Secondary structure prediction for circular RNAs

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There are circular RNAs?

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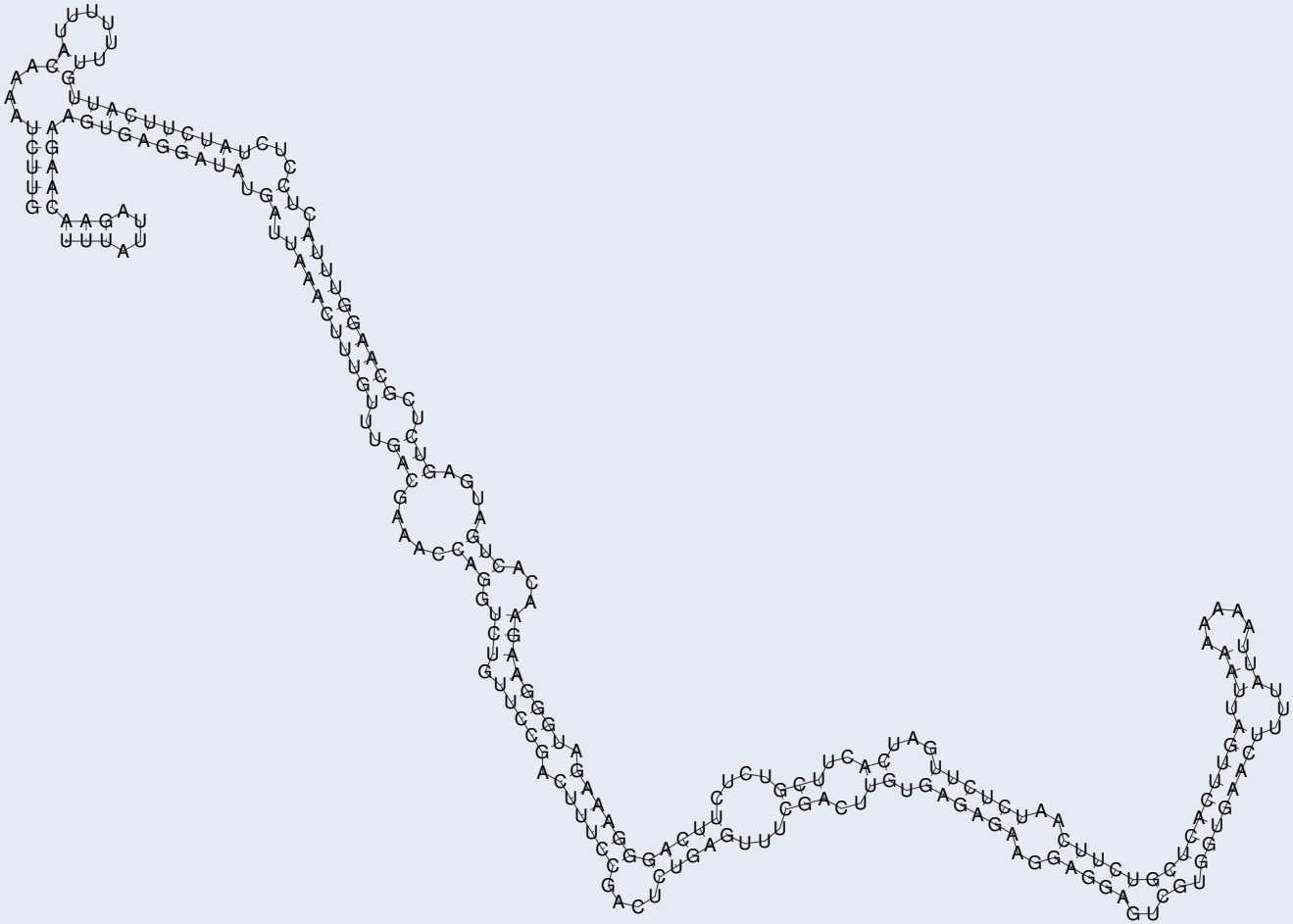
- Spliced group I introns form circles
- Some tRNA splicing products in archaea
- Box C/D RNAs in Pyrococcus furiosus
- Hepatitis δ Virus (HDV)
- Viroids
- Satellite RNAs
- RNA aptameres

More than 1300 circular viroid RNA genomes and related objects in the `Subviral RNA Database`

In almost all cases, circular RNAs code not for any mRNA but for their own structure

Predicted MFE secondary structure of *ASBVd*

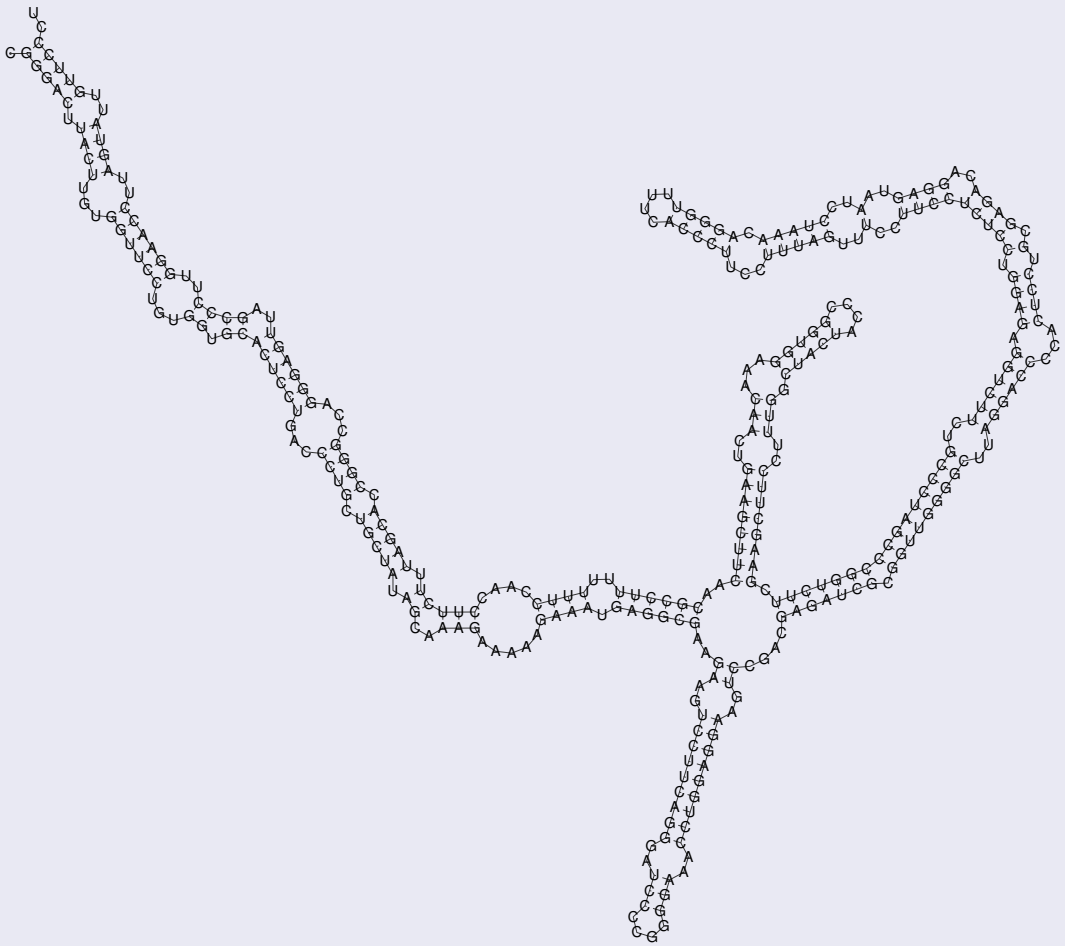


(Avocado Sunblotch Viroid)

Symptoms of *ASBVd* on fruit

(Image taken from "2006 Florida Plant Disease Management Guide: Avocado (Persea americana)", Palmateer, A.J. and Ploetz, R.C. and Harmon, P.F.)

Predicted MFE secondary structure of *CSVd*

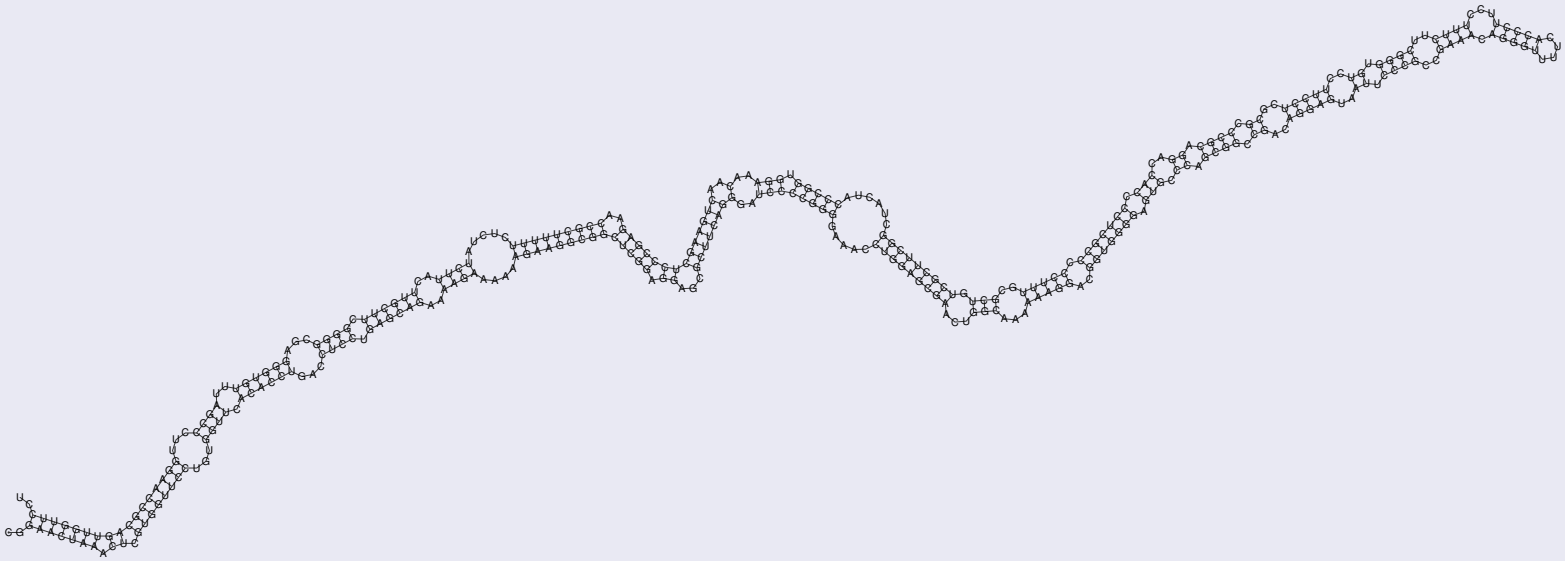


(Chrysanthemum Stunt Viroid)

Symptoms of *CSVd* infection on flowers of chrysanthemum cv. Gillglow

(Image taken by J. Dunez, France, Bugwood.org)

Predicted MFE secondary structure of *PSTVd*



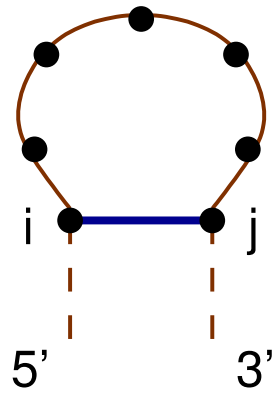
(Potato Spindle Tuber Viroid)

PSTVd on potato

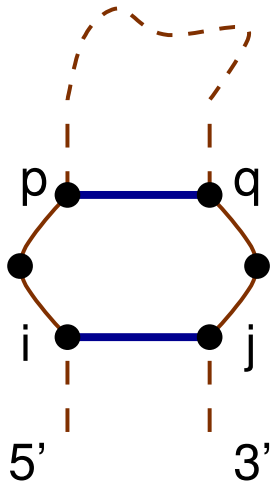
(Image taken by USDA ARS Archive, USDA Agricultural Research Service, USA, Bugwood.org)

Loop-based energy model:

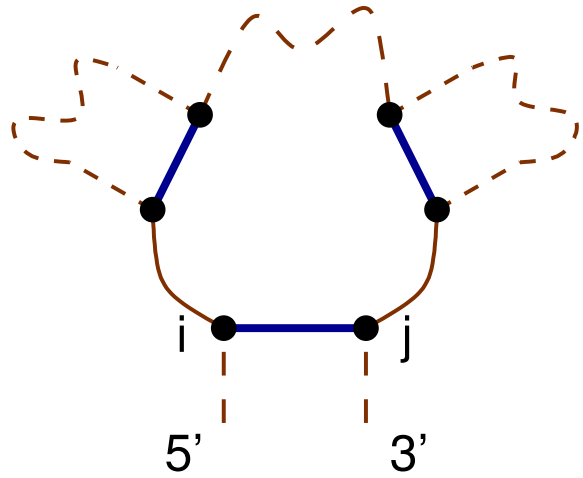
- free energy of a secondary structure is additively composed by free energy of its loops



Hairpin loop



Interior loop



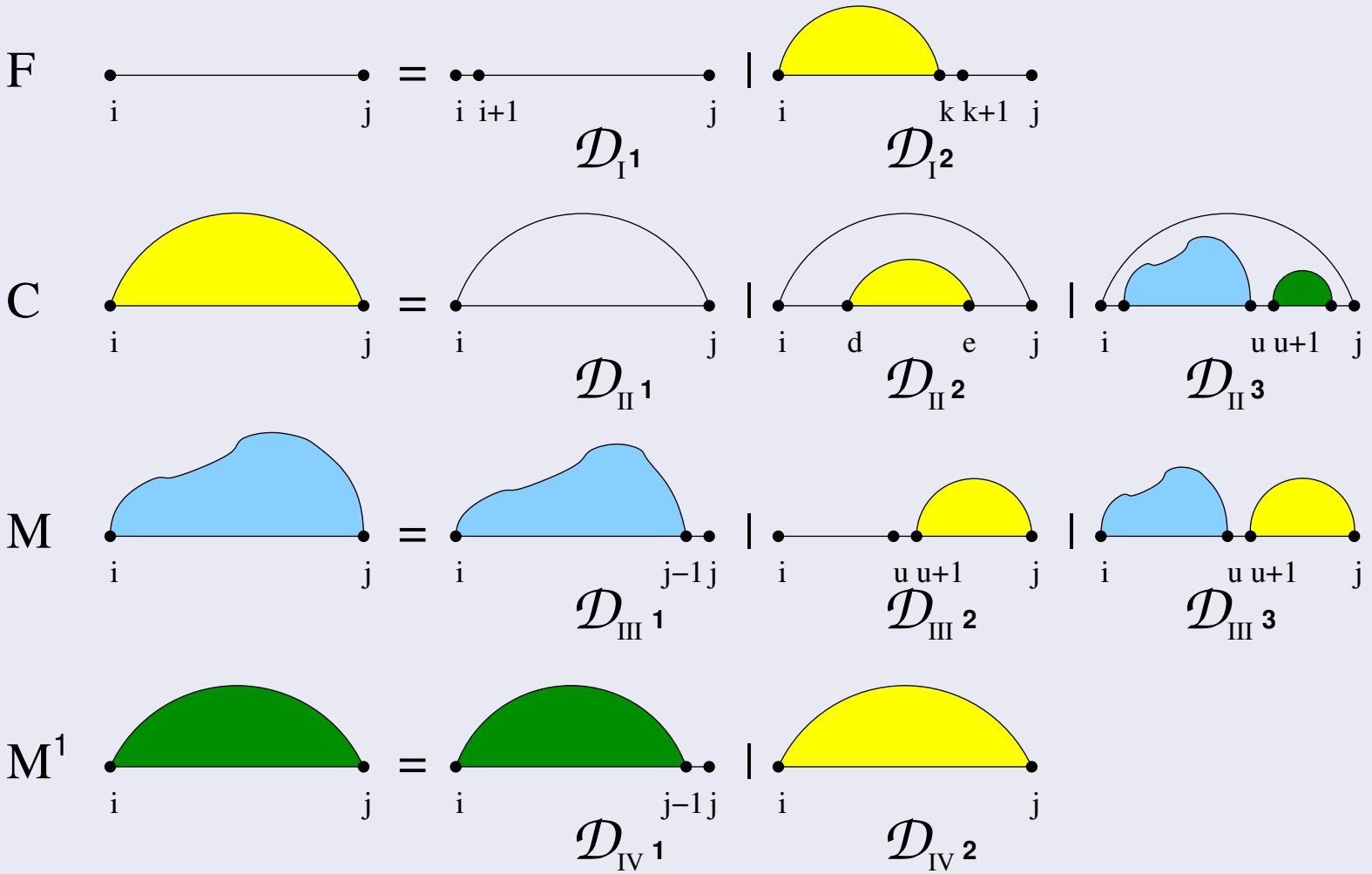
Multi loop

Dynamic programming algorithms:

Dynamic programming algorithms:

- optimal secondary structure according to free energy (MFE)
- suboptimal secondary structures with free energy in an interval arround MFE
- partition function and base pairing probabilities
- statiscally representative samples of the Boltzmann ensemble (stochastic backtracking)

Loop decomposition for linear RNAs



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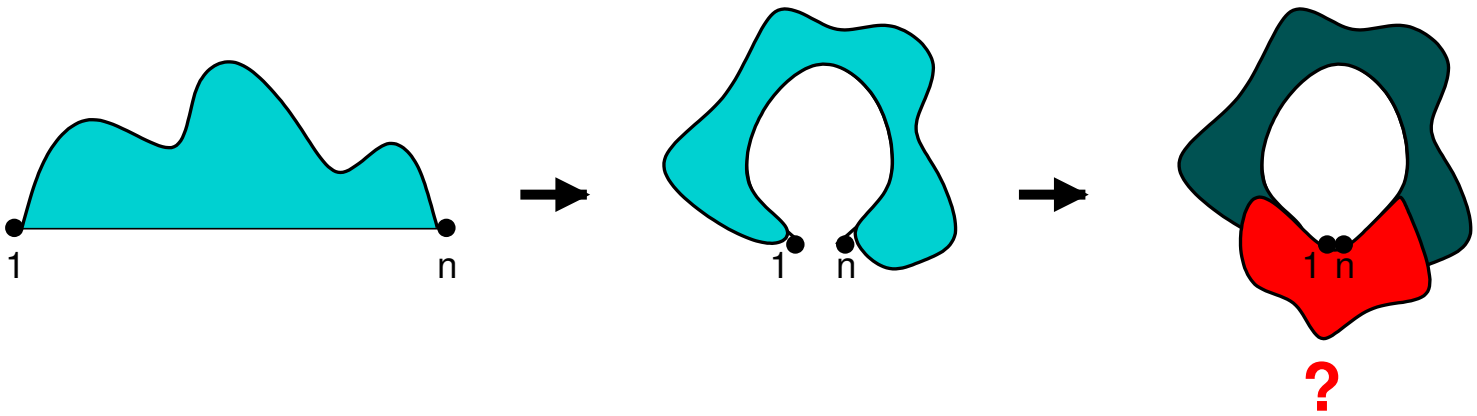

How to extend linear recursions for circular RNAs?

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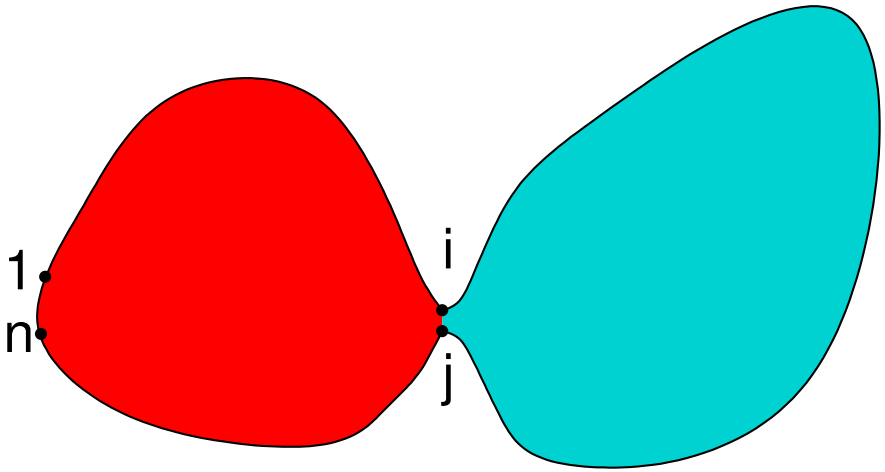
If at least one basepair exists:

(Hofmann et al., J. Biomol. Struct. Dyn., 1984)

- Take free energy of exterior loop and free energy of interior loop



$$\min_{i<j} \{C(j,i) + C(i,j)\}$$

- doubles computation time and memory requirements!

How to extend linear recursions for circular RNAs?
If at least one basepair exists:
(Zuker et al., Bulletin of Mathematical Biology, 1984)

- Concatenate sequence [1,n] on itself so that nucleotides $n+1, \ldots, 2n$ are the same as $1, \ldots, n$
- fill C matrix with linear algorithm and new condition:

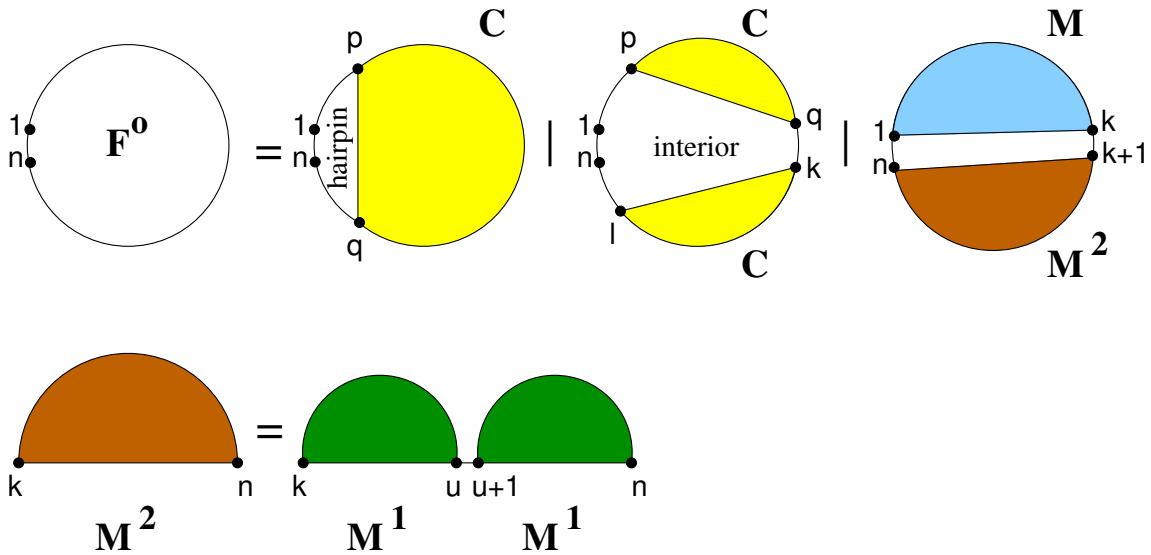
$$C(i,j) = \infty, \text{ if } j - i > n - 2$$

$$\min_{i<j} \{C(i,j) + C(j, i+n)\}$$

- quadruples the memory requirements and roughly triples computation time

How to extend linear recursions for circular RNAs?
If at least one basepair exists:
(Hofacker et al., Bioinformatics, 2005)

- Compute energy of the exterior loop as a kind of post-processing step



- $\mathcal{O}(n)$ additional memory, roughly doubling of computation time

Memory efficient algorithm was applied to:

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- partition function and base pairing prob. for aligned sequences
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These are now implemented in the `ViennaRNAPackage` and available in the programs: `RNAfold`, `RNAalifold` and `RNAsubopt`

What can one do now?

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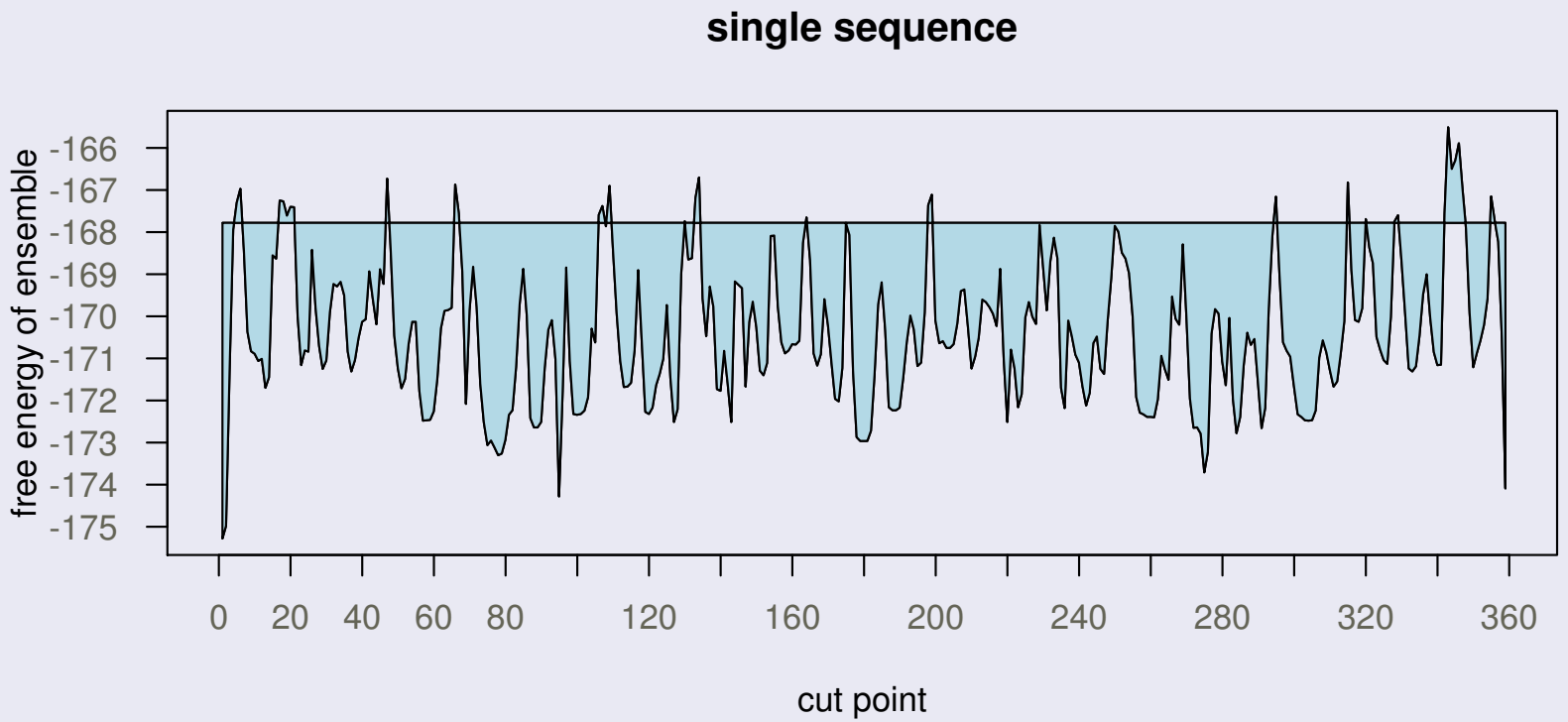
- predict secondary structures for even large circular RNAs
- compute partition function and base pairing probabilities
- generate samples of suboptimal secondary structures
- handle circular AND linear RNAs with the same tools
- ...

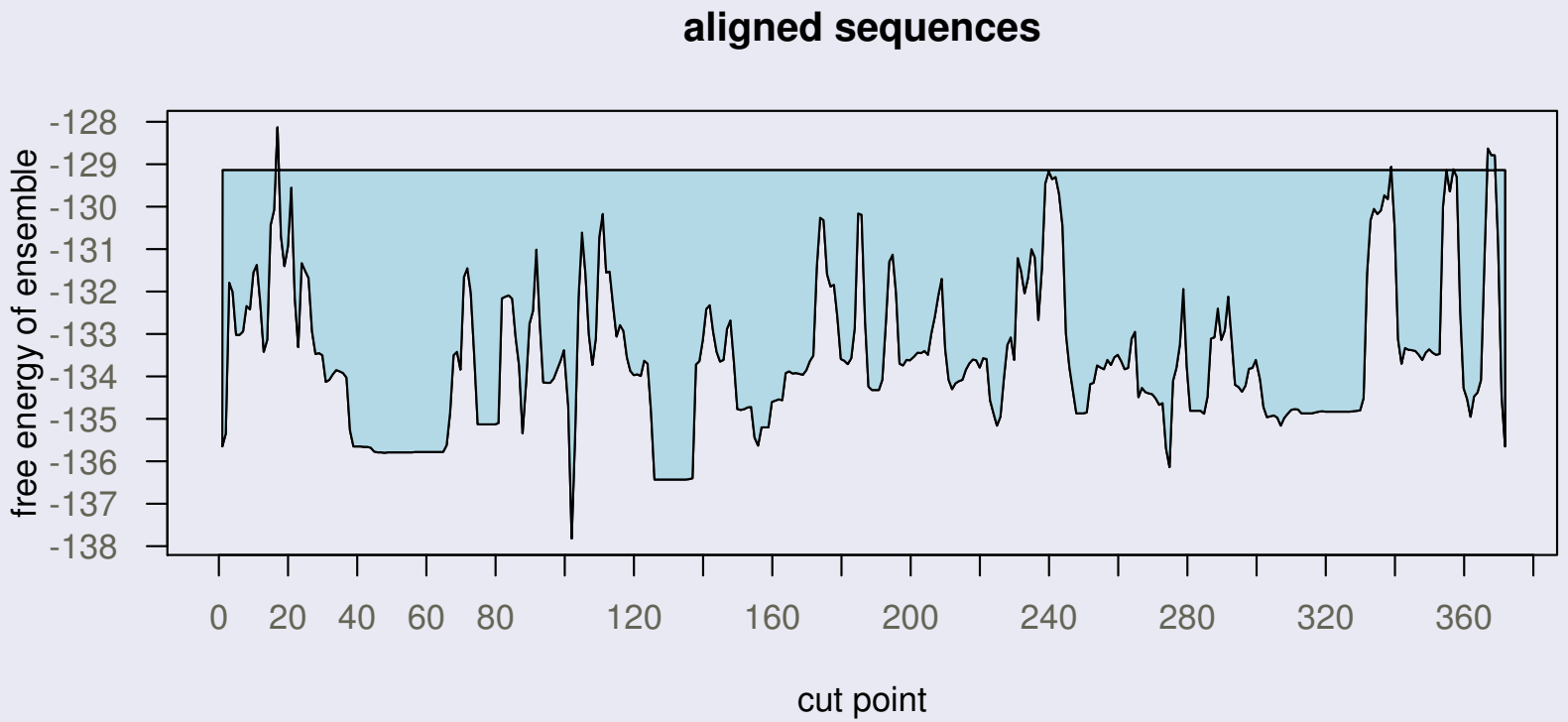
- *investigate if theres a difference between folding linear and circular (cut point specificity)*

Free energy of ensemble of an RNA sequence



Predicted Gibbs free energy $G = -kT \cdot ln(\mathcal{Q})$ compared to
$G^{\circ} = -167.78\ kcal/mol$
(*PSTVd, Acc. No. M16826*)

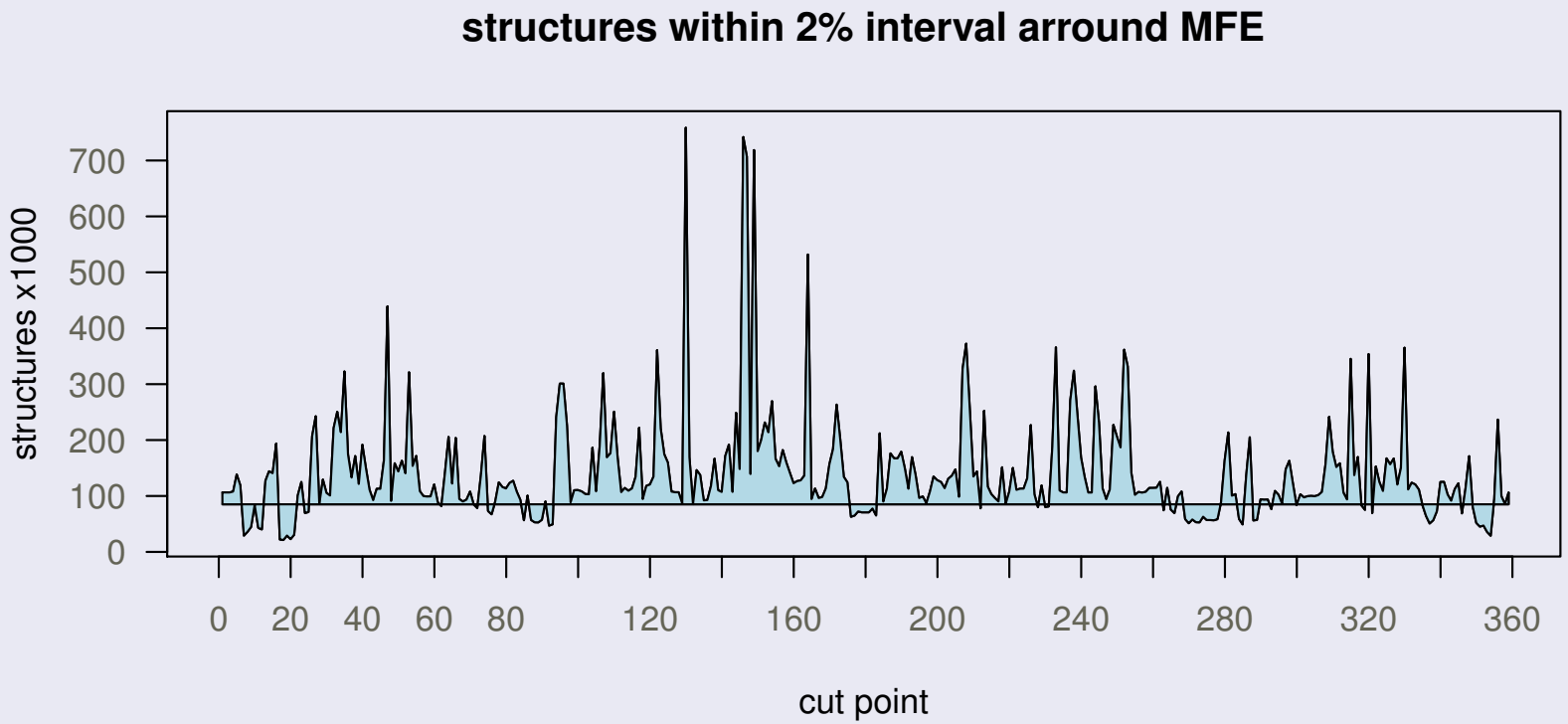
Free energy of ensemble of an alignment of RNA sequences



Predicted Gibbs free energy $G^{\mathcal{A}} = -kT \cdot ln(\mathcal{Q}^{\mathcal{A}})$ of an alignment compared to $G^{\mathcal{A}\circ} = -129.14\ kcal/mol$

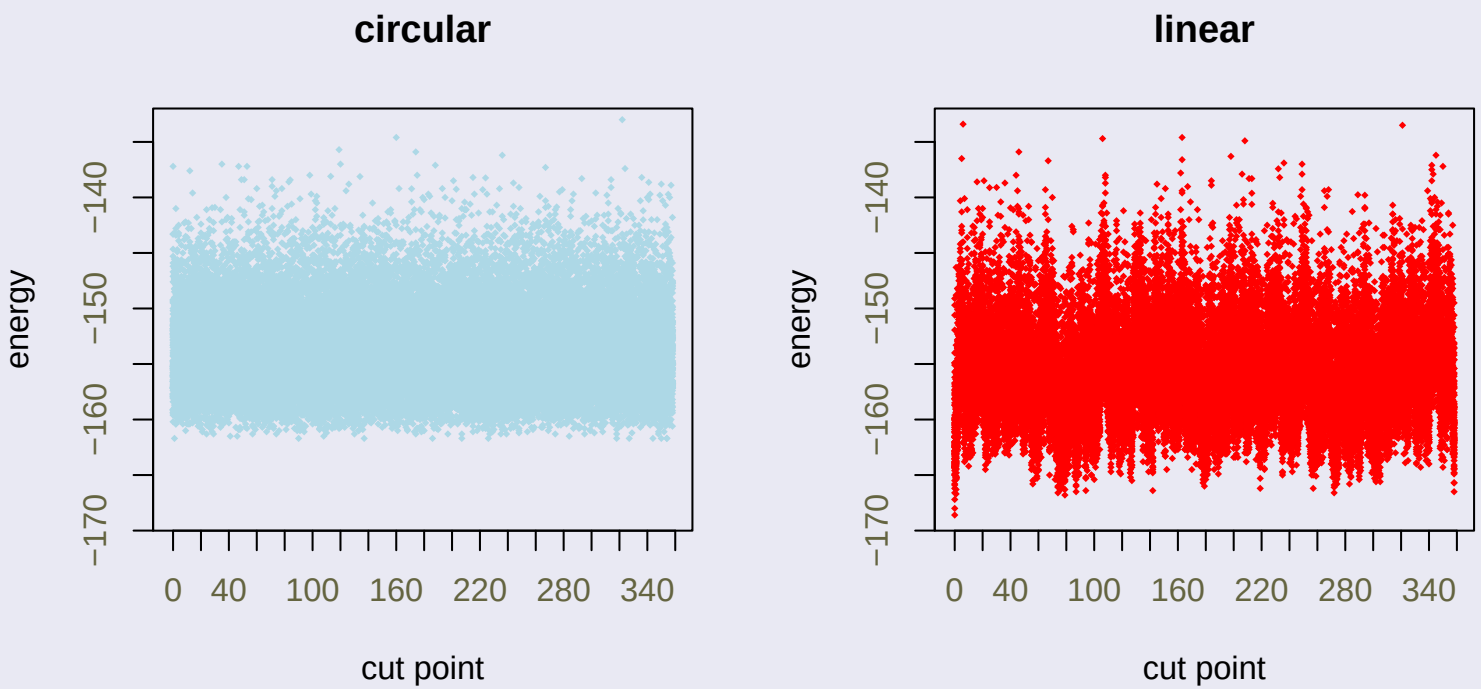
(134 *PSTVd* seq. from `Subviral RNA database`)

Suboptimal secondary structures



Number of secondary structures when folding linear compared to 85085 structures obtained by folding with circular extension (*PSTVd, Acc. No. M16826*)

Stochastic backtracking



Free energies in *kcal/mol* of stochastically sampled suboptimal structures (*PSTVd, Acc. No. M16826*)

Searching for multistable secondary structure states, folding paths and folding kinetics

- Viroids are known to exihibit different secondary structures, e.g. during replication and while acting pathogenic
- sequence too long for an analysis!
  e.g. PSTVd: 360nt, MFE: -160.3 kcal/mol

  Subopt Wuchty:
  24.000.000 within interval of 5.7 kcal/mol

  Stochastic backtracking:
  17.900.000 sampled
  11.000.000 after removing duplicates

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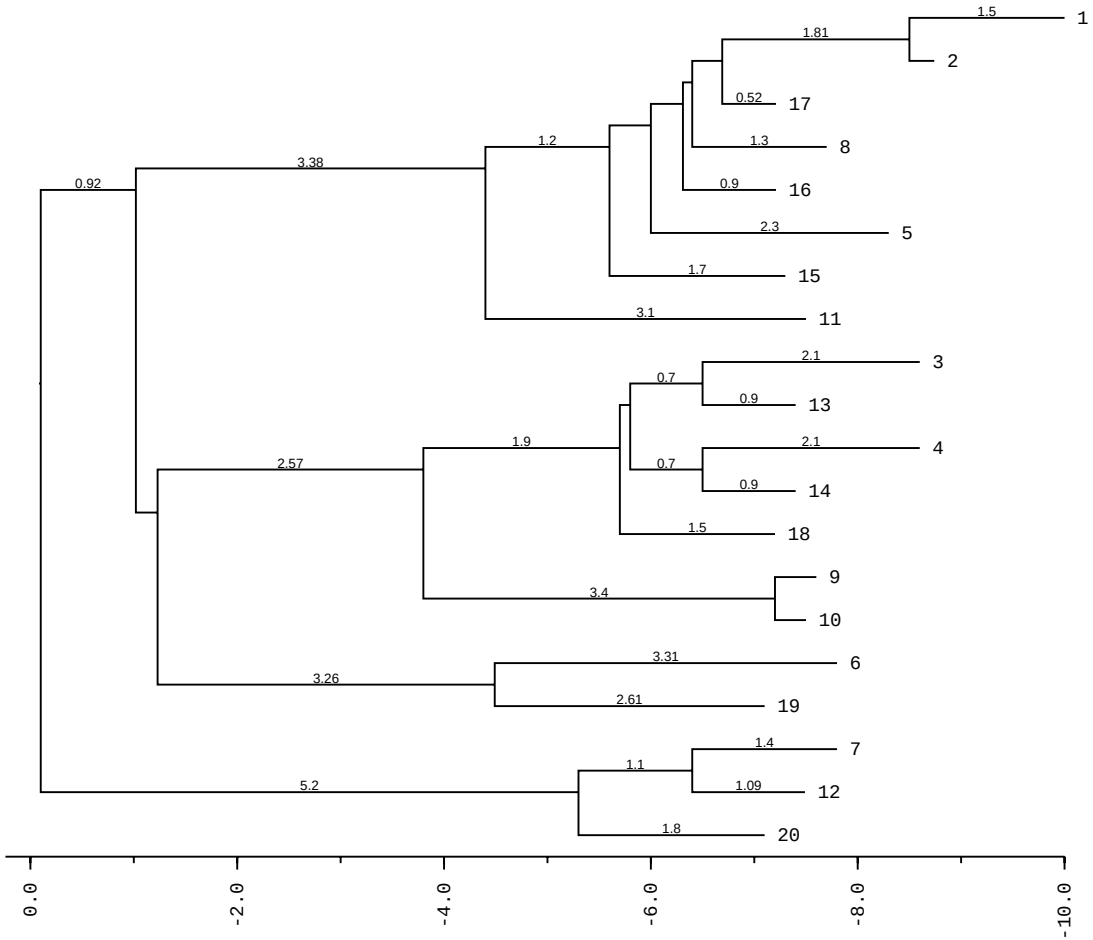
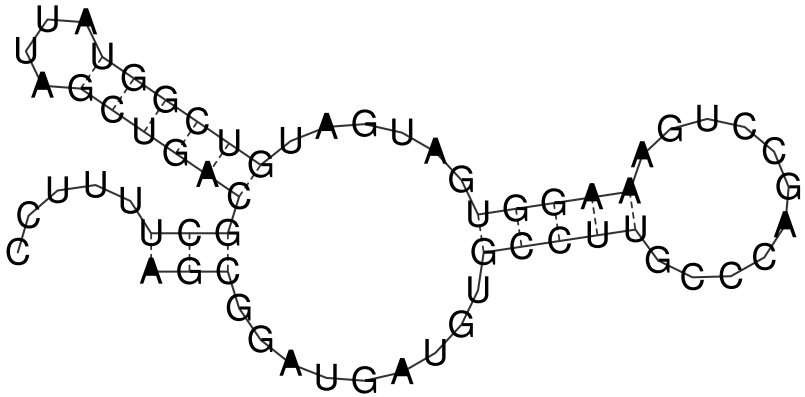
  Stochastic backtracking:
  17.900.000 sampled
  11.000.000 after removing duplicates
  this gets even worse with bigger samples ($>$ 50% duplicates)
  energy landscape is not well connected

Searching for multistable secondary structure states and folding paths and folding kinetics

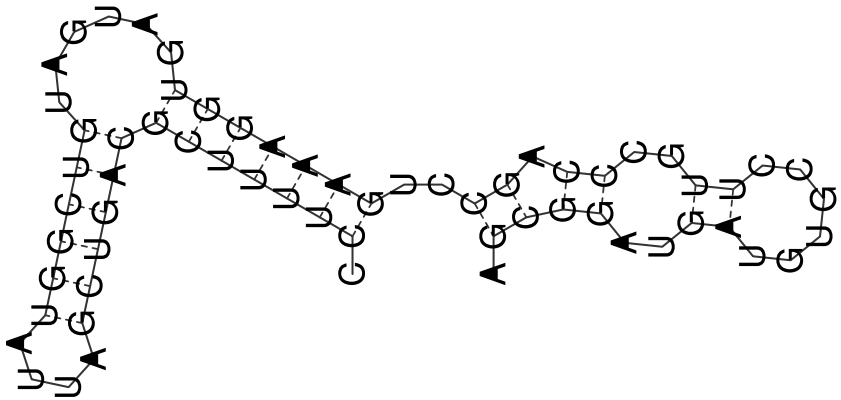
- small circular sRNA (sR29, 63nt) of *P. furiosus*
- `RNAsubopt` generated secondary structure space (6.5M structs)
- use these structures as input for barrier tree generation with `barriers` (20 local minima)
- select two stable structures (local minima) and compute the folding path


1.5
1
1.81
2
0.52
17
1.3
8
1.2
3.38
0.92
0.9
16
2.3
5
1.7
15
3.1
11
2.1
3
0.7
0.9
13
1.9
2.1
4
2.57
0.7
0.9
14
1.5
18
9
3.4
10
3.31
6
3.26
2.61
19
1.4
7
1.1
5.2
1.09
12
1.8
20
0.0
-2.0
-4.0
-6.0
-8.0
-10.0

select for example structure at local minimum 4

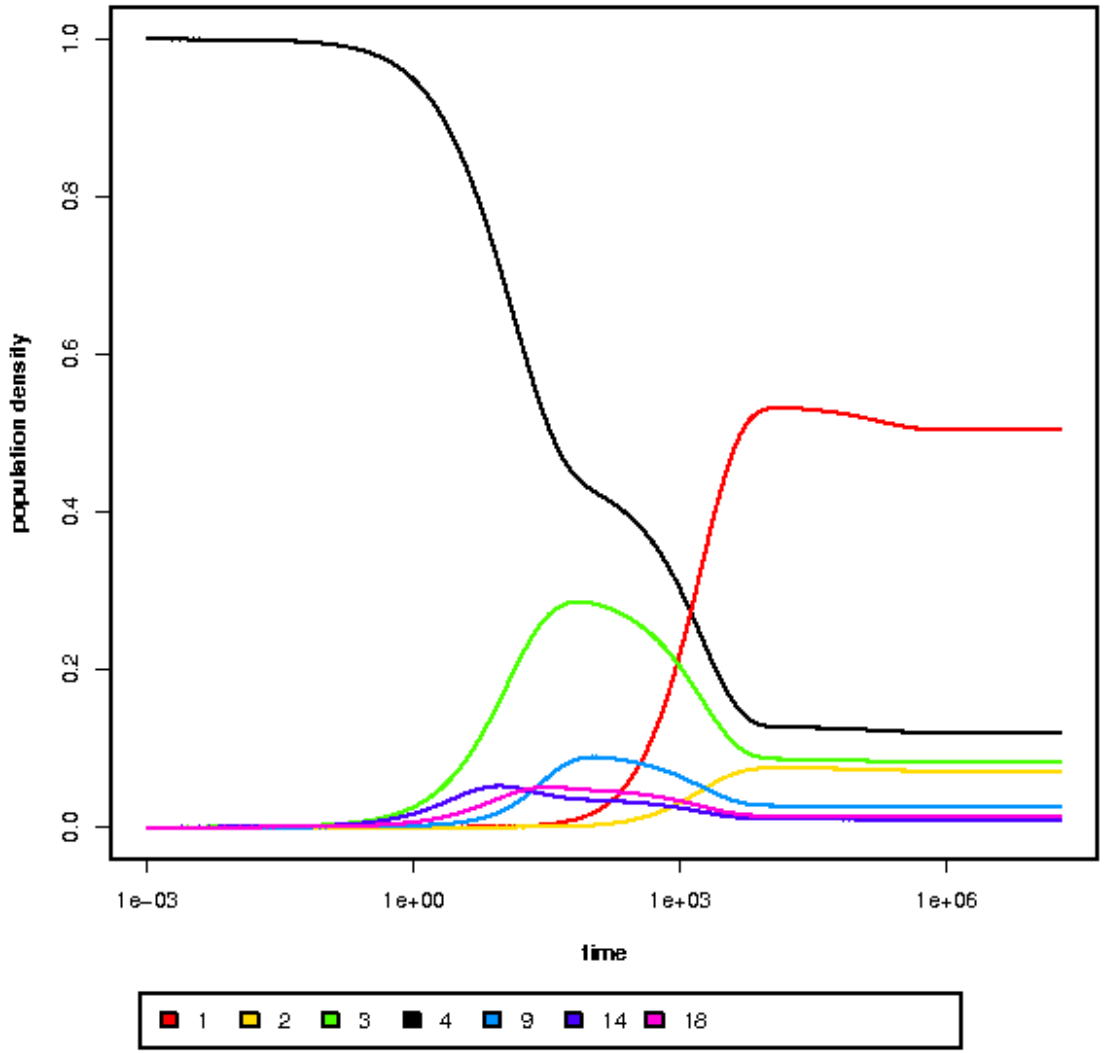


and investigate folding path to MFE structure 1



show visualization

Folding kinetics starting in macro state 4



More work to do:

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- Find efficient folding paths between two given strucures without the need for the entire sec. structure space
- find more small circular RNAs to investigate
- apply circular extensions to the design tools for Multistable RNAs

Thank you for your attention