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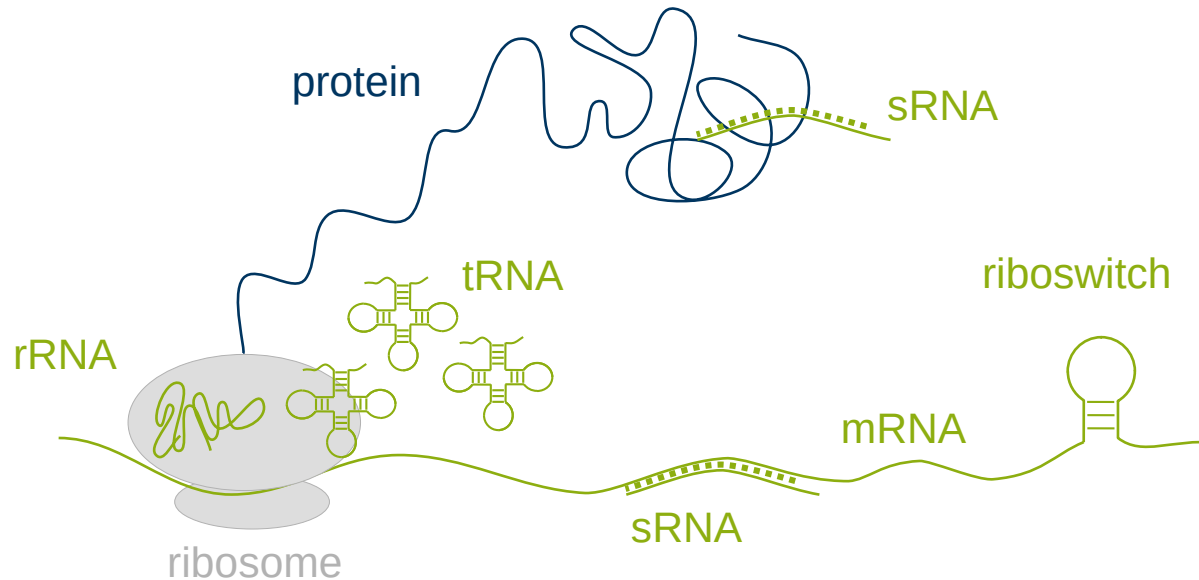
# RNA structuromics – *in vitro*, *in vivo* and *in silico*

Vivian Brandenburg | Microbial RNA Regulation – Virtual Conference | 04/01/2022

# RNA structuromics

## 'Transcriptome-wide RNA structure probing coupled with NGS'<sup>1</sup>

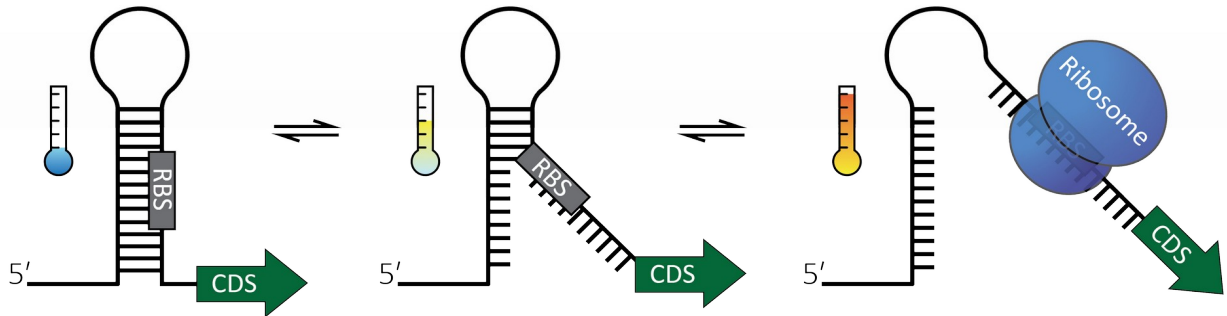
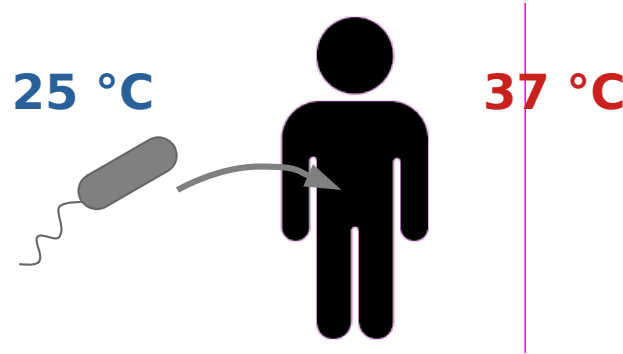
- Probe all RNAs in a cell at same time



<sup>1</sup> Kwok et al.: *Trends in Biochem* 2015

# *Yersinia pseudotuberculosis*

- Gram-neg.  $\gamma$ -proteobacterium
- Causes gut-associated diseases
- Senses host-invasion via temp. changes
  - > 300 genes differentially expressed<sup>1</sup>
- Involves RNA thermometers<sup>2</sup>
  - *LcrF*<sup>3</sup>: major virulence regulator



<sup>1</sup> Nuss *et al.*: *PLoS Gen* 2015

<sup>2</sup> Loh *et al.*: *Microbiol Spec* 2018

<sup>3</sup> Böhme *et al.*: *PLoS Path* 2012

# *In vitro* structuromics

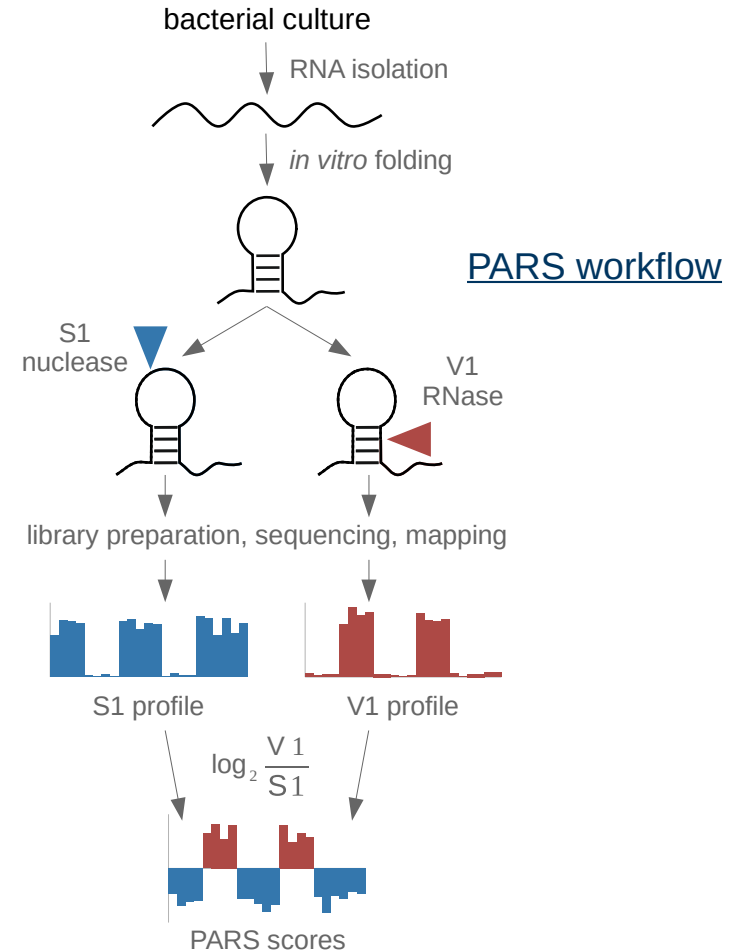
structure probing + NGS

- PARS<sup>1</sup>
  - Parallel analysis of RNA structure
  - Nuclease S1, RNase V1
- PIP-seq<sup>2</sup>
  - Protein interaction profile sequencing
  - RNase I, RNase V1
- FragSeq<sup>3</sup>
  - Fragmentation sequencing
  - Nuclease P1

1 Righetti *et al.*: *PNAS* 2016

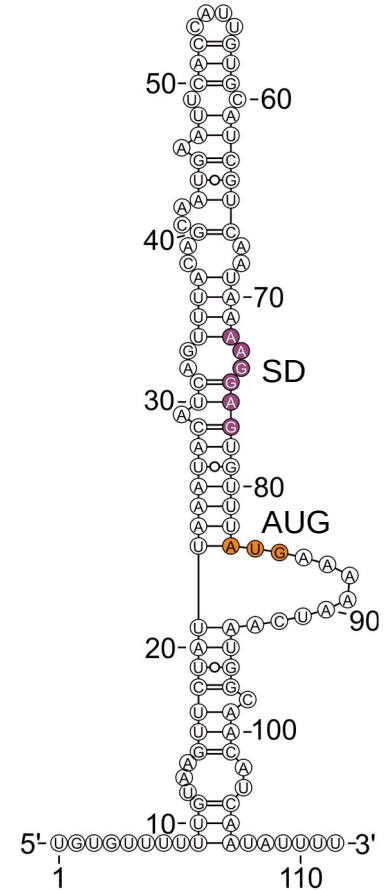
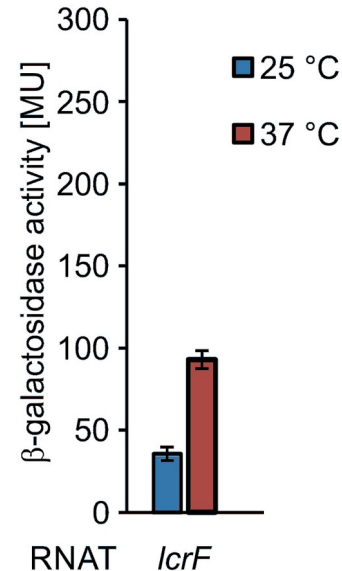
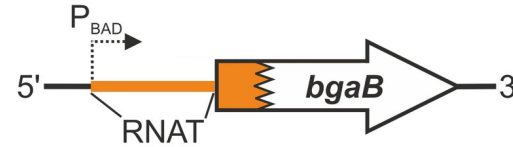
2 Silverman *et al.*: *Genome Biol* 2014

3 Underwood *et al.*: *Nat Meth* 2010



# PARS

- PARS in *Y. pseudotuberculosis* at 25 / 37 / 42 °C <sup>1</sup>
- Found at least 16 new RNATs
  - Oxidative stress response
  - Metabolism
  - Nutrient uptake
- e.g. *cnfY*<sup>2</sup>: multifunctional secreted toxin
  - Validated via  $\beta$ -galactosidase assay
  - **Essential** for virulence (mouse survival study)

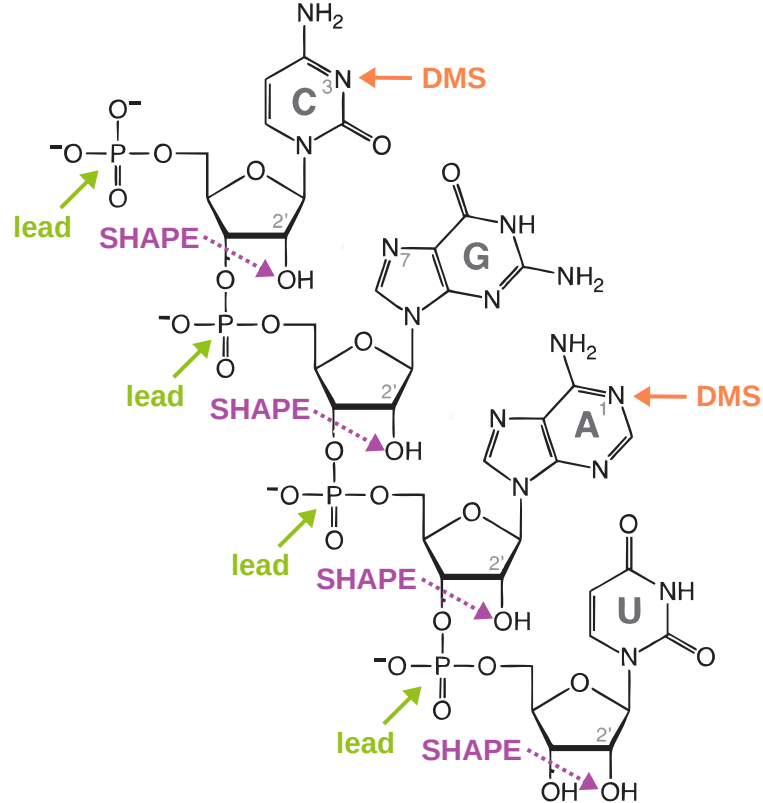


5'-UTR of *cnfY* at 25°C

<sup>1</sup> Righetti *et al.*: PNAS 2016

<sup>2</sup> Twittenhoff *et al.*: PloS Path 2020

# In vivo structuromics



- Chemical treatment + NGS
  - Chemical probes attack unpaired nt
  - No sample for paired nt
  - Compare to untreated control



- DMS-Seq<sup>1</sup>: Dimethyl sulfate
  - Methylation of A and C residues → RT drop-off



- SHAPE<sup>2</sup>: Selective 2'-hydroxyl acylation analyzed by primers extension
  - Acylation of ribose sugar → RT drop-off
  - E.g. 1M7 (1-methyl-nitroisatoic anhydride)



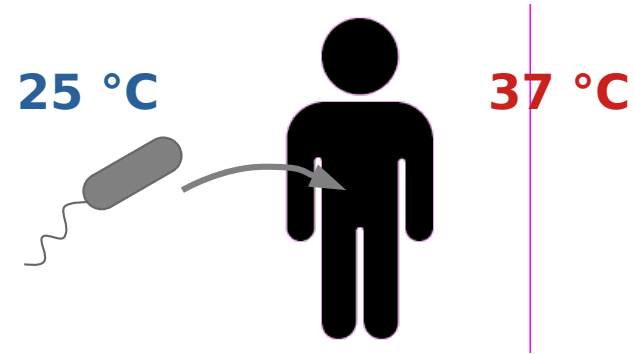
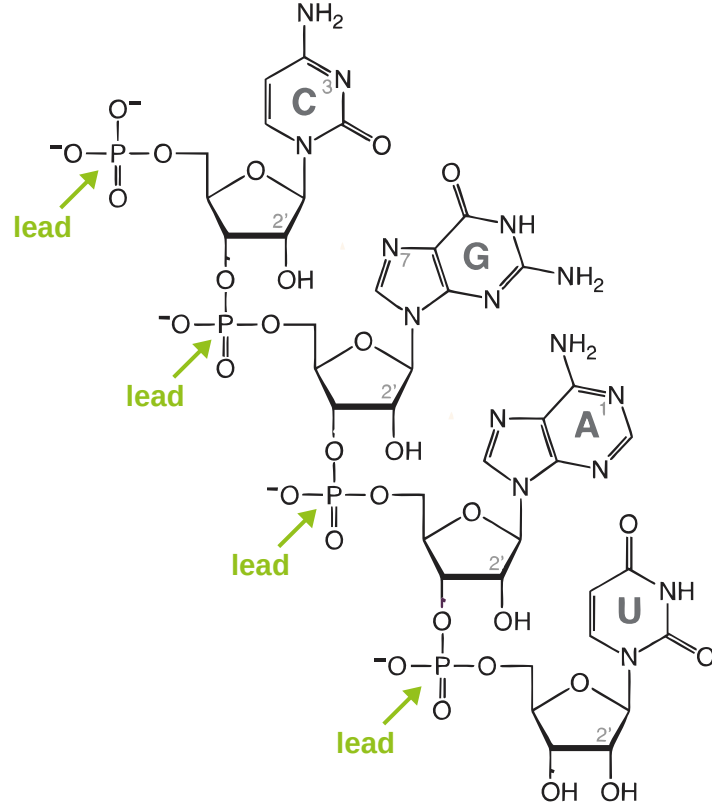
- Lead-Seq<sup>3</sup>: lead(II) acetate
  - Nucleophilic attack of P-group → RNA self-cleavage

1 Rouskin *et al.*: *Nature* 2014

2 Lucks *et al.*: *PNAS* 2011

3 Twittenhoff, Brandenburg, Righetti *et al.*: *NAR* 2020

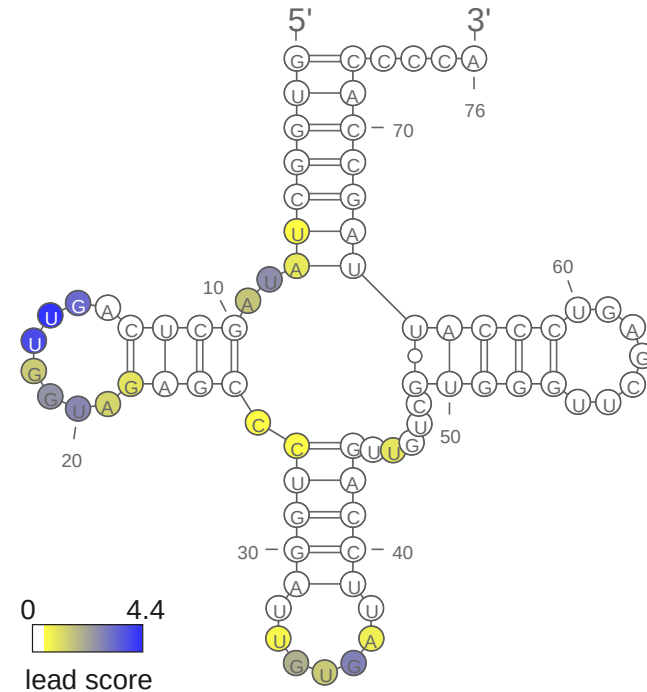
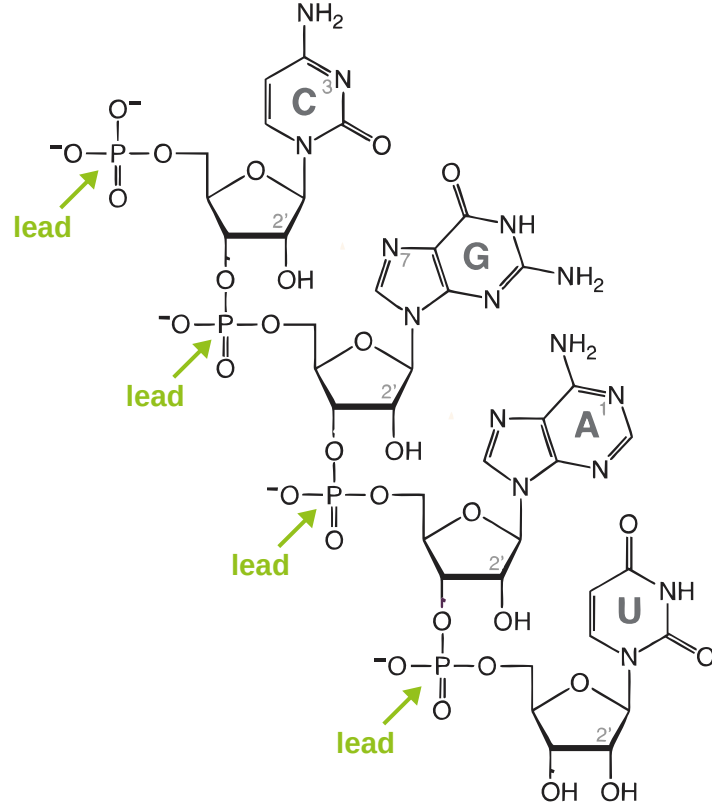
# In vivo structuromics



Lead-Seq on *Y. pseudotuberculosis* at 25 / 37 °C

Twittenhoff, Brandenburg, Righetti *et al.*: NAR 2020

# In vivo structuromics



tRNA<sup>Asp</sup> at 25 °C

Twittenhoff, Brandenburg, Righetti *et al.*: NAR 2020



# Lead-Seq: 16s-rRNA

lead cleavage<sup>1</sup> at

- 25 °C
- 37 °C
- 25/37 °C

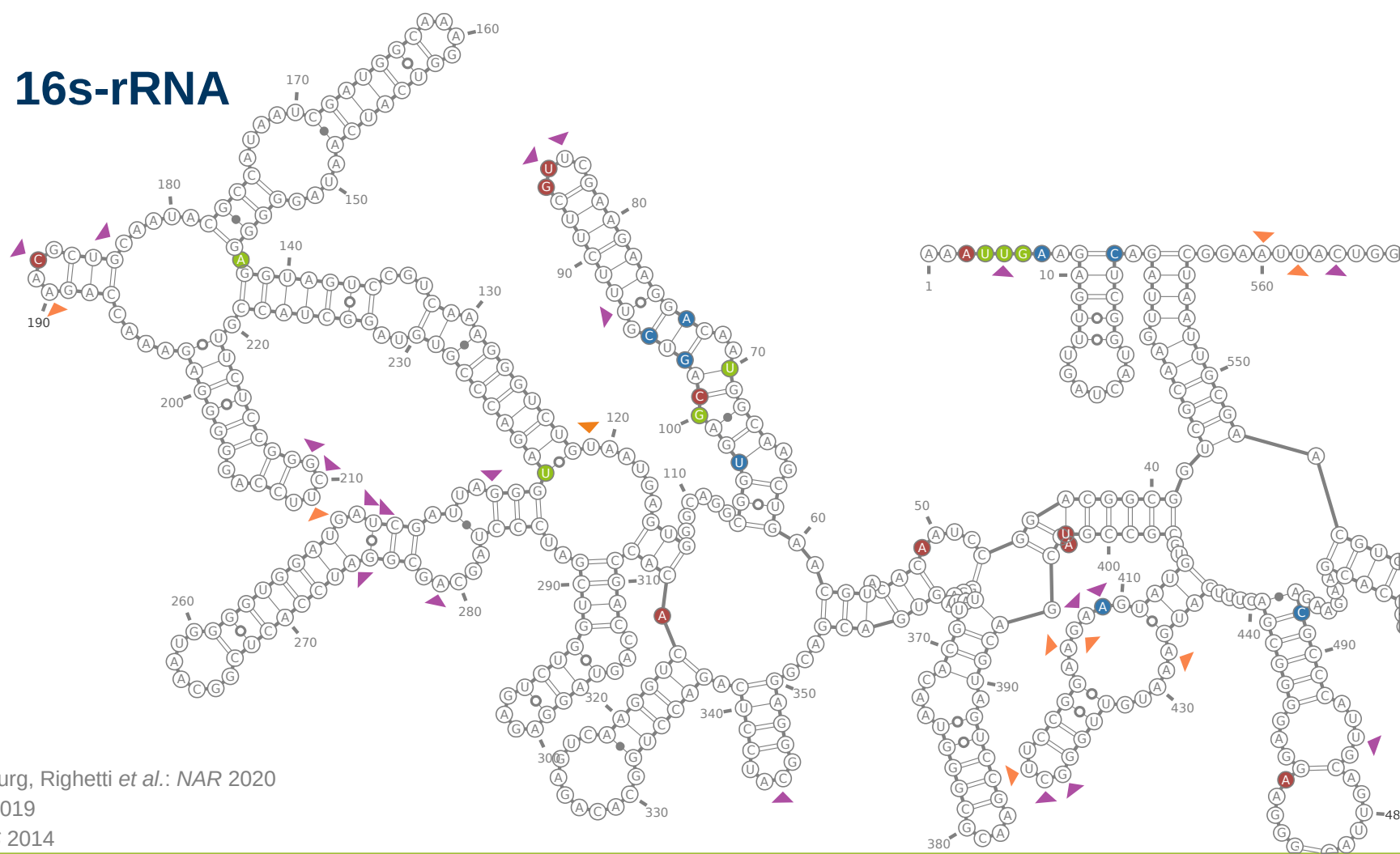
strong reactivity

- ▶ DMS<sup>2</sup>
- ▶ SHAPE<sup>3</sup>

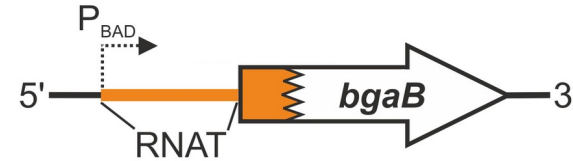
1 Twittenhoff, Brandenburg, Righetti *et al.*: NAR 2020

2 Mustoe *et al.*: PNAS 2019

3 McGinnis *et al.*: PNAS 2014

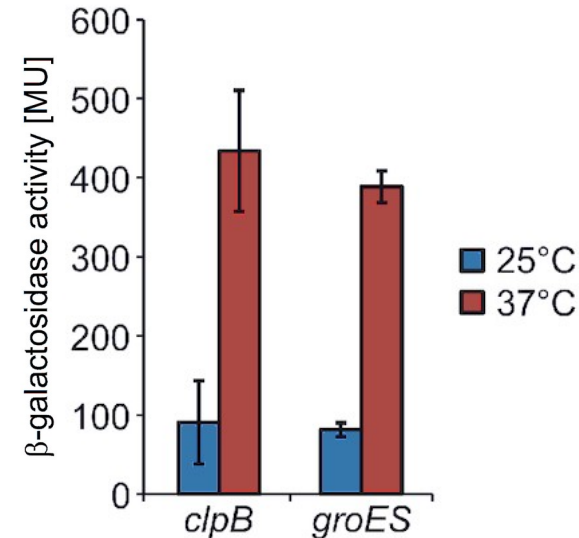


# Lead-Seq: RNA thermometers



Lead-Seq on *Y. pseudotub.* at 25 / 37 °C<sup>1</sup>

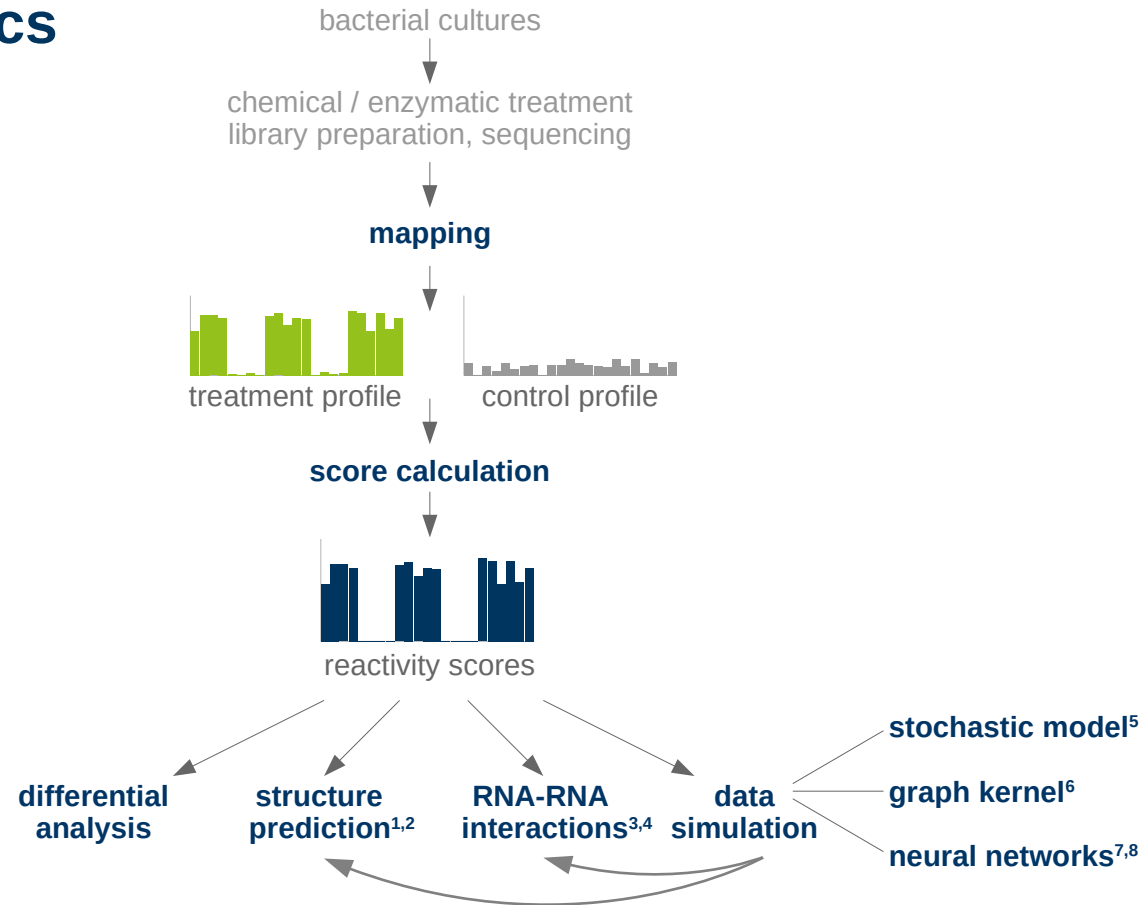
- ~ 80 RNAT candidates identified
  - 7 verified via  $\beta$ -gal. assay
  - 5 found in PARS<sup>2</sup>
  - At least 2 novel RNATs



<sup>1</sup> Twittenhoff, Brandenburg, Righetti *et al.*: *NAR* 2020

<sup>2</sup> Righetti *et al.*: *PNAS* 2016

# *In silico* structuromics



1 Deigan *et al.*: *PNAS* 2009

2 Washietl *et al.*: *NAR* 2012

3 Tafer *et al.*: *Bioinformatics* 2011

4 DiChiacchio *et al.*: *Bioinformatics* 2015

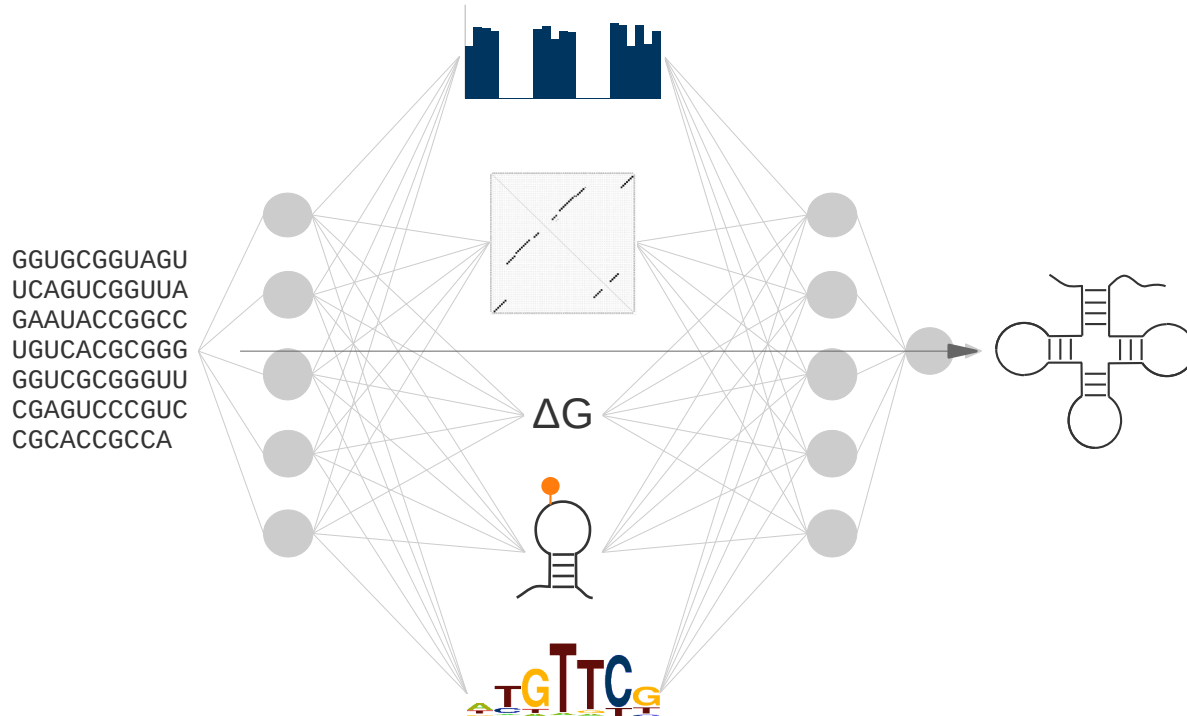
5 Sükösd *et al.*: *NAR* 2012

6 Mautner *et al.*: *Bioinformatics* 2019

7 Bliss *et al.*: *RNA Biol* 2020

8 Willmott *et al.*: *Comp Math Biophys* 2020

# Structuromics with Neural Networks



## RNA features

- Sequence
- Energy models
- Probing data
- RNA modifications
- Sequence motifs

## Neural networks

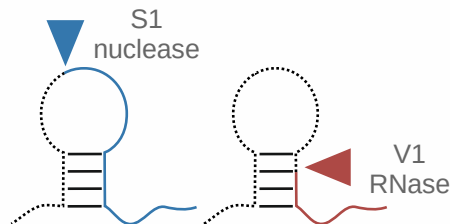
- Combine features & data types
- Automate feature extraction<sup>1</sup>

1 Quang & Xie: *NAR* 2015

# Structuromics

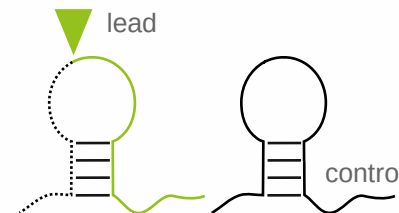
## In vitro

- Enzyme-based  
→ e.g. PARS
- Probes both types of pairing states



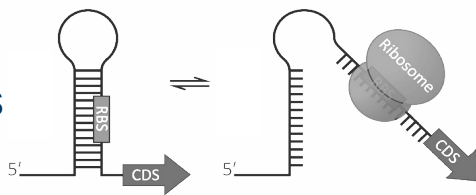
## In vivo

- Chemical-based  
→ e.g. Lead-Seq
- Folding under natural conditions

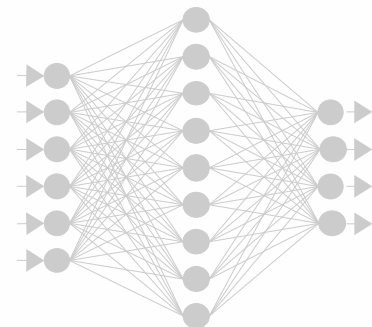


## In silico

- Differential analysis
- Data simulation
- Predict structures and interactions



- Neural Networks  
→ Combine knowledge  
→ Automate feature extraction



# Collaboration & Funding

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German Research Foundation

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