Detection and Rational Design of RNA Switches

Sebastian Will

Vienna RNA Meeting 2018

DISCLAIMER

MAY CONTAIN
TRACES OF
BIOINFORMATICS

My special perspective (on RNA research):

- **What can we learn** by applying *existing* computational tools?

    e.g. exploiting the `Vienna RNA package`

- **Which questions could be answered** by *novel* methods?

    e.g. comparing RNAs in my tool `LocARNA`

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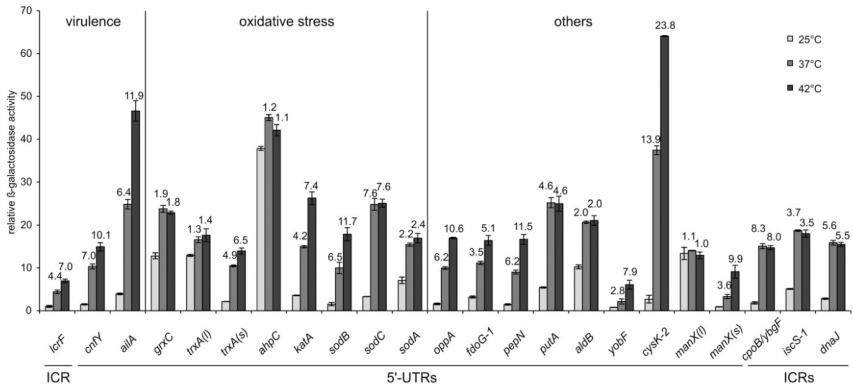
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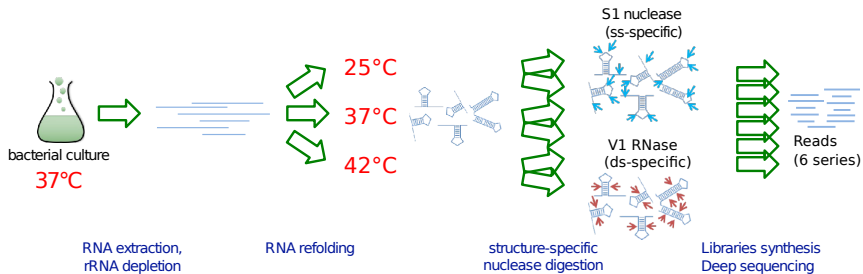**Today: What can Bioinformatics do** for RNA switches?

Finding Thermoswitches in
Y. Pseudotuberculosis

with F. Righetti *et al.* **The Temperature-responsive In-vitro RNA Structurome of Yersinia Pseudotuberculosis.** *PNAS*, 2016.
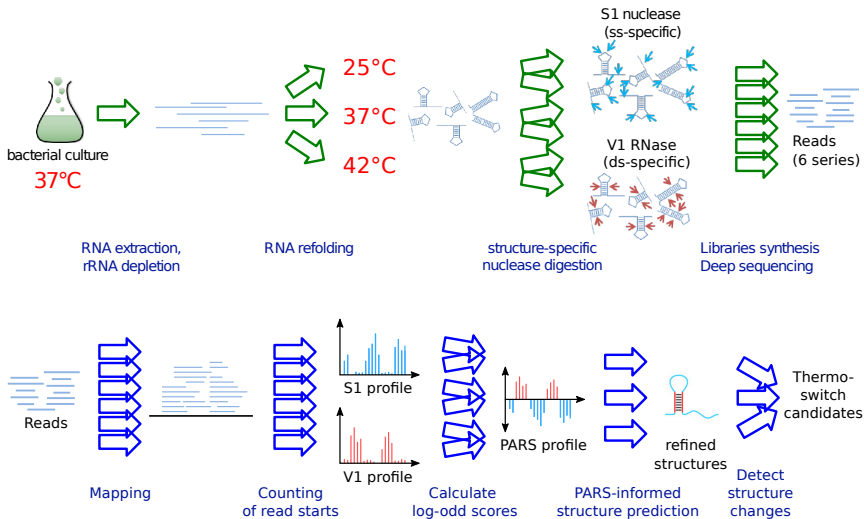
- we screened more than 1,750 RNAs at 25/37/42°C and selected *candidates* for thermoresponsive folding
- validated thermo-regulatory potential of 16 candidates

Genome-wide structure probing (PARS) at different temperatures

bacterial culture
37°C

RNA extraction,
rRNA depletion

25°C
37°C
42°C

RNA refolding

S1 nuclease
(ss-specific)

V1 RNase
(ds-specific)

structure-specific
nuclease digestion

Reads
(6 series)

Libraries synthesis
Deep sequencing

Genome-wide structure probing (PARS) at different temperatures

[adapted/extended from F. Righetti]

PARS at SD-region of selected RNA Thermometer candidates

Gene name	SD PARS*			PARS difference[†]		Thermal control[‡]
	25 °C	37 °C	42 °C	37–25 °C	42–25 °C	
cnfY	0.18	−0.37	−0.12	−0.55	−0.30	Y
ailA	0.09	0.61	−0.25	0.52	−0.34	Y
grxC	0.39	−0.56	−0.50	−0.95	−0.89	N
trxA	0.32	−0.53	−0.42	−0.85	−0.74	s:Y, l:N
ahpC	−0.60	−1.23	0.44	−0.63	1.04	N
katA	0.08	−0.37	−0.02	−0.45	−0.10	Y
sodB	0.00	−0.22	−0.29	−0.22	−0.29	Y
sodC	0.14	−0.08	−0.47	−0.22	−0.61	Y
sodA	0.09	0.07	0.02	−0.02	−0.07	N
oppA	0.64	−0.77	−0.13	−1.41	−0.77	Y
fdoG-1	0.55	−0.55	0.12	−1.10	−0.43	Y
pepN	0.36	−0.26	0.05	−0.62	−0.31	Y
putA	0.48	0.11	−0.05	−0.37	−0.53	Y
aldB	0.00	−0.27	0.22	−0.27	0.22	N
yobF	−0.08	0.01	0.02	0.09	0.10	Y
cysK-2	−0.04	0.07	−0.05	0.11	−0.01	Y
manX	−0.29	−0.11	−0.12	0.18	0.17	s:Y, l:N
cpoB/ybgF	0.86	−0.33	−0.20	−1.19	−1.06	Y
iscS-1	0.43	0.16	−0.22	−0.27	−0.65	Y
dnaJ	0.18	−0.05	−0.10	−0.23	−0.28	Y

Results for RNA thermometer candidate ailA

Two candidates associated with *Y.* virulence: ailA and cnfY.
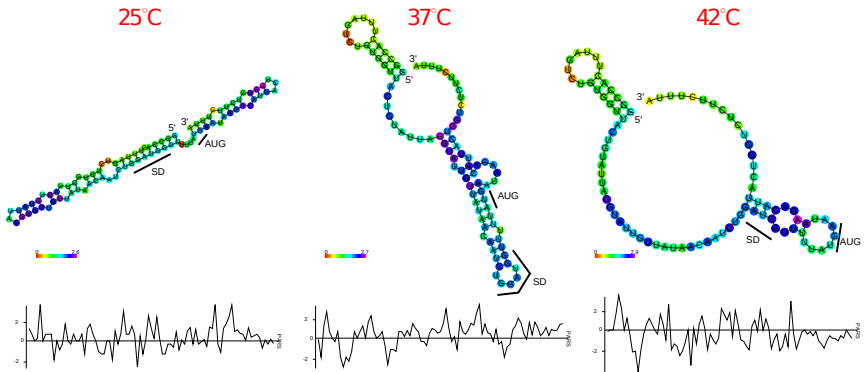
PARS profiles and PARS-guided predictions; color=entropy

Rational Design of Riboswitches

with G. Domin *et al.* **Applicability of a computational design approach for synthetic riboswitches.** *Nucleic acids research*, 2017.
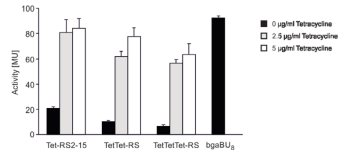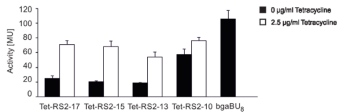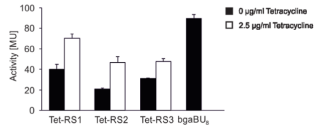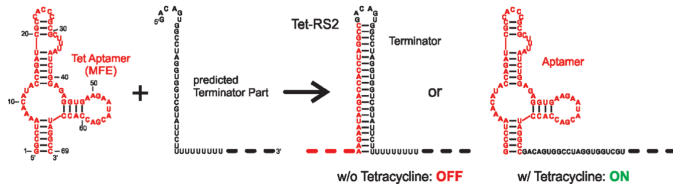
Goal: Design Riboswitch-systems to reprogram cells (here *E. coli*) to respond to small molecules (Theophylline, Tetracycline, Streptomycin)
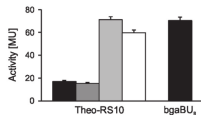

Terminator   Aptamer   mRNA of Reporter Gene
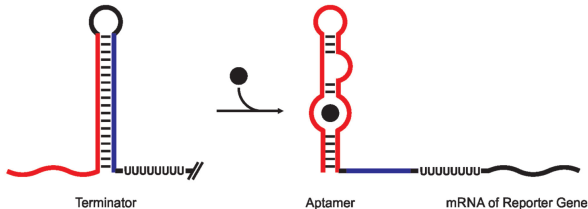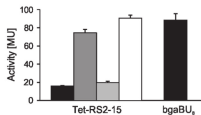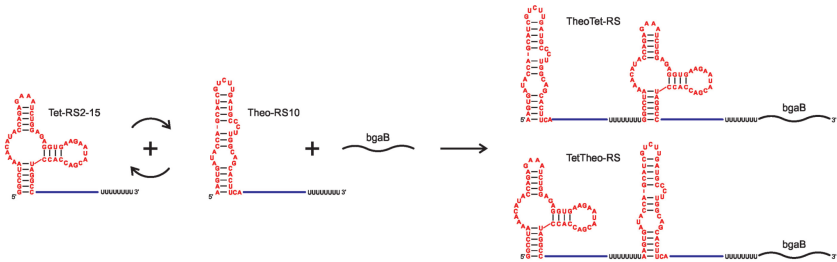
Tetracycline-RS Designs (Activity Tests)
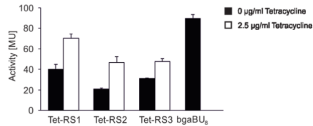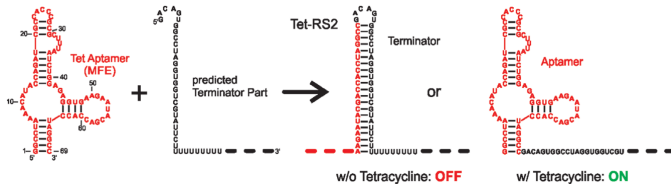
Tetracycline-RS Designs (Activity Tests)

Tandem-RS Designs for AND switch

Goal: Switch ON if Theophylline AND Tetracycline are both present
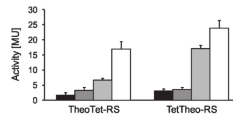
Bioinformatics challenge: Design candidate Riboswitch constructs "in-silico"
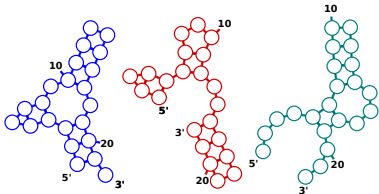
Novel Method for multi-target design of RNAs

with Stefan Hammer, Wei Wang and Yann Ponty. **Fixed-Parameter Tractable Sampling for RNA Design with Multiple Target Structures.** *RECOMB*, 2018.
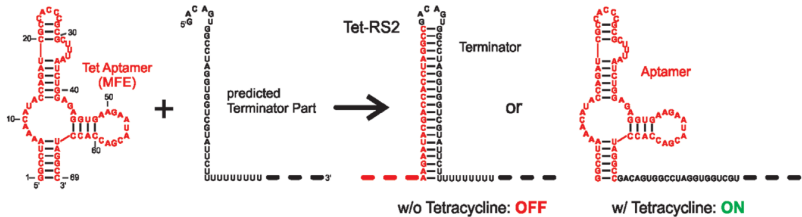
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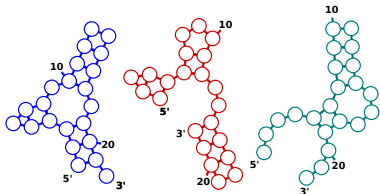
Multiple structures (*targets*)


```
(((((.)).(((..))).))).
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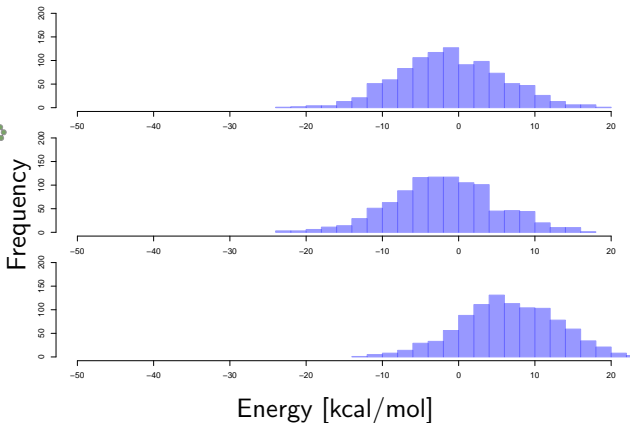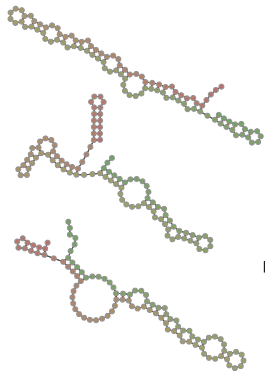
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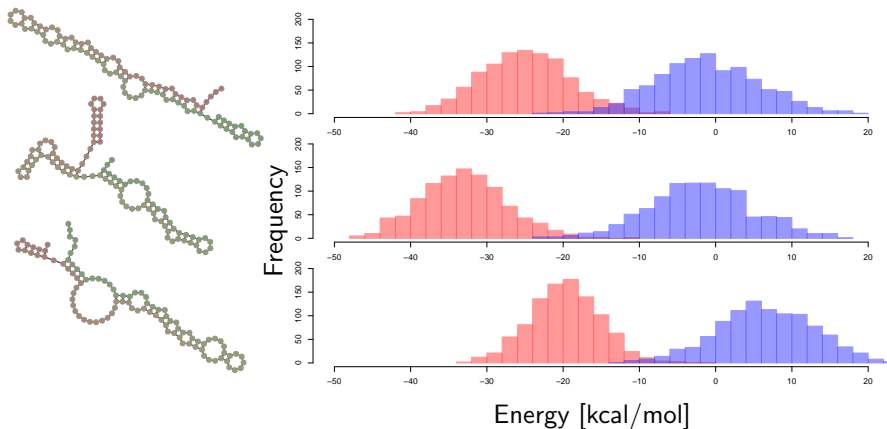
Task: generate seq's with *specific* properties

- low/specific energy for multiple structures
- specific GC content
- specific energy differences
- specific sequence/structure motifs

Approach:
defined "Boltzmann" **sampling**
of RNA sequences
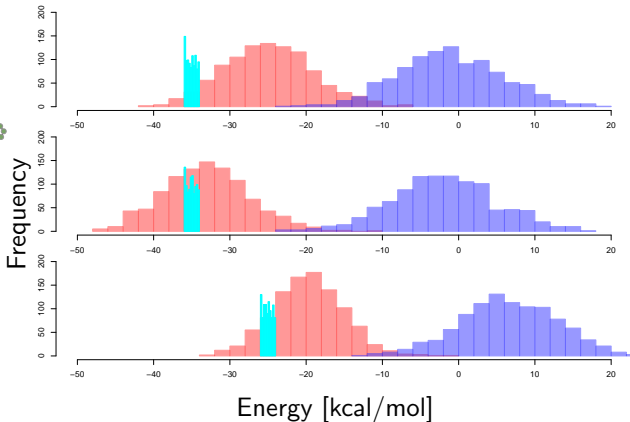
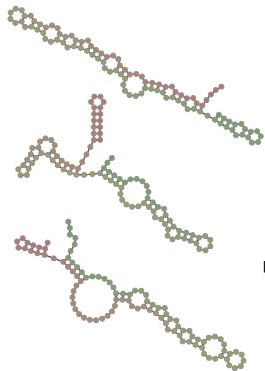# Multi-target design to three RNA structures

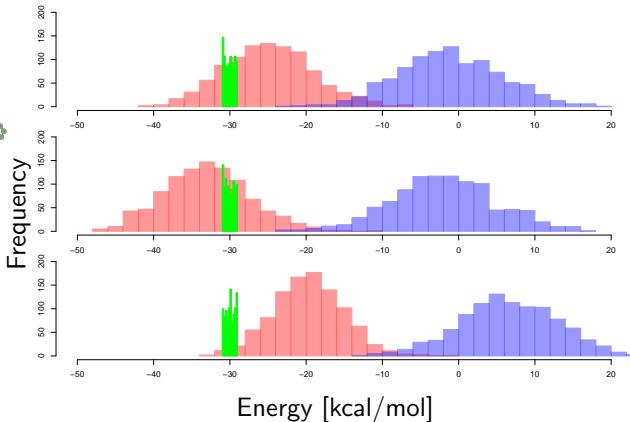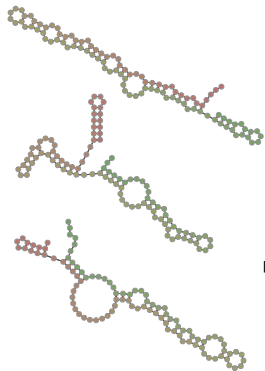# Multi-target design to three RNA structures

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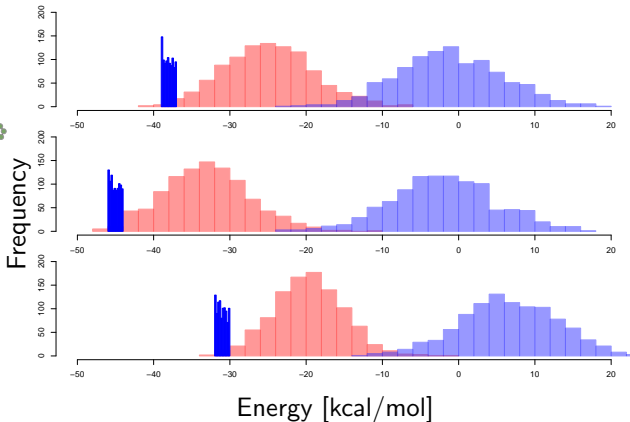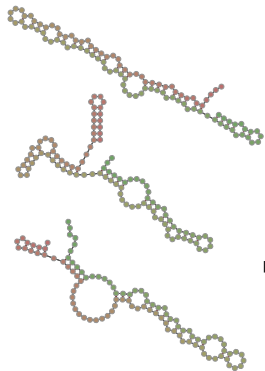
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# Multi-target design to three RNA structures

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Targeted samples: 1000 highly specific sequences; in minutes

- **Improves quality and feasibility of RNA Design**
    - e.g. for designing artificial riboswitches
- **Generic system to extend RNA Design . . .**
    - by including various desirable properties in the sampling
- **. . . and develop novel sampling-based tools**
    - design RNA alignments (target energies and evo-distances)
    - e.g. use to assess statistical significance
      and support the detection of potential RNA switches
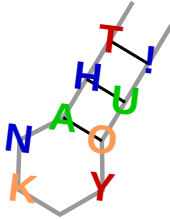
My co-authors / cooperation partners
Francesco Righetti, Aaron M. Nuss, Christian Twittenhoff, Sascha Beele, Kristina Urban, Stephan H. Bernhart, Peter F. Stadler, Petra Dersch, Franz Narberhaus; Gesine Domin, Sven Findeiß, Manja Wachsmuth, Mario Mörl; Stefan Hammer, Wei Wang, Yann Ponty

Team TBI of **Ivo Hofacker** at universität wien