

# SHAPE-Reactivity and RNA Structure

Winterseminar 2015

Roman Ochsenreiter, TBI

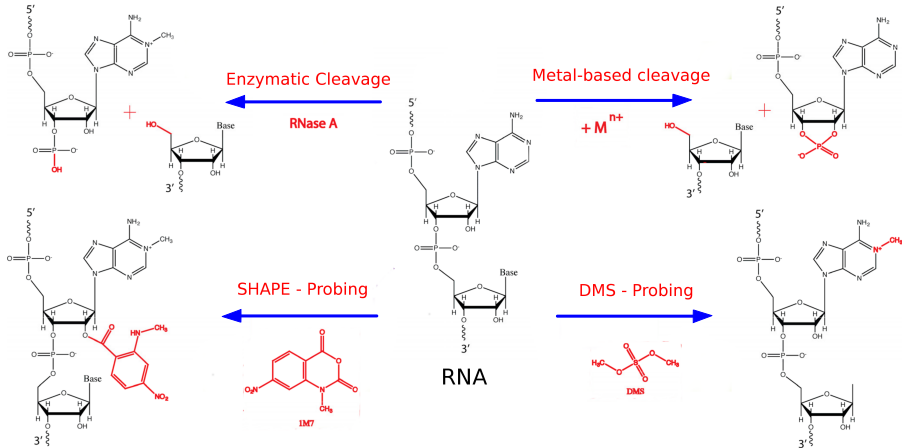
17.2.2015

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# RNA Probing

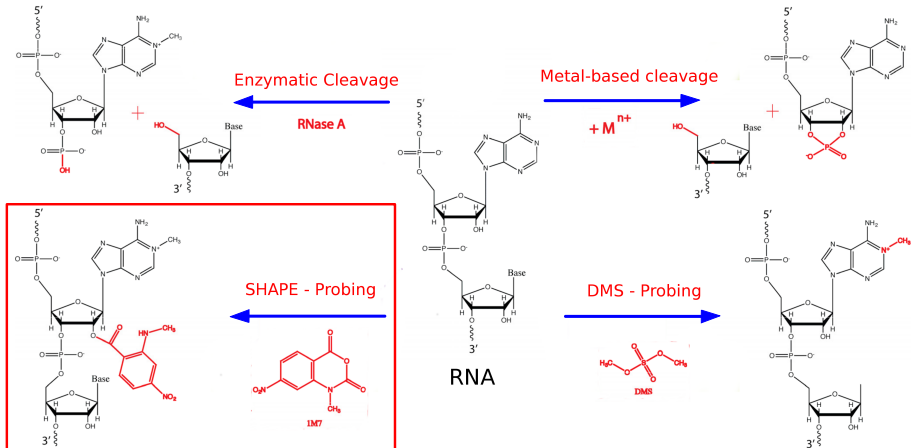
## Different methodological approaches



Modification rates depend on local RNA structure

# RNA Probing

## Different methodological approaches



Modification rates depend on local RNA structure

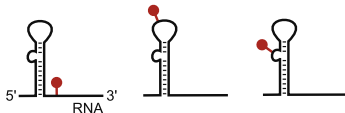
# SHAPE

## Investigating nucleotide flexibility

### Selective 2'-Hydroxyl Acylation analyzed by Primer Extension

**A**

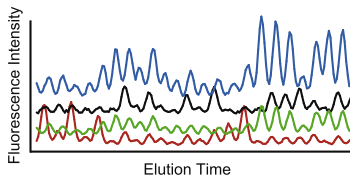
Selective 2'-Hydroxyl Acylation


**B**

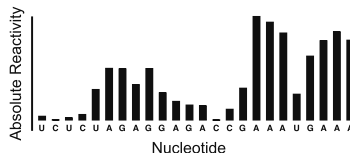
analyzed by Primer Extension


**C**

Raw capillary electrophoresis electropherogram


**D**

SHAPE reactivities (integrated peak areas)

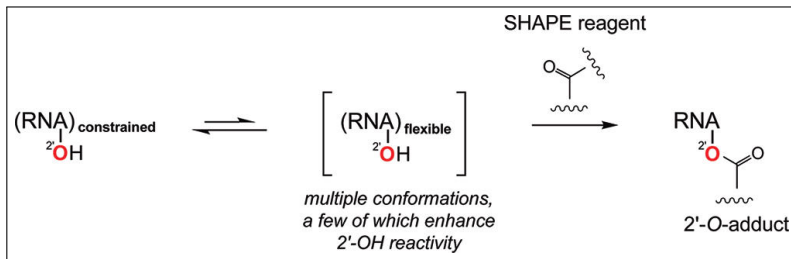


# SHAPE

## Why use SHAPE?

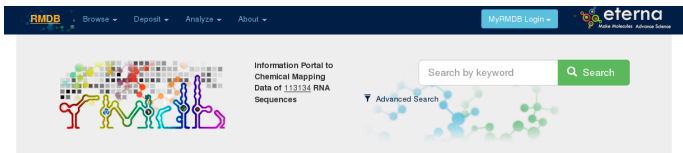
- Simple protocol
- Can be performed via high-throughput
- No intrinsically different reactivity of different bases
- Reactivity can be converted to Pseudo Free Energies for usage as folding constraints:

$$\Delta G_{SHAPE}(i) = m \ln(\text{SHAPE reactivity}(i) + 1) + b$$

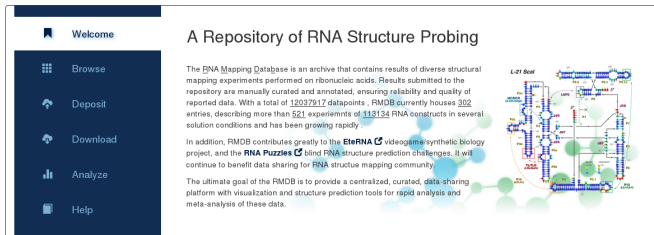


# SHAPE - Databases

- RMDB - Stanford RNA Mapping Database (> 100000 probing experiments)



RMDB has upgraded to [version 2.0 BETA](#) for online preview. More changes and updates are in progress.  
 Please report bugs and make suggestions. We really appreciate your feedback.



**Welcome**

- Browse
- Deposit
- Download
- Analyze
- Help

## A Repository of RNA Structure Probing

The RNA Mapping Database is an archive that contains results of diverse structural mapping experiments performed on ribonucleic acids. Results submitted to the repository are manually curated and annotated, ensuring reliability and quality of reported data. With a total of 12037917 datapoints, RMDB currently houses 302 entries, describing more than 521 experiments of 113134 RNA constructs in several solution conditions and has been growing rapidly.

In addition, RMDB contributes greatly to the Eterna videogame/synthetic biology project, and the RNA Puzzles blind RNA structure prediction challenges. It will continue to benefit data sharing for RNA structure mapping community.

The ultimate goal of the RMDB is to provide a centralized, curated, data-sharing platform with visualization and structure prediction tools for rapid analysis and meta-analysis of these data.

*L-01 Scat*



- “High-throughput” sequence design - by (many) hands
- Each design is scored by SHAPE-Probing
- Successful design rules are used for inverse folding algorithm
- EteRNA Score:

$$S_{EteRNA} = \frac{\sum_{i=1}^N \Delta}{N}, \quad \Delta \begin{cases} 1 & \text{if } r_i > 0.25 \text{ (designed to be unpaired)} \\ 1 & \text{if } r_i < 0.5 \text{ (designed to be paired)} \\ 0 & \text{otherwise} \end{cases}$$

$r_i$ : Reactivity of Nucleotide  $i$



# EteRNA

## EteRNA - "Workflow"

Other Ribosomal RNA - Difficulty Level 0

Total: 0 kcal

**MISSION!**

Your RNA must fold into the structure in white outline.

You must have 3 or more G-U pairs.

Click here to start!

romanoch

Me Puzzle Cloud Lab Community Help

romanoch

Chat Players Online (35)

your Windmill puzzle, maybe I spent total 48 hours on yours [0:55 AM]

wawan151: To get rid of 1 UG [0:55 AM]

Jieux: Then you make a harder one :) [0:55 AM]

wawan151: Time for me to sleep Jieux, I don't logoff. Happy snacking & be careful with your finger not press on the keyboard when fall asleep :) [1:05 AM]

## EteRNA - “Workflow”



# EteRNA

## EteRNA - "Workflow"

Other Ribosomal RNA - Difficulty Level 0

Total: 0 kcal

1/3

romanoch

Puzzle Cloud Lab Community Help

romanoch

Chat Players Online (35)

your Windmill puzzle, maybe I spent total 48 hours on yours [10:54 AM]  
**wawan151**: To get rid of 1 UG [10:55 AM]  
**Jieux**: Then you make a harder one :) [10:55 AM]  
**wawan151**: Time for me to sleep Jieux, I don't logoff. Happy snacking & be careful with your finger not press on the keyboard when fall asleep :) [11:05 AM]

# EteRNA

## EteRNA - "Workflow"

Other Ribosomal RNA - Difficulty Level 0

Total: -73.5 kcal

/3

romanoch

Puzzle Cloud Lab Community Help

romanoch

Chat Players Online (34)

your Windmill puzzle, maybe I spent total 48 hours on yours [0:34 AM]  
**wawyan151**: To get rid of 1 UG [0:35 AM]  
**Jieux**: Then you make a harder one :) [0:35 AM]  
**wawyan151**: Time for me to sleep Jieux, I don't logoff. Happy snacking & be careful with your finger not press on the keyboard when fall asleep :) [1:05 AM]

# EteRNA

## EteRNA - "Workflow"

Other Ribosomal RNA - Difficulty Level 0

Total: -73.7 kcal

0/3

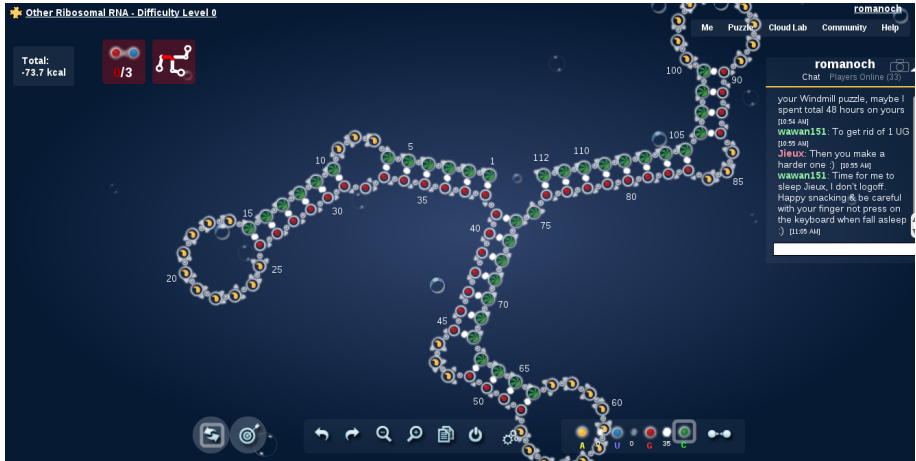
romanoch

Me Puzzle Cloud Lab Community Help

romanoch

Chat Players Online (33)

your Windmill puzzle, maybe I spent total 48 hours on yours [01:54 AM]  
**wawan151**: To get rid of 1 UG [01:55 AM]  
**Jleux**: Then you make a harder one :) [01:55 AM]  
**wawan151**: Time for me to sleep Jleux, I don't logoff. Happy snacking & be careful with your finger not press on the keyboard when fall asleep :) [01:55 AM]



# EteRNA

## EteRNA - "Workflow"

Other Ribosomal RNA - Difficulty Level 0

Total: -66.6 kcal

/3

romanoch

Puzzle Cloud Lab Community Help

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Chat Players Online (34)

your Windmill puzzle, maybe I spent total 48 hours on yours [0:55 AM]  
**wawani51**: To get rid of 1 UG [0:55 AM]  
**Jieux**: Then you make a harder one :) [0:55 AM]  
**wawani51**: Time for me to sleep Jieux, I don't logoff.  
 Happy snacking & be careful with your finger not press on the keyboard when fall asleep :) [1:05 AM]

# EteRNA

## EteRNA - "Workflow"

Other Ribosomal RNA - Difficulty Level 0

Total: -57.6 kcal

3/3

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Puzzle Cloud Lab Community Help

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Chat Players Online (33)

your Windmill puzzle, maybe I spent total 48 hours on yours [0:55 AM]  
**wawani51**: To get rid of 1 UG [0:55 AM]  
**Jleux**: Then you make a harder one :) [0:55 AM]  
**wawani51**: Time for me to sleep Jleux, I don't logoff.  
 Happy snacking & be careful with your finger not press on the keyboard when fall asleep :) [1:05 AM]

# EteRNA

## EteRNA - "Workflow"

Other Ribosomal RNA - Difficulty Level 0

Total: -57.5 kcal

**CLEARED!**

kisiel090	16902	4100
Zachary Titus	16902	4100
<b>roma...</b>	<b>16904</b>	<b>4095</b>
ModernArcheops	16904	4095
geoicons	16904	4095

Stay

Next Puzzle

romanoch

Me Puzzle Cloud Lab Community Help

romanoch

Chat Players Online (33)

your Windmill puzzle, maybe I spent total 48 hours on yours (0:51 AM)

wawan151: To get rid of 1 UG (0:25 AM)

Jieux: Then you make a harder one :D (0:55 AM)

wawan151: Time for me to sleep Jieux, I don't logoff. Happy snacking & be careful with your finger not press on the keyboard when fall asleep :D (1:25 AM)

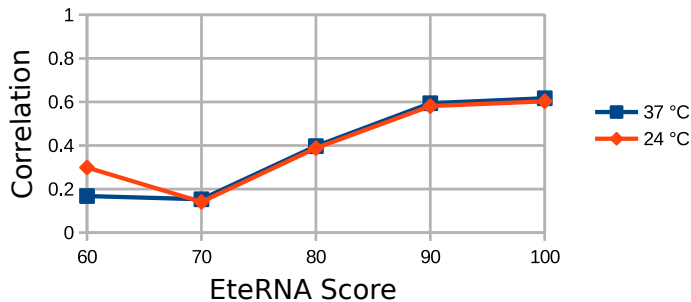


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# Scoring EteRNA Scores

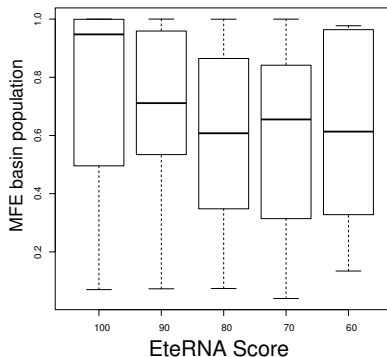
(Linear) Correlation of  $P(i \text{ is paired})$  vs.  $\text{Reactivity}(i)$



- Interesting drop in correlation with lower EteRNA-Scores
- Change of temperature has no effect

# Structural Ensembles and Probing

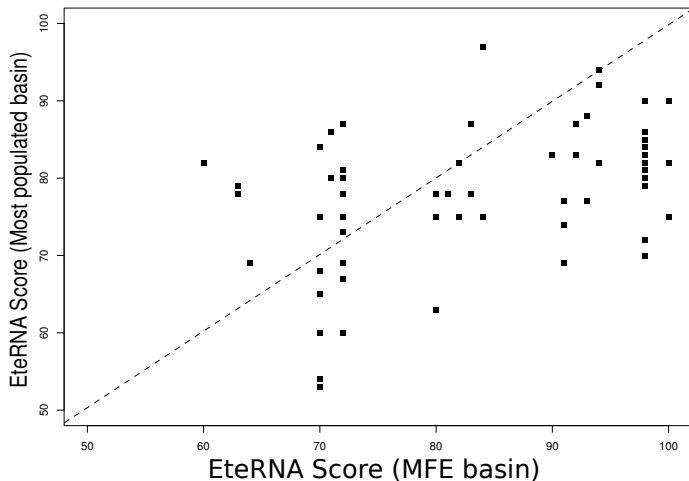
## Equilibrium Population vs. Probing Quality



- Lower EteRNA-scores are correlated to low MFE population rates

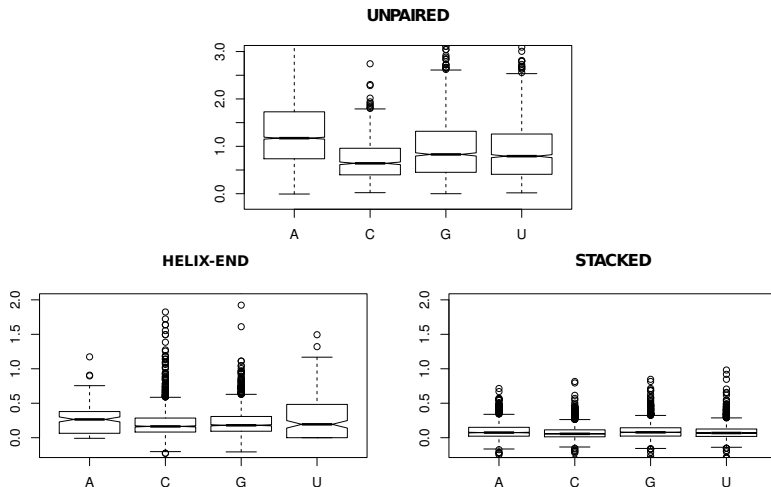
# Dominant structures

How well scores the dominant structure?



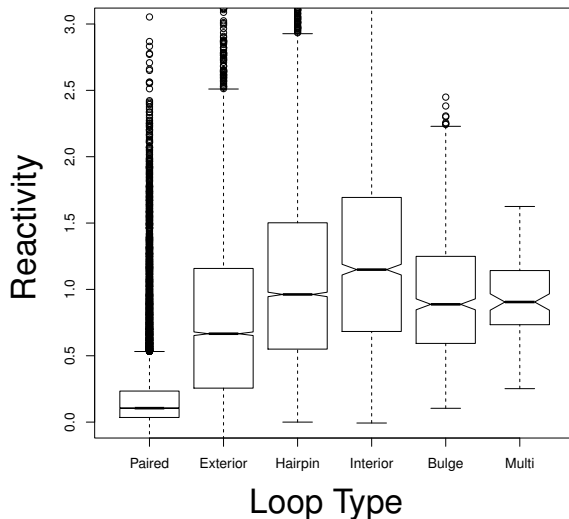
# Base-dependent Reactivity

Reactivity of all 4 bases in different structural contexts:



# Loop-Type and Reactivity

Reactivity of Nucleotides in all possible Loop-types:



# Take-Home message

- Large amounts of *SHAPE* data sets are freely available
- Reactivity reflects more than just nucleotide flexibility - also Loop-Types and Ensemble structures are important

# Thanks to..

Ivo Hofacker  
Christoph Flamm  
Sven Findeiss  
and  
YOU for your attention!