challenging simulations.
- **Mixed performance** on **RNA-protein targets**. Without template or literature information, RNA-protein docking presents a significant challenge.

Five representative targets



Four representative targets:

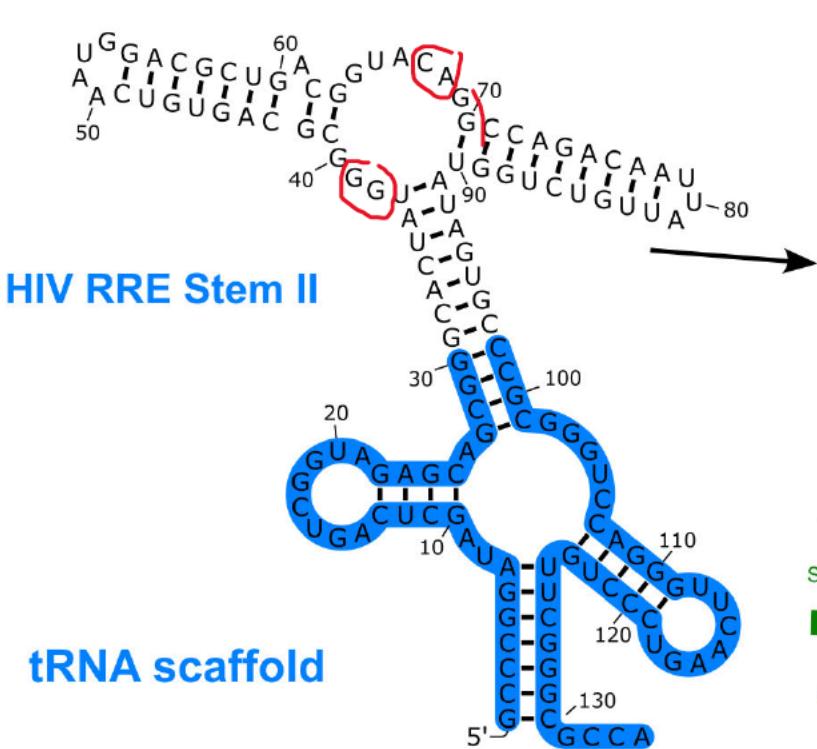
The Good:

- Two RRE SL2 targets
(our best models: 6.68 Å and 3.40 Å)
- RNA Origami dimer
(our best model: 19.17 Å)

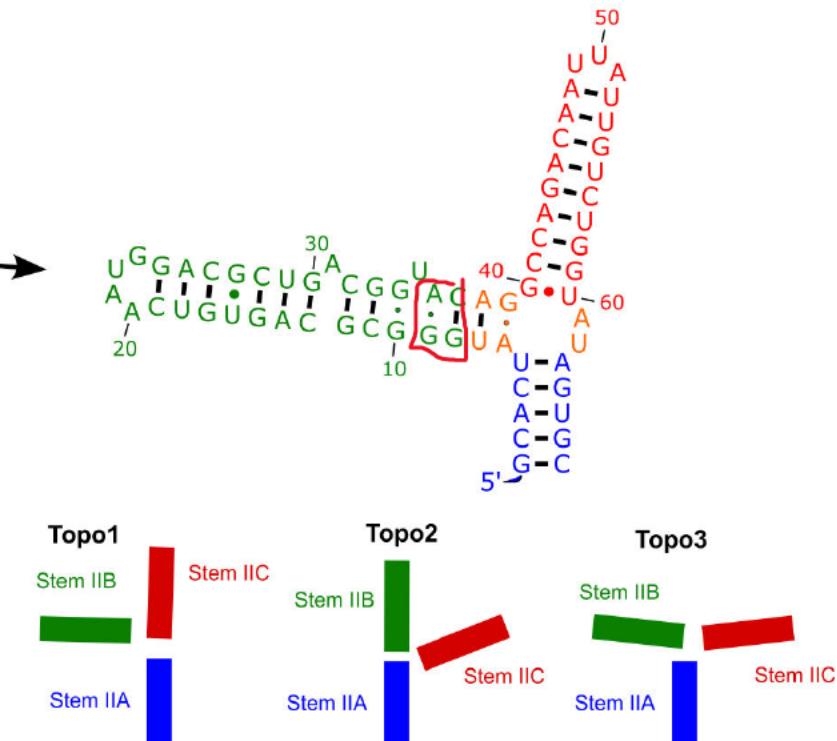
The Bad:

- raiA RNA
(our best model: 15.84 Å)
- GOLLD IncRNA
(our best model: 48.18 Å)

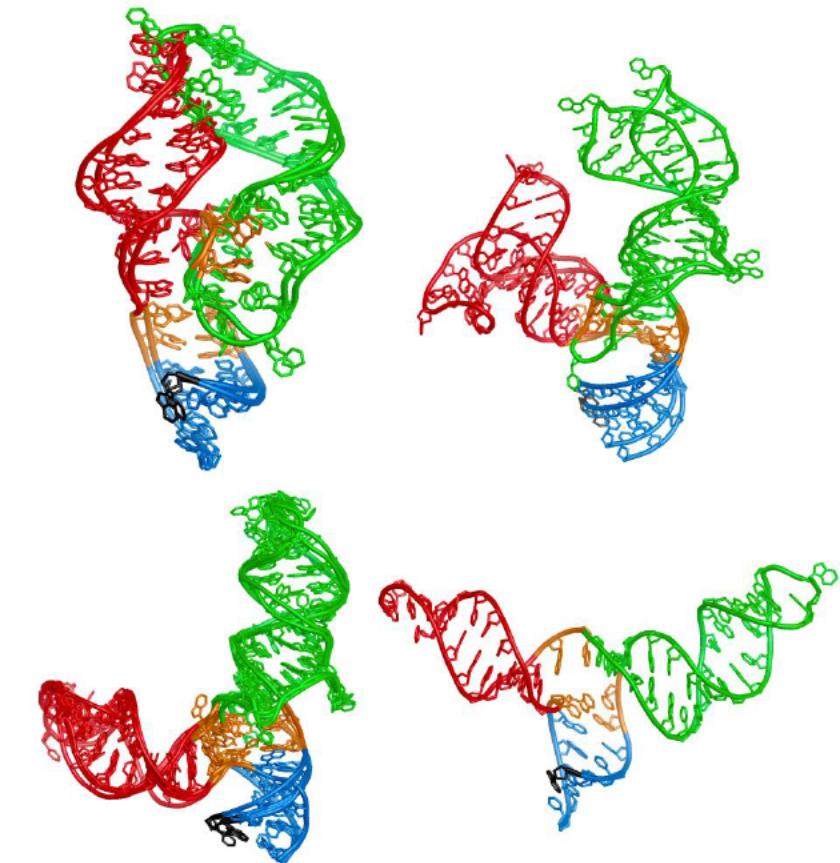
(1) RRE SLII (R1203): Importance of template refinement



The predicted 2D structure

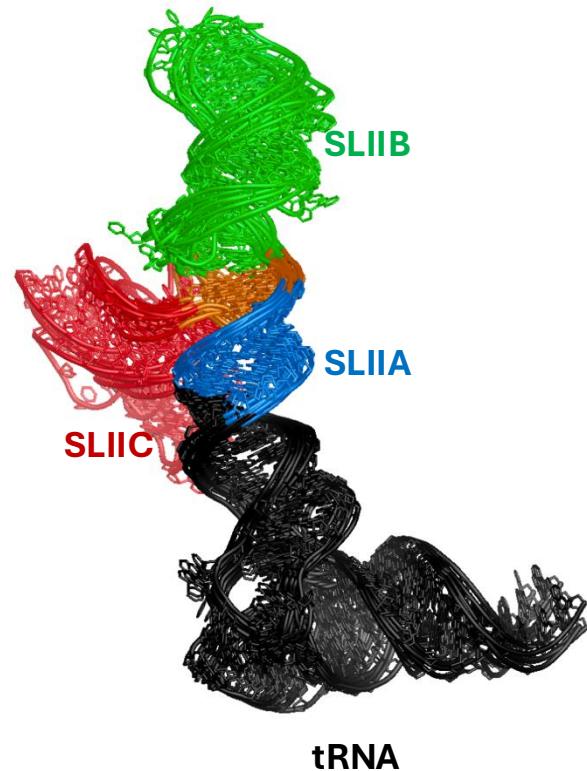


3 potential coaxial stacking modes

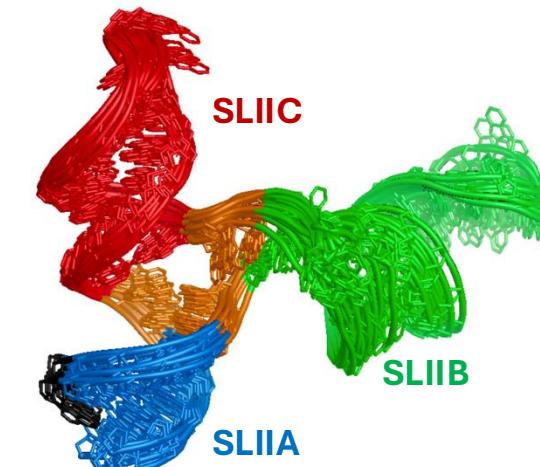


Rev response element (**RRE**)+Rev protein → transport viral mRNA out of nucleus

AF3 → different T-shaped topologies- w/wt tRNA scaffold

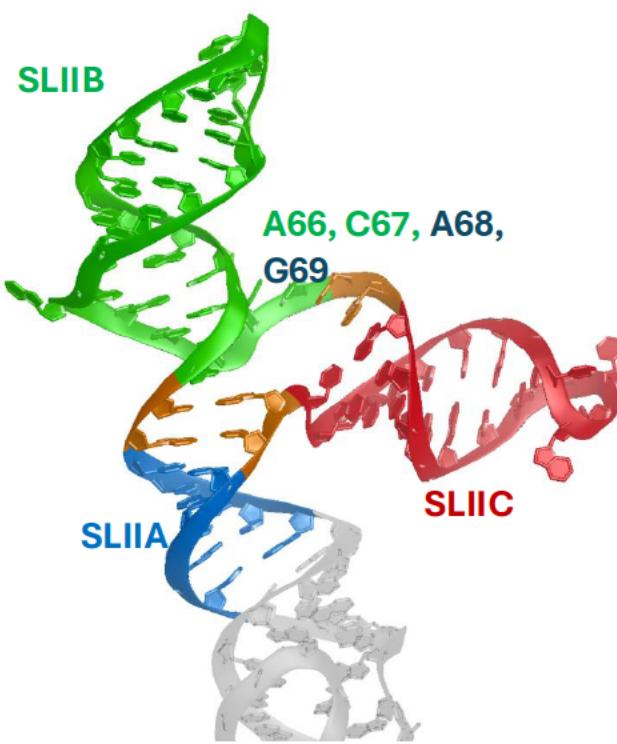


SLII with the tRNA scaffold
(R1203): Good

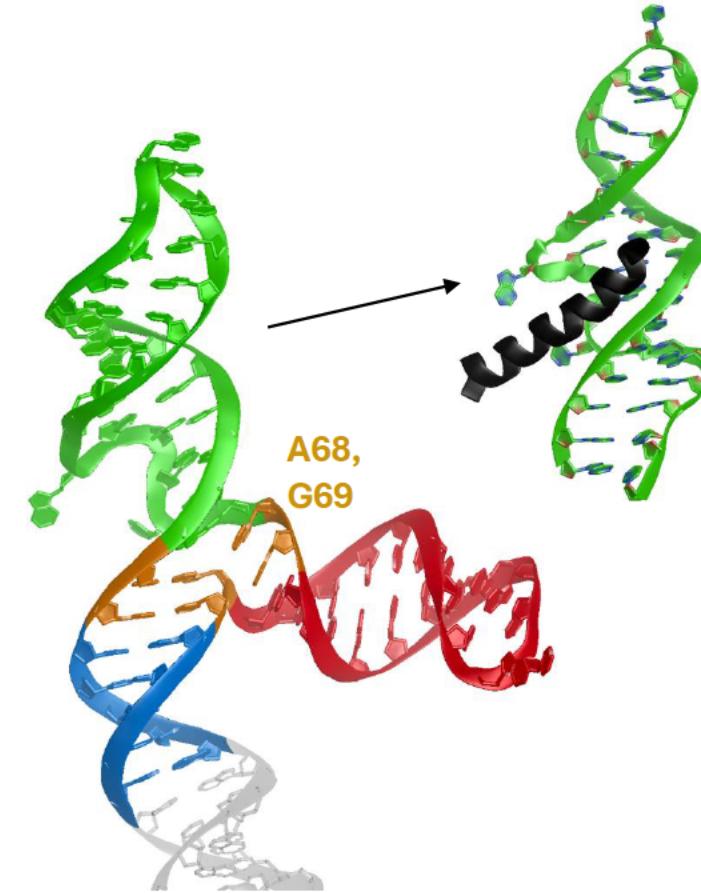


SLII without the tRNA scaffold
(R1203): Bad

(1) RRE SLII (R1203): Importance of template refinement



The native structure



The template we used:
RRE SLIIB with Rev peptide (PDB 1ETG)

The good: Vfold correctly predicted the coaxial stacking between SLIIA and SLIIB, as well as the T-shape of the 3WJ.

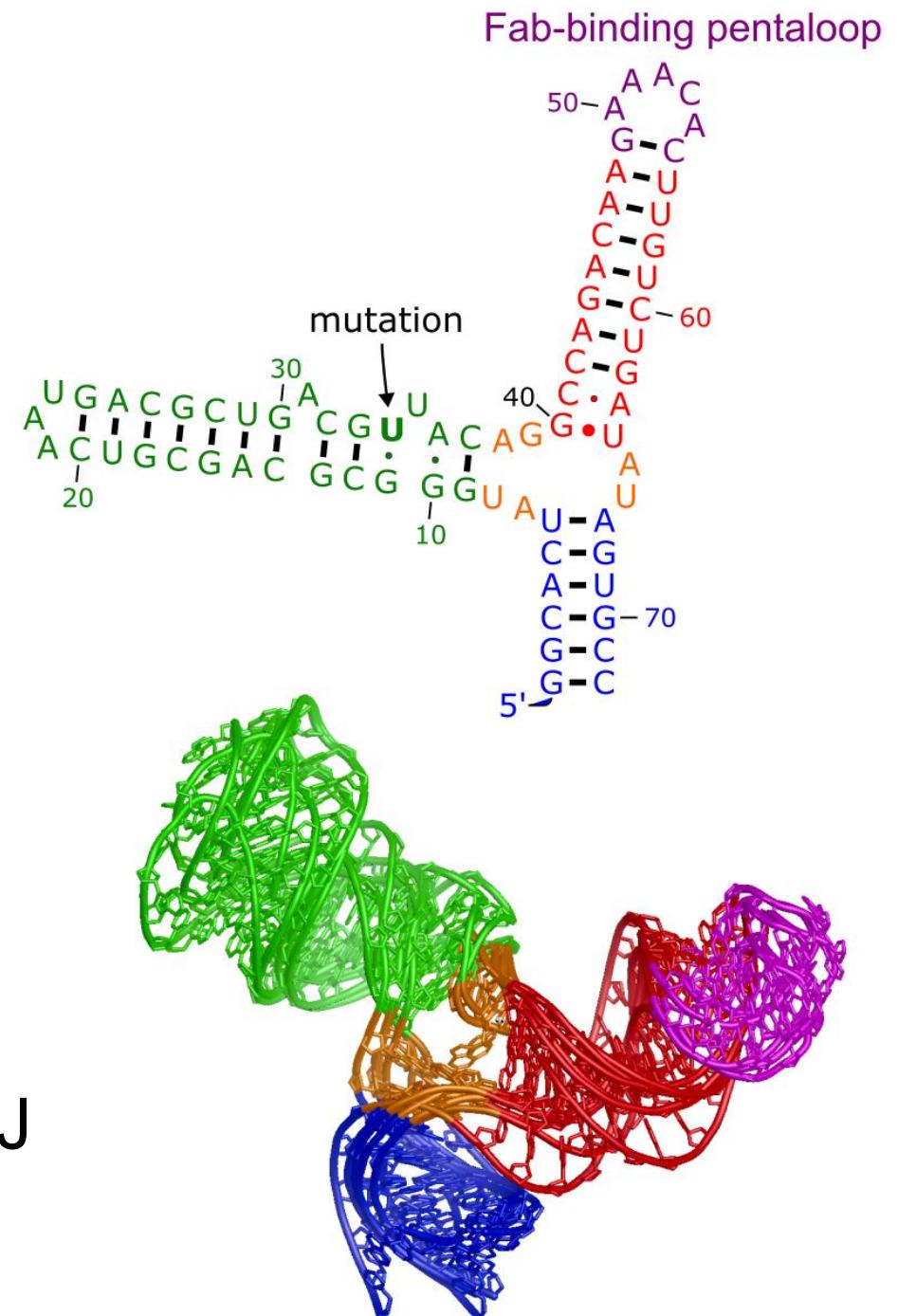
The bad: A66 and C67 form base pairs within SLIIB (following the template), but in the native structure, they flip out and stack with A68 and G69.

(2) RRE SLII (R1296): AF3-assisted sampling

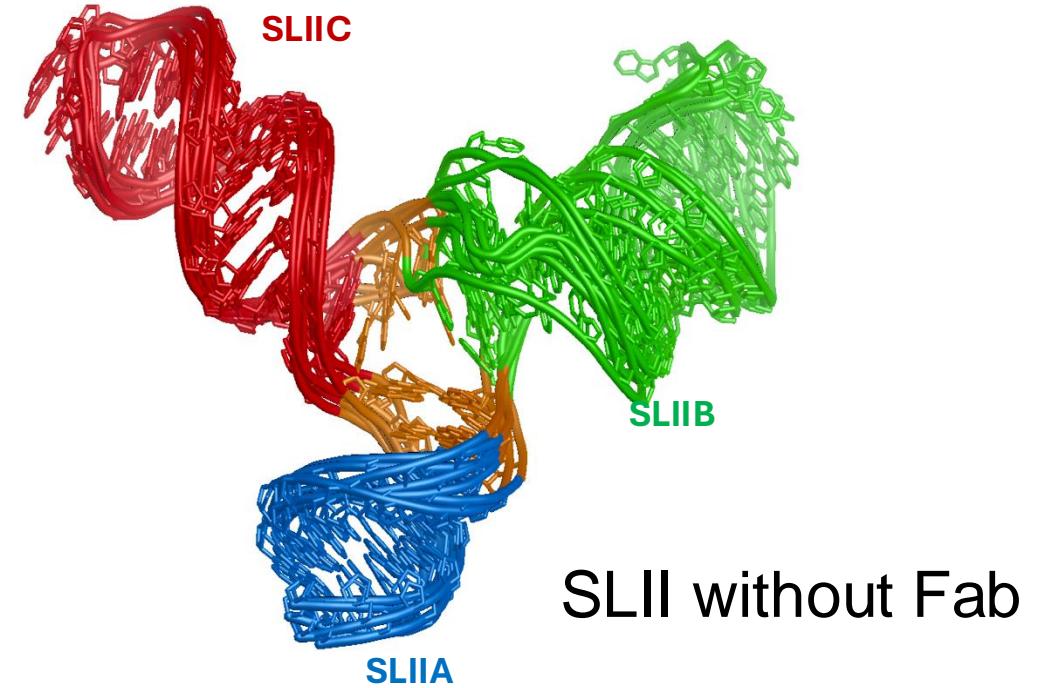
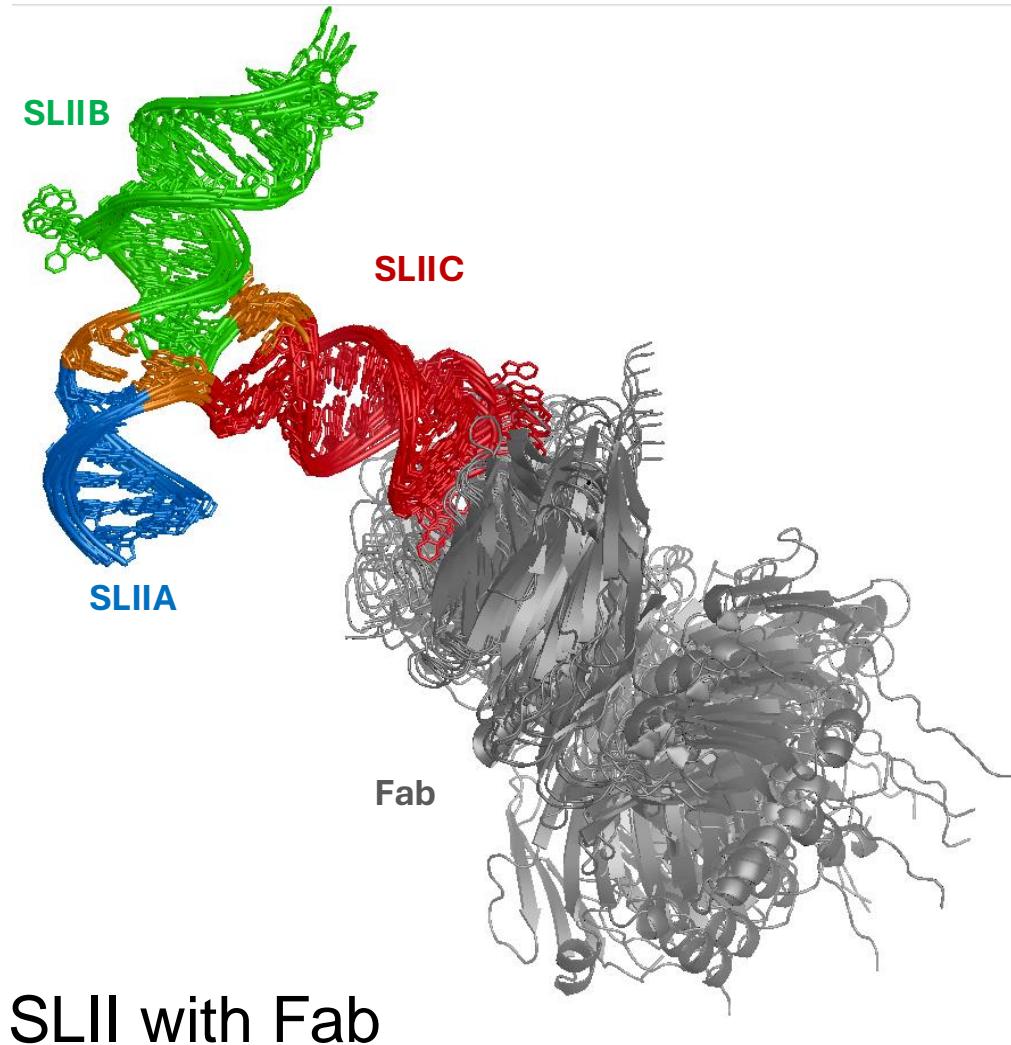
A single mutation →
Similar 2D but dramatically different 3D

The predicted 2D structure

Vfold suggests a highly dynamic 3WJ

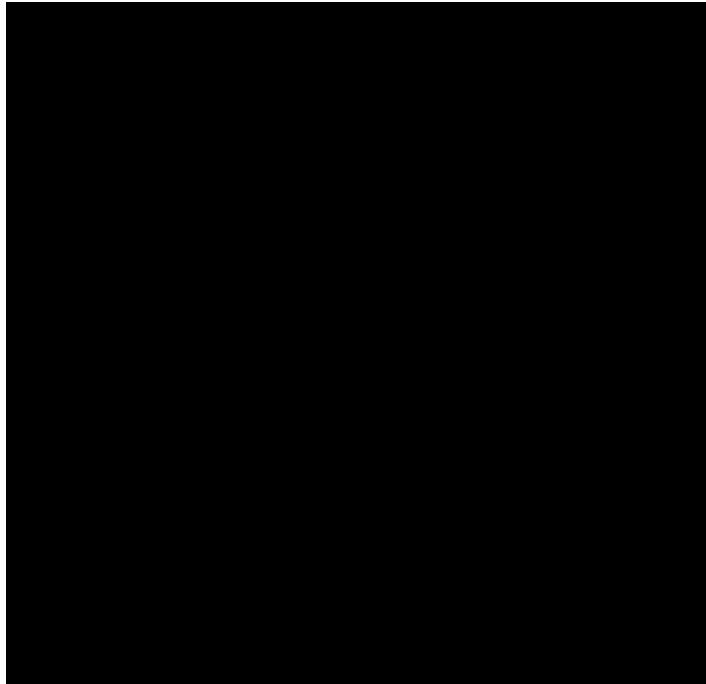


AF3 → Y-shaped topologies (Fab-free)

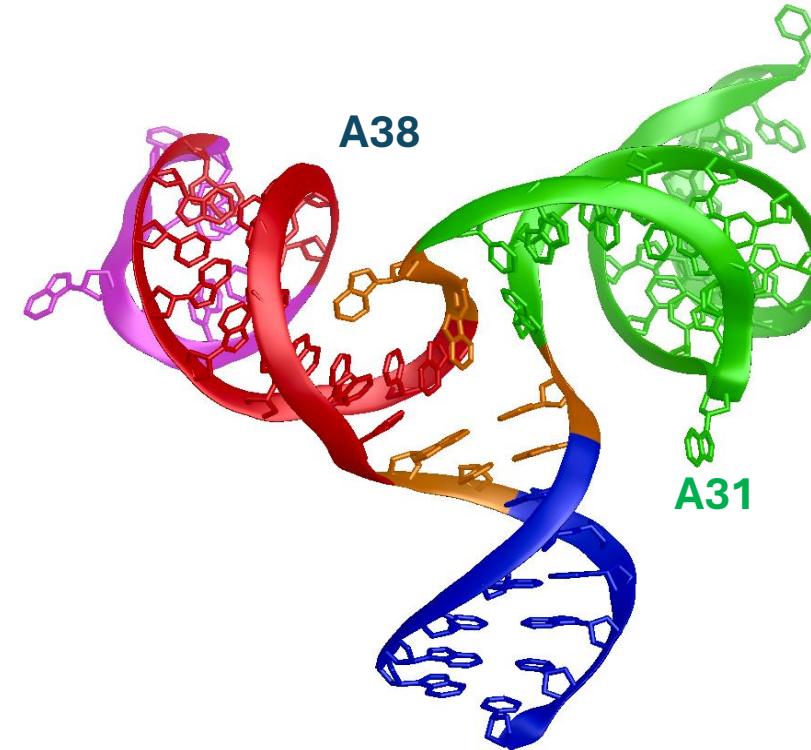


Y-shape: No coaxial stacking between
SLIIA, SLIIB, SLIIC
Absolutely dominant structure by AF3

(2) RRE SLII (R1296): AF3-assisted sampling



The native structure

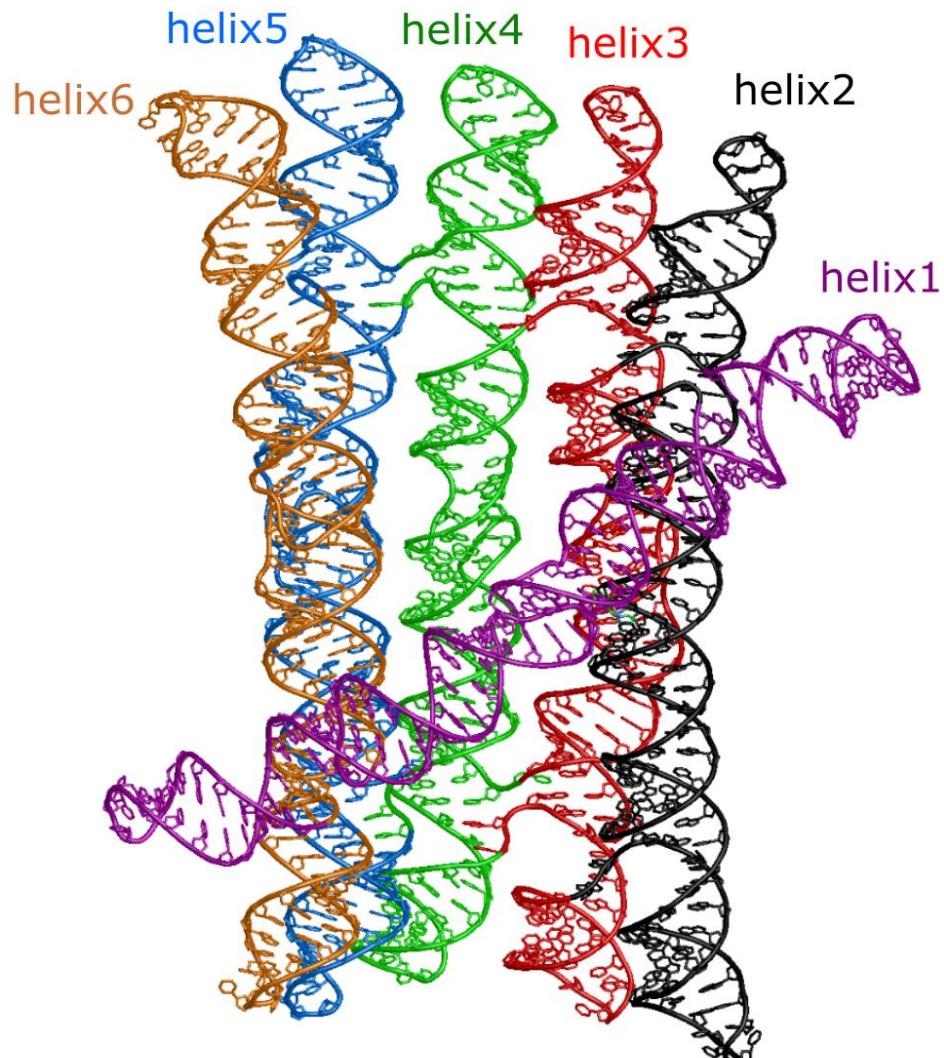


Our best model: 3.4 Å

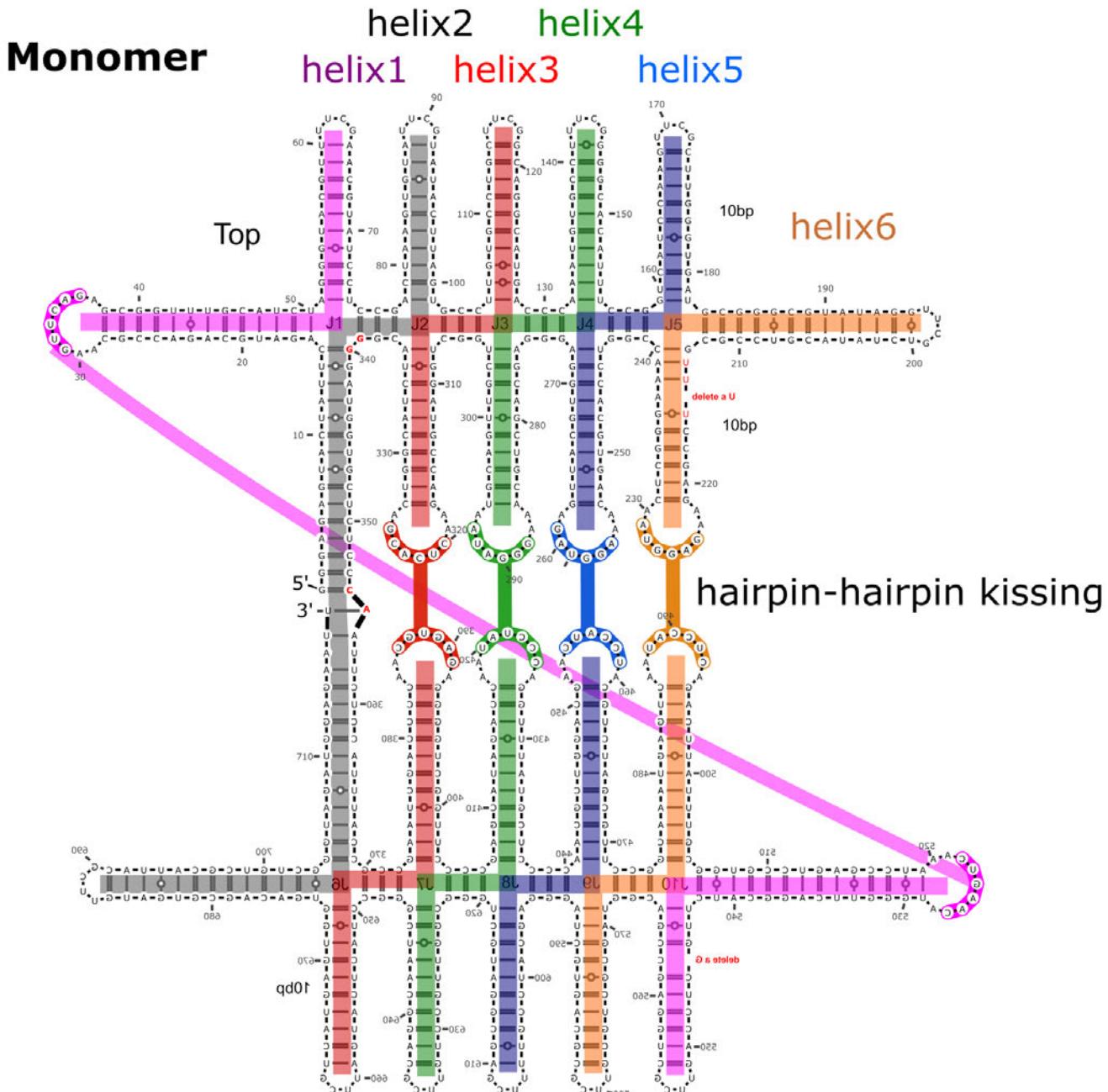
The good: Vfold correctly modeled the 3WJ conformation, in which the three arms do not exhibit coaxial stacking.

The bad: Vfold incorrectly predicts that A38 flip out to form base triples; additionally, we missed the A-minor interaction between A31 and SLIIA.

(3) RNA origami dimer (R1281)



Monomer:
6-helix bundle origami RNA (PDB 7PTK)

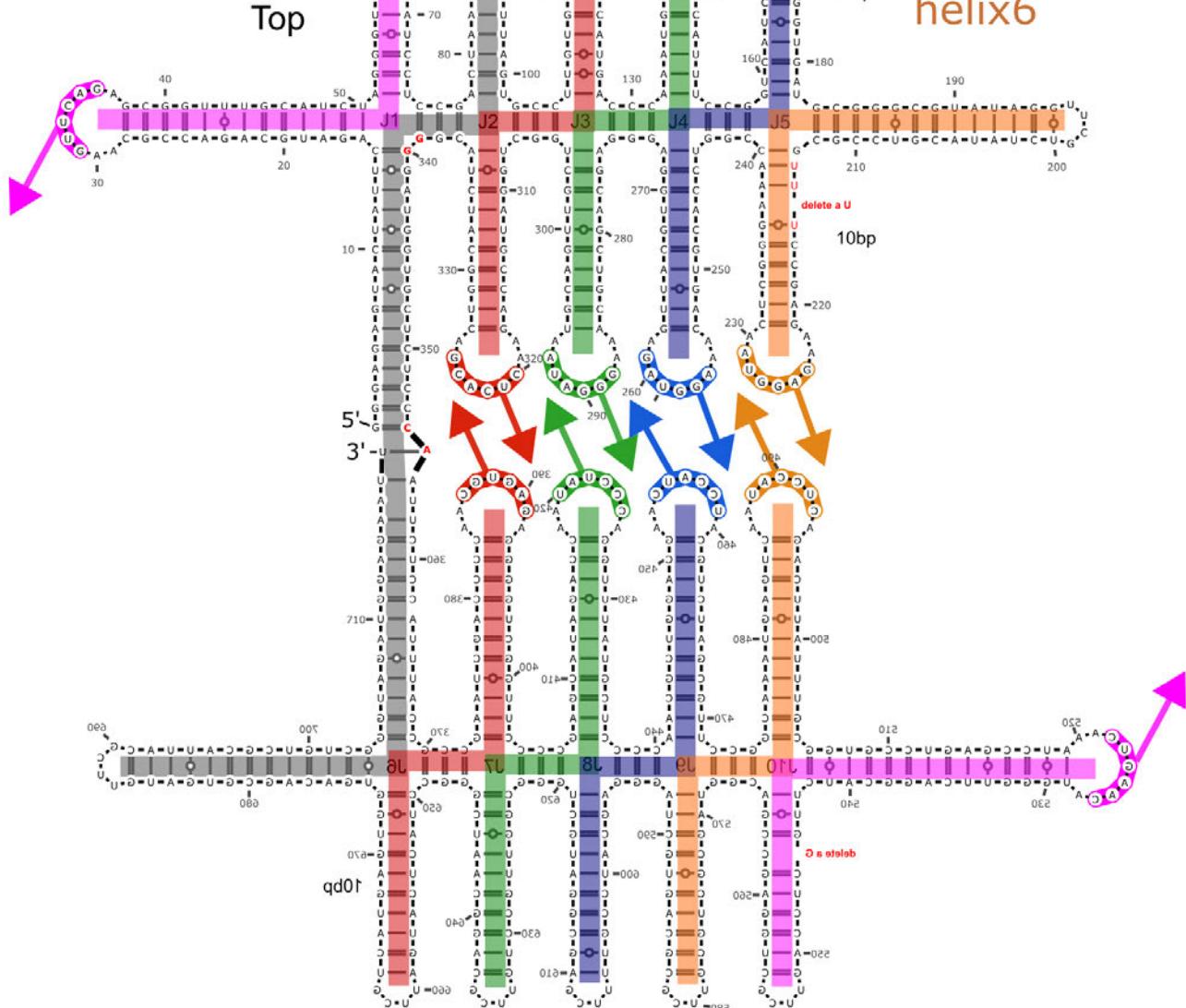


Mode 1

helix2 helix4
helix1 helix3 helix5

10bp

helix6



Low-barrier dimerization:

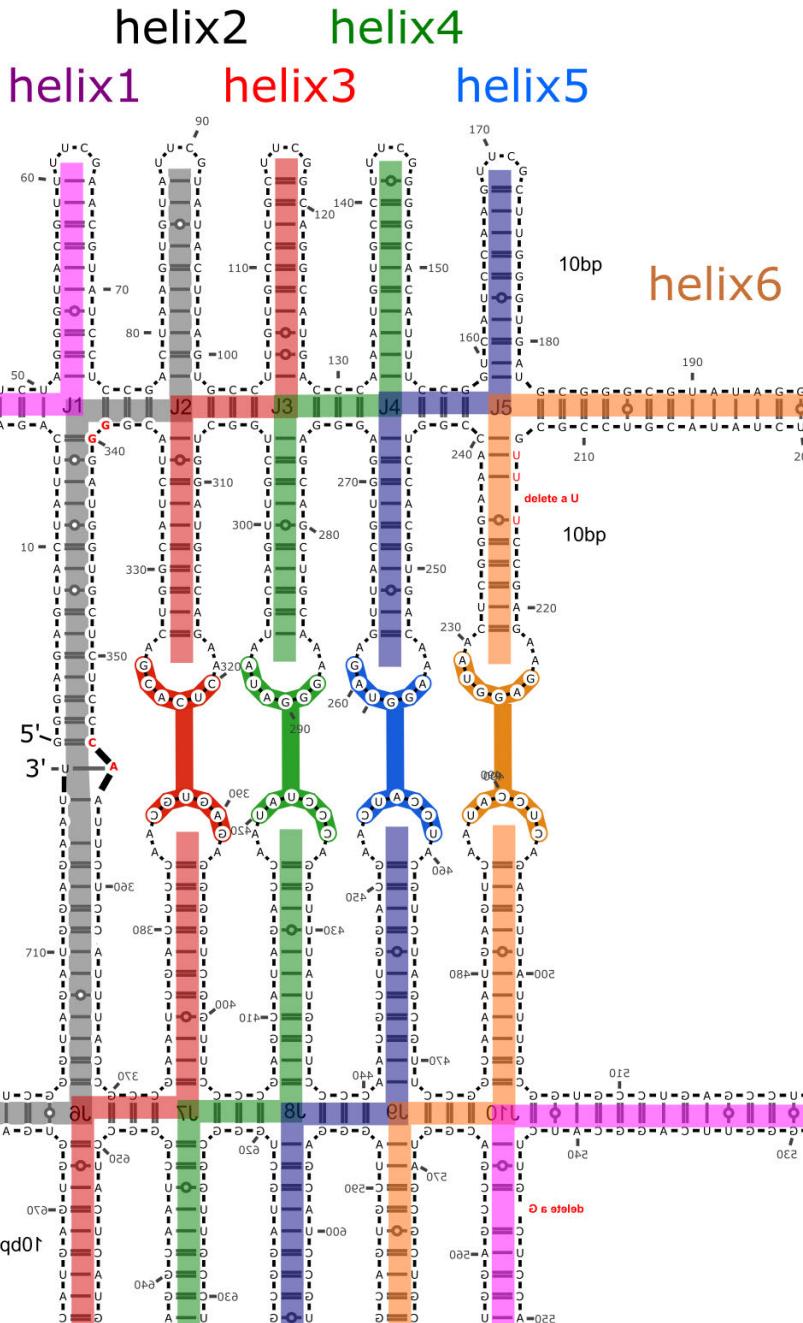
(A1-B1) + (A2-B2)

→

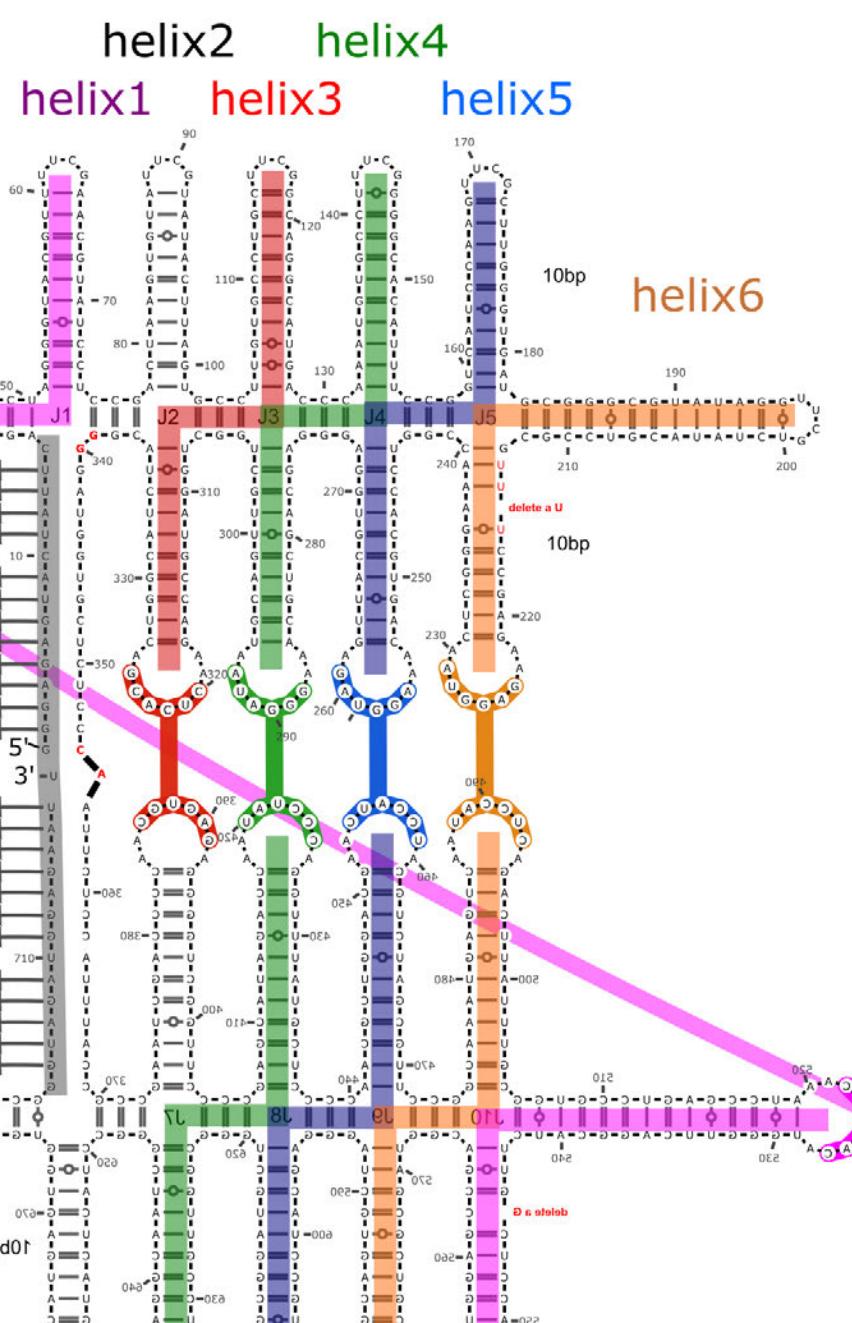
(A1-B2) + (A2-B1)

AF3 fails to generate a viable structure

Mode 2

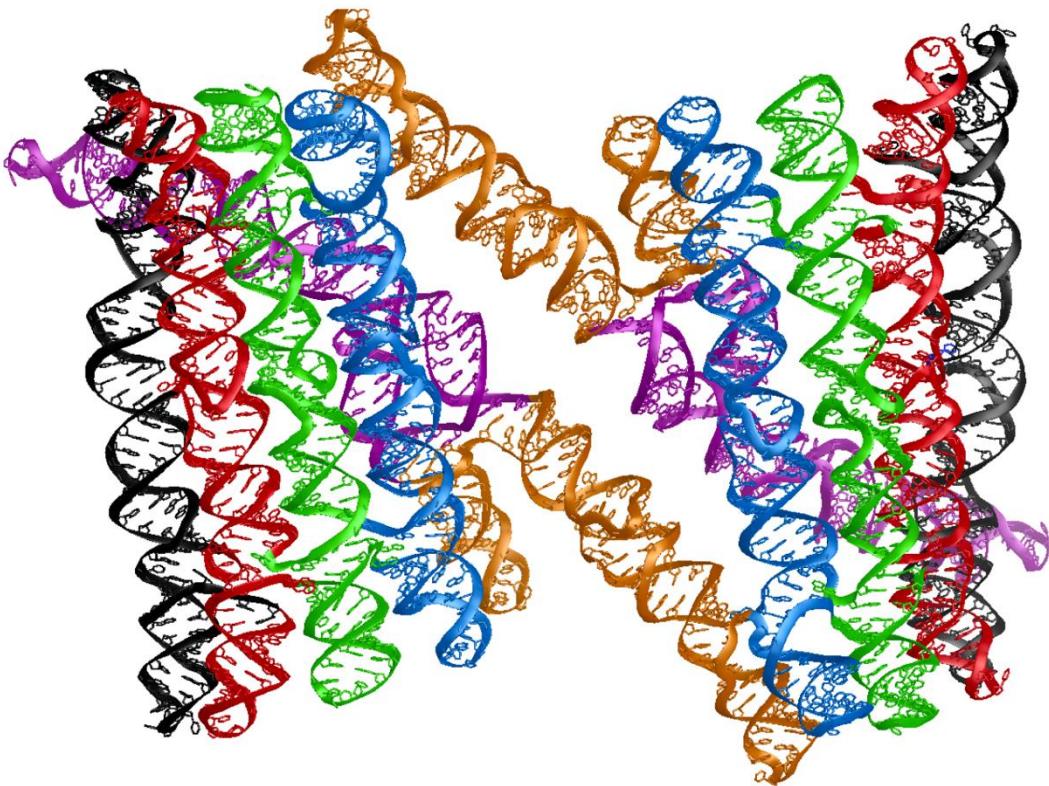


Mode 3

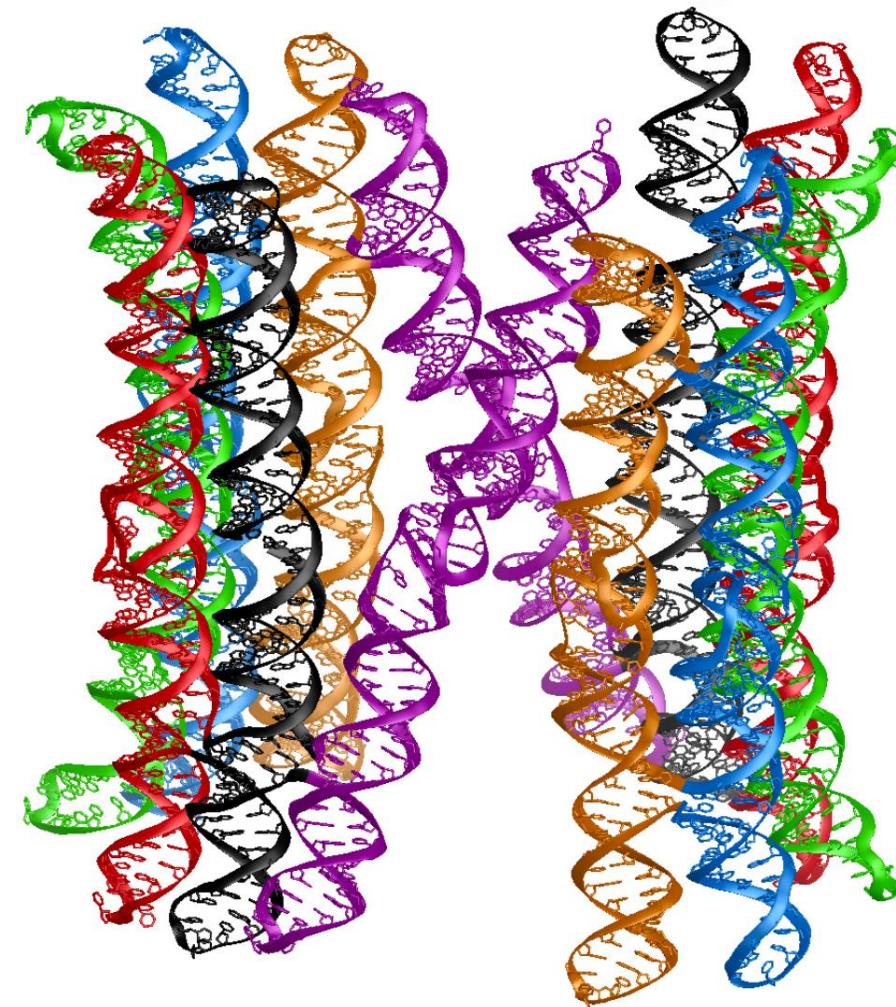


(3) RNA origami dimer (R1281)

helix6-helix6 kissing



helix1-helix1 kissing



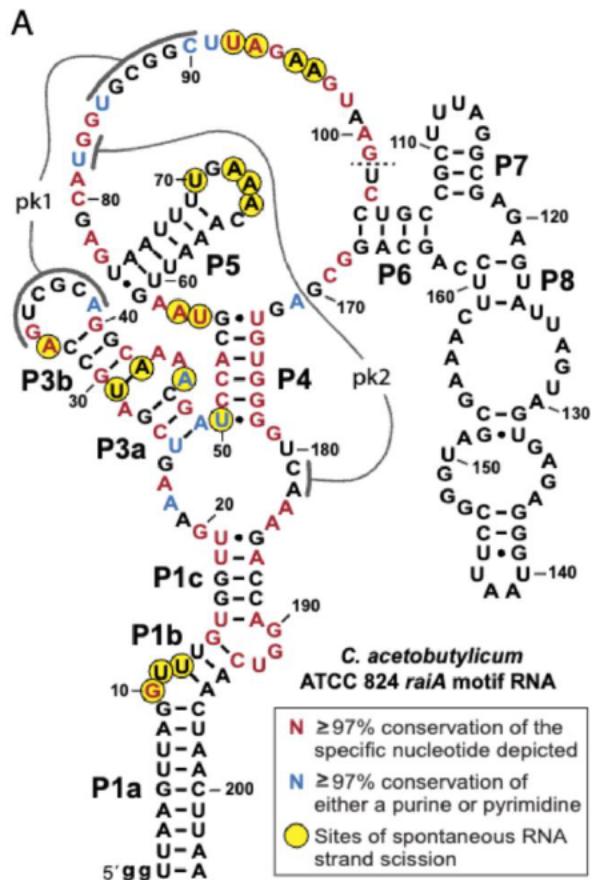
The native structure (dimer)
Mode 2 but for helix 6

Our best model (dimer) mode 2

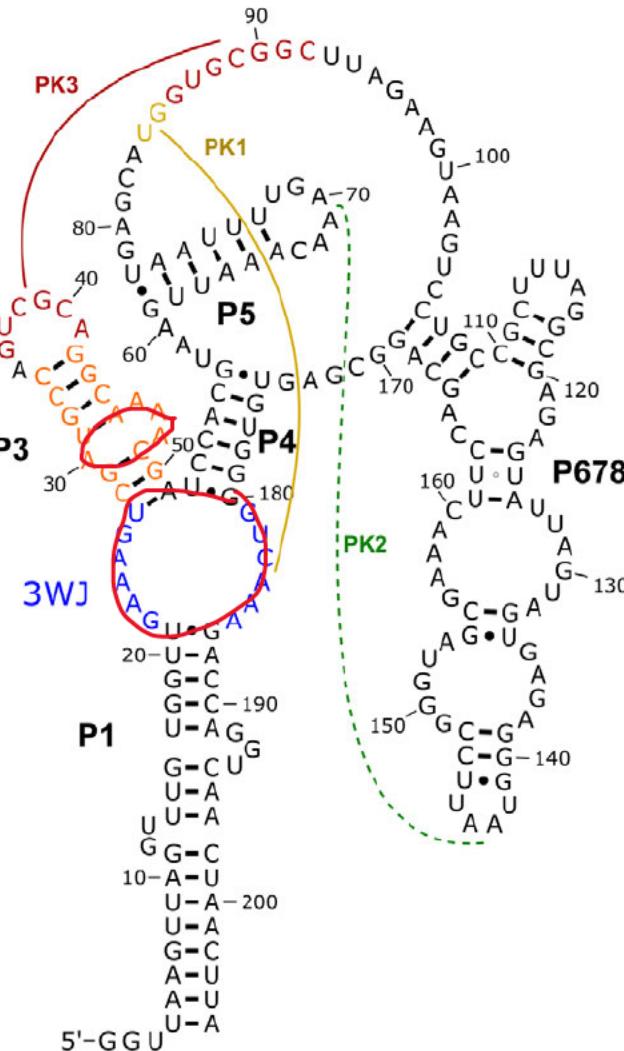
Monomer from our best model (red) against the native structure (green): 19.17 Å

(4) raiA RNA (R1242): good 2D → bad 3D

Structure information and in-line probing experiment on raiA motif from literature[1].



The 2D structure was predicted by combining literature info with our models.



The good: Vfold predicts good 2D structure

Specificity: 0.88 (ppv)

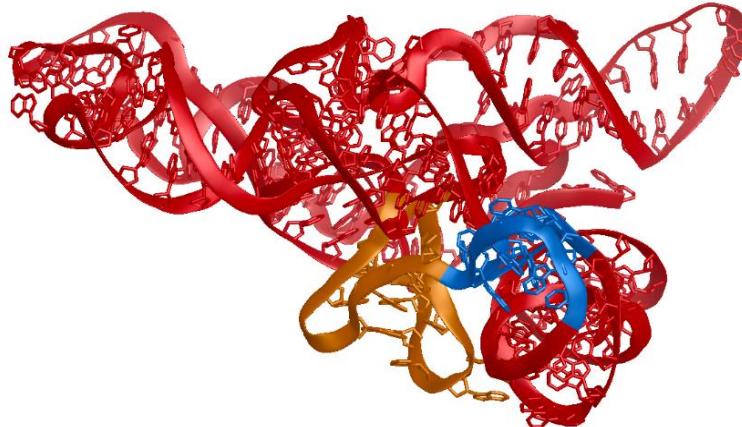
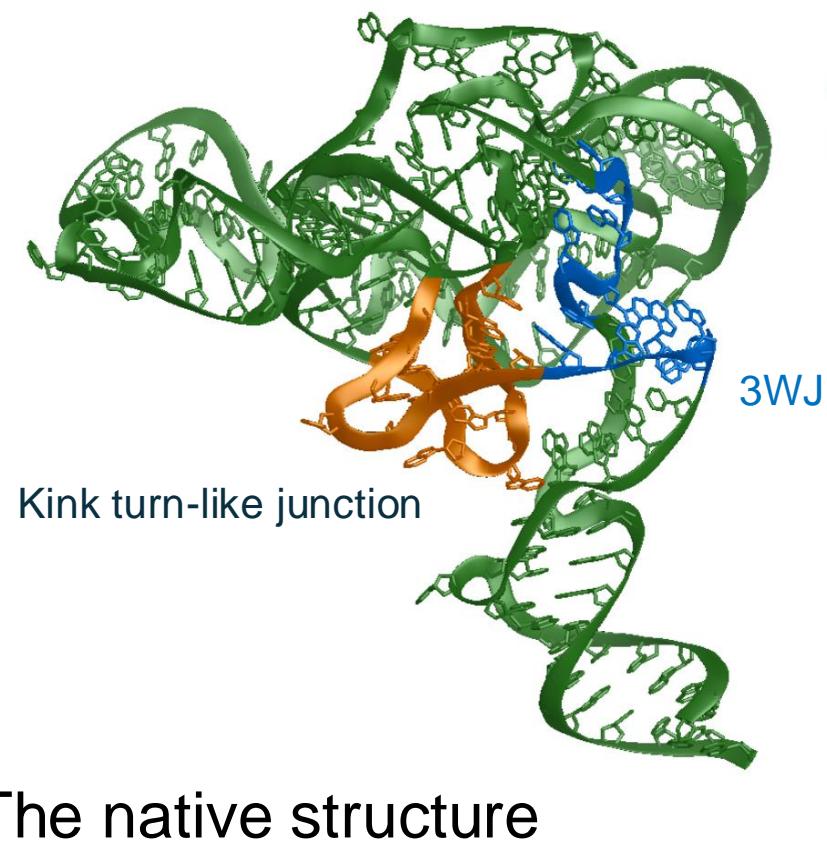
Sensitivity: 0.94 (recall)

F1-score: 0.91

The three pseudoknots are all correctly predicted.

[1] Soares, Lucas W., Christopher G. King, Chrishan M. Fernando, Adam Roth, and Ronald R. Breaker. (2024). PNAS

(4) raiA RNA (R1242): Good 2D → bad 3D

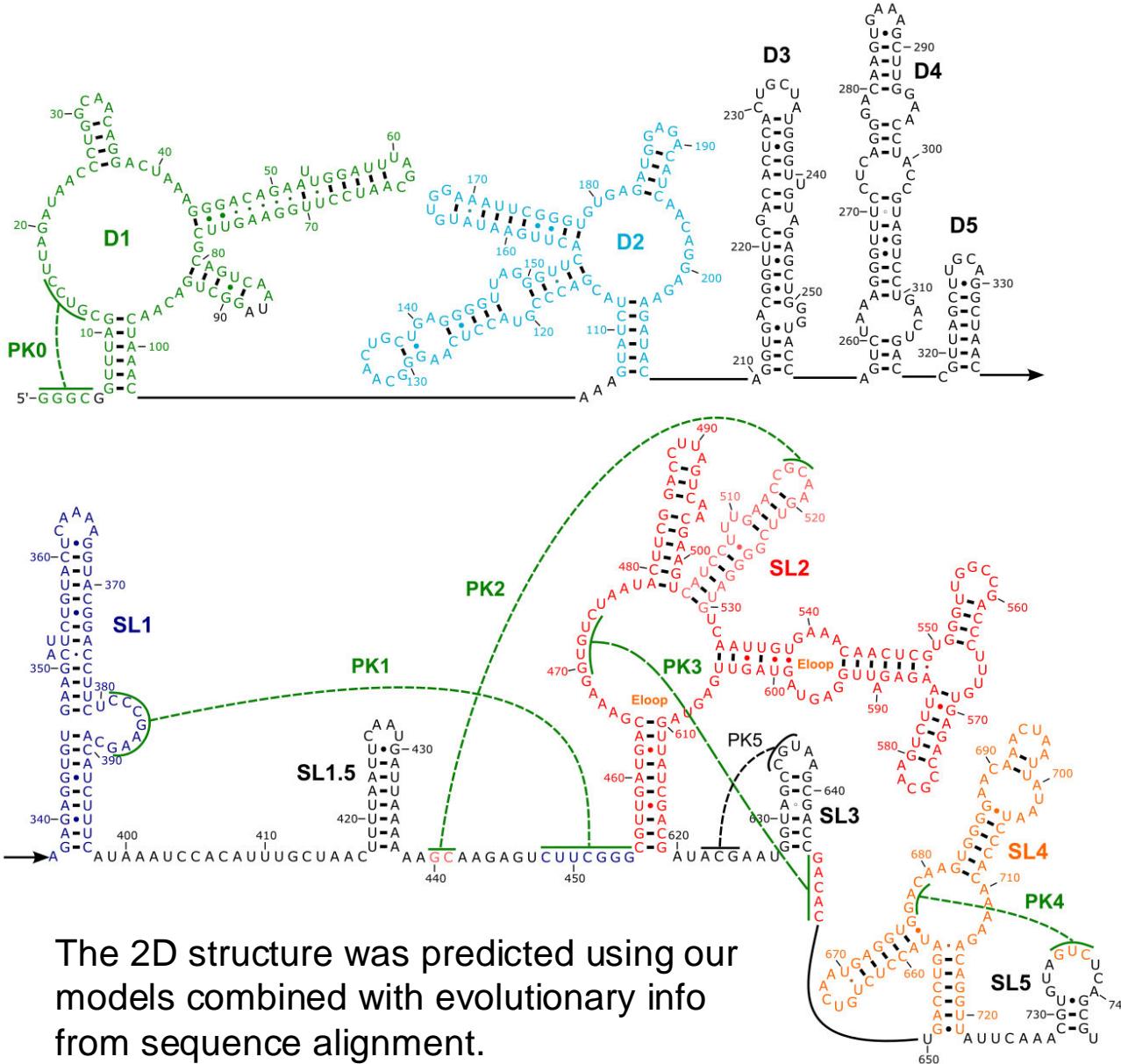


The bad: the kink turn-like junction and the large 3WJ

Vfold fails to generate the initial 3D structure

AF3 generated initial 3D fold + 2D structure → the predicted model

(5) GOLLD lncRNA (R1250): Structural rearrangement upon R-R binding



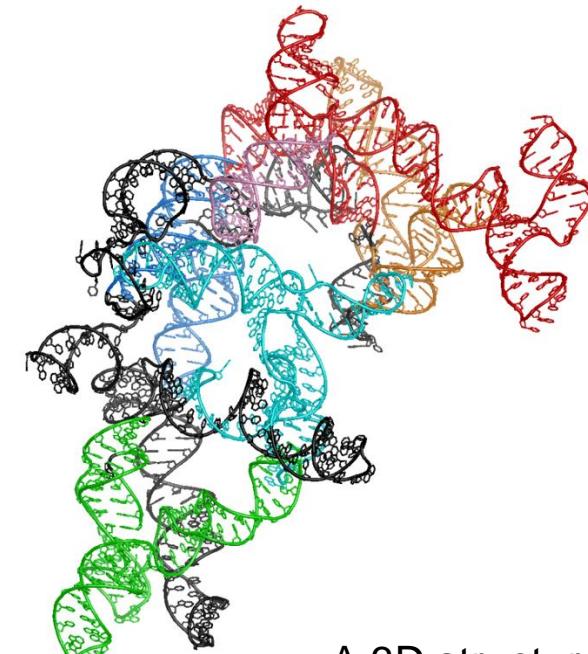
The bad: 2D structure prediction accuracy

Specificity: 0.55 (many false positives)

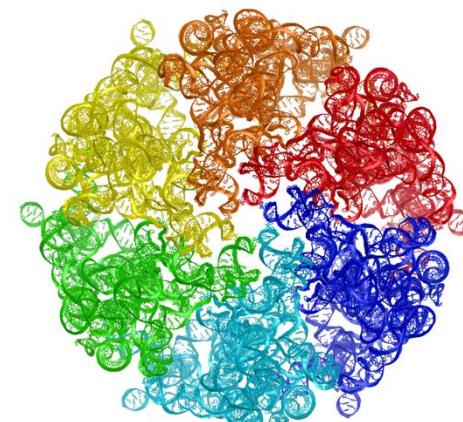
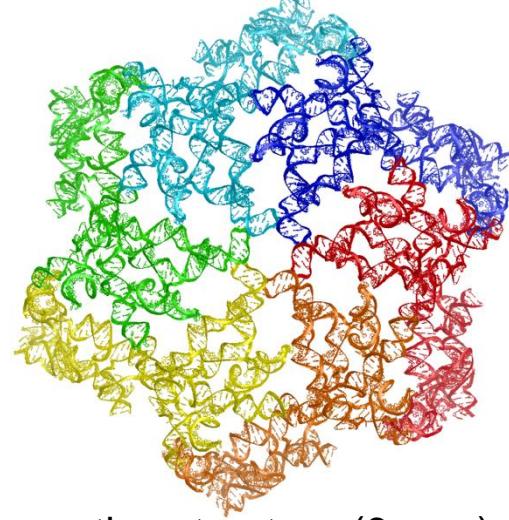
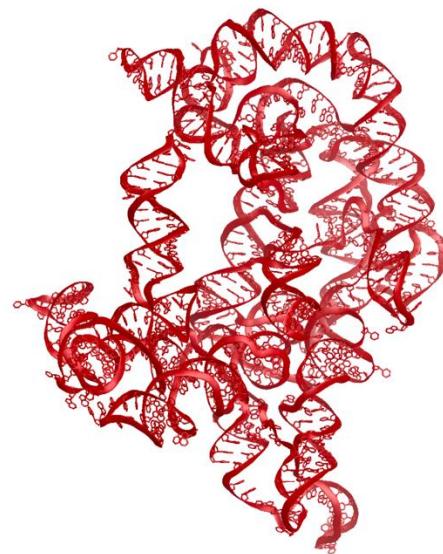
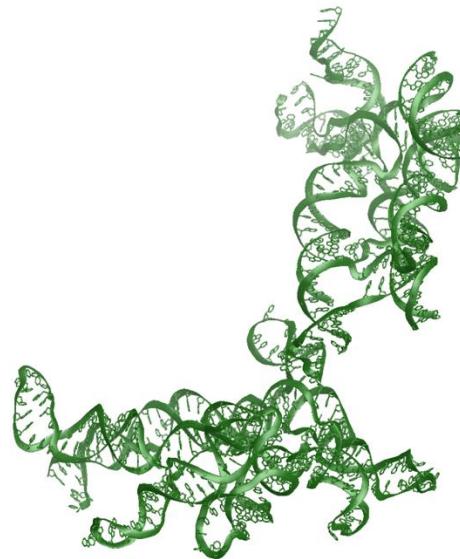
Sensitivity: 0.85

F1-score: 0.67

The formation of pseudoknots are correctly predicted, but with low specificity.



(5) GOLLD lncRNA (R1250): Structural rearrangement upon R-R binding



The bad: We first modeled the monomer structure and subsequently assembled the 6-mer structure through RNA-RNA docking. This resulted in overly compact monomer and 6-mer structures.

This target highlights the significant challenges in predicting large and multimeric RNA structures at both the 2D and 3D levels.

Perspectives

What we did right:

- Natural and small RNAs
- Conformation sampling using CGMD simulations
- Prediction of dimerization modes

What went wrong:

- Large RNA, both at 2D and 3D levels
- Multimeric RNAs: docking of monomeric RNA doesn't work
- RNA-Protein complexes

Future directions:

- New models for the simulations of large/multimeric RNAs
- Integration of machine learning-base models with simulation methods
- Improving RNA-protein docking, accounting for RNA flexibility

Acknowledgment

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