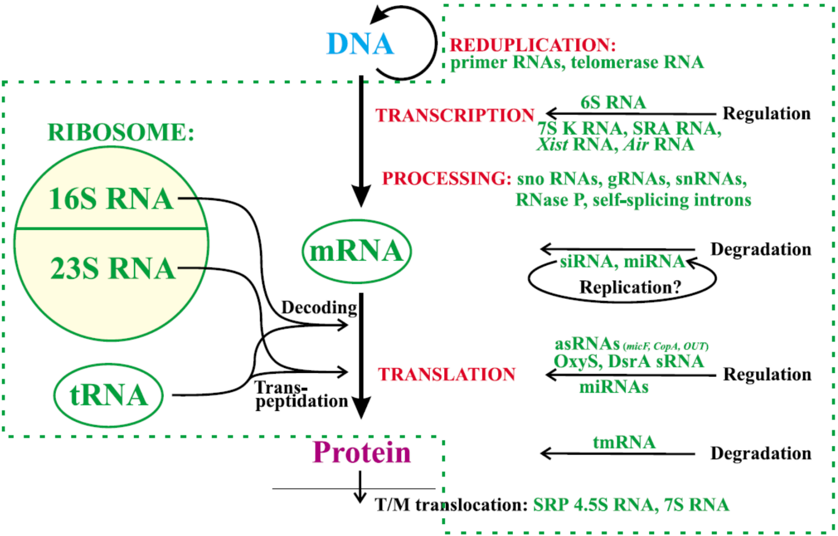
A Brief Overview of RNA Bioinformatics

Sebastian Will
University of Vienna

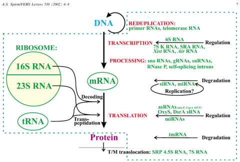
Freiburg sRNA Meeting 2019

Same Goal: Functions and Role of RNAs

A.S. Spirin/FEBS Letters 530 (2002) 4–8 7



Different Methods



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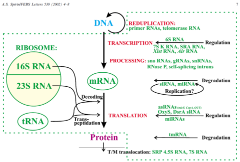
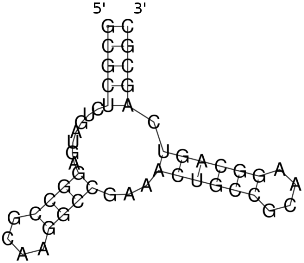


The Central Dogma (of RNA Bioinformatics)

Sequence $\Longrightarrow$ Structure $\Longrightarrow$ Function

5' - GCGCUCUGAUGAGGCCGCAAGGCCGAAACUGCCGCAAGGCAGUCAGCGC - 3'



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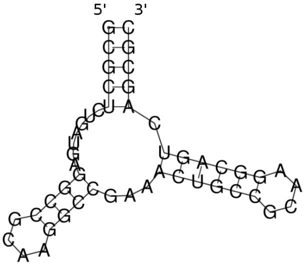
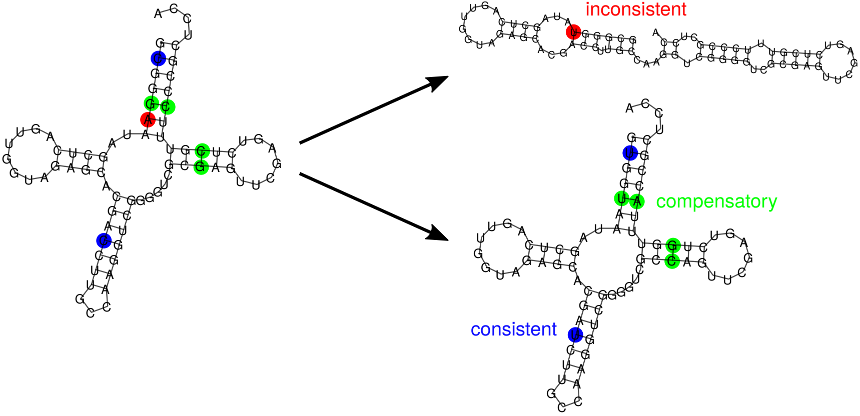


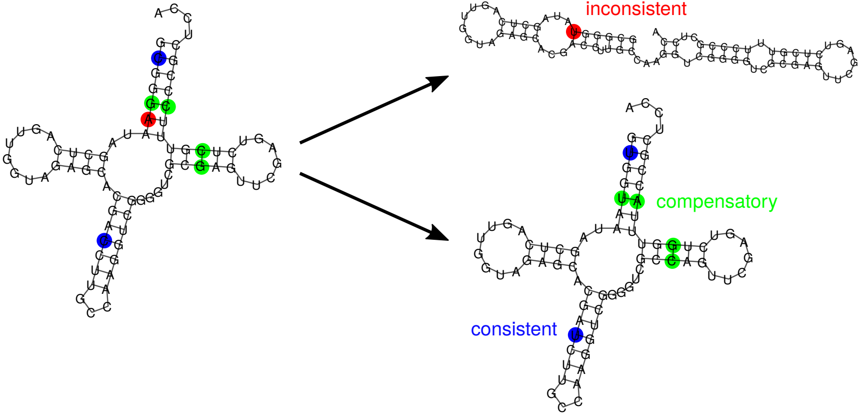
Structure as *Proxy* of Function

RNA Structure is Emergent



Almost identical sequences — very different structures
Very different sequences — same structure

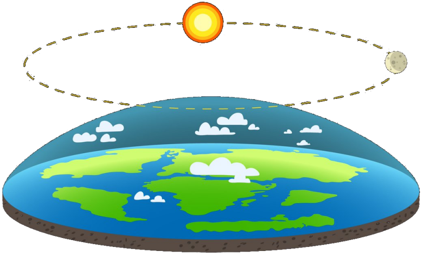
RNA Structure is Emergent



No Shortcut “Sequence $\Longrightarrow$ Function”

More Dogmas

The world is simple!*



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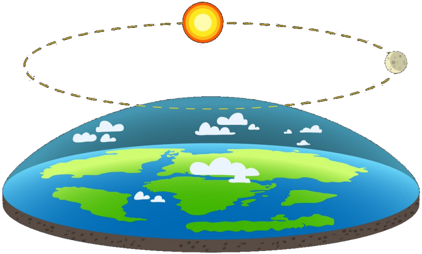
**in first approximation*



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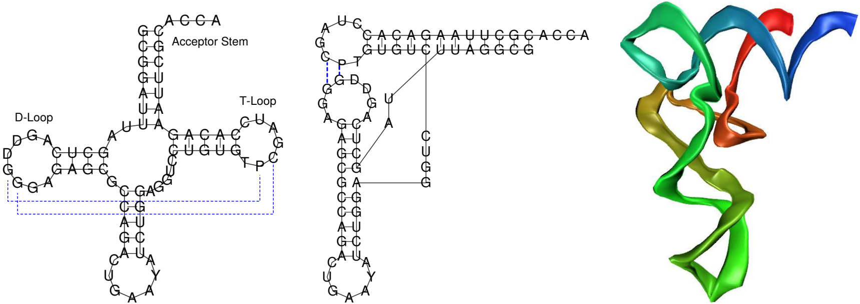


Viable Shortcut

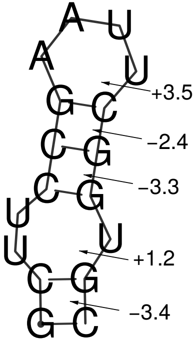
Sequence $\Longrightarrow$ 2D Structure $\Longrightarrow$ Function

Energies of RNA structures can be calculated

Nearest Neighbor Model (NNM)

- Free energies = sum of loop energies
- Loop energies measured experimentally (based on UV melting curves)
- Loop energies depend on
  - loop type
  - size
  - base composition

  ⇒ large energy parameter tables



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−4.4 kcal/mol

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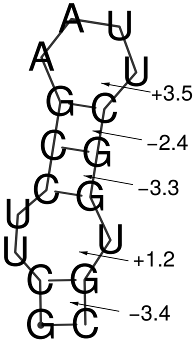

Total energy
−4.4 kcal/mol

+ distinguish RNA structures by free energy
+ define *minimum free energy (MFE)*
+ basis for entire tool set for RNA structure

- limitations (stay tuned)

From NNM to Structure Prediction

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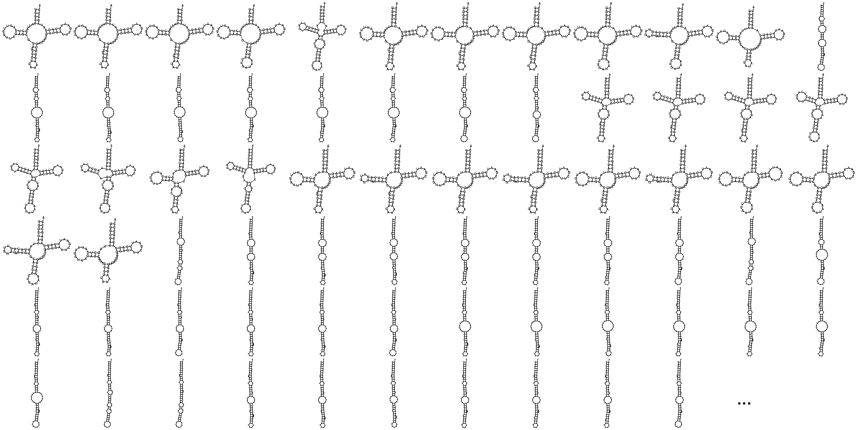

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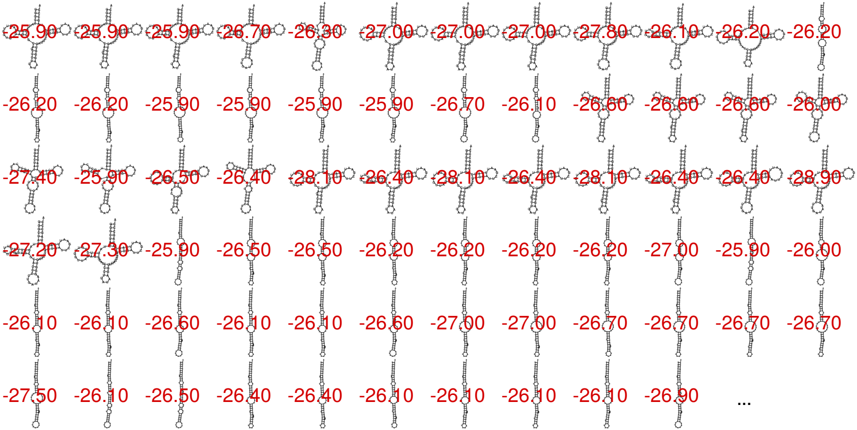

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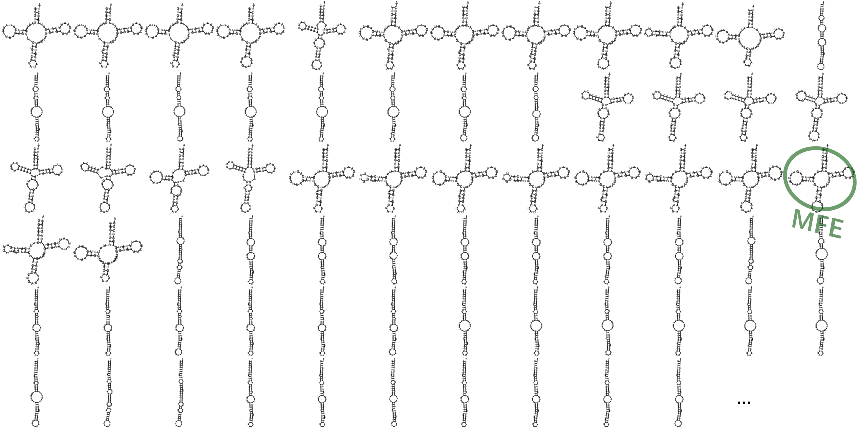
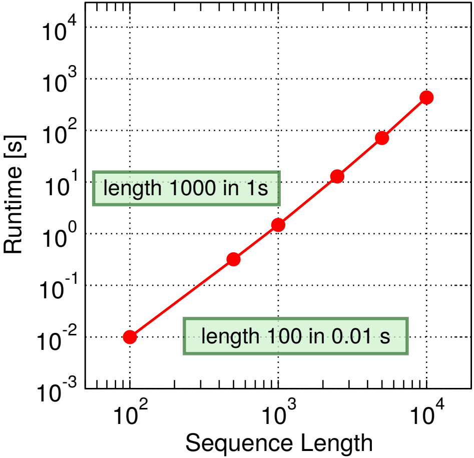


Structure Prediction: Fast and Accurate

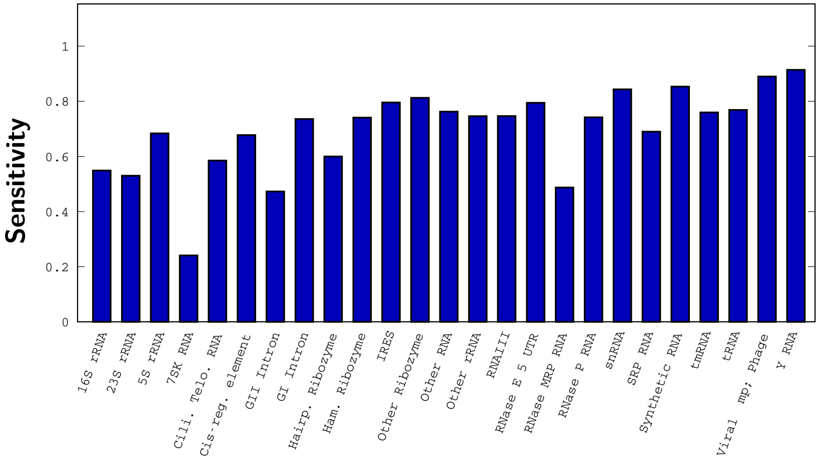
Performance of RNAFold (Vienna RNA Package 2.0)



[adapted from Vienna RNA Package 2.0, ALMOB 2011]

- **Very fast** folding algorithms: **0.01 seconds at length 100**

Structure Prediction: Fast and Accurate



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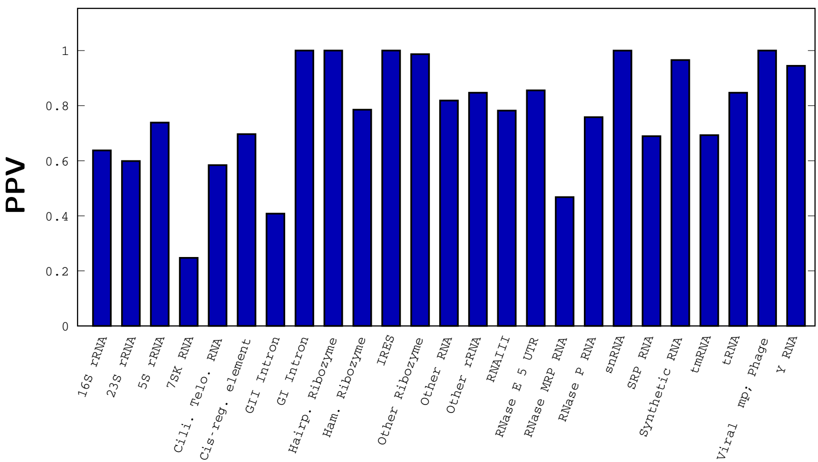
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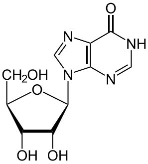
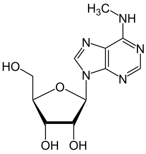
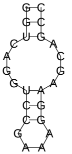
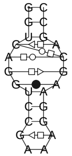
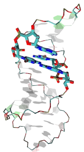
Limitations

- Modified bases  ,  , . . .

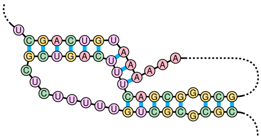
- Non-canonical base pairs

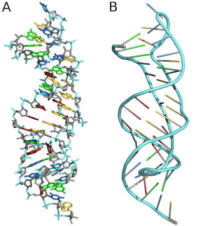




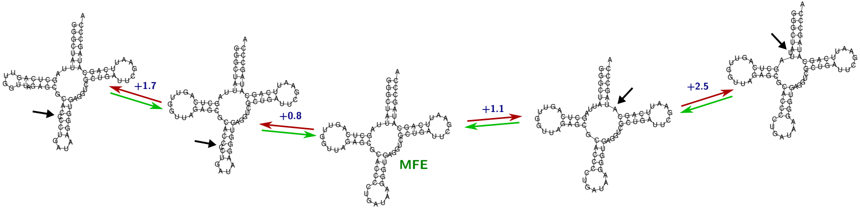


Pseudoknots





RNAs Refold at 'Room Temperature'



The MFE misleads! Look at

- suboptimal structures
- structure ensembles
- kinetics (co-transcriptional!)

Suboptimals and Probabilities

GGGCUAUUAGCUCAGUUGGUUAGAGCGCACCCCUGAUAAGGGUGAGGUCGCUGAUUCGAAUUCAGCAUAGCCCA

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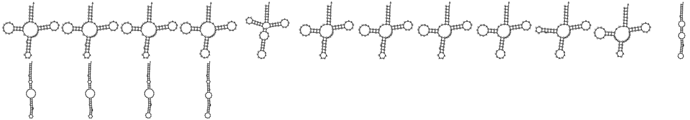

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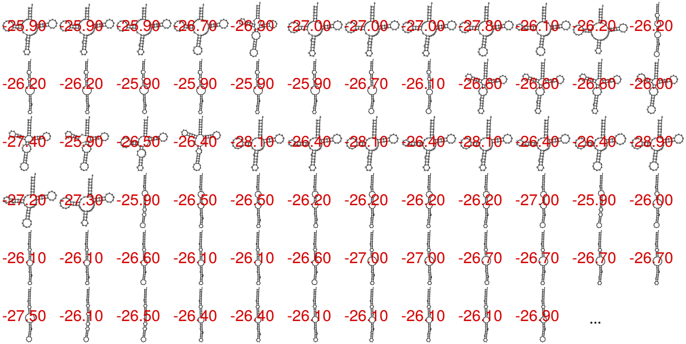

Energies → Structure Probabilities

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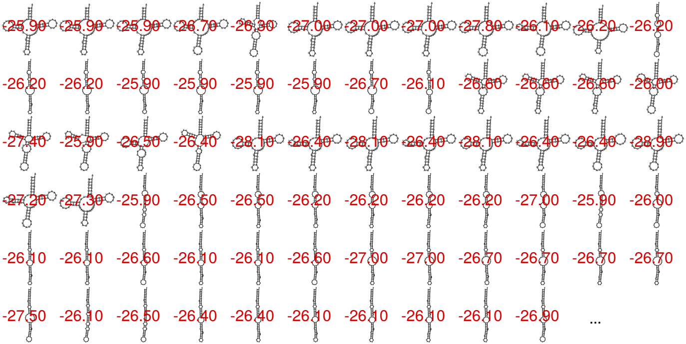
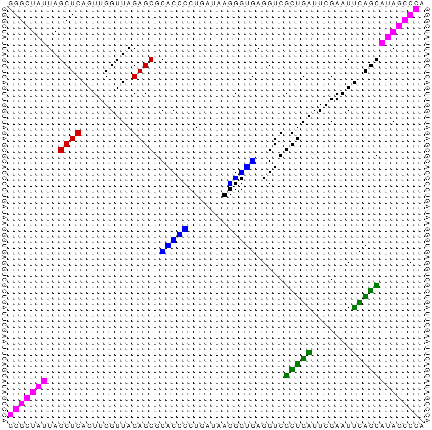
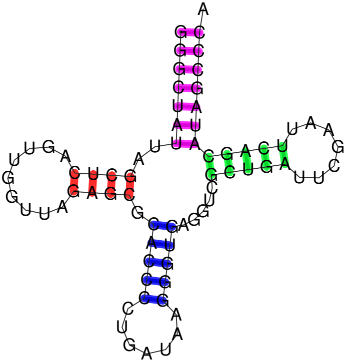


Energies → Structure Probabilities

Structure Probabilities → Base Pair Probabilities

Dotplots and Reliabilities





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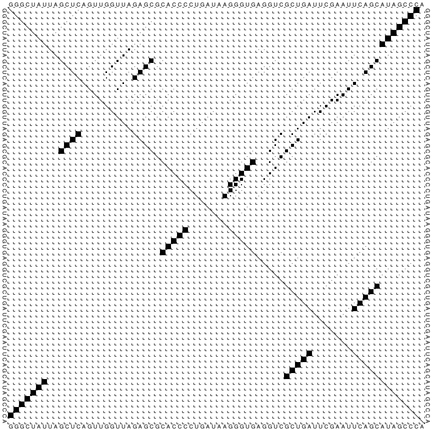
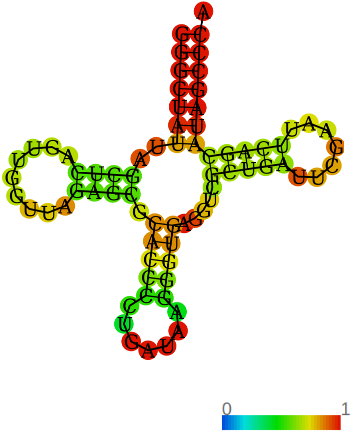
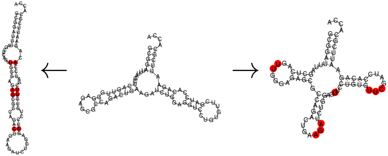




Integrating Prior Knowledge

- Knowledge on base pairing:



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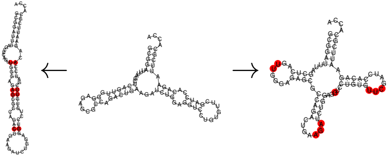
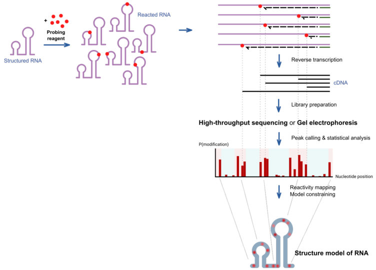
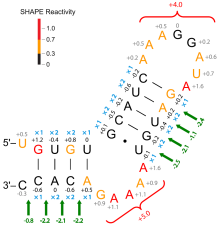


- Structure probing experiments (e.g. SHAPE)



Adapted from Ptrw08, A schematic figure explaining the steps in a typical chemical probing experiment to assay the structure of RNA molecules, CC BY-SA 4.0



$$\Delta G_{\text{NN}} = \sum \Delta G_{\text{stacks}} + \sum \Delta G_{\text{loops}}$$

$$\Delta G_{\text{SHAPE}} = \mathbf{1\times} \sum \Delta G_{\text{ends}} + \mathbf{2\times} \sum \Delta G_{\text{interior}}$$

$$\Delta G_{\text{total}} = \Delta G_{\text{NN}} + \Delta G_{\text{SHAPE}}$$

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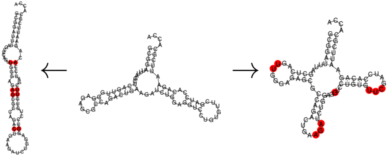

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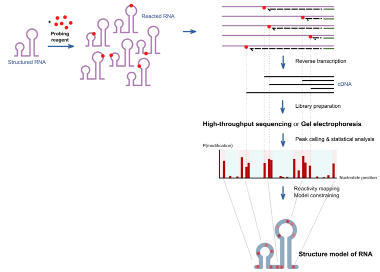

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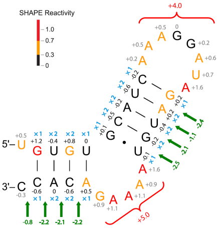

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- Homology

```
Examp1    ----------CCGG-AAA-CCGAACGCAGCACCGCGG------AU-CUGGAACGC--
Examp2    ----------CGCU-AG--AACAAC-------UAUCU------GU-AGCGCGAAAAC
Examp3    ---------AUUGUGUA--GCAUU------AGUUUGC-------GUGCAAAGAACGC
Examp4    -------UGCCAUCGCAUUAGCACC---U-AGCCGCAUUUUCUGGCGAUGAUG----
Examp5    AGCACCGAACCGCAU----GCGAACUGAG-AA--CGCAACC----AUGCGCGCACC-
```

Comparative Analysis with Alifold

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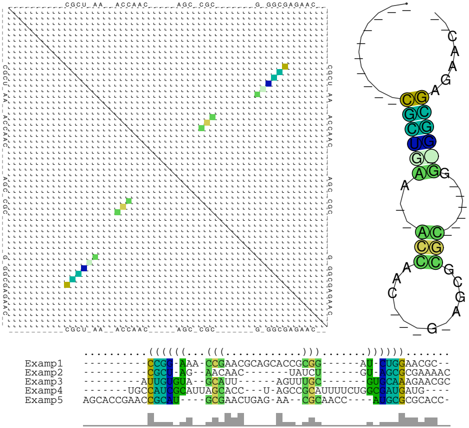
⇓ Alifold



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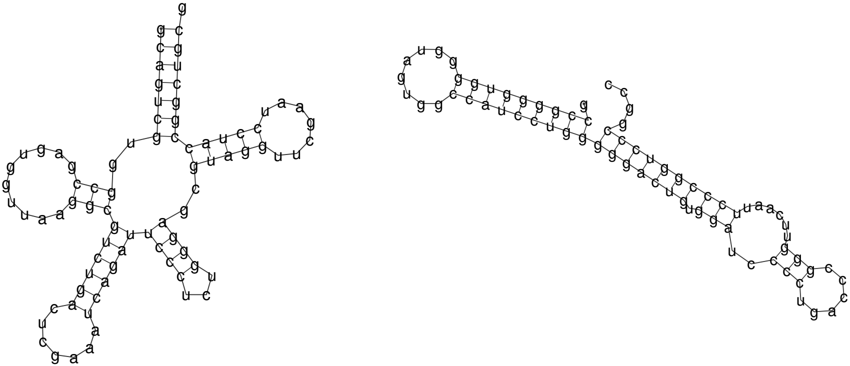
[Fig. adapted from Vienna RNA Package 2.0, ALMOB 2011, alifold exanple]

Simultaneous Alignment and Folding (with LocARNA)



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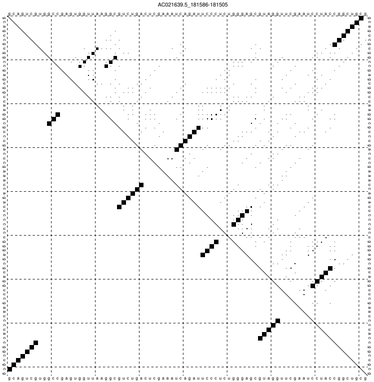

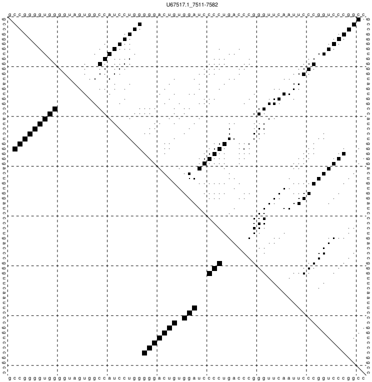

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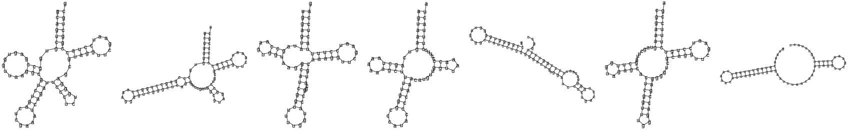

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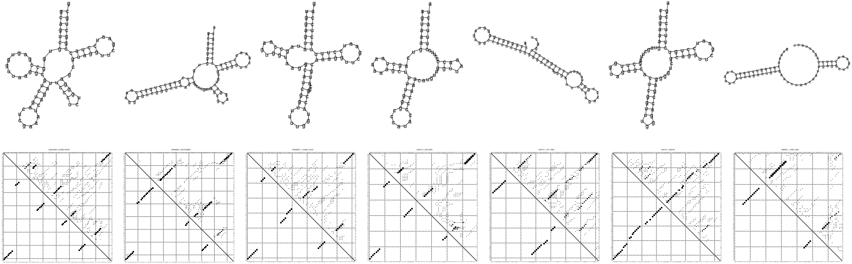

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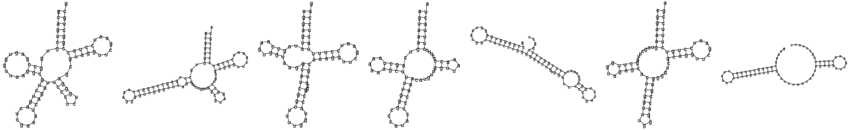

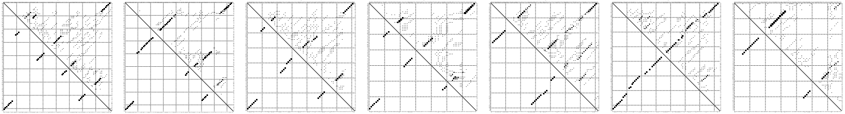

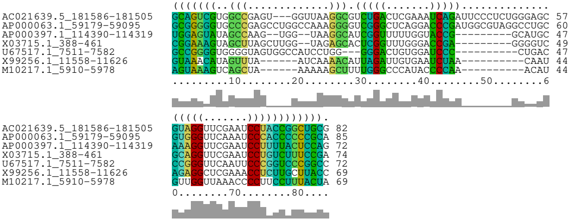

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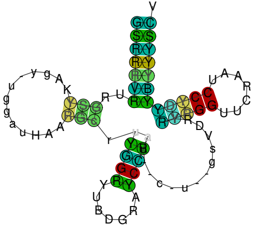
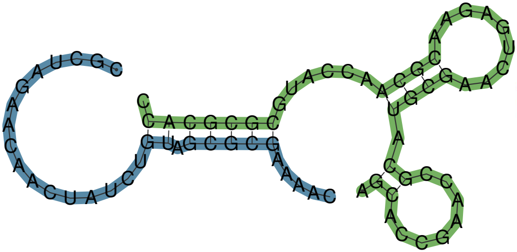
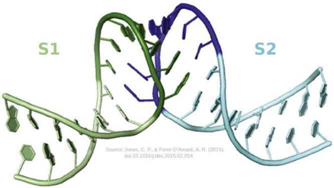
AP000063.1_59179-59095        GCGGGGGUGCCCGAGCCUGGCCAAAGGGGUCGGGCUCAGGACCCCGAUGGCGUAGGCCUGC 60
AP000397.1_114390-114319      UGGAGUAUAGCCAAG--UGG--UAAGGCAUCGGUUUUUGGUACCG----------GCAUGC 47
X03715.1_388-461              CGGAAAGUAGCUUAGCUUGG--UAGAGCACUCGGUUUGGGACCGA----------GGGGUC 49
U67517.1_7511-7582            GCCGGGGUGGGGUAGUGGCCAUCCUGG---GGGACUGUGGAUCCC----------CUGAC 47
X99256.1_11558-11626          GUAAACAUAGUUUA------AUCAAAACAUUAGAUUGUGAAUCUAA----------CAAU 44
M10217.1_5910-5978            AGUAAAGUCAGCUA------AAAAAGCUUUUGGGCCCAUACCCCAA----------ACAU 44
                              .........10........20........30........40........50........6

                              (((((.......))))))))))))).
AC021639.5_181586-181505      GUAGGUUCGAAUCCUACCGGCUGCG 82
AP000063.1_59179-59095        GUGGGUUCAAAUCCCACCCCCCGCA 85
AP000397.1_114390-114319      AAAGGUUCGAAUCCUUUUACUCCAG 72
X03715.1_388-461              GCAGGUUCGAAUCCUGUCUUUCCGA 74
U67517.1_7511-7582            CCGGGUUCAAUUCCCGGUCCCGGCC 72
X99256.1_11558-11626          AGAGGCUCGAAACCUCUUGCUUACC 69
M10217.1_5910-5978            GUUGGUUAAACCCCUUCCUUUACUA 69
                              0........70........80....
```

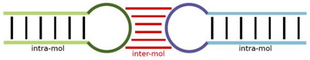


Interaction Prediction





Source: Jones, C. P., & Ferré-D'Amaré, A. R. (2015). doi:10.1016/j.tibs.2015.02.004



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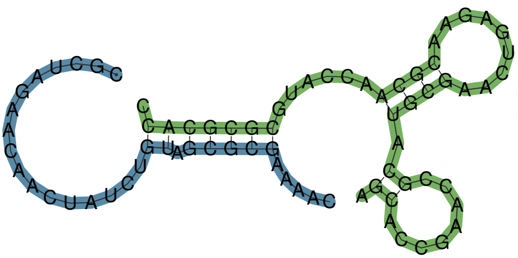
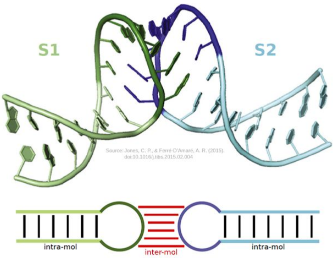


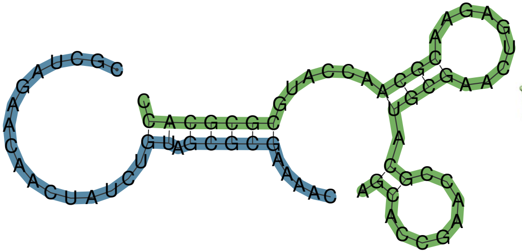


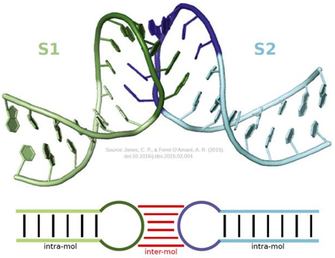
Source: Jones, C. P., & Ferré-D'Amaré, A. R. (2015). doi:10.1016/j.tibs.2015.02.004

- Similar to structure prediction: use NNM!

[Cofold example figure adapted from Vienna RNA Package 2.0, ALMOB 2011]

Interaction Prediction

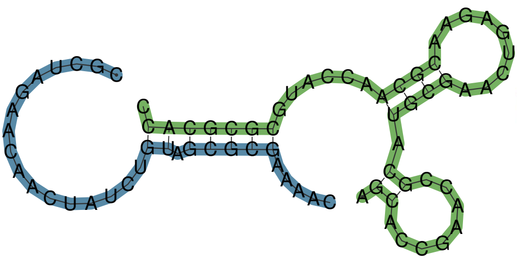


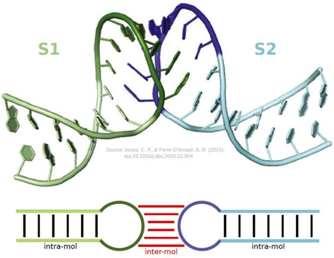


- Similar to structure prediction: use NNM!
- Predict intra- and inter-molecular structure
  - strong restrictions (cofold), **no** KHP → **fast**
  - more freedom (Alkan et al.), KHP → **slow**

[Cofold example figure adapted from Vienna RNA Package 2.0, ALMOB 2011]

Interaction Prediction





Source: Jones, C. P., & Ferré-D'Amaré, A. R. (2015). doi:10.1016/j.tibs.2015.02.004

- Similar to structure prediction: use NNM!
- Predict intra- and inter-molecular structure
  - strong restrictions (cofold), **no** KHP → **fast**
  - more freedom (Alkan et al.), KHP → **slow**
- IntaRNA: reasonable abstraction → **fast**
  - Use unpairing probabilities
  - E.g. genome-wide prediction of sRNA targets

[Cofold example figure adapted from Vienna RNA Package 2.0, ALMOB 2011]

RNA Bioinformatics—Take home

- **Secondary structure** as proxy for **RNA function**
- **Nearest neighbor model (NNM)** enables prediction of MFE structures and **probabilities**
- Solid fundament to construct methods for
  - Integrating prior knowledge
  - Simultaneous alignment and folding
  - Prediction of RNA interactions
  - ...pseudoknots, modifications, non-canonical base pairs, 3D structure, kinetics, design
- Building blocks of **pipelines to learn about RNA function**

  e.g. sRNA target prediction