Coarse grained RNA folding kinetics

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Why are we interested in this?

- RNAs with (long term stable) metastable structure states
- different functions coupled by change in conformation
- examples: RNA switches (thermometers, riboswitches, ...)

Questions arise:

- How does the structure (state) population density looks like in equilibrium?
- Starting from an initial population density, does the system reach its equilibrium directly (traps)?
- ...

RNA folding process in terms of a Markov process

- state space of allowed conformations (secondary structures)
- move-set defining elementary transitions between states (insert/deletion of base pairs)
- transition rates of allowed transitions (Metropolis/Kawasaki rule)

The master equation

$$\frac{d}{dt}\vec{p}(t) = \mathbf{R}\vec{p}(t) \quad \text{with formal solution} \quad \vec{p}(t) = e^{t\cdot\mathbf{R}} \cdot \vec{p}(0).$$

What we need is

- the initial population density $\vec{p}(0)$
- the transition rate matrix $\mathbf{R} = (r_{xy})$
- somebody who actually computes $\vec{p}(t)$

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- the transition rate matrix $\mathbf{R} = (r_{xy})$
- something that actually computes $\vec{p}(t)$ (`treekin`)

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- number of states grows exponentially with sequence length
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- Partition the state space into macrostates
- compute effective transition rates between the partitions
- diagonalize the rate matrix
- compute eigenvalues and eigenvectors
- solve master equation

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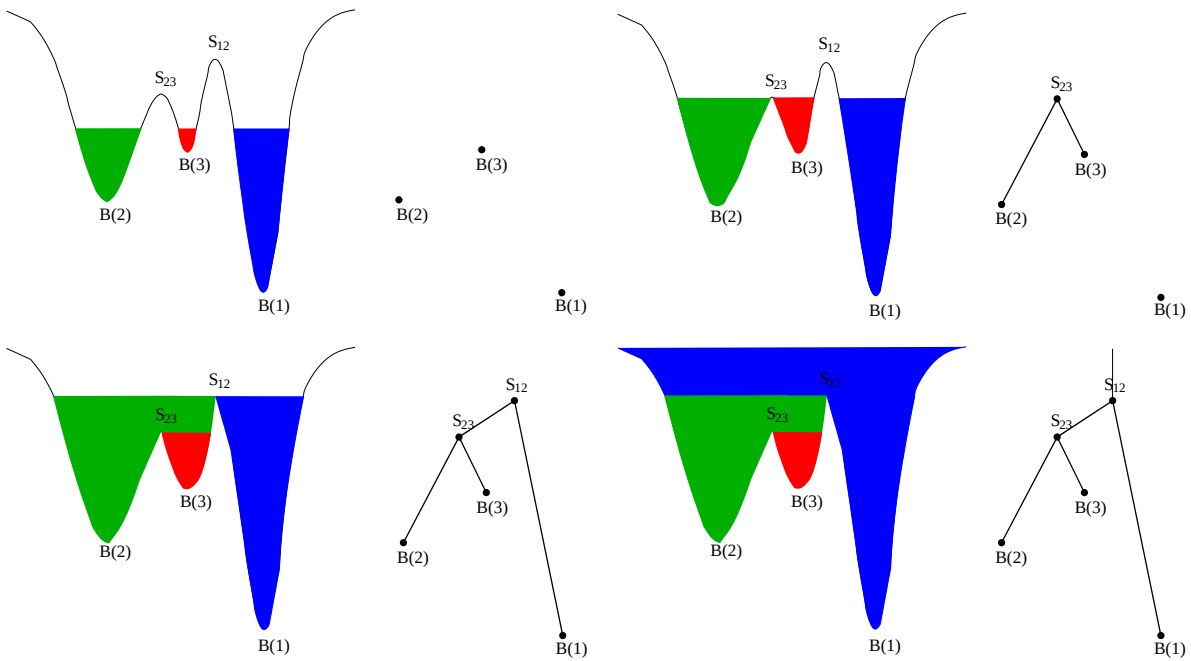
How to partition the state space anyway?

The flooding algorithm (Flamm et al. 2002)

- energy sorted list of structure states
- identification of all local minima
- identification of minimal saddle points connecting them
- assigning each structure to its respective gradient basin



How to obtain the rate matrix $\mathbf{R} = (r_{xy})$?

Estimation from rates between micro states k_{yx}:

$$\begin{aligned} r_{\beta\alpha} &= \sum_{x\in\alpha}\sum_{y\in\beta} k_{yx}\mathrm{Prob}[x|\alpha] \\ &\approx \sum_{x\in\alpha}\sum_{y\in\beta} k_{yx}\cdot\frac{e^{-\frac{E(x)}{kT}}}{Q_\alpha} \end{aligned}$$

with:

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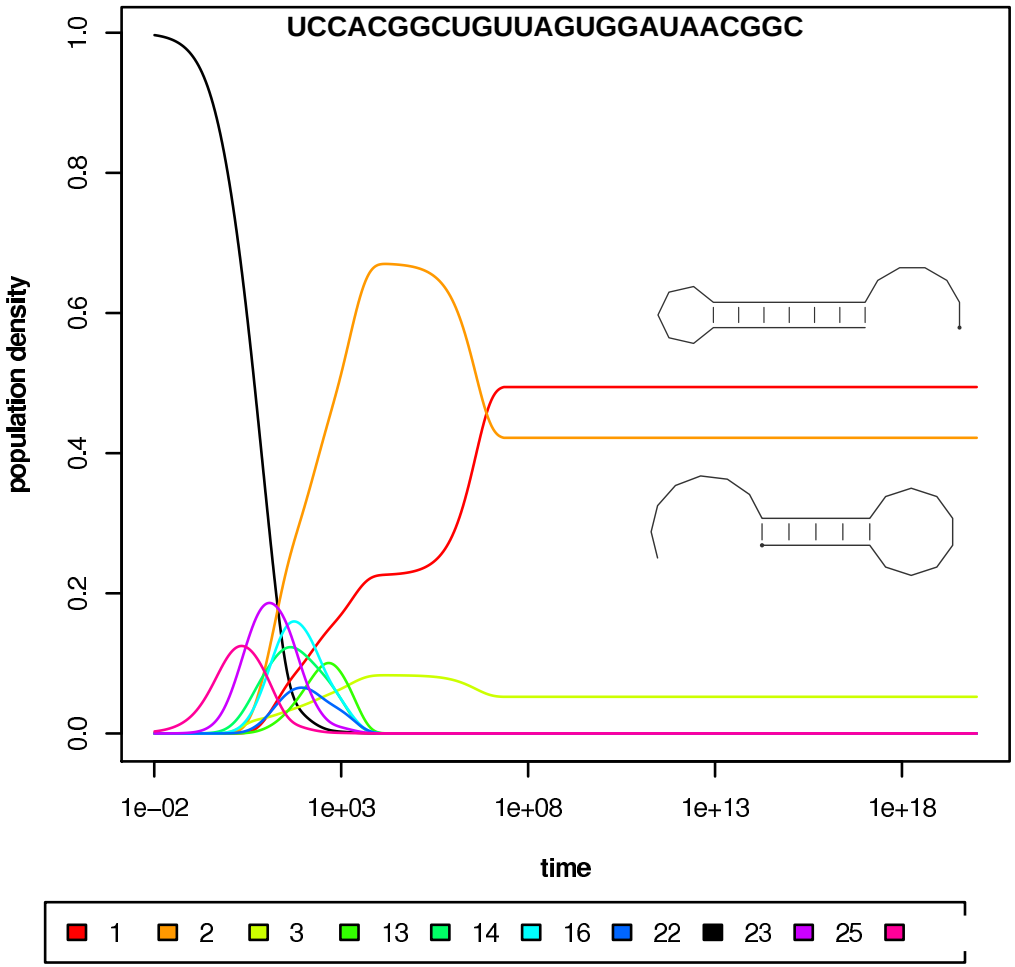
Limited to RNA molecules no longer than some 100 nt

small example with length = 25 nt, initial state = unfolded chain



What about sampling secondary structures from the state space according their Boltzmann probability to estimate partition functions and transition rates between macrostates?

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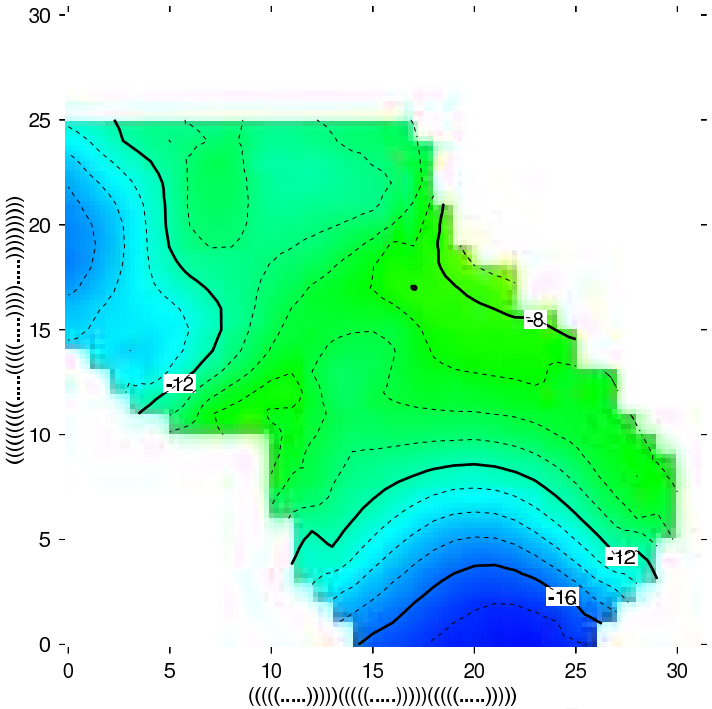
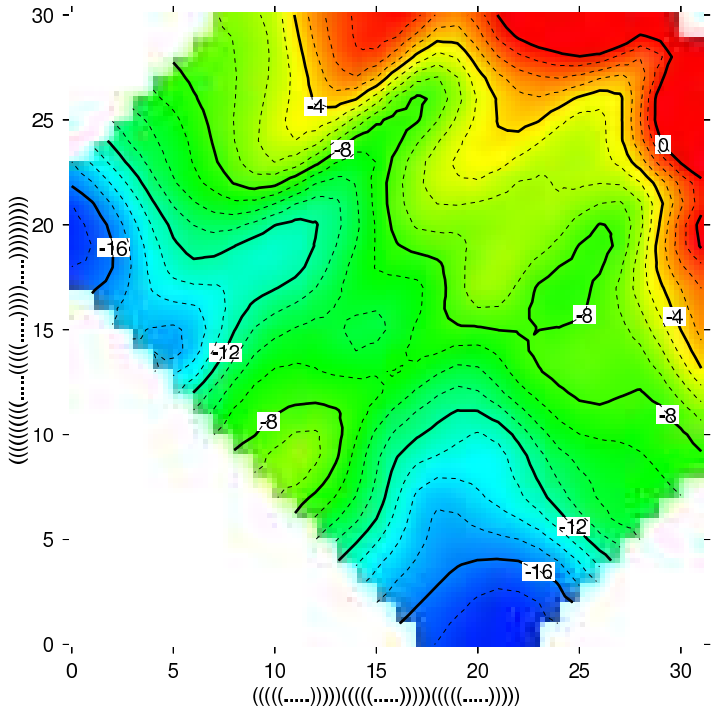
Sampling may not explore the landscape sufficiently!

MFE representatives with respect to two reference structures

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



Landscape projection obtained by sampling 10^7 structures from the ensemble



Landscape projection obtained by `RNA2Dfold`

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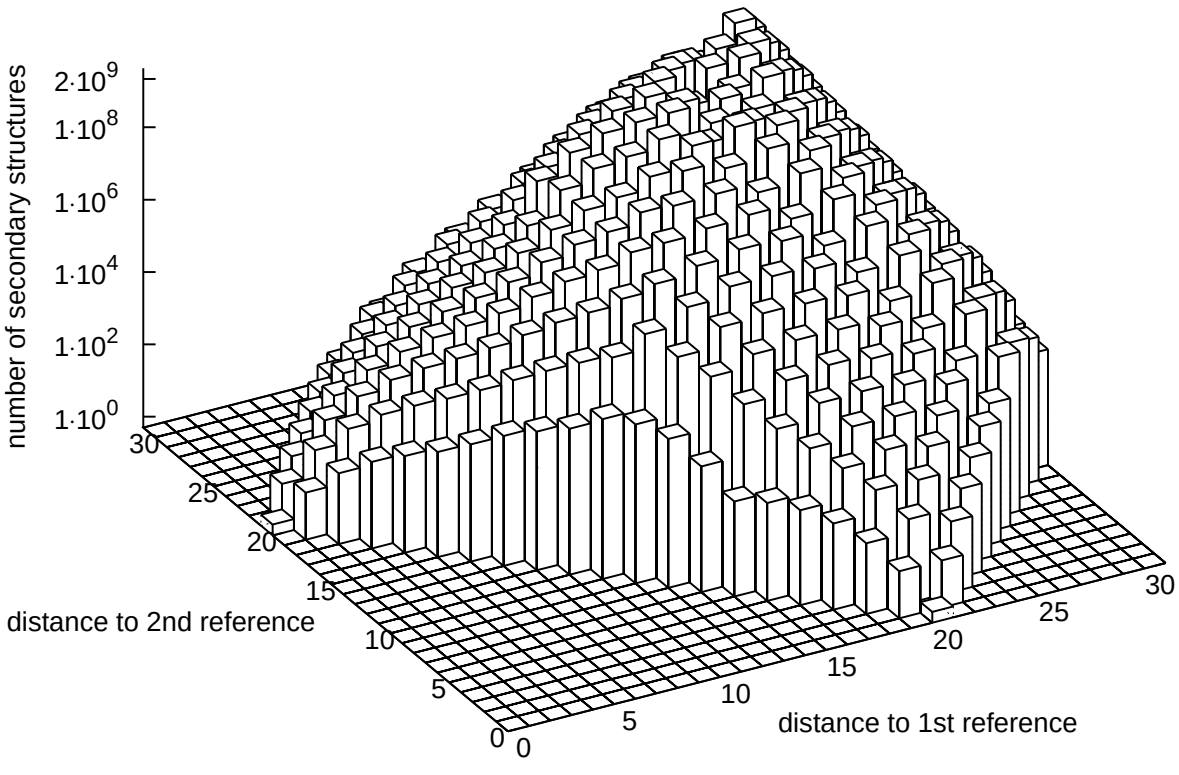

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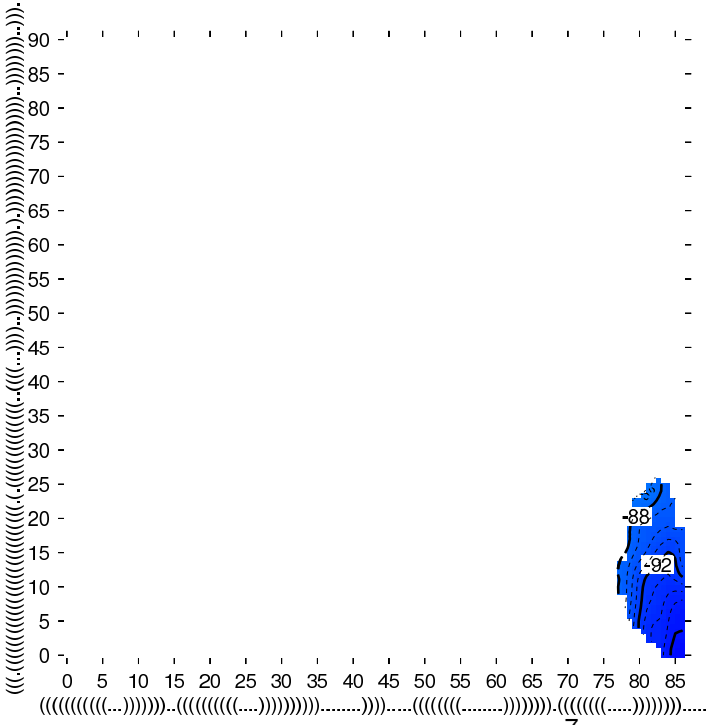

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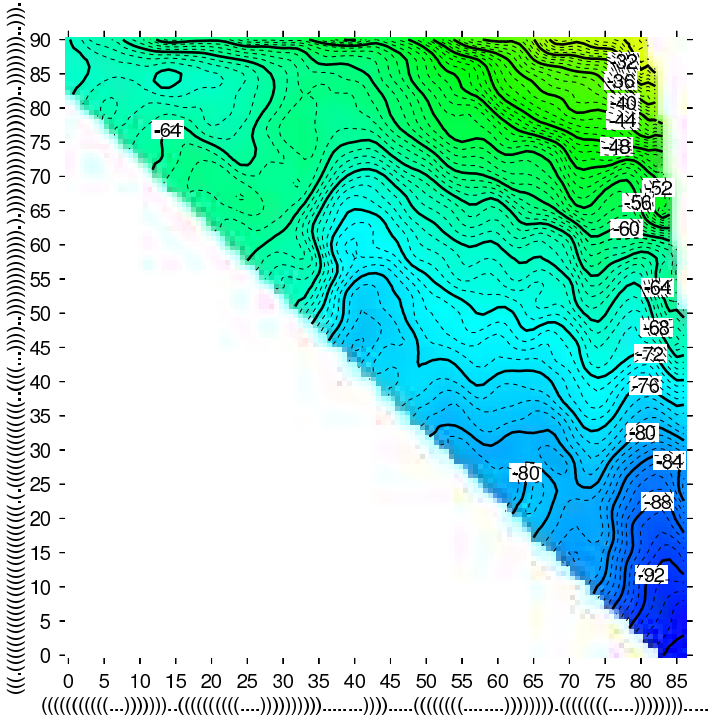

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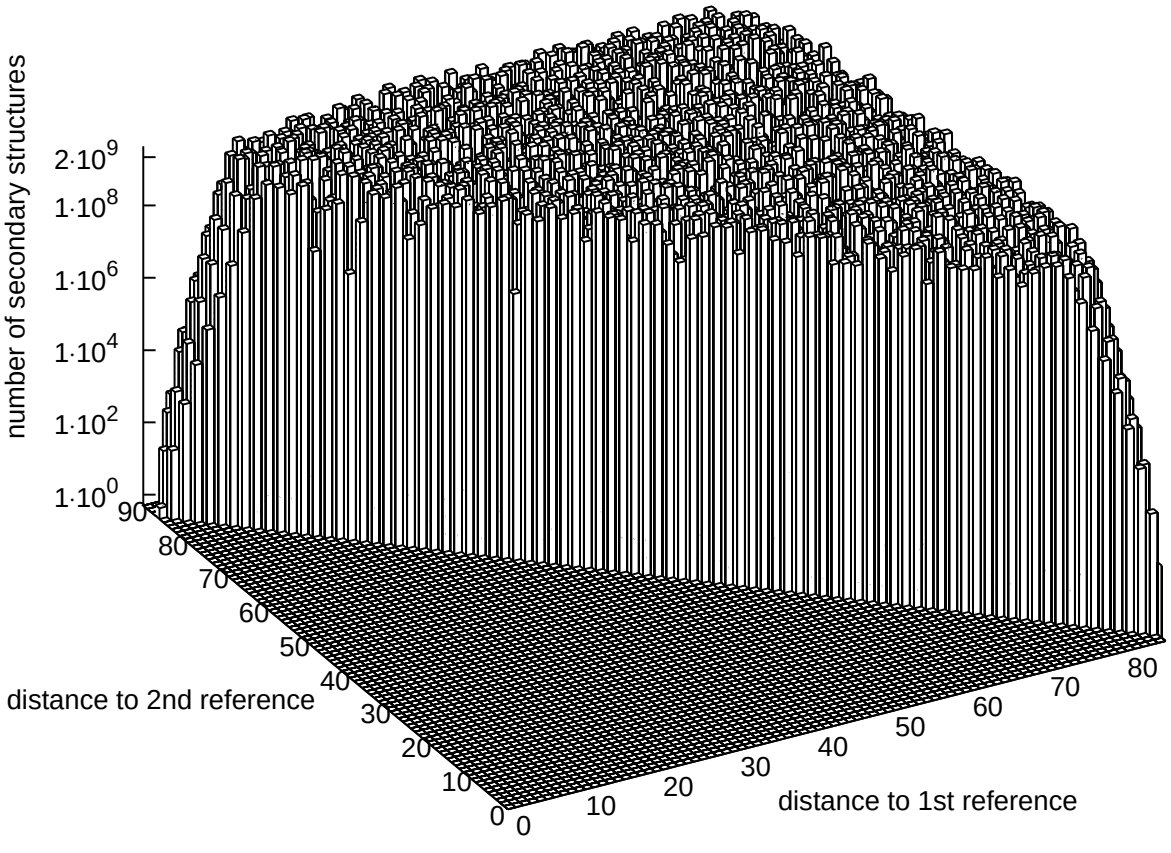

Simulating folding dynamics becomes easier with prior knowledge

- MFE structure is most probable in equilibrium (1^{st} reference)
- sometimes a metastable state is known (2^{nd} reference)
- partitioning into distance classes (κ, λ-neighborhoods) wrt. two reference structures
- MFEs and partition functions can be computed in $\mathcal{O}(n^7)$
- computable for sequence up to 500 nt on modern machines
- Boltzmann sampling from each κ, λ-neighborhood

How to obtain the rate matrix $\mathbf{R} = (r_{xy})$?

Approximation of the macro rates by Boltzmann sampling from each distance class S_α:

$$r_{\beta\alpha} \approx \frac{1}{|S_\alpha|} \sum_{x \in S_\alpha} \sum_{y \in \beta \cap \mathcal{N}(x)} k_{yx}$$

with:

$$k_{yx} = \begin{cases} e^{-\frac{E(y)-E(x)}{kT}} & \text{if } E(x) < E(y) \\ 1 & \text{otherwise.} \end{cases}$$

- detailed balance must not be effected by sampling errors
- sample size of 1000 per macro state proved sufficient for the examples tested

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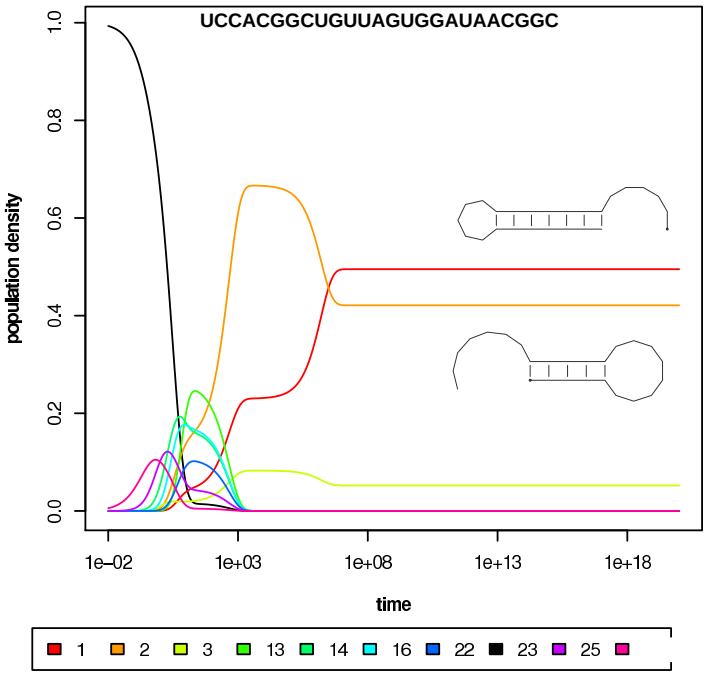
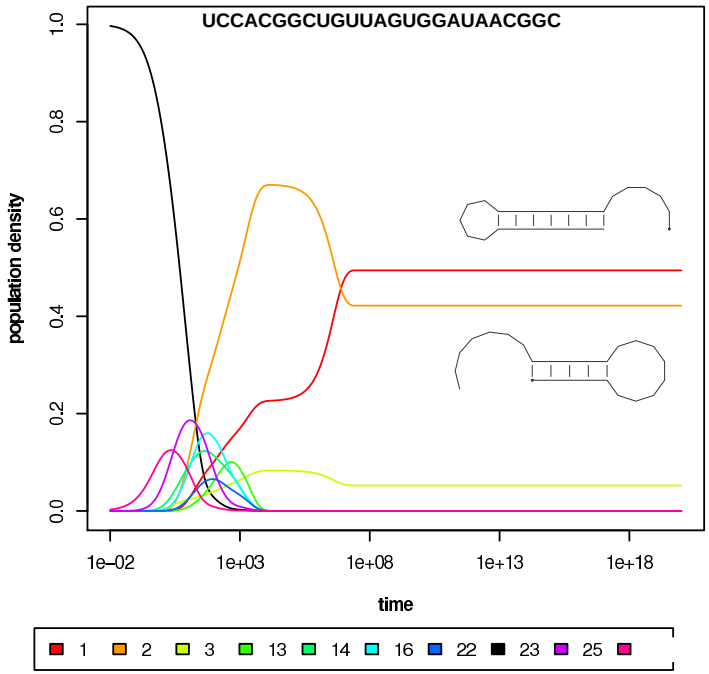


1000 samples per macrostate



Full kinetic with 10000 microstates sorted into same distance classes

This method may also work for other partitionings of the state space

RNA shapes

- no RNAshapes stochastic backtracking available
- expected behavior can be computed for previous example

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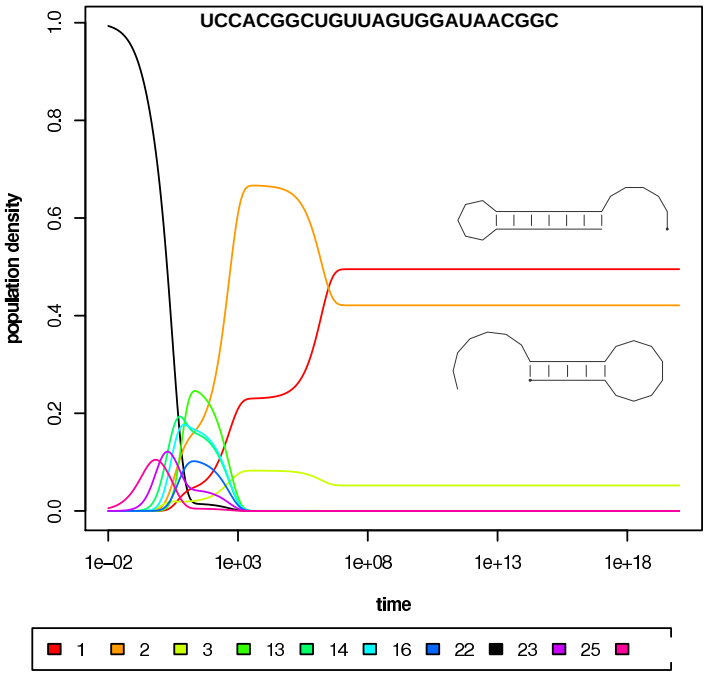
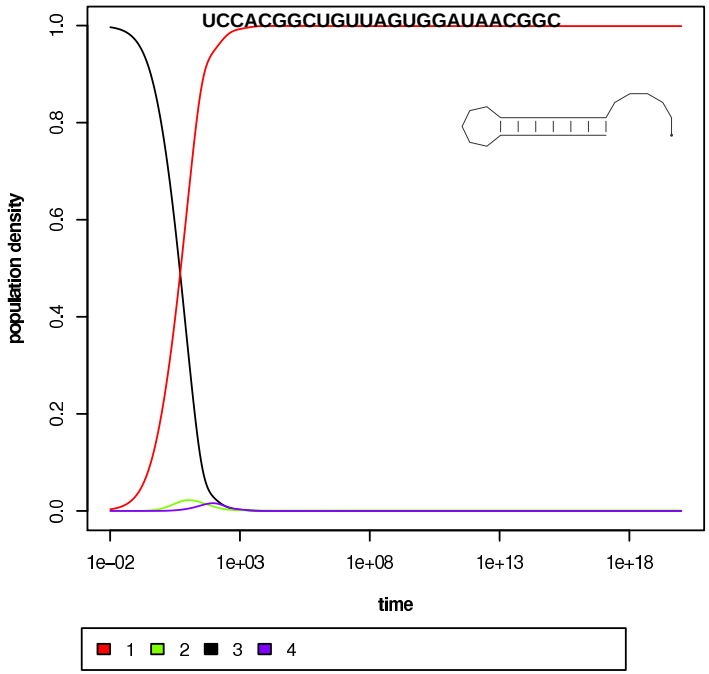
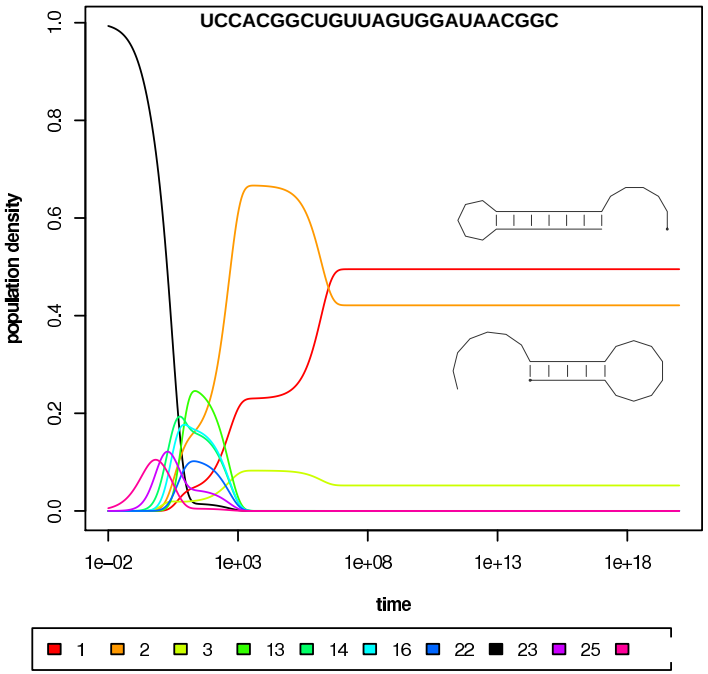


1000 samples per macrostate (κ, λ-approach)



Full kinetic sorted into shapes (abstraction level 5)

small example with length = 25 nt, initial state = unfolded chain



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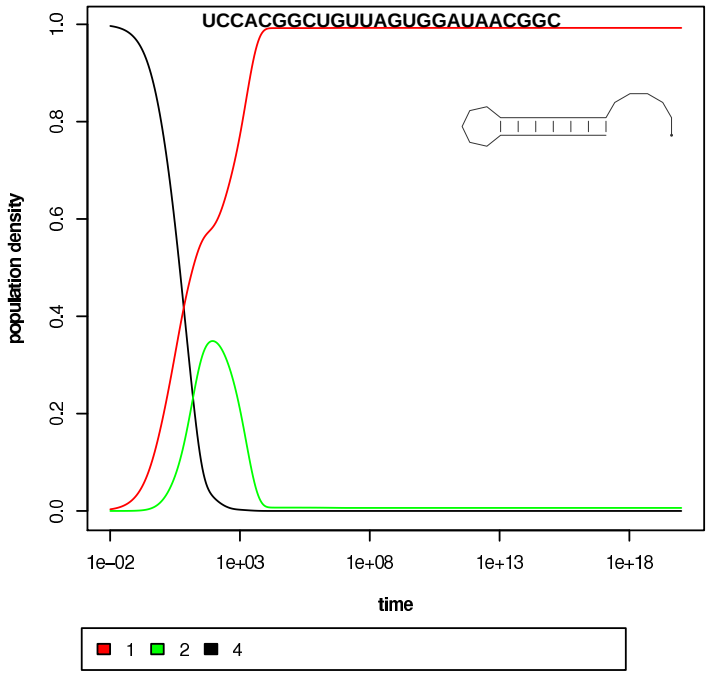
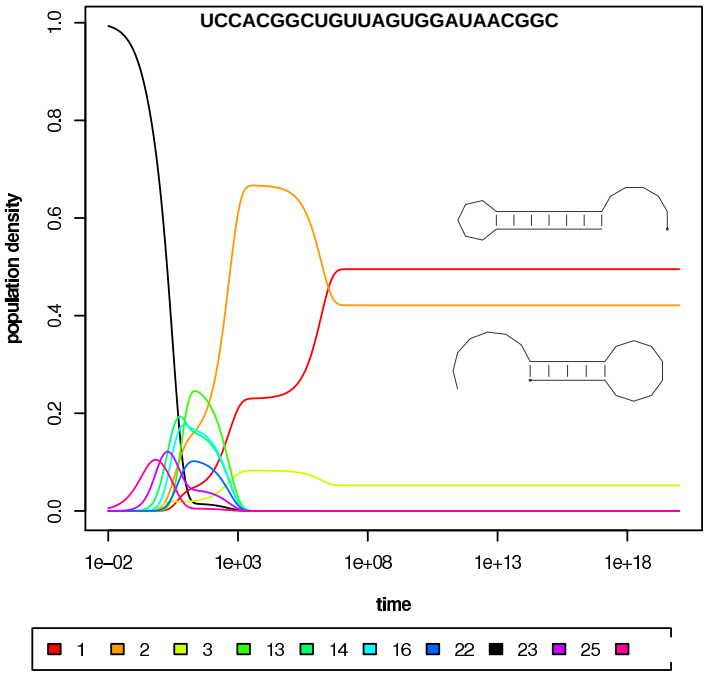
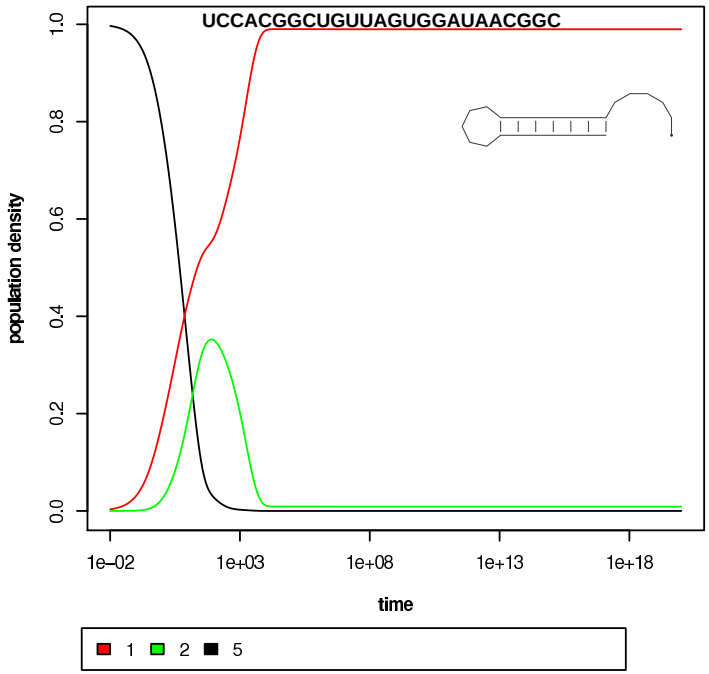


Full kinetic sorted into shapes (abstraction level 4)

small example with length = 25 nt, initial state = unfolded chain

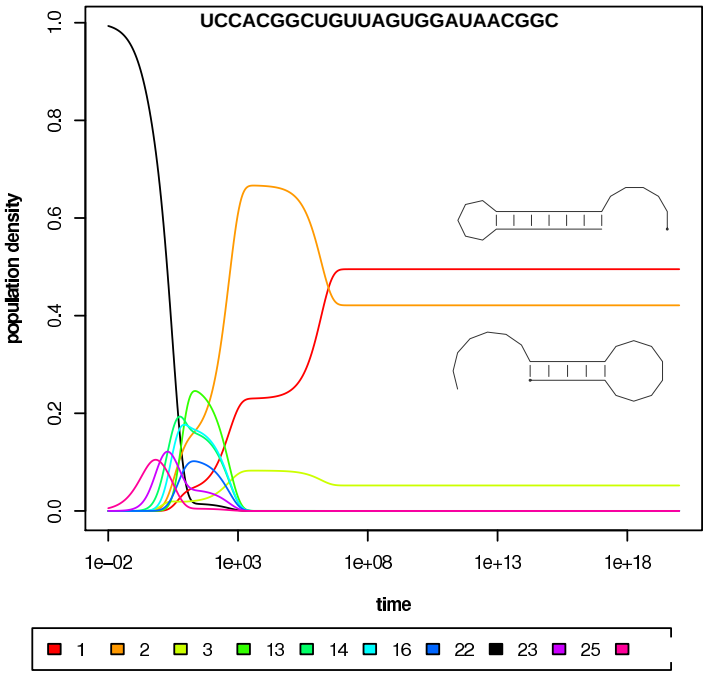


1000 samples per macrostate (κ, λ-approach)

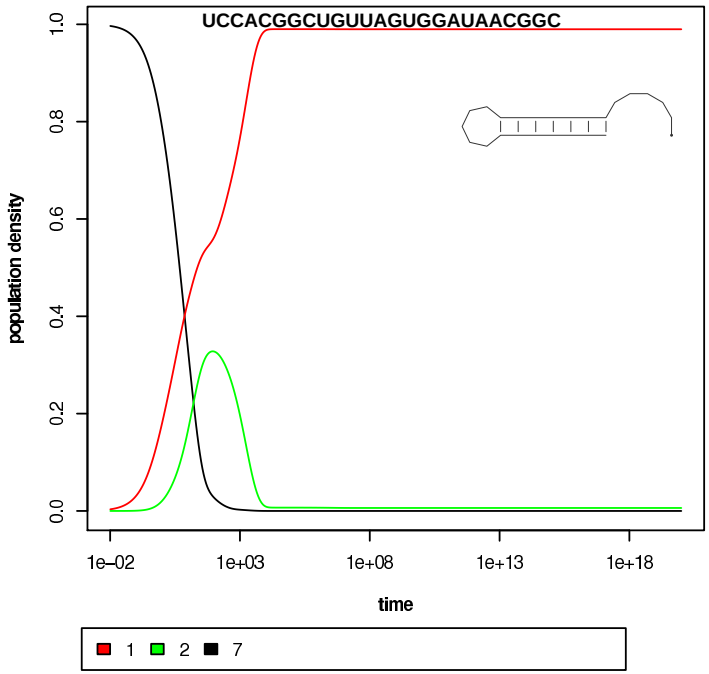


Full kinetic sorted into shapes (abstraction level 3)

small example with length = 25 nt, initial state = unfolded chain

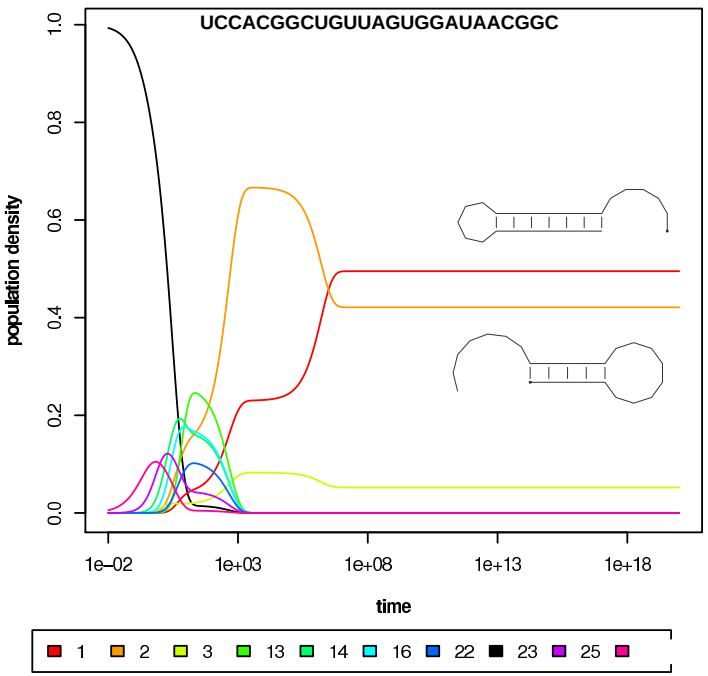


1000 samples per macrostate (κ, λ-approach)

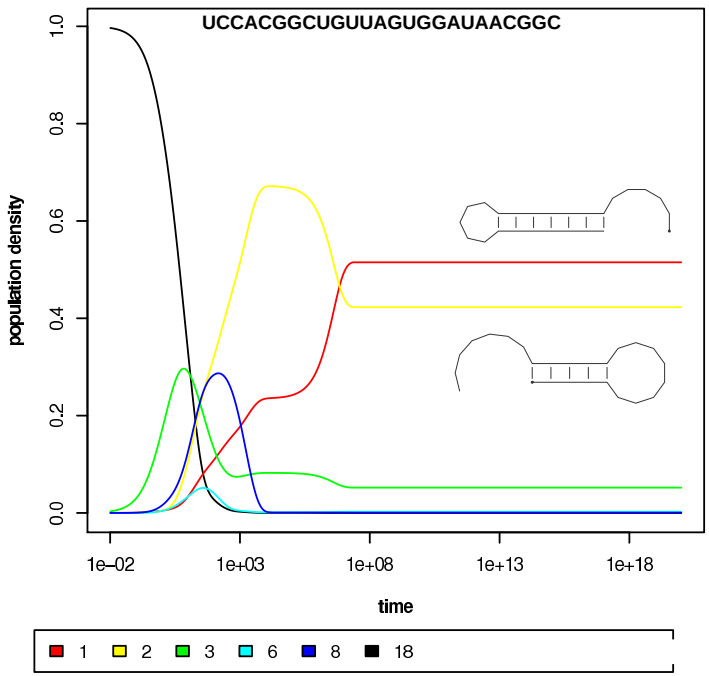


Full kinetic sorted into shapes (abstraction level 2)

small example with length = 25 nt, initial state = unfolded chain



1000 samples per macrostate (κ, λ-approach)



Full kinetic sorted into shapes (abstraction level 1)

To summarize

- prior knowledge can ease computational effort
- Boltzmann sampling may not explore important parts of the structure space
- sampling from distance classes implicitely explores more strucutral diversity
- significantly longer RNAs can be analyzed
- transition rate sampling may also work for RNAshapes partitioning

Thanks to:

Christoph Flamm
Christian Höner zu Siederdissen
Ivo Hofacker

...and You!

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