# COMP598: Advanced Computational Biology Research and Method

RNA folding dynamics

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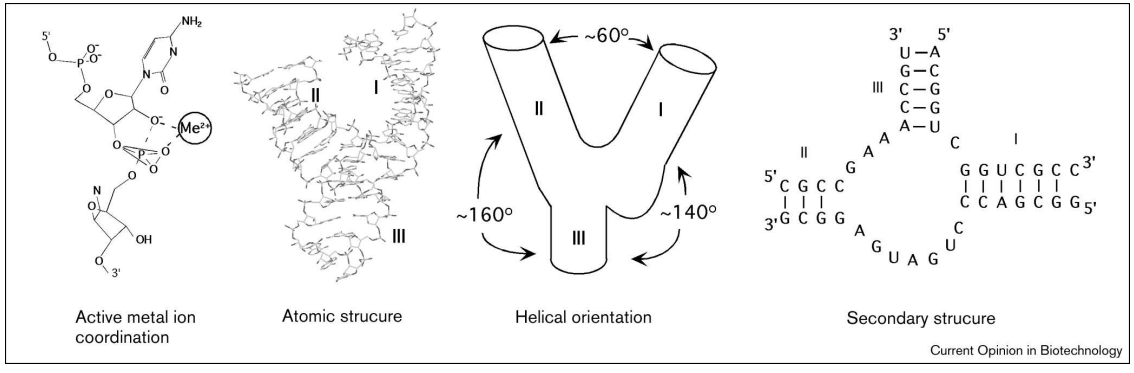
# The RNA secondary structure model

RNA secondary structure provide an ideal model to study biopolymer folding

- provide a biochemically useful structure description
- mathematically and computationally easy to handle
- energy model based on carefully measured parameters
- efficient algorithms for structure prediction



# Computing RNA secondary structures

Most *equilibrium* properties can be computed exactly and efficiently by dynamic programming

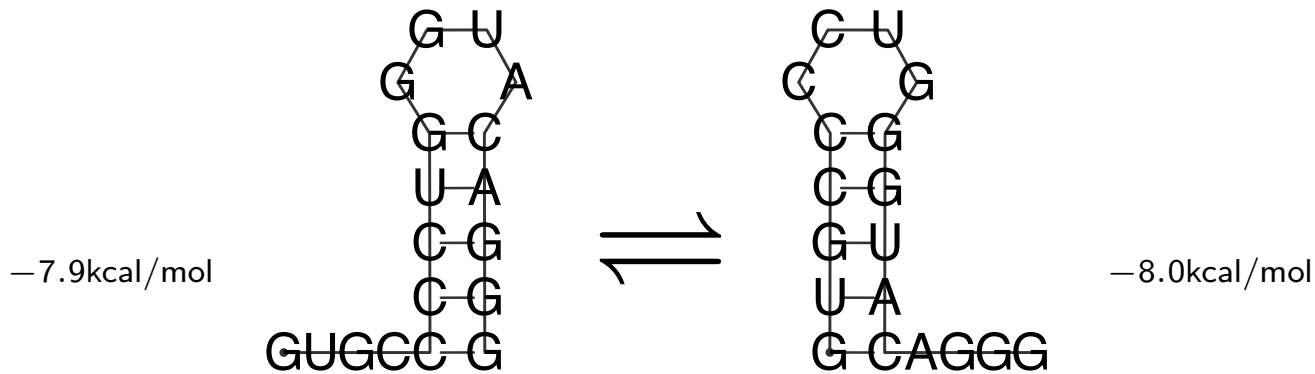
- Minimum free energy structure (Zuker & Stiegler '81)
- Suboptimal structures
  - representative suboptimal structures (Zuker '89)
  - all structures within an energy range (Wuchty et.al. '99)
  - Boltzmann weighted samples (Ding & Lawrence '03)
- Partition function and base pair probabilities (McCaskill '90)
- Density of states (Cupal '96)
- Minimum free energy with pseudoknots (Rivas & Eddy '99)

Free software available in the `Vienna RNA Package` at
`http://www.tbi.univie.ac.at/~ivo/RNA/`

# Thermodynamic vs. Kinetic Folding

Equilibrium properties can be calculated efficiently
But what about dynamics?

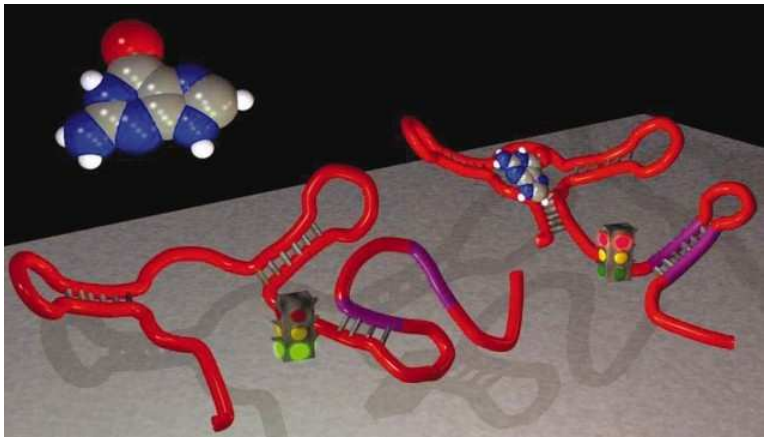
- On what time scale is equilibrium reached?
- How fast/slow is re-folding between dissimilar structures?
- What structures are populated initially?

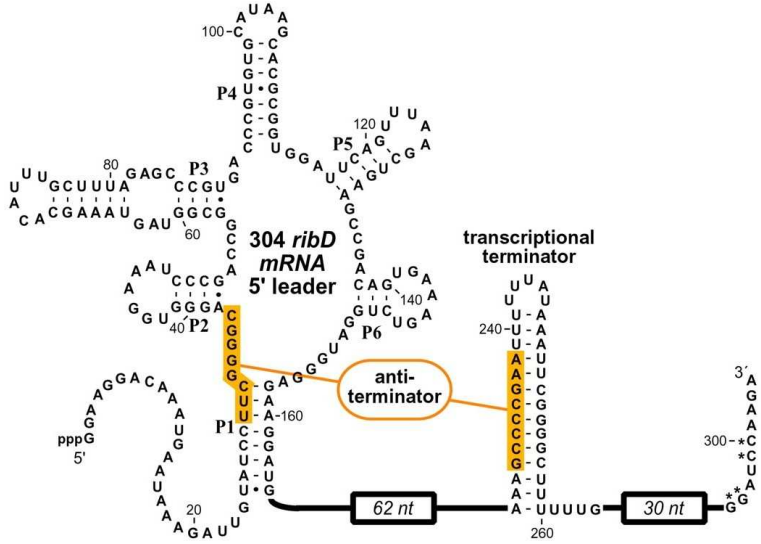


# Structural changes are common in functional RNA

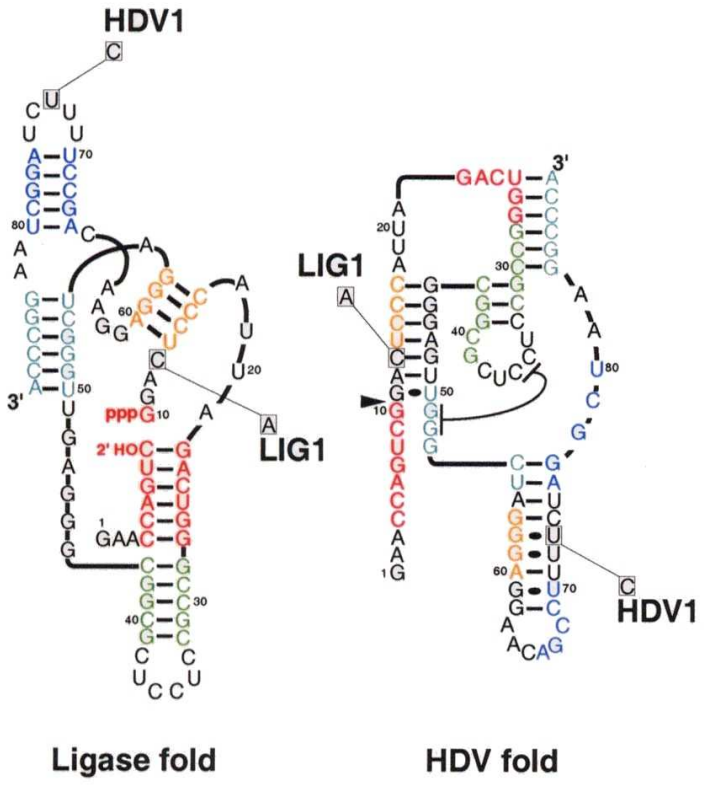
**RNA switches** toggle between active and inactive states by changing conformation.
Used especially to control mRNA translations; triggered by:

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

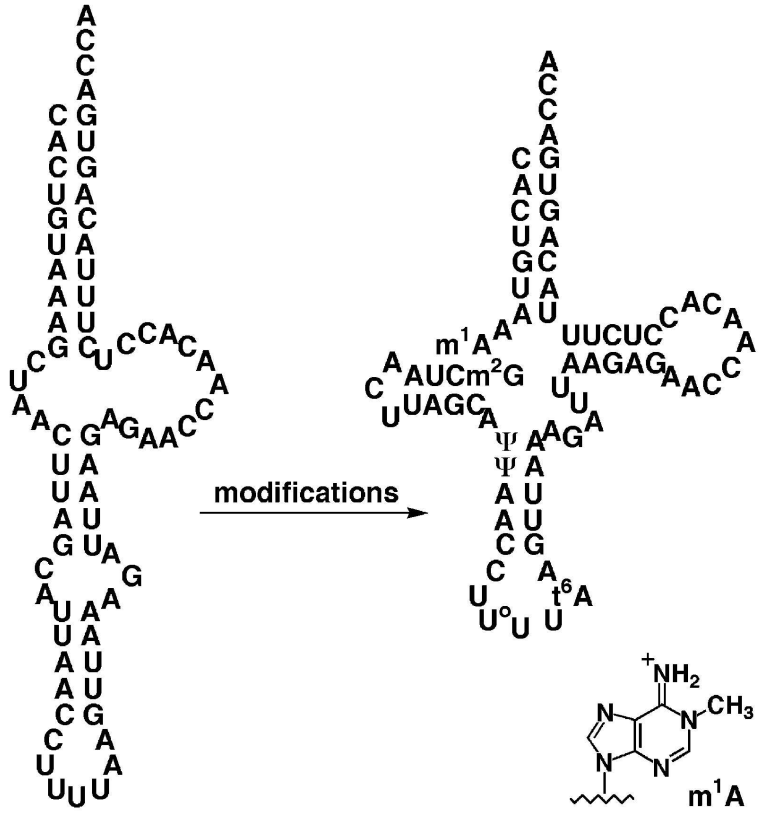




# Examples of RNA switches




A Ribozyme with two functions (Schultes & Bartel 2000)




Chemical modification triggers the cloverleaf fold of a tRNA (Helm & Giegé 1999)

# Predicting dynamics of RNA folding

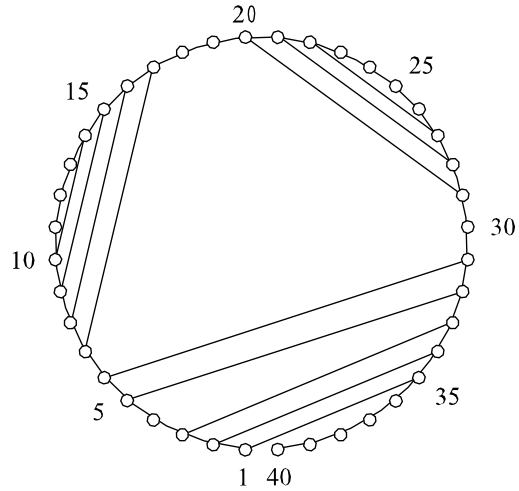
Folding dynamics described by a Morkov process with master equation

$$\frac{\mathrm{d}p_x}{\mathrm{d}t} = \sum_{y \in X} r_{xy} p_y(t)\,, \qquad \text{with } r_{xx} = -\sum_{y \neq x} r_{yx}.$$

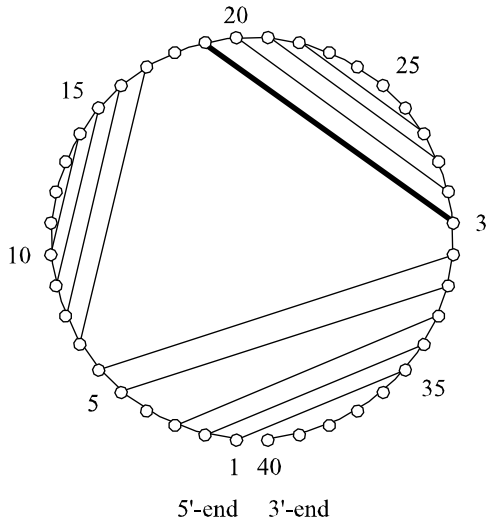
- Integration of the master equation (toy models only).
- Stochastic folding simulations. Needs many trajectories.
- Qualitative analysis of the energy landscape to identify possible traps (local minima). $\longrightarrow$ coarse grained versions of the Markov process

Need to model the rate $r_{xy}$. For small moves Metropolis rule is sufficient.

# Elementary move set for RNA secondary structures




Add base pair

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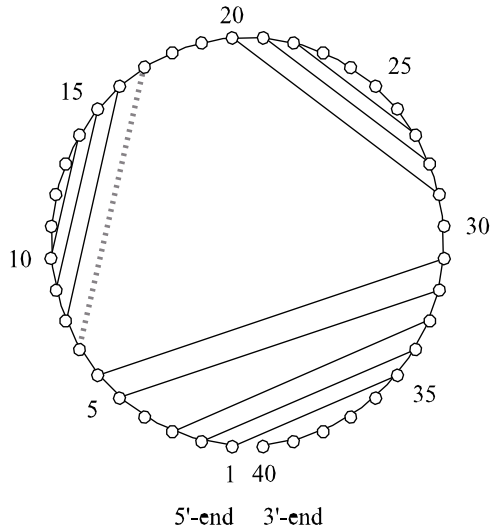
(((•((((((•••))))))•(((••••)))•)))))•••••

Remove base pair

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# Kinetic Folding Algorithm

Simulate folding kinetics by a Monte-Carlo type algorithm:

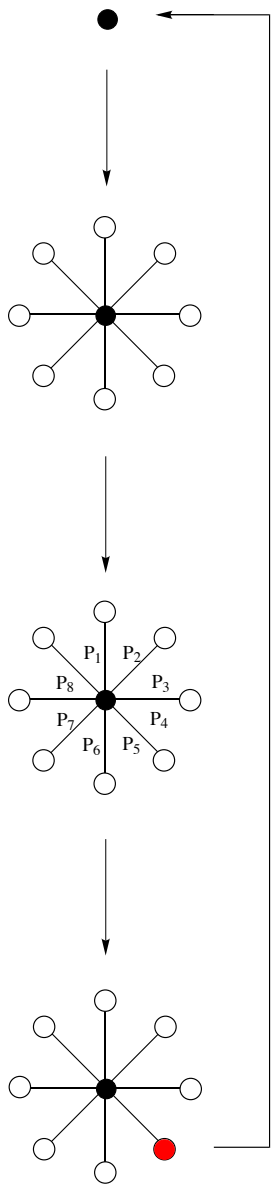
Generate all neighbors using the move-set

- Basepair Insertion
- Basepair Deletion

Assign rates to each move, e.g.

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

Advance clock $1/\sum_i P_i$.
select a move with probability proportional to its rate



# Characterization of Landscapes

A landscape consists of a configuration space $V$, a move set within that configuration space and an energy function $f : V \to \mathbb{R}$.

Simplest move set for secondary structures: opening and closing of pairs.

Speed of optimization depends on the *roughness* of the Landscape.
Measures of roughness suggested in the literature:

- Number of local optima
- Correlation lengths (e.g. along a random walk)
- Lengths of adaptive walks
- Folding temperature vs. glass temperature $T_f / T_g$
- Energy barriers between the local optima. Especially, the maximum barrier height ("depth" in SA literature)

# Energy barriers

$$E[s,w] = \min\left\{ \max\left[ f(z) \middle| z \in \mathbf{p} \right] \;\middle|\; \mathbf{p} : \text{path from } s \text{ to } w \right\},$$

$$B(s) = \min\left\{ E[s,w] - f(s) \middle| w : f(w) < f(s) \right\}$$

Depth and Difficulty
(borrowed from simulated annealing theory)

$$\mathrm{D} = \max\left\{ B(s) \middle| s \text{ is not a global minimum } \right\}$$

$$\psi = \max\left\{ \frac{B(s)}{f(s) - f(\min)} \middle| s \text{ is not a global minimum} \right\}$$

# Calculating barrier trees

## The flooding algorithm:

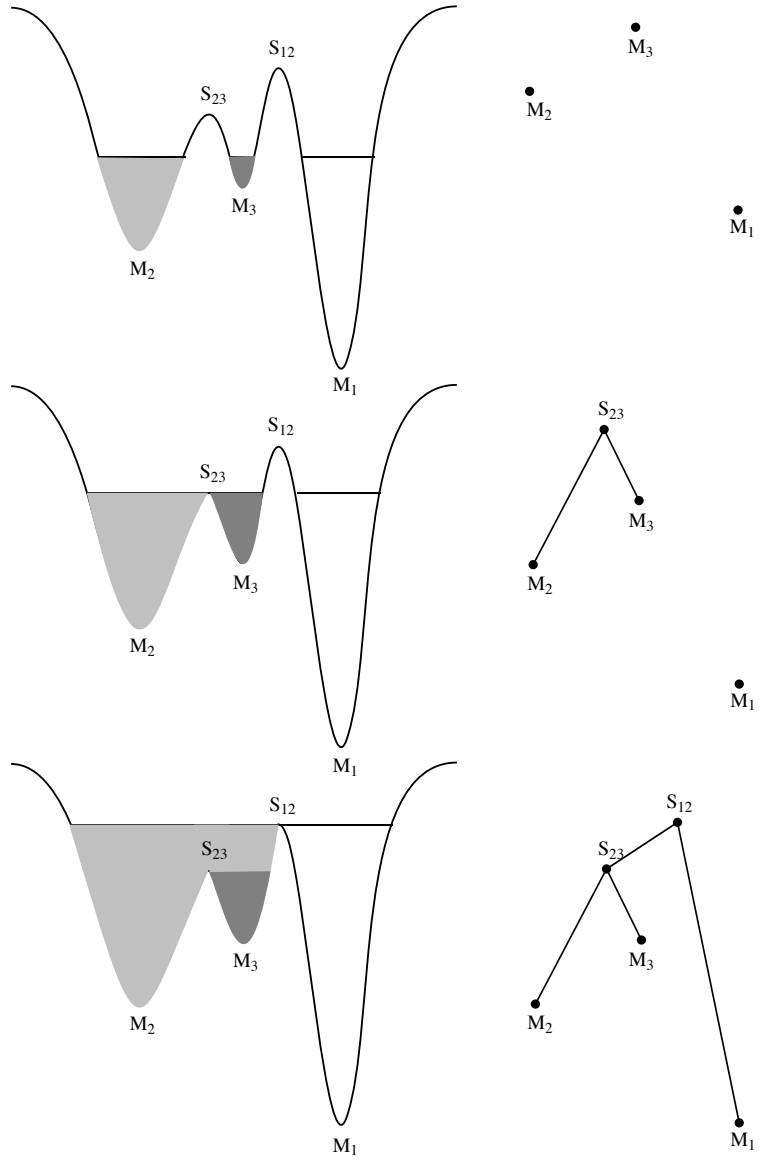
Read conformations in energy sorted order.

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. Basins $B(s_1), \ldots, B(s_k)$ are then united and are assigned to the deepest of local minimum.

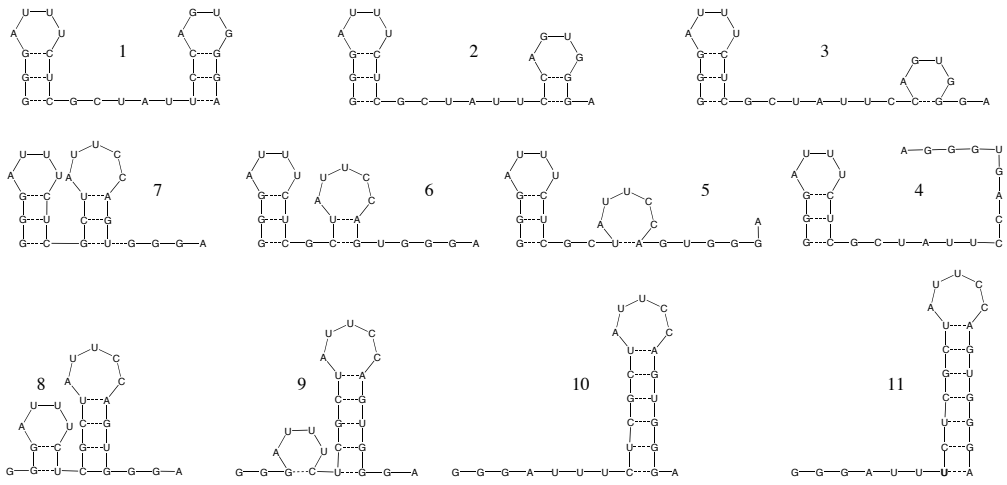


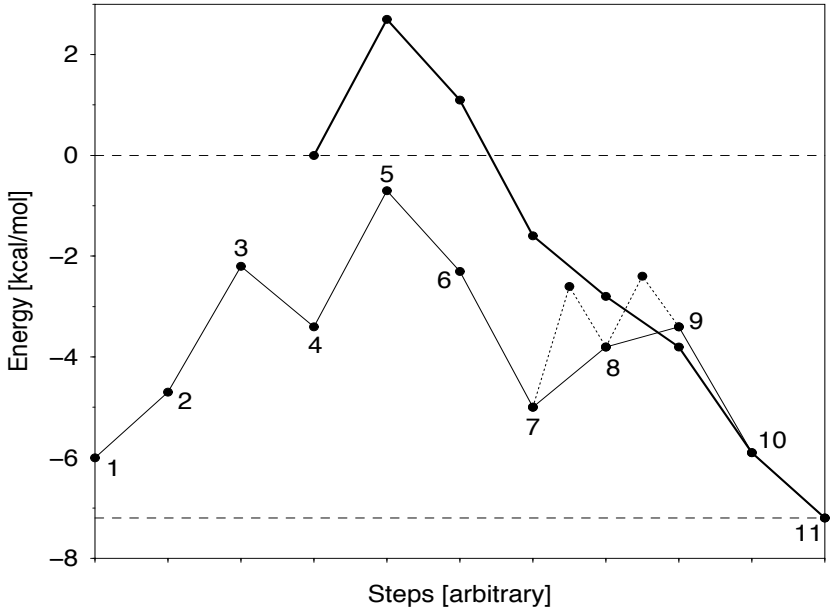
# Information from the Barrier Trees

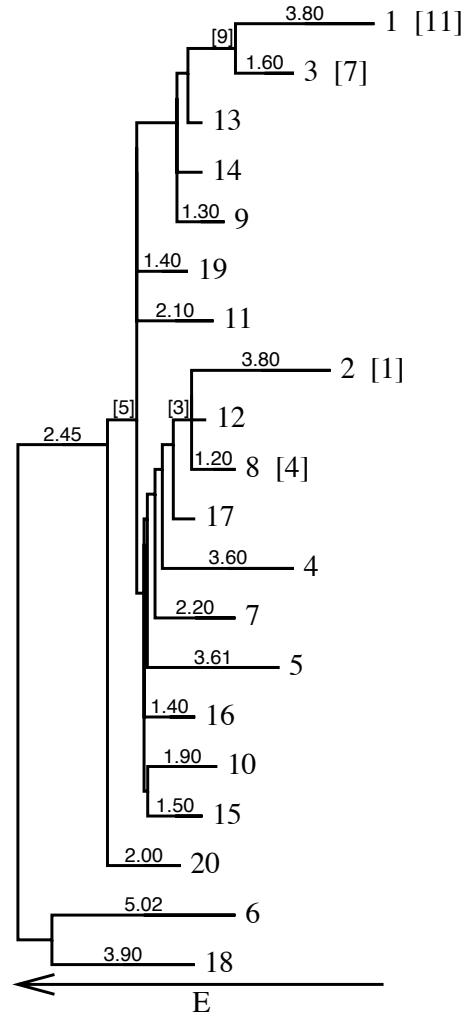
- Local minima
- Saddle points
- Barrier heights
- Gradient basins
- Partition functions and free energies of (gradient) basins
- Effective refolding rates between gradient basins
- Optimal refolding paths
- Depth and Difficulty of the landscape

A *gradient basin* is the set of all initial points from which a gradient walk (steepest descent) ends in the same local minimum.

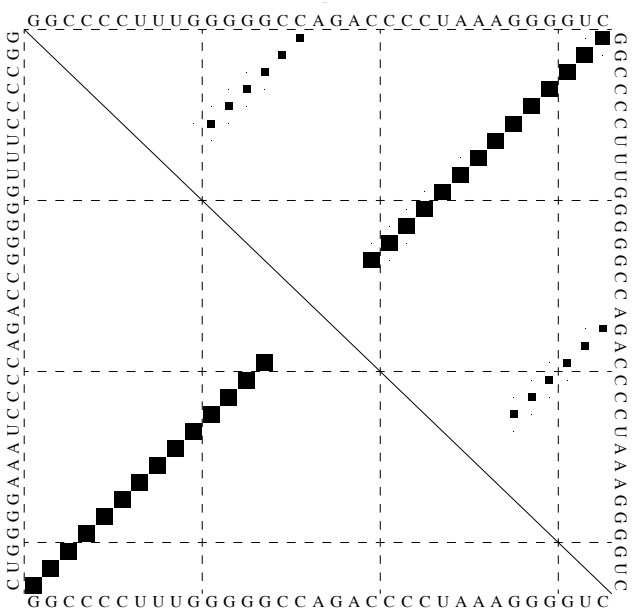
# Energy Landscape of a Toy Sequence

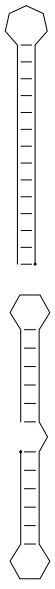


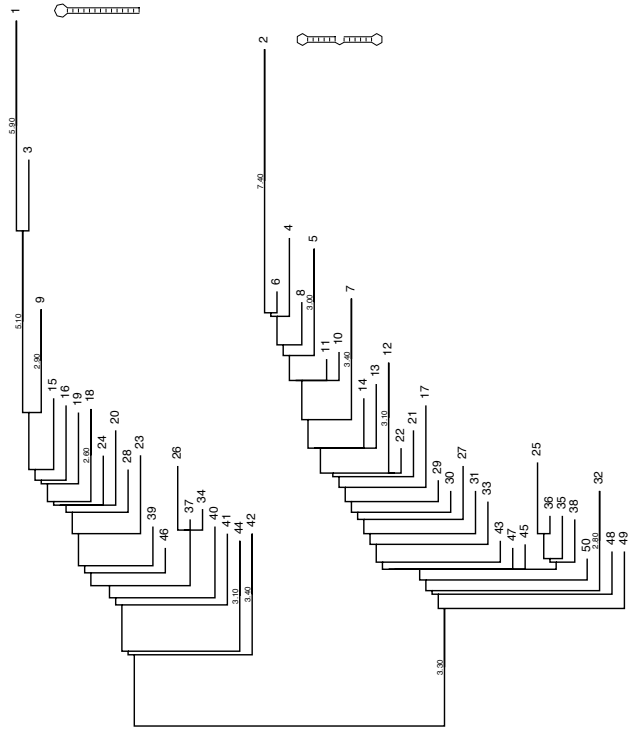
# A Designed Bi-stable Sequence



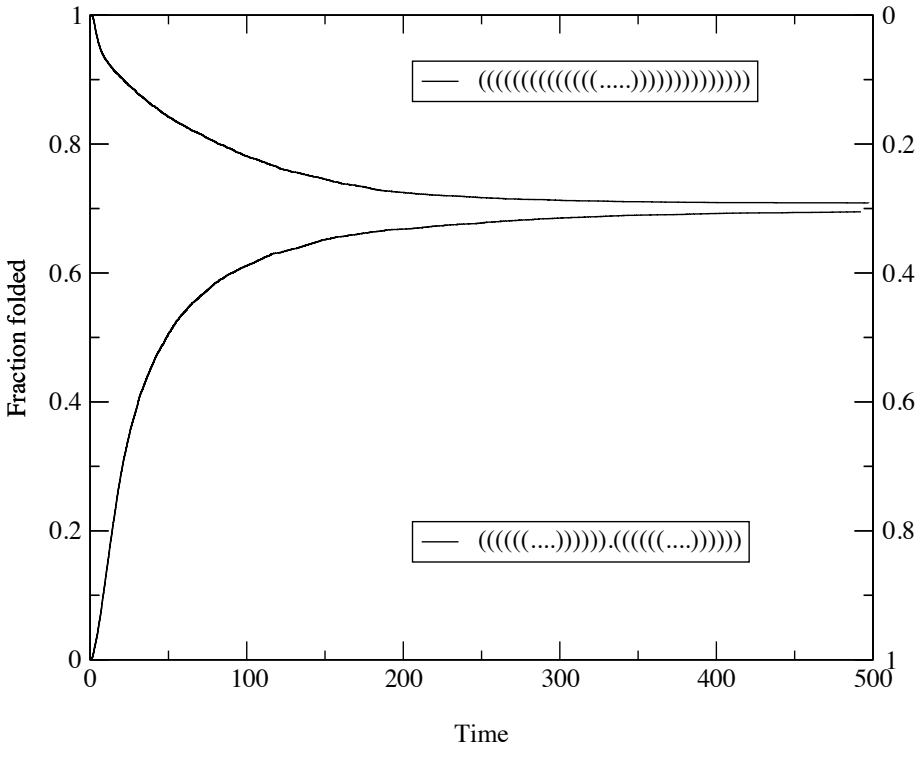



-23.8 kcal

-23.0 kcal



# Barrier Tree and refolding Path

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((((((....)))))).((((((....)))))) -23.00
(((((.(...)))))).((((((....)))))) -17.50
((((.((...)))))).((((((....)))))) -17.50
(((.(((...))))).((((((....)))))) -17.50
.((.(((...))))).((((((....)))))) -13.70
.(.(((...))))).((((((....)))))) -13.70
.(.(((...))))).).((((((....)))))) -14.30
...((((...)))).((((((....)))))) -14.10
....(((...))).....((((((....)))))) -12.10
.....(...)).....((((((....)))))) -09.20
.....(.....).....((((((....)))))) -08.40
.................((((((....)))))) -09.80
.........(.....).((((((....)))))) -08.60
.........((...)).((((((....)))))) -10.30
........(((...))).((((((....)))))) -11.40
.......((((...)))).((((((....)))))). -09.90
......(((((...))))).((((((....)))))).. -09.10
.(...)(((((...))))).((((((....)))))).. -06.20
.(....(((((...))))).((((((....)))))). -04.00
((....(((((...))))).((((((....)))))) -04.70
(((...(((((...))))).((((....)))))) -04.50
((((..(((((...))))).((((....)))))) -04.50
(((((.(((((...))))).((((....)))))) -04.50
((((((((((...)))))).........)))))) -09.09
((((((((((...)))).........)))))) -09.69
((((((((((...))).........)))))) -10.09
((((((((((...))).(.....).)))))) -09.50
((((((((((...)).(.....)))))))))) -09.80
((((((((((...))(.....)))))))))) -09.50
((((((((((...))((....)))))))))) -11.30
((((((((((.....)((....)))))))))) -09.60
((((((((.(....)((....)))))))))) -08.70
((((((((((....).(....)))))))))) -08.30
((((((((((....).......)))))))))) -07.94
((((((((((...........)))))))))) -14.48
((((((((((.........)))))))))) -17.60
((((((((((.......)))))))))) -20.70
((((((((((((.....)))))))))))) -23.80
```

- The two component structure is kinetically prefered, because both hairpins act as nucleation centers
- For the full length chain 75% of trajectories reach the two component stucture first
- Much stronger effect for co-transcriptional folding:
  only 1 in 1000 trajectories ends in the one component structure

# 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

How can we optimally choose the macro-states?
Use the gradient basins around each local minimum.

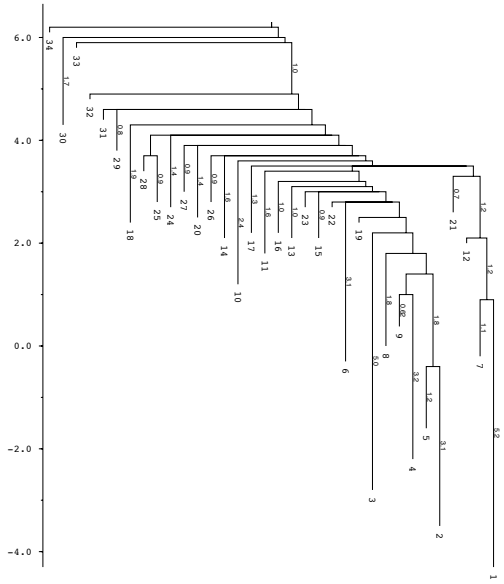
Transition rates could follow an Arrhenius rule
$r_{\beta\alpha} = \exp\left(-(E^*_{\beta\alpha} - G_\alpha)/RT\right)$.

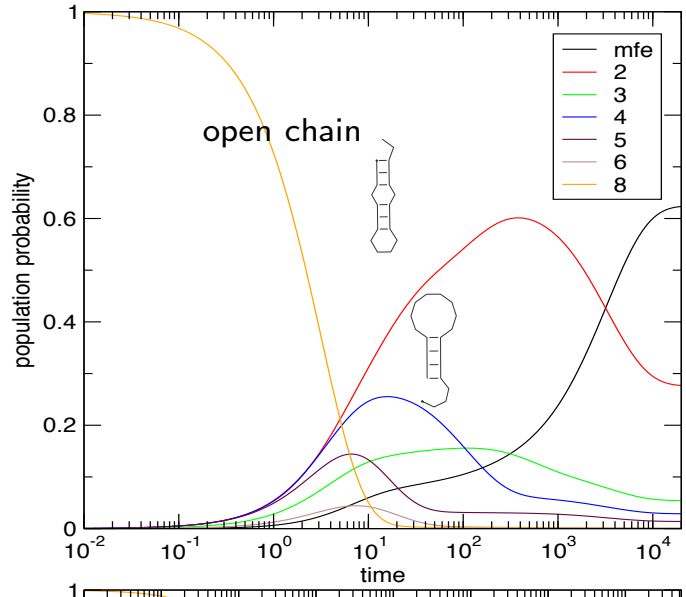
Or compute macro state rates from microscopic ones

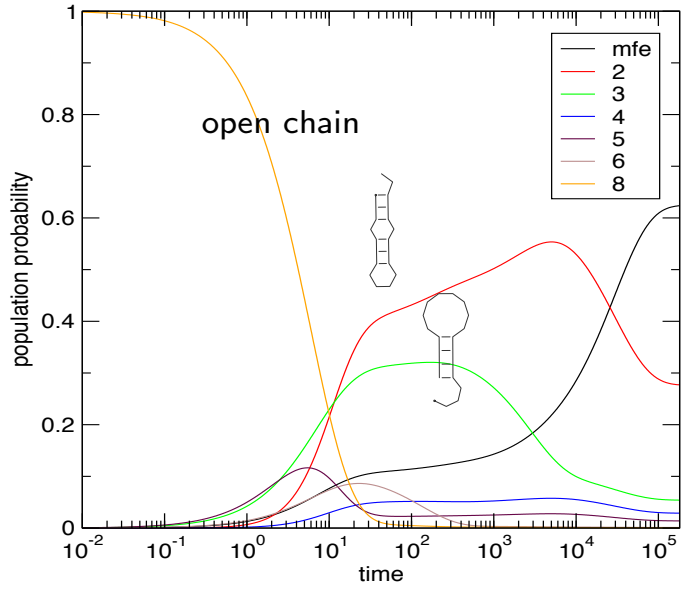
$$r_{\beta\alpha} = \sum_{y\in\beta}\sum_{x\in\alpha} r_{yx}\mathrm{Prob}[x|\alpha] = \frac{1}{Z_\alpha}\sum_{y\in\beta}\sum_{x\in\alpha} r_{yx}e^{-E(x)/RT}$$

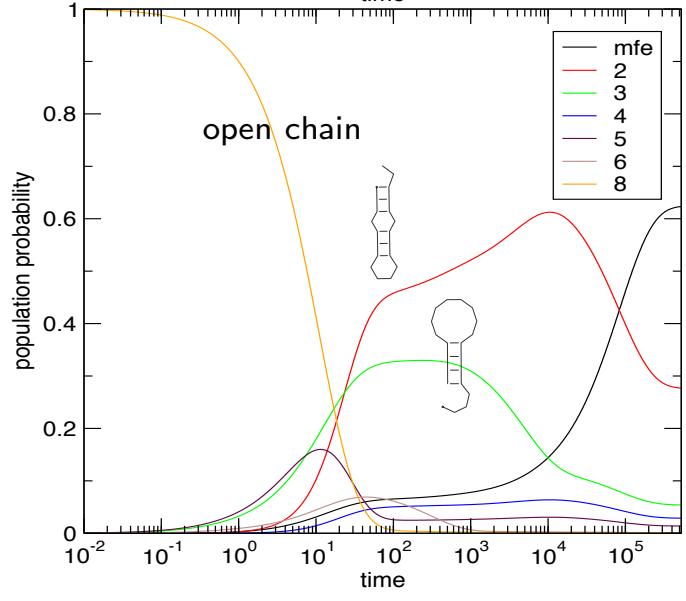
assuming local equilibrium.

# Coarse grained dynamics vs. full dynamics





# Folding during Transcription

- RNA is transcribed at a rate of only 30–40 nucleotides per second
- The nascent chain starts folding as soon as its leaves the ribosome
- Stem 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

# Kinetic Folding Algorithm

Simulate folding kinetics by a Monte-Carlo type algorithm:

Generate all neighbors using the move-set

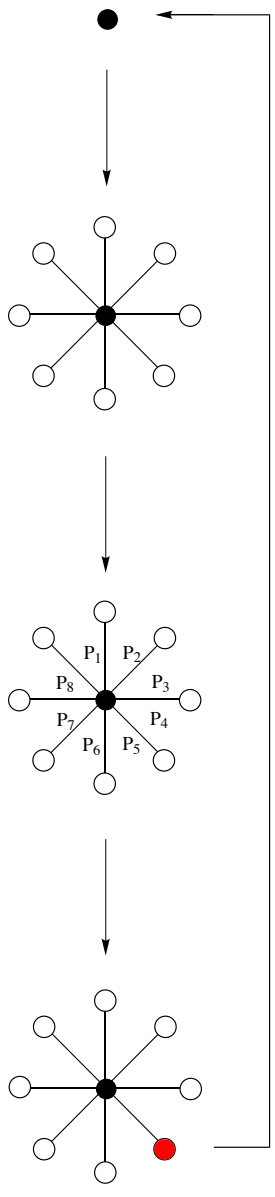
- Basepair Insertion
- Basepair Deletion

Assign rates to each move, e.g.

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

Advance clock $1/\sum_i P_i$.

select a move with probability proportional to its rate



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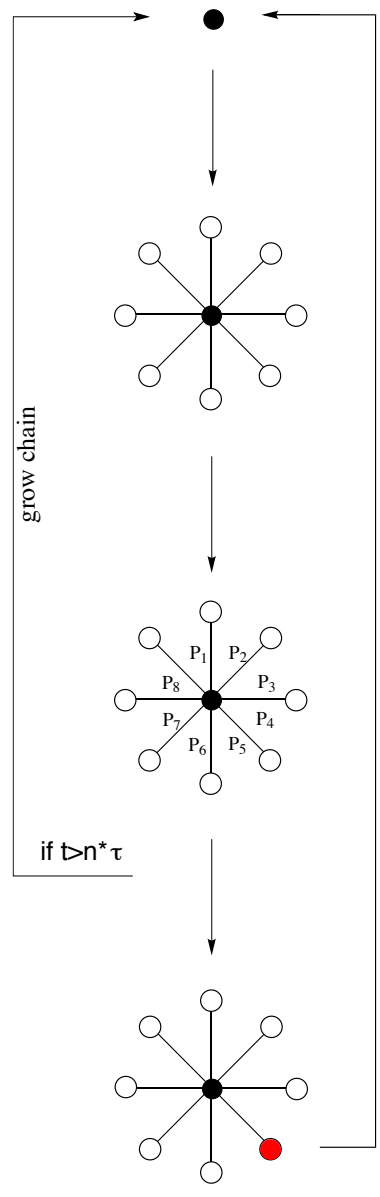
Assign rates to each move, e.g.

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

Advance clock $1/\sum_i P_i$.
extend chain by one if $t > n \cdot \tau$ else
select a move with probability proportional to its rate



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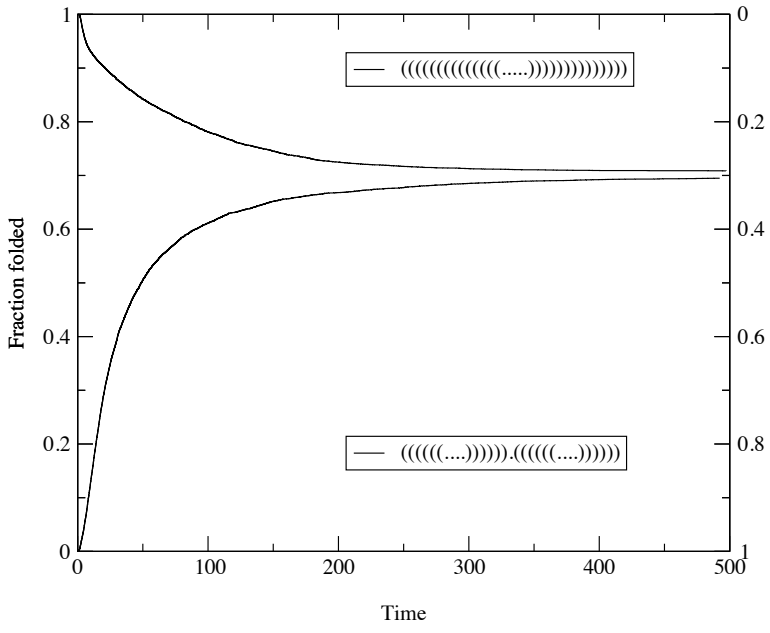


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((((((....)))))).((((((....)))))) -23.00
(((((.(...)))))).((((((....)))))) -17.50
((((.((...)))))).((((((....)))))) -17.50
(((.(((...))))).((((((....)))))) -17.50
.((.(((...))))).((((((....)))))) -13.70
.(.((((...)))))..((((((....)))))) -13.70
.(.((((...)))))).((((((....)))))) -14.30
...((((...)))).((((((....)))))) -14.10
....(((...))).((((((....)))))) -12.10
.....(((...))).((((((....)))))) -09.20
.....(.....).((((((....)))))) -08.40
.................((((((....)))))) -09.80
.........(.....).((((((....)))))) -08.60
.........((...)).((((((....)))))) -10.30
.........(((...))).((((((....)))))) -11.40
.......((((...)))).((((((....)))))). -09.90
......(((((...))))).((((....)))))... -09.10
.(...)(((((...))))).((((....)))))... -06.20
.(....(((((...))))).((((....)))))). -04.00
((....(((((...))))).((((....)))))) -04.70
(((...(((((...))))).-(((....))))) -04.50
((((.-(((((...))))).-.((....))))) -04.50
(((((.(((((...))))).-..(....))))) -04.50
((((((((((...)))))).........)))))) -09.09
((((((((((...)))).........)))))) -09.69
((((((((((...))).........)))))) -10.09
((((((((((...)))(.....).)))))) -09.50
((((((((((...)).(.....)))))))) -09.80
((((((((((...))(......)))))))) -09.50
((((((((((...))((....)))))))) -11.30
((((((((((.....)((....)))))))) -09.60
(((((((((.(....)((....)))))))) -08.70
((((((((((....).(....)))))))) -08.30
((((((((((....).......)))))))) -07.94
((((((((((...........)))))))) -14.48
((((((((((.........)))))))) -17.60
((((((((((.......)))))))) -20.70
((((((((((.....)))))))) -23.80
```

- The two component structure is kinetically prefered
- From the open chain 75% of trajectories end in the two component stucture
- Much stronger effect for co-transcriptional folding:
  only 1 in 1000 trajectories ends in the one component structure

# Some Examples

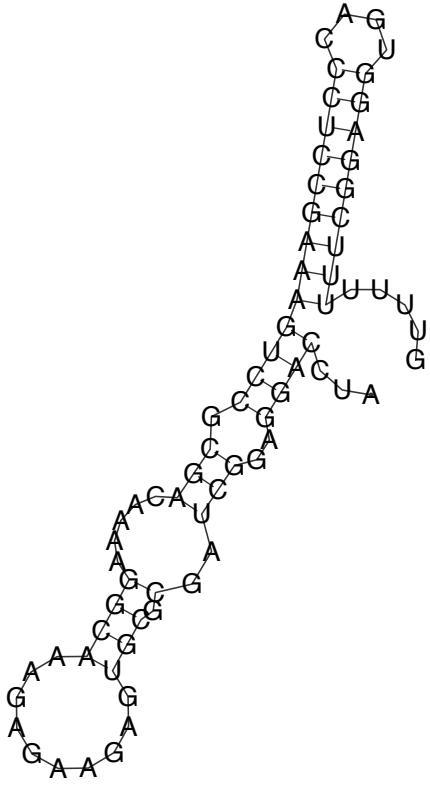
Effect of co-transcriptional folding for some bi-stable structures taken from the PARNAS web site.

| name | full seq | slow | fast | very fast | equil. | maxB [1] |
|---|---|---|---|---|---|---|
| MS2 | 69/31 | 99.6/0.4 | 59/41 | 76/24 | 99.9/0.1 | 8.1 |
| S15 | 60/40 | 99.7/0.3 | 99.5/0.5 | 60/40 | 99/1 | 6.24 |
| dsrA | 32/68 | 63/37 | 42/58 | 65/35 | 62/38 | 7.8 |
| attenuator | 85/15 | 99.9/0.1 | 25/75 | 69/31 | 94/6 | 13.7 |

With realistically slow transcription rate, co-transcriptional folding often leads to equilibrium.

[1] kcal/mol

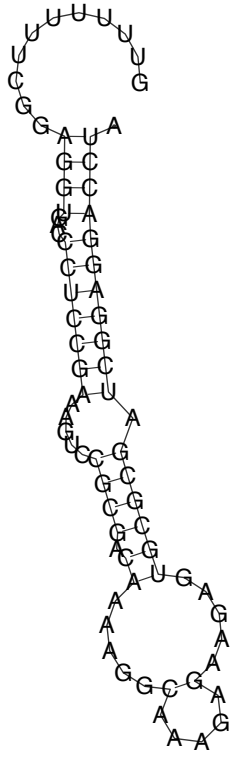
# Attenuator example



−22.9kcal/mol

13.7kcal

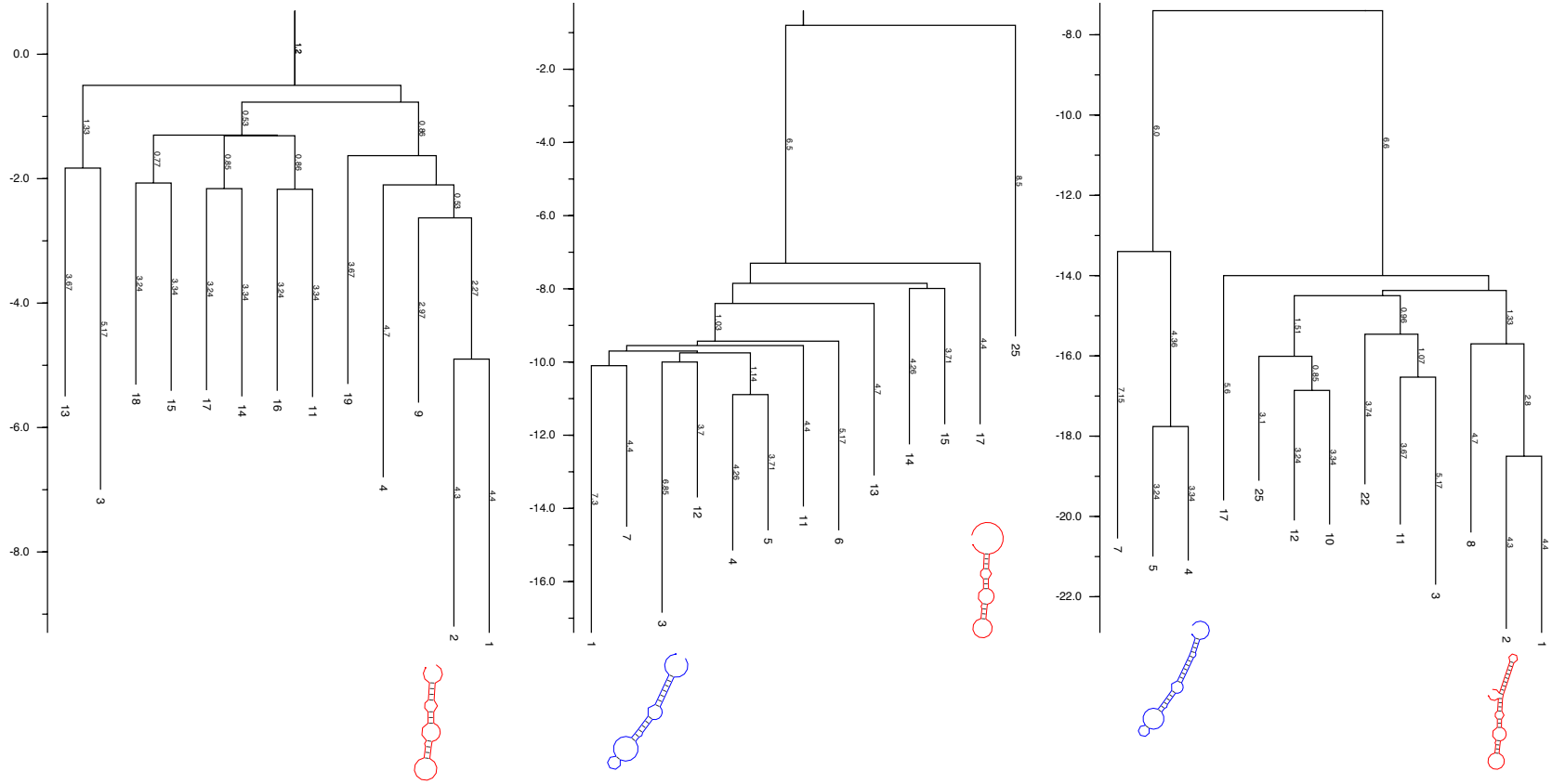
$\rightleftharpoons$



−21.1kcal/mol

# Barrier Trees of Growing Sequence

$n = 50$ $n = 60$ $n = 70$

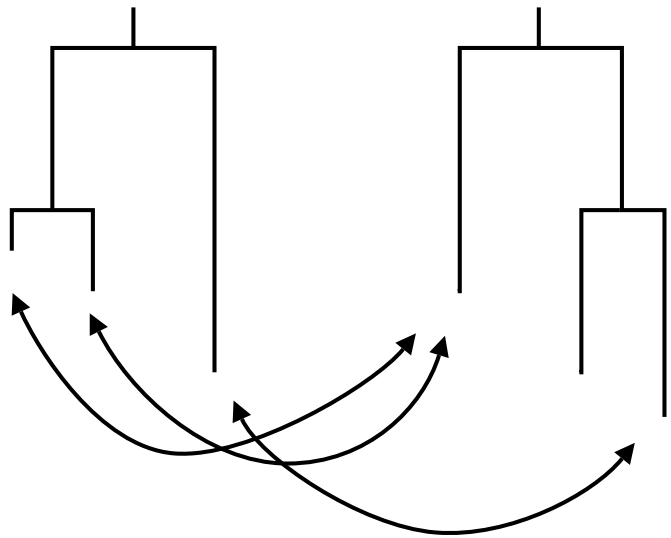


```
AUCCAGGAGGCUAGCGCGUGAGAAGAGAAACGGAAAACAGCGCCUGAAAGCCUCCCAGUGGAGGCUUUUUUU
...((((..(((..(.(((.........)))).....))).)))).....  (-9.3)

.....((((((.((((((.....(.....).....)).))))).....))))))).....  (-17.4)
...((((..(((..(.(((.........)))).....))).)))).............  ( -9.3)

...((((..(((..(.(((.........)))).....))).))))(((((((((...)))))))))..  (-22.9)
.((((((((((.((((((.....(.....).....)).))))).....))))))).)))........  (-21.1)
```

# Mapping between Barrier Trees

Each structure $x$ at length $n$ corresponds to an extended structure $x\bullet$ at length $n+1$.
For a minimum $m$, the correponding minimum $m'$ can be found by a gradient walk starting with $m\bullet$.

- Two minima may be mapped to the same minimum in the $n+1$ landscape.
- In addition new minima may appear.



# Mapping between Barrier Trees Example

`bar_map.pl` computes the mapping between a sequence of bar files

```
> bar_map.pl attenuator_*.bar
```

```
 44    46    48    50    52    54    56    58    60    62    64    65    66    67    68   70
  6 ->  1 ->  1 ->  1 ->  1 -> 17 -> 16 -> 25 -> 25 ~> 26 ~> 27 ->  7 ->  3 ->  1 ~>  1 ~> 1
  7 ~>  2 ~>  2 ~>  2 ~>  2 ~> 18 ~> 18 ~> 25 -> 25 ~> 26 ~> 27 ->  7 ->  3 ->  1 ~>  1 ~> 1
    ->  5 ->  4 ->  4 -> 12 ~> 18 ~> 18 ~> 25 -> 25 ~> 26 ~> 27 ->  7 ->  3 ->  1 ~>  1 ~> 1
    ->  8 ->  5 ->  5 -> 16 ~> 18 ~> 18 ~> 25 -> 25 ~> 26 ~> 27 ->  7 ->  3 ->  1 ~>  1 ~> 1
        ....
                                                                  ->  8 ~>  4 ~>  2 ~>  2 ~> 2
  1 ->  7 ~> 14 -> 14 ~>  9 -> 20 -> 20 ~>  1 ->  1 ~>  1 ->  1 ->  1 ->  1 ->  3 ->  3 -> 4
  4 -> 14 ~> 14 -> 14 ~>  9 -> 20 -> 20 ~>  1 ->  1 ~>  1 ->  1 ->  1 ->  1 ->  3 ->  3 -> 4
    -> 19 -> 11 -> 11 ~>  9 -> 20 -> 20 ~>  1 ->  1 ~>  1 ->  1 ->  1 ->  1 ->  3 ->  3 -> 4
          -> 15 -> 15 ~>  9 -> 20 -> 20 ~>  1 ->  1 ~>  1 ->  1 ->  1 ->  1 ->  3 ->  3 -> 4
                     ->  3 ->  1 ->  1 ->  1 ->  1 ~>  1 ->  1 ->  1 ->  1 ->  3 ->  3 -> 4
                     ->  6 ->  4 ~>  1 ->  1 ->  1 ~>  1 ->  1 ->  1 ->  1 ->  3 ->  3 -> 4
                     ->  7 ->  5 ~>  1 ->  1 ->  1 ~>  1 ->  1 ->  1 ->  1 ->  3 ->  3 -> 4
        ....
  2 ~>  9 ~> 17 ~> 17 ~> 10 ~> 21 ~> 21 ~>  2 ~>  2 ~>  2 ~>  2 ~>  2 ~>  2 ~>  4 ~>  4 ~> 5
          -> 16 ~> 16 ~> 10 ~> 21 ~> 21 ~>  2 ~>  2 ~>  2 ~>  2 ~>  2 ~>  2 ~>  4 ~>  4 ~> 5
                     ->  4 ~>  2 ~>  2 ~>  2 ~>  2 ~>  2 ~>  2 ~>  2 ~>  2 ~>  4 ~>  4 ~> 5
                     ->  8 ~>  6 ~>  2 ~>  2 ~>  2 ~>  2 ~>  2 ~>  2 ~>  2 ~>  4 ~>  4 ~> 5
                     -> 21 -> 11 ->  6 ->  6 ->  6 ~>  5 -> 11 -> 13 -> 15 -> 19 ~>  4 ~> 5
                           -> 19 ~>  2 ~>  2 ~>  2 ~>  2 ~>  2 ~>  2 ~>  2 ~>  4 ~>  4 ~> 5
```

# Coarse grained Simulation with Chain Growth

How to generalize the coarse grained simulations for co-transcriptional folding

1. Simulate folding on barrier tree of size $n$ for time $\tau$
2. map final population to size barrier tree of size $n + 1$
3. use mapped population as initial condition for next simulation

Not yet implemented...

# Summary

- Folding dynamics can be simulated through either explicit MC simulation or coarse grained computation on the barrier tree.
- Both approaches can be generalized to co-transcriptional folding
- Co-transcriptional folding can focus the outcome on just one structure
- Results can depend strongly on transcription speed
- Need to fix our time-scale by comparison with experiment

# Kinetic Folding of RNA Secondary Structures


Christoph Flamm, Walter Fontana, Ivo L. Hofacker, Peter Schuster. *RNA folding kinetics at elementary step resolution.* RNA **6**:325-338, 2000

Christoph Flamm, Ivo L. Hofacker, Sebastian Maurer-Stroh, Peter F. Stadler, Martin Zehl. *Design of multistable RNA molecules.* RNA **7**:325-338, 2001

Christoph Flamm, Ivo L. Hofacker, Peter F. Stadler, Michael T. Wolfinger. *Barrier trees of degenerate landscapes.* Z.Phys.Chem. **216**:155-173, 2002

Michael T. Wolfinger, W. Andreas Svrcek-Seiler, Christoph Flamm, Ivo L. Hofacker, Peter F. Stadler. *Efficient computation of RNA folding dynamics.* J.Phys.A: Math.Gen. **37**:4731-4741, 2004

## The Folding Algorithm

A sequence I specifies an energy ordered set of compatible structures $\mathfrak{S}(I)$:

$$\mathfrak{S}(I) = \{S_0, S_1, \ldots, S_m, \mathbf{O}\}$$

A trajectory $\mathfrak{T}_k(I)$ is a time ordered series of structures in $\mathfrak{S}(I)$. A folding trajectory is defined by starting with the open chain **O** and ending with the global minimum free energy structure $S_0$ or a metastable structure $S_k$ which represents a local energy minimum:

$$\mathfrak{T}_0(I) = \{\mathbf{O}, S(1), \ldots, S(t-1), S(t), S(t+1), \ldots, \mathbf{S_0}\}$$
$$\mathfrak{T}_k(I) = \{\mathbf{O}, S(1), \ldots, S(t-1), S(t), S(t+1), \ldots, \mathbf{S_k}\}$$

Transition probabilities $P_{ij}(t) = \mathcal{Prob}\{S_i \rightarrow S_j\}$ are defined by

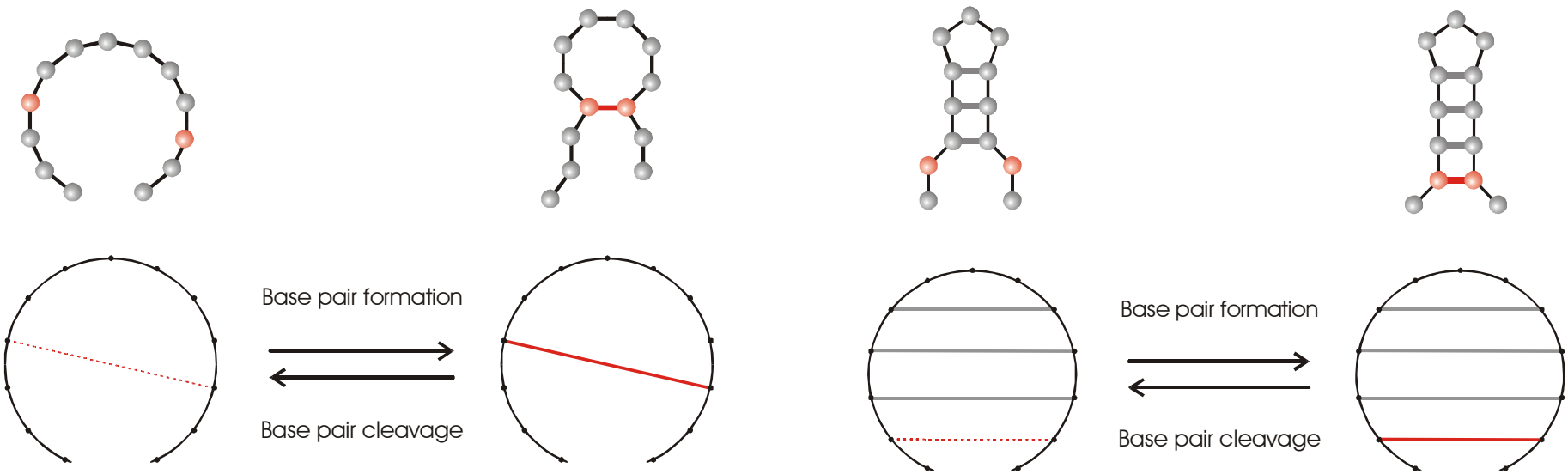
$$P_{ij}(t) = P_i(t)\, k_{ij} = P_i(t) \exp(-\Delta G_{ij}/2RT) / \Sigma_i$$

$$P_{ji}(t) = P_j(t)\, k_{ji} = P_j(t) \exp(-\Delta G_{ji}/2RT) / \Sigma_j$$

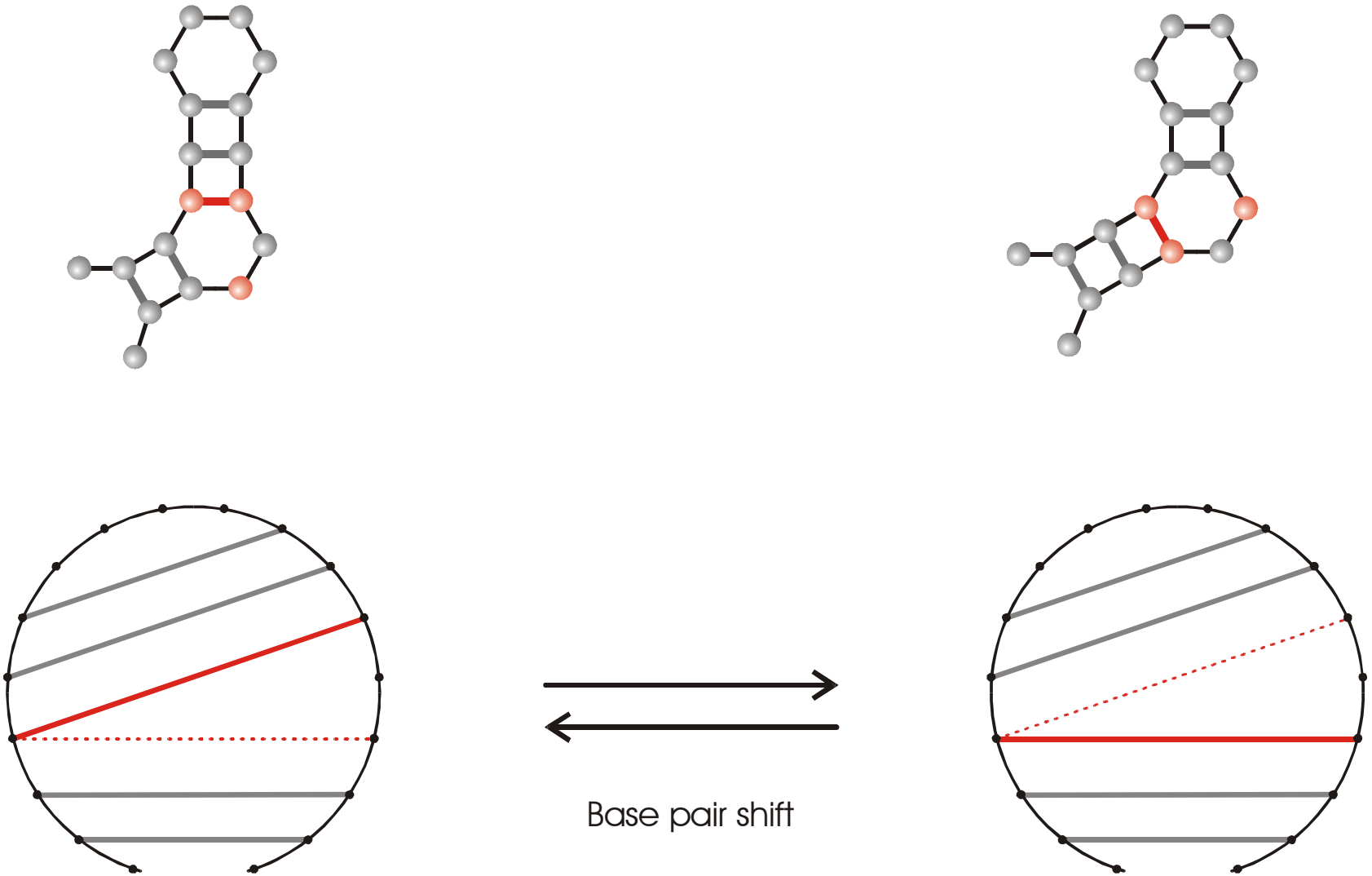
$$\Sigma_k = \sum_{k=1, k\neq i}^{m+2} \exp(-\Delta G_{ki}/2RT)$$

The symmetric rule for transition rate parameters is due to Kawasaki (K. Kawasaki, *Diffusion constants near the critical point for time depen-dent Ising models*. Phys.Rev. **145**:224-230, 1966).

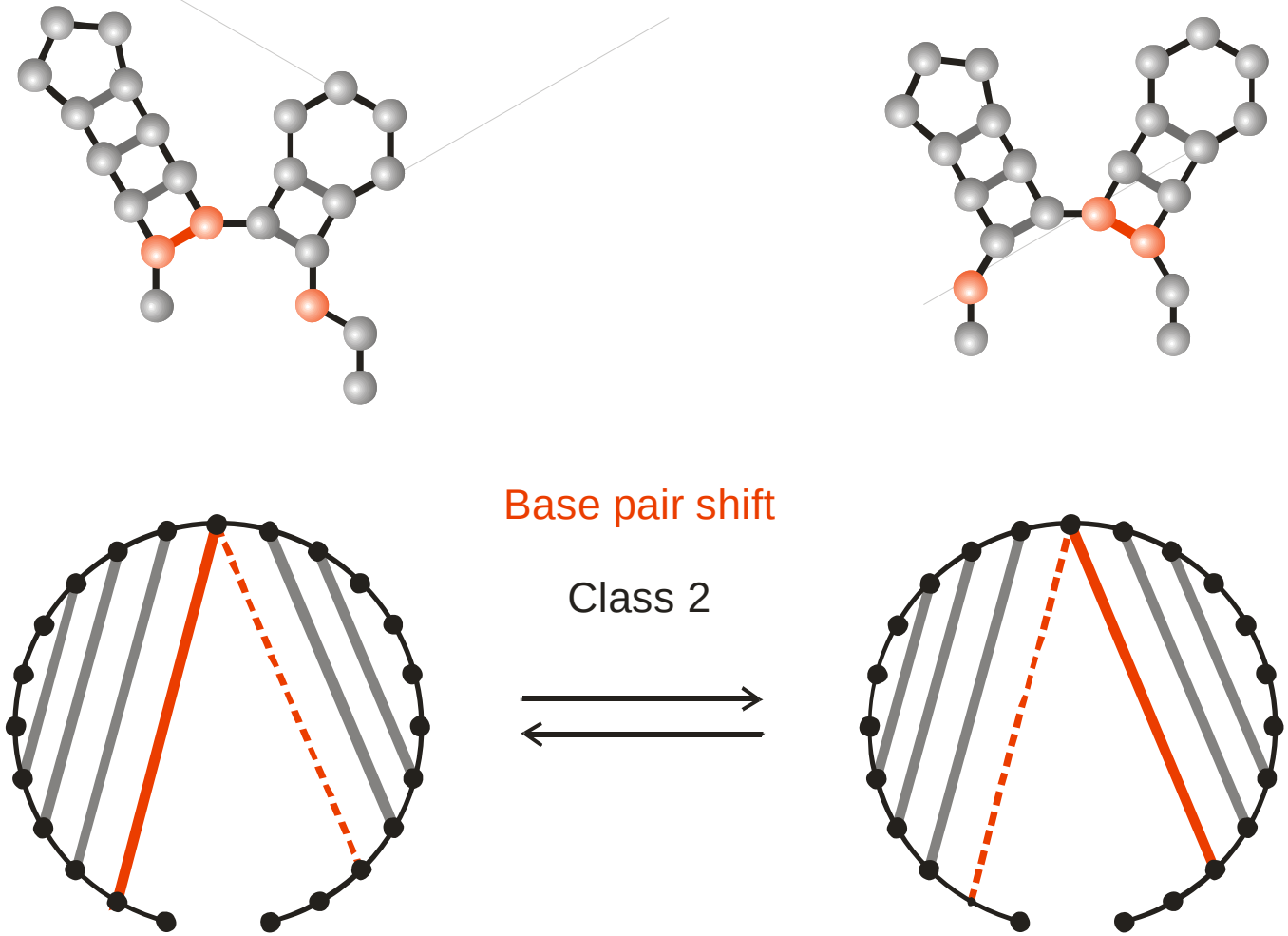
Formulation of kinetic RNA folding as a stochastic process




Base pair formation and base pair cleavage moves for nucleation and elongation of stacks

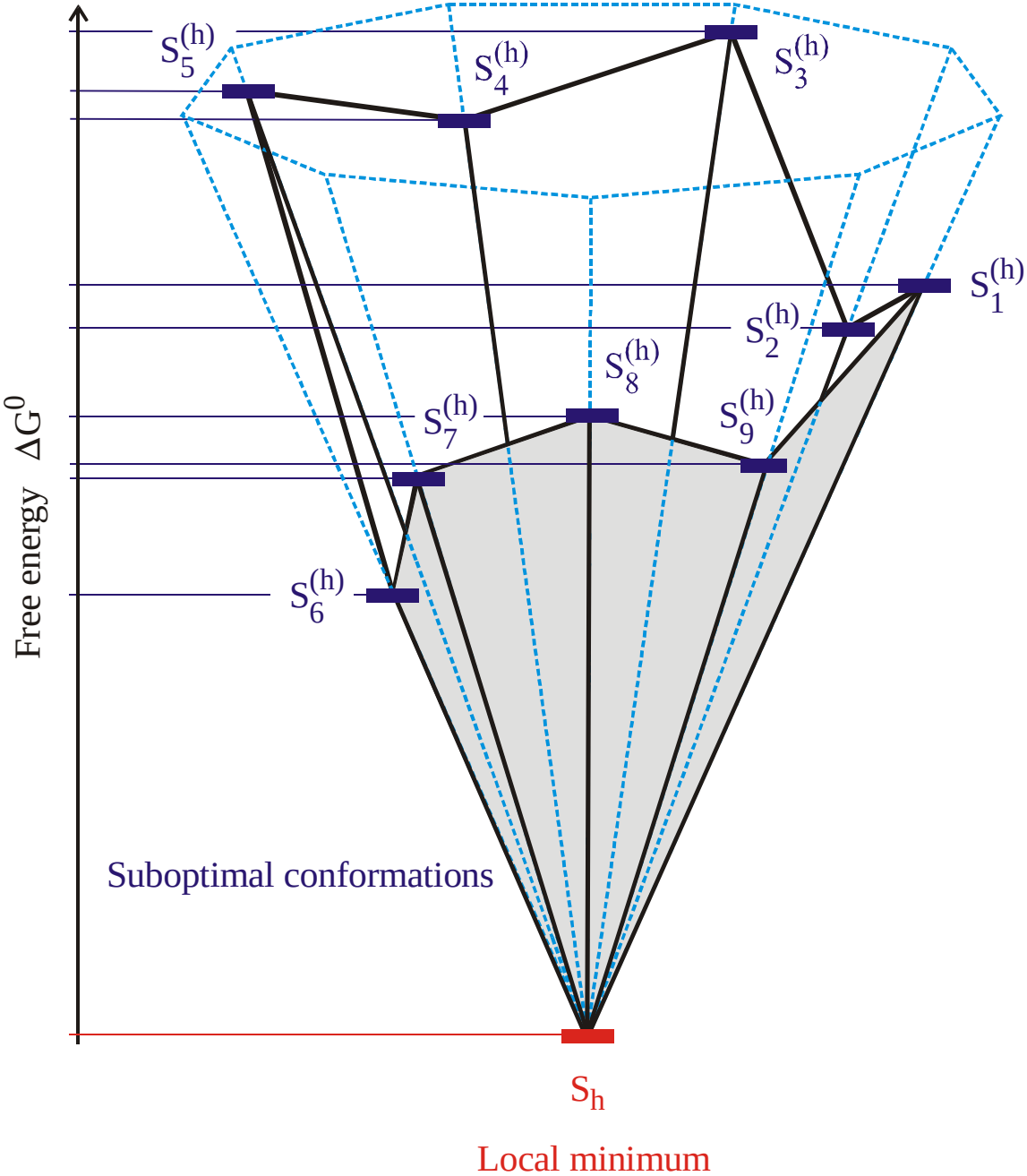


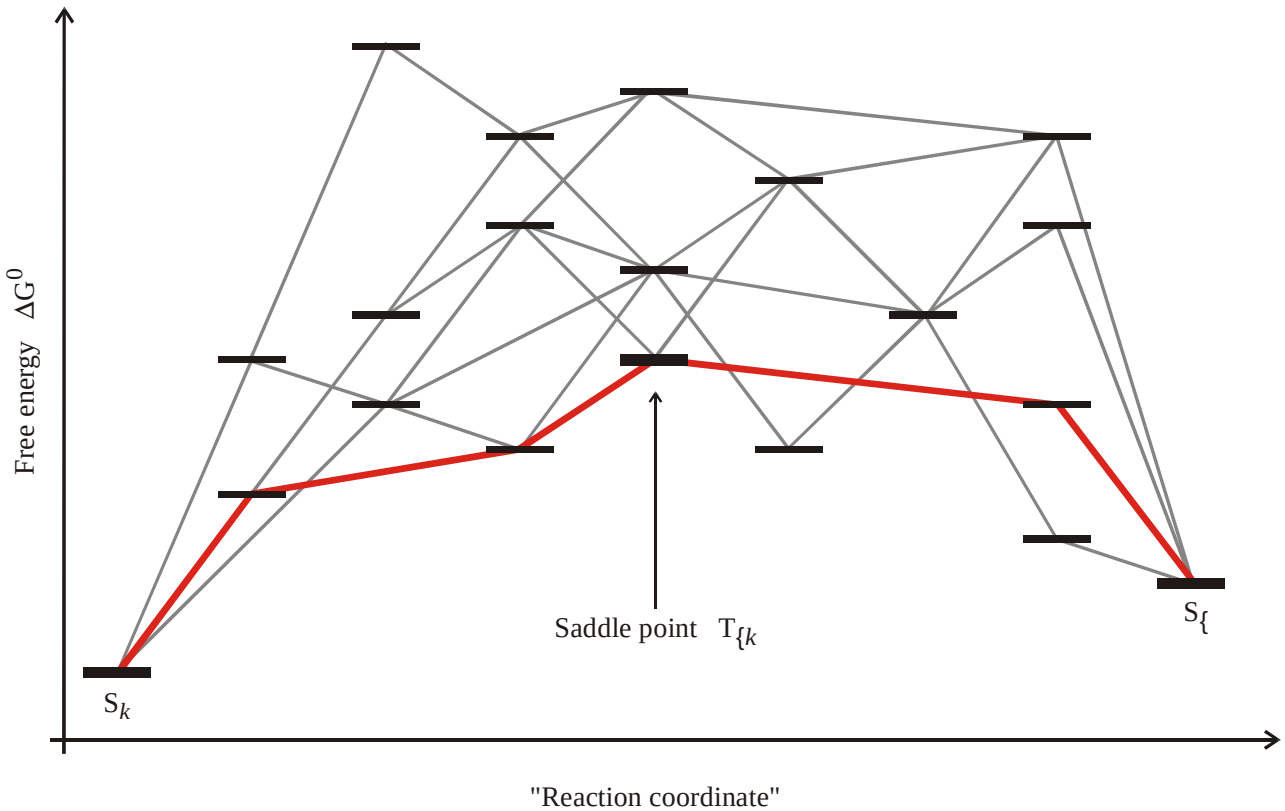

Base pair shift move of class 1: Shift inside internal loops or bulges

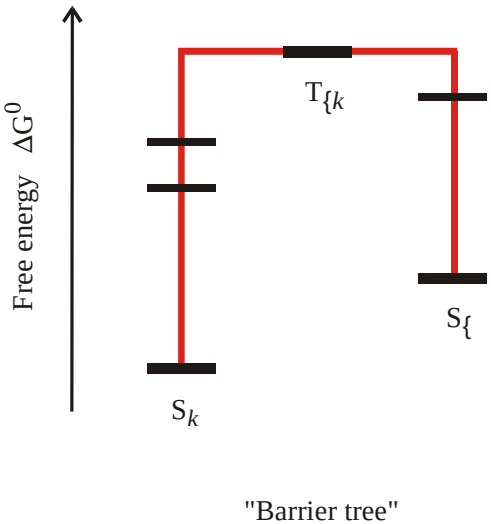



**Base pair shift move of class 2: Shift involving free ends**

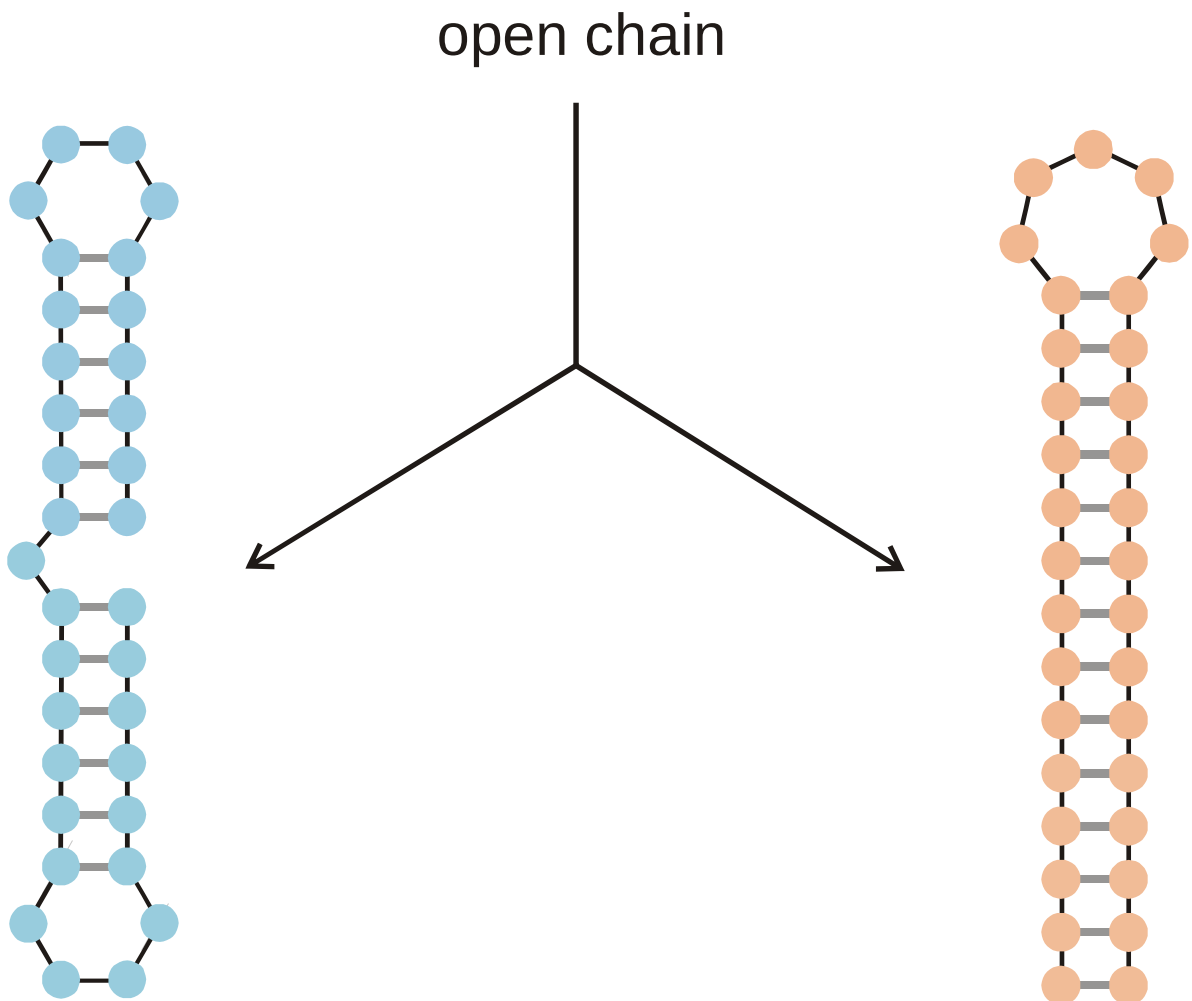
Search for local minima in conformation space



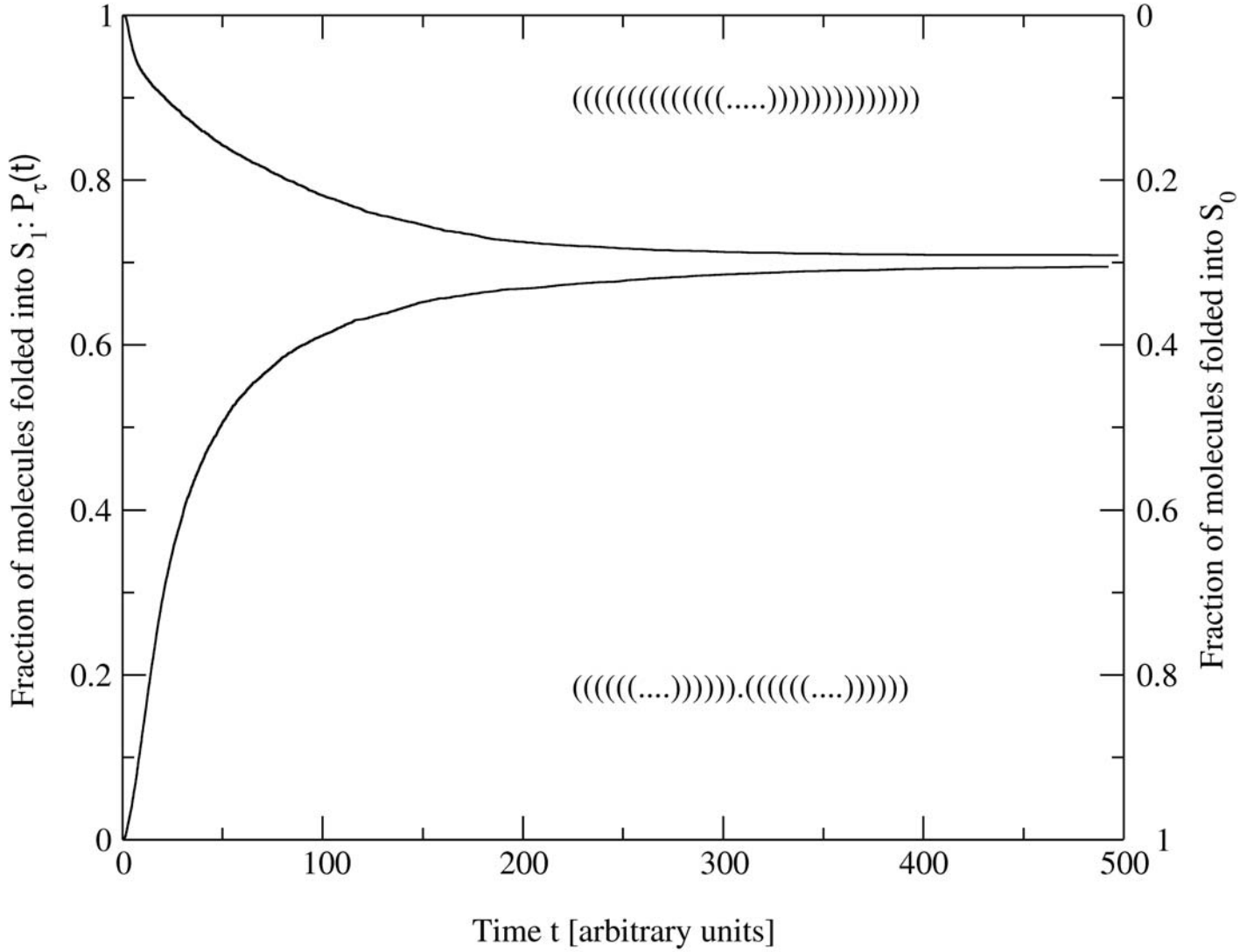


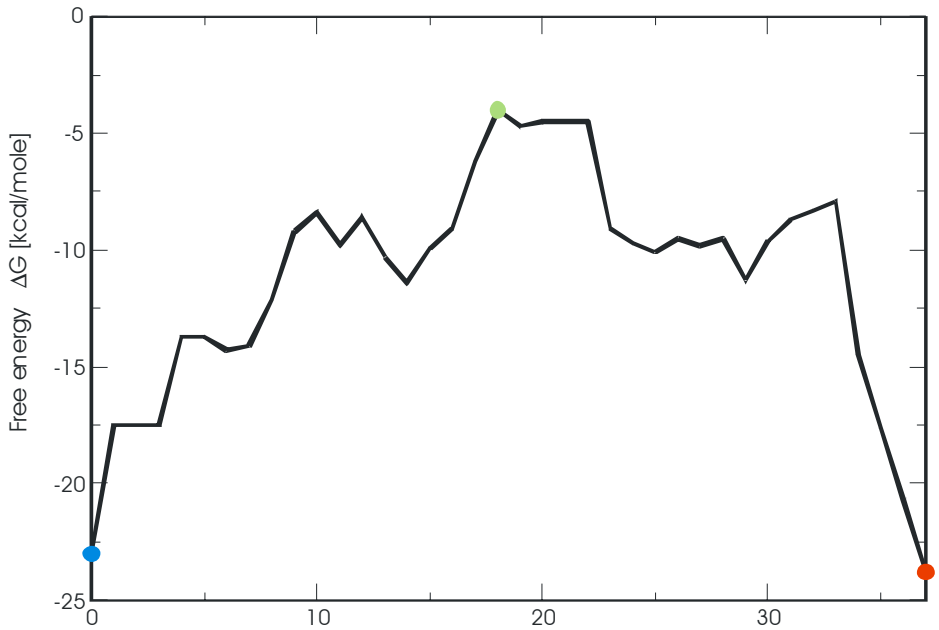

Definition of a ‚barrier tree‘




A nucleic acid molecule folding in two dominant conformations

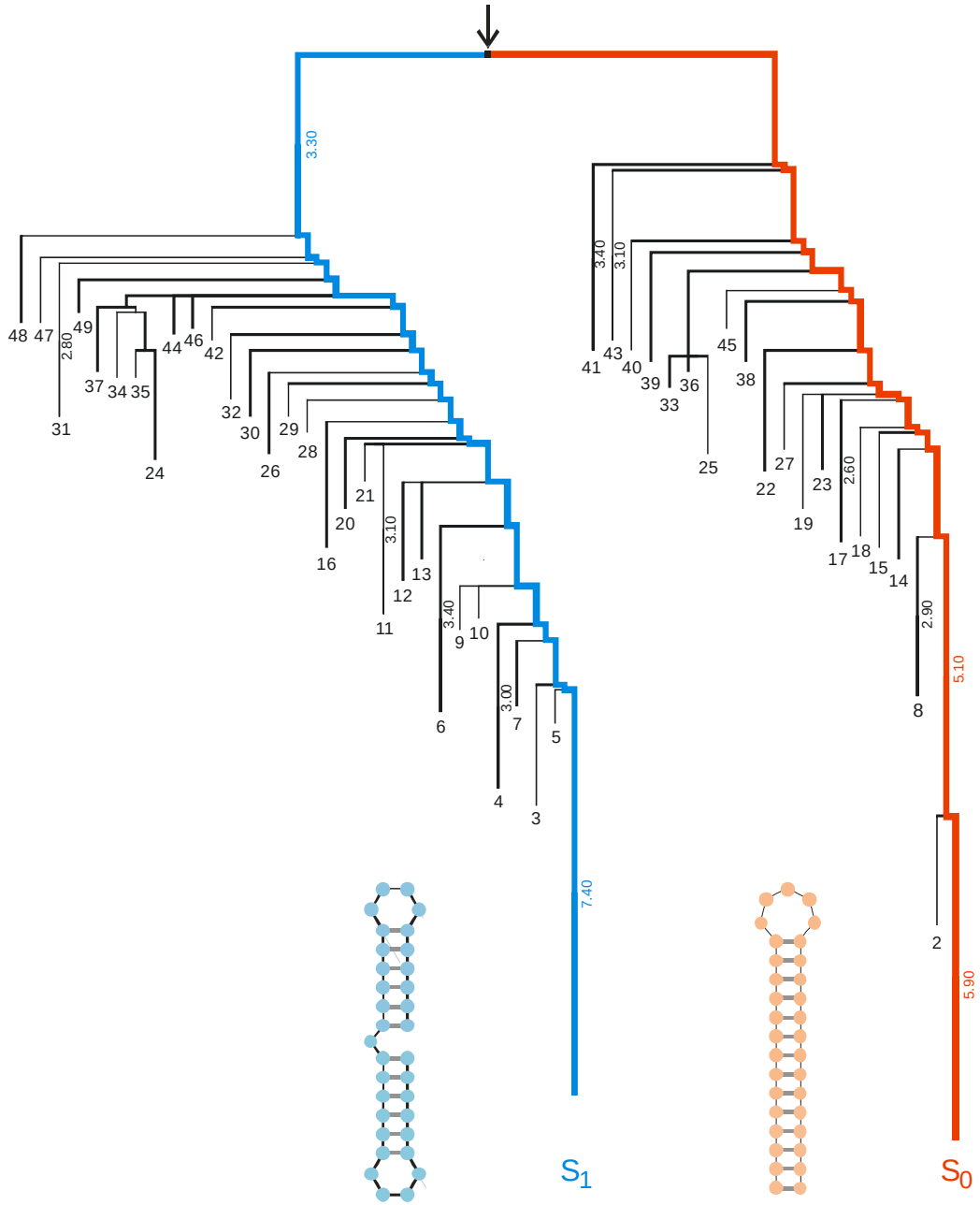



Folding dynamics of the sequence **GGCCCCUUUGGGGGCCAGACCCCUAAAAAGGGUC**



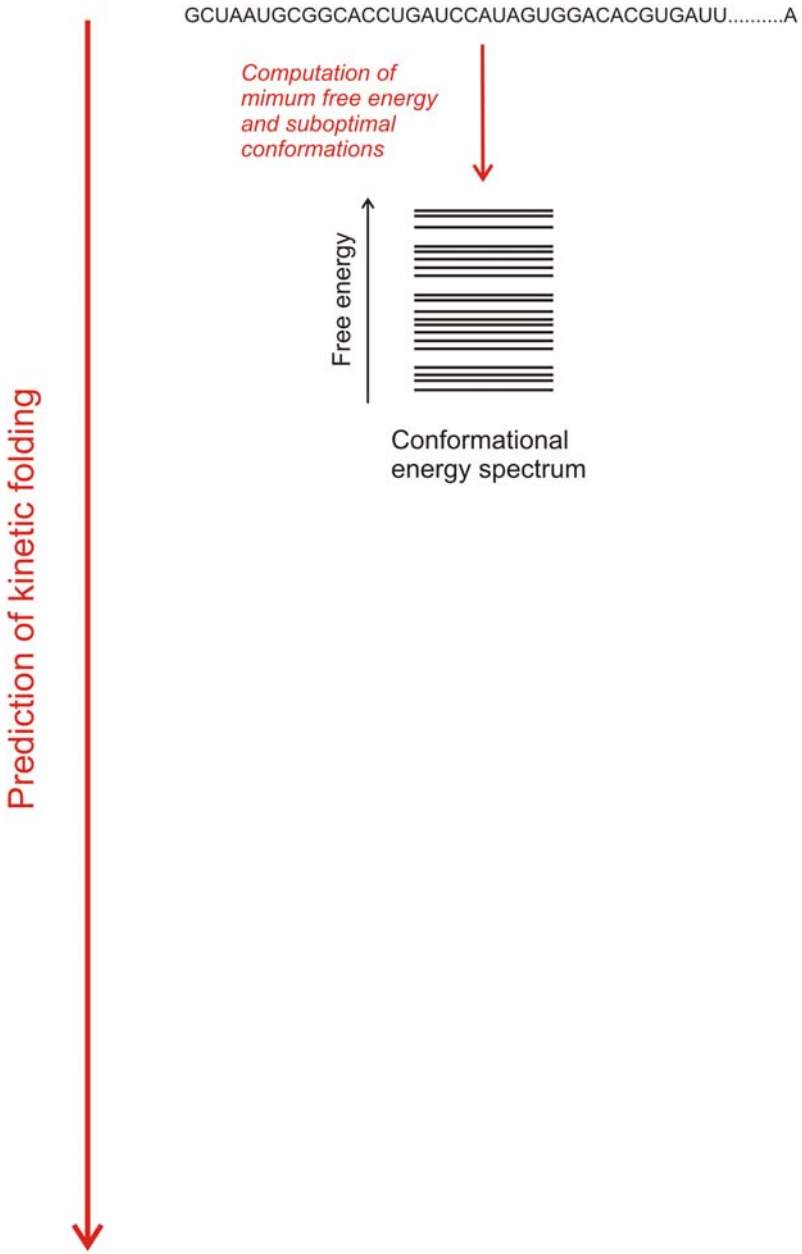

The folding path from $S_1$ to $S_0$

| Structure | ΔG [kcal/mole] |
|---|---|
| ((((((....)))))).((((((....)))))) | -23.00 |
| (((((.(...)))))).((((((....)))))) | -17.50 |
| ((((.((...)))))).((((((....)))))) | -17.50 |
| (((.(((...)))))).((((((....)))))) | -17.50 |
| .((.(((...))))).((((((....)))))) | -13.70 |
| .(.(((...))))).((((((....)))))) | -13.70 |
| .(.((((...)))).).((((((....)))))) | -14.30 |
| ...((((...)))).((((((....)))))) | -14.10 |
| ....(((...))).....((((((....)))))) | -12.10 |
| .....((...)).....((((((....)))))) | -09.20 |
| .....(.....).....((((((....)))))) | -08.40 |
| ..................((((((....)))))) | -09.80 |
| .........(.....).((((((....)))))) | -08.60 |
| .........(...)).((((((....)))))) | -10.30 |
| .........(((...)))((((((....)))))) | -11.40 |
| .......(((...)))).((((((....)))))). | -09.90 |
| ......((((...)))))((((....)))))... | -09.10 |
| .(...)((((...)))))((((....)))))... | -06.20 |
| .(....((((...))))).((((....)))))). | -04.00 |
| ((....((((...))))).((((....)))))) | -04.70 |
| (((...((((...))))).((....)))))) | -04.50 |
| ((((..((((...))))).((....)))))) | -04.50 |
| (((((.((((...))))).(....)))))) | -04.50 |
| ((((((((((...))))).........)))))) | -09.09 |
| ((((((((((...)))).........)))))) | -09.69 |
| ((((((((((...))).........)))))) | -10.09 |
| ((((((((((...)))(.....).)))))) | -09.50 |
| ((((((((((...)).(.....)))))))) | -09.80 |
| ((((((((((...))(......)))))))) | -09.50 |
| ((((((((((...))(....)))))))) | -11.30 |
| ((((((((((.....)(....)))))))) | -09.60 |
| (((((((((.(....)(....)))))))) | -08.70 |
| ((((((((((....).(....)))))))) | -08.30 |
| ((((((((((....).......)))))))) | -07.94 |
| ((((((((((..........)))))))))) | -14.48 |
| ((((((((((.........)))))))))) | -17.60 |
| ((((((((((.......)))))))))) | -20.70 |
| ((((((((((((.....)))))))))))) | -23.80 |

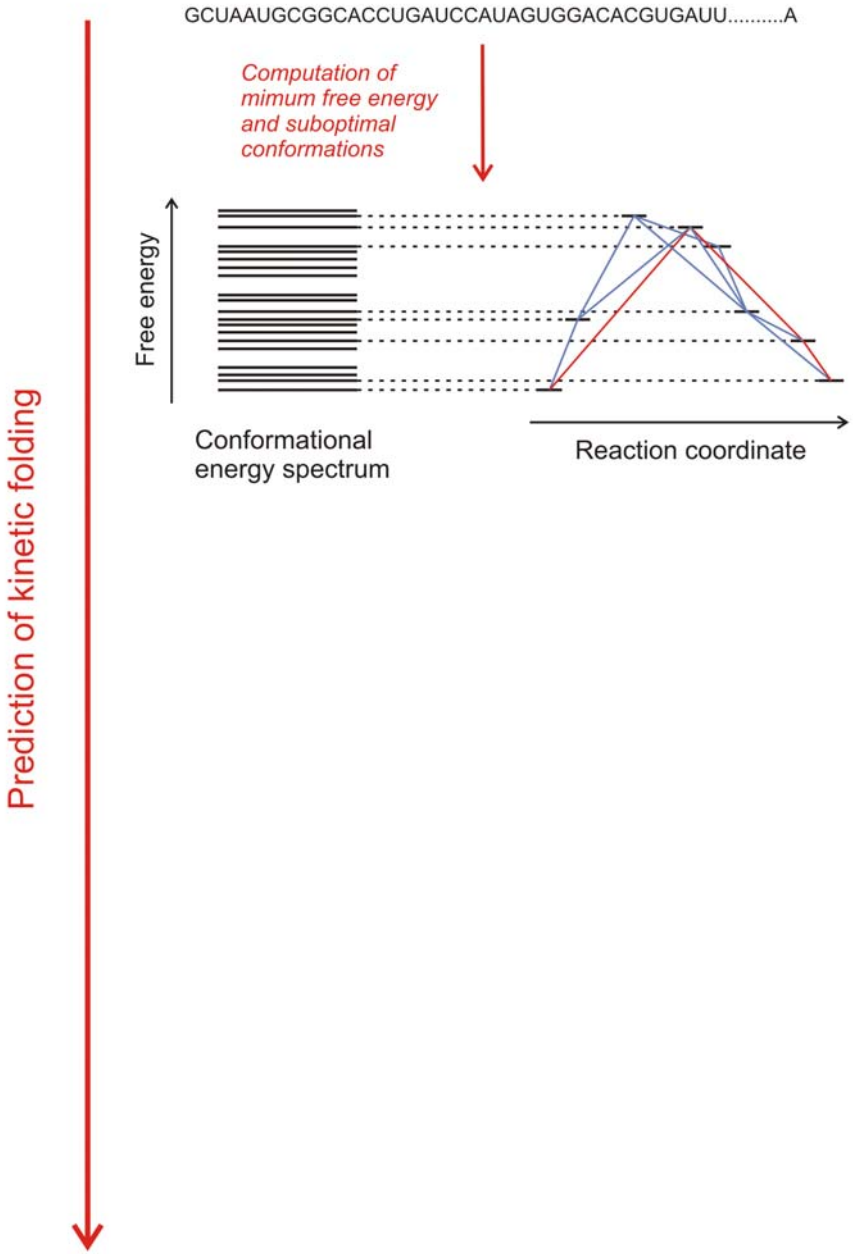



The barrier tree connecting $S_1$ and $S_0$

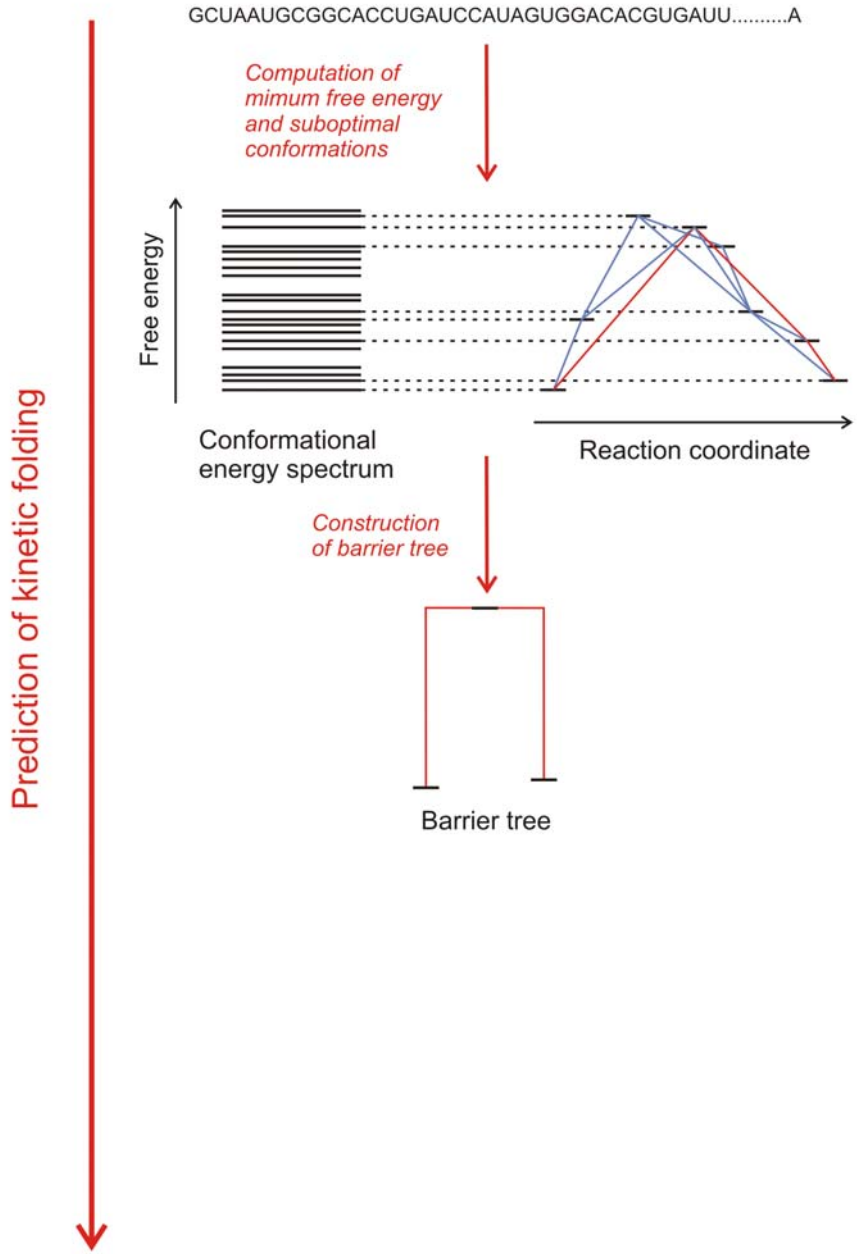

Prediction of RNA kinetic folding of secondary structures based on Arrhenius kinetics



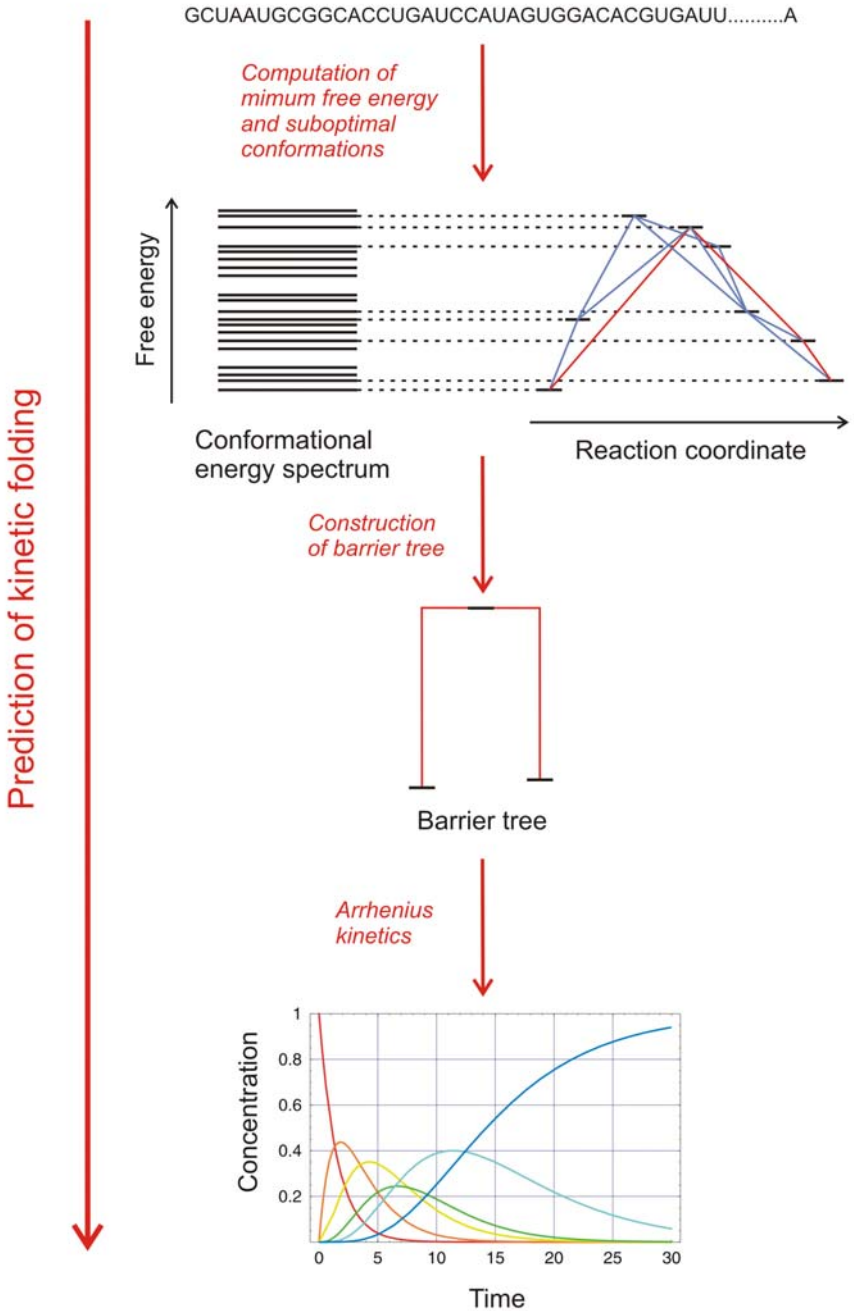

Prediction of RNA kinetic folding of secondary structures based on Arrhenius kinetics




Prediction of RNA kinetic folding of secondary structures based on Arrhenius kinetics

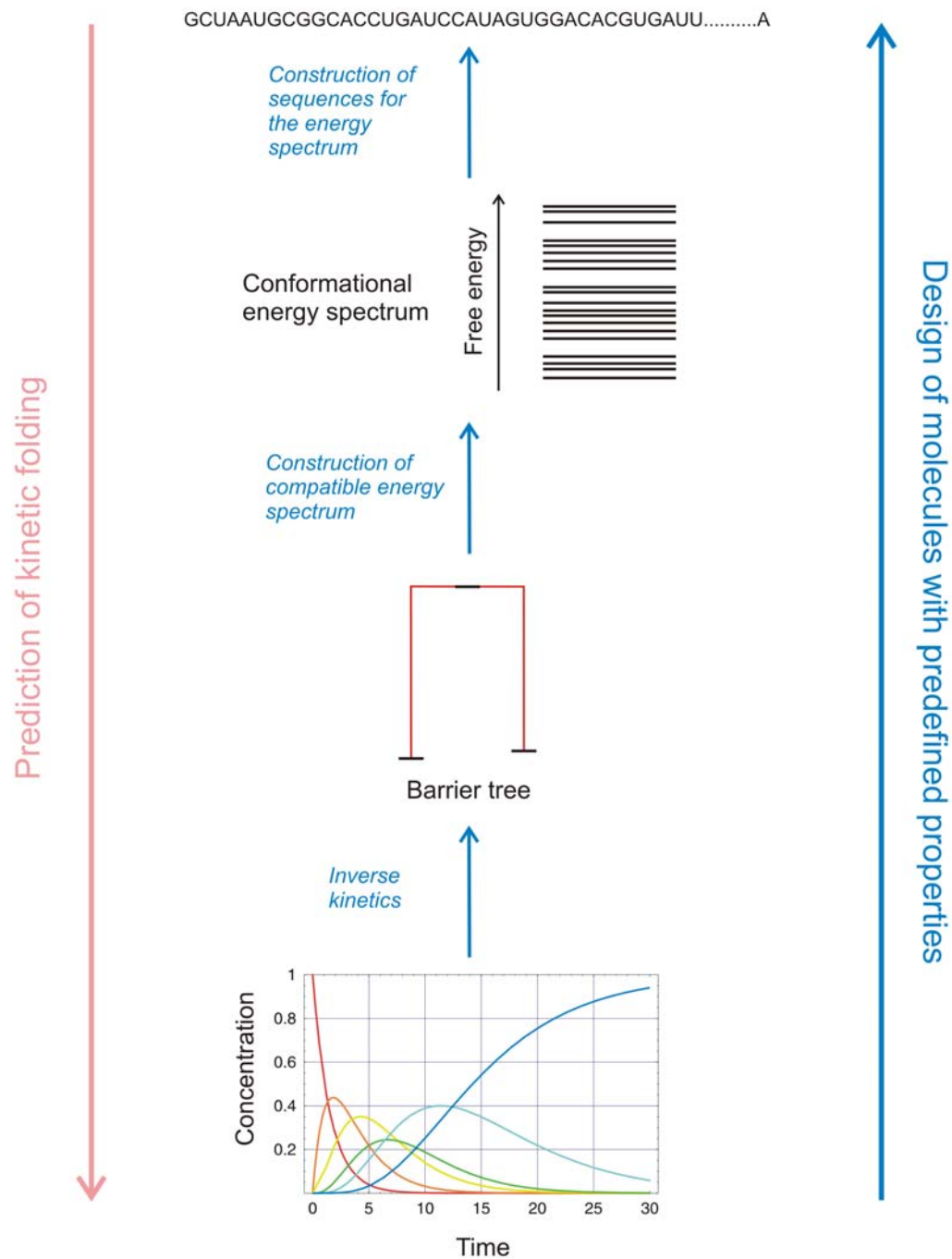


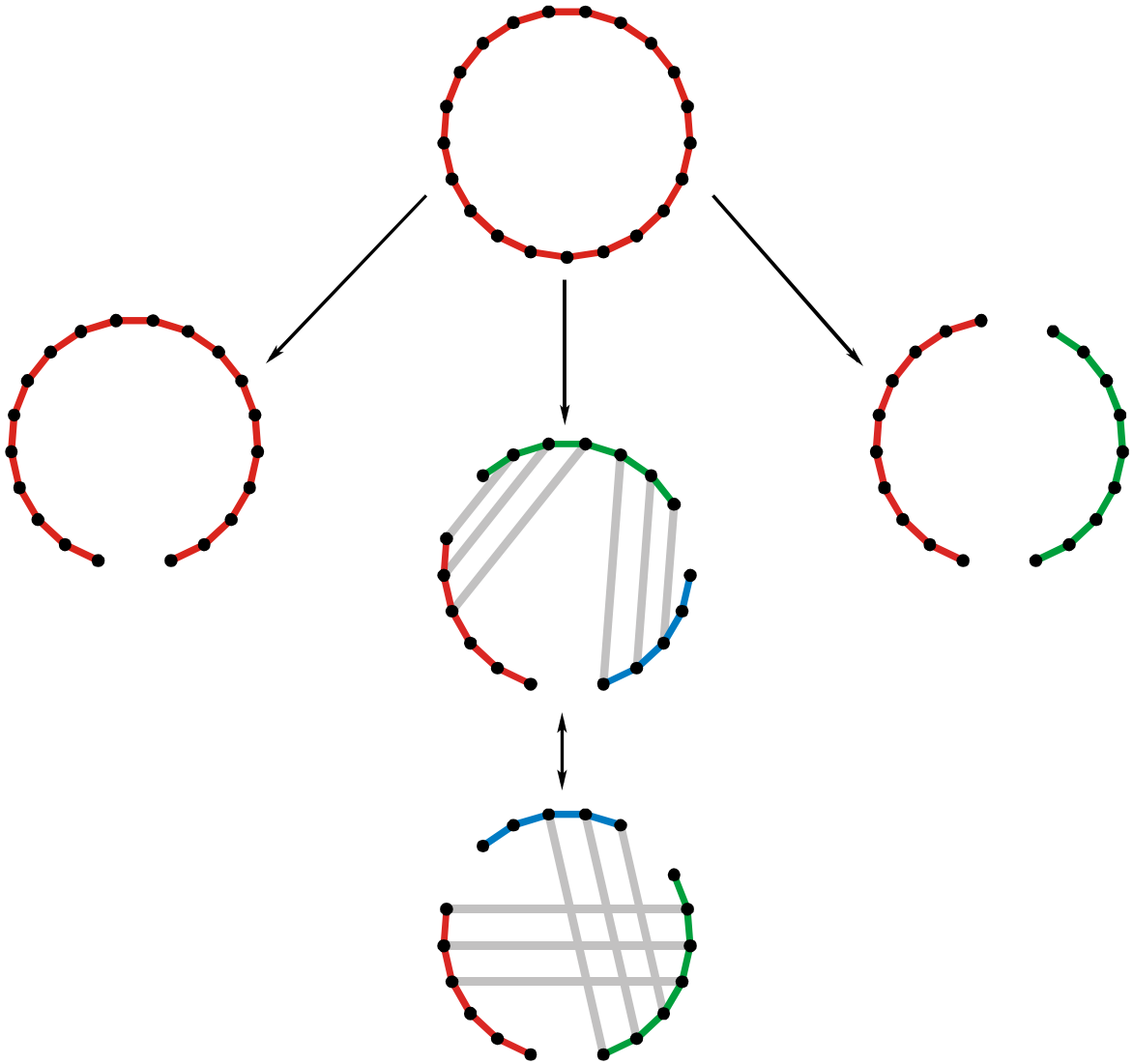

Prediction of RNA kinetic folding of secondary structures based on Arrhenius kinetics



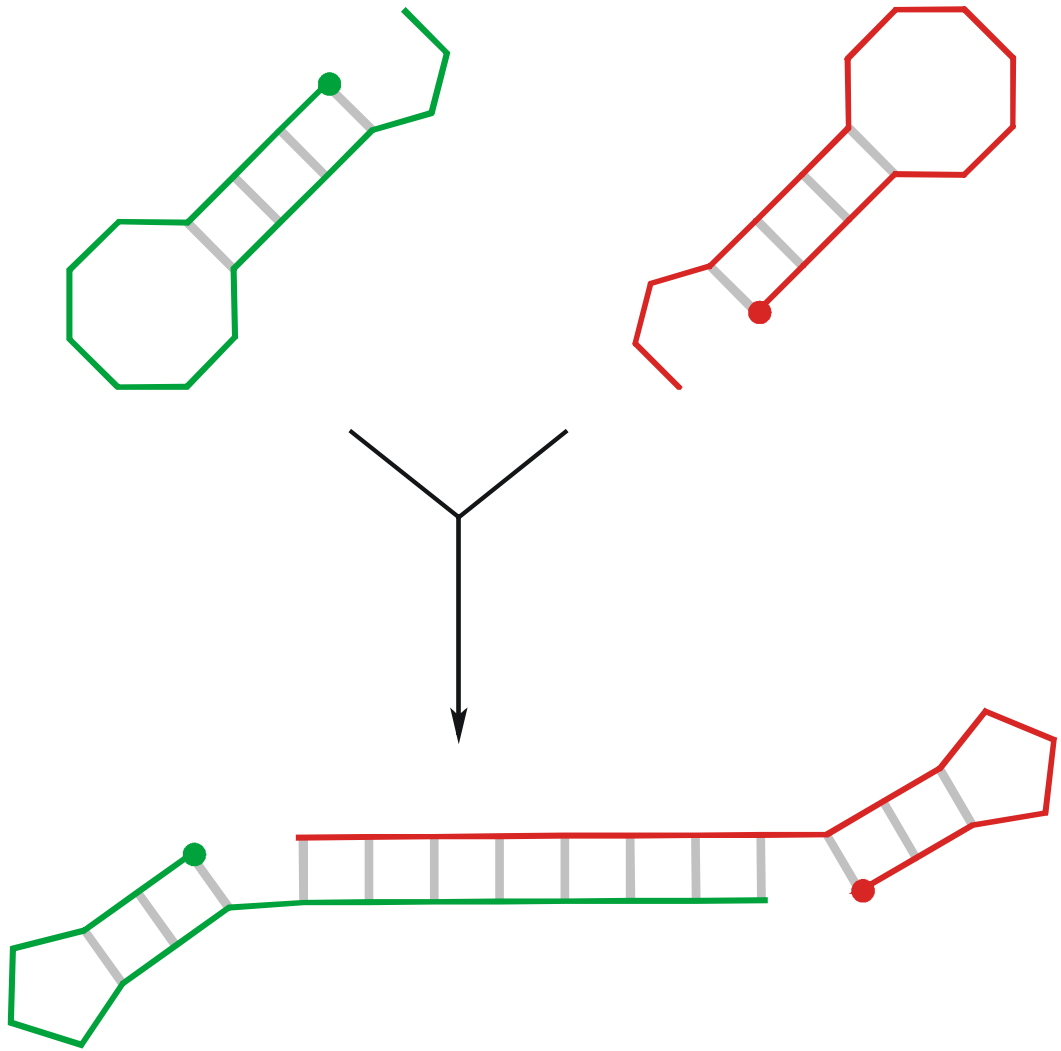

Prediction of RNA kinetic folding of secondary structures based on Arrhenius kinetics

Design of RNA molecules with
with predefined folding kinetics

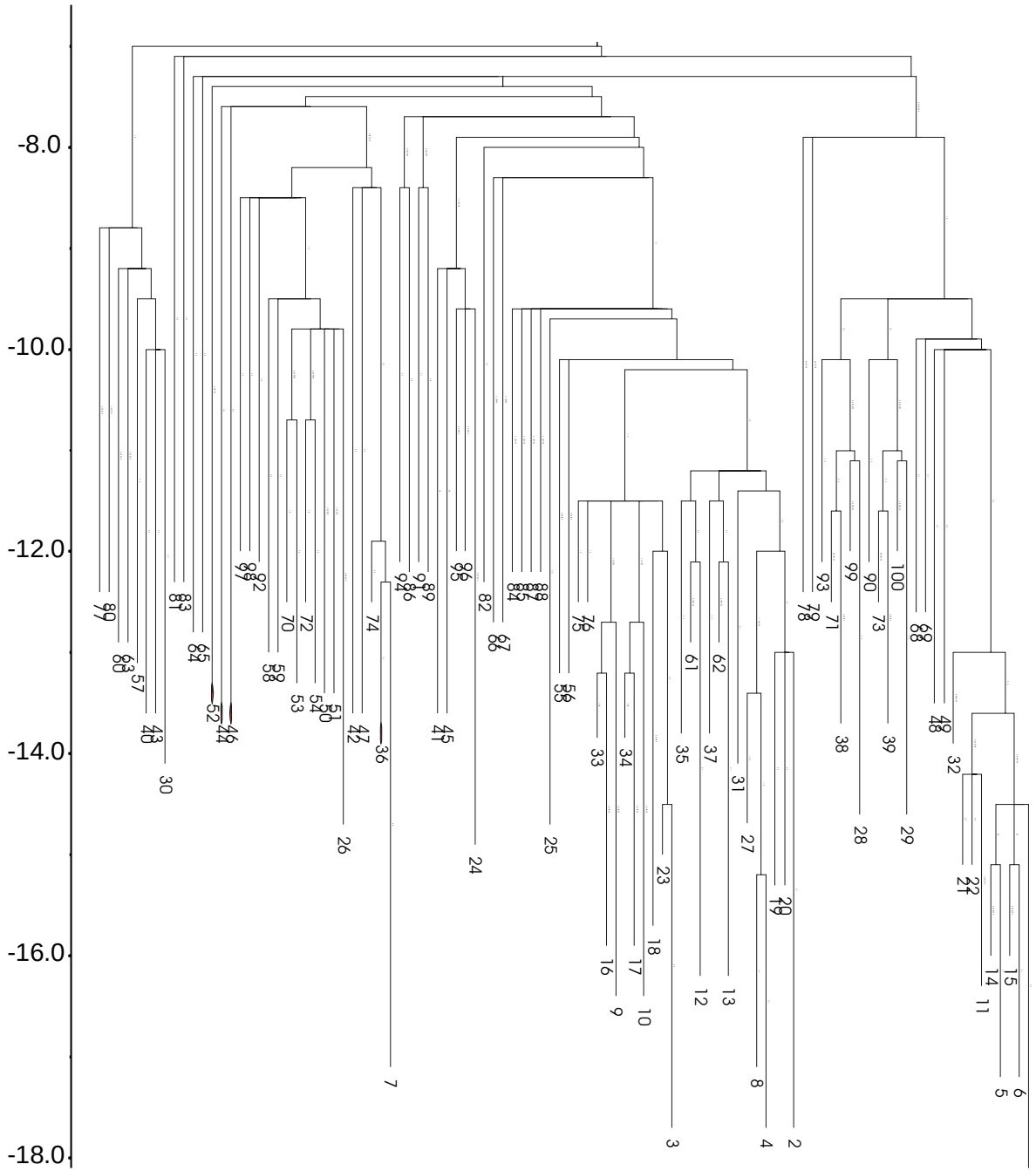




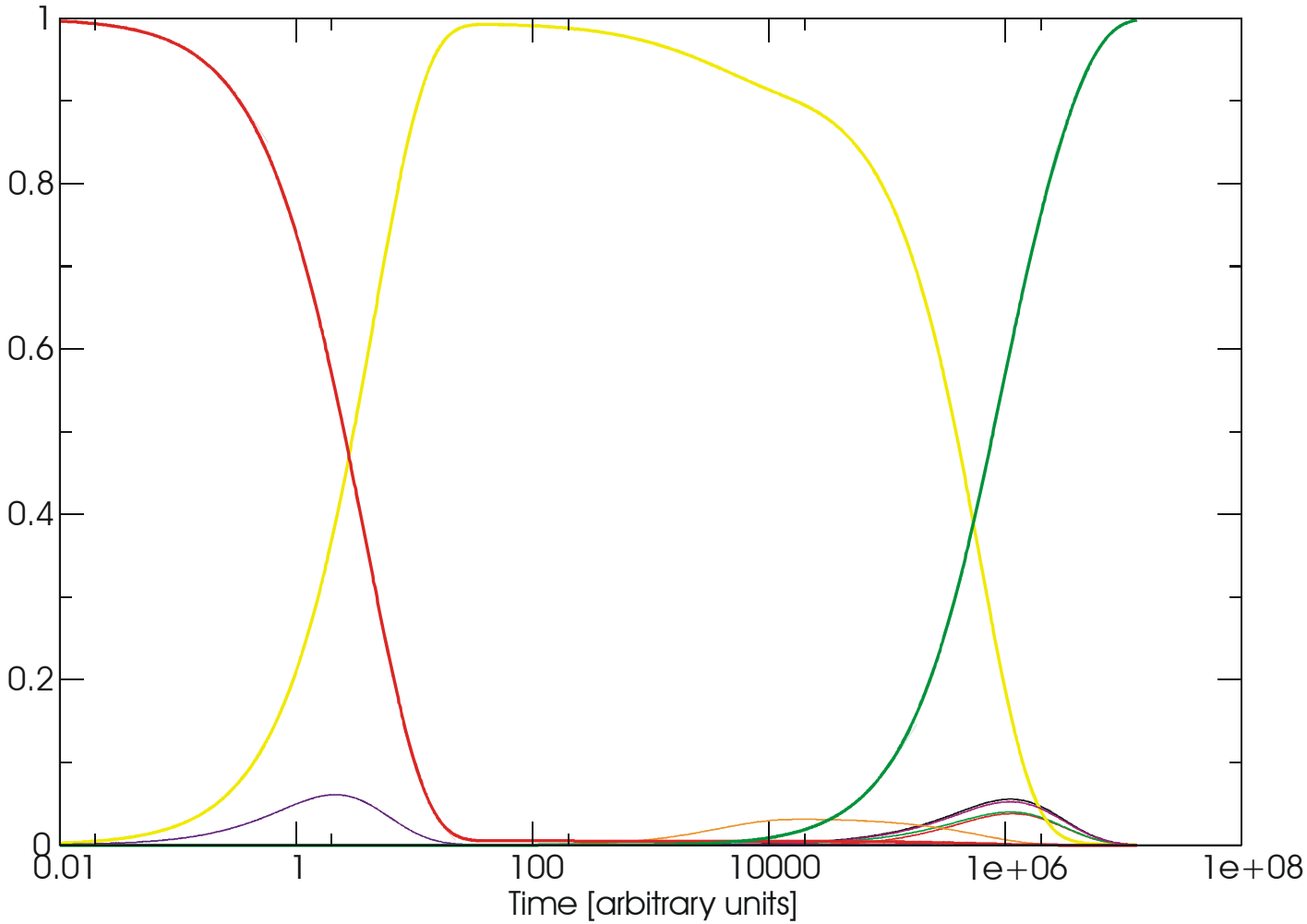
Cofolding two or three nucleic acid molecules



An example for ‘symmetric’ cofolding of two molecules




Cofolding tree




Cofolding kinetics