Computational RNA Design

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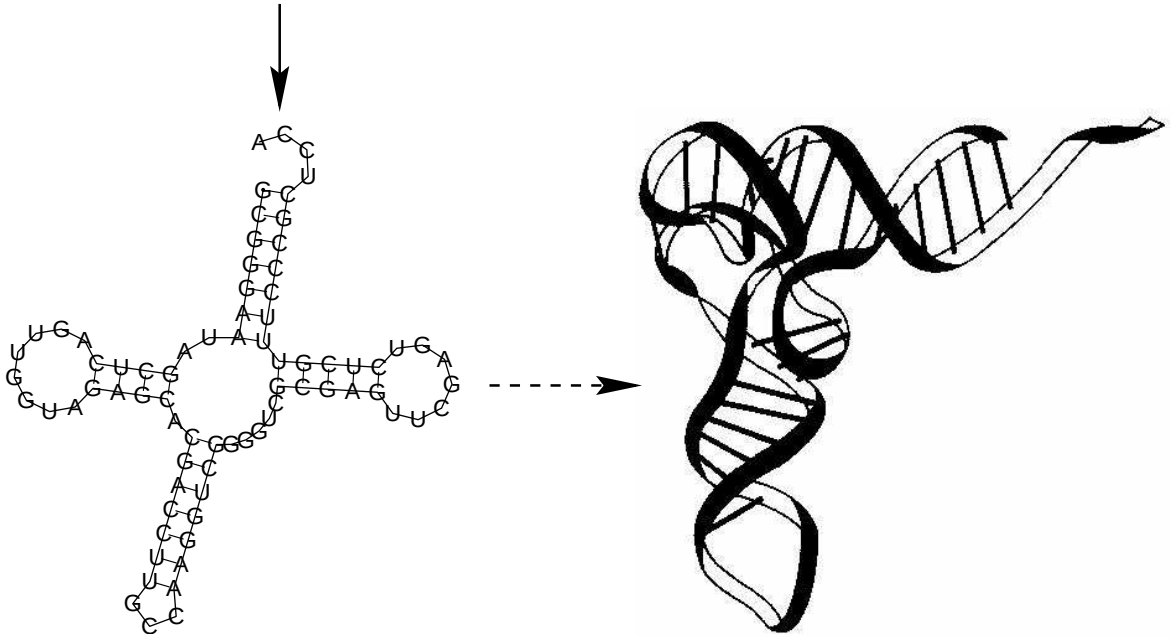
From Sequence to Shape and Back

A historical note: why inverse folding is easy in practice ...

The starting point

GCGGGAAUAGCUCAGUUGGUAGAGCACGACCUUGCCAAGGUCGGGGUCGCGAGUUCGAGUCUCGUUUCCCGCUCCA



Sequence-structure relationships in RNA

Sequence-Structure Map of RNA

1. *Redundancy:* Many more sequences than structures
2. *Sensitivity:* Small changes in the sequences may lead to large changes in the structure
3. *Neutrality:* A substantial fraction of mutations does not alter the structure.

Implications:

1. *Neutral Networks:* $S(\psi)$ forms a connected "percolating" network in sequence space for all "common" structures.
2. *Mutual Accessibility:* The neutral networks of any two structures almost touch each other somewhere in sequence space.

- RNA sequence evolve drift-like while maintaining secondary structure
- Substitution pattern reflects selective constraints on the structure

Proc.Roy.Soc.B **255** 279-284 (1994), Proc. Natl. Acad. Sci. USA **93**, 397-401 (1996),

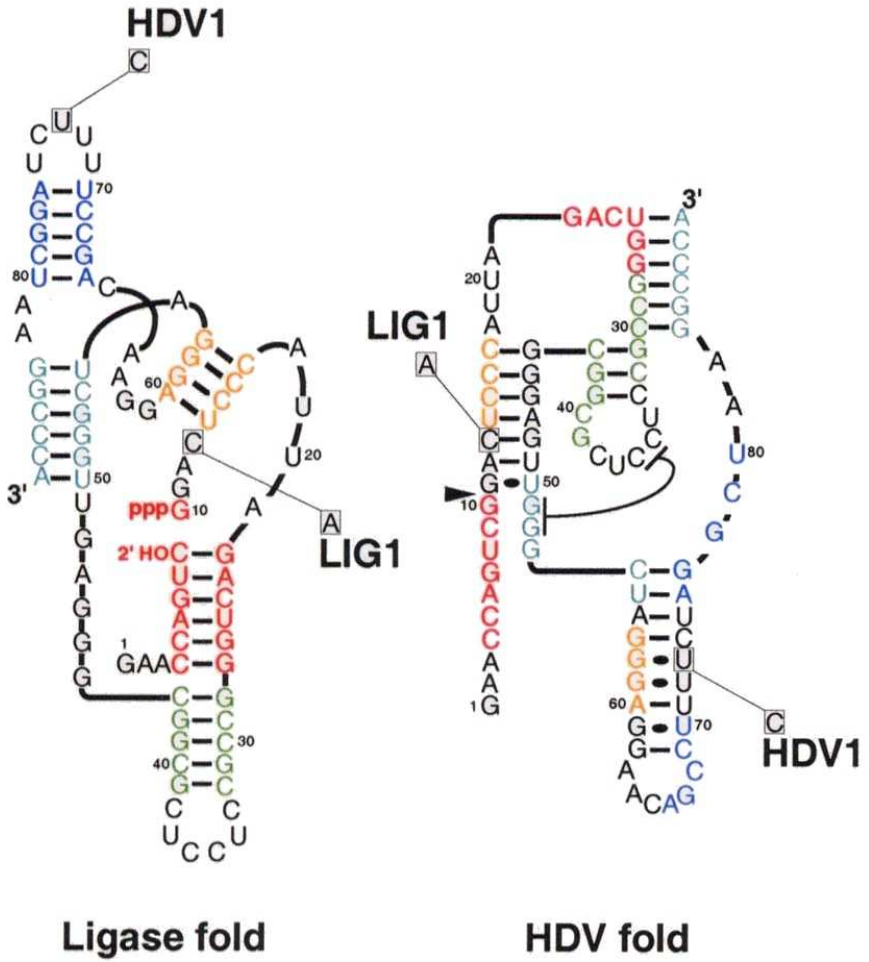
Bull. Math. Biol. **59**, 339-397 (1997), RNA **7**: 254-265 (2000).

Implementations

Several programs are available that implement simple inverse folding:

- `RNAinverse` (Hofacker *et al.* 1994)
  unbiased search using adaptive walks and a simple hierarchical problem decomposition
- `RNAdesigner` (Andronescu 2004)
  Uses sequence bias in paired/unpaired regions, more sophisticated decomposition to speed up search
- `INFO-RNA` (Busch & Back 2006)
  Uses a sequence with minimal energy for the target structure as starting point (strong sequence bias)
- `NUPACK` (Zadeh, Wolf, Pierce 2011)
  Allows design of multi-strand structures
- `RNAiFOLD` (Garcia-Martin, Clote, Dotu 2013)
  uses constraint programming or a large neighborhood search heuristic

One Sequence, Two target Structures



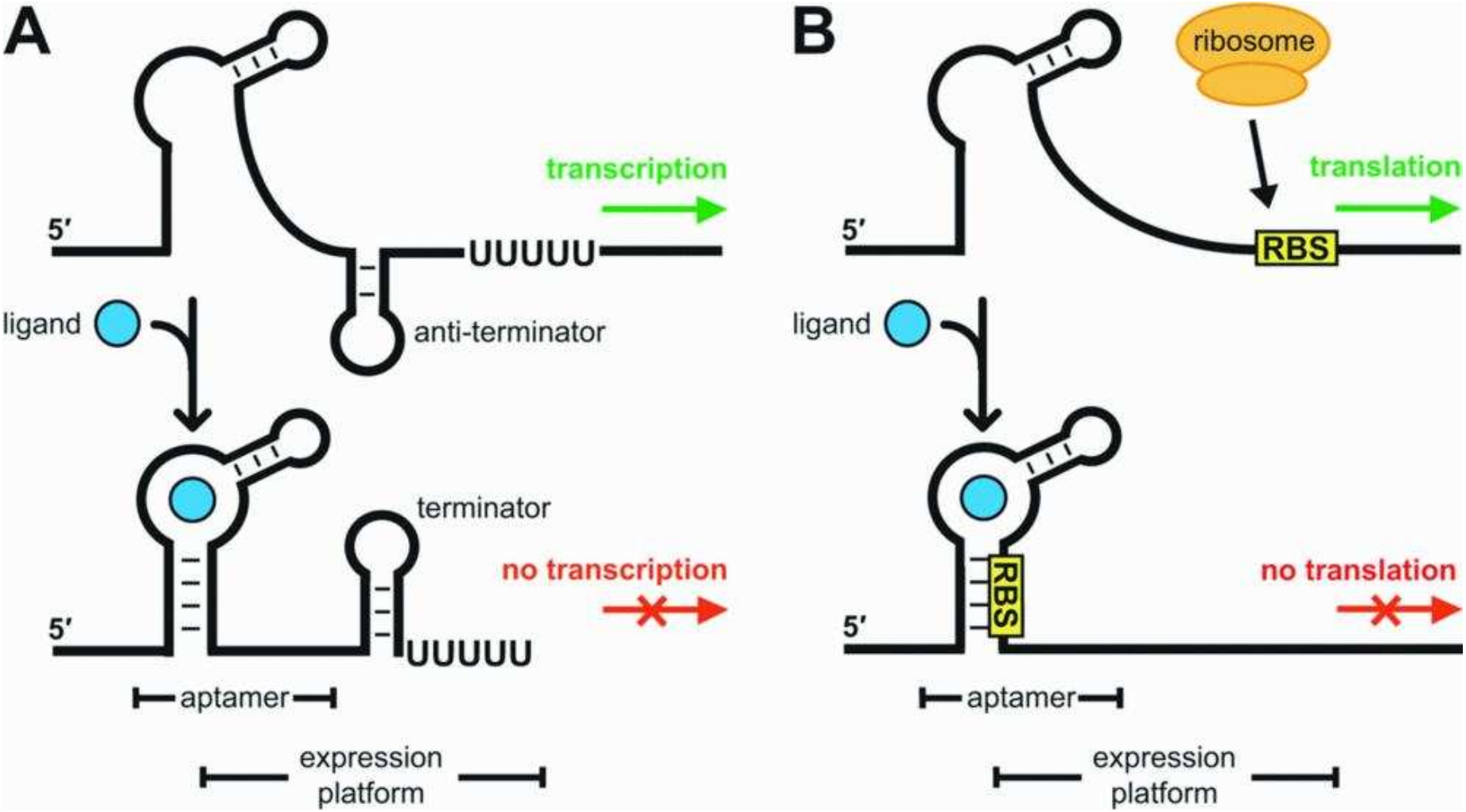
Schultes, EA & Bartel, DP; Science (2000), **289**:448-452

Design of Artificial Riboswitches

- Riboswitches are a convenient gadget in synthetic biology
- Task: combine ligand-specific sensor with an effector (i.e., some form of a regulatory element)
- Question: to what extent is this really modular?
- Idea: use RNA structure prediction to model the interplay of sensor and effector

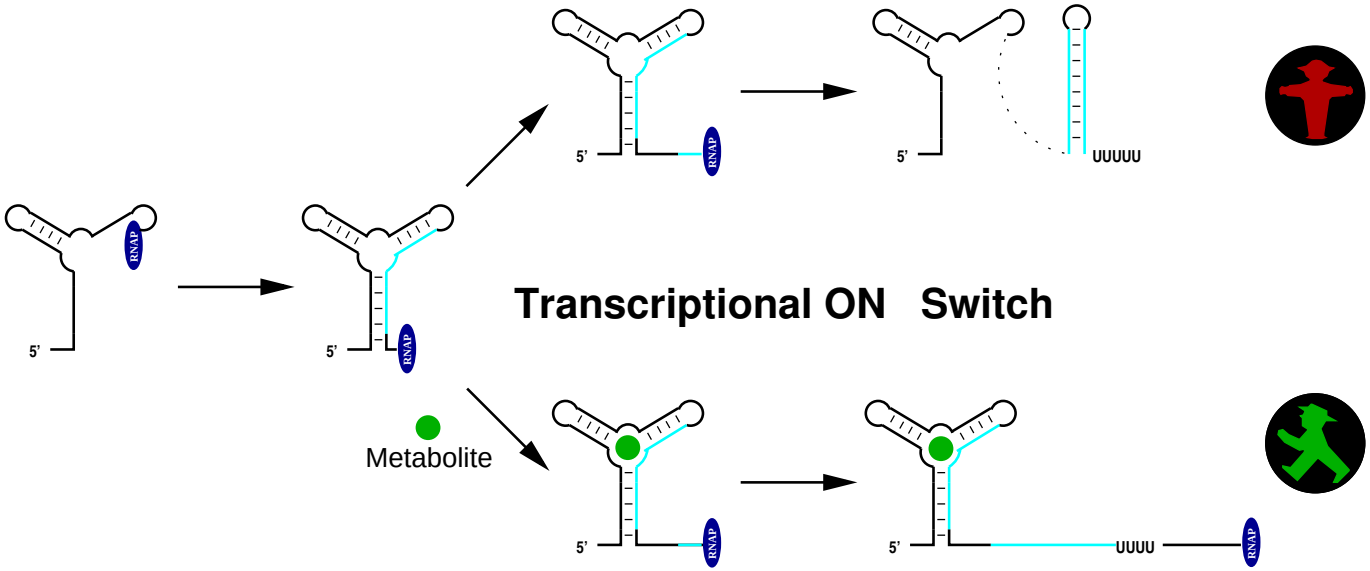
Riboswitches: Regulators of Gene Expression

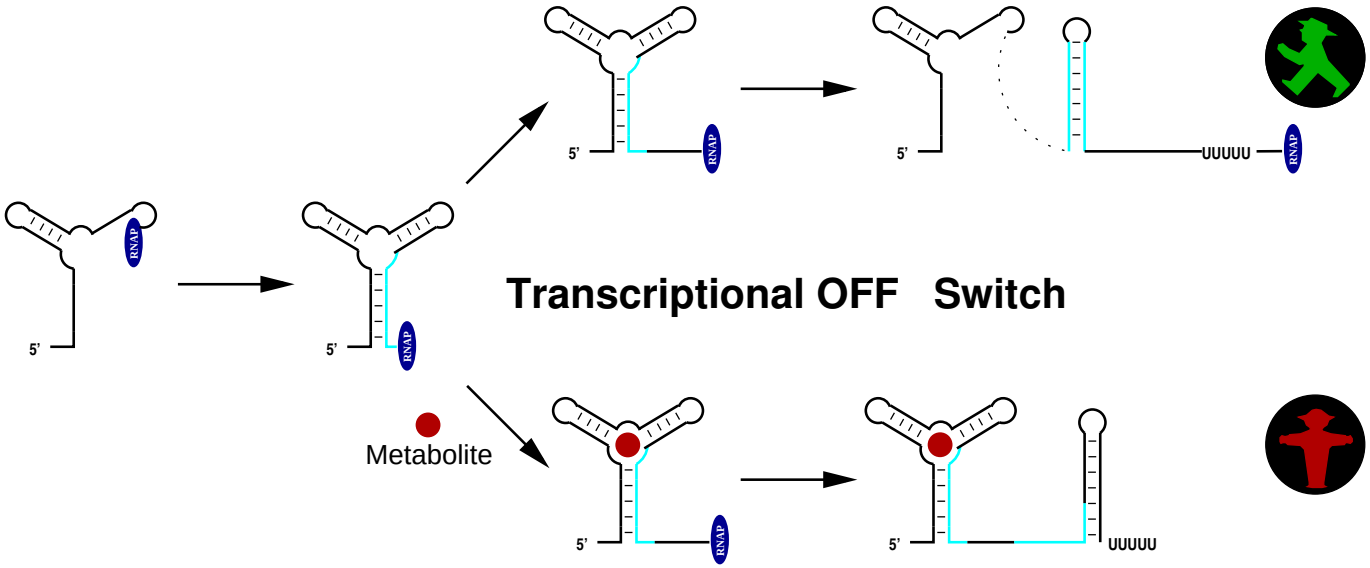
Transcriptional *versus* translational riboswitch



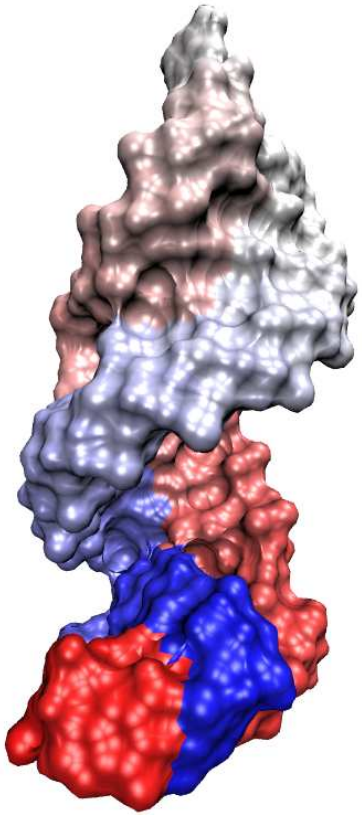
Kim & Breaker, *Biol. Cell* (2008)

Ribo-Switching of Transcription

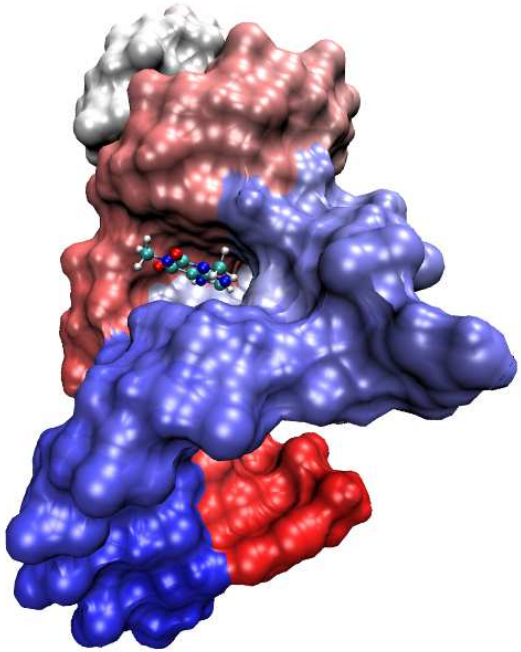




Theophylline Aptamer

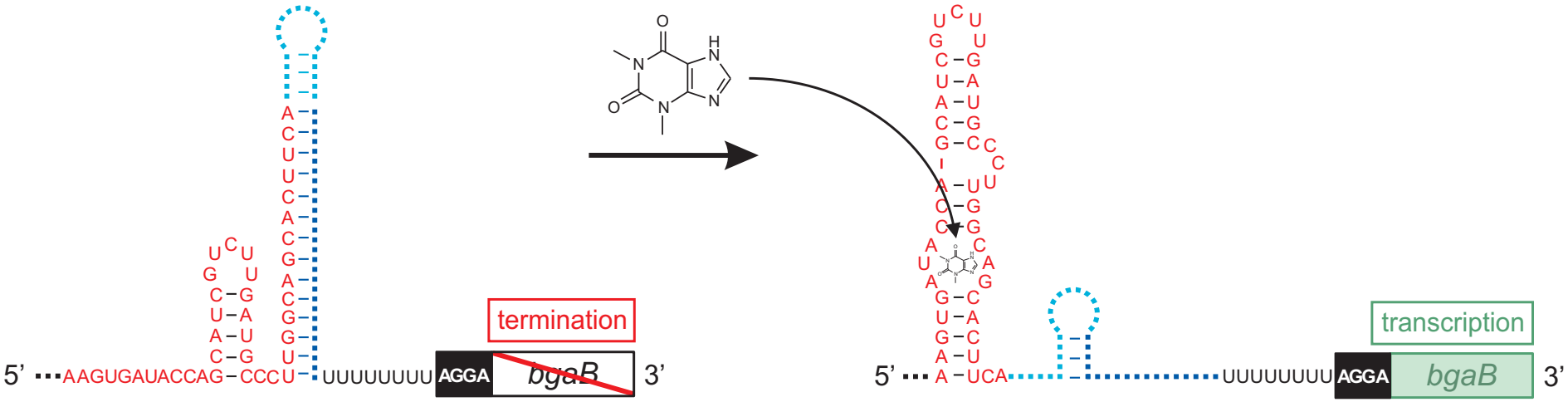


Unbound aptamer
Model predicted using Rosetta



Theophylline bound aptamer
Crystal Structure
(PDB-ID 1O15)

Design Idea



Goal: a theophylline triggered on-switch

Essence of the Multistable Design Problem

- Design a sequence that *compatible* with not just one but *several* target structures
- Each target should be almost a ground state
- **Questions:**
  - When can this be solved?
  - How can we include ligang specificity
- First step: generate sequences that are compatible with all design goals.
- 2nd step: optimize the sequences toward the design goal(s)

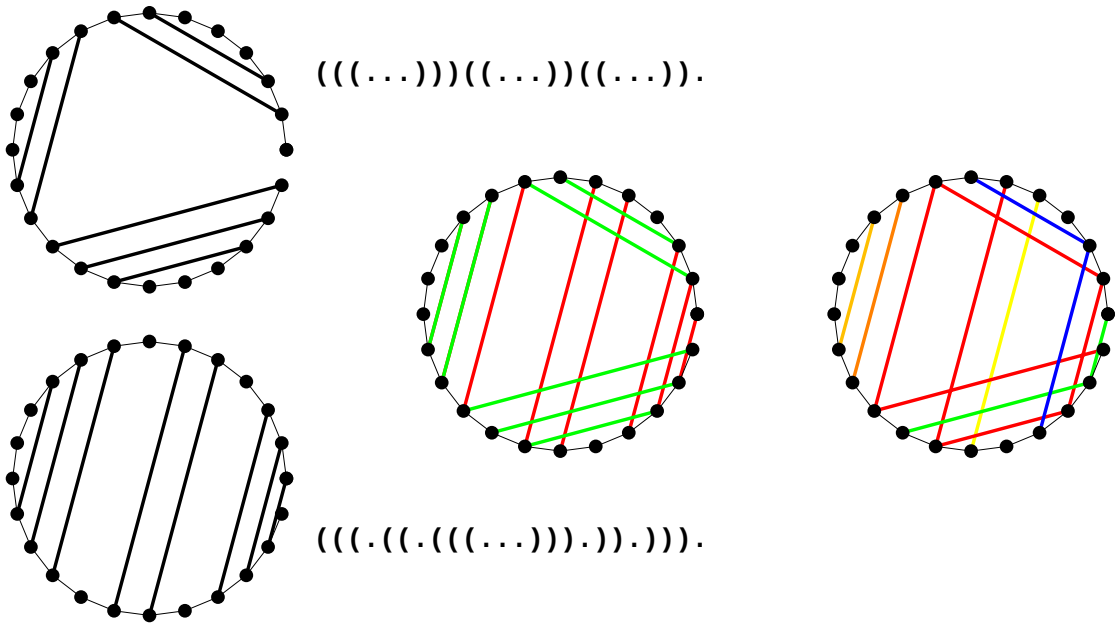
Bi-Stable Structures

Given two structures $\mathcal{S}_1$ $\mathcal{S}_2$, are there sequences compatible to both?

intersection theorem:

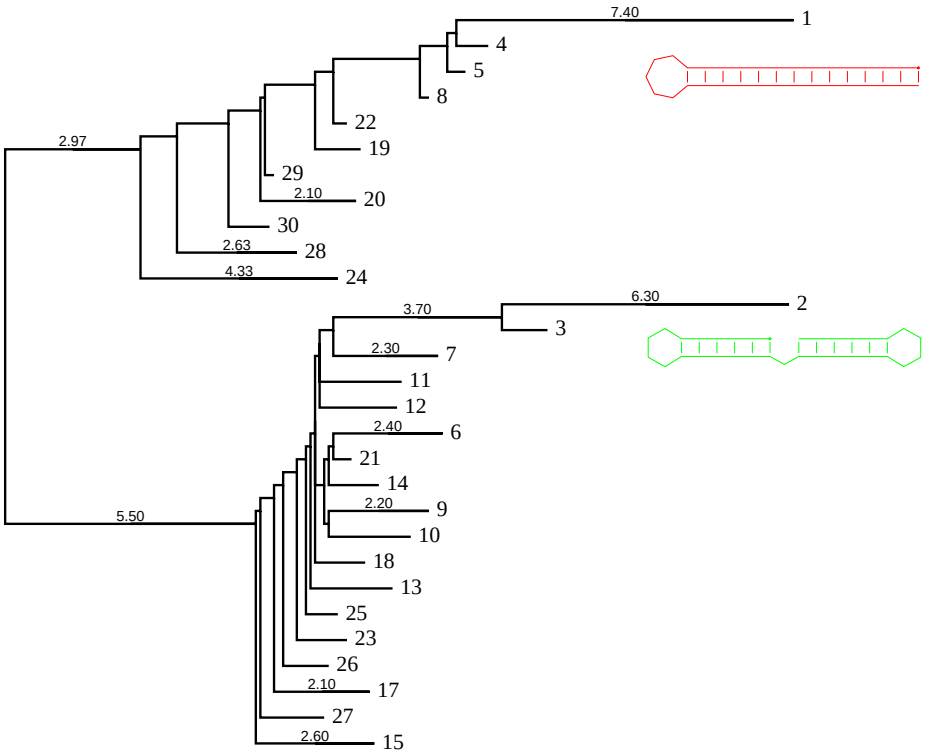
$$\mathbf{C}[\mathcal{S}_1] \cap \mathbf{C}[\mathcal{S}_2] \neq \emptyset$$

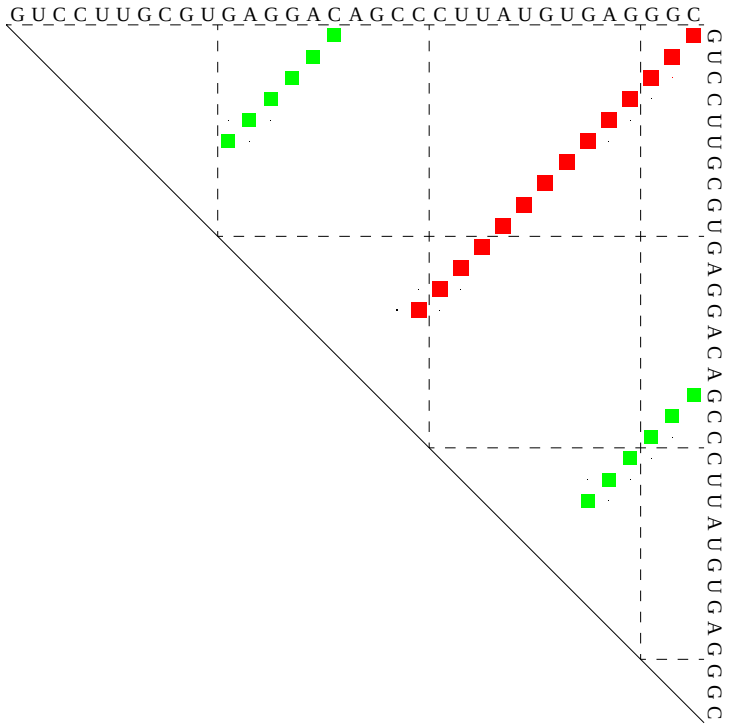
Proof: Dependency graph decomposes into paths and cycles of even length



the alternating sequence AUAUAU... is compatible with each path and cycle.

Examples of bistable structures





$$\Xi(x) = E(x, \Omega_1) + E(x, \Omega_2) - 2G(x) + \xi \left(E(x, \Omega_1) - E(x, \Omega_2)\right)^2$$

Multi-Stable Structures

Generalization to multiple Targets:

Theorem. There is a sequence satisfying each secondary structure constraints $S_1, S_2, \ldots, S_M$ if and only if the overlap graph $S_1 \cup S_2 \cup \cdots \cup S_M$ is bipartite.

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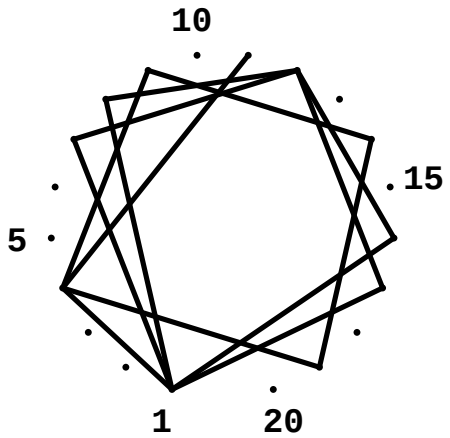
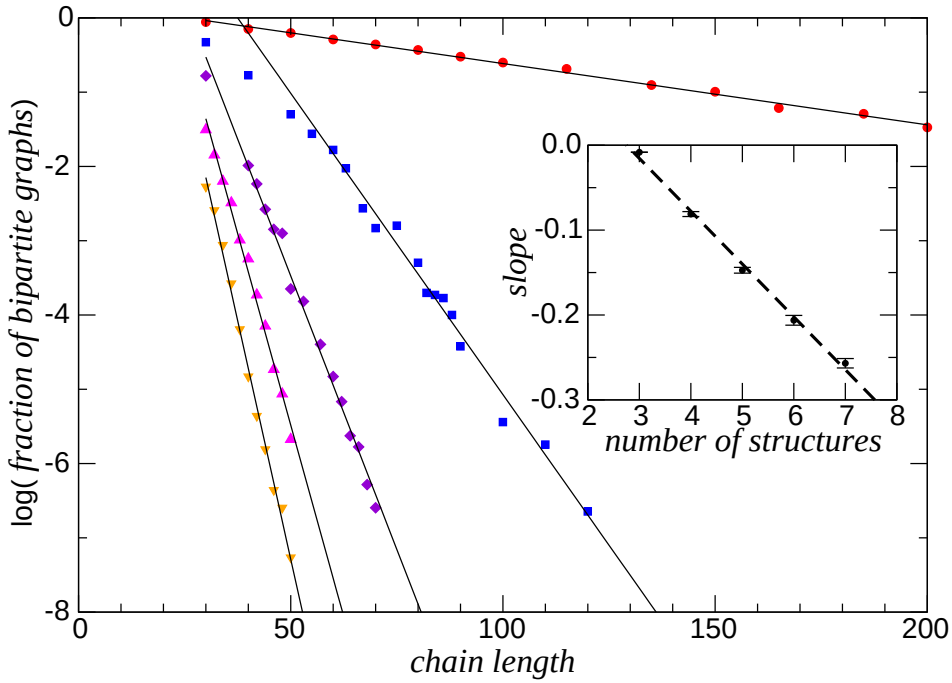




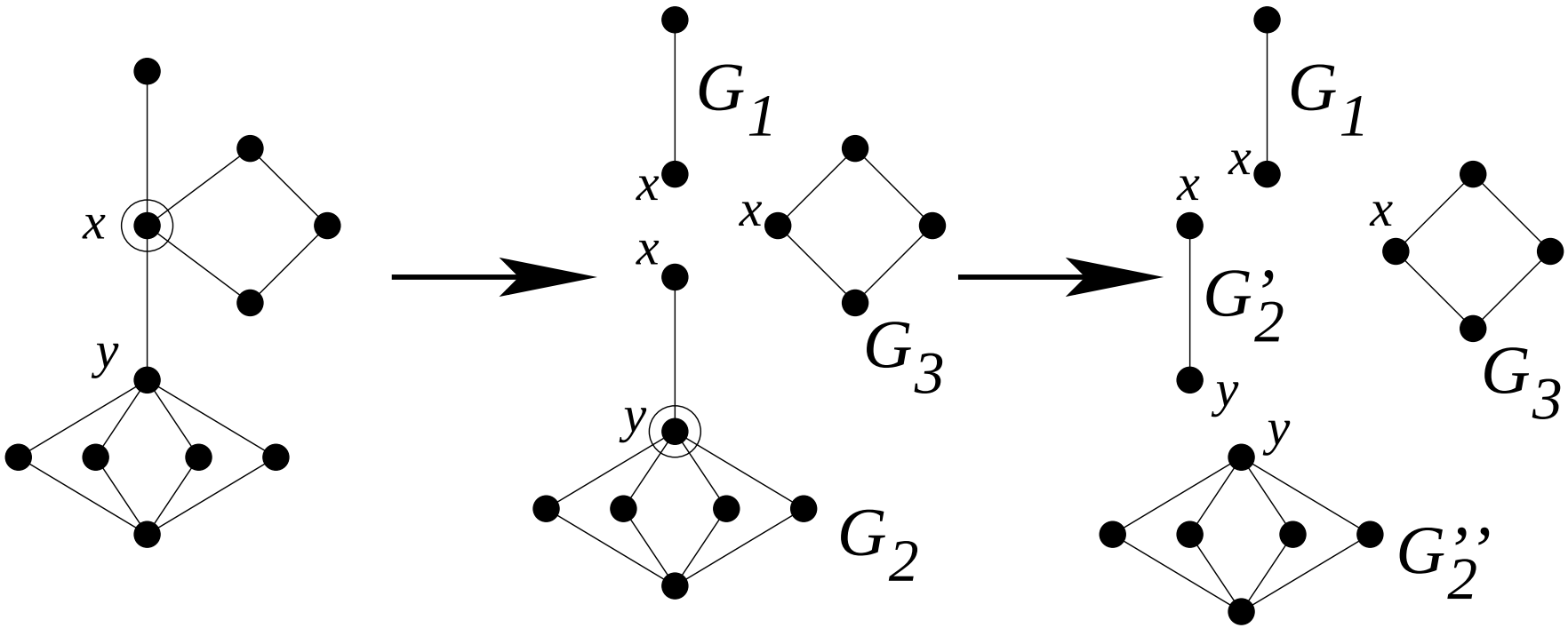
Solving Multi-Constraint Design Problems

- one possibility: constraint programming [Dotu's work]
- stochastic heutristics
  - Complex search space. Only $\mathbf{C} := \bigcap_{i=1}^{M} \mathbf{C}(\Omega_i)$ allowed
  - How to choose a good (fair) starting position?
    simple for $M = 2$: constraints are path and cycles. Simple recursions to sample uniformly from $\mathbf{C}$
  - Difficult for $M > 2$: need more complex descompositions of graphs

How to sample uniformly?

Use the overlap graph: it defines the dependence structure of nucleoides.

- Zeroth Step: connected components are indepenently sampled.
- First Step: block decomposition of the overlap graph.
  Color every block separately with fixed colors at the cut points

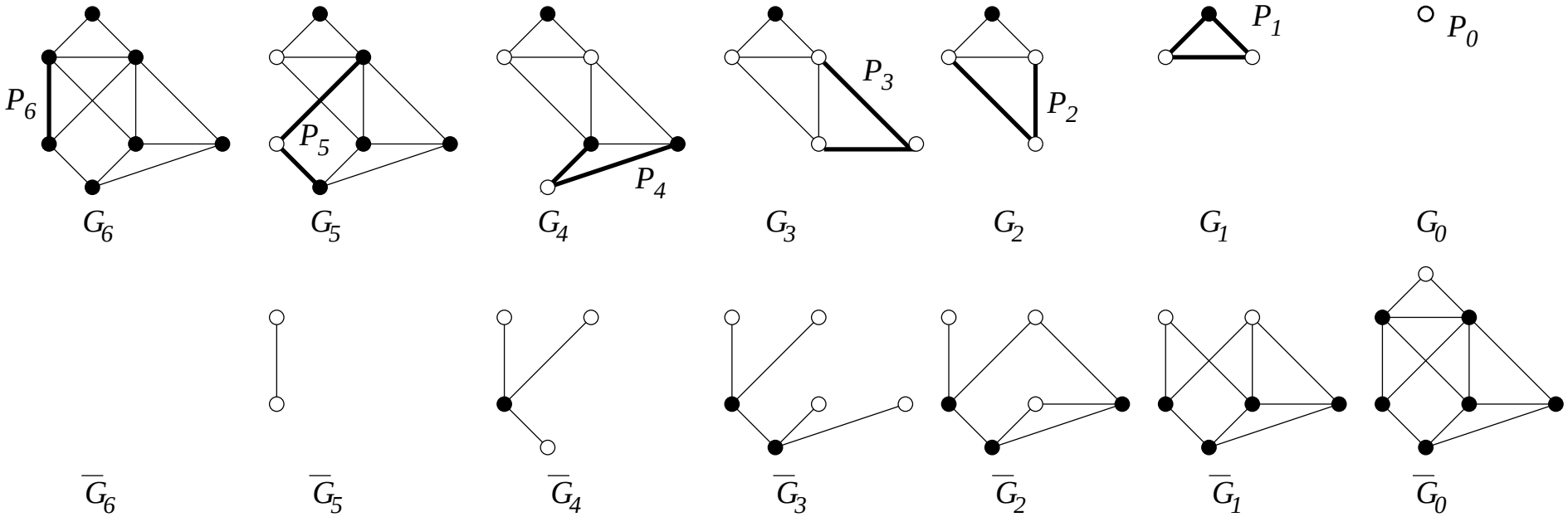


- Second Step: Color Blocks by Dynamic Programming

Biopolymers **99**: 1124-1136 (2013)

Coloring dense blocks: Ear decomposition

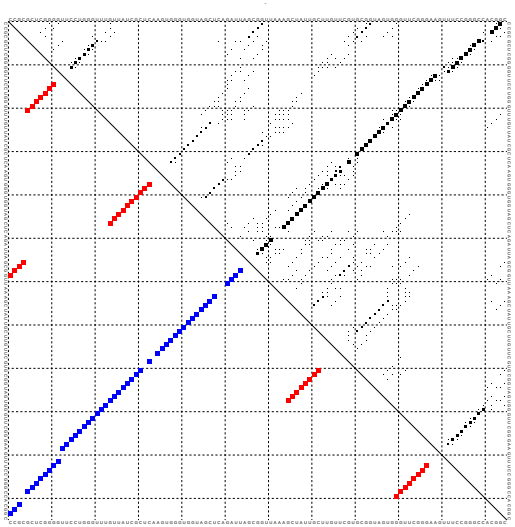
ear decomposition

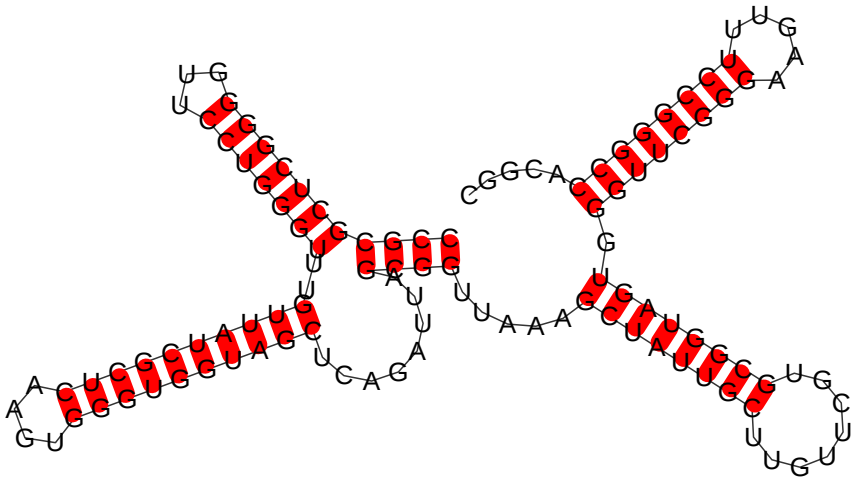


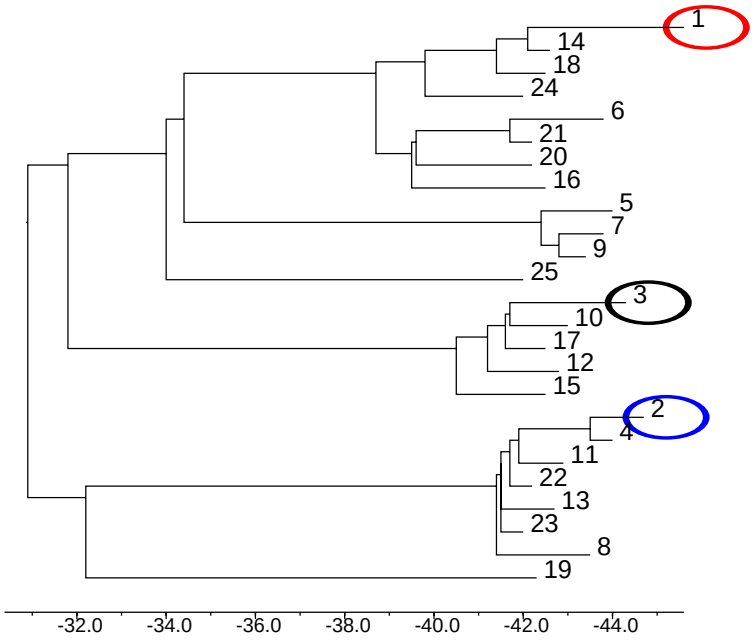
complement graph with attachment vertices

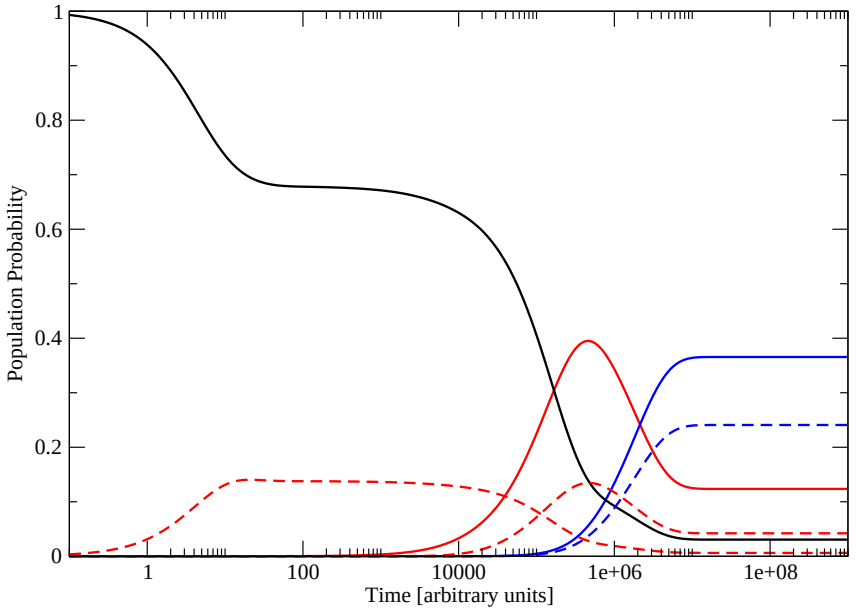
- dynamic programming approach to count colorings with given color combinations at the attachment vertices.
- memory exponential in the maximum number of attachment vertices α, CPU time in the maximum size of the union of attachment vertices in consecutive steps β

A design for the SV11 switch

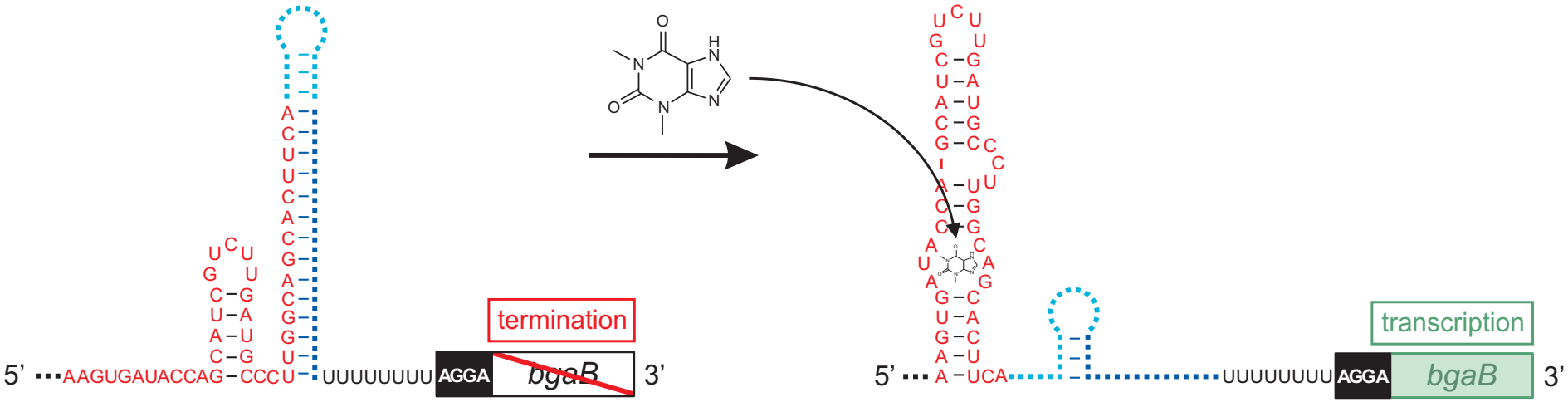






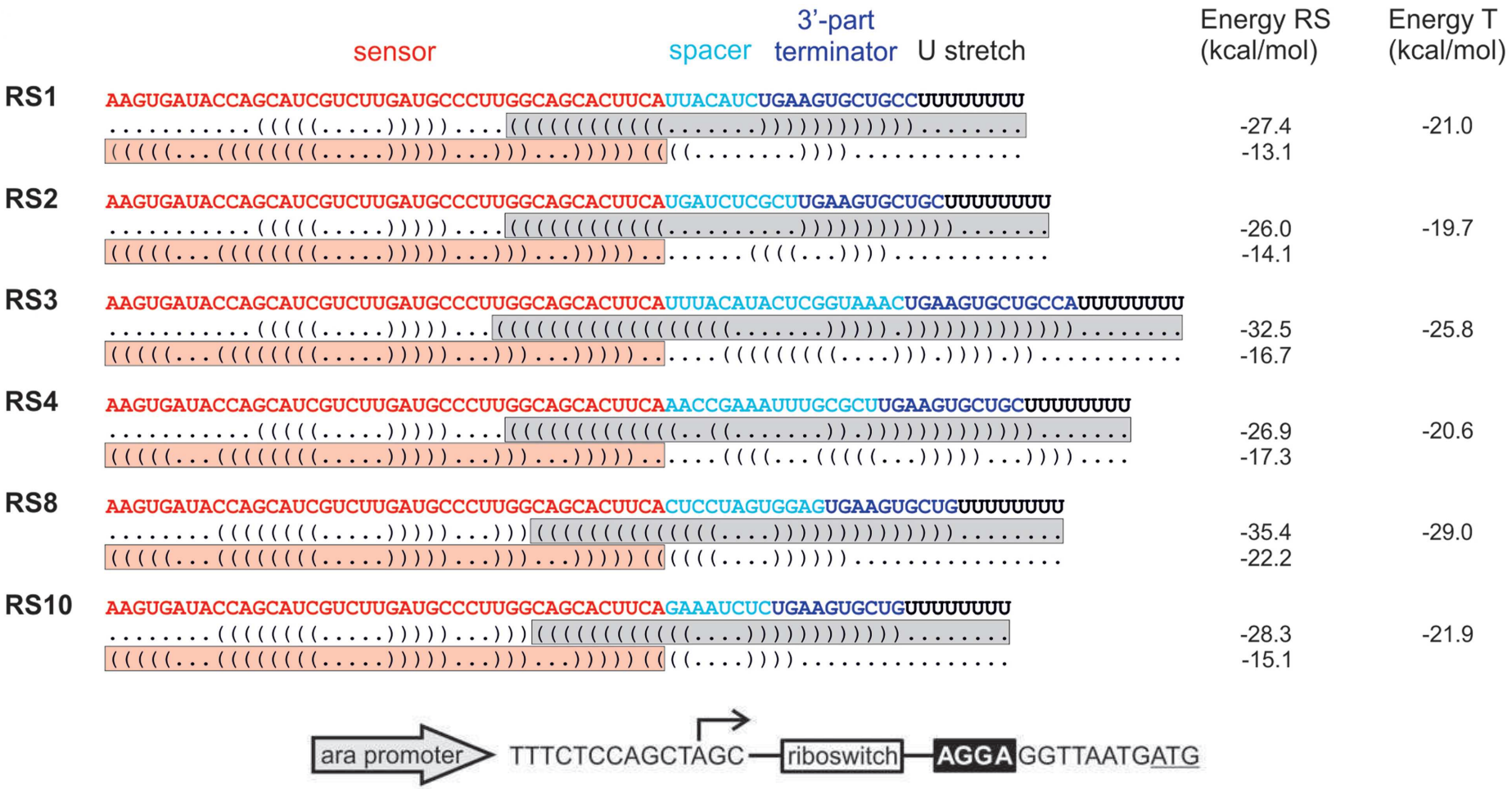


Back to the Theophylline Switch



Goal: a theophylline triggered on-switch

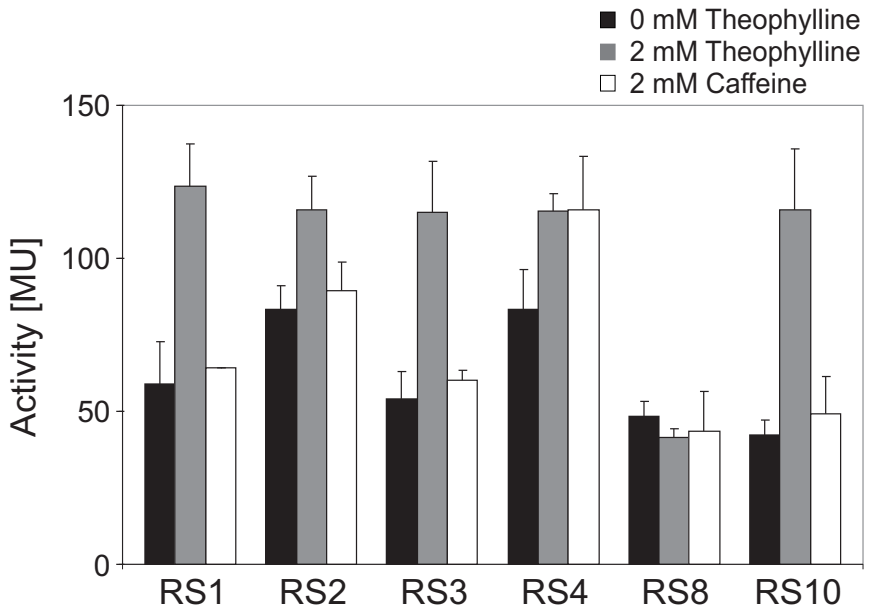
Designed Theophylline Switches



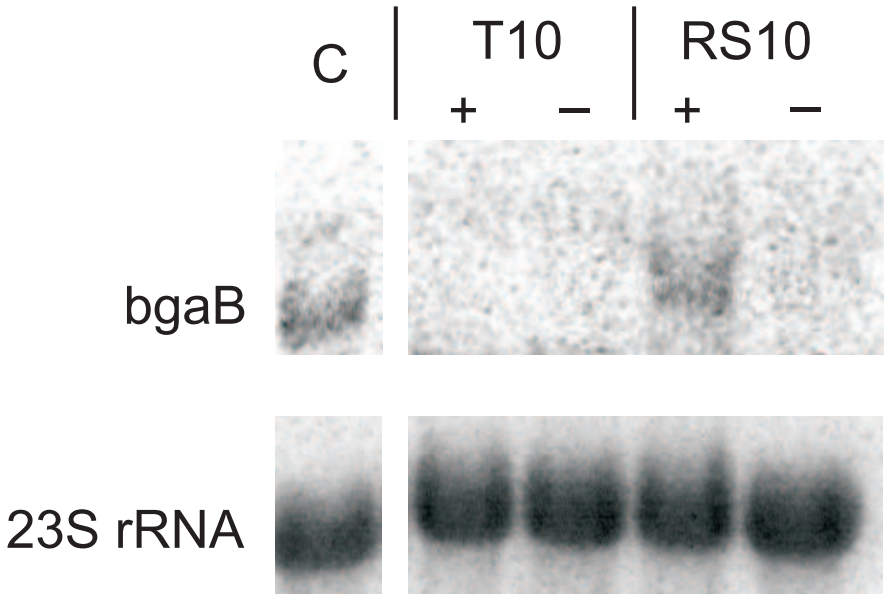
Transcribed from arabinose promoter of plasmid pBAD, using β-galactosidase as reporter gene.

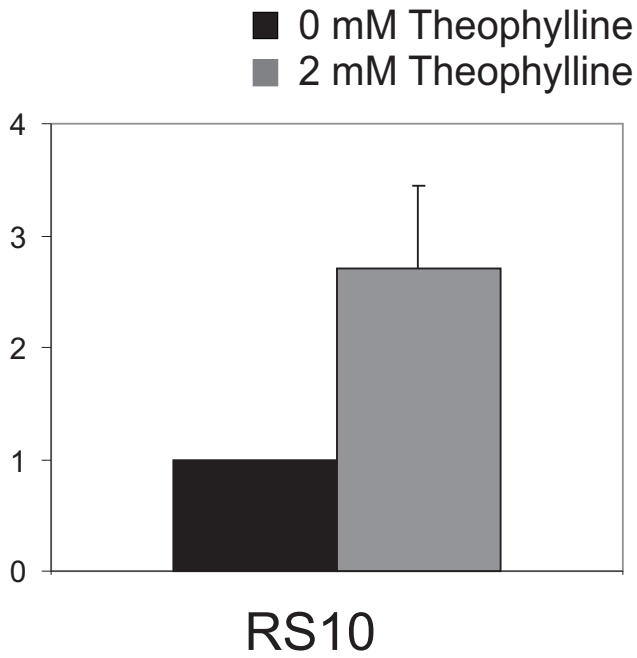
Construct Expression

	sensor	spacer	3'-part terminator	poly(U)
RS1:	aptamer-	UUACAUC----------	-UGAAGUGCUGCC	--UUUUUUUU
RS2:	aptamer-	UGAUCUCGCU--------	-UGAAGUGCUGC	---UUUUUUUU
RS3:	aptamer-	UUUACAUACUCGGUAAAC	-UGAAGUGCUGCCA	-UUUUUUUU
RS4:	aptamer-	AACCGAAAUUUGCGCU--	-UGAAGUGCUGC	---UUUUUUUU
RS8:	aptamer-	CUCCUAGUGGAG------	-UGAAGUGCUG	----UUUUUUUU
RS10:	aptamer-	GAAAUCUC----------	-UGAAGUGCUG	----UUUUUUUU



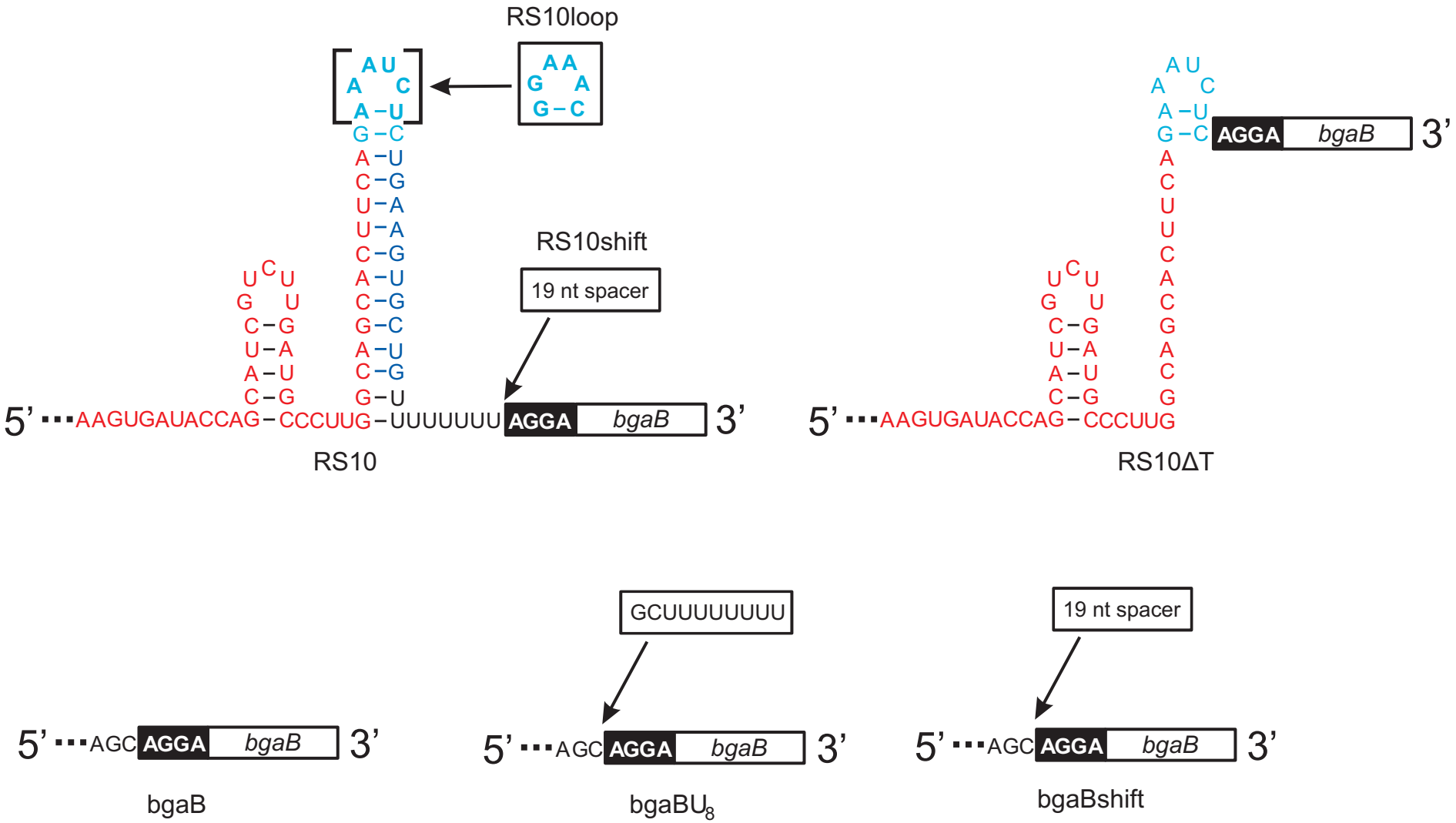
Transcriptional Switching



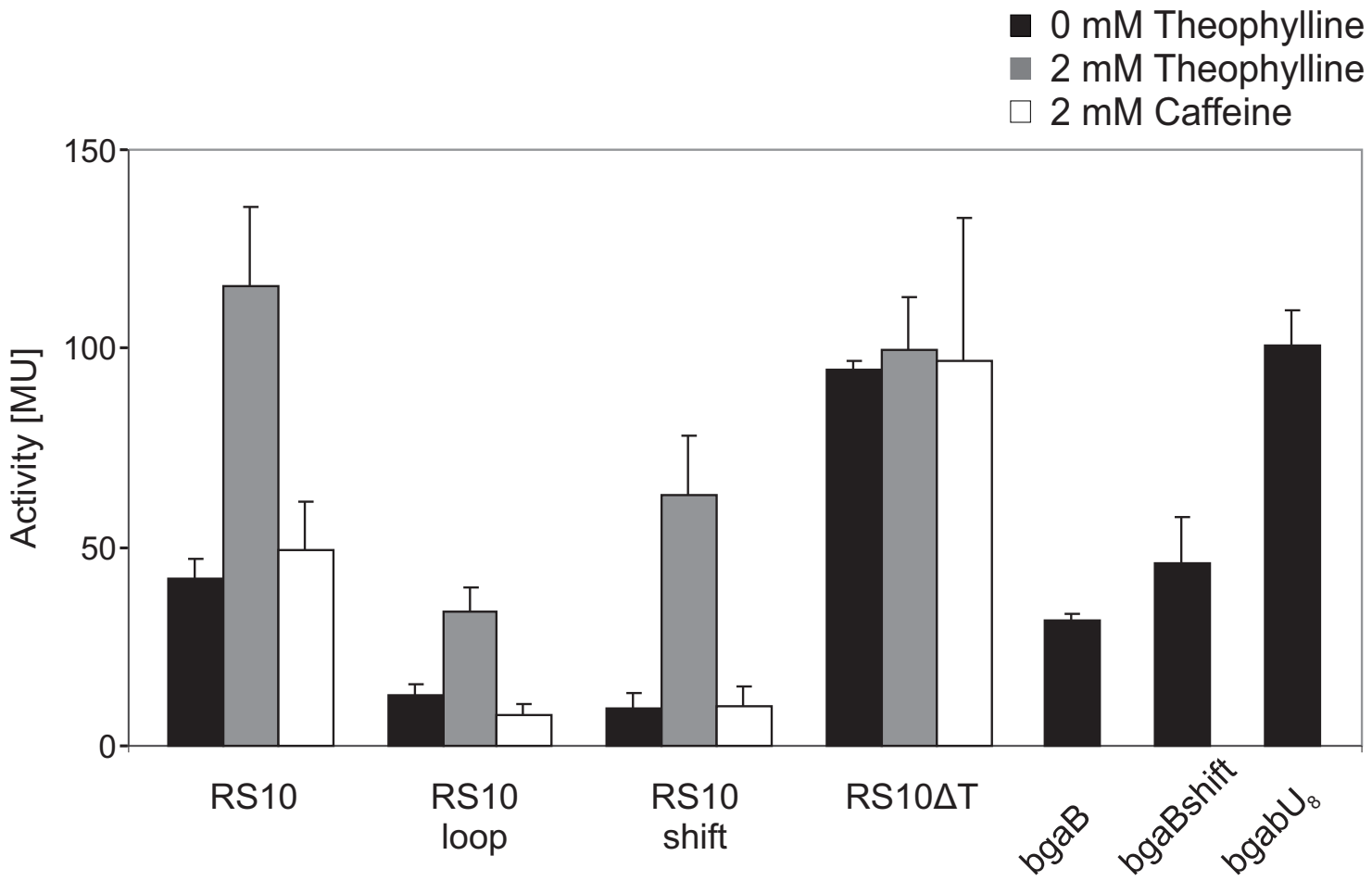


Northern blot of RS10 and terminator T10

Optimizing the Switch

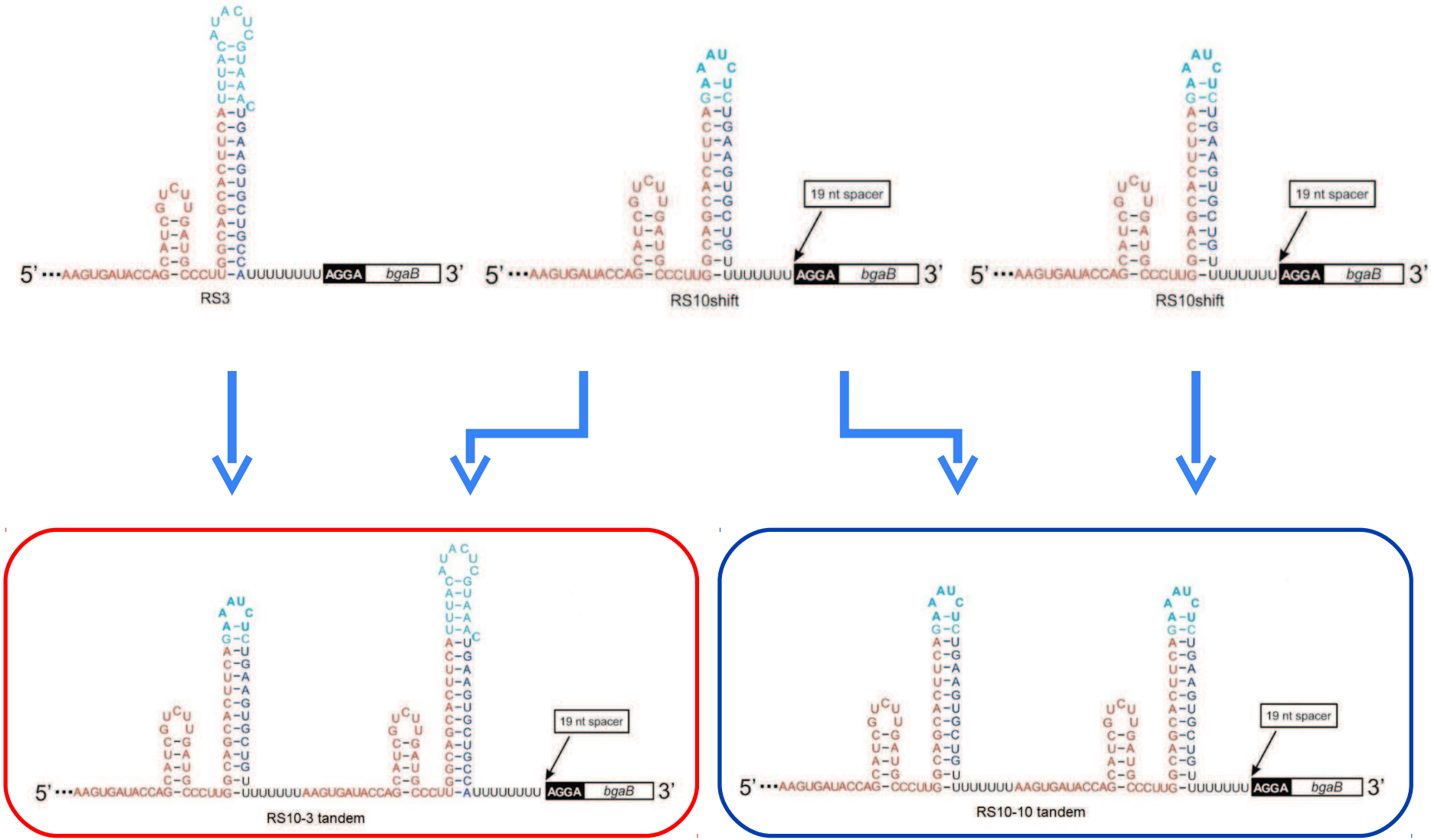


Optimizing the Switch

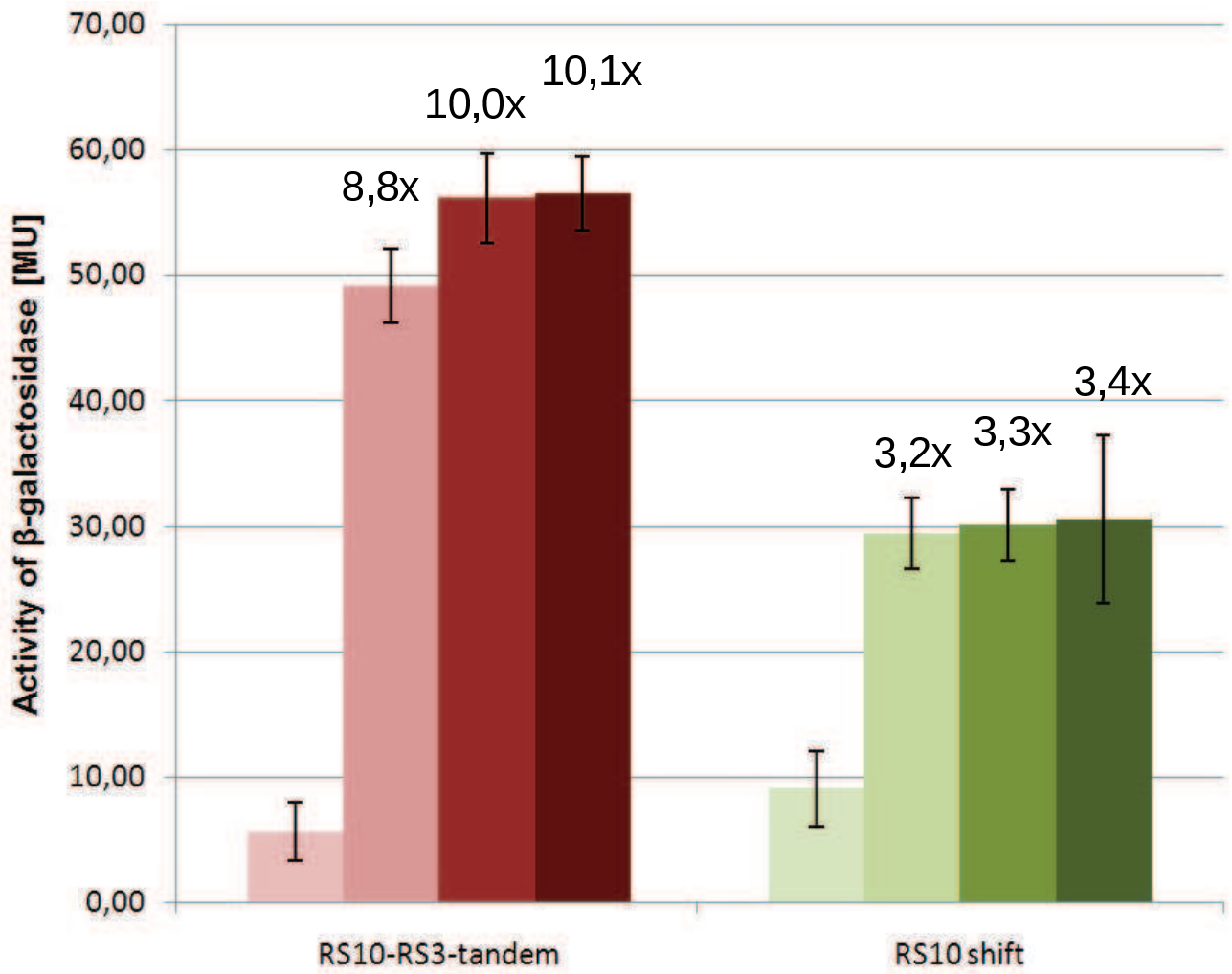


Activities of optimized constructs

Working in Tandem ...



... works even better



A more principled way to include ligand binding

- Known/measured binding energy $-\varepsilon$ of the ligand to a particular structural motif Ψ necessary for binding.
- without ligand: we want structural feature Ω
- with ligand: we want structural feature Ψ
- Compute the partition function $Z[\Psi]$ over all structures with feature Ψ.

  Partition function over structures without feature Ψ is $Z[\neg\Psi] := (Z - Z[\Psi])/Z$.
- Binding distorts the ensembl of structure when the ligand is present: $Z_L = Z[\neg\Psi] + Z[\Psi]\exp(-\varepsilon)$
- Objectives
  - without ligand: $p_0(\Omega) := Z[\Omega]/Z \to max$ and $p_0(\Psi)$ should be small
  - with ligand: $p_L(\Psi) := Z[\Psi]\exp(-\varepsilon)/Z_L \to$ max and $p_L(\Omega)$ should be small.

    ...easy if Ψ and Ω are mutually exclusive, otherwise we also need the partition function $Z(\Omega \wedge \Psi)$.

Using Constraints ...

Modified folding algorithms that scores certain structures differently
$Z\{\psi; e\}$ scores a (set of) pattern(s) ψ (in practice e.g. the loop forming the binding pocket) with bonus energies e
Key relationship:

$$\frac{[RNA \cdot L]}{[RNA]\,[L]} = K = \frac{Z\{\psi; e\}}{Z z_L}$$

Set $z_L = 1$ for a small molecular ligand and gauge the binding energies accordingly.

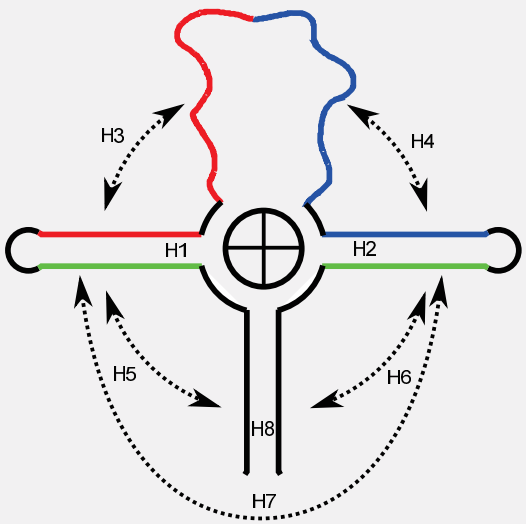
$$Z\{\psi; e\} \approx Z[\neg\Psi] + Z[\Psi]\exp(-\varepsilon)$$

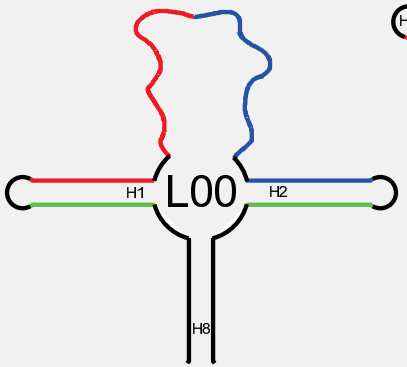
in the more general model with (soft) constraints we may include a more elaborate parametrization that includes e.g. a set of variant binding site structures ...
Details of the theory (and implementations) are still being developed ... see Ronny's talk

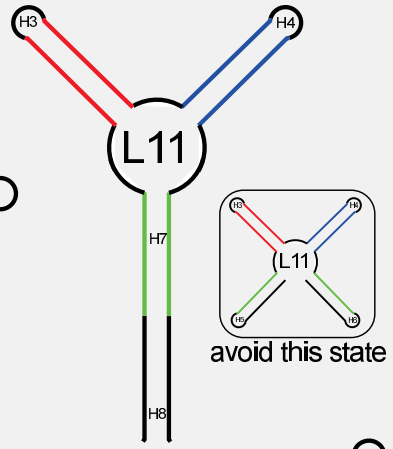
Designing an Integrated XOR Switch

Idea: Combination of two adptamers interacting non-linearly

	Lig1	Lig2	AT	T	
L00	0	0	0	1	OFF
L10	1	0	1	0	ON
L01	0	1	1	0	ON
L11	1	1	0	1	OFF







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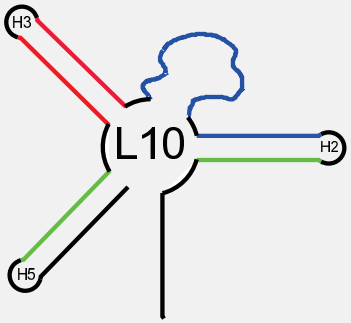
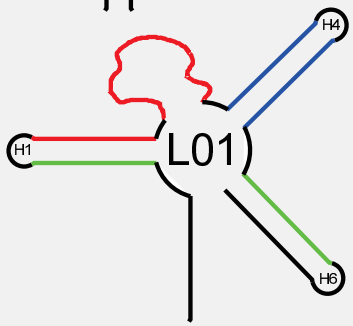
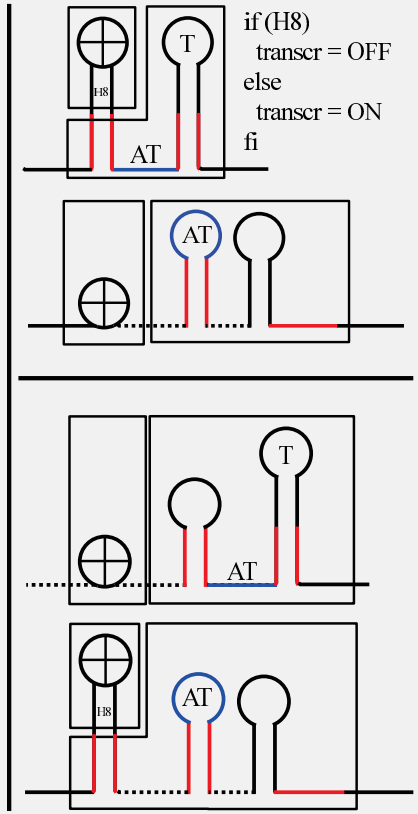
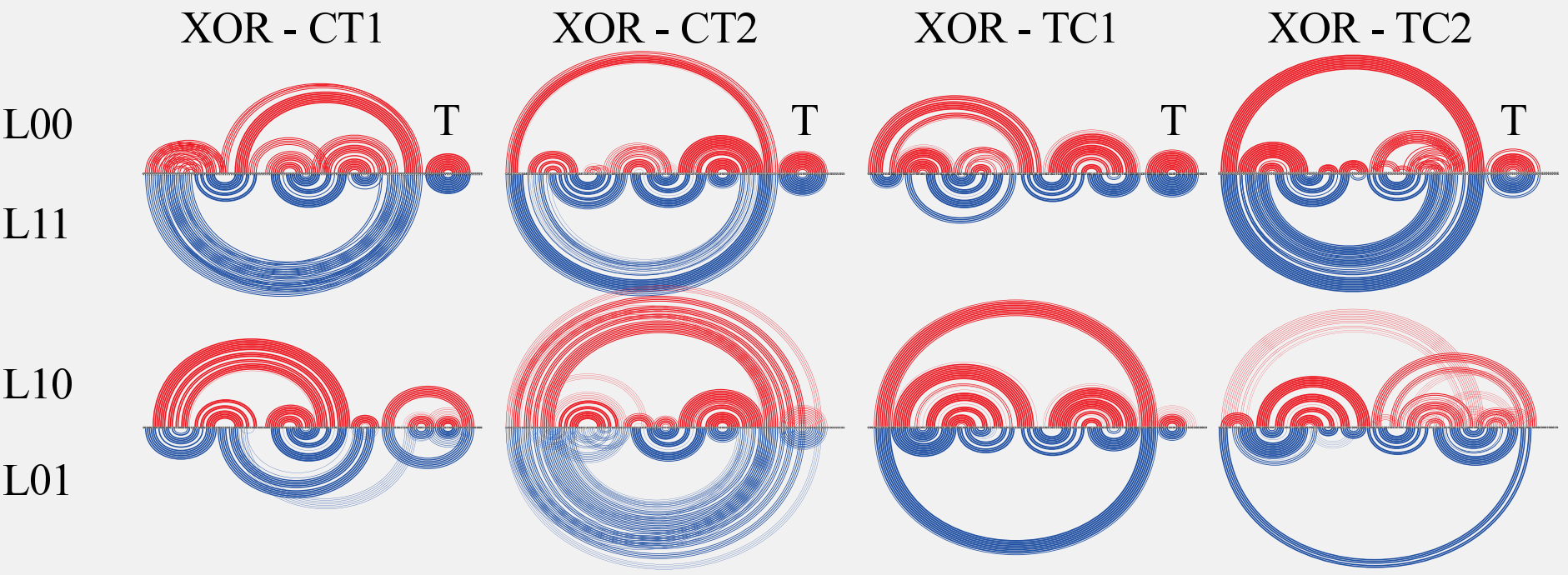




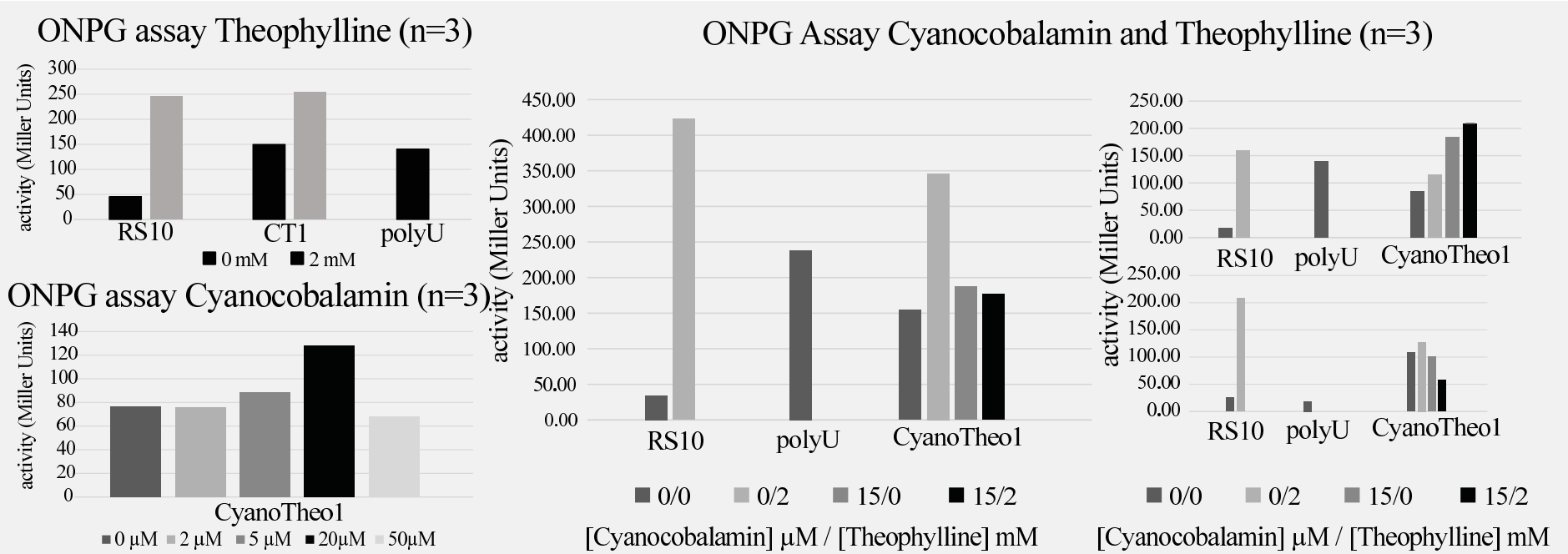


Designing an Integrated XOR Switch

4 computational designs for the cobalamin and theophyllin aptamers

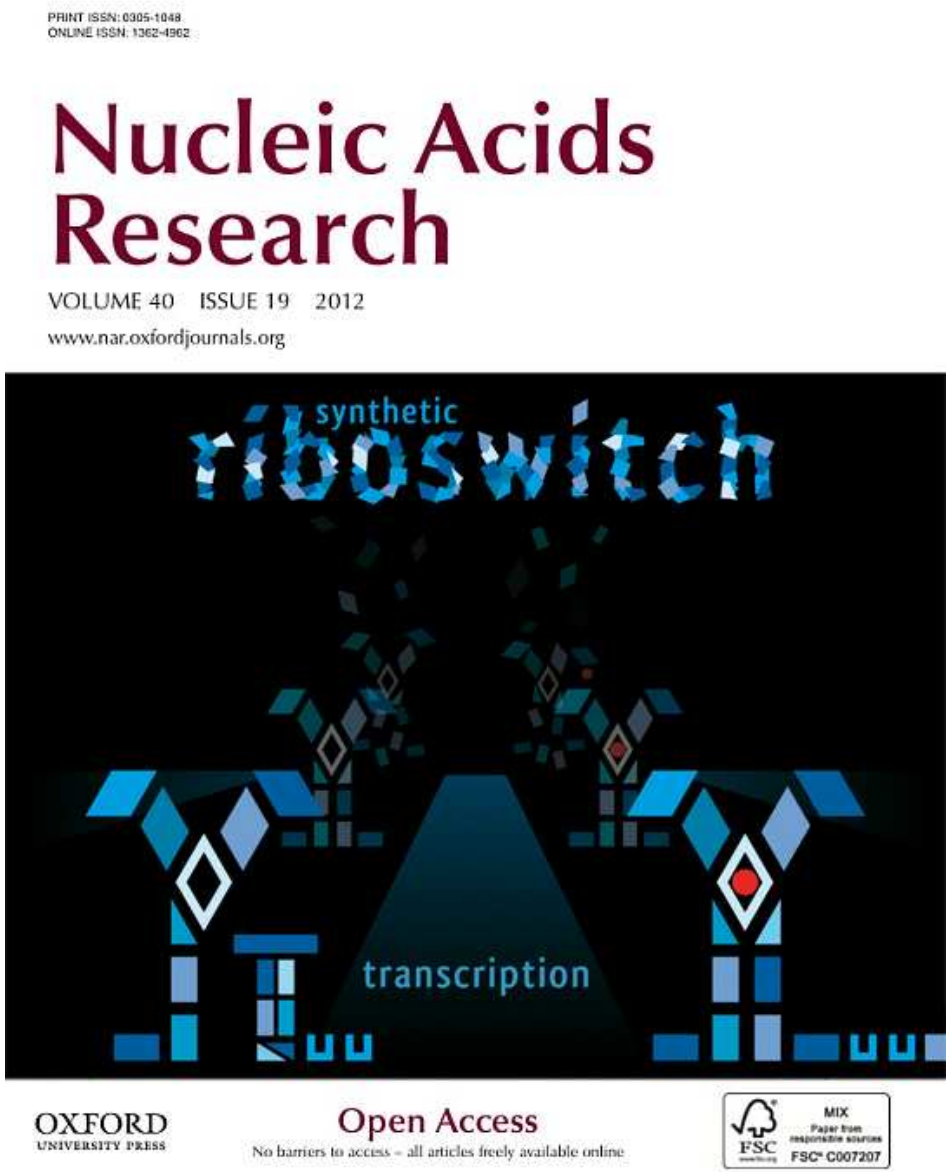


Initial experimental tests



... are promising ... but a lot of optimization will still be necessary

Acknowledgements



Funding: DFG

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