COBRA
COMPUTATIONAL BIOLOGY
RESEARCH AND ANALYTICS

Unravelling RNA function through structure prediction

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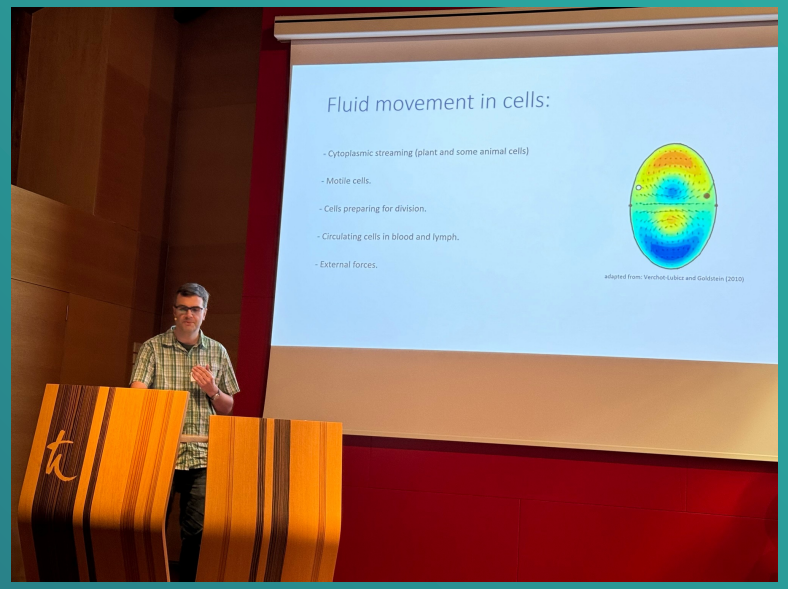

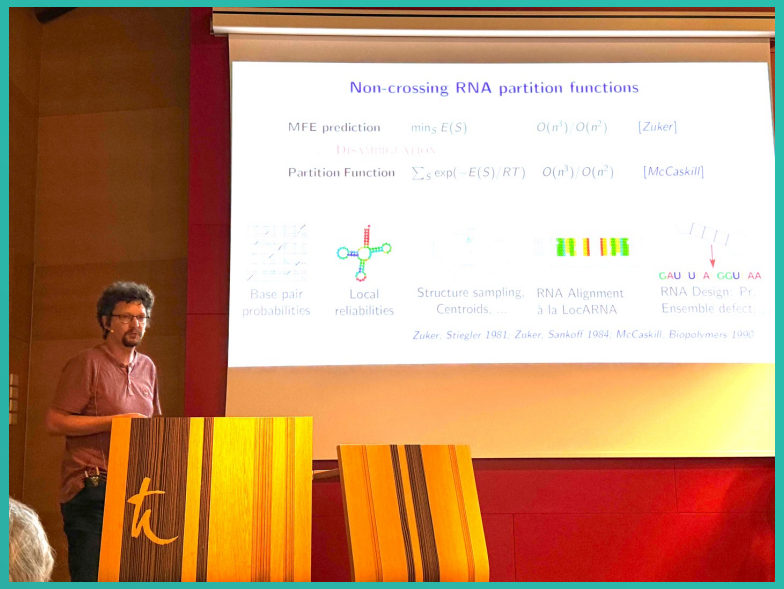

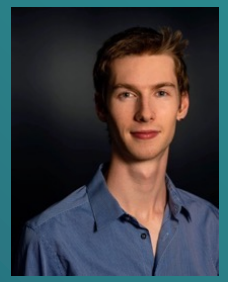

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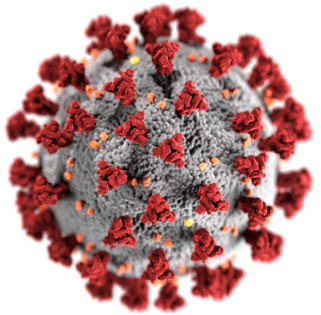
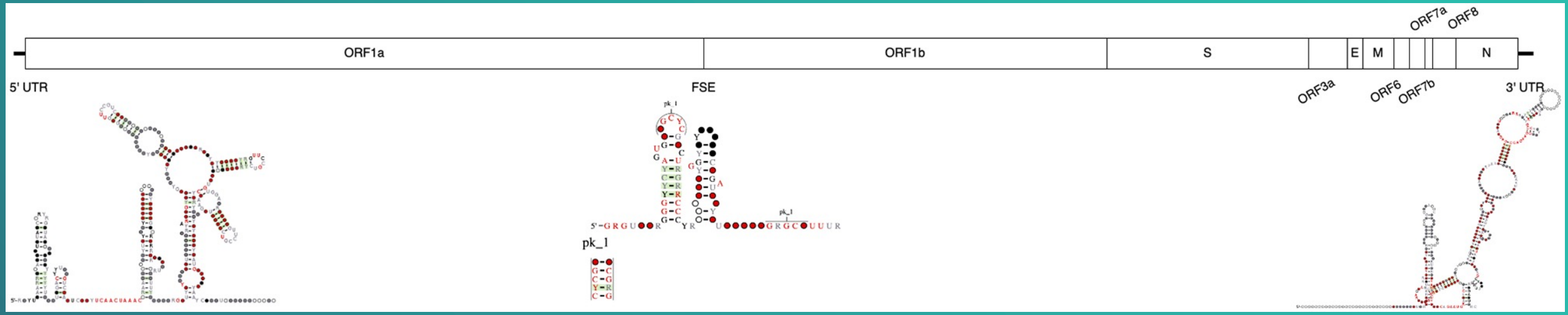
Known Example!



- SARS-CoV-2
  - A positive sense single stranded RNA
  - Length: 30k base



Structure information from experimental data

- Incarnato laboratory [Manfredonia et al. 2020]
  - (in vivo and in vitro) SHAPE and DMS
  - 87 regions (not 3'UTR or FSE)
- Pyle laboratory [Huston et al. 2021]
  - (in vivo) SHAPE
  - 3' UTR and FSE, and dispersed throughout ORF1ab
- Cliff Zhang's laboratory [Sun et al. 2021]
  - (in vivo click) SHAPE
  - 37 structural elements
  - 5' UTR but not pseudoknot 3' UTR, and no mention of FSE.

Structure information from computational work

- Moss laboratory [Andrews et al. 2020]
  - ScanFold-based computational pipeline
  - 8 regions
- Pyle laboratory [de Cesaris et al. 2021]
  - SuperFold
  - 61% of the genome as being base paired
- Das laboratory [Rangan et al. 2020]
  - combination of CONTRAfold and RNAz
  - 44 regions (components of 5' UTR, FSE, and 3' UTR)
- Mathews laboratory [Li et al. 2021]
  - LinearTurboFold
  - 50 elements, 26 of which were not in previous studies

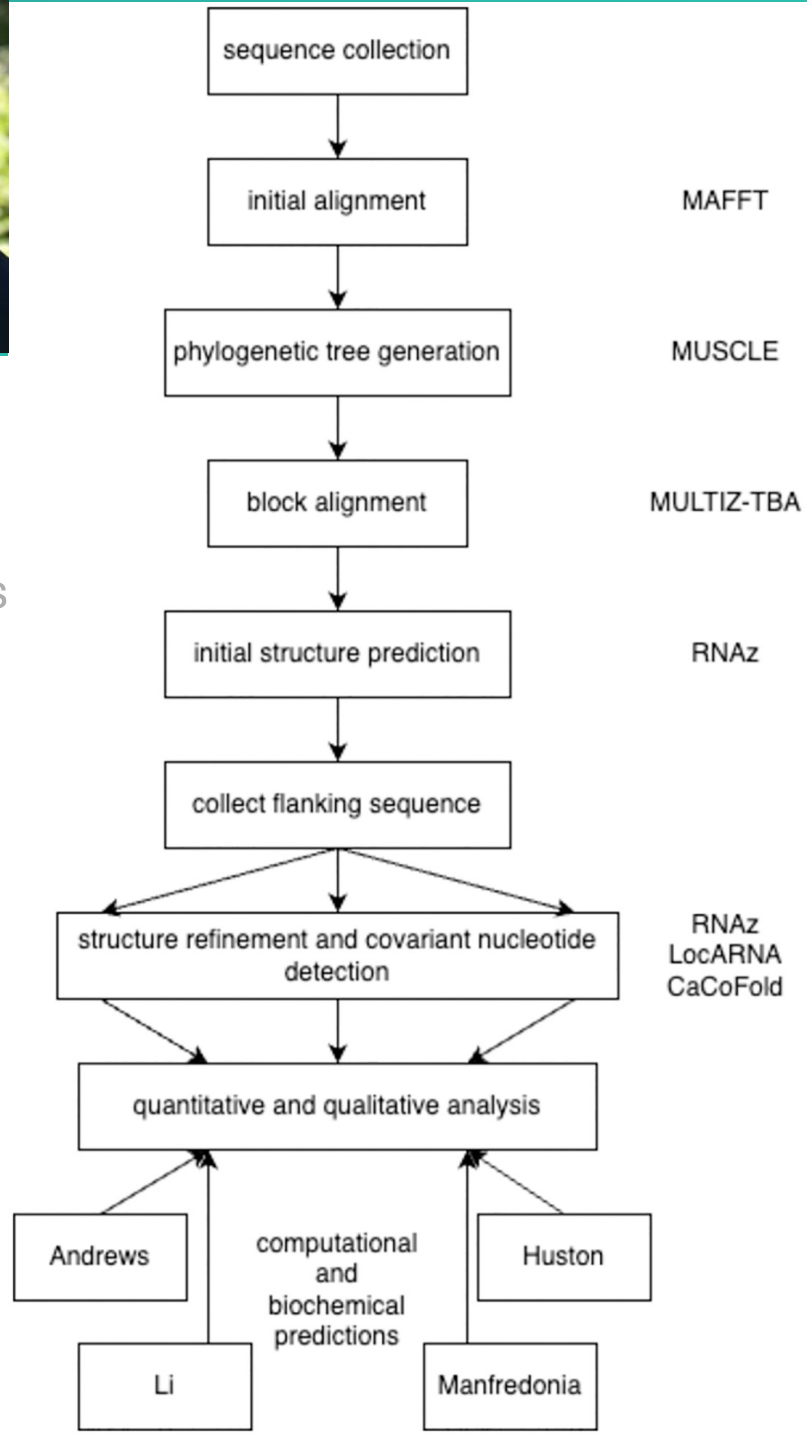
- RNA Puzzles [Gumna et al. 2022]
  - 74 3D models of UTRs, including some predicting pseudoknots in 3' UTR, and some extended 5' UTR structures beyond the currently accepted 5 stem loops

- Little is known about structures in coding regions

Structural Pipeline

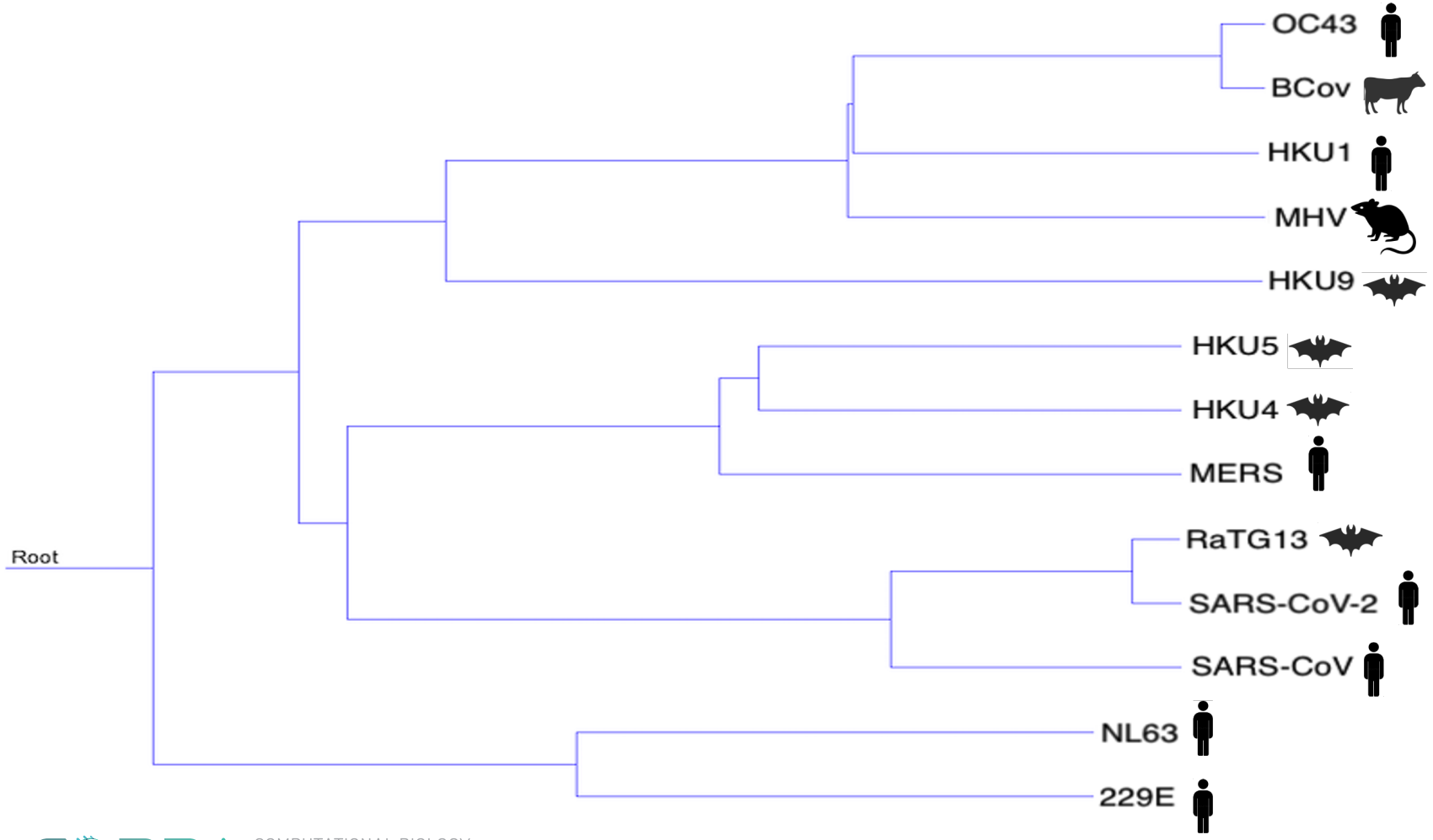
- Started with a known pipeline
  - Conserved structures in vertebrates [Thiel et al. Genes 2018]
  - Conserved structures in non-coding RNA [Will et al. Genome Res 2013]

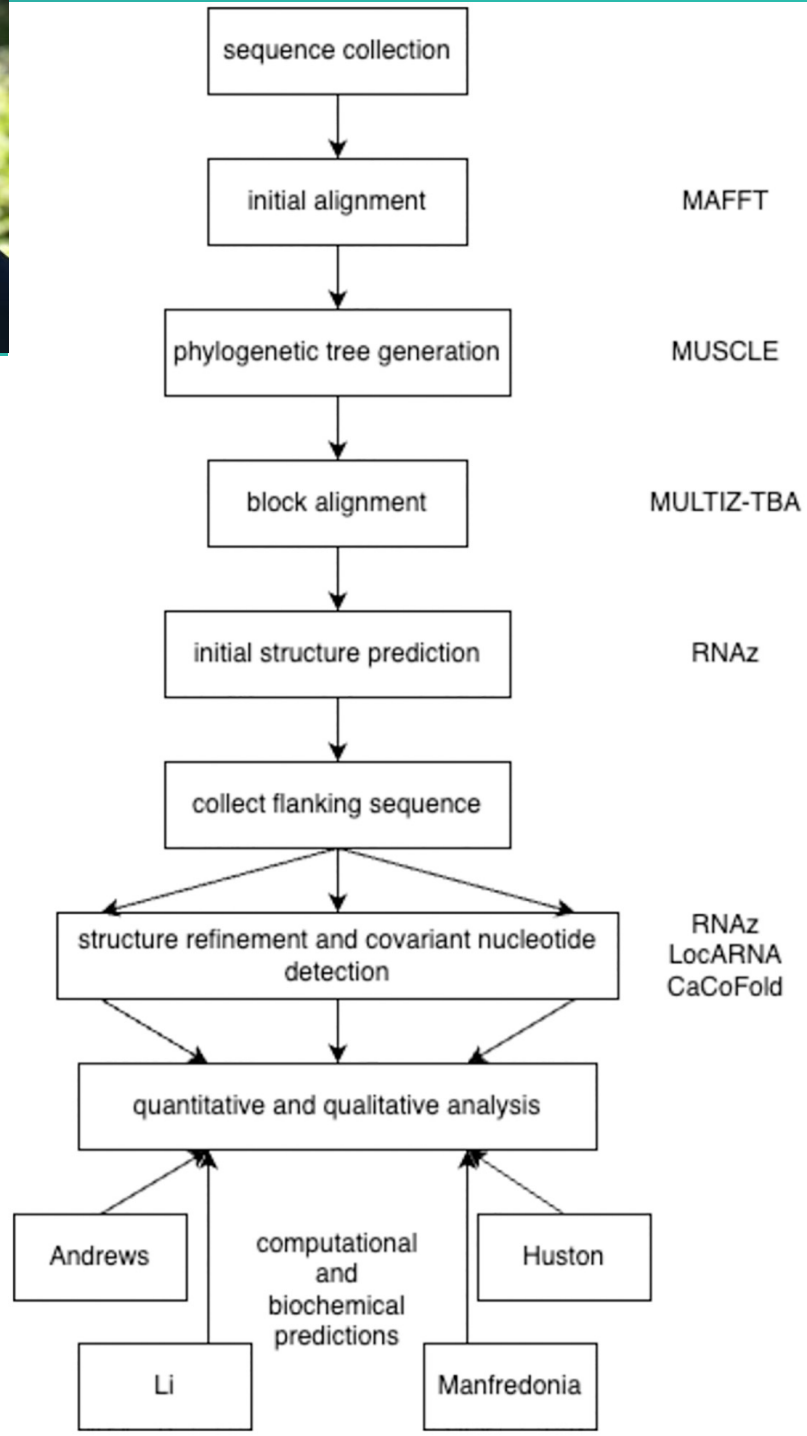
- Modified it for shorter sequences
  - More stringent criteria
  - Added Cacofold [Rivas PLoS Comp Bio. 2020]
  - Cross referenced findings with others



Ziesel and Jabbari 2024

Structural Pipeline





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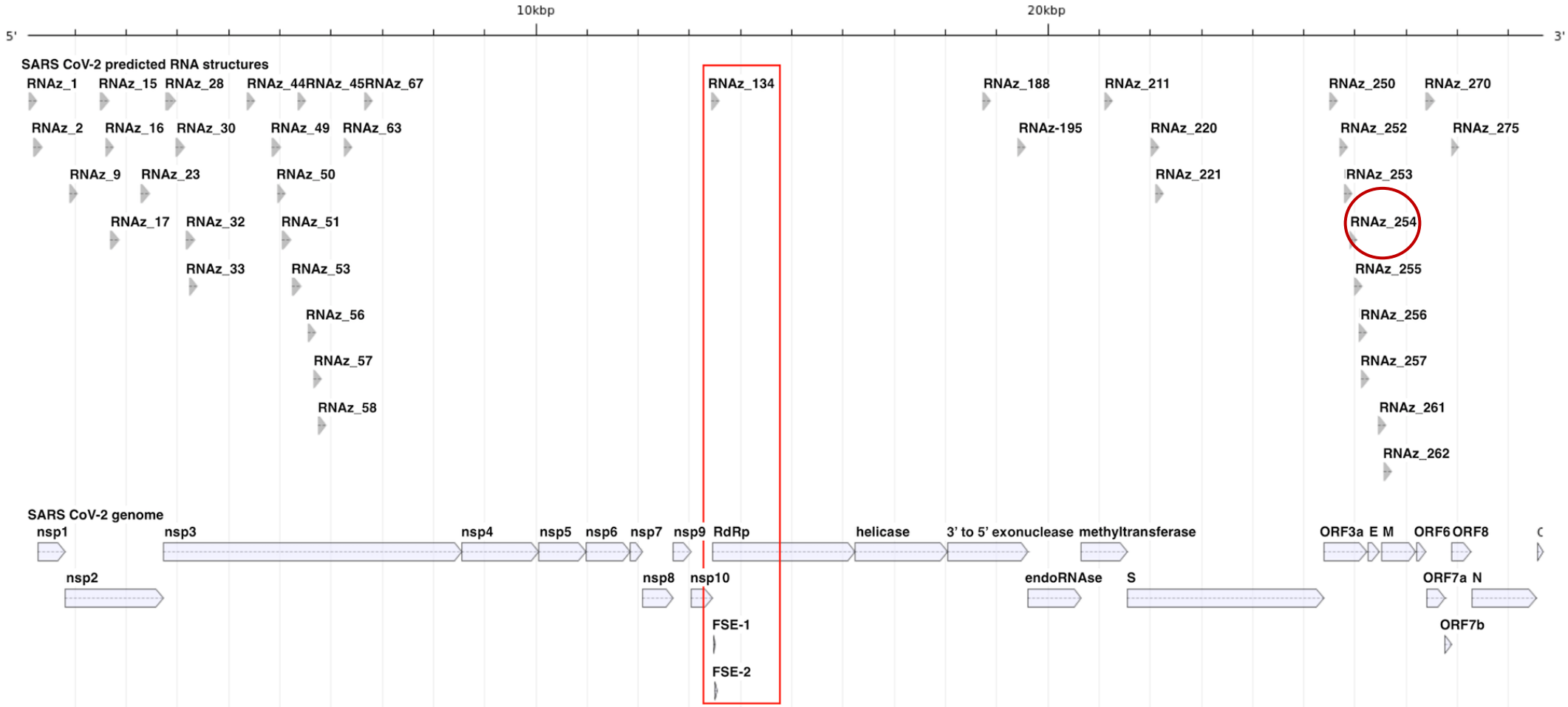
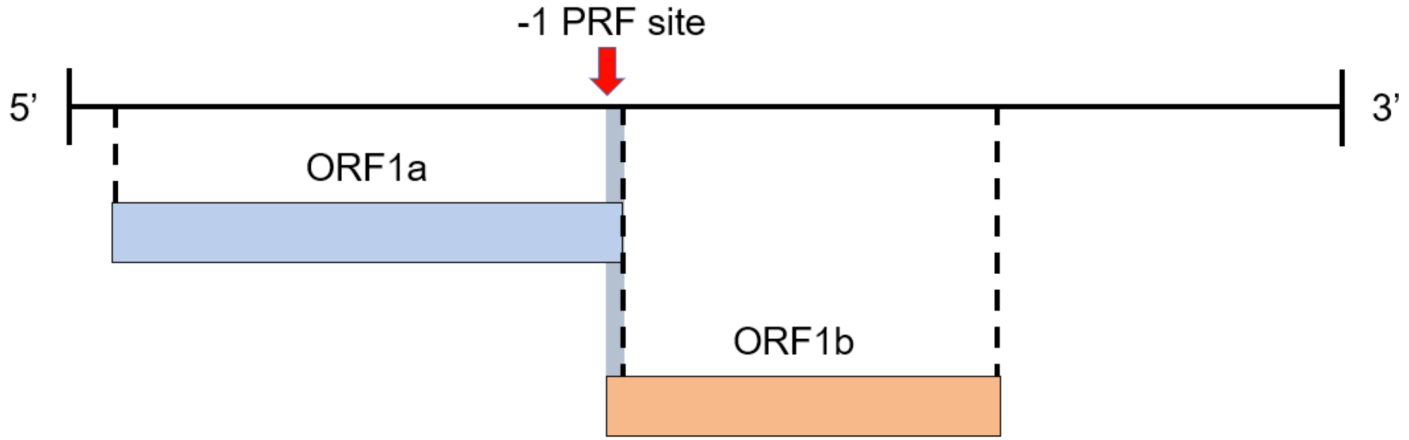
Ziesel and Jabbari 2024

Structure Comparison



Frameshifting Pseudoknot

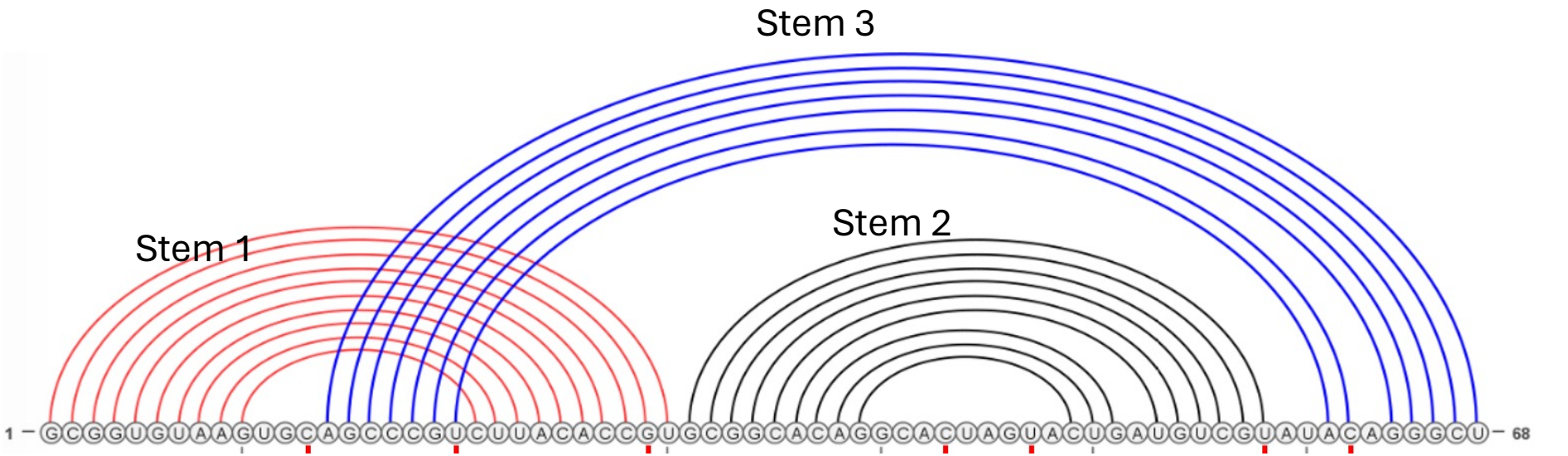
- Key in replication of coronaviruses: *ribosomal frameshifting*
- SARS-CoV-2 has 2 ORF
  - Normally only one translated
  - Pseudoknot facilitates frameshifting and translation of both!



What we know

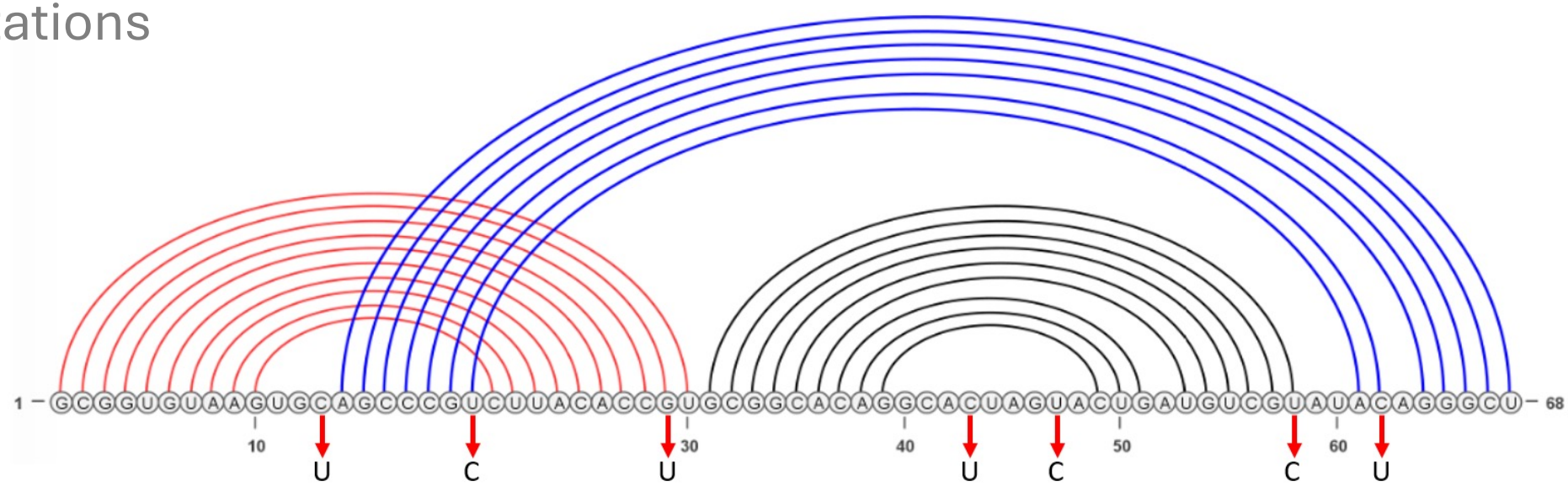
- SARS-CoV −1 PRF conserved in SARS-CoV-2
  - only a single base difference

- Pseudoknot plasticity



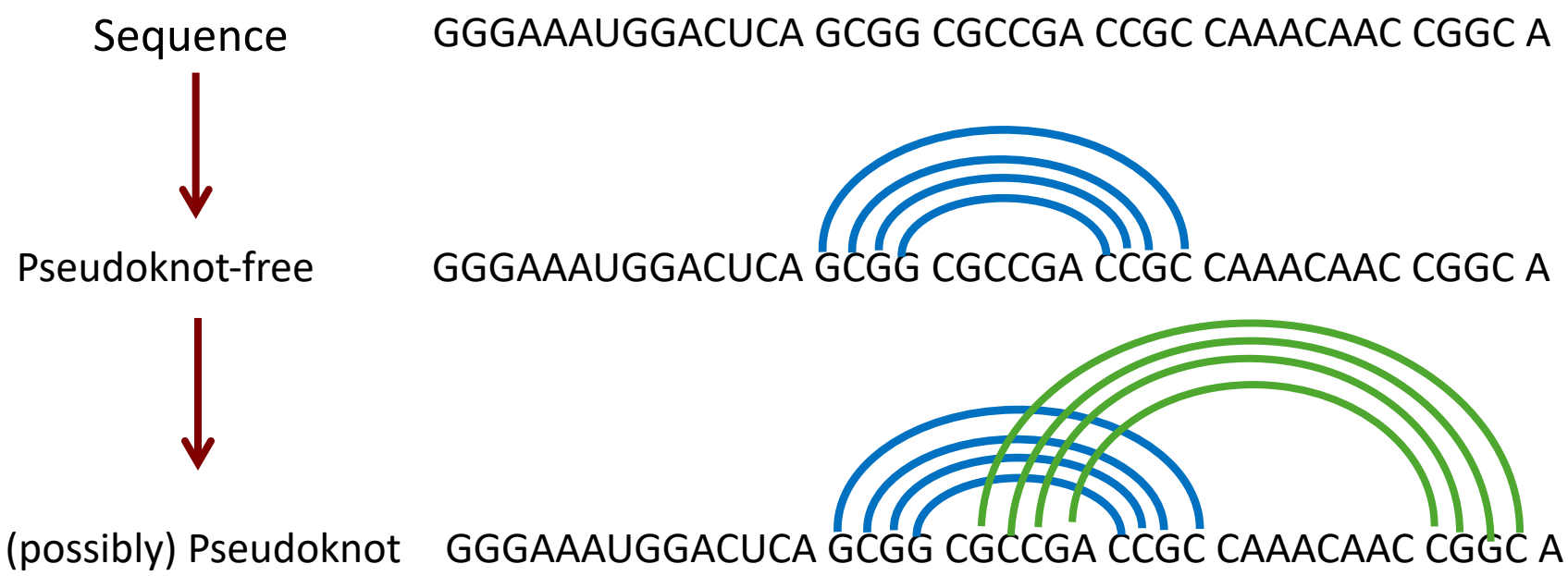
What we don’t know

- Characterization of the structure and its pathways
  - a sampling of land- scape of previously unidentified–non-native structures
  - Following a hierarchical folding view
  - Incorporating experimental data (SHAPE)
- Effects of mutations



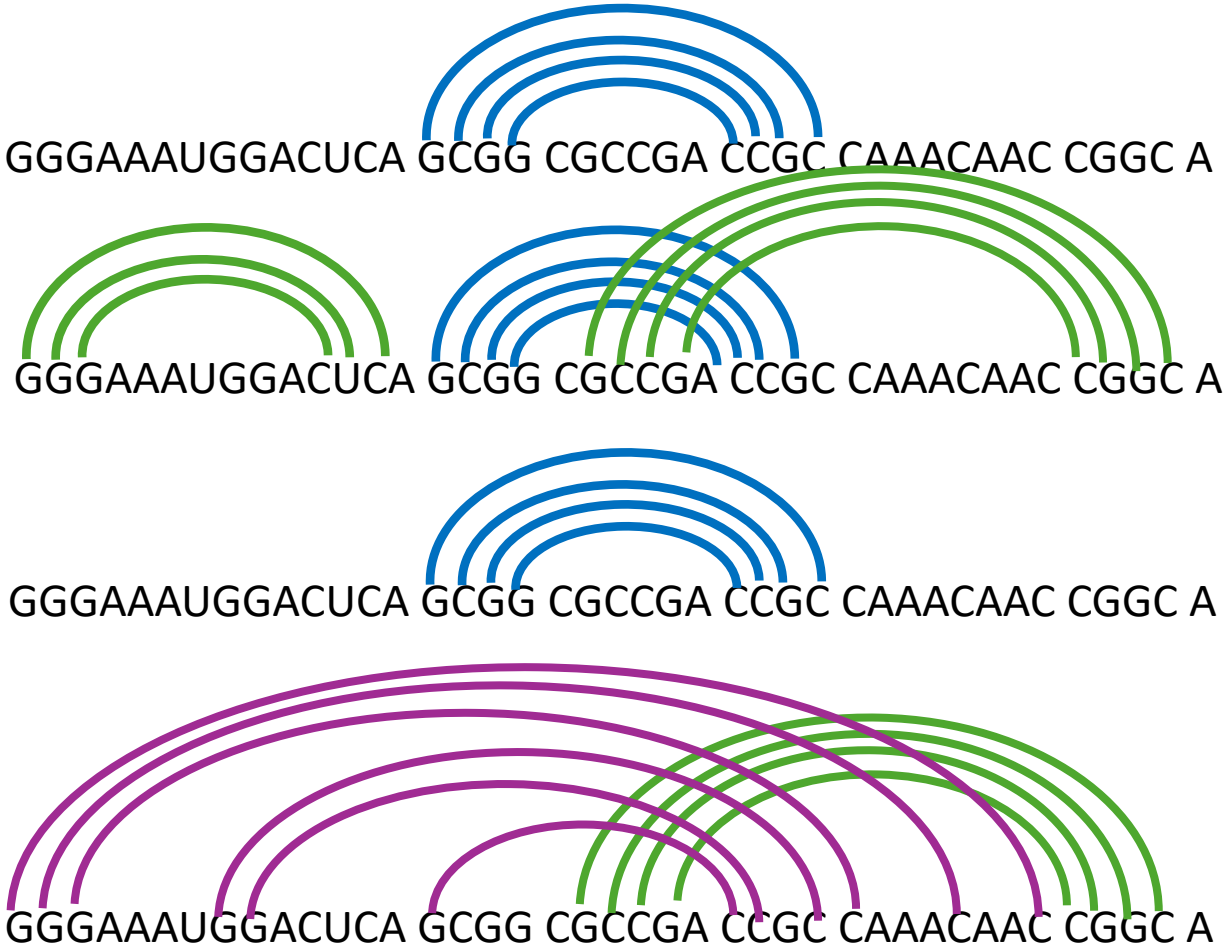
Hierarchical Folding

- Evidence that RNA structure formation is hierarchical [Tinoco & Bustamante 1999, Cho et al. 2009, Bailor et al. 2010]



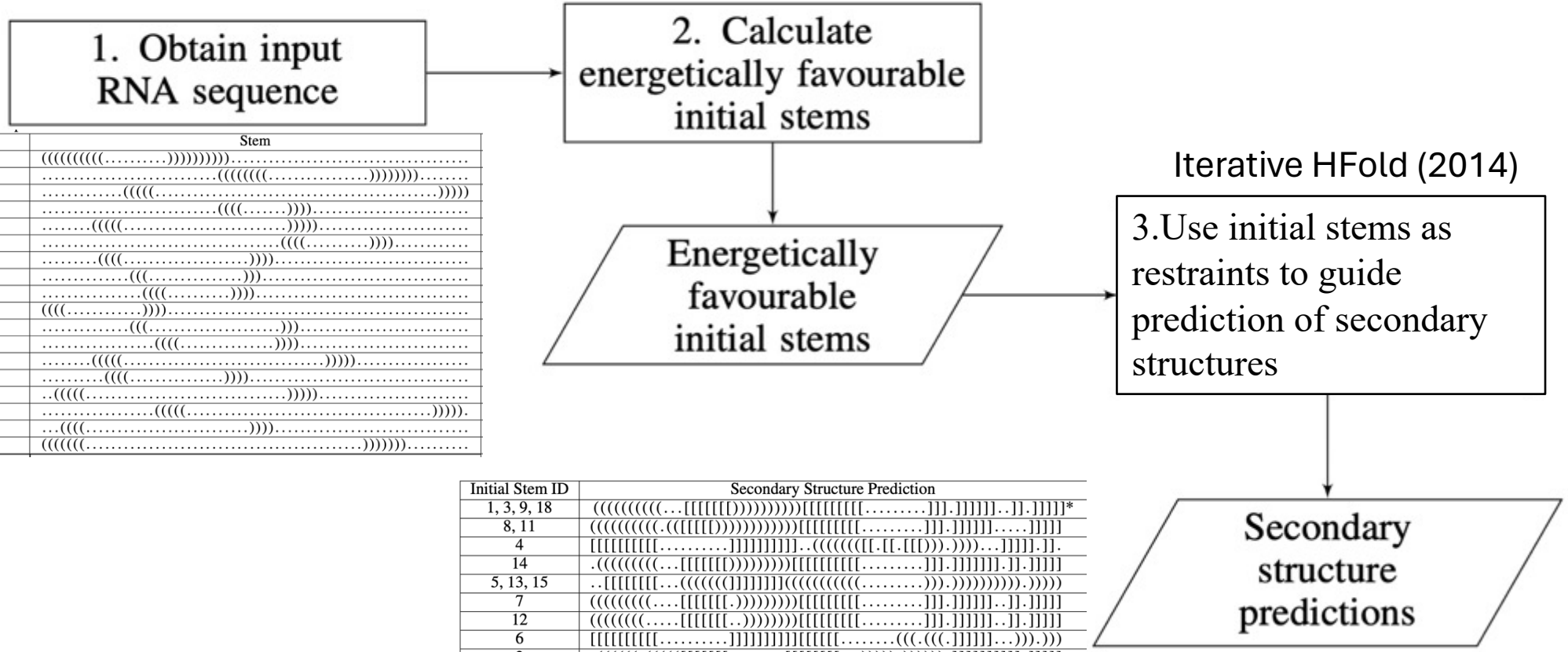
Hierarchical folding-based methods

- HFold [Jabbari et al. 2008]
  - Input: RNA Seq + a pk-free structure
  - Input structure as a **constraint**
  - Output: MFE given input
- Iterative HFold [Jabbari et al. 2014]
  - Input: RNA Seq + a pk-free structure
  - Input structure as a **restraint**
  - Output: MFE guided by the input



SARS-CoV-2 PRF Structure Prediction

GCGGUGUAAGUGCAGCCCGUCUUACACCGUGCGGCACAGGCACUAGUACUGAUGUCGUAUACAGGGCU

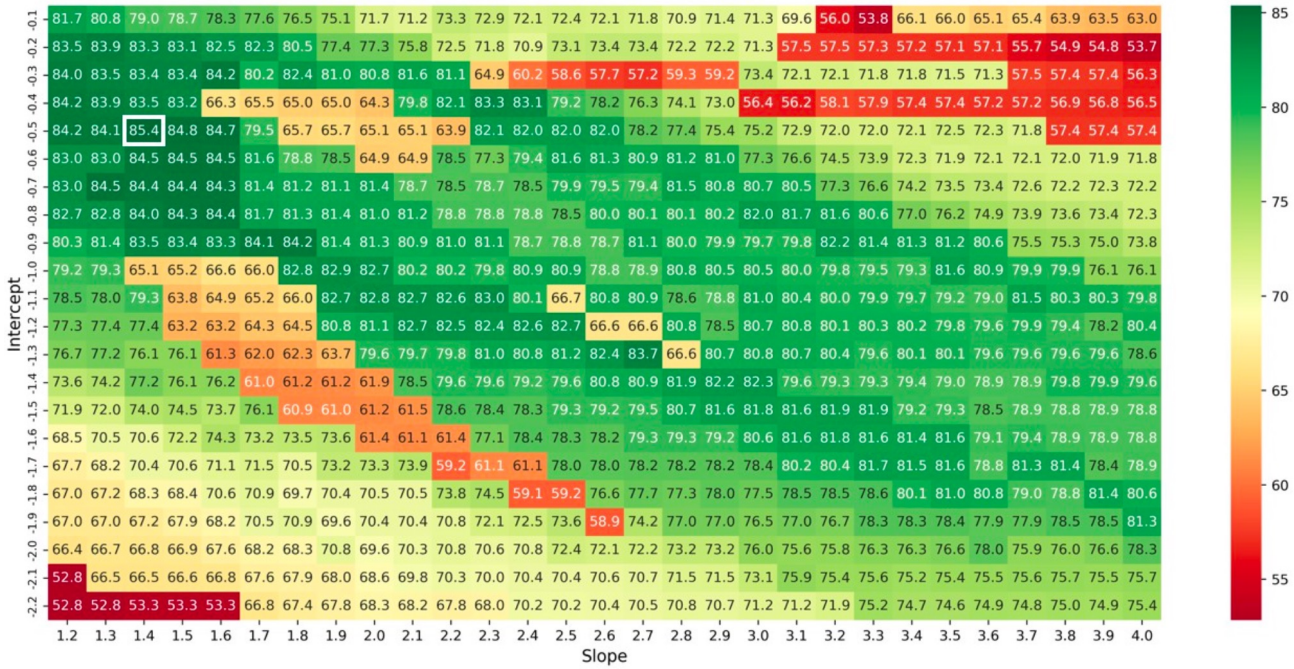


ID	Stem
1	(((((((((.....))))))))).....
2	.....(((((((.....))))))).....
3	.....(((((.....)))))
4	.....((((.....)))).....
5	.....(((((.....))))).....
6	.....((((.....)))).....
7	.....((((.....)))).....
8	.....(((.....))).....
9	.....((((.....)))).....
10	((((.....)))).....
11	.....(((.....))).....
12	.....((((.....)))).....
13	.....(((((.....))))).....
14	.....((((.....)))).....
15	..(((((.....))))).....
16	.....(((((.....))))).
17	...((((.....)))).....
18	(((((((.....)))))).....

Initial Stem ID	Secondary Structure Prediction
1, 3, 9, 18	((((((((((...[[[[[[[)))))))))))[[[[[[[[[.....]]].]]]]]]..]].]]]]]*
8, 11	((((((((((.(([[[[[)))))))))))))[[[[[[[[[.....]]].]]]]]].....]]]]]
4	[[[[[[[[[[.....]]]]]]]]]]..(((((((([.[[.[[[))).))))...]]]]].]].
14	.((((((((...[[[[[[[)))))))))[[[[[[[[[[.....]]].]]]]]]].]].]]]]]
5, 13, 15	..[[[[[[[[...(((((((]]]]]]]]((((((((((.....))).))))))))).)))))
7	((((((((....[[[[[[[.)))))))))[[[[[[[[[.....]]].]]]]]]..]].]]]]]
12	((((((((.....[[[[[[[..)))))))))[[[[[[[[[.....]]].]]]]]]..]].]]]]]
6	[[[[[[[[[[.....]]]]]]]]]]]][[[[[[.....(((.(((.]]]]]]...))).)))
2	.(((((((.((((([[[[[[[.....[[[[[[[[...)))))).))))))).]]]]]]]]]].]]]]]
10	.....(((((([[[[[[[.....[[[[[[[[[[[)))))....]]].]]]]]]]]]].]]]]]
16	[[[[[[[.....((((()]]]]]]]][[[[[[[[[.....]]].]]]]]]....))))).
17	.....[[[[(((((....(((((.((((.]]]]..))))..)).....)))....)))))
Input sequence	GCGGUGUAAGUGCAGCCCGUCUUACACCGUGCGGCACAGGCACUAGUACUGAUGUCGUAUACAGGGCU

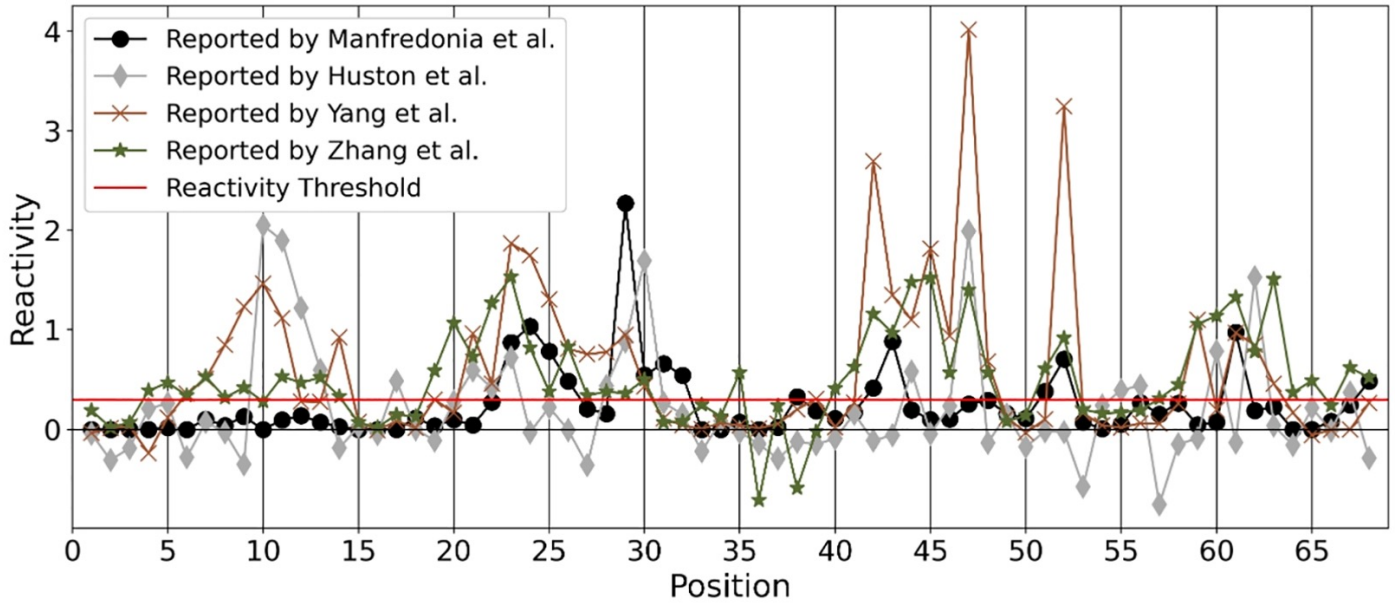
Incorporating SHAPE

- ShapeKnots data
  - 5 RNAs > 300
  - 5 riboswitches
  - 4 hard to predict
  - 3 RNAs in interactions
- Extended
  - 6 from RNA Mapping Database
  - 3 riboregulators
  - SARS-CoV2 3’ and 5’ UTR
  - ribonuclease domain of Bacillus subtilis

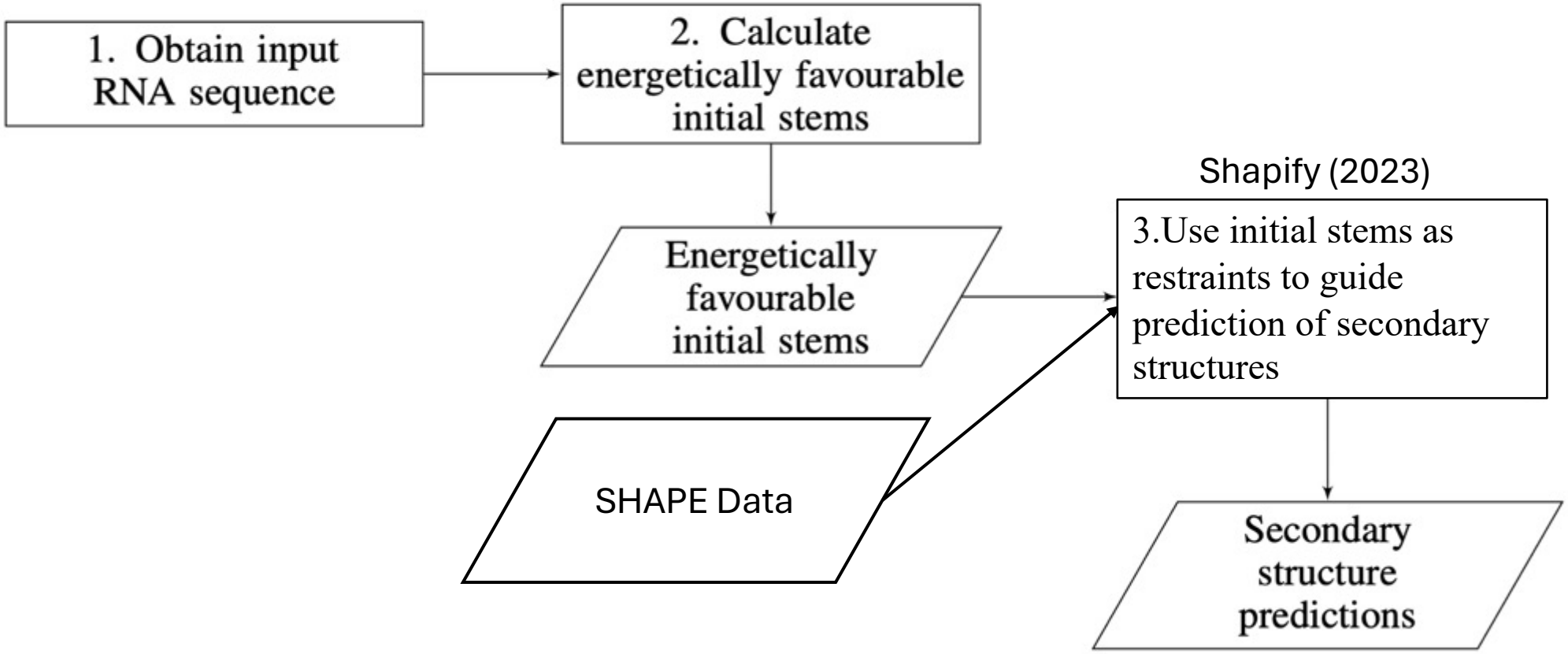


Shapify [Trinity et al. 2023]

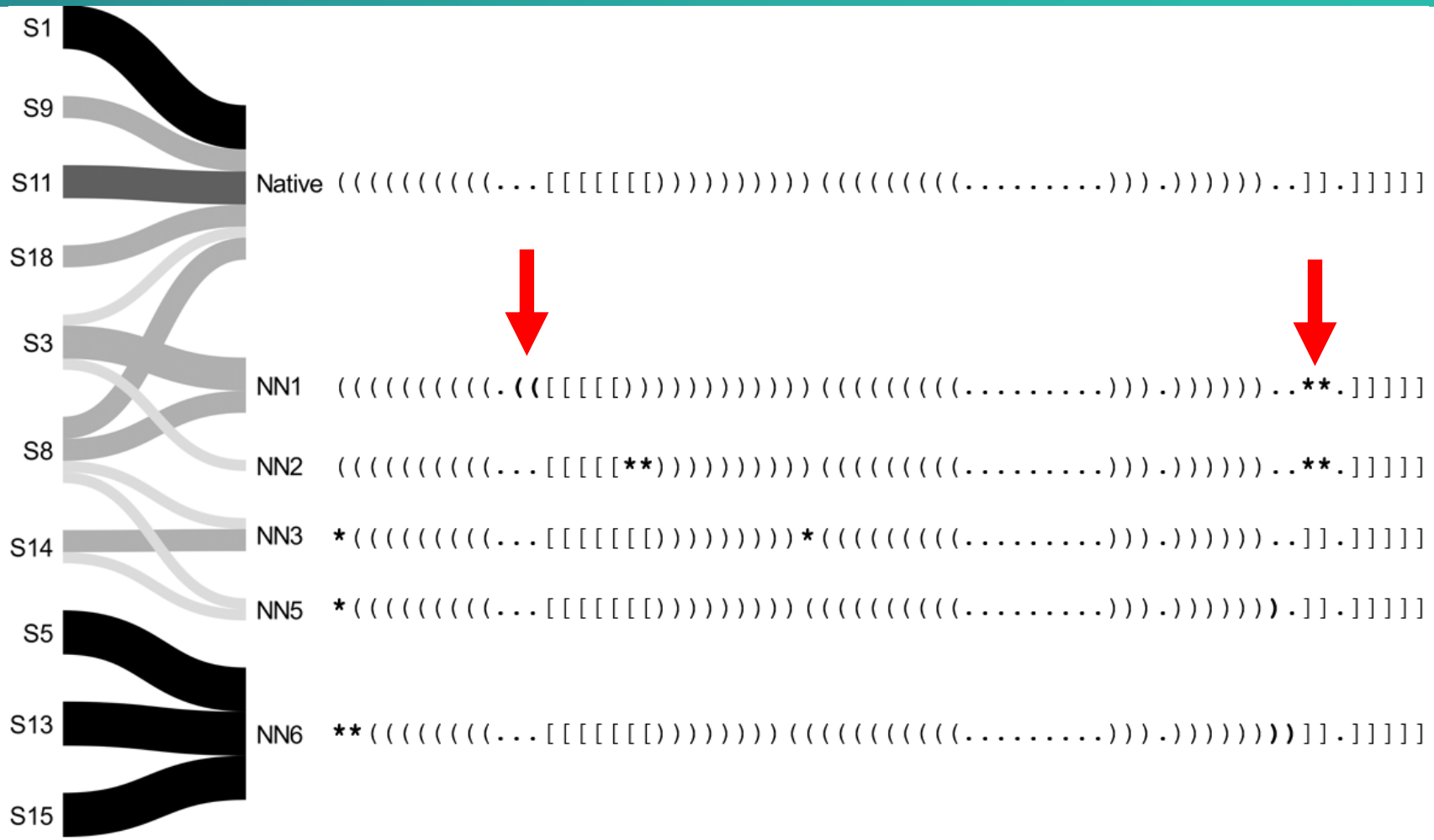
- Huston 2021 (in vivo, genome-wide)
- Manfredonia 2020 (in vivo, genome-wide)
- Yang 2021 (in vivo, genome-wide)
- Zhang 2021 (in vitro, PRF)



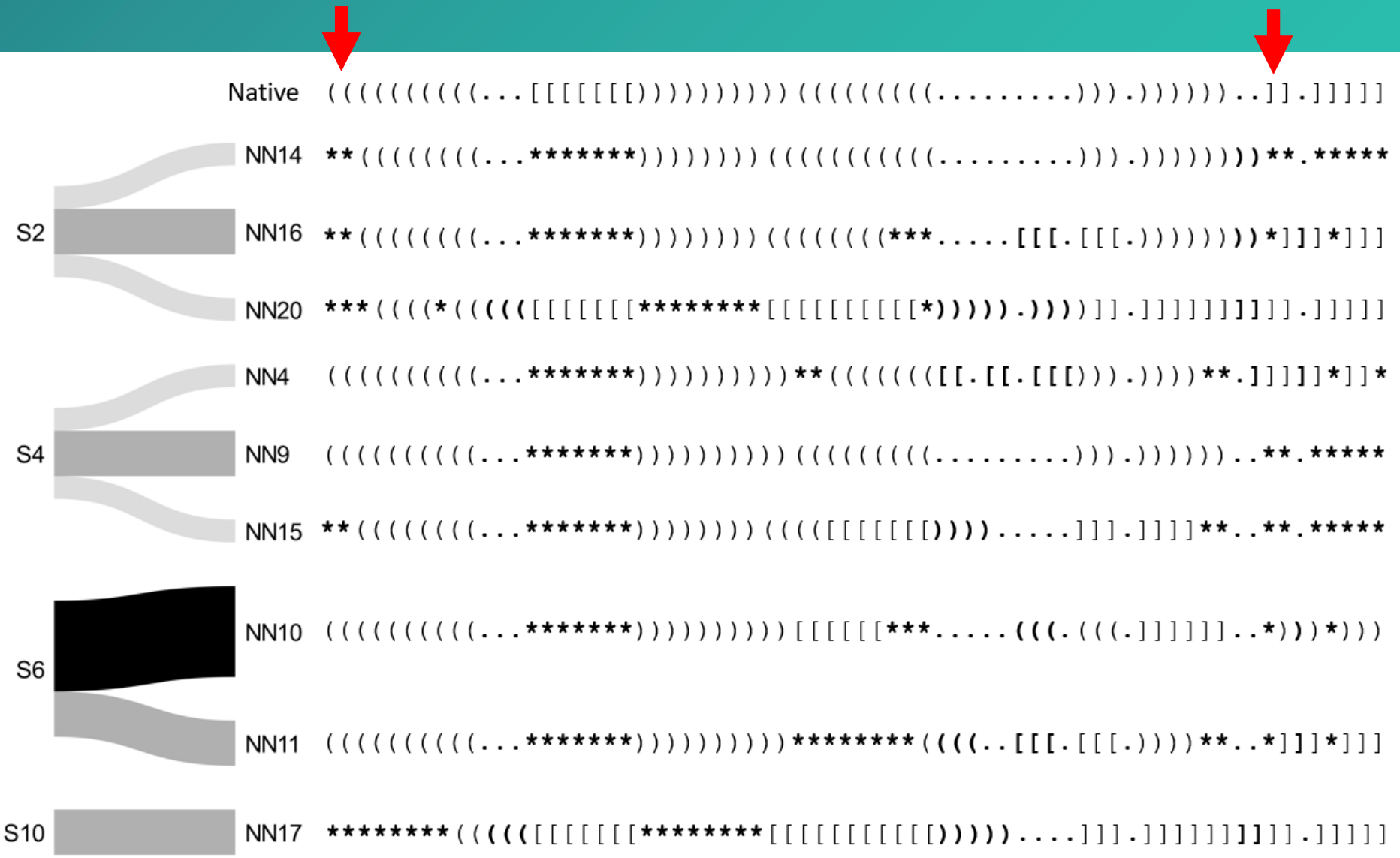
SARS-CoV-2 PRF Structure Prediction



Native-adjacent Structures

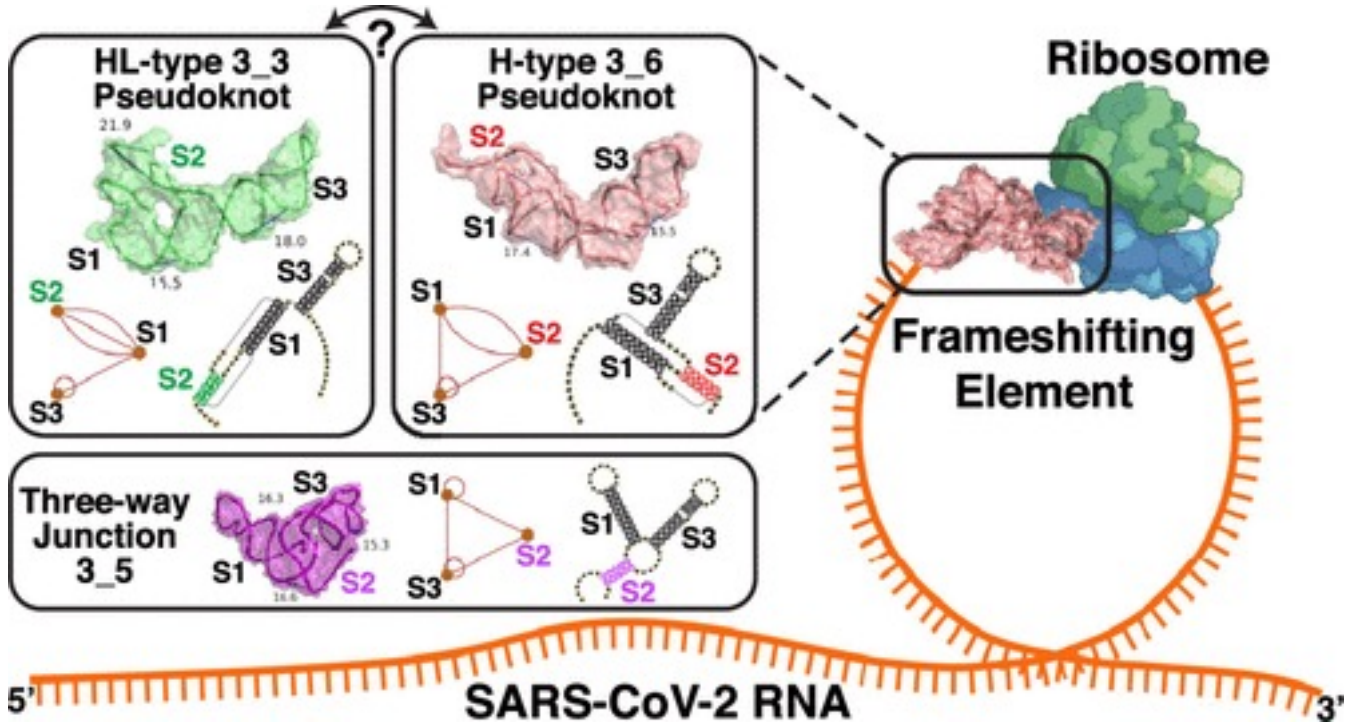


Native Non-adjacent Structures



What others found

- Schlick et al. identified 3 motifs [J Am Chem Soc. 2021]
  - SHAPE
  - ‘3_6’ motif corresponds with NN1, NN2, and NN4.
- Bhatt et al. [Science 2021]
  - cryo-EM
  - Structure that matched ‘3_6’ motif



Dual Graphs

2_1

((((.....))))((((.....))))

2_2

((((...((((...))))...))))

2_3

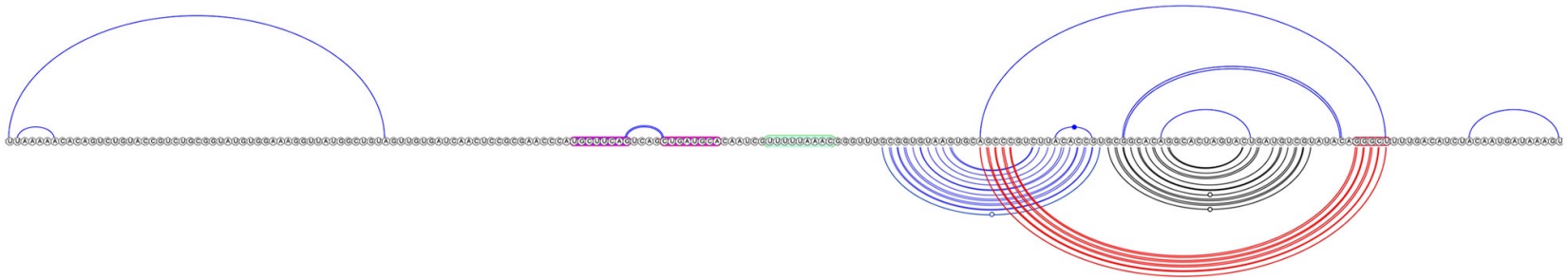
((((...[[[[...))))...]]]]

Tying the Knot [Trinity et al. 2024]

- Expanded FSE window size
  - 90 to 222 nt

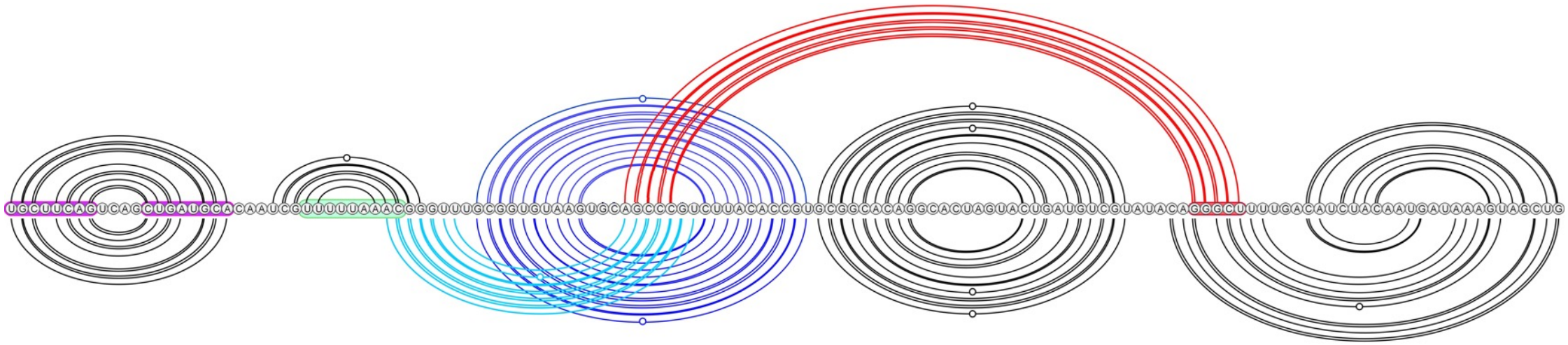
$$\text{Free energy per nt} = \frac{\text{Structure free energy}}{\text{Sequence length}}$$

- Considered covariation
  - KnotAli [Gray et al. 2020]
  - MSA obtained from Schlick et al. JACS 2021
- Considered refolding



144 nt window

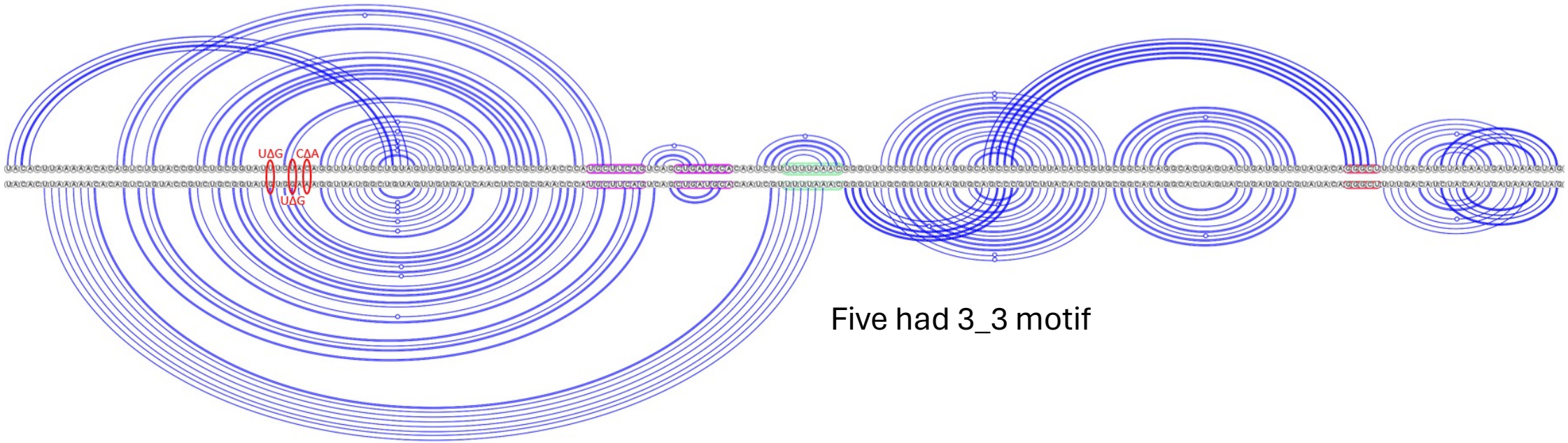
3_6 motif – Native structure



3_3 motif

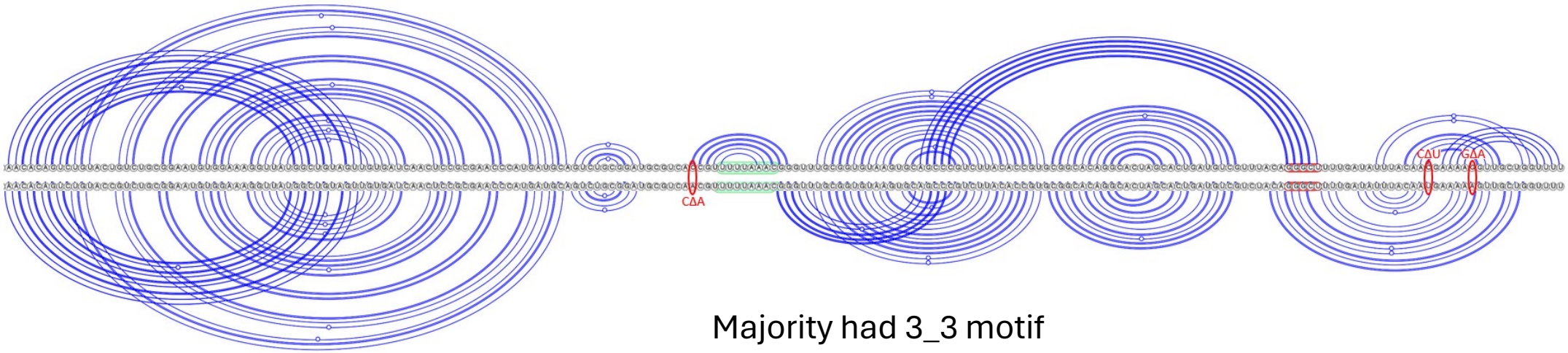
Trinity et al. 2024

Incorporating Covariation – 7 SARS-CoV2 sequences



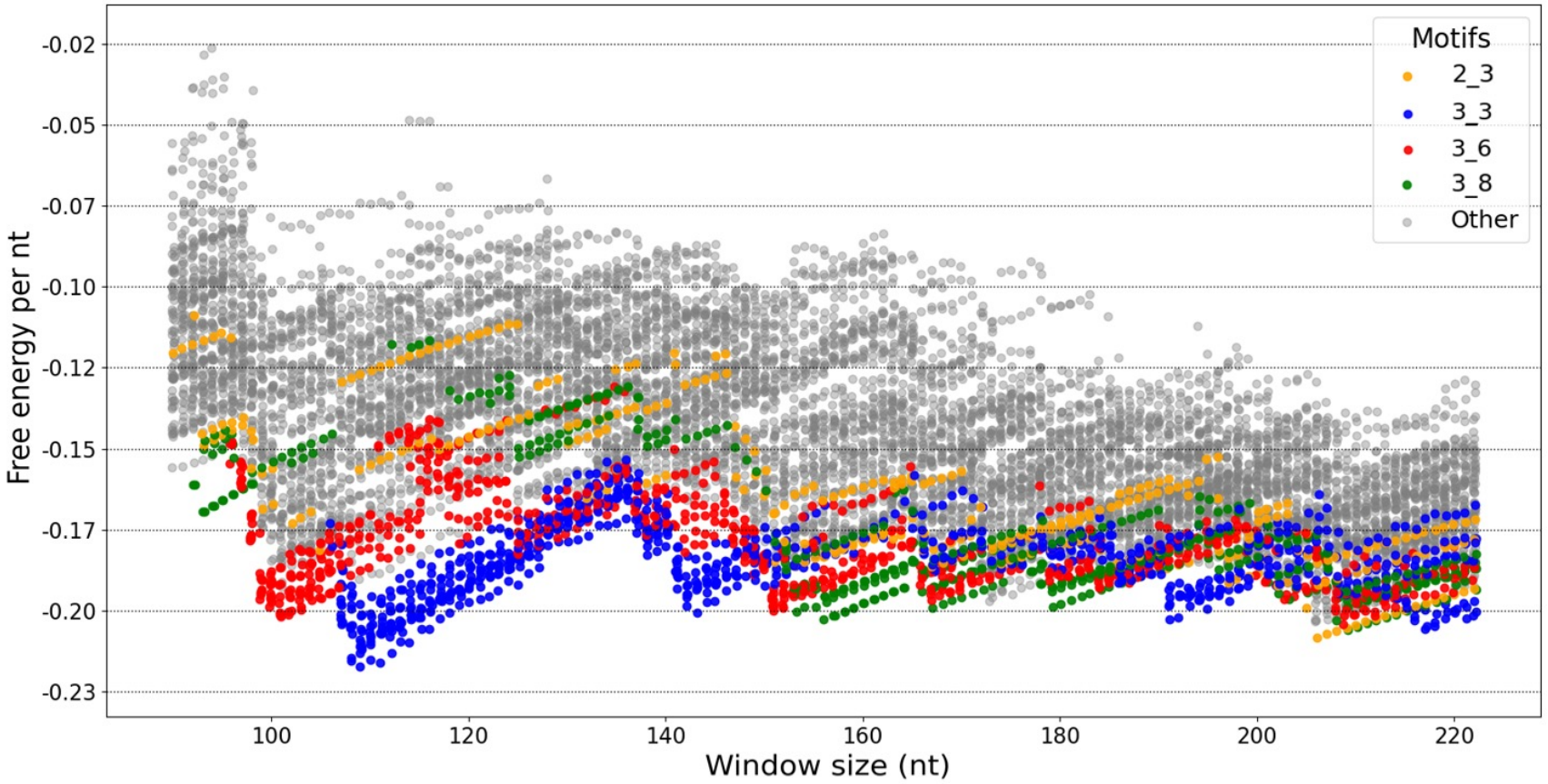
Five had 3_3 motif

Incorporating Covariation – sarbecoviruses



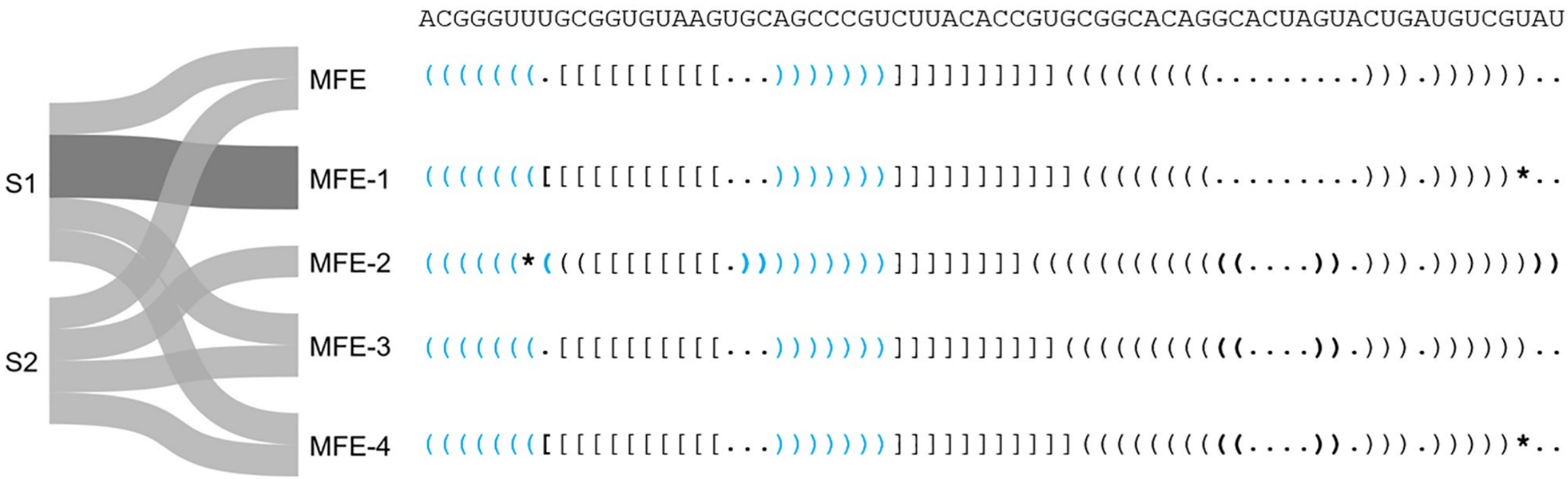
Majority had 3_3 motif

Multiple Structures – Varying Window Size and SHAPE

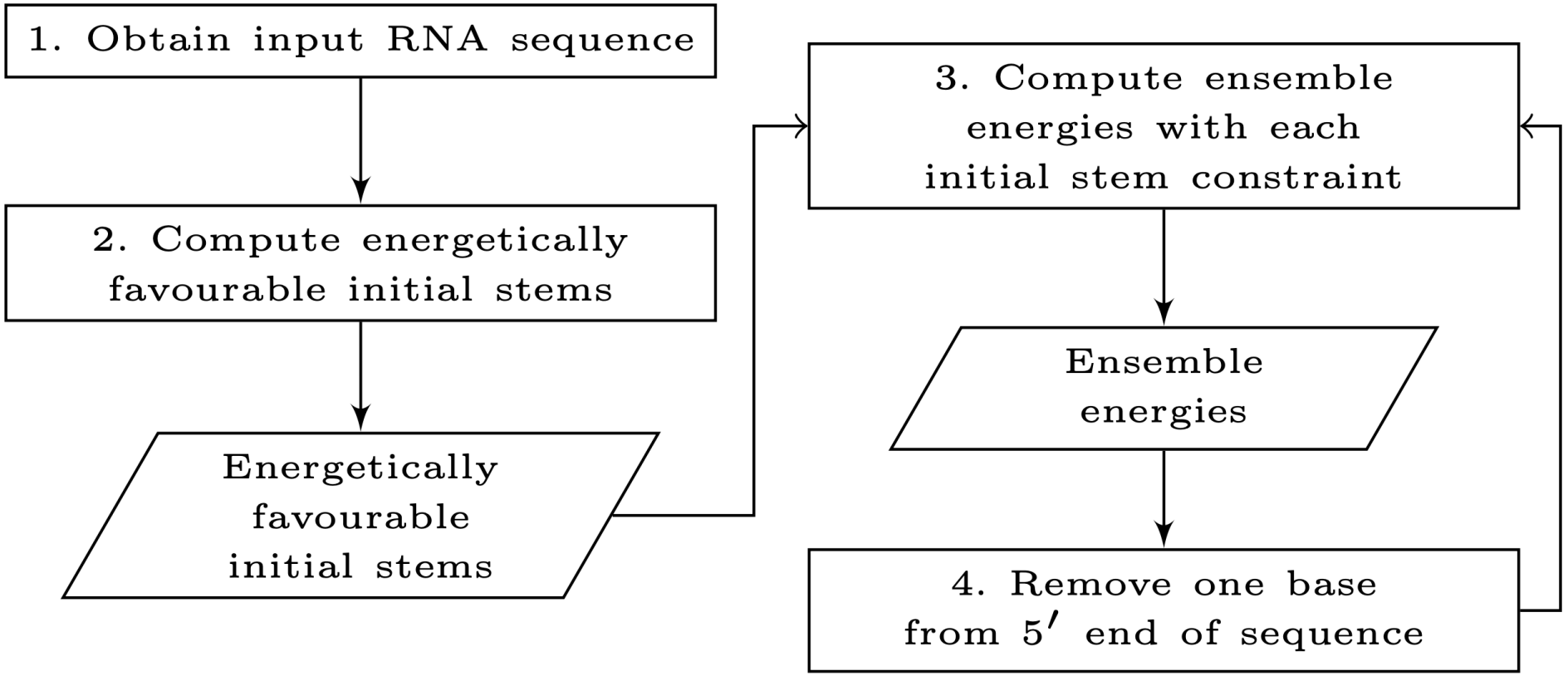


~11k sequences

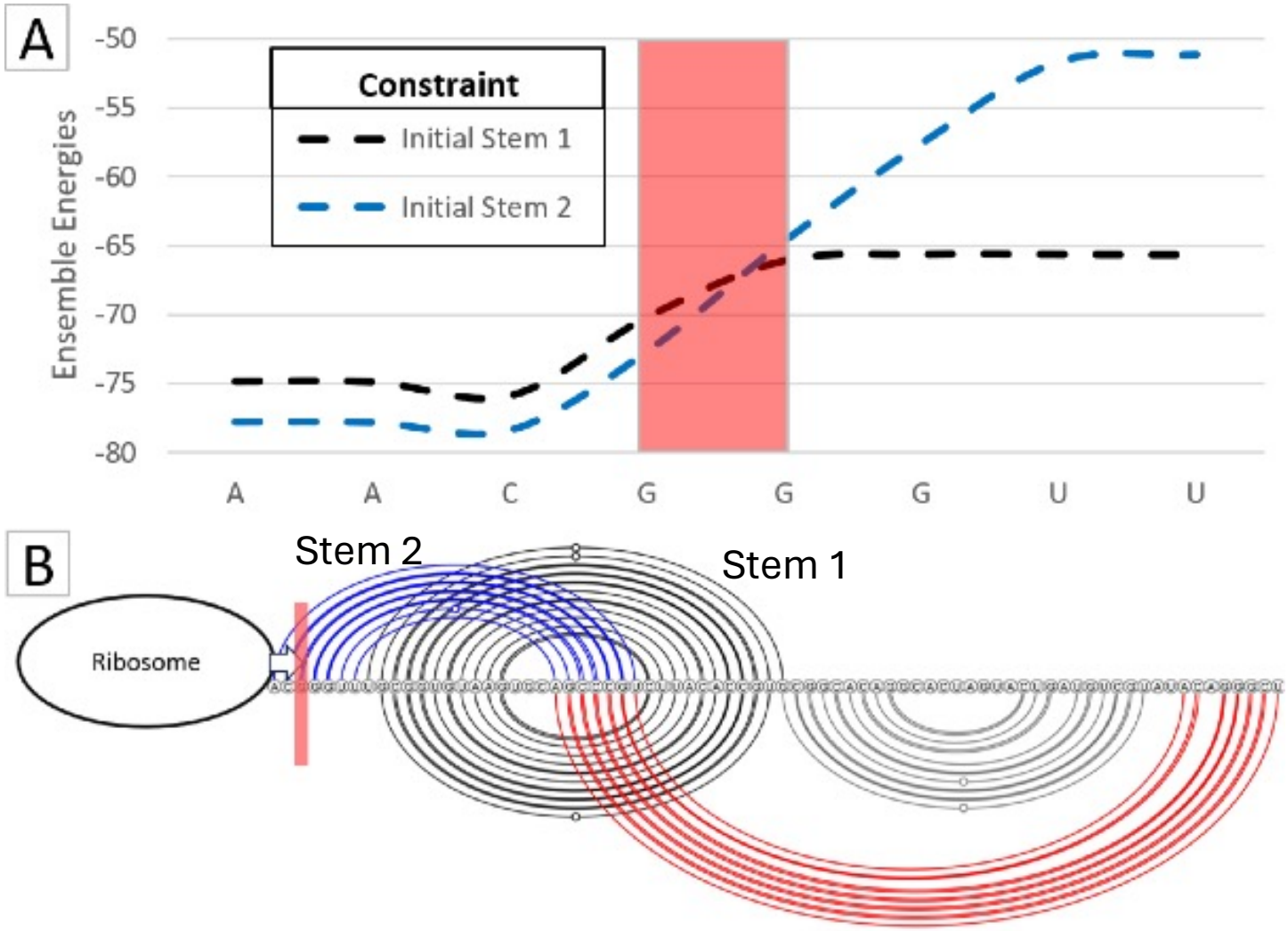
Path convergence for 3_3 motif



Simulating refolding– CParty [Trinity et al. 2024, submitted]



Decreasing sequence length from 77 to 70



CParty

Iterative HFold

What we found

- Conserved Similarity
- Structural paths
- SHAPE incorporation
- Covariations and length dependence
- Motif transitions

We are hiring PhD students and Postdocs!

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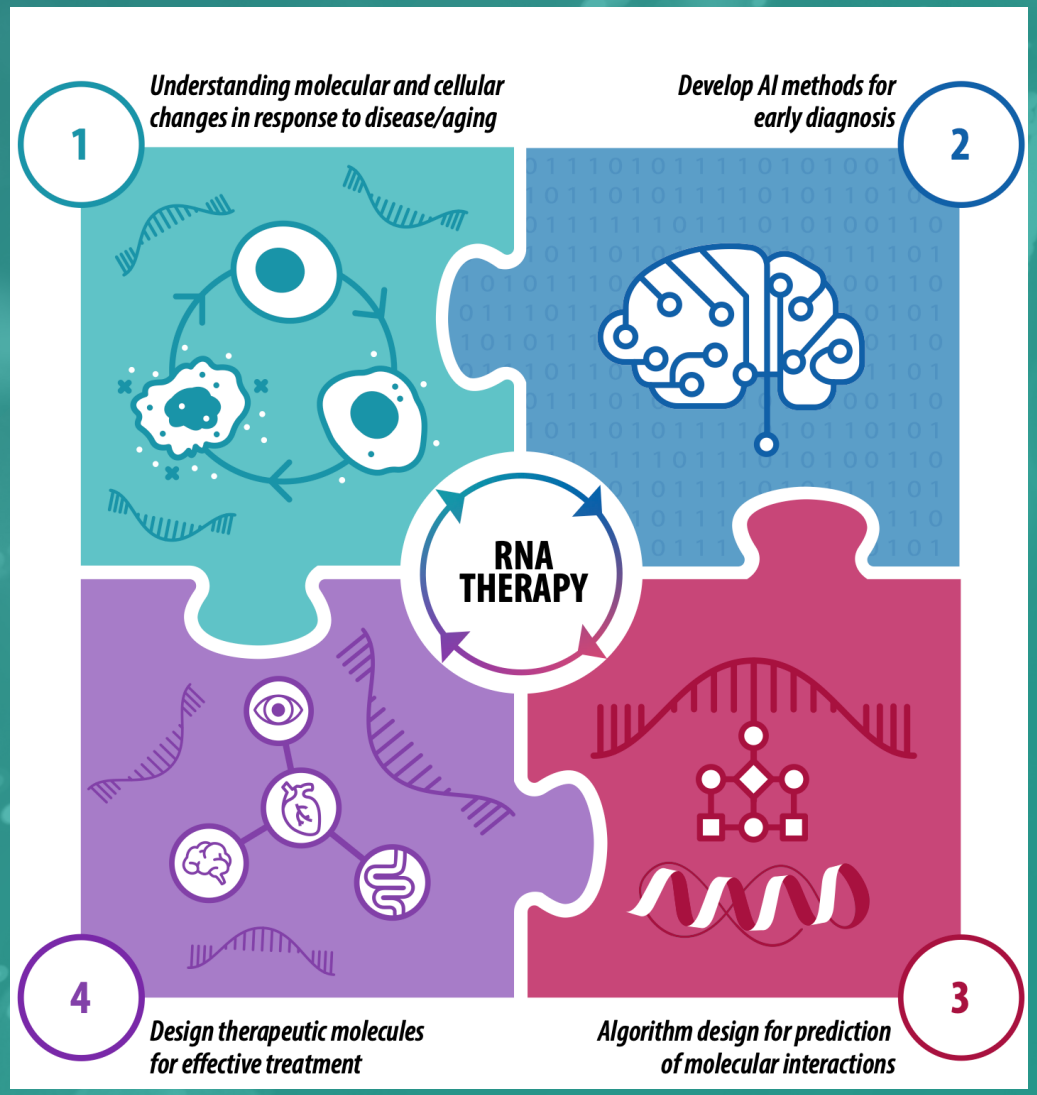

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Thank you!

Sebastian Will

Ulrike. Stege

Yan Ponty

All members of the COBRA Lab

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