Detection and Rational Design of RNA Switches

Sebastian Will

Vienna RNA Meeting 2018
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

Today: What can Bioinformatics do for RNA switches?
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**Today:** What can Bioinformatics do for RNA switches?
Finding Thermoswitches in Y. Pseudotuberculosis


- we screened more than 1,750 RNAs at 25/37/42°C and selected candidates for thermo-responsive folding
- validated thermo-regulatory potential of 16 candidates
Genome-wide structure probing (PARS) at different temperatures

- **bacterial culture** at 37°C
- RNA extraction, rRNA depletion
- RNA refolding
- S1 nuclease (ss-specific)
- V1 RNase (ds-specific)
- 25°C, 37°C, 42°C
- RNA refolding
- Structure-specific nuclease digestion
- Libraries synthesis
- Deep sequencing
- Reads (6 series)

Mapping
Reads
Counting
of read starts
Calculate
log-odd scores
S1 profile
V1 profile
PARS profile
PARS-informed
structure prediction
Thermo-switch
candidates
refined
structures
Detect
structure
changes

(adapted/extended from F. Righetti)
Genome-wide structure probing (PARS) at different temperatures

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Reads
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Calculate log-odd scores
PARS profile
V1 profile
PARS profile
Thermo-switch candidates
Detect structure changes

[adapted/extended from F. Righetti]
# PARS at SD-region of selected RNA Thermometer candidates

<table>
<thead>
<tr>
<th>Gene name</th>
<th>SD PARS* 25 °C</th>
<th>SD PARS* 37 °C</th>
<th>SD PARS* 42 °C</th>
<th>PARS difference 37–25 °C</th>
<th>PARS difference 42–25 °C</th>
<th>Thermal control†</th>
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<td>−0.02</td>
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Results for RNA thermometer candidate ailA

Two candidates associated with *Y. virulence*: ailA and cnfY.

25°C

37°C

42°C

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)
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”

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<tr>
<th>Tetracycline Sensor</th>
<th>Spacer</th>
<th>3'-Part Terminator</th>
<th>U Stretch</th>
<th>Energy RS (kcal/mol)</th>
<th>Energy T (kcal/mol)</th>
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Novel Method for multi-target design of RNAs

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.

Multiple structures *(targets)*

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

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

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

....(((((.)))....))....
Novel Method for multi-target design of RNAs


**Multiple structures** *(targets)*

```
((((((.)).(((..))).)))).
((.))((...))..(((..)))
....((((((..)))...))...)
```

**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

Boltzmann sample: 1000 low energy sequences; generated in seconds
Targeted samples: 1000 highly specific sequences; in minutes
Multi-target design to three RNA structures

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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 of Ivo Hofacker at Universität Wien