Computational approaches to RNA Folding Kinetics

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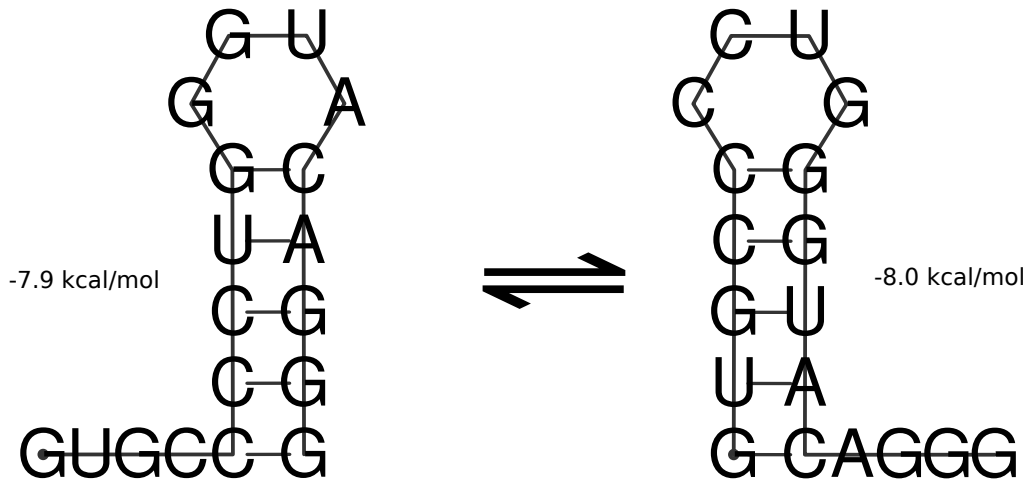
Thermodynamic vs. Kinetic Folding

Equilibrium properties for RNA secondary strutcures can be calculated efficiently

But what about dynamics?

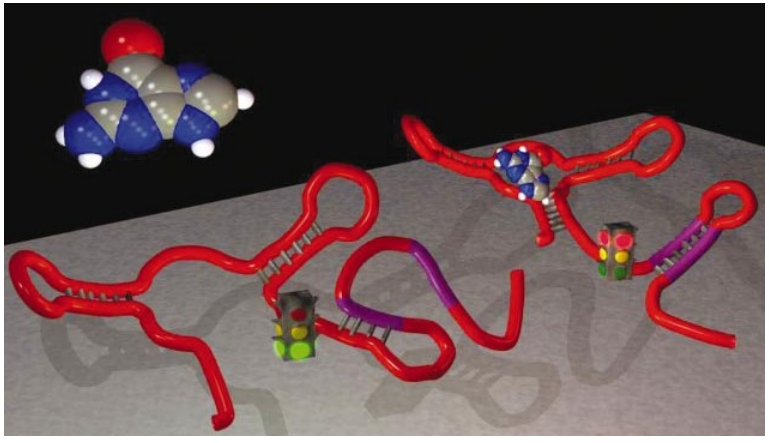
- On what time scale is equilibrium reached?
- How fast/slow is re-folding between dissimilar structures?
- What structures are populated initially?

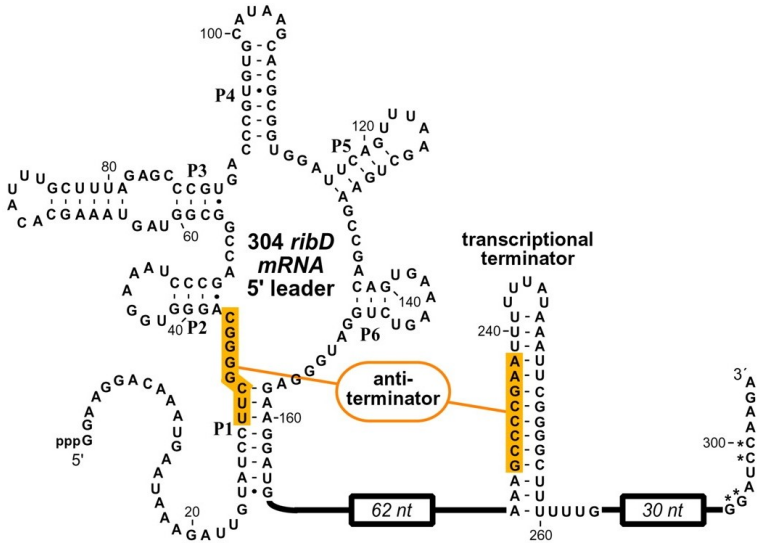


Structural changes are common in functional RNA

RNA switches toggle between active and inactive states by changing conformation.
Used especially to control mRNA translations; triggered by:

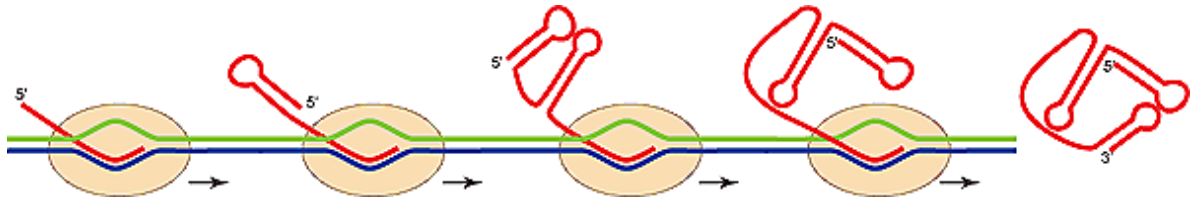
- binding of proteins or small ligands
- chemical modification, e.g. tRNA
- temperature dependent switches
- timed mRNA switches, e.g. HOK





Folding during Transcription

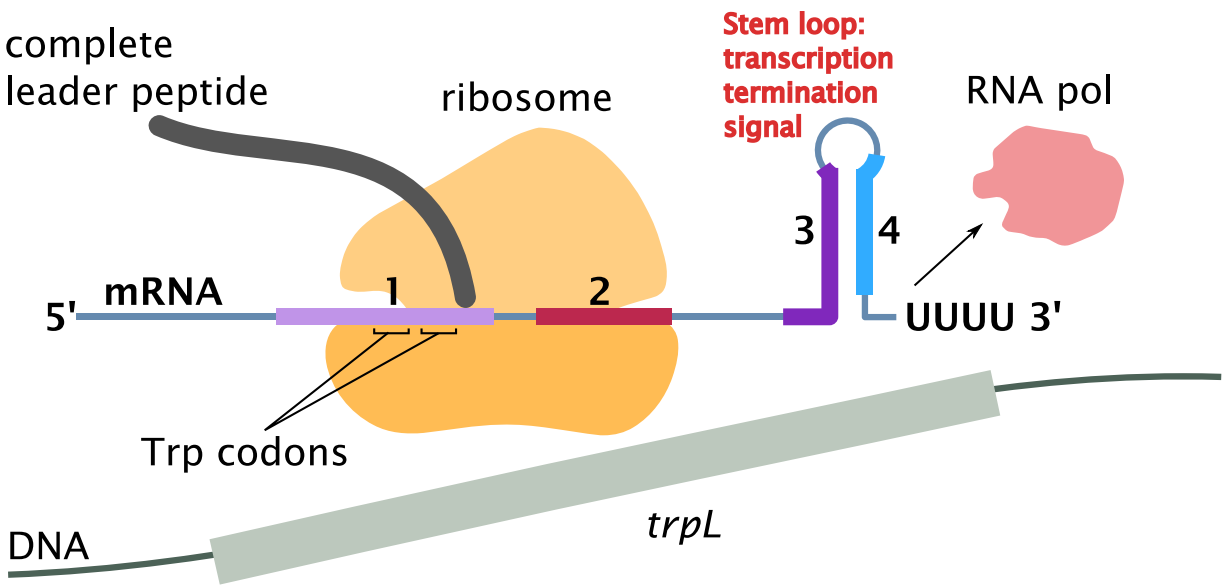
Almost all RNA structures may be affected by co-transcriptional folding:



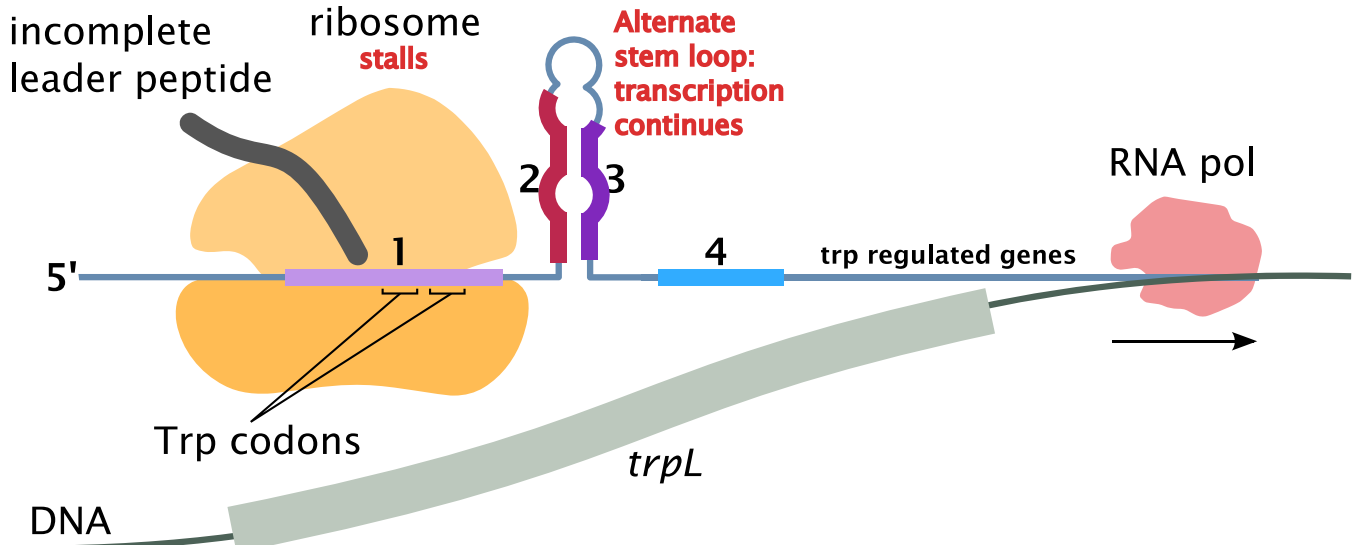
- RNA is transcribed at a rate of only 25–50 nucleotides per second
- The nascent chain starts folding as soon as its leaves the ribosome
- Stems formed by the incomplete chain may be too stable to refold later on
- Co-transcriptional folding may drive the folding process to a well-defined folded state (possibly different from the MFE)
- An energy barrier of 5kcal/mol is sufficient to prevent refolding during extension

Regulation of the Trp Operon

High level of tryptophan

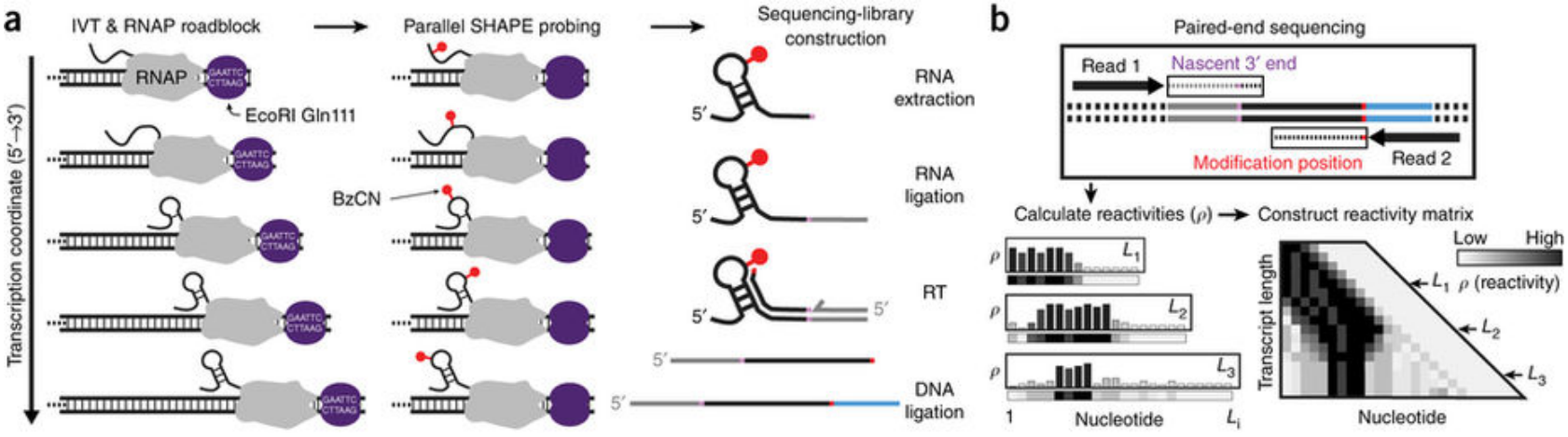


Low level of tryptophan



Co-Transcriptional Structure Probing

Co-transcriptional is becoming experimentally accesssible



Watters et al, Nat. Struct. Biol. 2016

Folding Dynamics as Markov Process

Let's compute prob. $P_x(t)$ of observing structure x at time t. Given transition rates k_{xy}, this gives rise to a *Markov process* with master equation

$$\frac{dP_x(t)}{dt} = \sum_{y \neq x} [P_y(t) k_{xy} - P_x(t) k_{yx}].$$

or in matrix form, with $k_{xx} = -\sum_{x \neq y} k_{yx}$:

$$\frac{d}{dt} P(t) = \mathbf{K} P(t).$$

A *formal* solution can be written simply

$$P(t) = e^{t \cdot \mathbf{K}} P(0)$$

Way too many states to solve directly (10^{17} for a tRNA)

Three Strategies for Predicting Folding Kinetics

- Folding trajectories via Monte-Carlo simulation
  - Time-consuming
  - Need statistics over many trajectories
  - Non-trivial to analyze and interpret
  - `kinfold`, `KineFold`
- Coarse grained dynamics via Barriers / Treekin / Barmap
  - Identify local minima, assign macro-states
  - Energy barriers and transition rates (`barriers`)
  - Solve $P_x(t)$ on coarse grained landscape (`treeekin`)
  - Extend sequence and transfer population to next landscape (`barmap`)
- Heuristic landscape construction
  - Model landscape by small set of representative structures
  - Estimate energy barriers and rates
  - Can be nicely combined with co-transcriptional folding `DrTransformer`

Folding Dynamics as Markov Process

But, for a tRNA the dimension of K is about $10^{17} \times 10^{17}$
The formal solution is therefore of limited use.
We can:

- Solve toy models by integration of the master equation
- Perform stochastic folding simulations.
  Needs many trajectories.
- Reduce the number of conformations by coarse graining
  i.e. lump structures together into *macro states*
- Just try to compute a single best folding pathway.

Stochastic Simulations

Simulate folding kinetics by Gillespie (rejectionless Monte Carlo) algorithm :

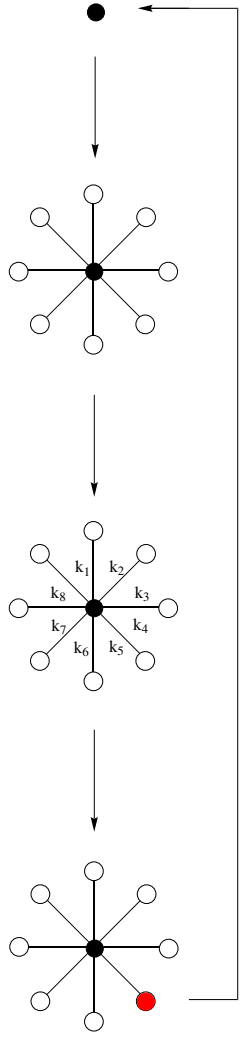
Generate all neighbors using a move-set
Close base pair – Open base pair

Assign rates to each move, e.g.:

$$k_i = \Gamma \cdot \min\left\{1, \exp\left(-\frac{\Delta E}{kT}\right)\right\}$$

Select a move i with probability $\propto k_i$

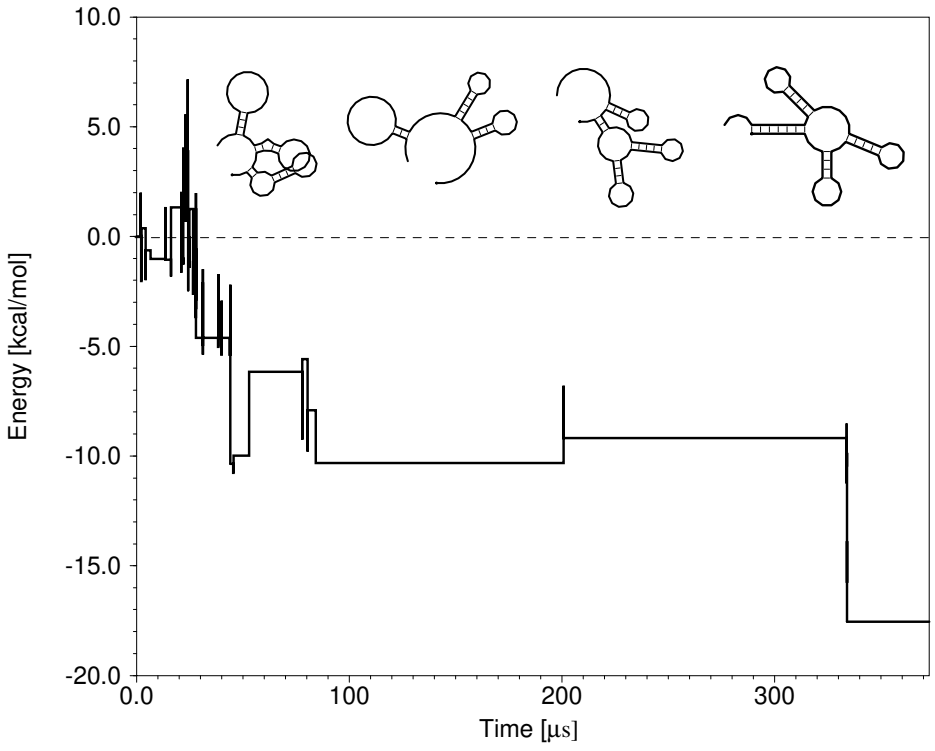
Advance clock by $1/\sum_i k_i$ (on average).



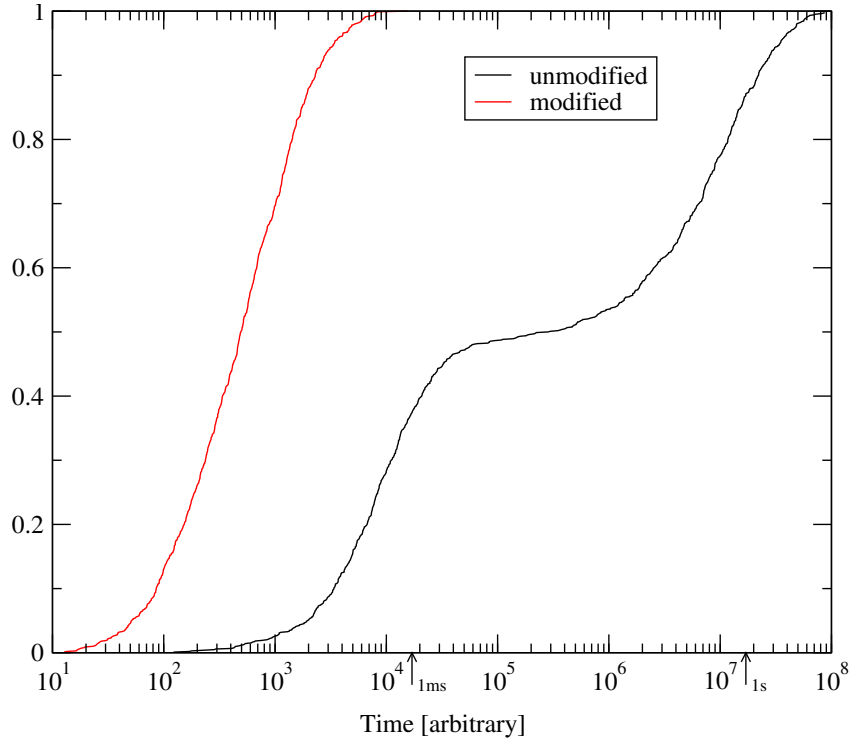
- computationally somewhat expensive
- need to analyze many trajectories
- easy to include co-transcriptional folding

Simulated folding of $tRNA^{phe}$

Many trajectories have to be collected in order to do statistics.



energy profile of a single trajectory

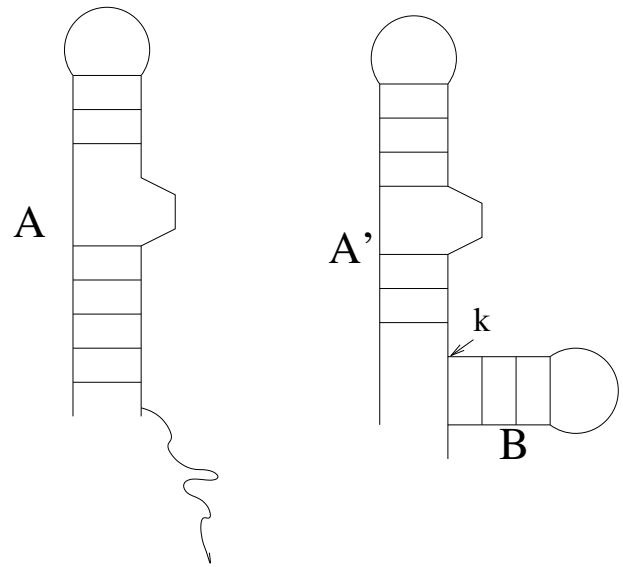


distribution of first passage times

Folding Simulation using Isambert's `Kinefold`

- Use opening/closing of entire helices as move set
- Allows pseudo-knots,
- Suitable for RNAs up to several hundred nt.

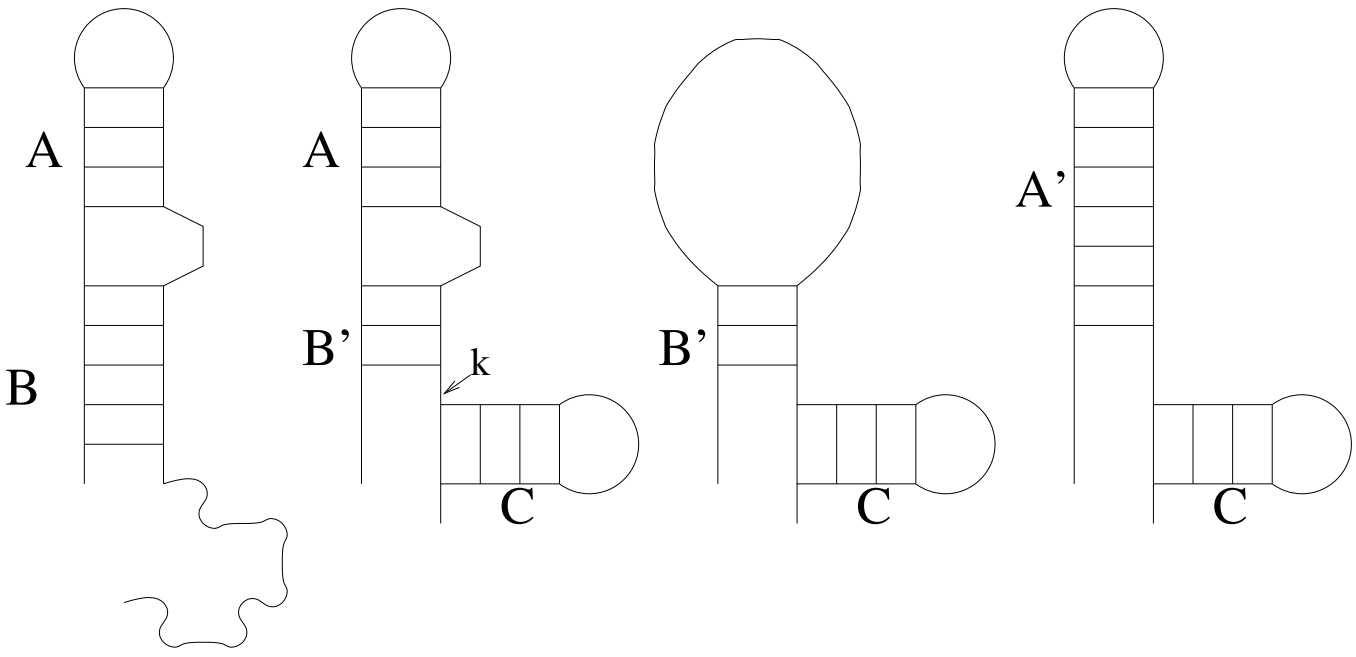
Helix moves require a local conflict resolution after each step



Web service available at `http://kinefold.curie.fr`

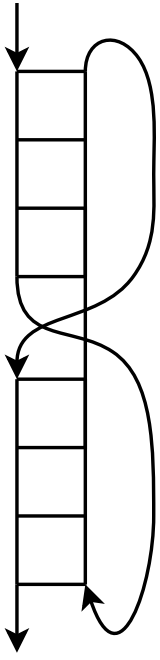
Potential problems with Conflict Resolution

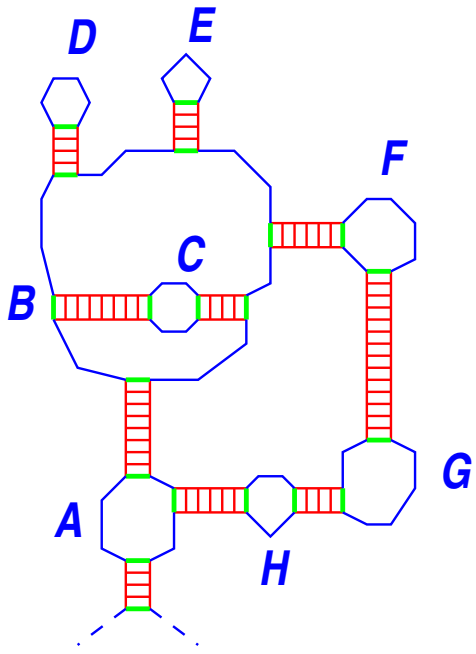
Maintaining detailed balance with helix moves is non-trivial:

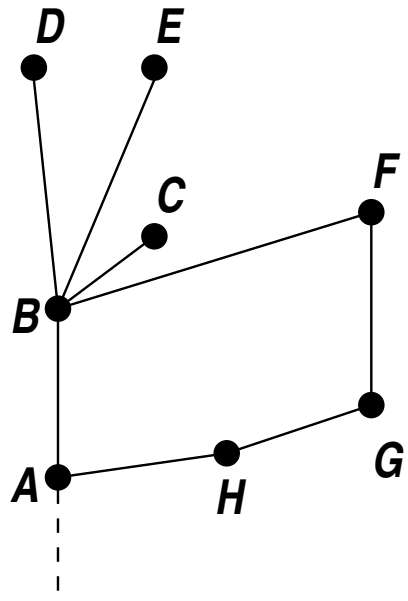


Pseudo-knots

- Pseudo-knots do not pose a problem for folding simulations.
- Still requires accurate pseudo-knot energies







- Frequently only H-type knots are considered.
- Kinefold allows complex pseudo-knots whose entropy is approximated by a cross-linked "Gaussian gel"

Kinetic Rate Models

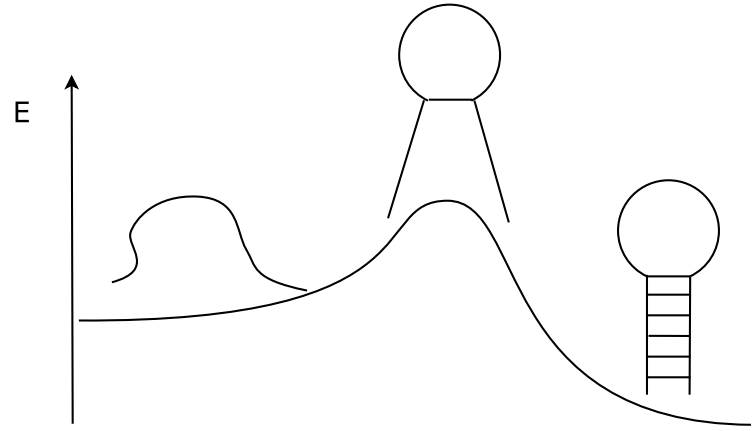
The simplest rate model satisfying detailed balance is the Metropolis rule

$$k_{xy} = \Gamma \cdot \max\left(1,\ e^{(\Delta G(x) - \Delta G(y))/RT}\right)$$

More accurate models define a transition state with free energy $\Delta G^{\dagger}$ and Arrhenius rates:

$$k_{xy} = \Gamma \exp\left(-(\Delta G^{\dagger}_{xy} - \Delta G(x))/RT\right)$$

This is essential for large moves (e.g. helix moves).



Abstract Definition of Landscapes

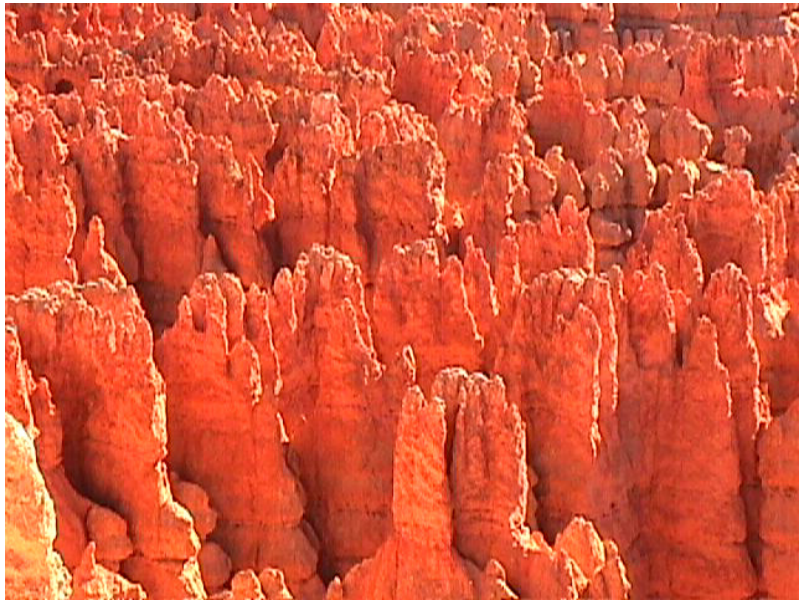
A **landscape** is a triple $(V, \mathcal{X}, f)$ where

- V is a set of *configurations*.
  E.g.: RNA sequences, tours of a travelling salesman, spin configurations,

  secondary structures of given RNA molecule;
- f is a *cost* or *fitness function* $f : V \to \mathbb{R}$;
- $\mathcal{X}$ is a way of defining "nearness", "closeness", "dissimilarity", or "accessibility" among the configurations.

  E.g. an *adjacency relation* (thus a graph), *transition matrix* (defining a Markov chain), or a *(pre)topology* on V.

Ruggedness



Rugged: Bryce Canyon UT

Smooth: Capulin Volcano NM

Measures of Ruggedness:

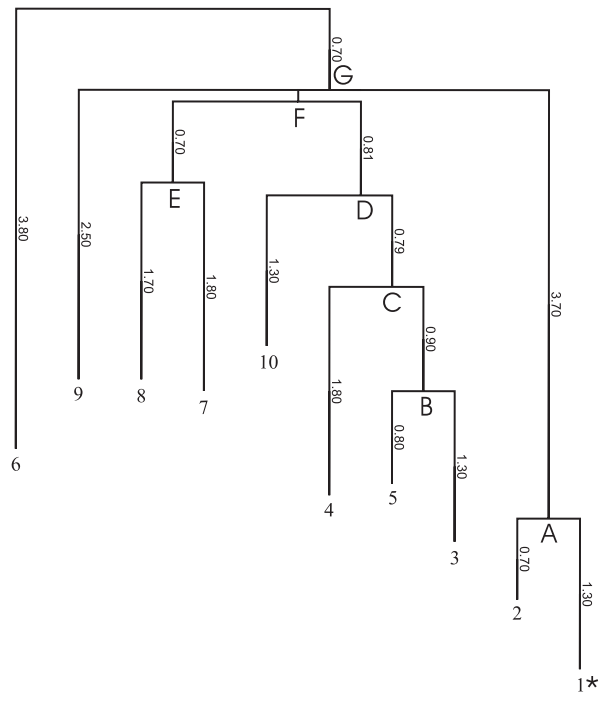
- Number of Local Minima and Maxima
- Correlation length
- Basin sizes

- Length of Adaptive Walks

RNA Landscape Analysis

Barrier trees

- Contains all local minima as leafs
- Barrier heights and saddles between minima
- Groups structures into *macro states*
- Transition rates between macro states $\rightarrow$ coarse grained dynamics
- Time and space proportional to the size of the landscape
  Limited to RNA < 100nt
- Sampling based heuristics for longer RNAs



Flamm et al, Z. Phys. Chem. (2002), Kucharík et al, Bioinformatics (2014, 2016)

Calculating barrier trees

The flooding algorithm:

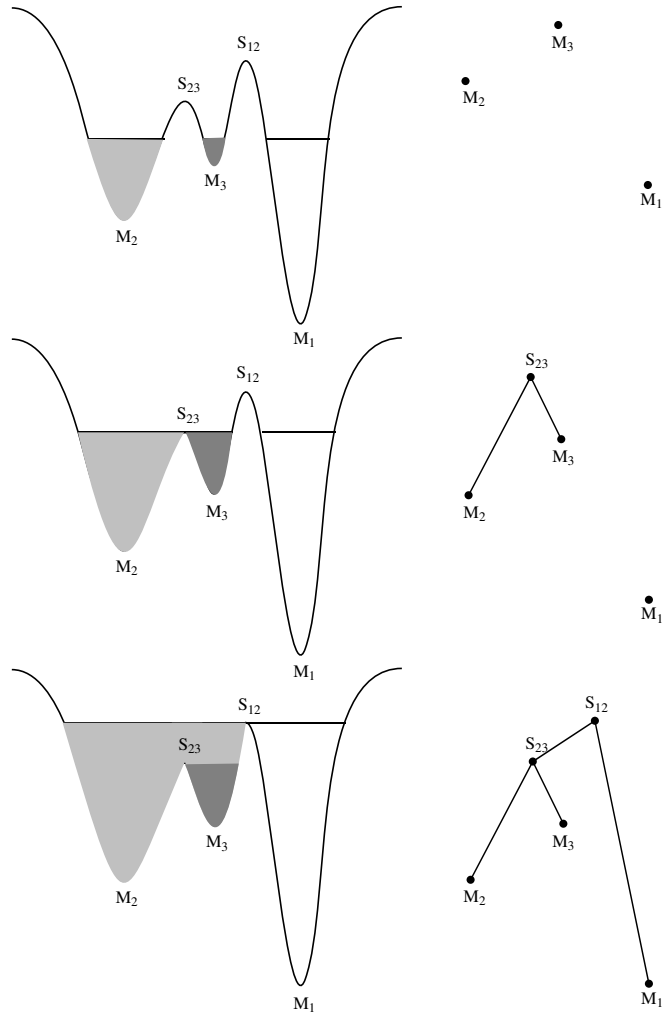
Read conformations in energy sorted order.

For each confirmation x we have three cases:

(a) x is a *local minimum* if it has no neighbors we've already seen

(b) x belongs to basin $B(s)$, if all known neighbors belong to $B(s)$

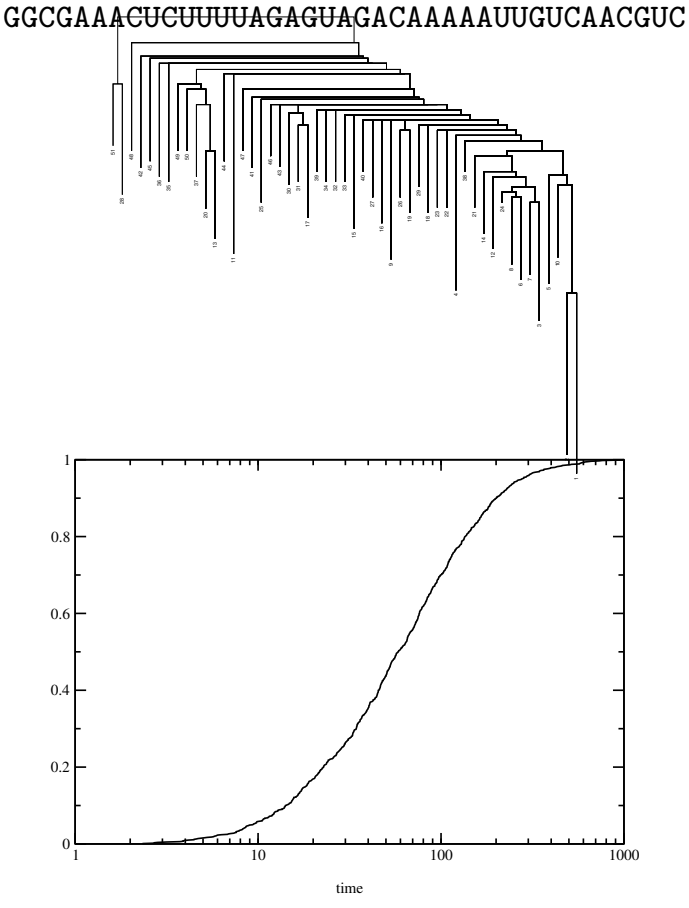
(c) if x has neighbors in several basins $B(s_1) \ldots B(s_k)$ then it's a *saddle point* that *merges* these basins.

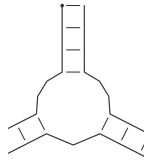


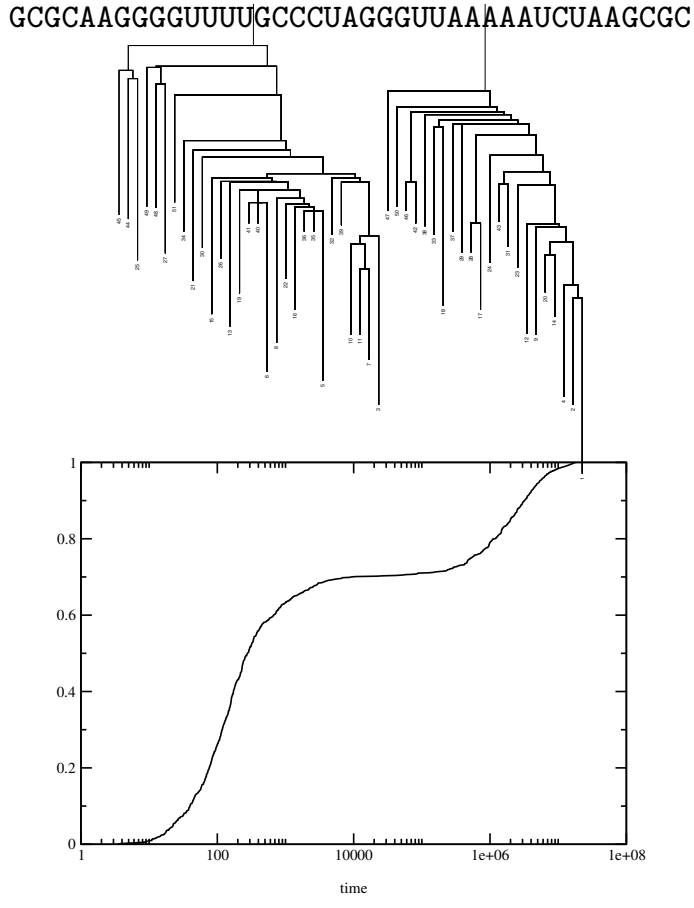
The barriers program

- Computes all local minima
- Barrier heights and saddle points between minima
- Optimal refolding paths between any two minima
- Groups structures into *macro states* connected to each minimum
- Computes effective transition rates between macro states $\rightarrow$ coarse grained dynamics can be computed without simulation
- Time and space $\mathcal{O}(N \cdot n)$ for an RNA of length n with N structures. However, N grows exponentially

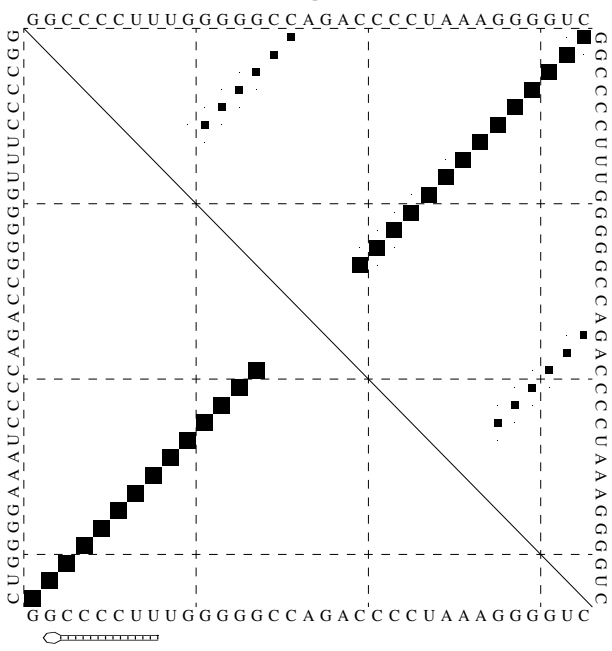
Fast Folder vs. Slow Folder

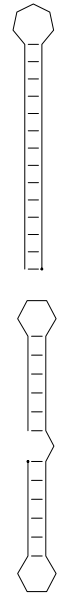






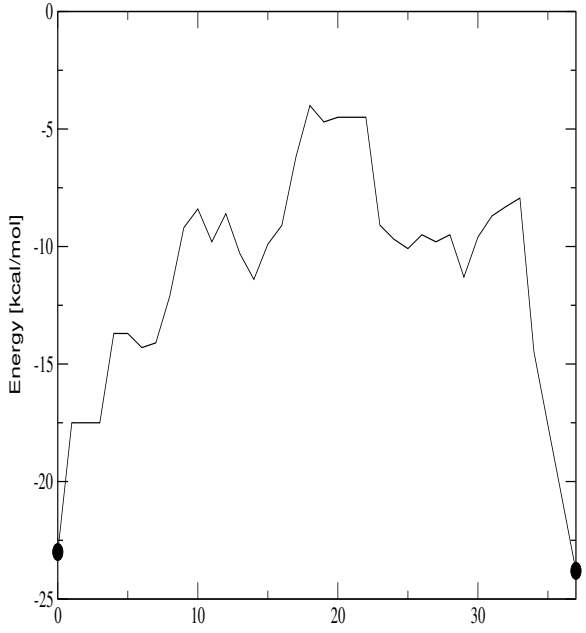
A designed bi-stable Sequence

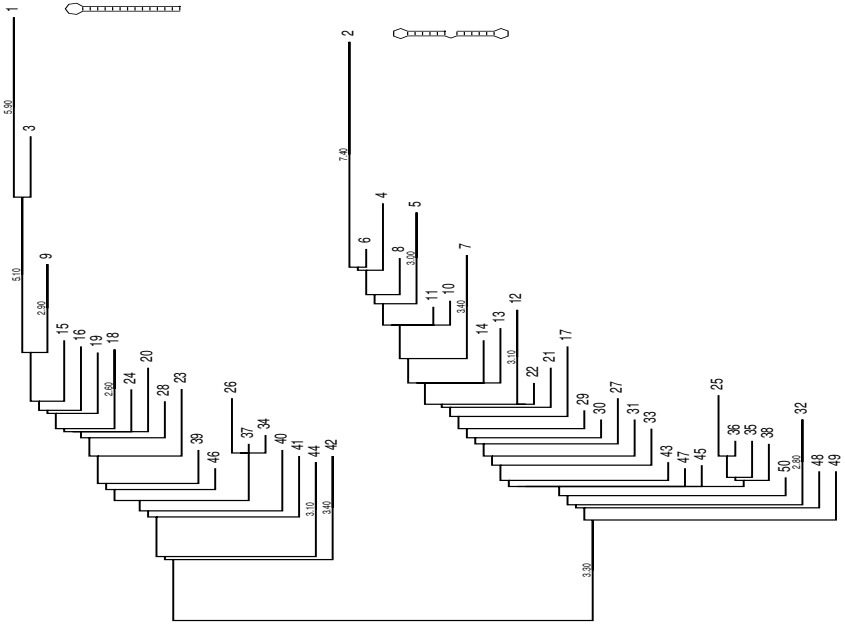




-23.8 kcal

-23.0 kcal





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.(.(((...))))).((((((....))))))  -13.70
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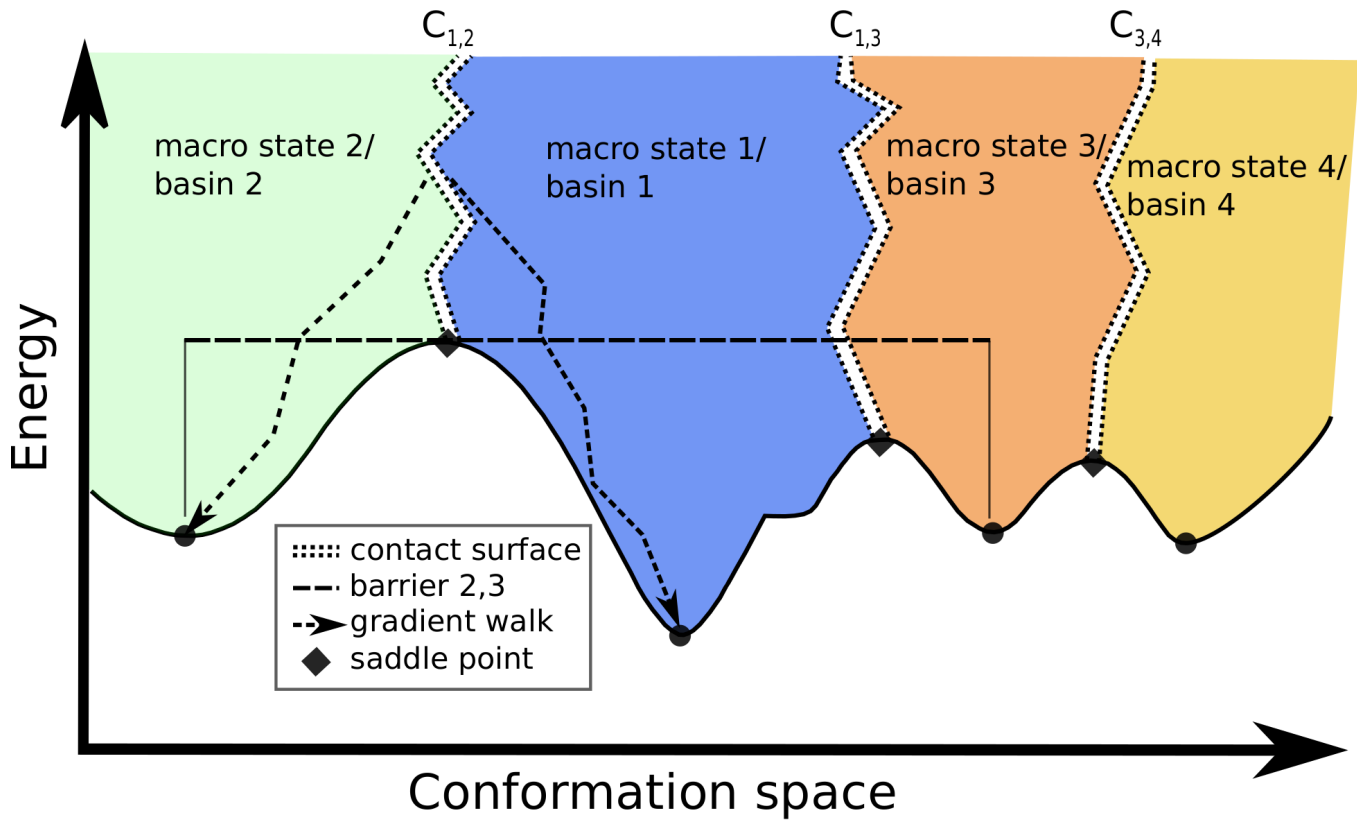
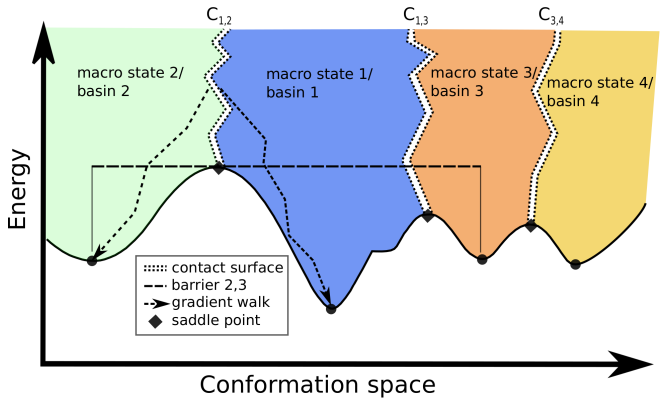
Coarse Graining the Landscape



Coarse Graining the folding dynamics

For a reduced description we need

- macro-states that form a partition of full configuration space
- transition rates between macro states
- macro-states defined via gradient walks



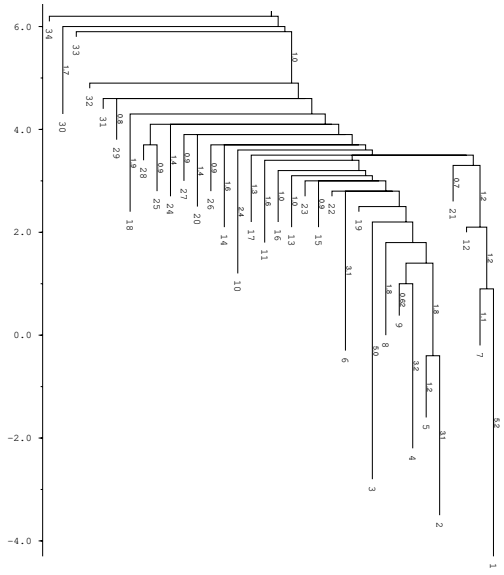
Transition rates could follow an Arrhenius rule
$r_{\beta\alpha} = \exp\left(-(E^*_{\beta\alpha} - G_\alpha)/RT\right)$.

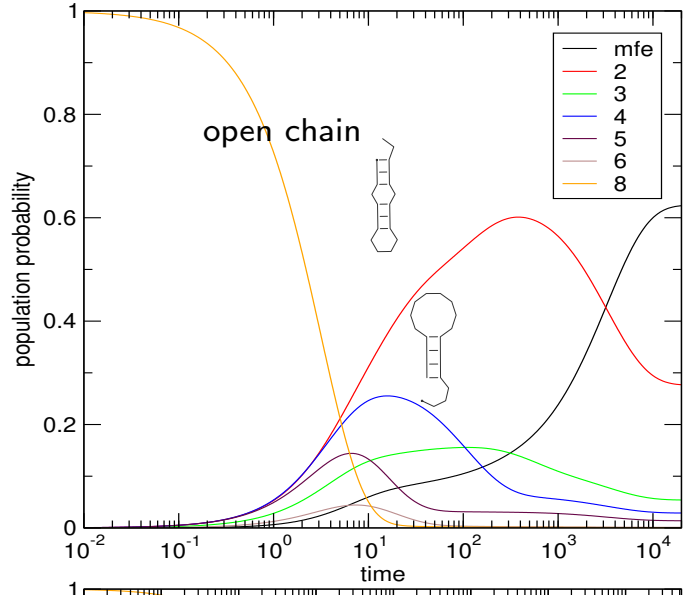
Better: include *all* transition states

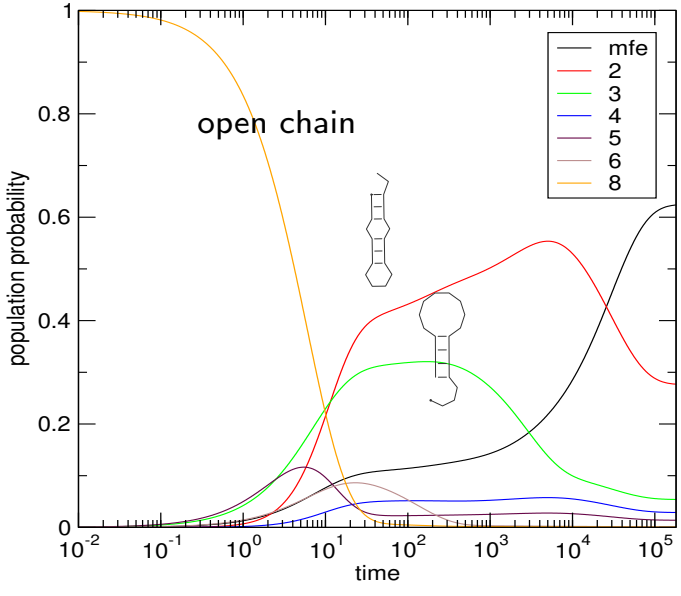
$$r_{\beta\alpha} = \sum_{y\in\beta}\sum_{x\in\alpha} r_{yx}\mathrm{Prob}[x|\alpha] \approx \frac{1}{Z_\alpha}\sum_{y\in\beta}\sum_{x\in\alpha} r_{yx}e^{-E(x)/RT}$$

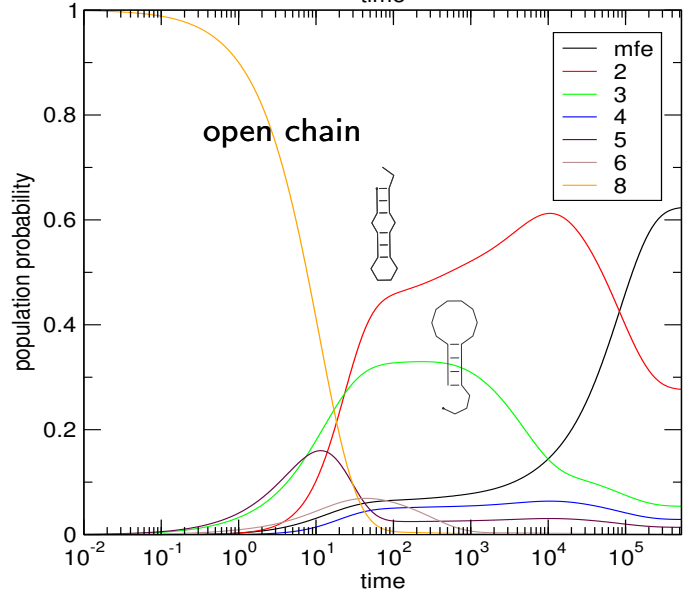
assuming local equilibrium.

Coarse grained dynamics vs. full dynamics





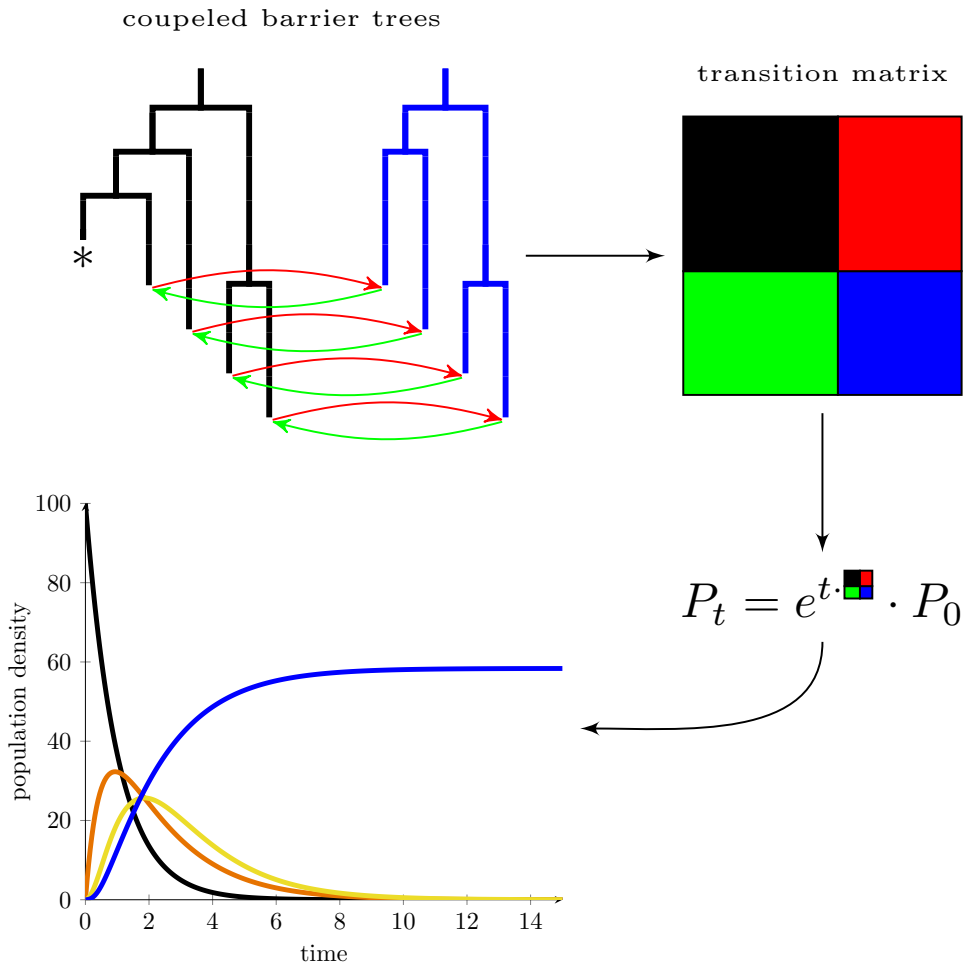




How to include Ligand Binding ?

- Need to know binding motif and binding rates from experiment
- Simple strategy:
  - Add binding energy $\theta = RT \ln \frac{K_d}{c^{\ominus}}$ to every binding competent structure
  - Assumes infinite ligand concentration and infinitely fast binding
- Treat binding / unbinding events explicitly
  - Barrier trees for bound and unbound states
  - Usual rates within bound / unbound structures
  - Concentration dependent rate of complex formation
    $k_{\text{off}} = k_{\text{on}} e^{-\theta/RT}, \quad r = k_{\text{on}} \cdot C$

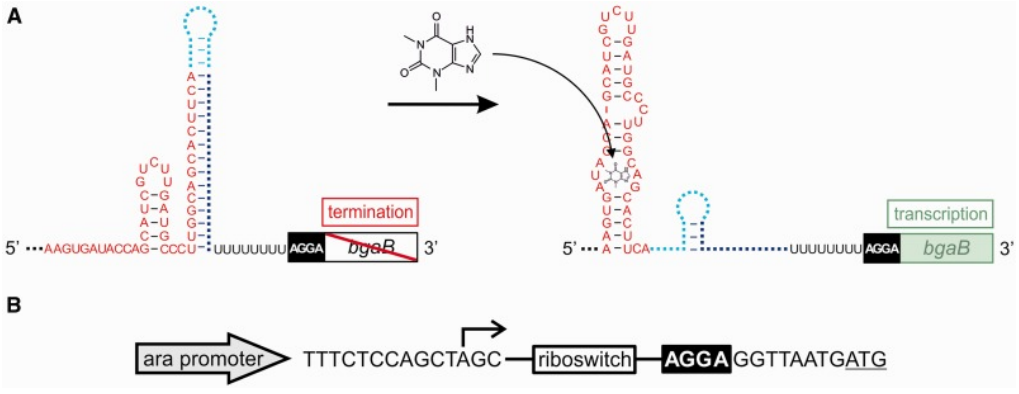
How to include Ligand Binding ?



Kühnl et al, BMC Bioinf. (2017), Wolfinger et al. Methods (2018)

An Artifical Riboswitches

A designed *transcriptional* switch



Wachsmuth et al, NAR (2013)

- Theophylline binding to the aptamer inhibits terminator hairpin
- How to model the effect of the ligand?
- *Co-transcriptional* folding
  Terminator can act only if it is formed fast enough

An Artifical Riboswitches

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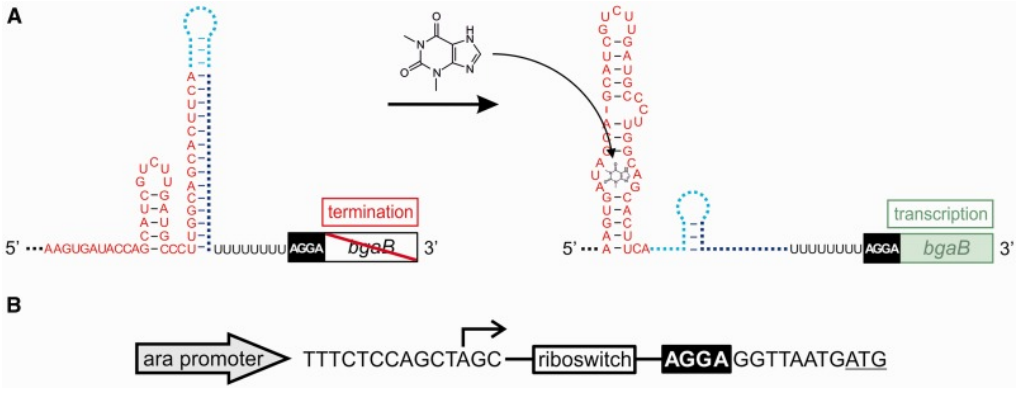

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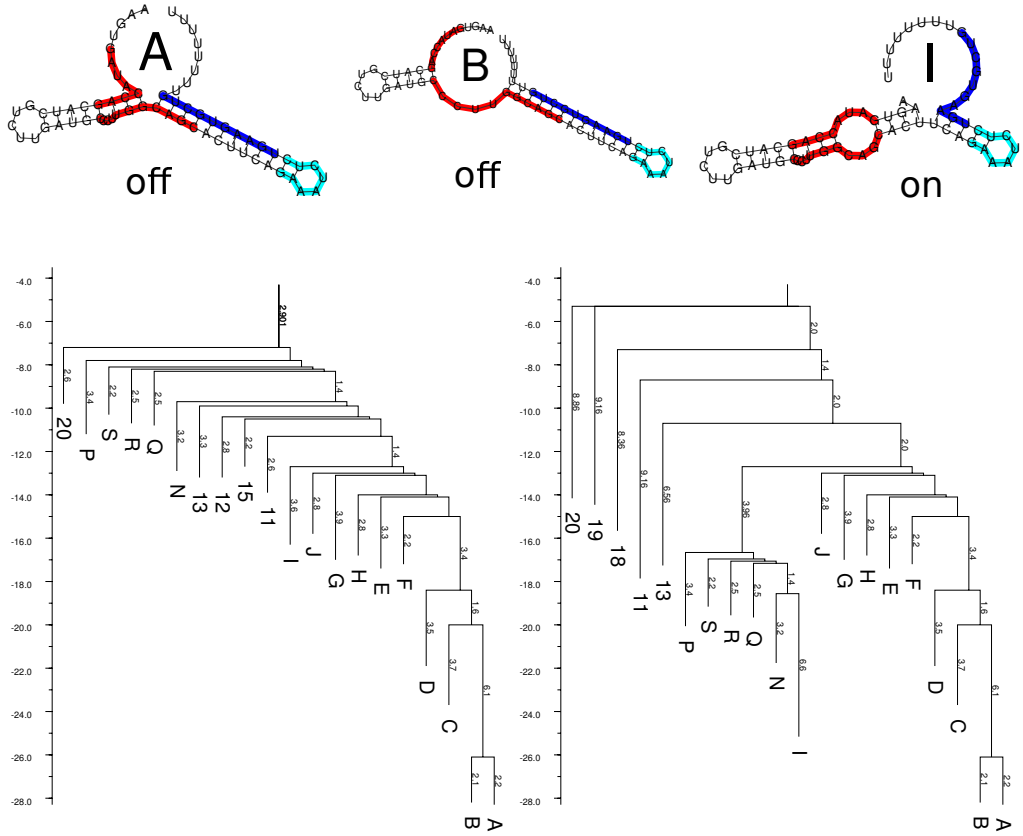
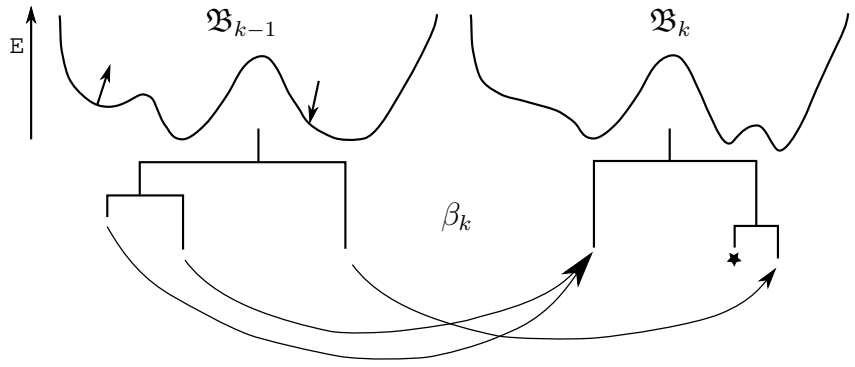
Barrier Tree for RS10 with and without Theophylline



- Binding motif and K_d measurements
- Binding-competent structures are stabilized by about 8.9kcal/mol
- $\Rightarrow$ Distortion of the folding landscape by ligand

Co-transcriptional with BarMap

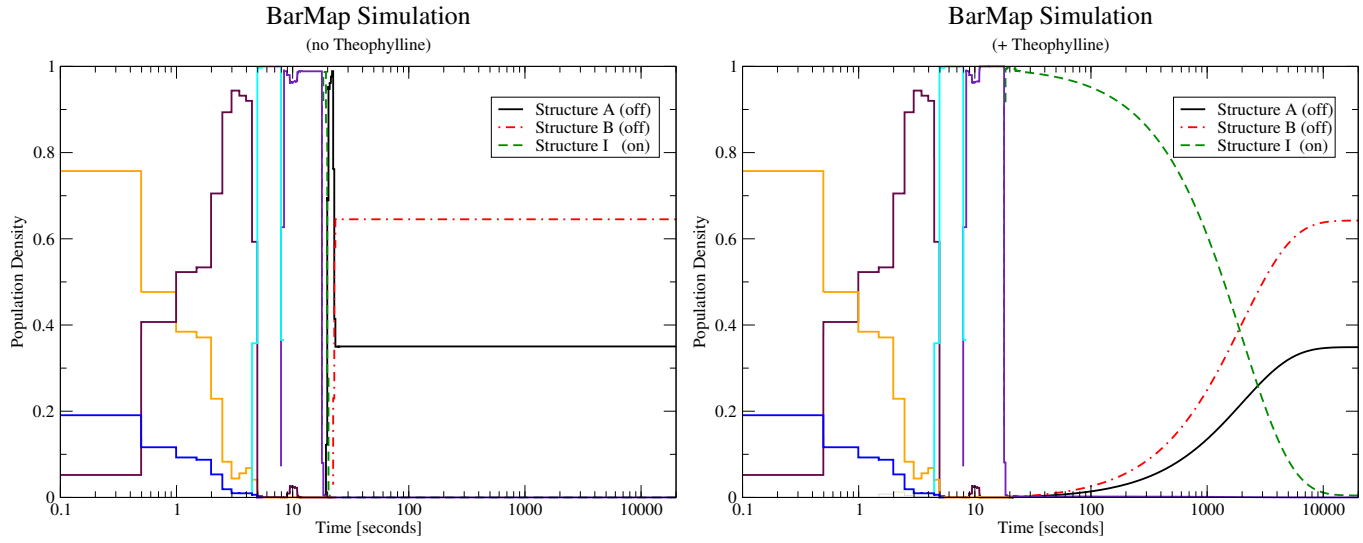
Each extension of the RNA structure modifies the landscape:



- Compute barrier trees for each sequence length $1 \ldots n$
- Compute a mapping between the minima of subsequent landscapes
- Compute dynamics piece-wise:
  - Compute dynamics on landscape for length k
  - Transfer population to landscape of length $k + 1$

Hofacker et al., RNA (2010)

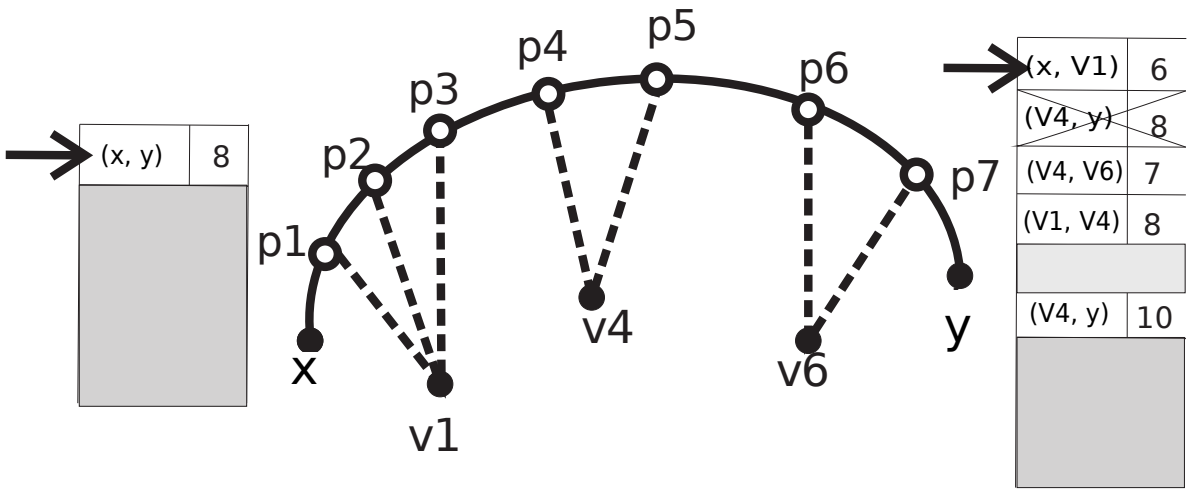
Co-transcriptional of the RS10 Riboswitch



- Without theophylline, the RNA is in equilibrium at the end of transcription
  Terminator is formed, transcription terminates
- With theophylline, almost 100% in state I (on-state)
- Only few of the initial designs show switching behavior

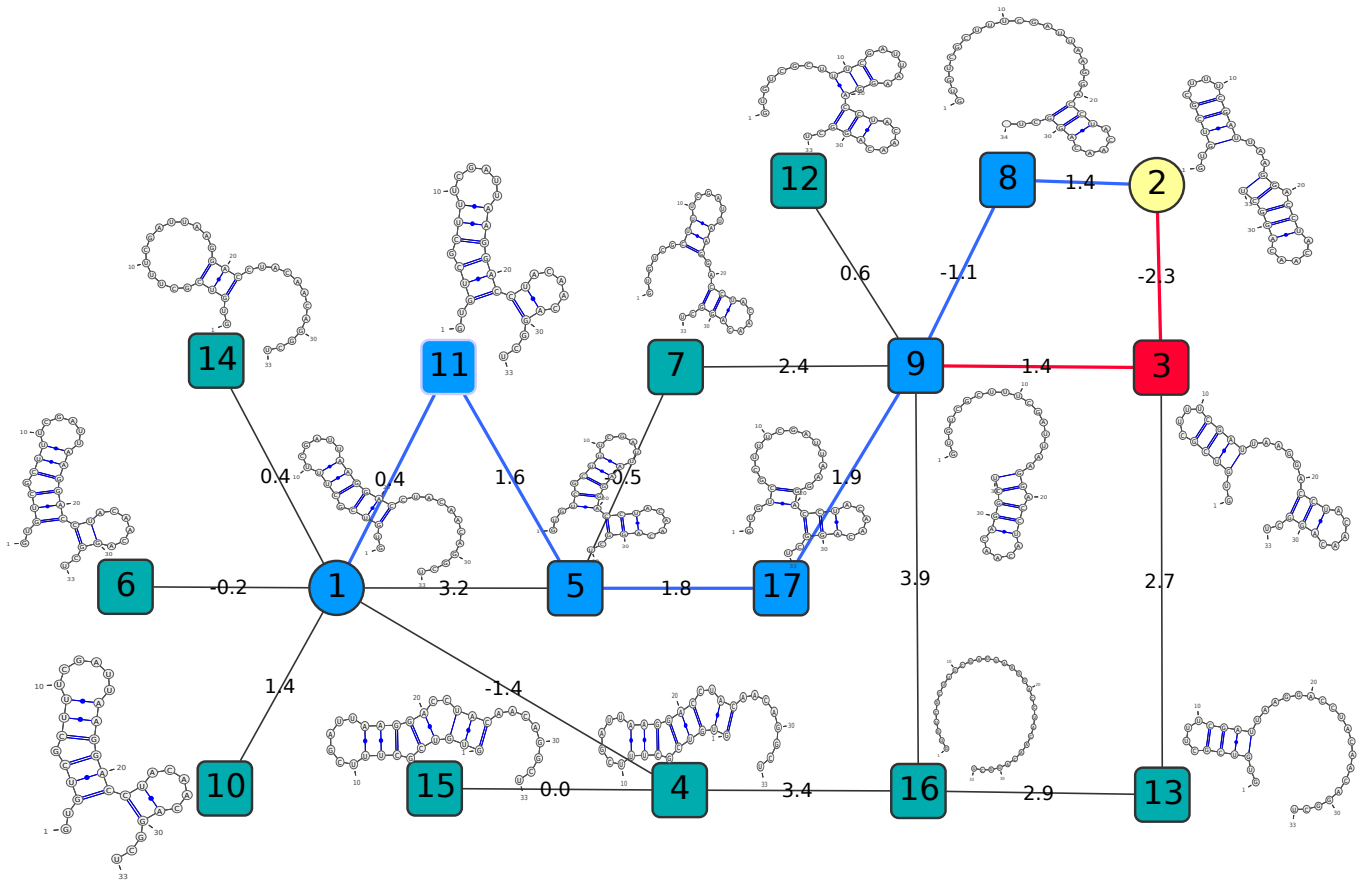
Approximation of Basins and Barriers

- Idea: sample local minima and connect them by **direct paths**
- Sampling:
  - sample secondary structures from a Boltzmann ensemble
  - use adaptive or gradient walk to find the corresponding minimum
- Construct connecting paths recursively: subdivide estimates at intermediate minima



$\Longrightarrow$ Basin hopping graph of the landscape

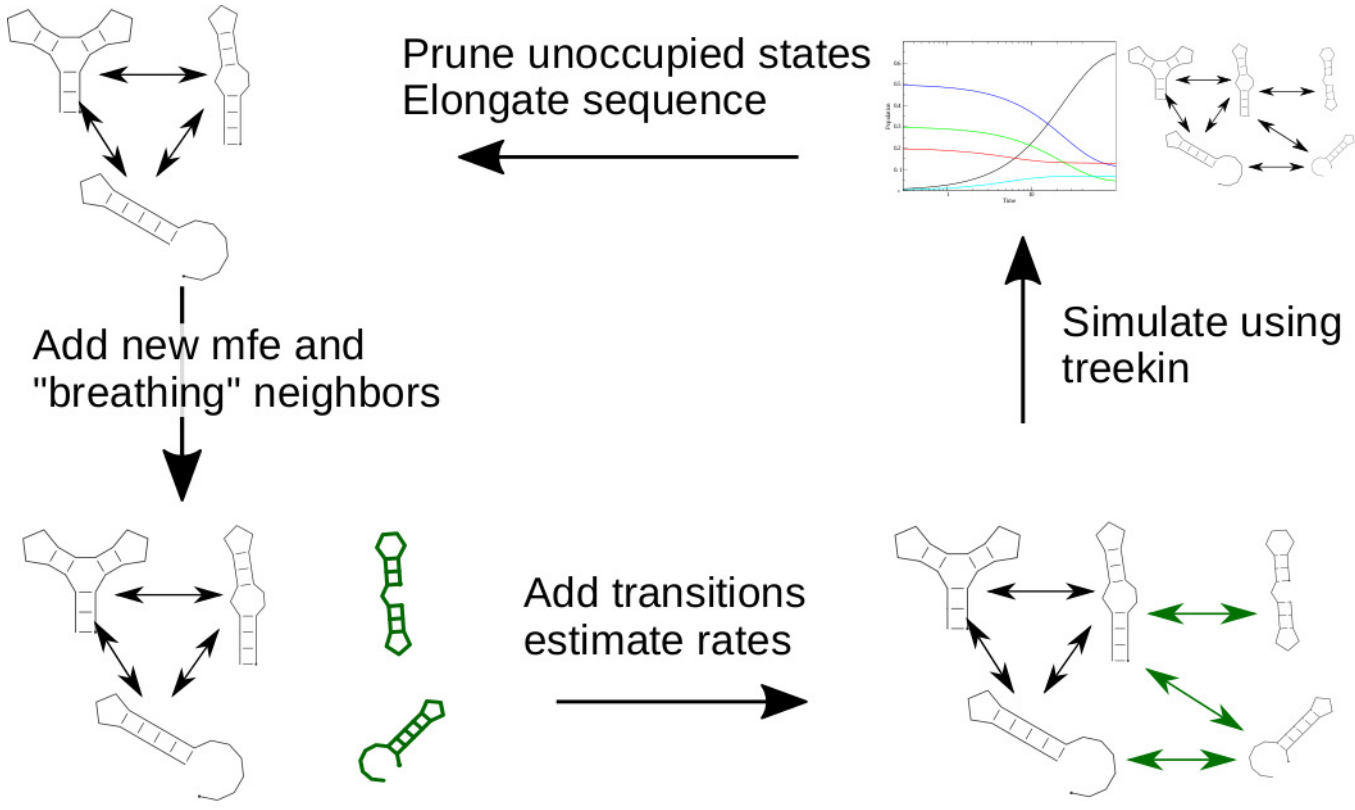
Basin Hopping Graph



Kucharík et al, Bioinformatics (2014, 2016)

DrTransformer: Ultrafast co-transcriptional Folding

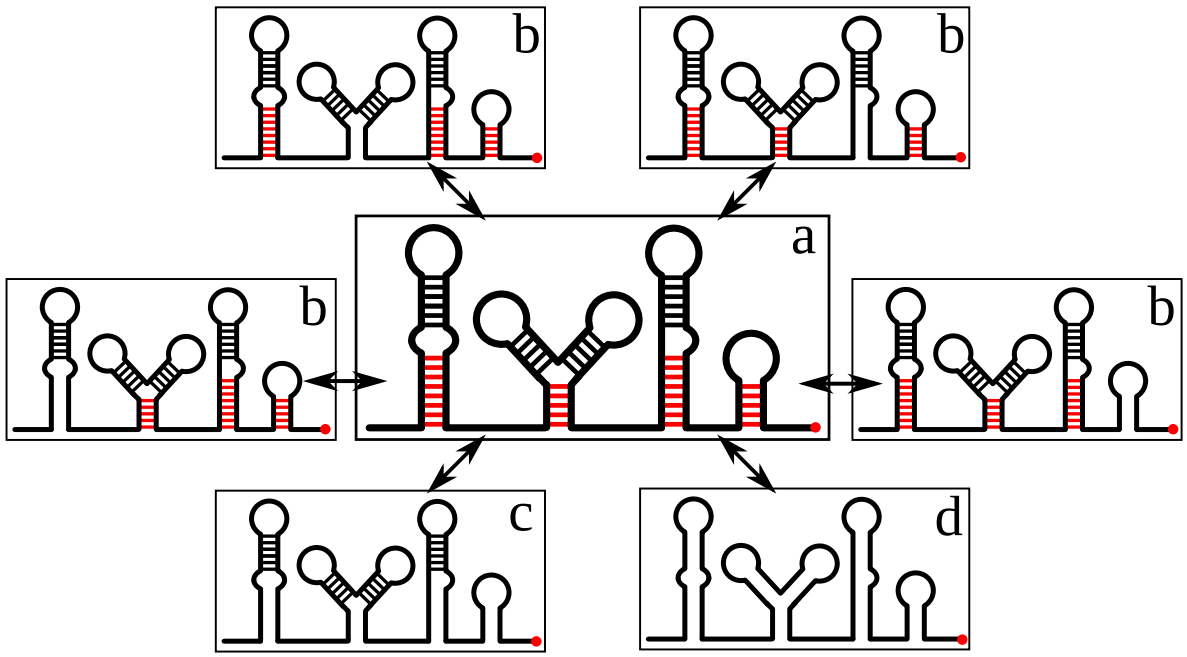
- Simulate a **small** network consisting only of the most relevant structural states
- Evolve network as RNA grows



DrTransformer: "Breathing" neighbors

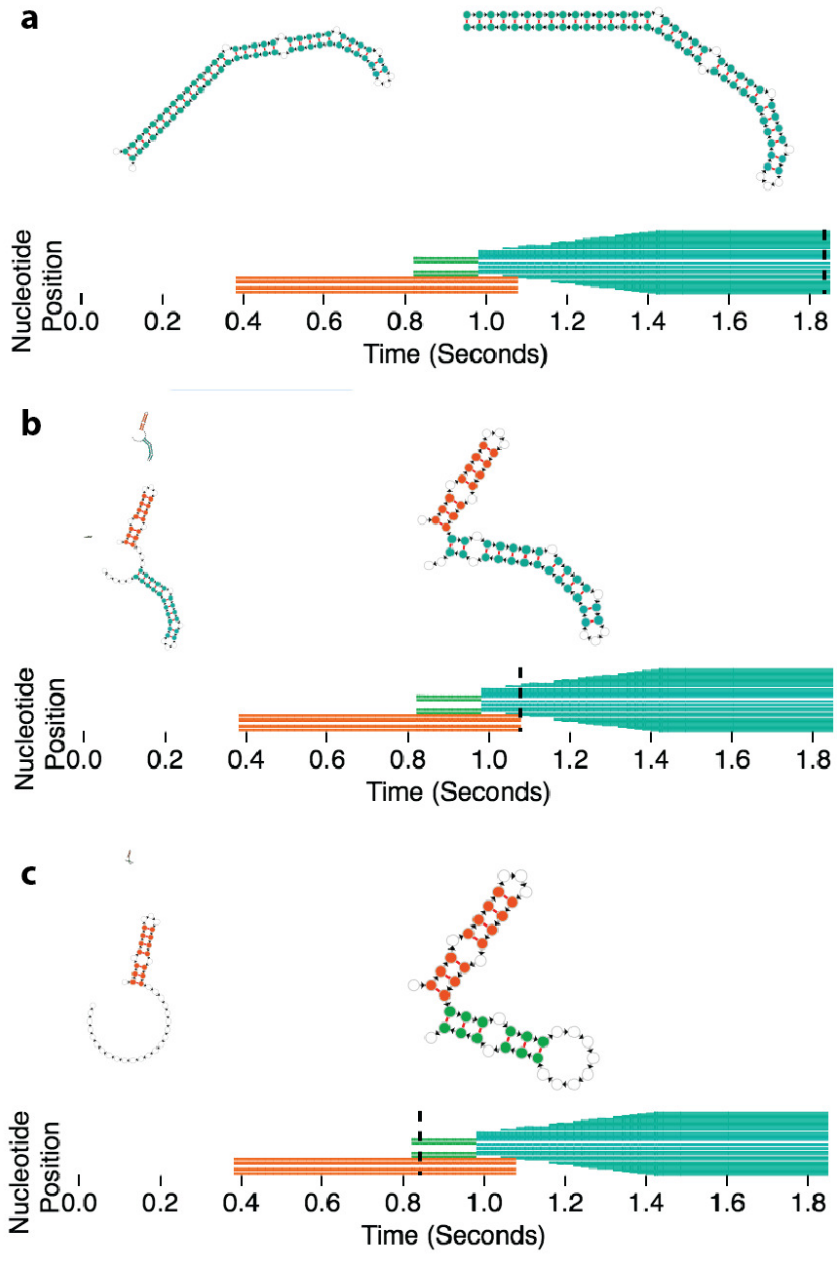
Which new structures should be added after an elongation step?

- Elongation can only effect the surroundings of the exterior loop
- Partially unfold all helices that protrude from exterior loop
- Use constrained folding to re-fold exterior loop surroundings



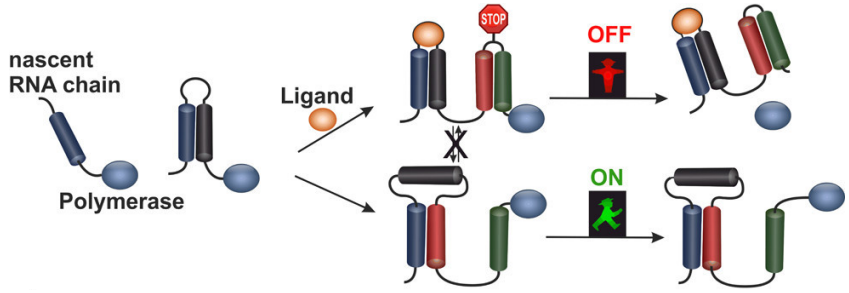
DrTransformer Visualization

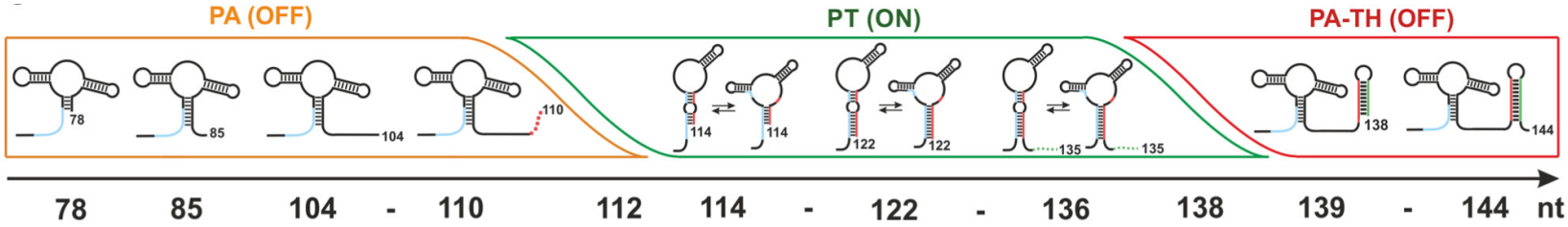
- Simple webinterface
- Interactive visualization Javascript and SVG
- Structure ensemble as function of time



Example: The dG-Riboswitch

- Aptamer for 2'deoxyguanosin
- Binding leads to transcription termination
- NMR analysis (Schwalbe lab): Ground state structure contains terminator even without ligand

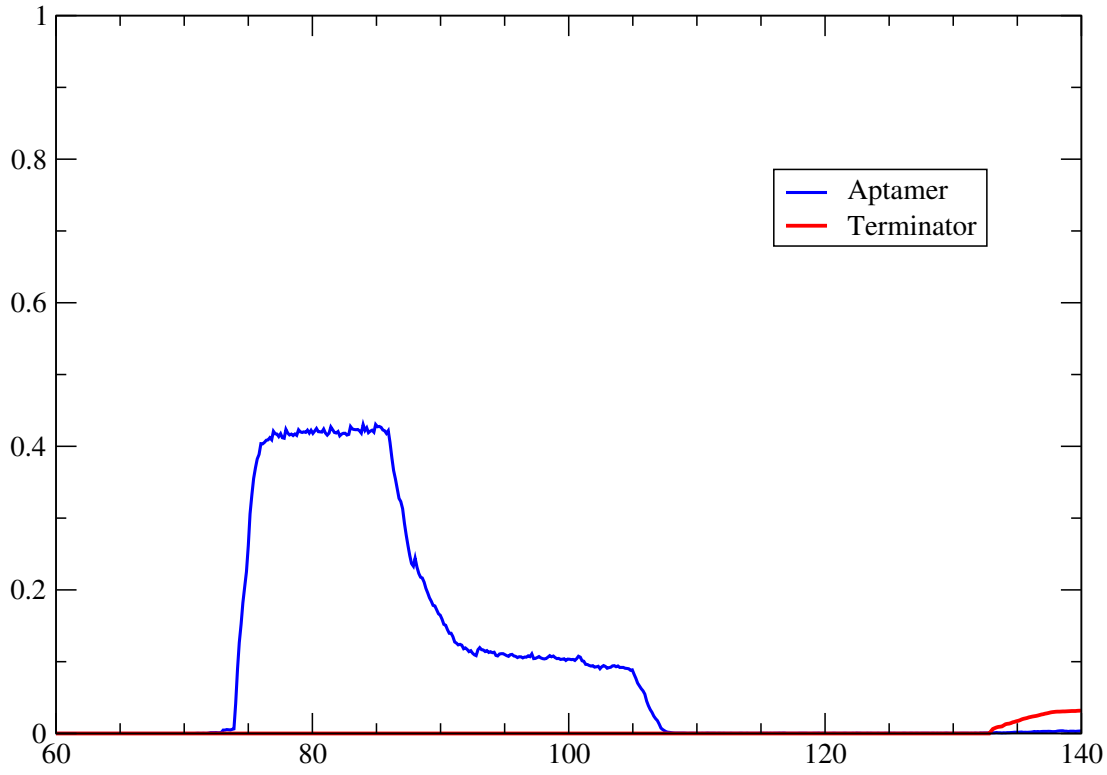




Helmling et al, JACS (2017)

Kinfold simulation of the dG Riboswitch

- 10000 Kinfold trajectories (186 cpu hours)
- Classify each structure as aptamer and/or terminator
- Simulation with ligand: Add a bonus of 8kcal/mol for each binding competent structure



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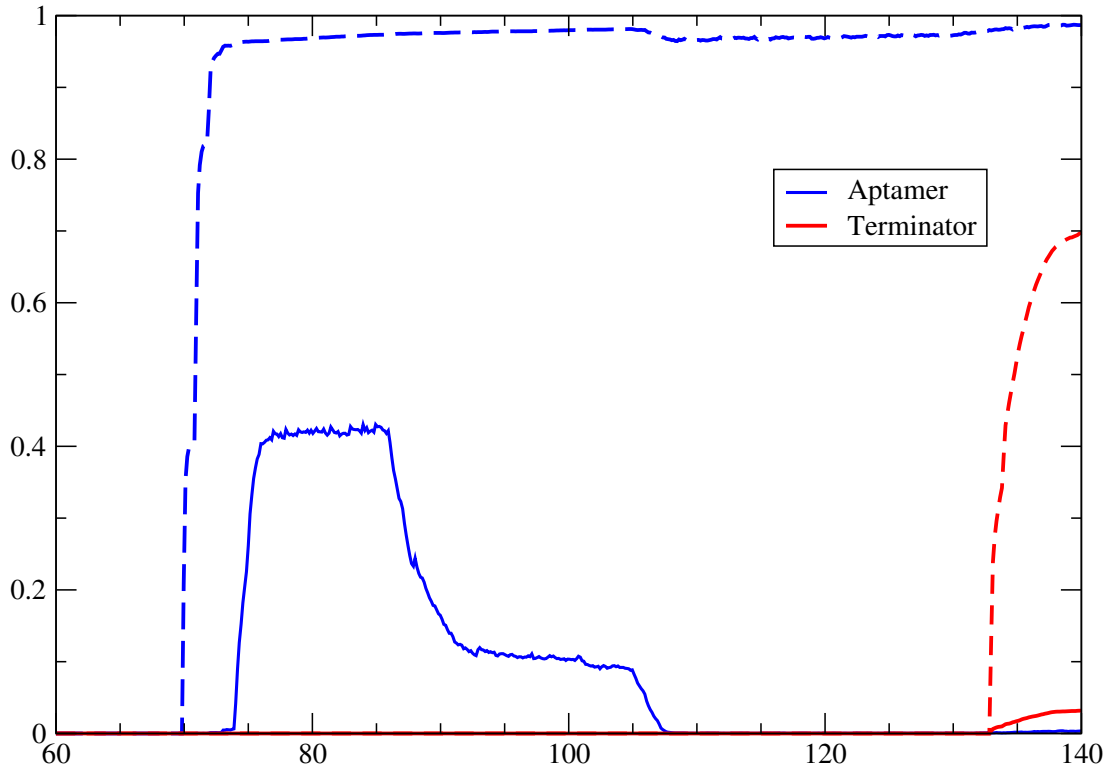
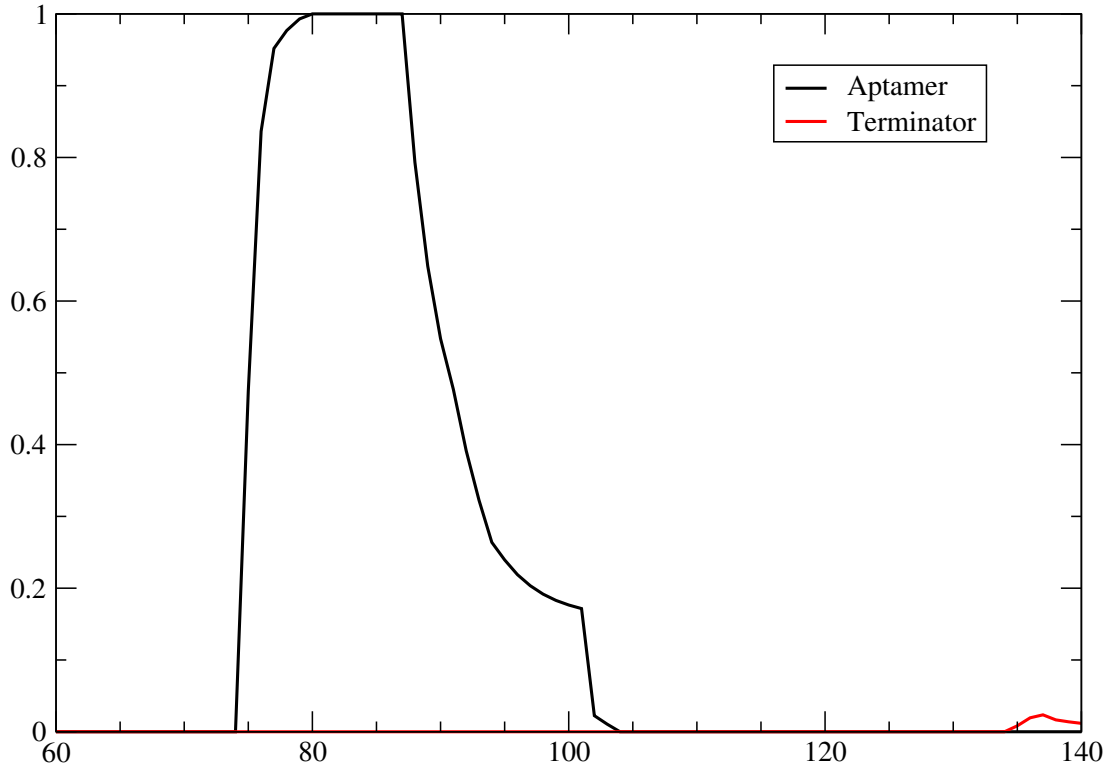
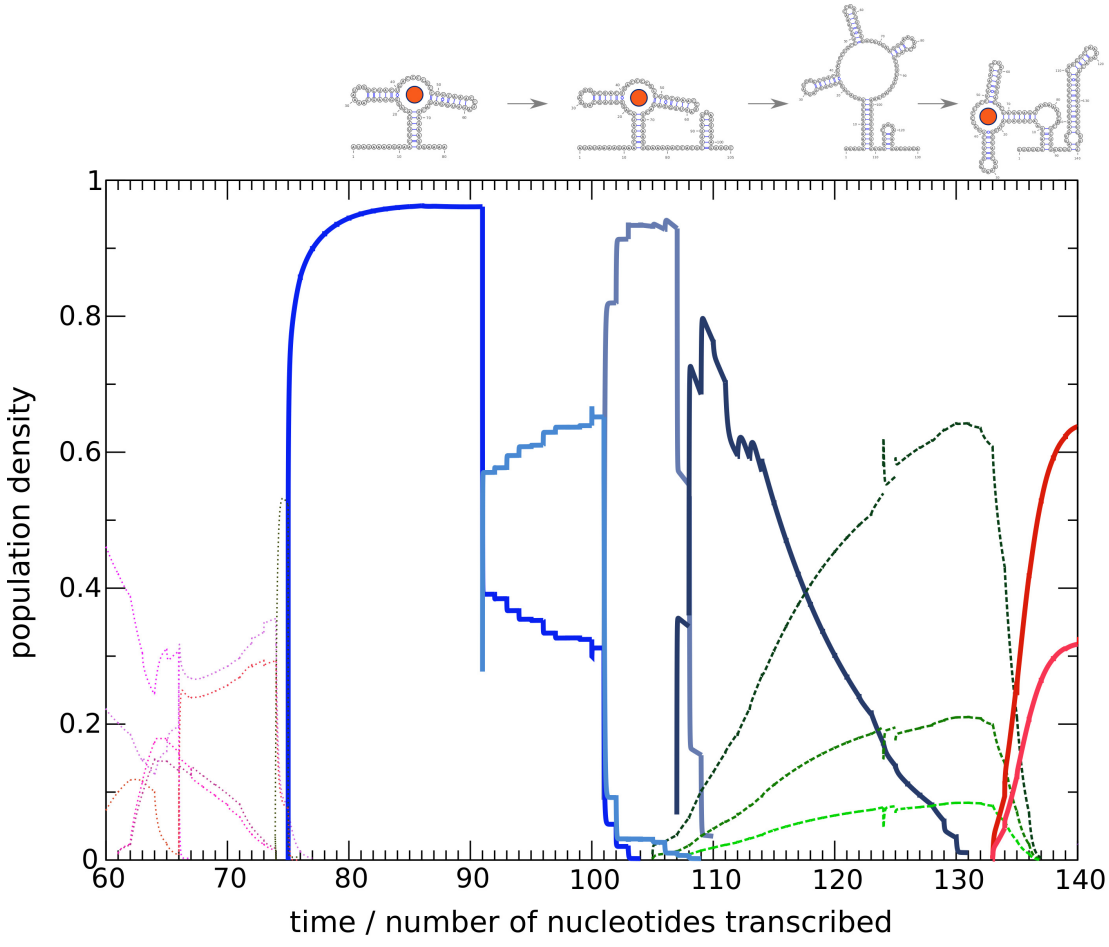


DrTrafo simulation of the dG Riboswitch

- Only 1 run needed (3 cpu sec)
- Classify each structure as aptamer and/or terminator
- Final state 1% population in terminator
- Simulation with ligand not yet possible



BarMap simulation of the dG Riboswitch



Simulation at 25C, transcription speed 25 nt/sec, ligand concentration of 1mM

Take home messages

- RNAs don't always reach their MFE or equilibrium state in reasonable time.
- Co-transcriptional folding essential to regulatory elements such as riboswitches
- Predicting kinetics is much harder than predicting equilibrium
- Previous methods too slow too cumbersome
- Faster, easy to interpret methods, now available

Acknowledgments

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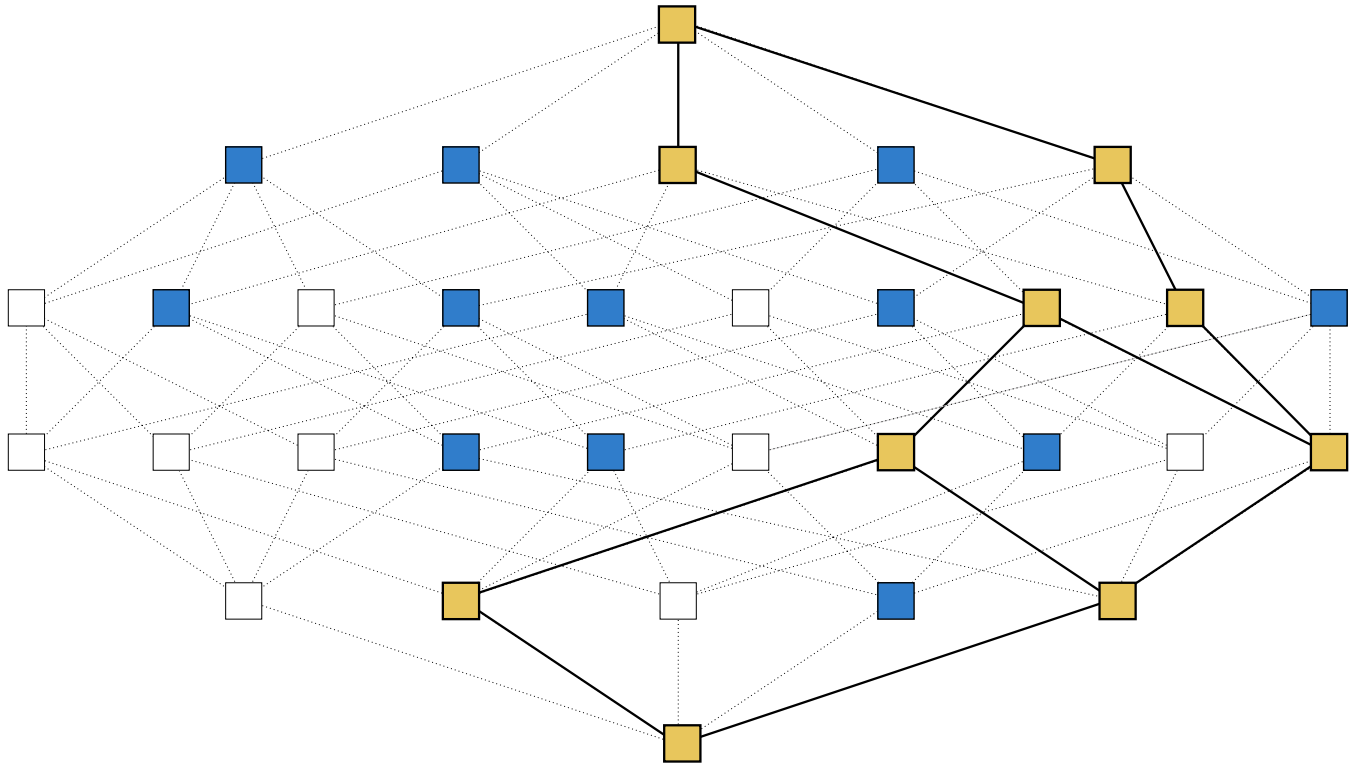
Stefan **Badelt** (now CalTech, `DrTransformer`)
Peter Kerpedjiev (now Harvard, DrTransformer visualization)
Stefan **Hammer** (visualization)

H. Schwalbe, B. Fürtig, Ch. Helmling (Frankfurt, (dG-ribsowitch)

The findpath re-folding path heuristic

Perform a bounded breadth first search of direct paths.



- Only consider **direct** paths, i.e. where distance decreases with each step.
- Up to $D(x, y)!$ direct paths.
- Bound the search by keeping only m best candidates from each distance class.