

Design of artificial RNA-switches

A coarse-grained method for RNA refolding

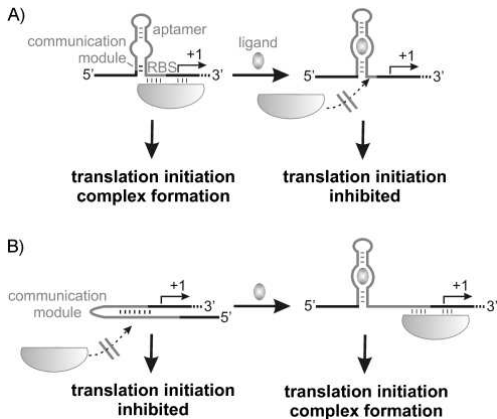
Stefan Badelt

Institute for Theoretical Chemistry
Theoretical Biochemistry Group

February 19, 2010

What is a Riboswitch?

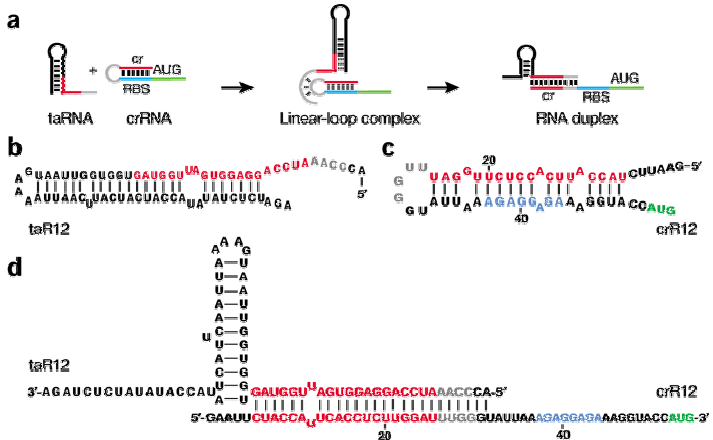
- Mechanism:



Markus Wieland and Jörg S. Hartig. Artificial Riboswitches: Synthetic mRNA-Based Regulators of Gene Expression. *ChemBioChem* 9,1873-1878 (2008)

What is a Riboswitch?

- Artificial riboregulation:



Isaacs et al. Engineered riboregulators enable post-transcriptional control of gene expression. *Nat. Biotechnology* 22,841-847 (2004)

Concept:

- Generate two sequences (crRNA, taRNA) that...
 - have certain constraints (AUG, AGGAGG, ...)
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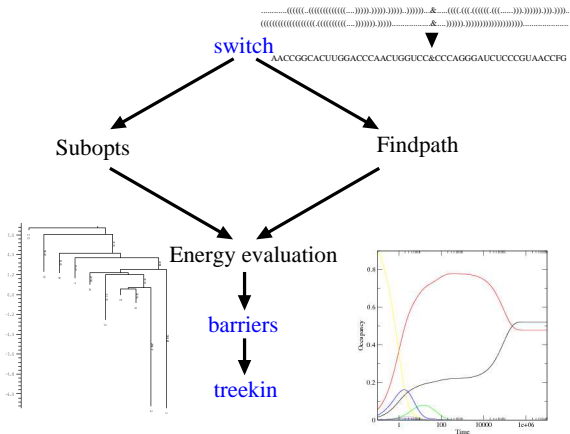
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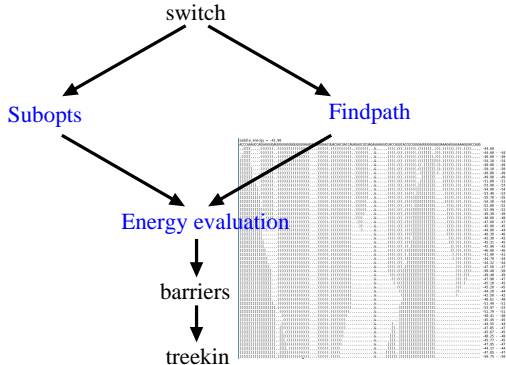
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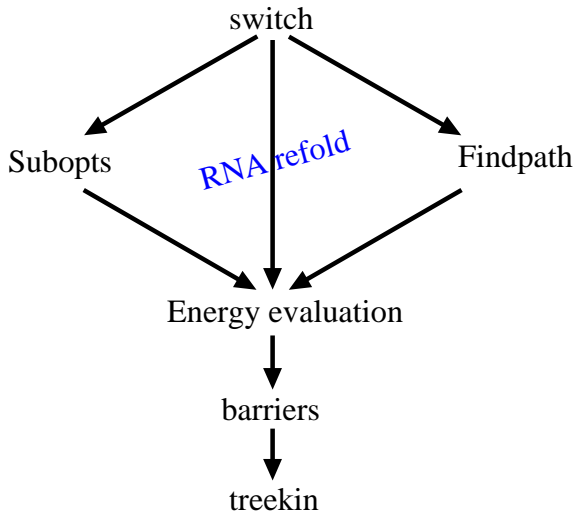
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- Find *all possible* refolding-schemata for trans-activation
- Assign energies to pseudo-knot transition states
- Compute
 - local minima, energy-barriers \Rightarrow [barriers](#)
 - folding dynamics by local minima \Rightarrow [treekin](#)

Overview

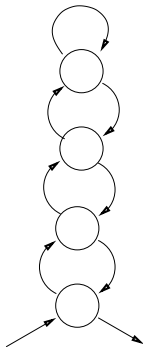
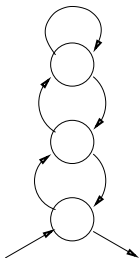






.....(((((((.....))))))))).....

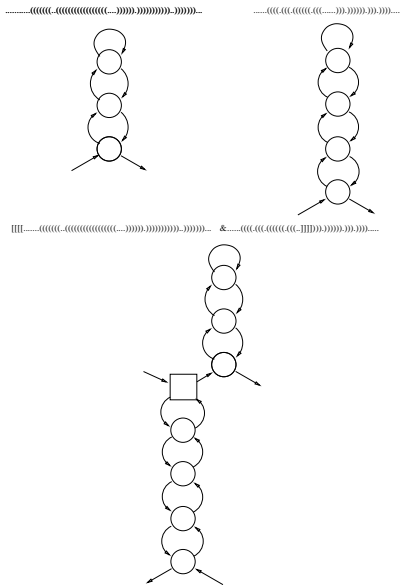
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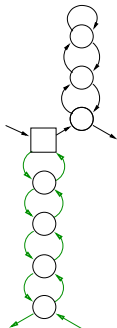
$$\text{ARC-ARRAY} = [\text{PrevVert}, \text{SEQ}, \text{NextVert}]$$

$$\text{VERT-ARRAY} = \begin{bmatrix} \text{PrevArc}, \text{SEQ}, \text{NextArc}, (\text{KG}) \\ \text{PrevArc}, \text{SEQ}, \text{NextArc}, (\text{KG}) \end{bmatrix}$$

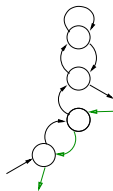
RNArefold



[[[[.....((((((-((((((((((((((((.....))))))))).....))]]]]]]..... &.....((((((-((((((((((((((((.....))))))))).....))]]]]]].....



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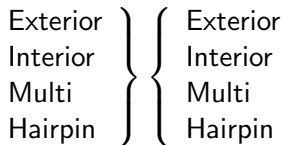
- Input:
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- Generate all Neighbours +/- one Vertex
- Evaluate the Structures (discard waste)
- Repeat Neighbourgeneration **while** ...
- Output:
 - Refolding-Scheme(s) $St1 \Rightarrow St2$
 - Energy-Barrier separating the states
 - ...

- Forbid intramolecular pseudo knots

- Sum up Secondary Structures

$$(((\dots[[[([\dots)])])&..]]]).. = (((\dots\dots\dots)))&\dots\dots + \dots\dots[[[([\dots&..]]])]$$

- Assign Penalties for Loop-Interaction-Type

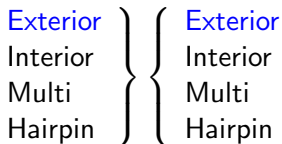


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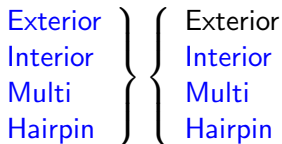


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- Assign Penalties for Loop-Interaction-Type



- HP size 7
 - no bases paired
 - 4 bases paired (loss of entropy)
- ⇒ treat as a worm-like-chain
- ⇒ compute persistence length
- ⇒ compute radius of gyration [RG]
- ⇒ assign penalty in relation to the change of RG.

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 - refolds two interacting sequences
 - can handle all types of intermolecular pk structures

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Thank you for your attention!