Projection of the RNA secondary structure space: The κ, λ - neighborhood

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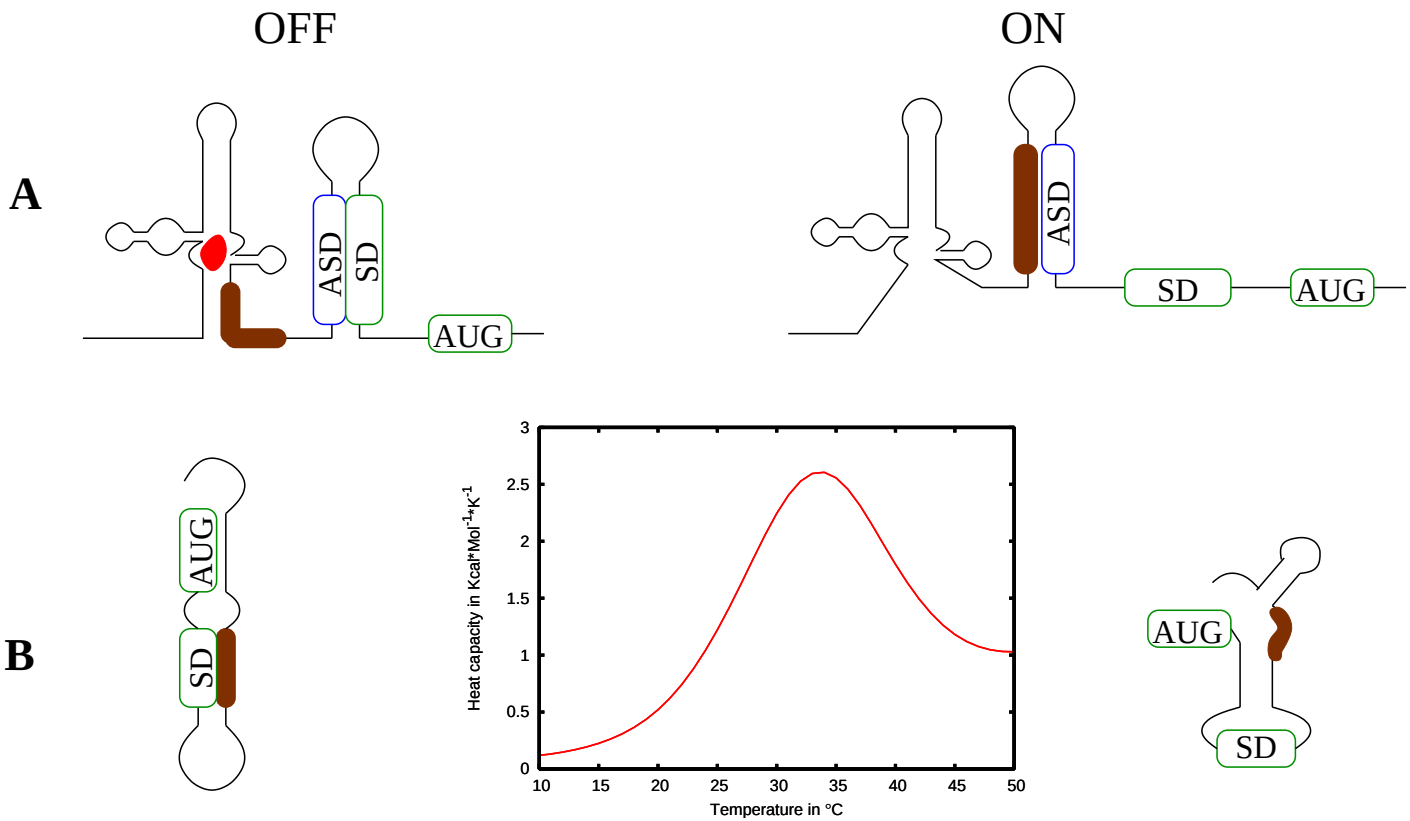
1. RNA structures and energy landscapes
   - Motivation
   - RNA structures
   - Loop decomposition
   - Nearest neighbor energy model
   - Secondary structure free energy landscape

2. The κ, λ - neighborhood of two secondary structure states
   - Representatives of the κ, λ - neighborhood
   - Complexity and implementation
   - Application and interpretation
   - Estimation of barrier heights using refolding paths

Gene regulation by RNA structure changes

- 5'-UTR element controlling translation
- metabolite sensors, (Riboswitch, A)
- temperature sensors, (RNA thermometer, B)

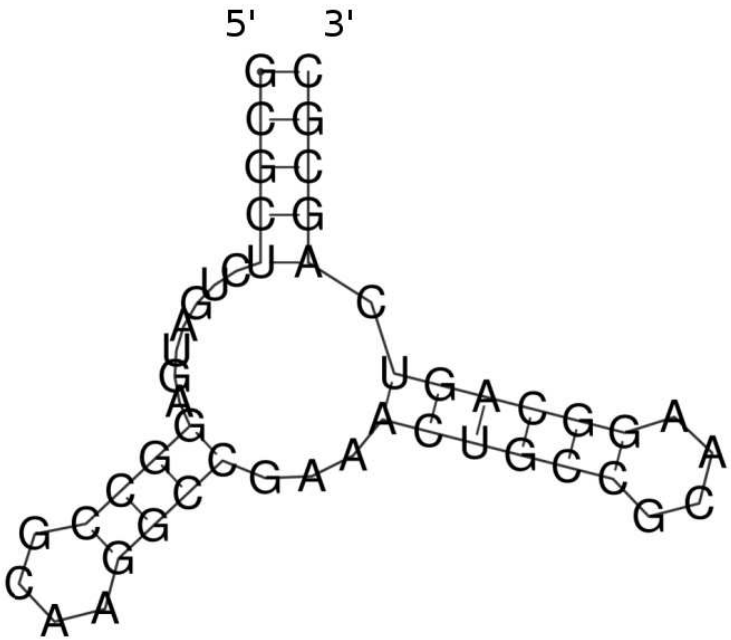


adapted from Henkin, T (2008) *Genes & Development* **22**:3383-3390

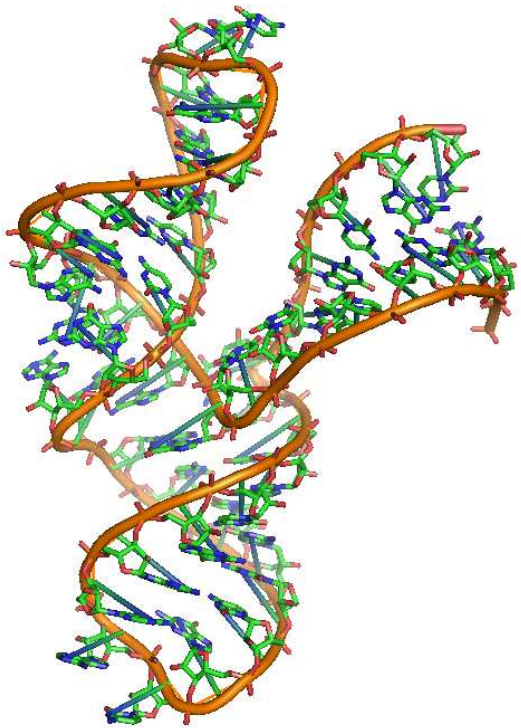
Primary structure

5' - GCGCUCUGAUGAGGCCGCAAGGCCGAAACUGCCGCAAGGCAGUCAGCGC - 3'

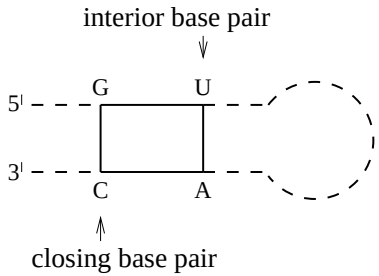
Secondary structure



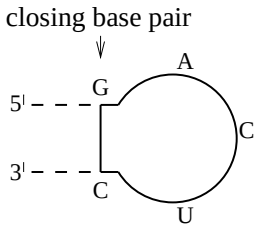
Tertiary structure



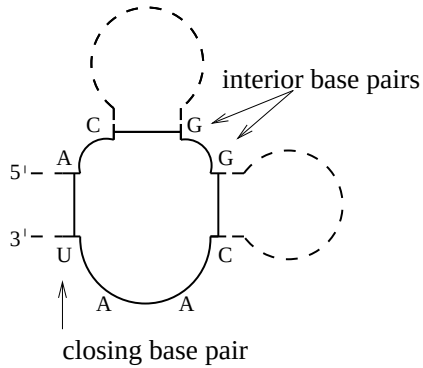
Secondary structures can be uniquely decomposed into loops



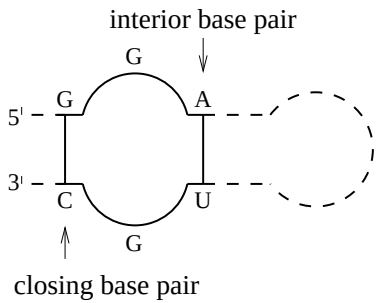
stacking pair



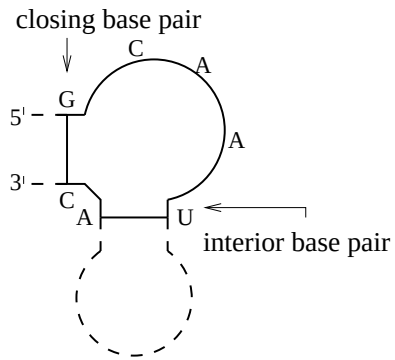
hairpin loop



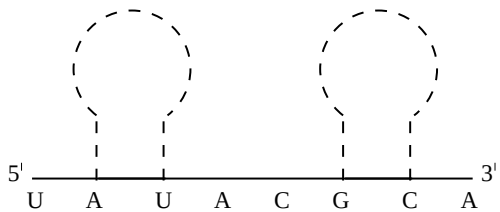
multi loop



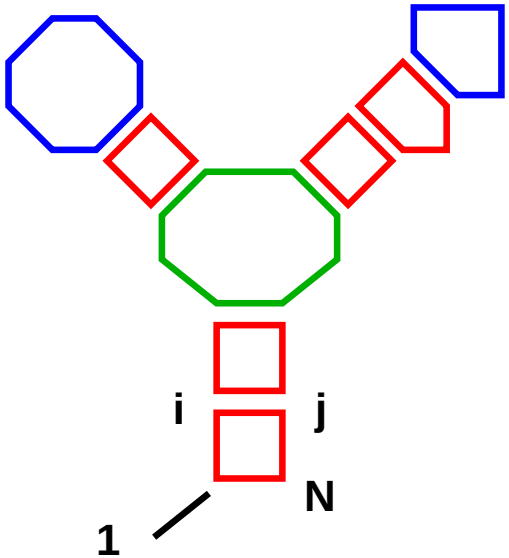
interior loop



bulge



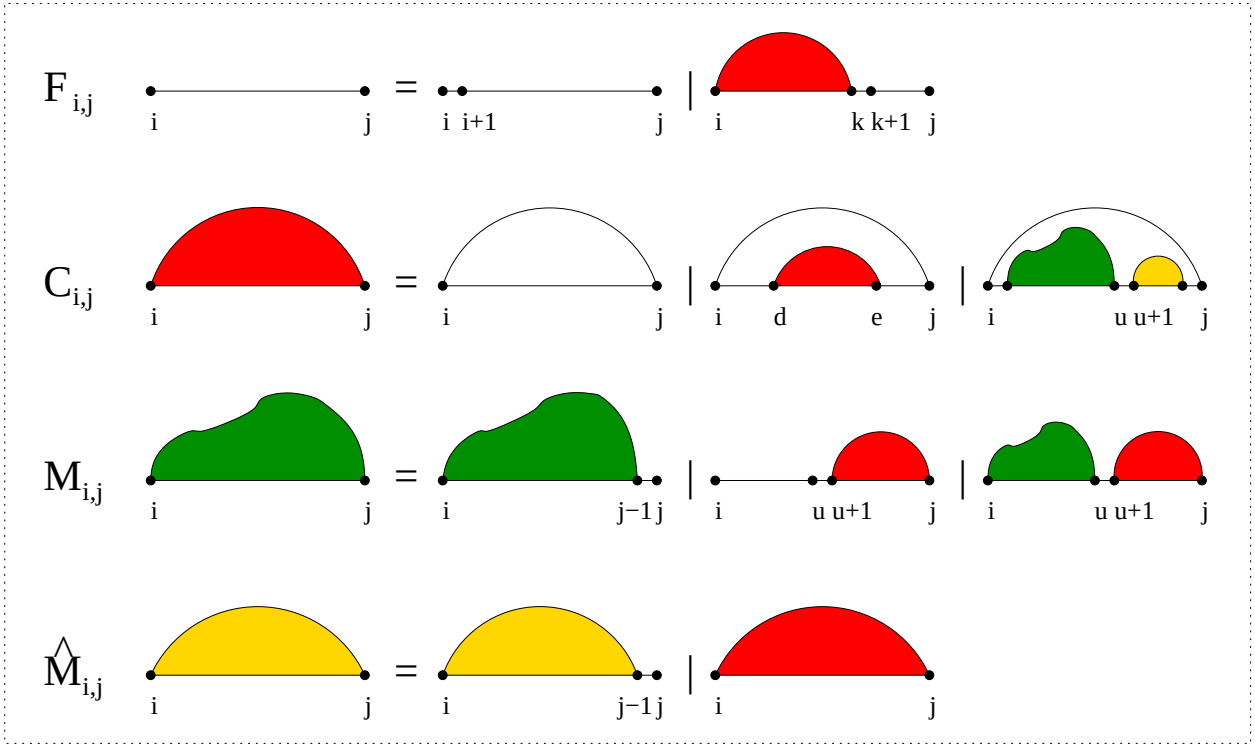
exterior loop



$$E(\mathcal{S}) = \sum_{L \in \mathcal{S}} E(L)$$

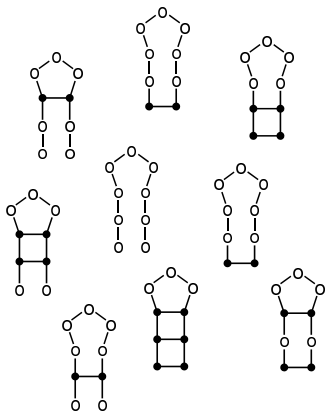
- The free energy of a secondary structure is the sum of the free energy of the loops its composed of
- Loop energies depend on loop type, loop size and sequence
- Energy parameters are measured experimentally or extrapolated by mathematical models

MFE dynamic programming algorithm

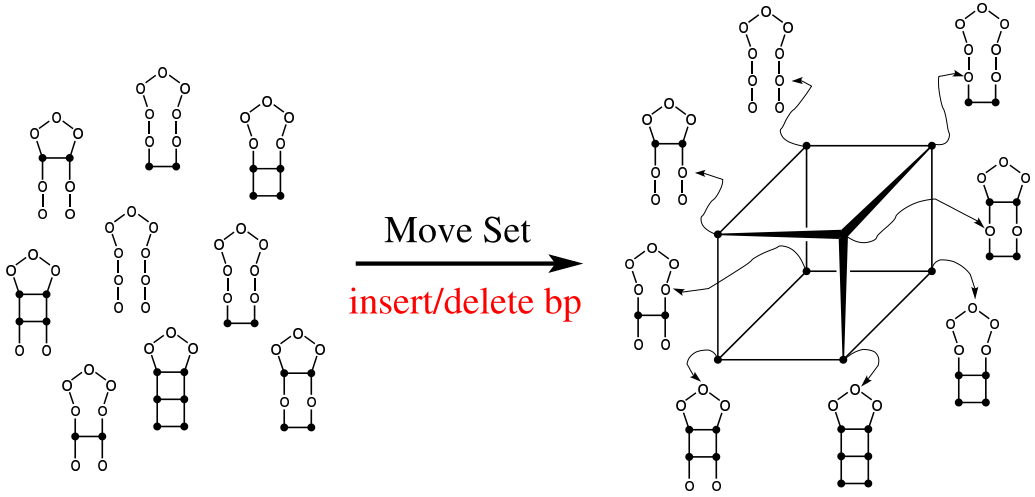


$\mathcal{O}(n^3)$ in time, $\mathcal{O}(n^2)$ in memory

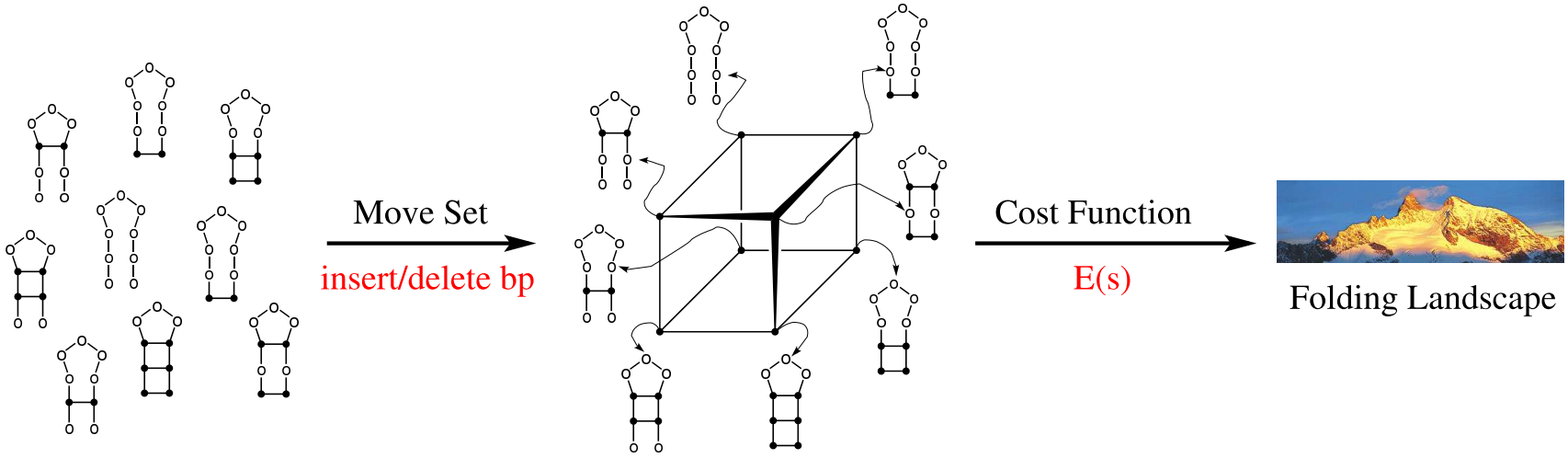
RNA free energy landscape



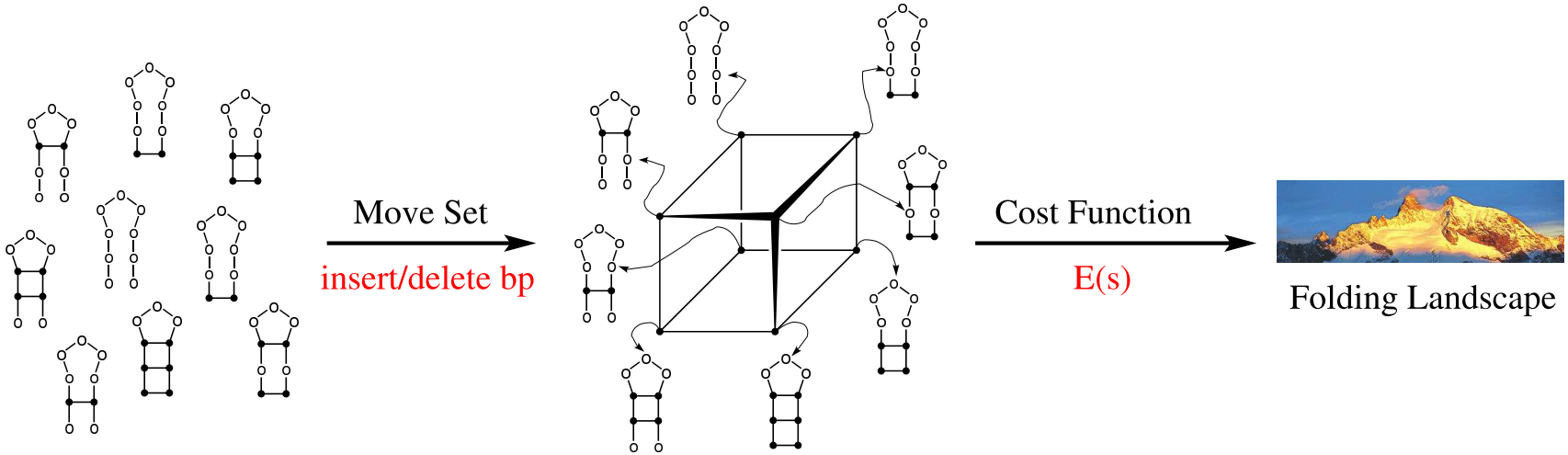
RNA free energy landscape



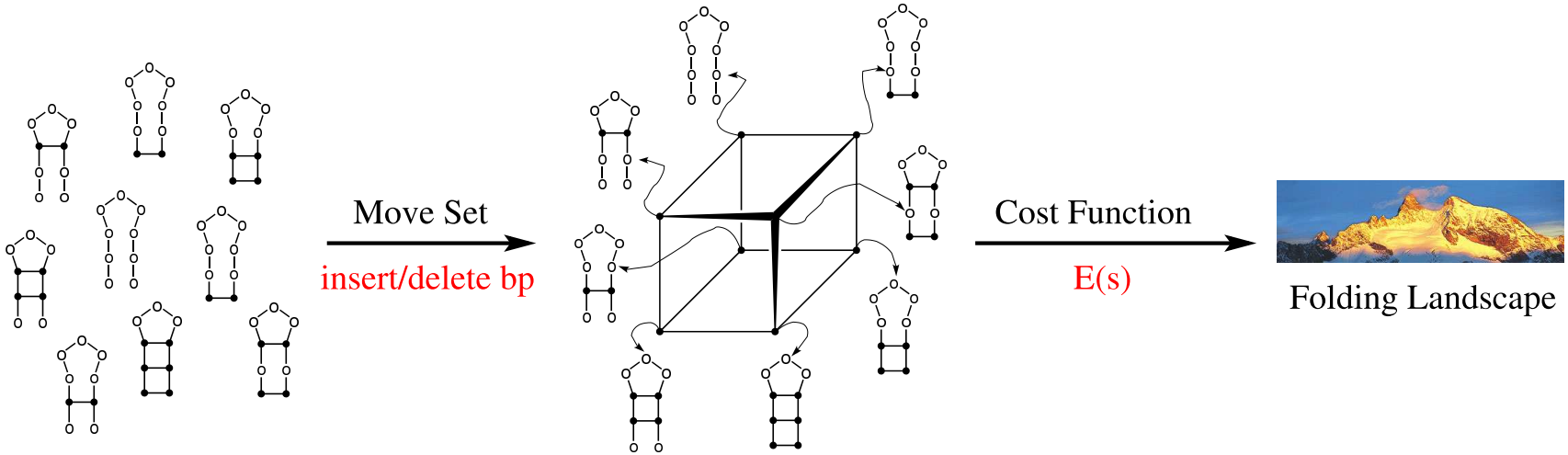
RNA free energy landscape

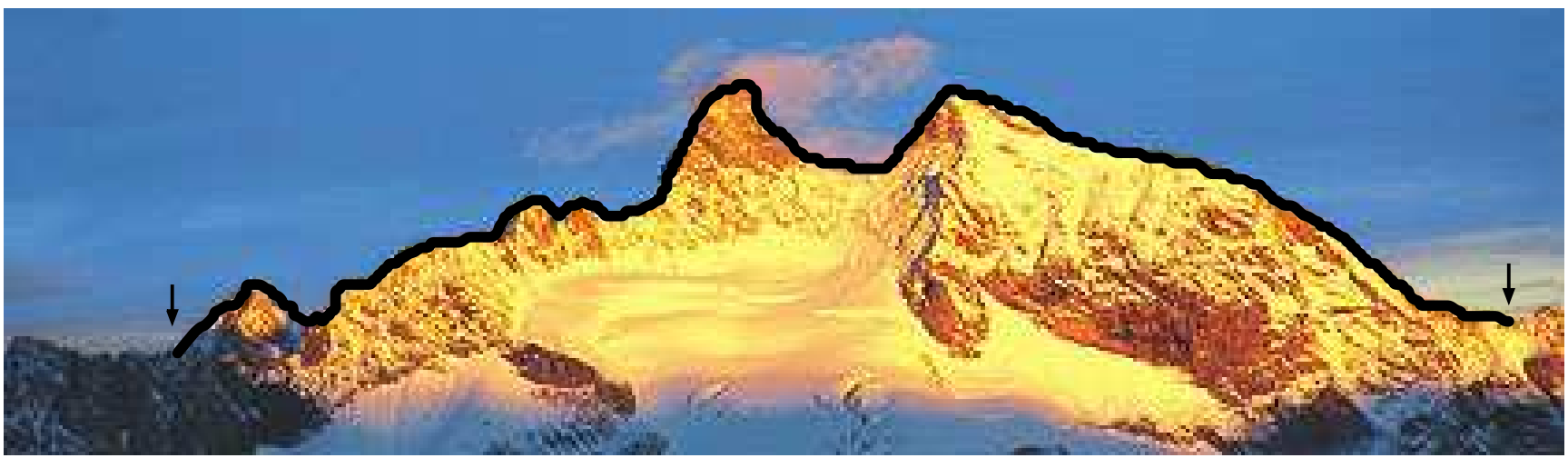


RNA free energy landscape



RNA free energy landscape





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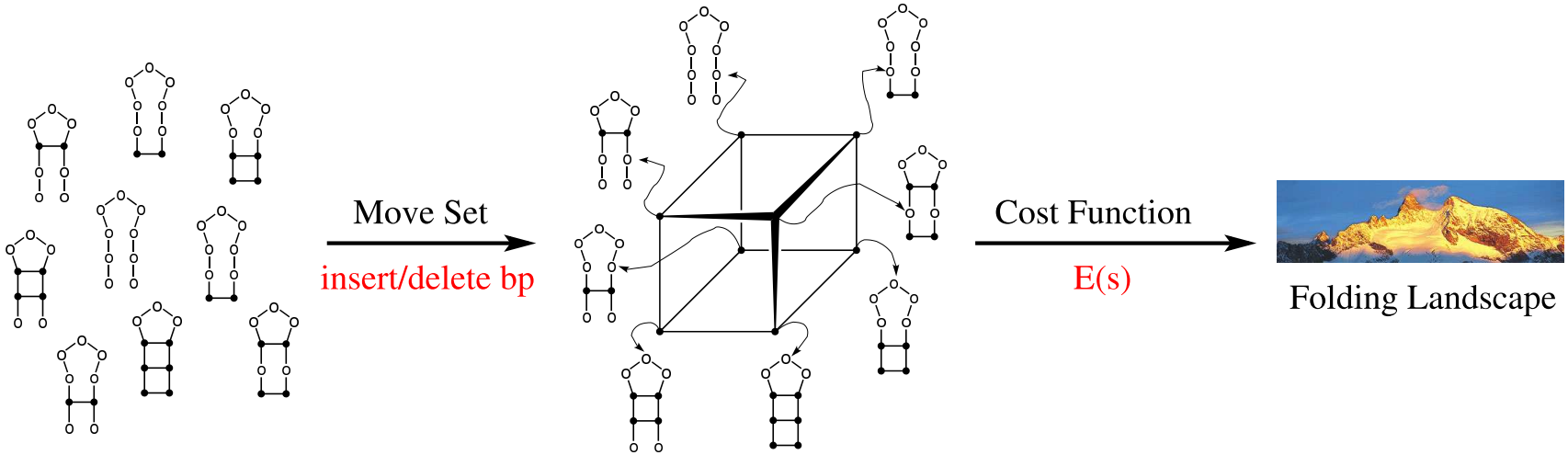
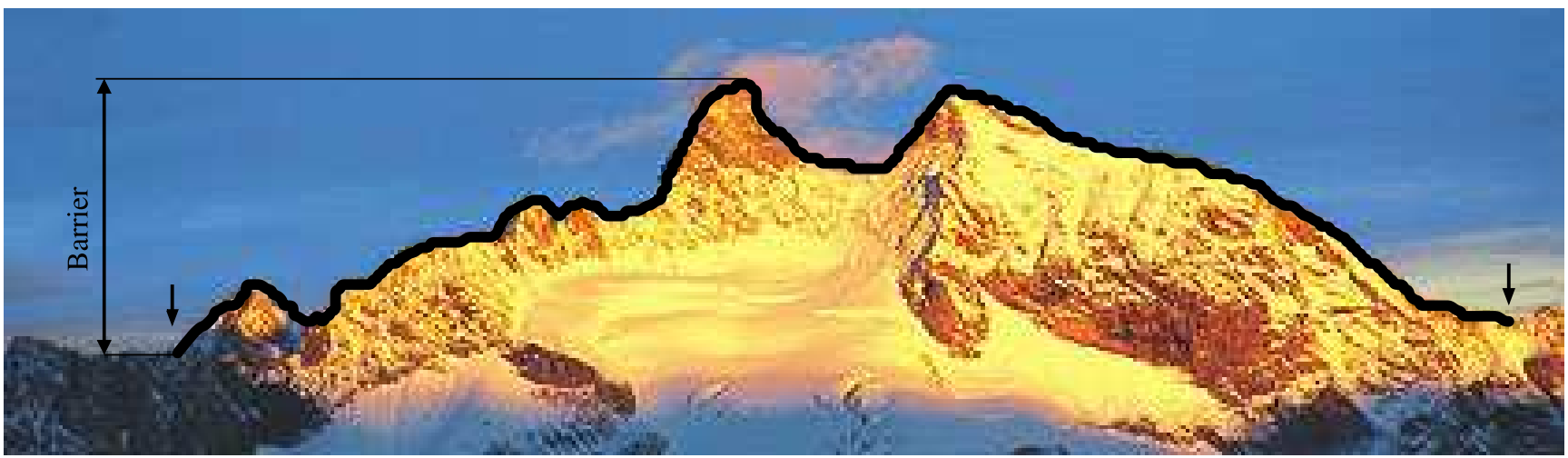
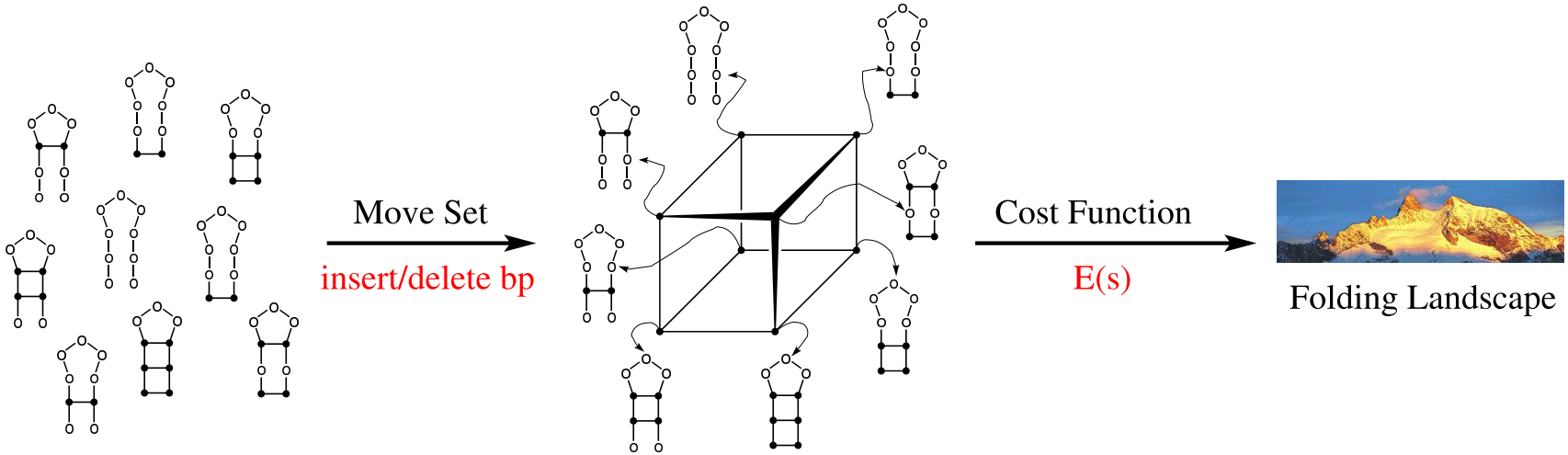
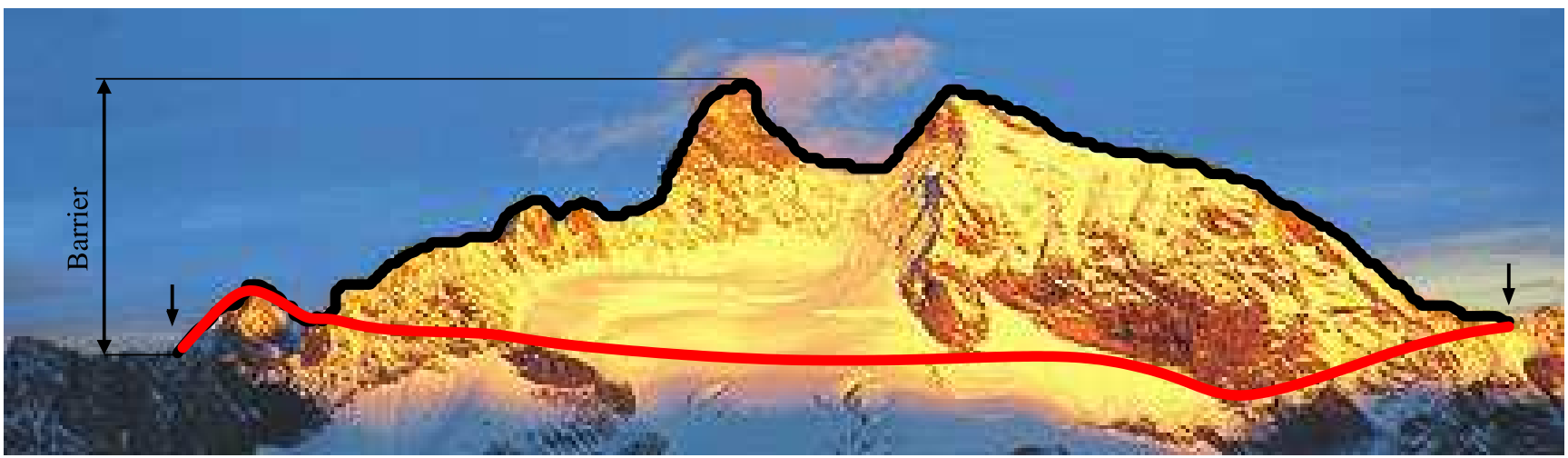




RNA free energy landscape





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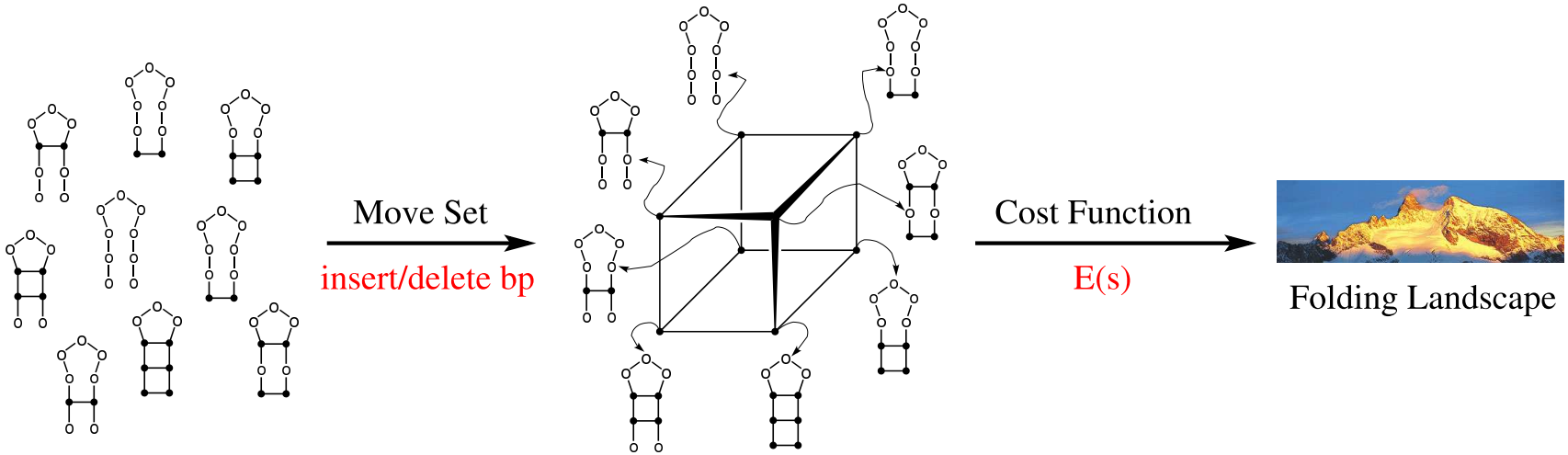

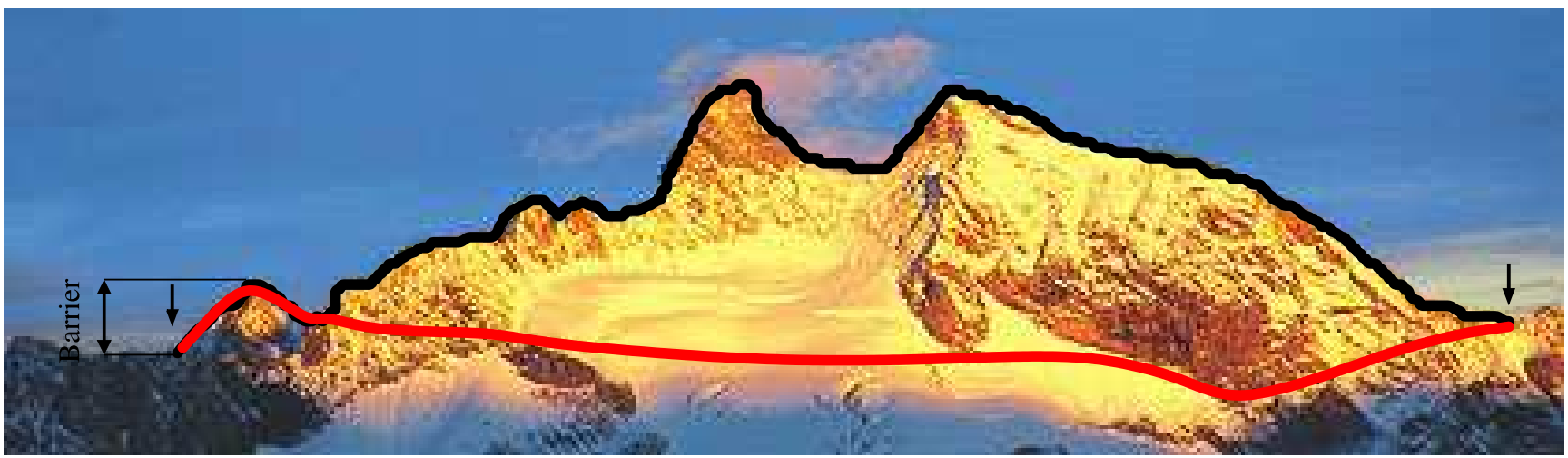

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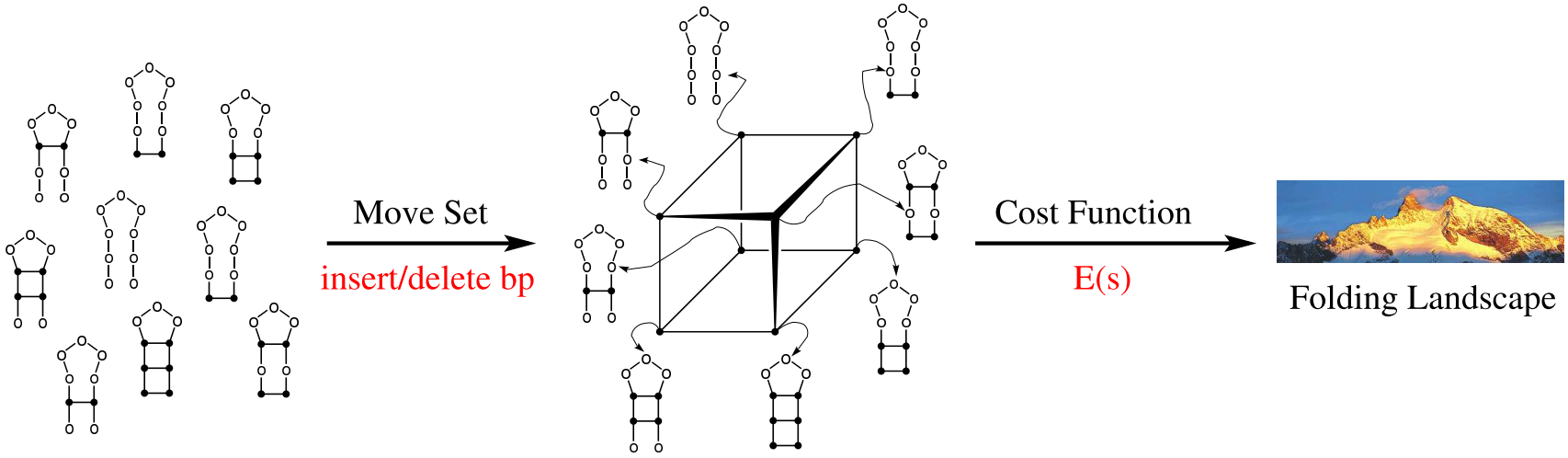
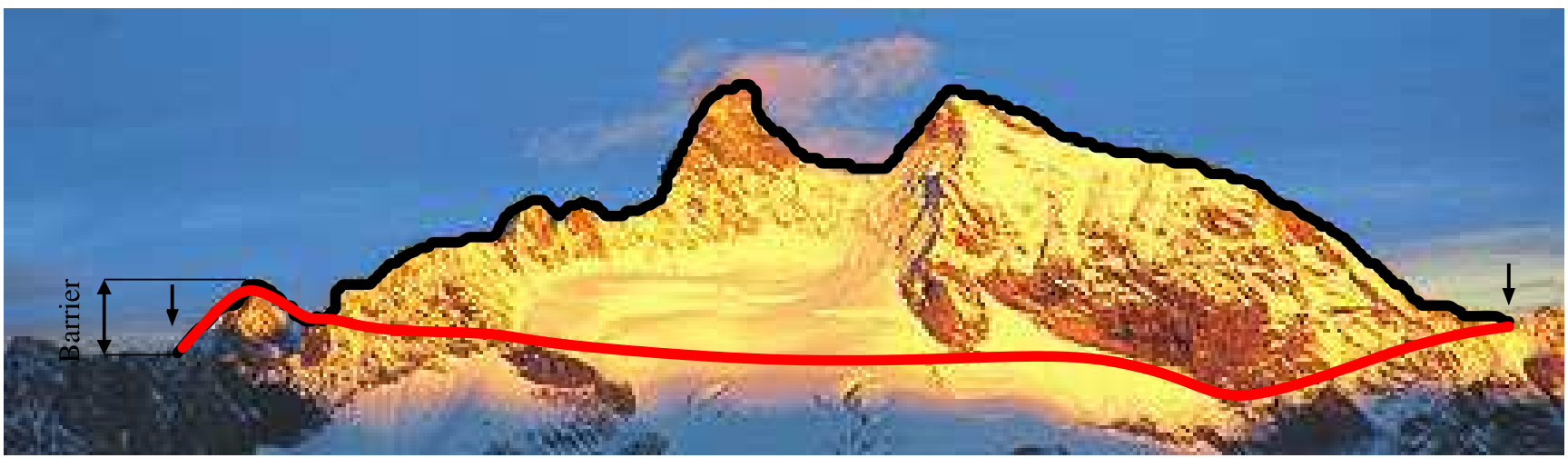
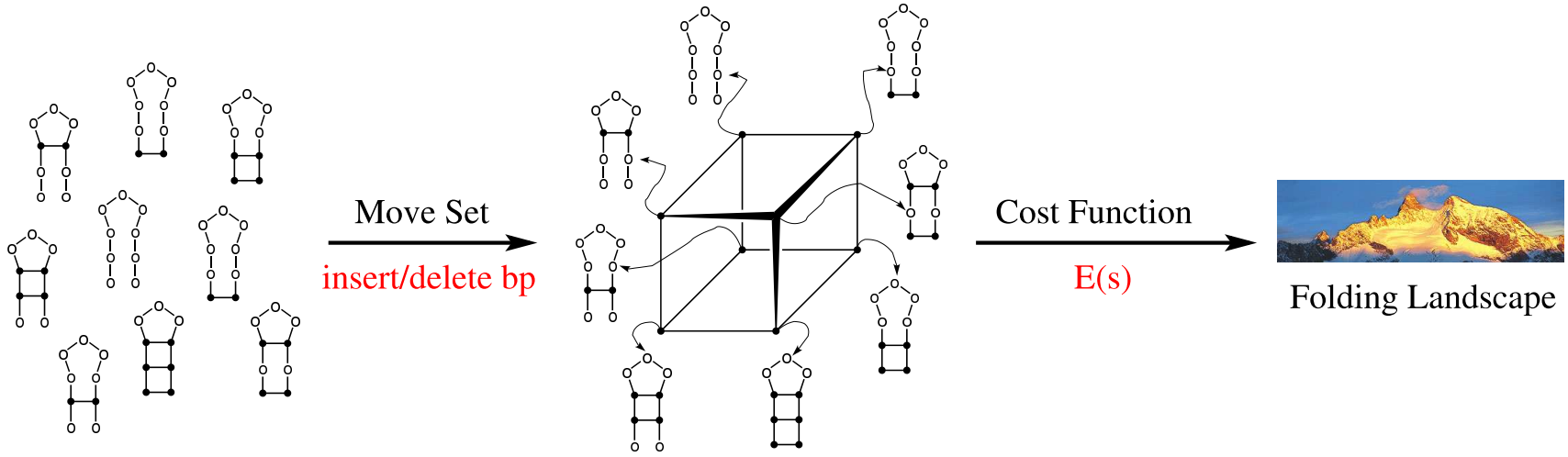
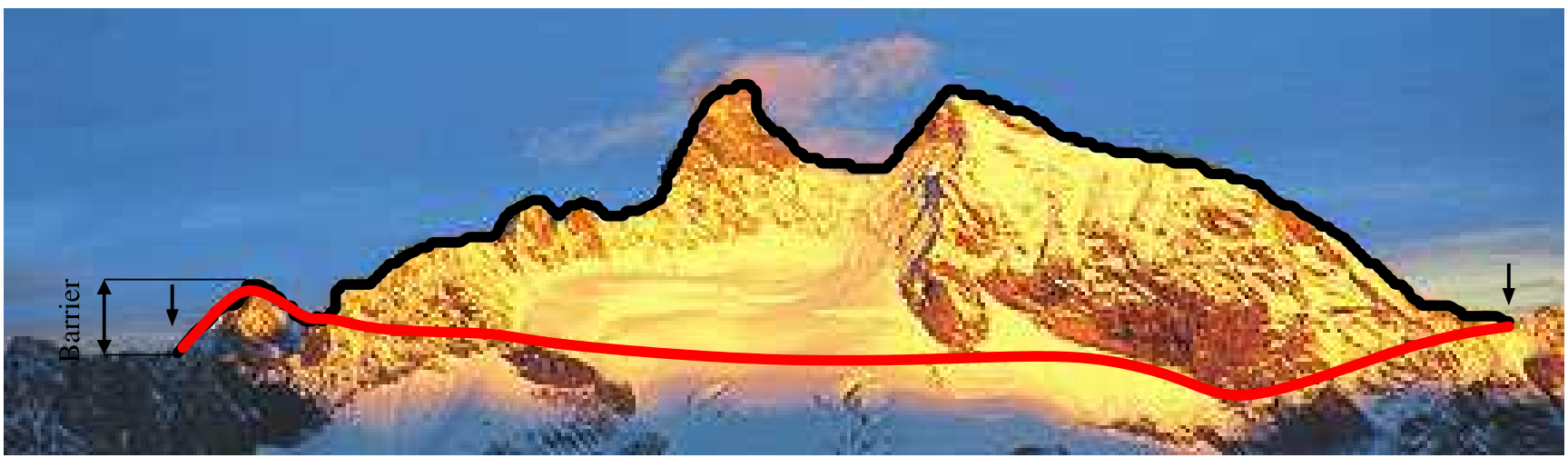




Free energy landscape is a high-dimensional irregular space

RNA free energy landscape





Does a projection into a low dimensional space with few states reveal insights of the high-dimensional irregular landscape?

κ, λ - **neighborhood**

- fixed reference structures s_1 and s_2
- s is a κ, λ - neighbor $\Leftrightarrow d_{BP}(s, s_1) = \kappa \wedge d_{BP}(s, s_2) = \lambda$
- partitioning of the landscape into κ, λ - neighborhoods

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Questions:

- What are the states with certain free energy (e.g. MFE)?
- What is the partition function?
- How many states?

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- partitioning of the landscape into κ, λ - neighborhoods

Questions:

- What are the states with certain free energy (e.g. MFE)?
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Classified dynamic programming

- Density of states Cupal, J et al. (1996) *Computer Science and Biology* **96**: 184-186
- RNAshapes Steffen, P et al. (2006) *Bioinformatics* **22**(4): 500-503
- RNAbor Freyhult, E et al. (2007) *Bioinformatics* **23**(16): 2054-2062

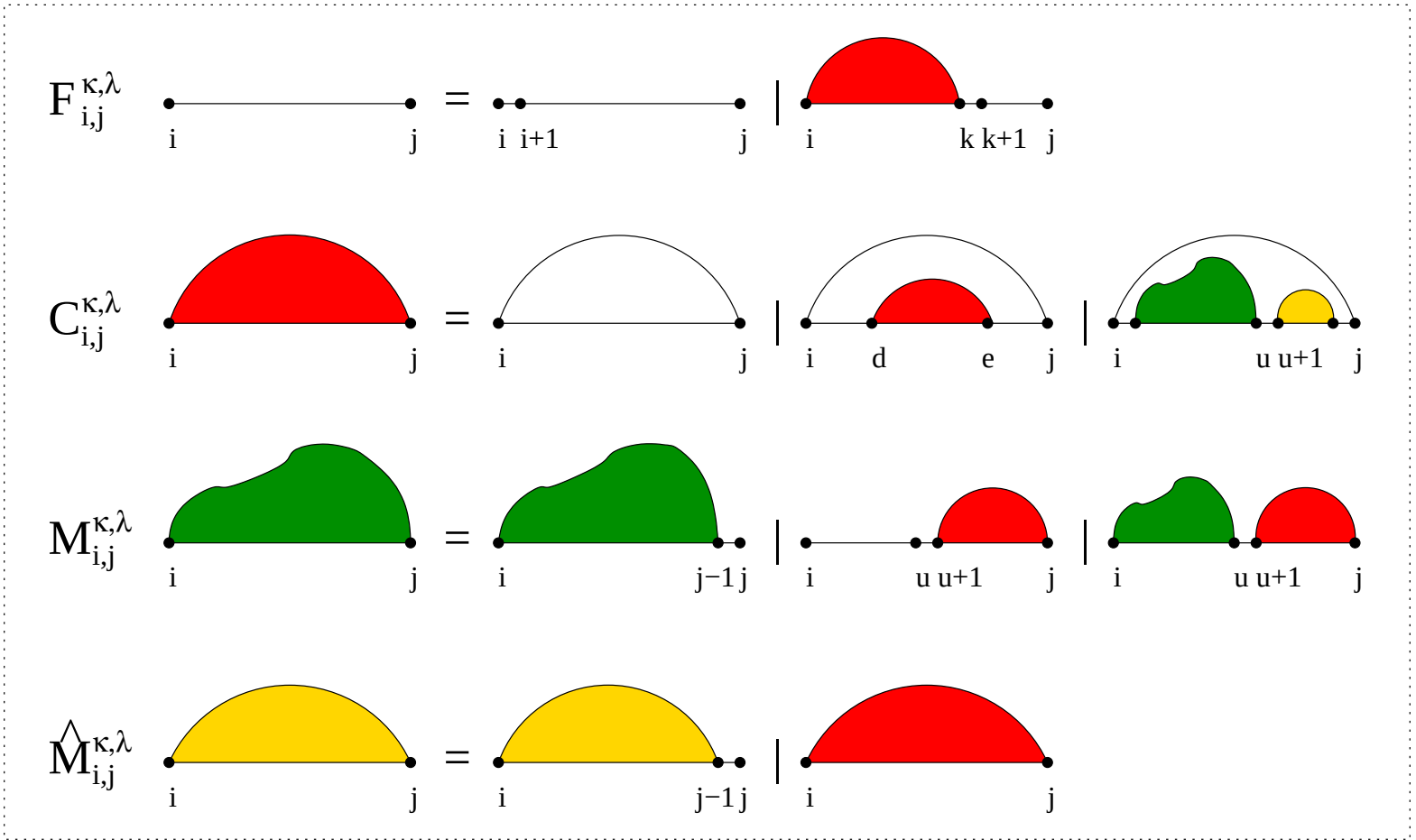
Structure decomposition scheme for κ, λ-neighbors

$$
\begin{aligned}
F_{i,j}^{\kappa,\lambda} &= \min \left\{ \begin{array}{l} F_{i,j-1}^{\kappa-\delta_1^1(i,j),\lambda-\delta_1^2(i,j)}, \\ \min\limits_{i\le u<j} \min\limits_{\substack{\omega_1+\hat{\omega}_1=\kappa-\delta_2^1(i,j,u) \\ \omega_2+\hat{\omega}_2=\lambda-\delta_2^2(i,j,u)}} F_{i,u-1}^{\omega_1,\omega_2} + C_{u,j}^{\hat{\omega}_1,\hat{\omega}_2} \end{array} \right. \\
C_{i,j}^{\kappa,\lambda} &= \min \left\{ \begin{array}{l} \mathfrak{H}(i,j,\kappa,\lambda), \\ \min\limits_{i<p<q<j} \left\{ C_{p,q}^{\kappa-\delta_3^1(i,j,p,q),\lambda-\delta_3^2(i,j,p,q)} + \mathcal{I}(i,j,p,q) \right\}, \\ \min\limits_{i<u<j} \min\limits_{\substack{\omega_1+\hat{\omega}_1=\kappa-\delta_4^1(i,j,u) \\ \omega_2+\hat{\omega}_2=\lambda-\delta_4^2(i,j,u)}} \left\{ M_{i+1,u}^{\omega_1,\omega_2} + \hat{M}_{u+1,j-1}^{\hat{\omega}_1,\hat{\omega}_2} + a \right\} \end{array} \right. \\
M_{i,j}^{\kappa,\lambda} &= \min \left\{ \begin{array}{l} M_{i,j}^{\kappa-\delta_1^1(i,j-1),\lambda-\delta_1^2(i,j)} + c \\ \min\limits_{i\le u<j} \left\{ (u-i)\cdot c + C_{u,j}^{\kappa-\delta_5^1(i,j,u),\lambda-\delta_5^2(i,j,u)} + b \right\}, \\ \min\limits_{i\le u<j} \min\limits_{\substack{\omega_1+\hat{\omega}_1=\kappa-\delta_2^1(i,j,u) \\ \omega_2+\hat{\omega}_2=\lambda-\delta_2^2(i,j,u)}} \left\{ M_{i,u-1}^{\omega_1,\omega_2} + C_{u,j}^{\hat{\omega}_1,\hat{\omega}_2} + b \right\}, \end{array} \right. \\
\hat{M}_{i,j}^{\kappa,\lambda} &= \min \left\{ \begin{array}{l} C_{i,j}^{\kappa,\lambda} + b \\ \hat{M}_{i,j-1}^{\kappa-\delta_1^1(i,j),\lambda-\delta_1^2(i,j)} + c, \end{array} \right.
\end{aligned}
$$

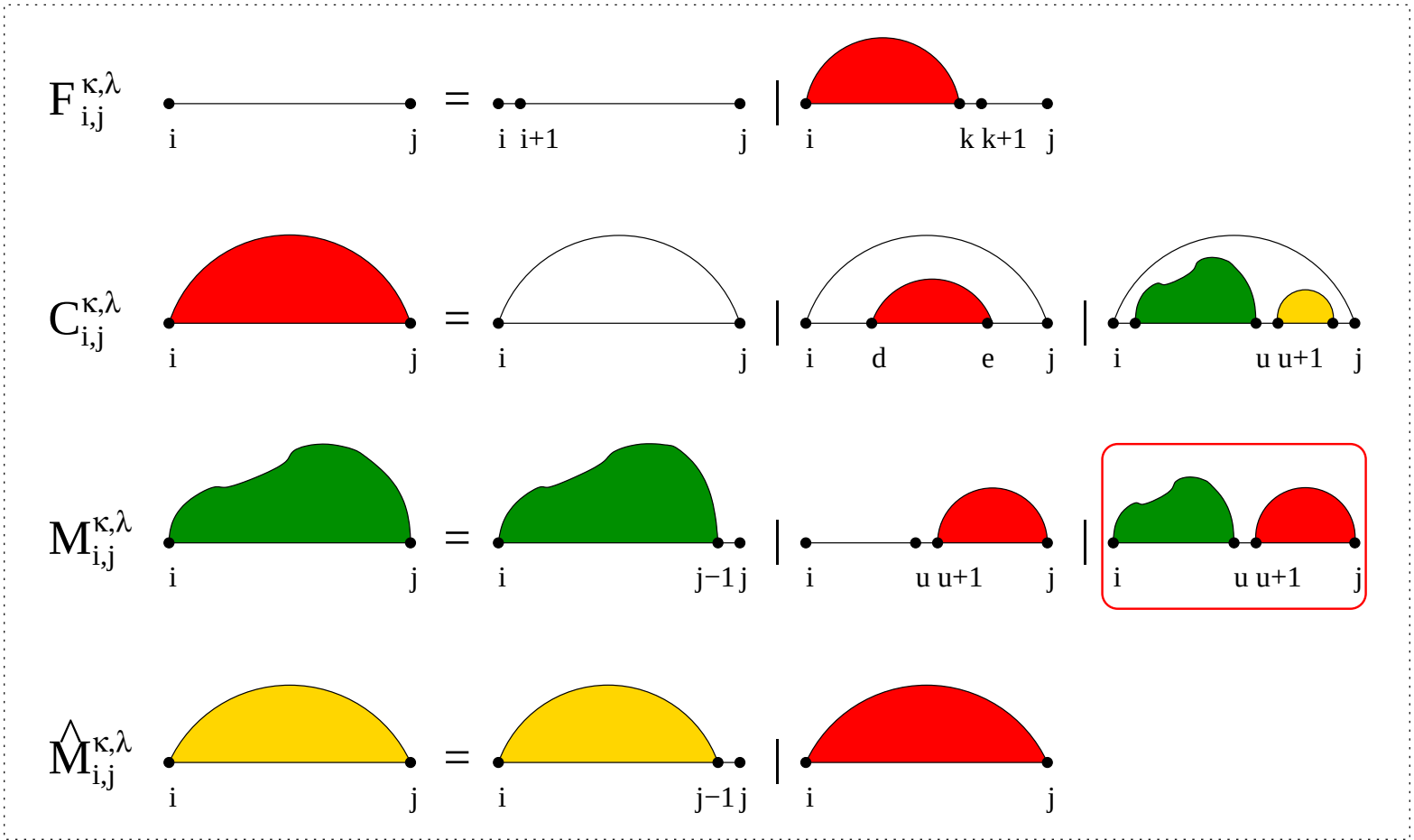
with:

$$
\begin{aligned}
\delta_1^x(i,j) &= d_{\mathrm{BP}}(s_x[i,j], s_x[i,j-1]) \\
\delta_2^x(i,j,u) &= d_{\mathrm{BP}}(s_x[i,j], s_x[i,u-1] \cup s_x[u,j]) \\
\delta_3^x(i,j,p,q) &= d_{\mathrm{BP}}(s_x[i,j], \{(i,j)\} \cup s_x[p,q]) \\
\delta_4^x(i,j,u) &= d_{\mathrm{BP}}(s_x[i,j], \{(i,j)\} \cup s_x[i+1,u] \cup s_x[u+1,j-1]) \\
\delta_5^x(i,j,u) &= d_{\mathrm{BP}}(s_x[i,j], s_x[u,j])
\end{aligned}
$$

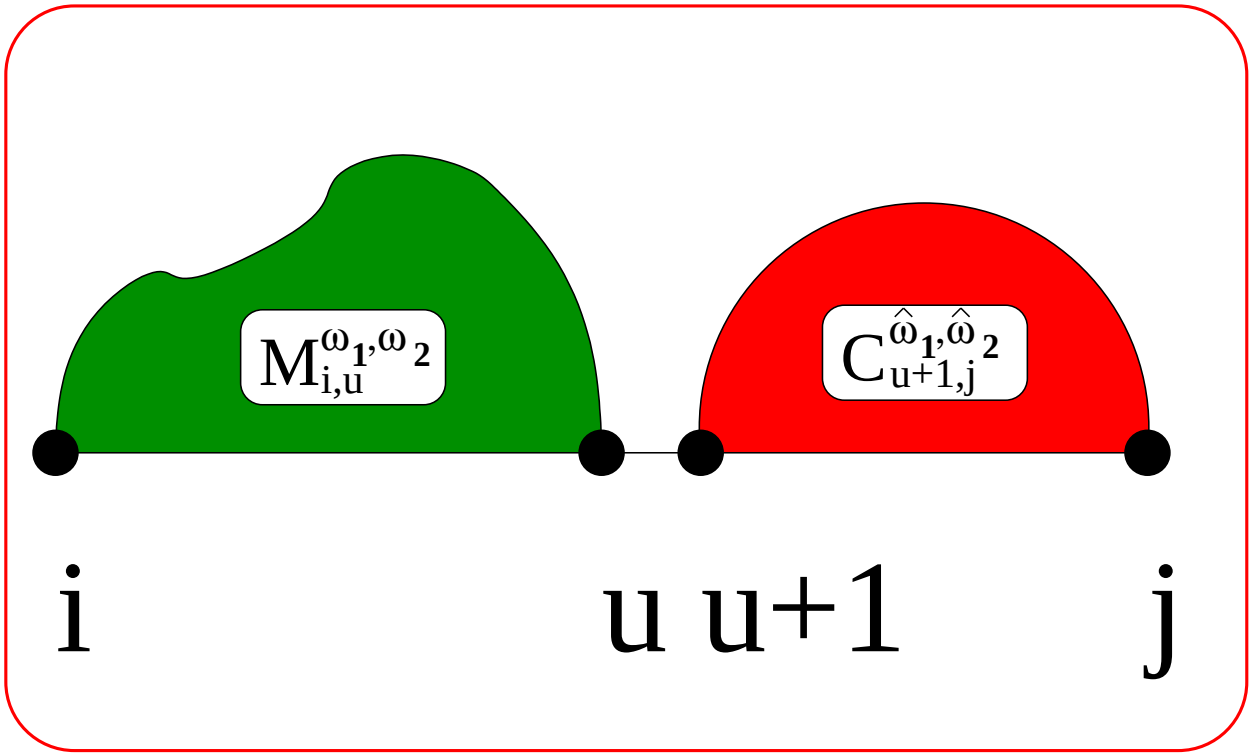
Structure decomposition scheme for κ, λ-neighbors



Structure decomposition scheme for κ, λ-neighbors



Example: multi-loop decomposition



$$\omega_1 + \hat{\omega}_1 = \kappa - d_{\mathrm{BP}}(s_1[i,j], s_1[i,u] \cup s_1[u+1,j])$$
$$\omega_2 + \hat{\omega}_2 = \lambda - d_{\mathrm{BP}}(s_2[i,j], s_2[i,u] \cup s_2[u+1,j])$$

Complexity

- $\mathcal{O}(n^7)$ in time
- $\mathcal{O}(n^4)$ in memory

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- $\mathcal{O}(n^4)$ in memory

Implementation

Naïve:

- more than 3.5h runtime for sequence of length $100nt$
- more than 30GB RAM for sequences with length $> 200nt$

Complexity

- $\mathcal{O}(n^7)$ in time
- $\mathcal{O}(n^4)$ in memory

Implementation

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- more than 3.5h runtime for sequence of length $100nt$
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This does not look promising :(

Complexity

- $\mathcal{O}(n^7)$ in time
- $\mathcal{O}(n^4)$ in memory

Implementation

Naïve:

- more than 3.5h runtime for sequence of length $100nt$
- more than 30GB RAM for sequences with length $> 200nt$

BUT: Sparse matrices (less than 2% entries with values $\neq \infty$)

Complexity

- $\mathcal{O}(n^7)$ in time
- $\mathcal{O}(n^4)$ in memory

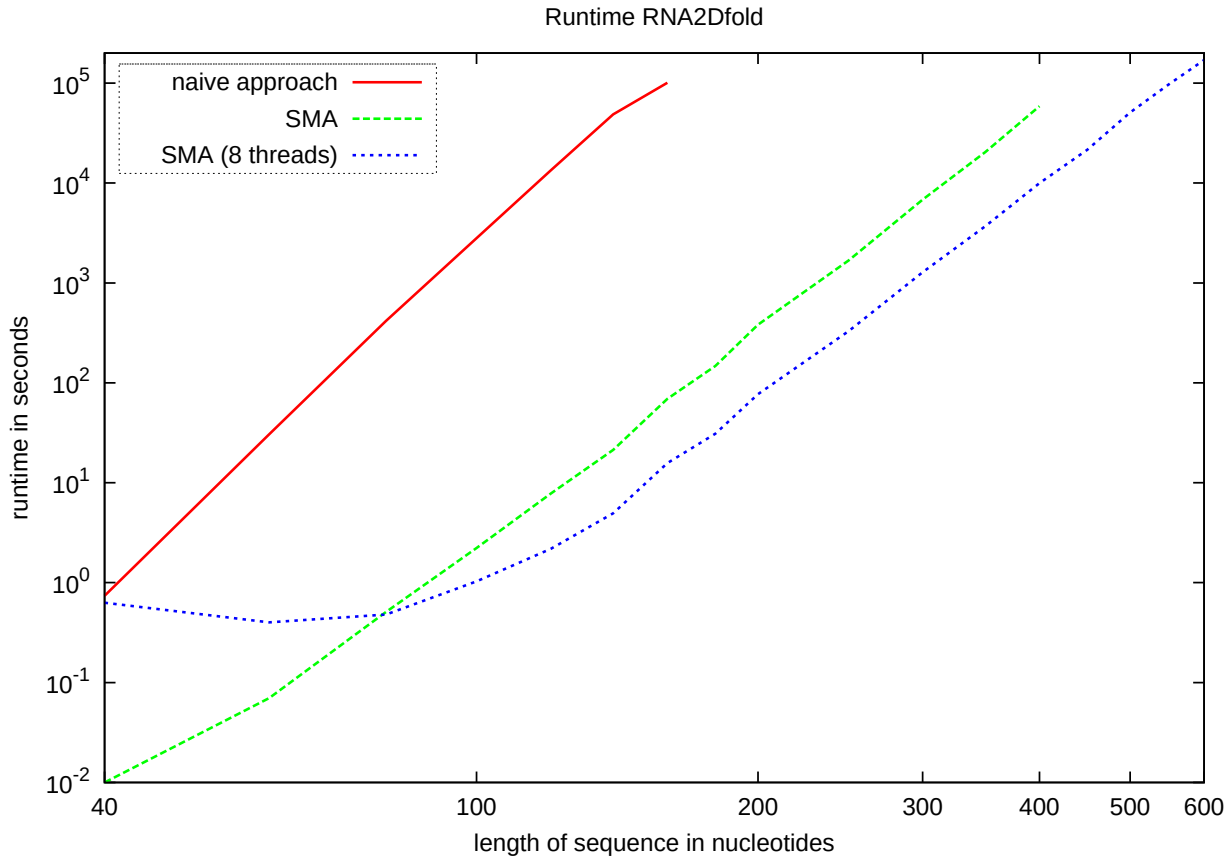
Implementation

Naïve:

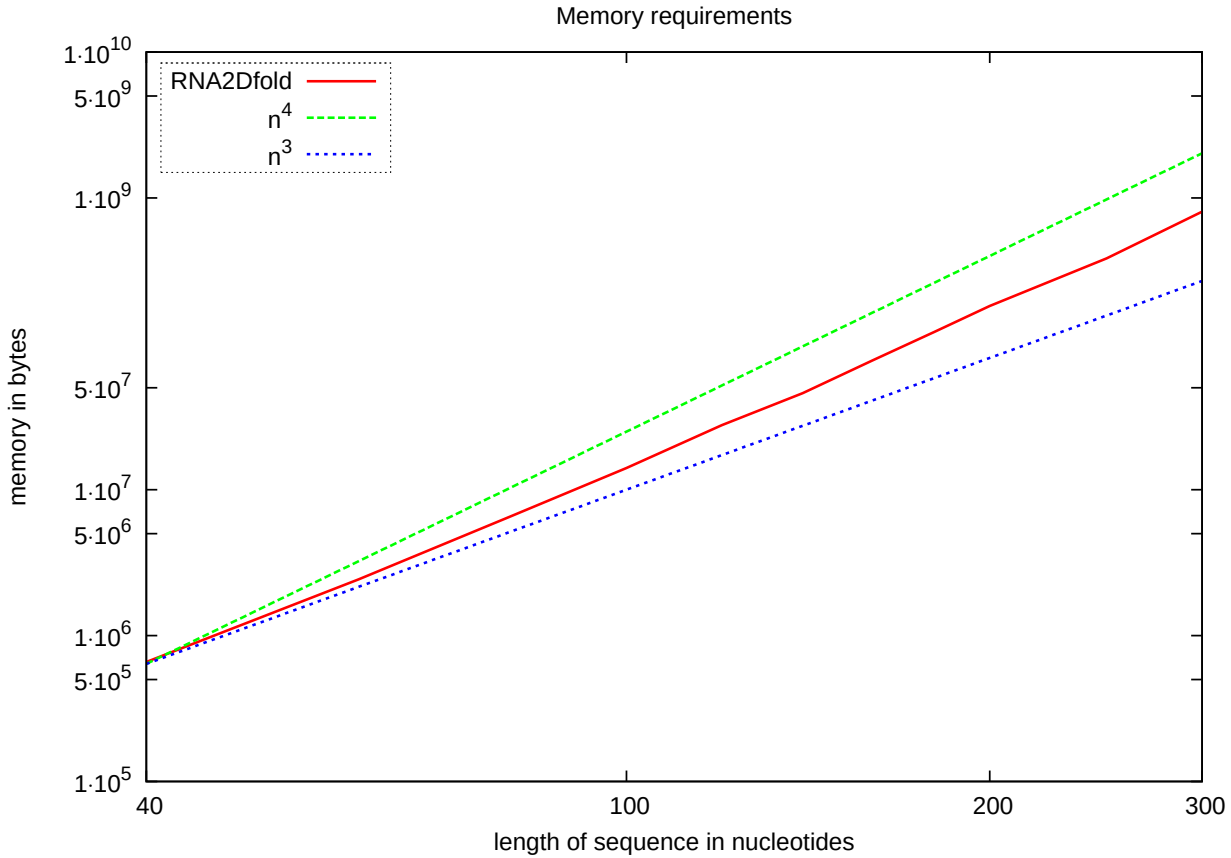
- more than 3.5h runtime for sequence of length $100nt$
- more than 30GB RAM for sequences with length $> 200nt$

`RNA2Dfold`

- exploits sparse matrices and parallel computation of diagonal elements with `OpenMP`
- MFE computation
- Partition function computation
- Boltzmann sampling



Runtimes for dual quad-core Intel® Xeon® E5450 @3.00GHz, 32GB RAM


Memory requirements
RNA2Dfold
n^4
n^3
memory in bytes
1·10^10
5·10^9
1·10^9
5·10^7
1·10^7
5·10^6
1·10^6
5·10^5
1·10^5
40
100
200
300
length of sequence in nucleotides

Output of RNA2Dfold

```
GGGCGCGGUUCGCCCUCCGCUAAAUGCGGAAGAUAAAUUGUGUCU
(((((.....)))))(((((.....)))))(((((.....)))))  (-18.20) <mfe>
(((((.....)))))(((((.....)))))(((((.....)))))  (-18.20) <ref 1>
.............................................  (  0.00) <ref 2>
free energy of ensemble = -19.32 kcal/mol
```

k	l	P(nb)	P(struct in nb)	P(structure)	E_min	E_gibbs	structure
0	15	0.16350376	1.00000000	0.16350396	-18.20	-18.20	(((((.....)))))(((((.....)))))(((((.....)))))
1	14	0.13710099	0.52985725	0.07264404	-17.70	-18.09	(((((.....)))))(((((.....))))).(((.....))).
2	13	0.03870938	0.31496460	0.01219207	-16.60	-17.31	(((((.....)))))(((((.....))))).(((.....))).
2	15	0.00043959	0.78124709	0.00034343	-14.40	-14.55	(((((.....)))))(((((.....)))))(((.(....)))))
3	12	0.00549664	0.31651335	0.00173976	-15.40	-16.11	((((.....))))(((((.....))))).(((.....))).
3	14	0.00050399	0.30274973	0.00015258	-13.90	-14.64	(((((.....)))))(((((.....))))).(((.(....))).
4	11	0.00055642	0.23315097	0.00012973	-13.80	-14.70	((((.....)))).(((.....)))).((((.....))).
4	13	0.00013334	0.19204623	0.00002561	-12.80	-13.82	(((((.....)))))(((((.....)))).)(((.....))).
4	15	0.00001102	0.74654403	0.00000822	-12.10	-12.28	(((((.....)))))(((((.....)))))((((...))..)))
5	10	0.01691144	0.99730503	0.01686584	-16.80	-16.80	((((.....))))(((((.....)))))..............
5	12	0.00001727	0.21163330	0.00000365	-11.60	-12.56	((((.....))))(((((.....))))).(((.....))).
5	14	0.00001787	0.24052012	0.00000430	-11.70	-12.58	(((((.....)))))(((((.....)))))(((.....))..))
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.
20	11	0.00001320	0.20013684	0.00000264	-11.40	-12.39	(((((((....)))...(((.....)))..........))))))
20	13	0.00459594	0.37854283	0.00173976	-15.40	-16.00	.(((((((((.....(((.....))))).....))))))))).
20	15	0.18615427	0.39023612	0.07264404	-17.70	-18.28	((((((((((.....(((.....)))))).....))))))))))
21	6	0.00000000	0.11205340	0.00000000	-6.10	-7.45	..((((((........)))).....)).....................
21	8	0.00000000	0.27124082	0.00000000	-5.90	-6.70	.(((((((........))(......).............))))).
21	10	0.00000041	0.34501497	0.00000014	-9.60	-10.26	(((((((........)))(.....)).............))))))
21	12	0.00003261	0.25218449	0.00000822	-12.10	-12.95	.(((((((((.....(((.....)))).....)))))))))).
21	14	0.00907139	0.36701076	0.00332929	-15.80	-16.42	((((((((((.....(((.....)))).....))))))))))
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.
27	16	0.00000011	0.42869149	0.00000005	-8.90	-9.42	((((((((((.(..((.(((.....)).).)).).))))))))))
28	13	0.00000063	0.51237067	0.00000032	-10.10	-10.51	((((((((((.((.....)).....(...)..))))))))))
28	15	0.00000000	0.31993086	0.00000000	-5.60	-6.30	((((((((((.(..((.(((.......).).)).).))))))))))
29	14	0.00000024	0.16525529	0.00000004	-8.80	-9.91	((((((((((..(.(.(........).)).)..)...))))))))
29	16	0.00000000	0.50536309	0.00000000	-3.80	-4.22	((((((((((.(.(.(((.....)).).).).).))))))))))
30	15	0.00000015	0.43442428	0.00000006	-9.10	-9.61	((((((((((.((.....)).....(((....).))))))))))))
31	16	0.00000000	0.31578542	0.00000000	-5.80	-6.51	((((((((((.((..(((.....)).))..).).))))))))))

Output of RNA2Dfold

```
GGGCGCGGUUCGCCCUCCGCUAAAUGCGGAAGAUAAAUUGUGUCU
(((((.....)))))(((((.....)))))(((((.....)))))  (-18.20) <mfe>
(((((.....)))))(((((.....)))))(((((.....)))))  (-18.20) <ref 1>
.............................................  (  0.00) <ref 2>
free energy of ensemble = -19.32 kcal/mol
```

k	l	P(nb)	P(struct in nb)	P(structure)	E_min	E_gibbs	structure
0	15	0.16350376	1.00000000	0.16350396	-18.20	-18.20	(((((.....)))))(((((.....)))))(((((.....)))))
1	14	0.13710099	0.52985725	0.07264404	-17.70	-18.09	(((((.....)))))(((((.....))))).(((.....))).
2	13	0.03870938	0.31496460	0.01219207	-16.60	-17.31	(((((.....)))))(((((.....))))).(((.....))).
2	15	0.00043959	0.78124709	0.00034343	-14.40	-14.55	(((((.....)))))(((((.....)))))(((.(....)))))
3	12	0.00549664	0.31651335	0.00173976	-15.40	-16.11	((((.......))))(((((.....))))).(((.....))).
3	14	0.00050399	0.30274973	0.00015258	-13.90	-14.64	(((((.....)))))(((((.....))))).(((.(....))).
4	11	0.00055642	0.23315097	0.00012973	-13.80	-14.70	((((.......)))).(((.....)))).(((.......))).
4	13	0.00013334	0.19204623	0.00002561	-12.80	-13.82	(((((.....)))))(((((.....)))).)(((.......))).
4	15	0.00001102	0.74654403	0.00000822	-12.10	-12.28	(((((.....)))))(((((.....)))))((((...))..)))
5	10	0.01691144	0.99730503	0.01686584	-16.80	-16.80	((((((.....))))))(((((.....)))))..............
5	12	0.00001727	0.21163330	0.00000365	-11.60	-12.56	((((.......))))(((((.....))))).)(((.....))).
5	14	0.00001787	0.24052012	0.00000430	-11.70	-12.58	(((((.....)))))(((((.....)))))(((.....))..))
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.
20	11	0.00001320	0.20013684	0.00000264	-11.40	-12.39	(((((((....))...(((.....)))..........))))))
20	13	0.00459594	0.37854283	0.00173976	-15.40	-16.00	.(((((((((.....(((.....))))).....))))))))).
20	15	0.18615427	0.39023612	0.07264404	-17.70	-18.28	((((((((((.....(((((.....)))))).....))))))))))
21	6	0.00000000	0.11205340	0.00000000	-6.10	-7.45	..((((((........))))).....)).....
21	8	0.00000000	0.27124082	0.00000000	-5.90	-6.70	.(((((((........)))(.......).....)))))).
21	10	0.00000041	0.34501497	0.00000014	-9.60	-10.26	(((((((........)))(.....)).....))))))
21	12	0.00003261	0.25218449	0.00000822	-12.10	-12.95	.((((((((.....(((.....)))).....)))))))).
21	14	0.00907139	0.36701076	0.00332929	-15.80	-16.42	((((((((((.....(((.....)))).....))))))))))
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.
27	16	0.00000011	0.42869149	0.00000005	-8.90	-9.42	((((((((((.(..((.(((.....)).).)).).))))))))))
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29	16	0.00000000	0.50536309	0.00000000	-3.80	-4.22	((((((((((.(.(.(((.....)).).).).).))))))))))
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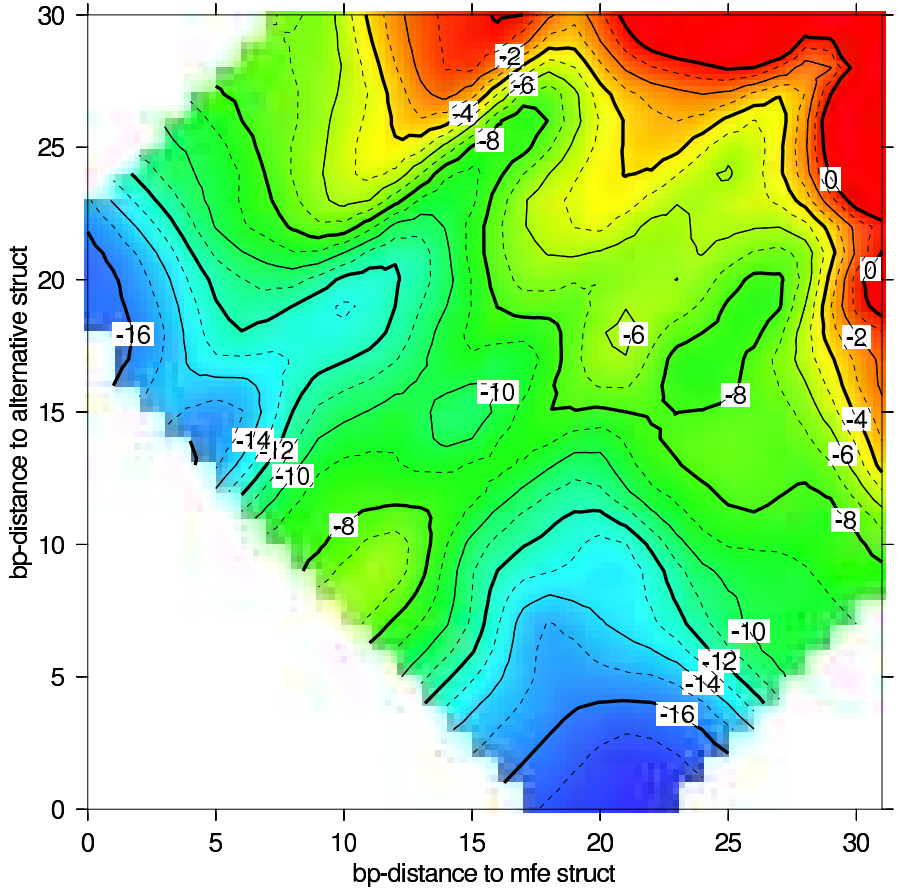
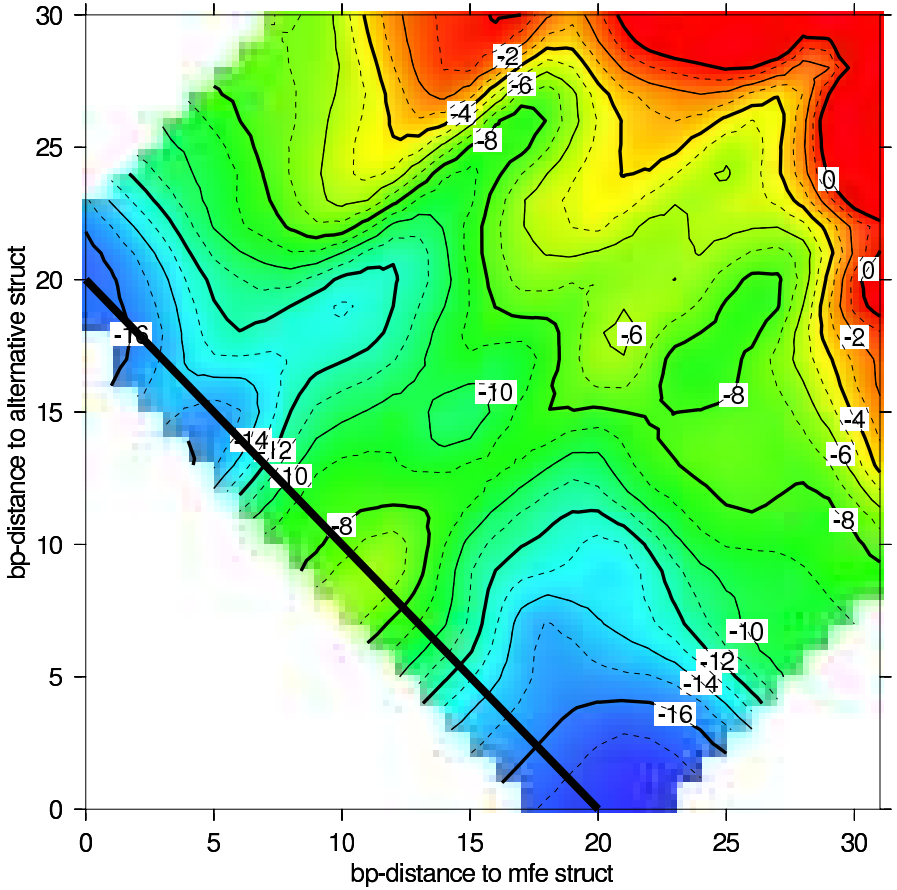
Height map of κ, λ-neighborhood MFEs



MFE structure vs. alternative structure

Estimating barrier height

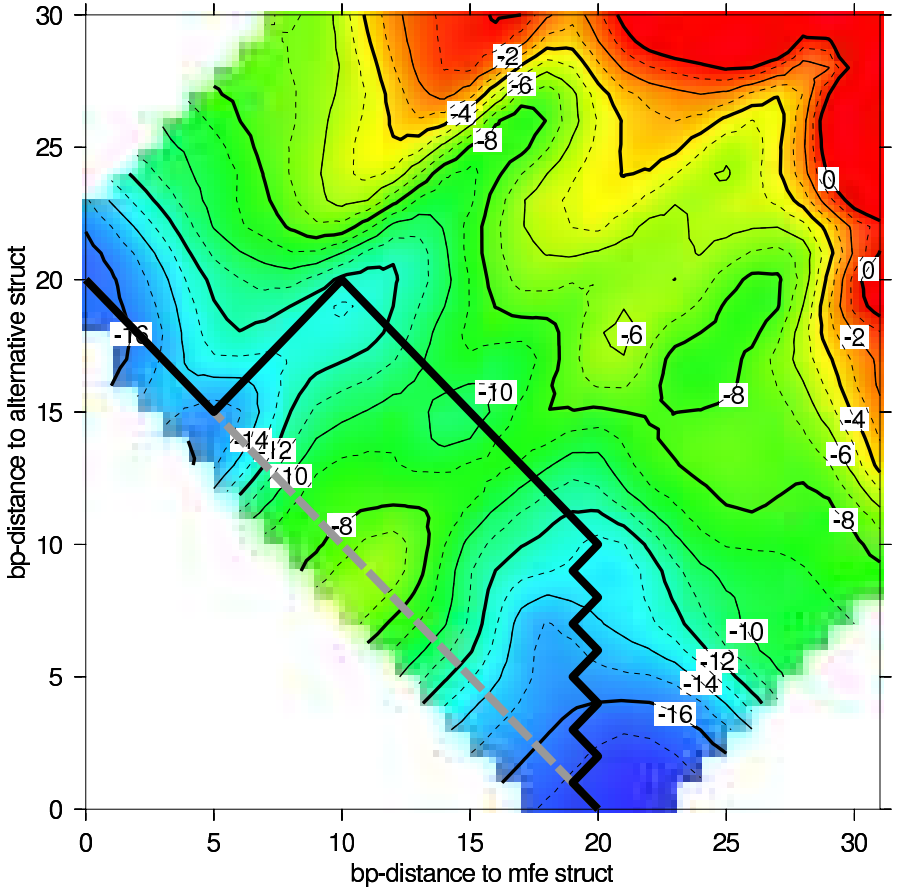


Direct path

best saddle energy ≥ -4.9 kcal/mol

lower bound of barrier ≥ 13.3 kcal/mol

Estimating barrier height



Best path in κ, λ-neighborhood (Ford-Bellman algorithm)

$$\text{best saddle energy} \geq -8.2 \text{ kcal/mol}$$

$$\text{lower bound of barrier} \geq 10.0 \text{ kcal/mol}$$

Finding (energetically) best transition path

- exact solution for small sequences (exhaustive enumeration)
- several heuristics, e.g. Morgan & Higgs '98, Flamm et al. '01
- consider only shortest (direct) paths in most cases
- direct paths (NP hard even for Nussinov energy model)[1]
- indirect paths (NP hard)[1]

Direct paths

- perform poorly when states have great distance
- stabilizing base pairs are neglected
- may estimate barrier too high

[1] Maňuch, J et al. (2009) *Proc. of the 15th Intl. Meeting on DNA Computing and Molecular Programming*

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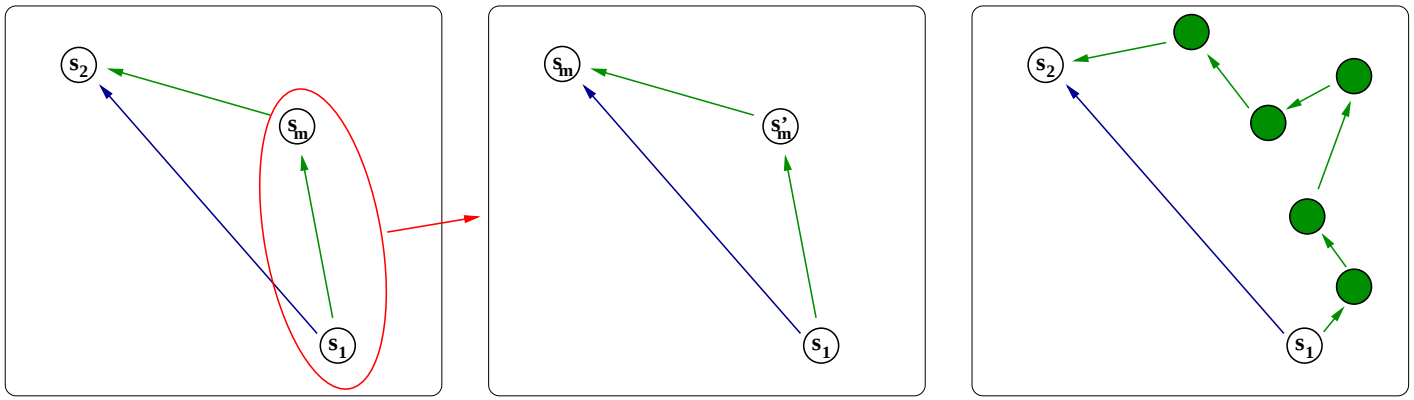
How to exploit the vast amount of general paths?

[1] Maňuch, J et al. (2009) *Proc. of the 15th Intl. Meeting on DNA Computing and Molecular Programming*

Constructing general- from direct-refolding paths

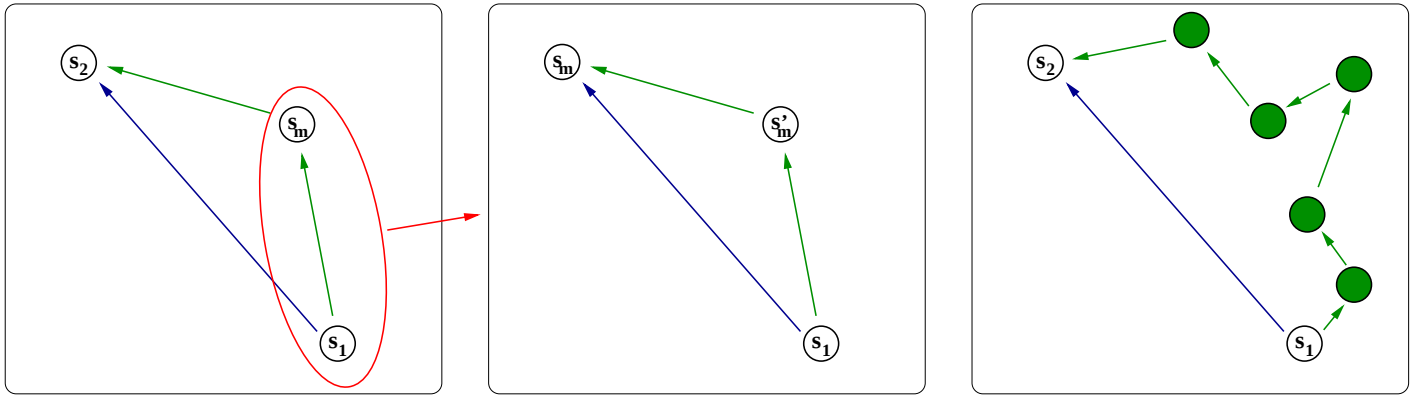
`Pathfinder`



1. generate best direct folding path p_d between s_1 and s_2
2. find x other structures s_{x_m} (meshpoint x)
3. generate best direct paths p_{x_1} from s_1 to s_{x_m} and p_{x_2} from s_{x_m} to s_2
4. concatenate paths $p_x = p_{x_1} + p_{x_2}$
5. accept if $B(p_x) < B(p_d)$
6. if more refinement required, level p_{x_1} and p_{x_2} the same way
7. otherwise return p_x with lowest barrier

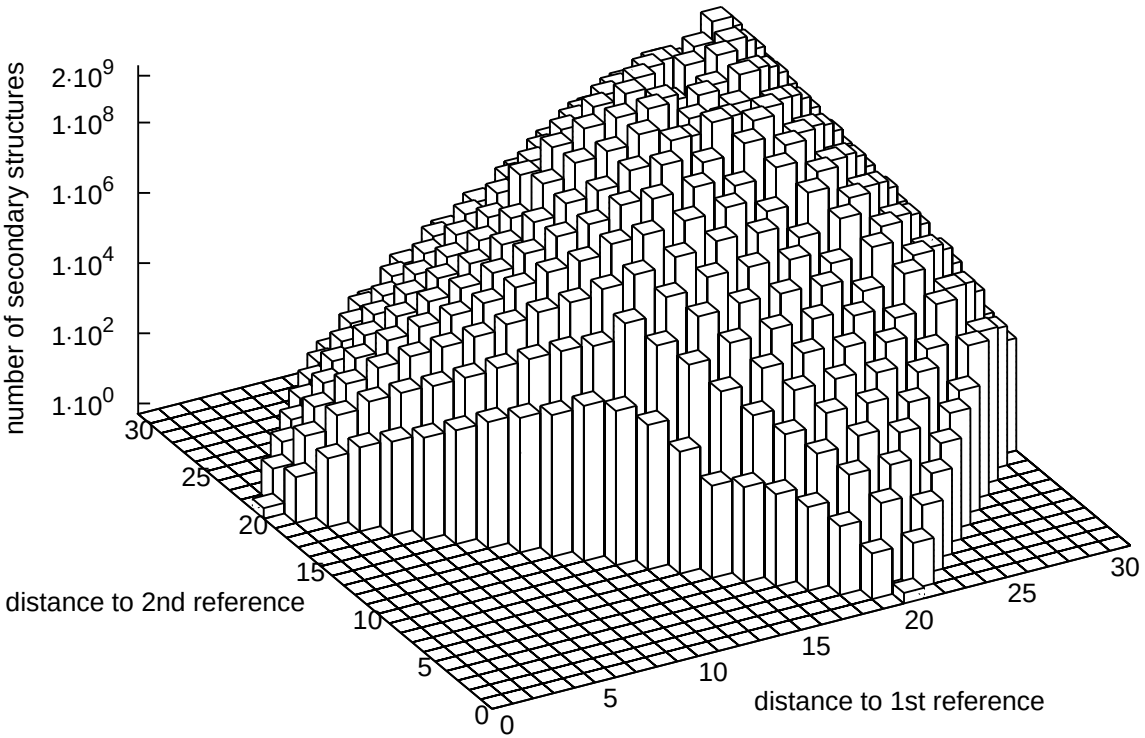
Constructing general- from direct-refolding paths

Pathfinder

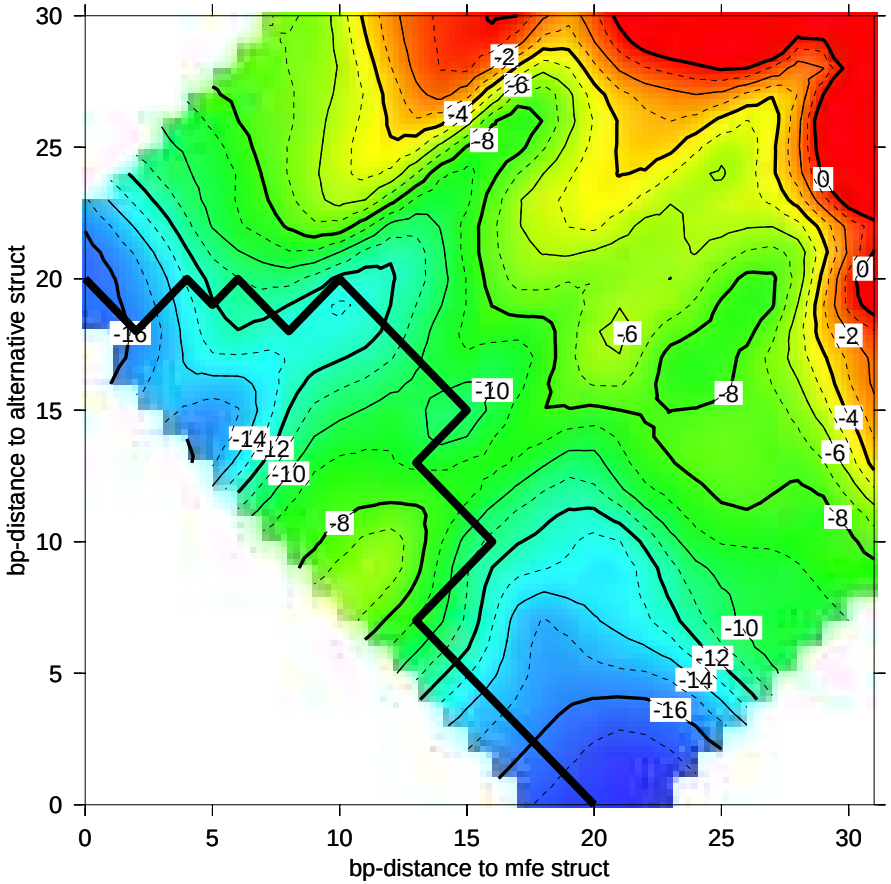


1. generate best direct folding path p_d between s_1 and s_2
2. find x other structures s_{x_m} (meshpoint x, κ, λ MFE representatives)
3. generate best direct paths p_{x_1} from s_1 to s_{x_m} and p_{x_2} from s_{x_m} to s_2
4. concatenate paths $p_x = p_{x_1} + p_{x_2}$
5. accept if $B(p_x) < B(p_d)$
6. if more refinement required, level p_{x_1} and p_{x_2} the same way
7. otherwise return p_x with lowest barrier

Embedding refolding paths



Embedding refolding paths

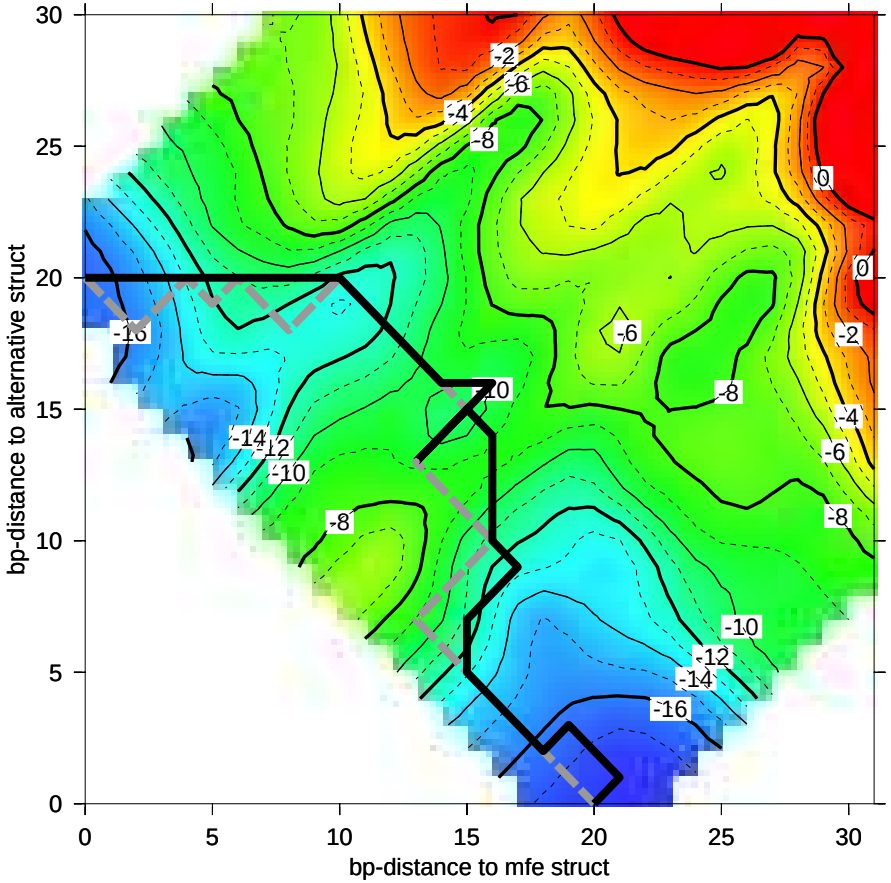


indirect path obtained by the `Pathfinder` algorithm

saddle energy $= -7.5$ kcal/mol

barrier estimate $= 10.7$ kcal/mol

Embedding refolding paths



best path computed by exhaustive enumeration

saddle energy $= -7.5$ kcal/mol

real barrier $= 10.7$ kcal/mol

Conclusion

- 2D projection of high-dimensional folding space into κ, λ-neighborhoods
- Produces qualitative pictures of the energy landscape
- `RNA2Dfold` is fast enough to treat most biologically interesting RNAs by exploiting sparseness and parallelization
- Starting point for the recognition of RNA switches or for coarse grained folding simulations.
- Intermediate node suggestion for indirect folding path construction

Thanks to:

Stephan Bernhart
Christoph Flamm
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Ivo Hofacker
Andrea Tanzer

...and You!

`RNA2Dfold` α-release available:
`http://www.tbi.univie.ac.at/~ronny/RNA/`

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