Co-transcriptional Folding Kinetics and Riboswitch Modeling

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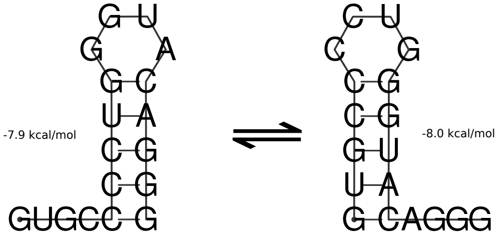
Thermodynamic vs. Kinetic Folding

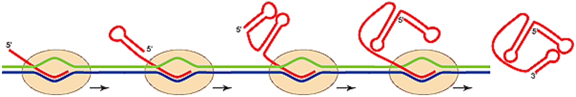
Equilibrium properties for RNA secondary strutcures can be calculated efficiently
But what about dynamics?

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



Folding during Transcription

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



- RNA is transcribed at a rate of 25–50 nucleotides per second
- Nascent chain starts folding as soon as its leaves the ribosome
- Helices once formed 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 can prevent refolding during extension

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`

Stochastic Simulations

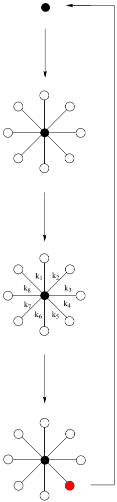
Simulate folding kinetics by Gillespie (rejectionless Monte Carlo) algorithm :

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).

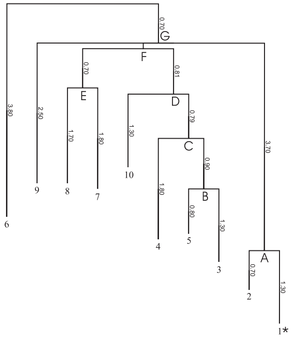


- ☹ computationally somewhat expensive
- ☹ need to analyze many trajectories
- ☺ easy to include co-transcriptional folding

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
  → 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)

Calculating barrier trees

The flooding algorithm:

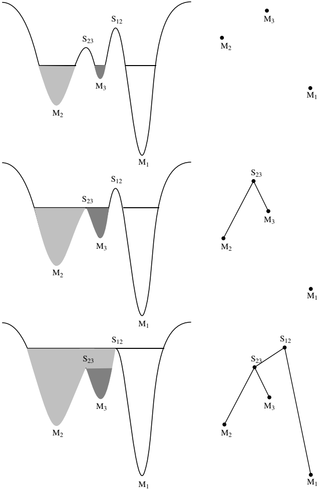
Read conformations in energy sorted order.

For each confirmation x we have three cases:

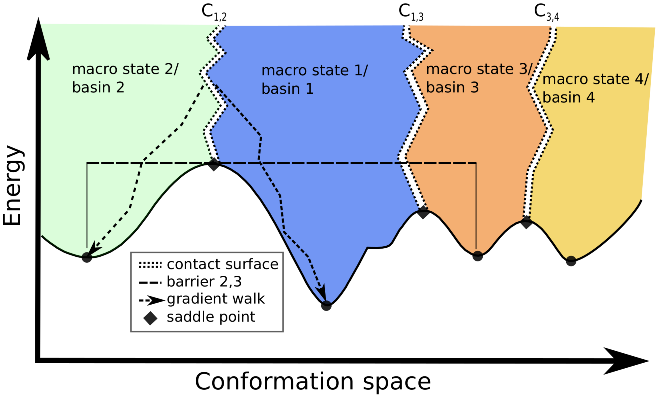
(a) x is a *local minimum* if it has no neighbors we've already seen

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

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



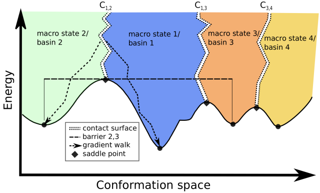
Coarse Graining the Landscape



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

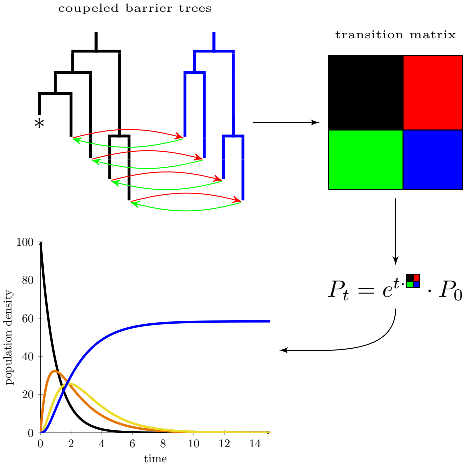
$$r_{\beta\alpha} = \sum_{y\in\beta}\sum_{x\in\alpha} r_{yx}\mathrm{Prob}[x|\alpha] \approx \frac{1}{Z_\alpha}\sum_{y\in\beta}\sum_{x\in\alpha} r_{yx}e^{-E(x)/RT}$$

assuming local equilibrium.

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
    $k_{\text{off}} = k_{\text{on}} e^{-\theta/RT}, \quad r = k_{\text{on}} \cdot C$

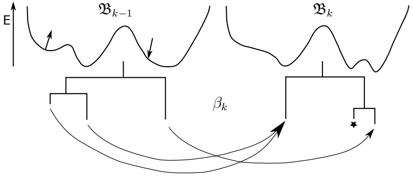
How to include Ligand Binding ?



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

Co-transcriptional folding with BarMap

Each extension of the RNA structure modifies the landscape:

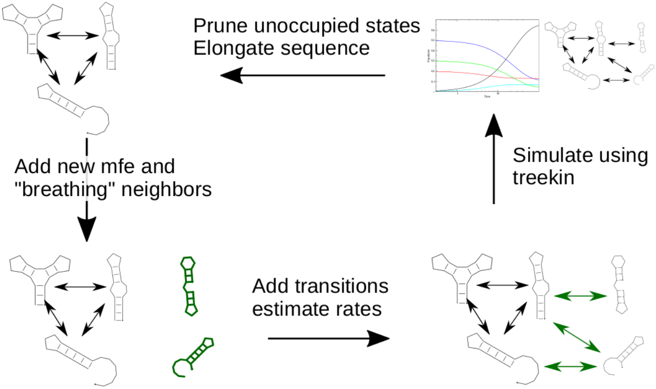


- Compute barrier trees for each sequence length $1 \dots n$
- Compute a mapping between the minima of subsequent landscapes
- Compute dynamics piece-wise:
  - Compute dynamics on landscape for length k
  - Transfer population to landscape of length $k+1$

Hofacker et al., RNA (2010)

DrTransformer: Fast co-transcriptional Folding

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



Prune unoccupied states
Elongate sequence

Add new mfe and
"breathing" neighbors

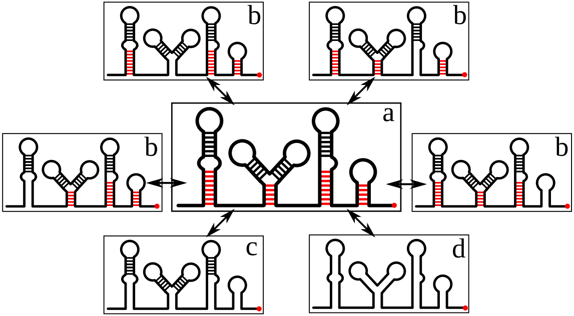
Add transitions
estimate rates

Simulate using
treekin

DrTransformer: “Breathing” neighbors

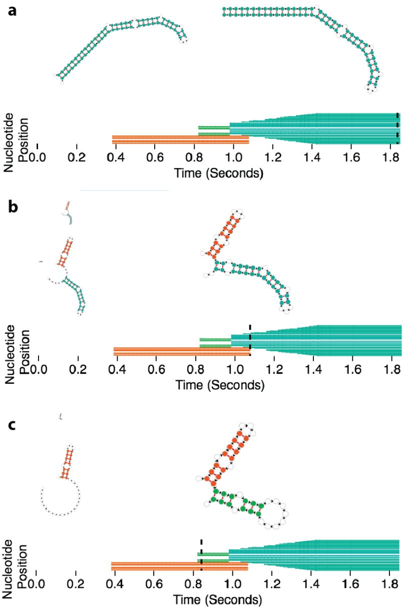
Which new structures should be added after an elongation step?

- 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



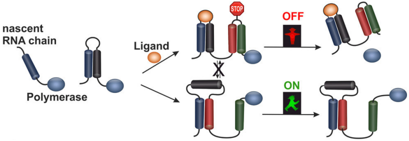
DrTransformer Visualization

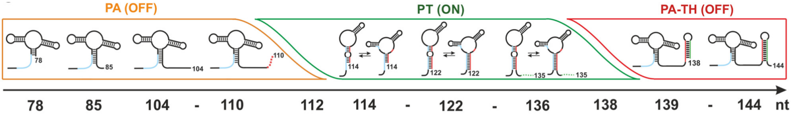
- Simple webinterface
- Interactive visualization Javascript and SVG
- Structure ensemble as function of time



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

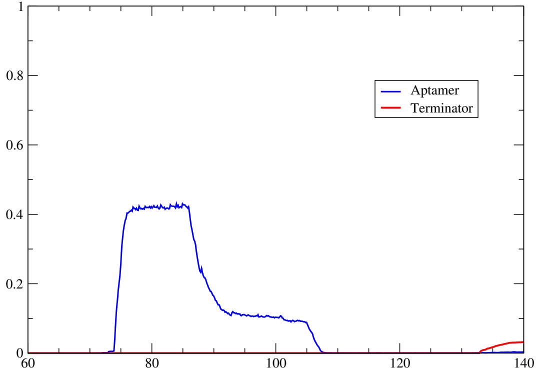




Helmling et al, JACS (2017)

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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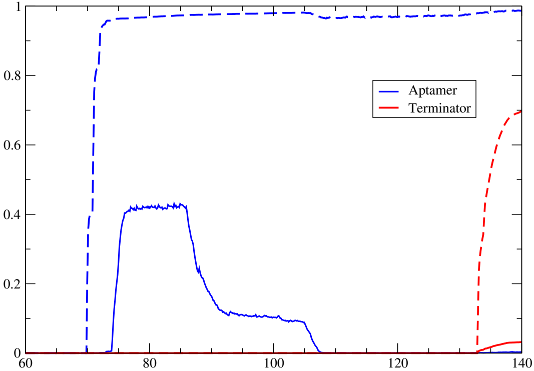
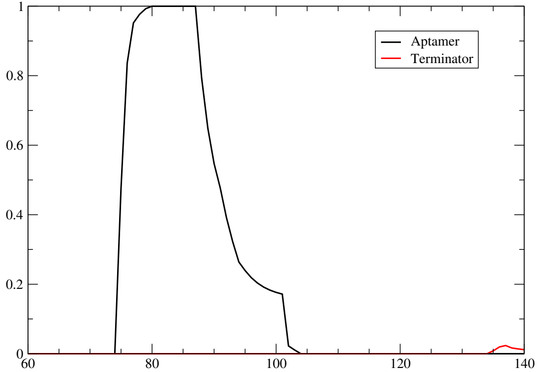
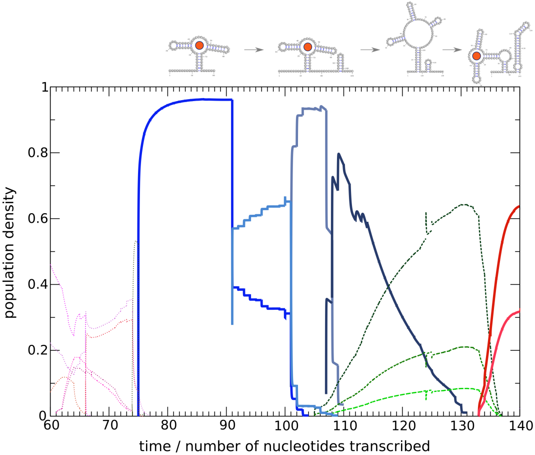


DrTrafo simulation of the dG Riboswitch

- 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

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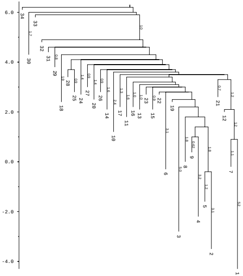
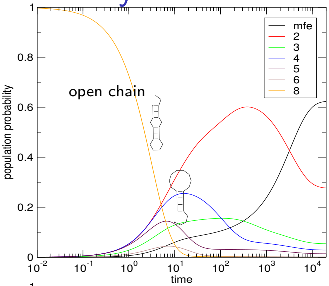
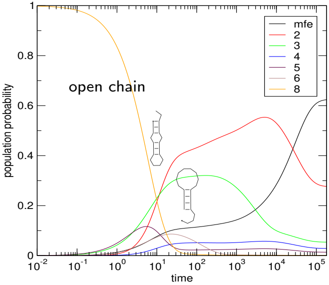
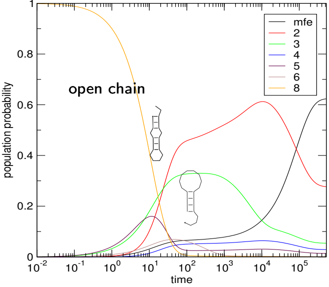
Coarse grained dynamics vs. full dynamics



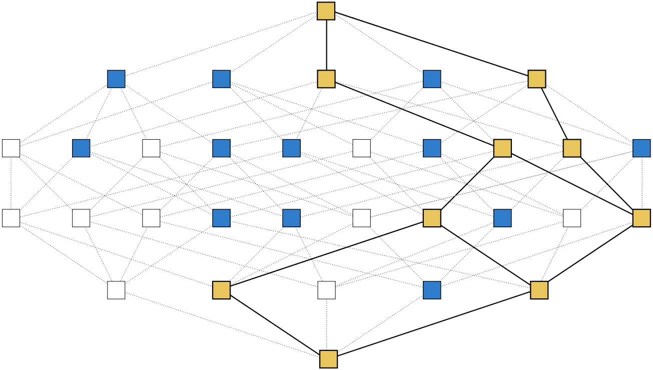






The findpath re-folding path heuristic

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.