Designing RNA Structure and Function
A Toolbox for Synthetic Biology

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Synthetic Biology – From understanding to application

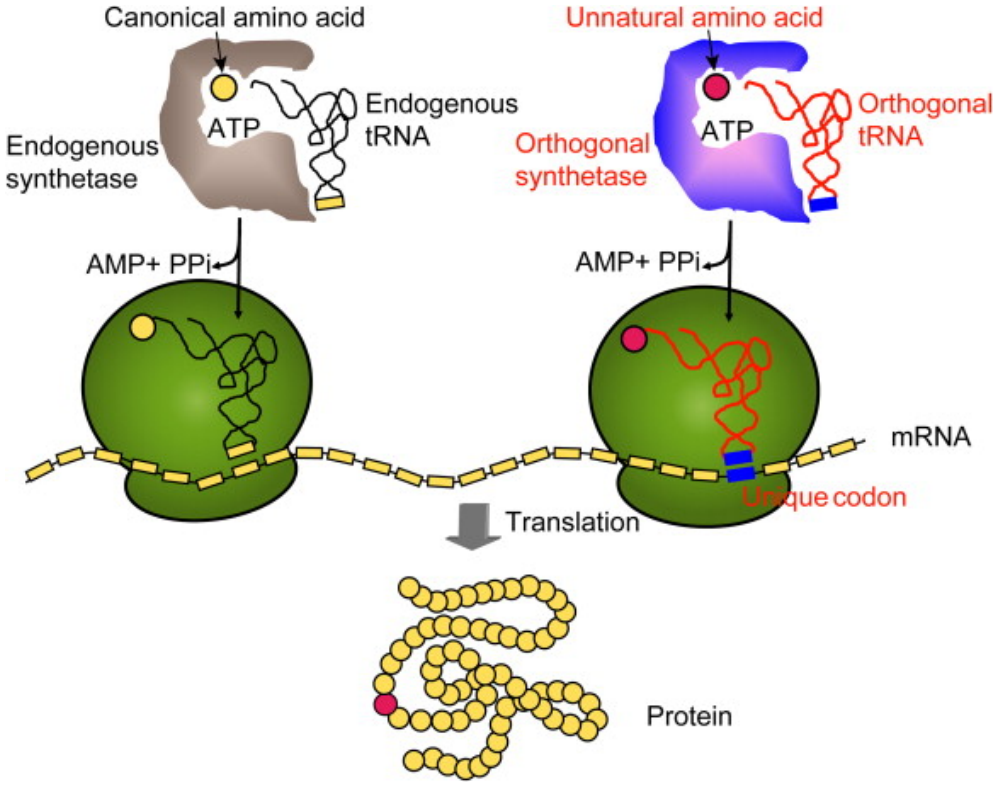
DKFZ-Heidelberg, 09.– 11.12.2013

Web-Page for further information:

http://www.tbi.univie.ac.at/~pks

Prologue

The goals of synthetic biology



A general method for genetically encoding unnatural amino acids in live cells.

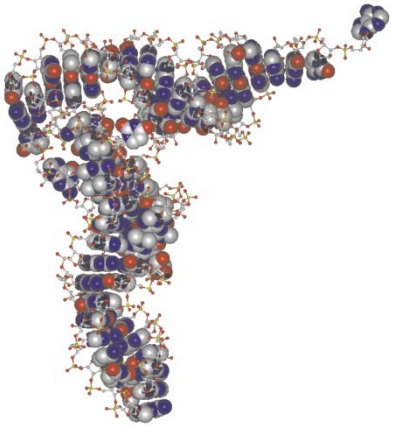
Qian Wang, Angela R. Parrish, Lei Wang. Expanding the genetic code for biological studies. *Chemistry & Biology* **16**:323-336, 2009.

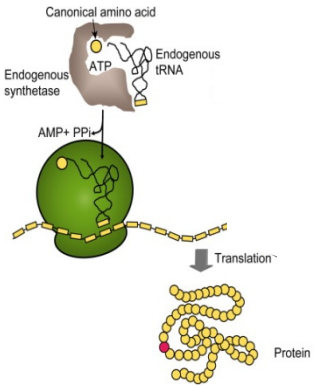
Lei Wang, Peter G. Schultz. Expanding the genetic code. Angew.Chem.Int.Ed. 44:34-66, 2005.

1. One RNA sequence – one structure
2. Many RNA sequences – one structure
3. One RNA sequence – many structures
4. RNA switches

1. **One RNA sequence – one structure**
2. Many RNA sequences – one structure
3. One RNA sequence – many structures
4. RNA switches

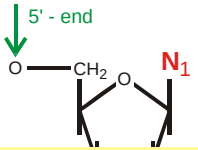
GCGGA ··· UUGCACCA



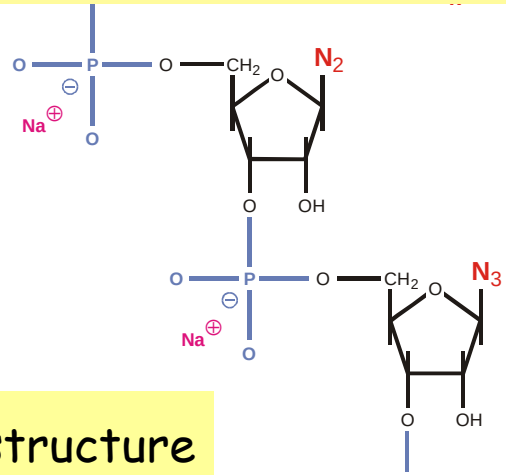


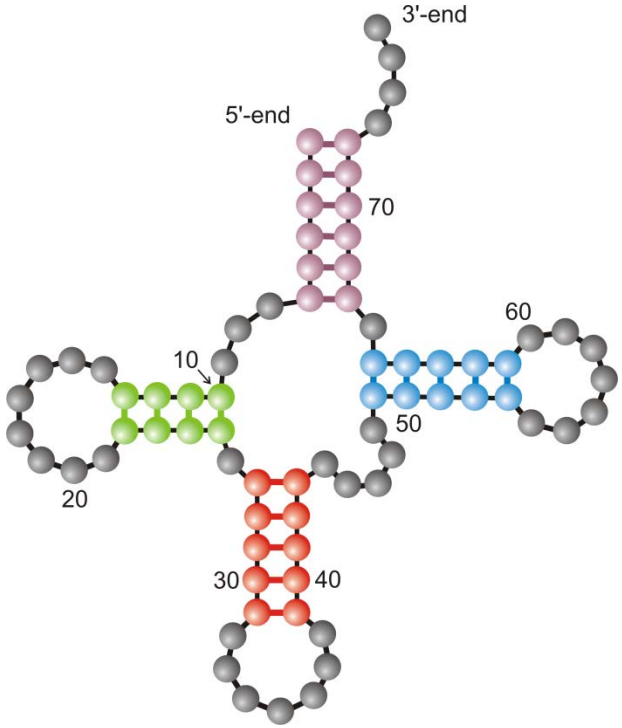
one sequence ⇒ one structure ⇒ function

The paradigm of structural biology

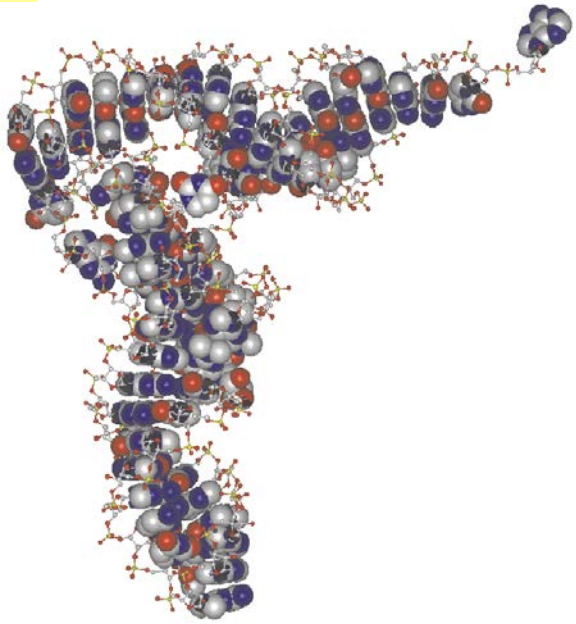


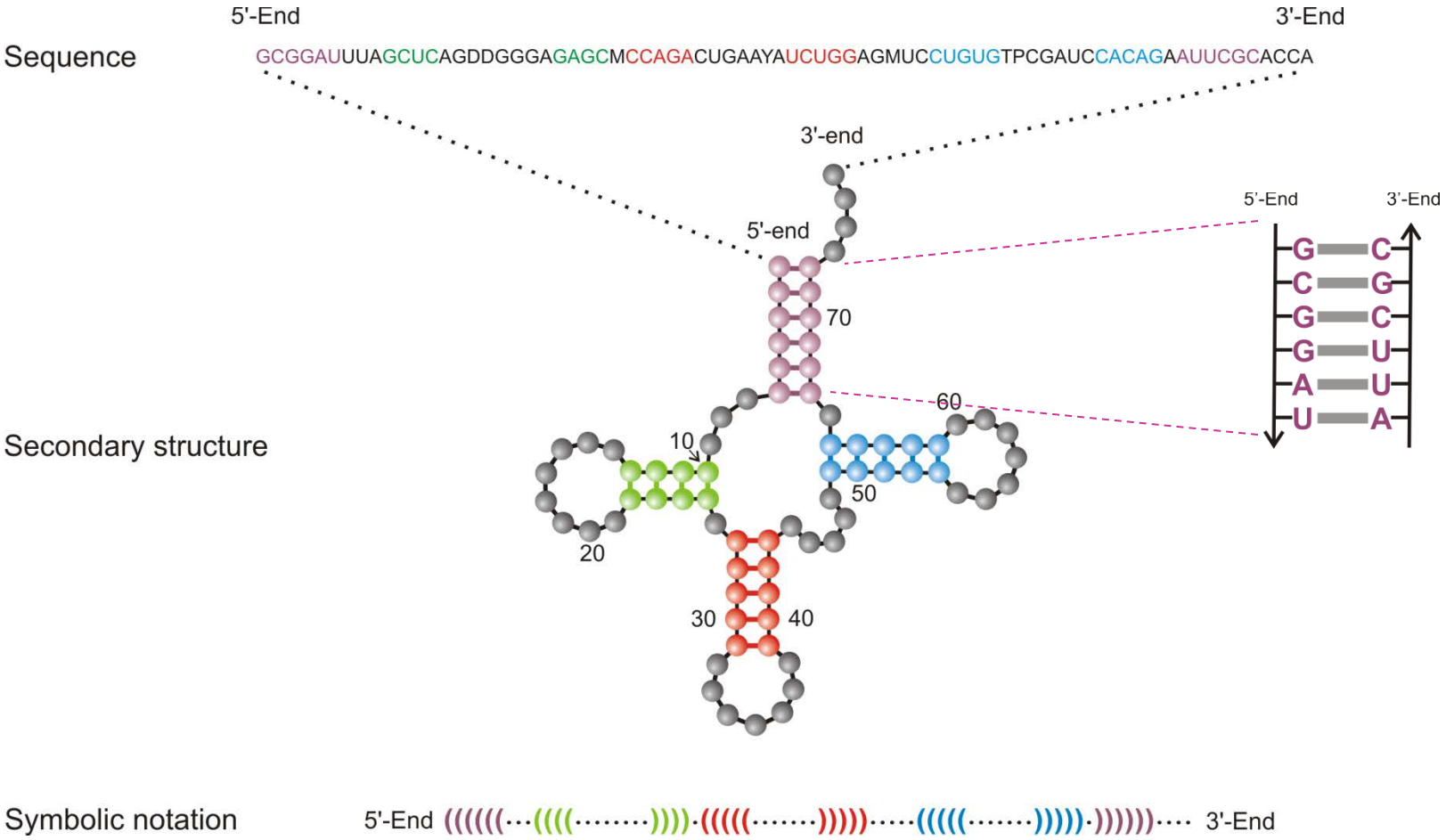
5'-end GCGGAUUUAGCUCAGUUGGGAGAGCGCCAGACUGAAGAUCUGGAGGUCCUGUGUUCGAUCCACAGAAUUCGCACCA 3'-end





Definition of RNA structure





Criterion: Minimum free energy (mfe)

Rules: _ (_) _ ∈ {**AU**,**CG**,**GC**,**GU**,**UA**,**UG**}

A symbolic notation of RNA secondary structure that is equivalent to the conventional graphs

RNA sequence GUAUCGAAAUACGUAGCGUAUGGGGAUGCUGGACGGUCCCAUCGGUACUCCA

RNA folding:

Structural biology, spectroscopy of biomolecules, understanding **molecular function**

Biophysical chemistry: thermodynamics and kinetics

Empirical parameters

Vienna RNA-Package

Version 2.0

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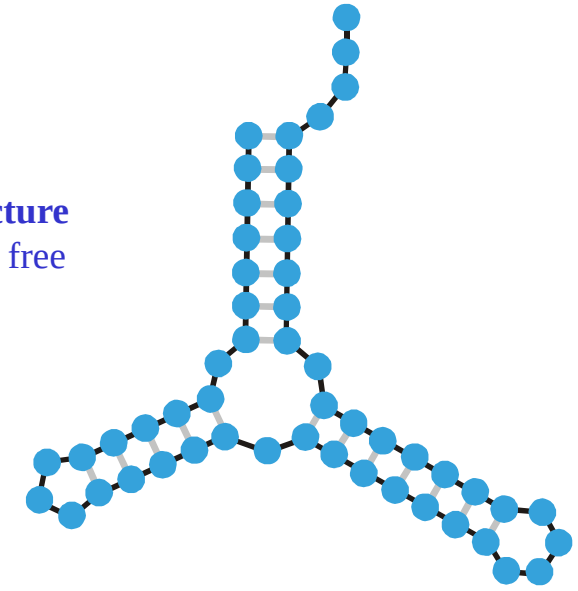
RNA structure of minimal free energy



Sequence, structure, and design

RNA folding into secondary structures

Michael S. Waterman, T. F. Smith. 1978. RNA secondary structures: A complete mathematical analysis.
Math.Biosci. **42**:257-266.

Ruth Nussinov, A. B. Jacobson. 1980.
Fast algorithm for predicting the secondary structure of single-stranded RNA.
Proc.Natl.Acad.Sci. USA **77**:6309-6313.

Michael Zuker, P. Stiegler. 1981.
Optimal computer folding of large RNA sequences using thermodynamics and auxiliary information.
Nucleic Acids Res. **9**:133-148.

Ivo L. Hofacker, Walter Fontana, Peter F. Stadler, Sebastian Bonhoeffer, Manfred Tacker, Peter Schuster. 1994.
Fast folding and comparison of RNA secondary structures.
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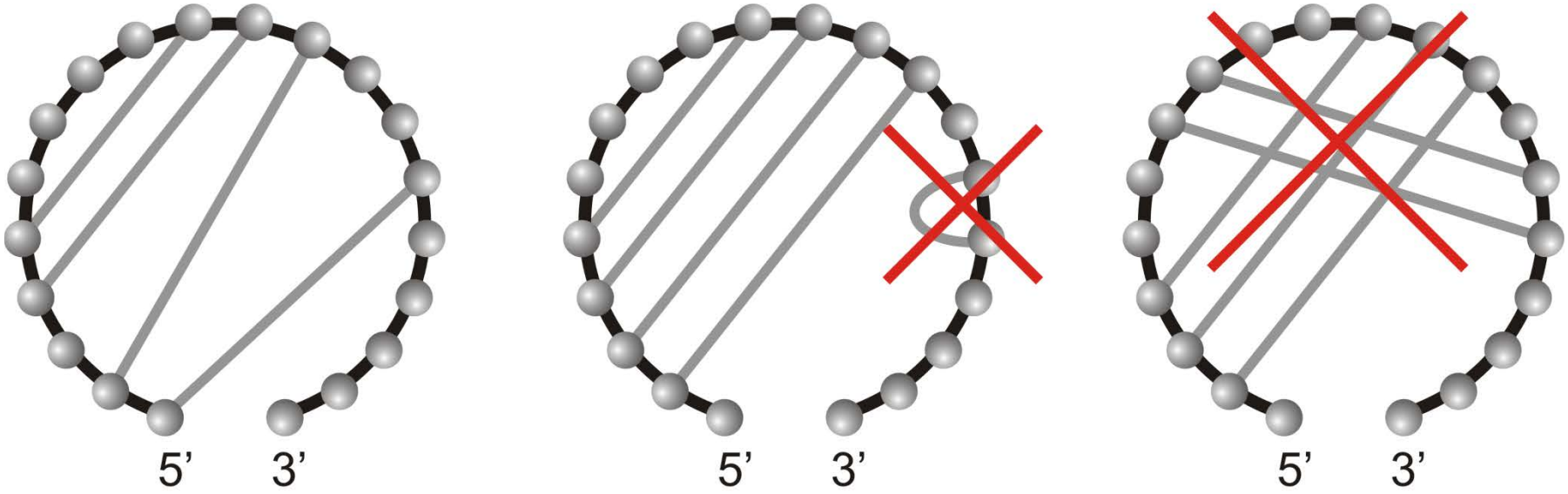


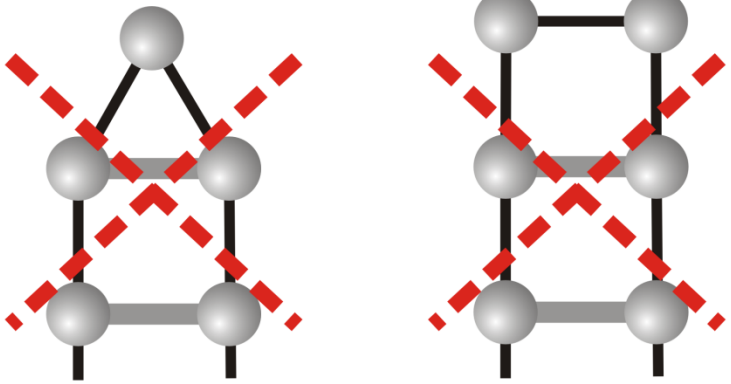
Base pairing

No nearest neighbor pair rule

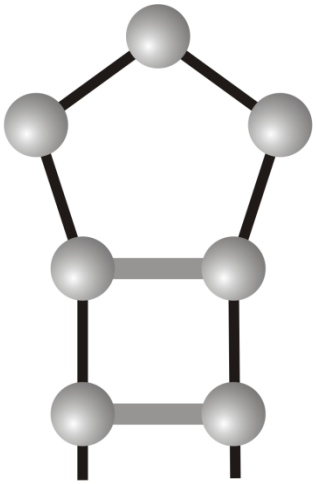
No pseudoknot rule

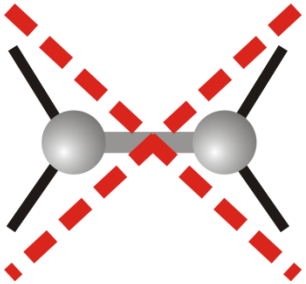
Base pairs $\in$ {**AU**,**CG**,**GC**,**GU**,**UA**,**UG**}

Conventional definition of RNA secondary structures

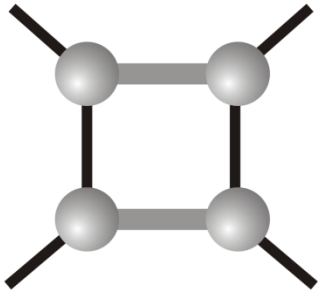


Impossible (extremely high free energies)
for steric reasons





High free energies because of lack of stacking and
very rare in minimum free energy structures



Restrictions on physically acceptable mfe-structures: $\lambda \geq 3$ and $\sigma \geq 2$

Monatshefte für Chemie 125, 167–188 (1994)

Monatshefte für Chemie
Chemical Monthly

Printed in Austria

Fast Folding and Comparison of RNA Secondary Structures

I. L. Hofacker[1,*], W. Fontana[3], P. F. Stadler[1,3], L. S. Bonhoeffer[4], M. Tacker[1] and P. Schuster[1,2,3]

[1] Institut für Theoretische Chemie, Universität Wien, A-1090 Wien, Austria
[2] Institut für Molekulare Biotechnologie, D-07745 Jena, Federal Republic of Germany
[3] Santa Fe Institute, Santa Fe, NM 87501, U.S.A.
[4] Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, U.K.

Summary. Computer codes for computation and comparison of RNA secondary structures, the Vienna RNA package, are presented, that are based on dynamic programming algorithms and aim at predictions of structures with minimum free energies as well as at computations of the equilibrium partition functions and base pairing probabilities.

An efficient heuristic for the inverse folding problem of RNA is introduced. In addition we present compact and efficient programs for the comparison of RNA secondary structures based on tree editing and alignment.

All computer codes are written in ANSI C. They include implementations of modified algorithms on parallel computers with distributed memory. Performance analysis carried out on an Intel Hypercube shows that parallel computing becomes gradually more and more efficient the longer the sequences are.

Keywords. Inverse folding; parallel computing; public domain software; RNA folding; RNA secondary structures; tree editing.

RNA sequence GUAUCGAAAUACGUAGCGUAUGGGGAUGCUGGACGGUCCCAUCGGUACUCCA

RNA folding:

Structural biology, spectroscopy of biomolecules, understanding **molecular function**

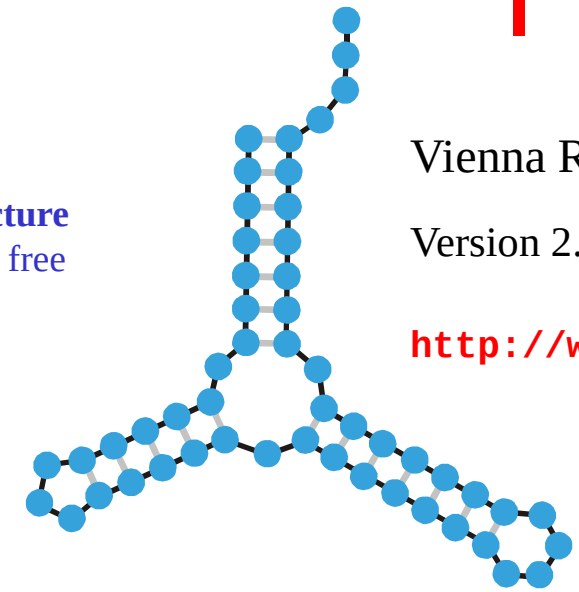
Iterative determination of a sequence for the given secondary structure

Inverse Folding Algorithm

Inverse folding of RNA:

Biotechnology, **design of biomolecules** with predefined structures and functions

RNA structure of minimal free energy



Vienna RNA-Package

Version 2.0

`http://www.tbi.univie.ac.at`

Sequence, structure, and design

Inverse folding algorithm

$\mathbf{I_0} \rightarrow I_1 \rightarrow I_2 \rightarrow I_3 \rightarrow I_4 \rightarrow \ldots \rightarrow I_k \rightarrow I_{k+1} \rightarrow \ldots \rightarrow \mathbf{I_t}$

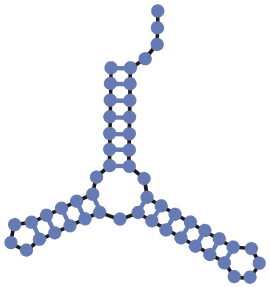
$\mathbf{S_0} \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_4 \rightarrow \ldots \rightarrow S_k \rightarrow S_{k+1} \rightarrow \ldots \rightarrow \mathbf{S_t}$

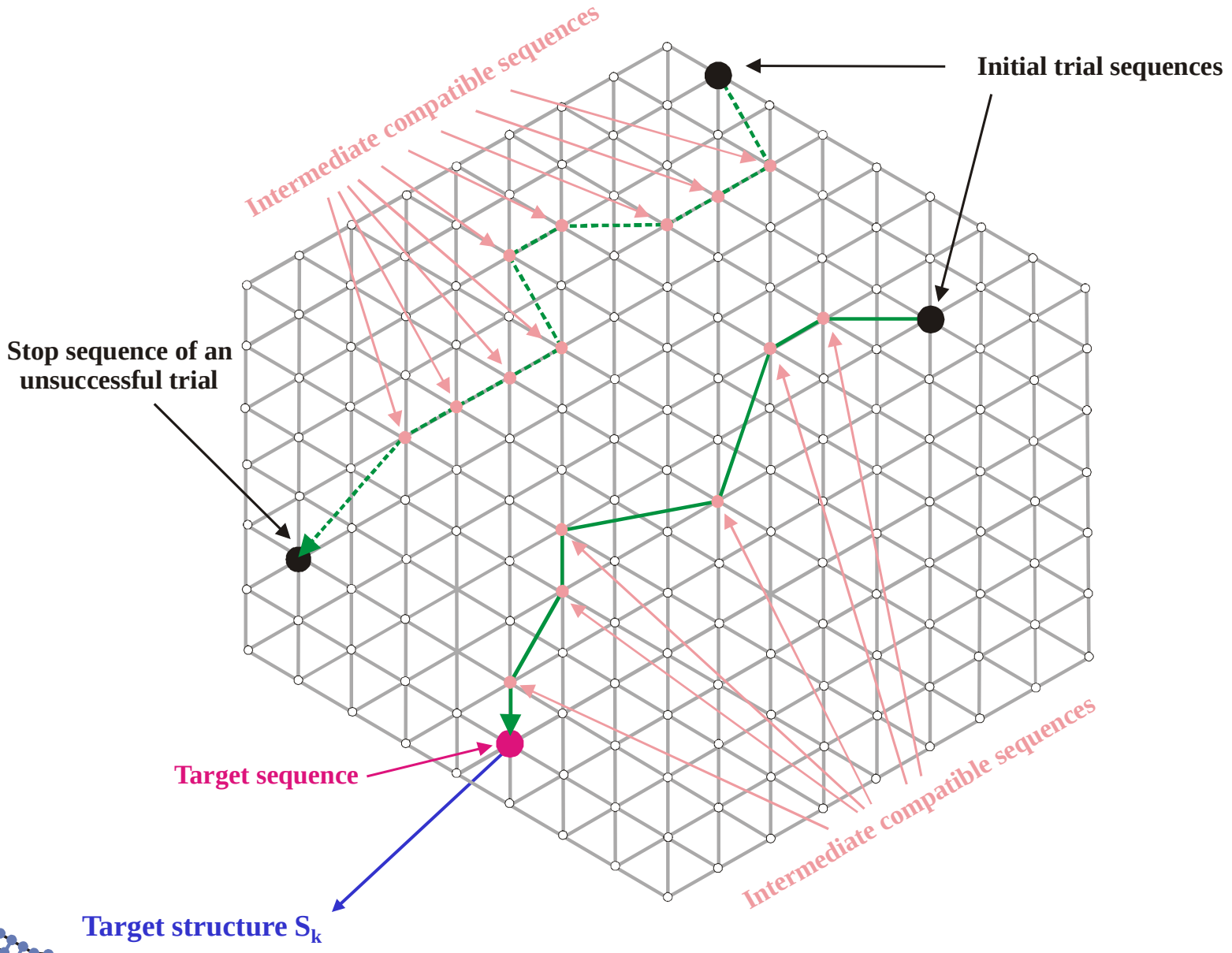
$I_{k+1} = \mathfrak{M}_k(I_k)$ and $\Delta d_S(S_k,S_{k+1}) = d_S(S_{k+1},S_t) - d_S(S_k,S_t) < 0$

$\mathfrak{M}$ ... base or base pair mutation operator

$d_S(S_i,S_j)$ ... distance between the two structures S_i and S_j

‚Unsuccessful trial' ... termination after n steps





Approach to the **target structure S_k** in the inverse folding algorithm

RNA inverse folding: Secondary structures ⇒ sequences

Mirela Andronescu, Antony P. Fejes, Frank Hutter, Holger H. Hoos, Anne Condon. 2004.
A new algorithm for RNA secondary structure design.
J. Mol. Biol. **336**:607-624.

Department of Computer Science
University of British Comlumbia
Vancouver, BC, Canada

Robert M. Dirks, Milo Lin, Erik Winfree, Niles A. Pierce. 2004.
Paradigms for computational nucleic acid design.
Nucleic Acids Research **32**:1392-1403.

California Institute of Technology
Pasadena, CA, USA

Rune B. Lyngsø, James J.W. Anderson, Elena Sizikova, Amarendra Badugu, Thomas Hyland, Jotun Hein. 2012.
fRNAkenstein: Multiple traget inverse RNA folding.
BMC Bioinformatics **13**:e260.

Department of Statistics
University of Oxford

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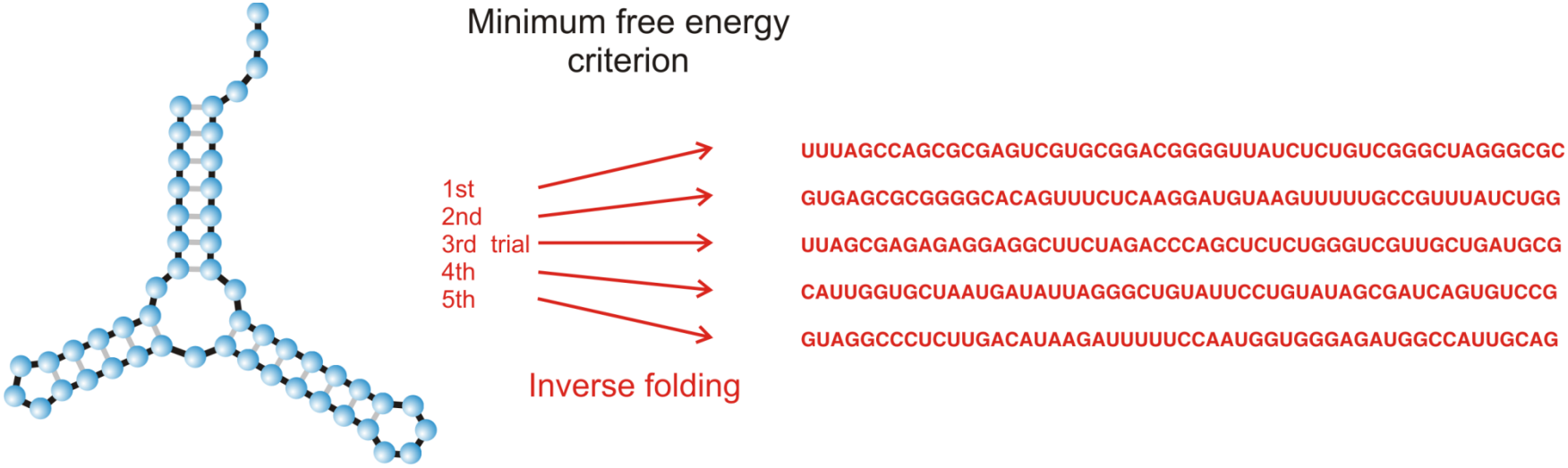
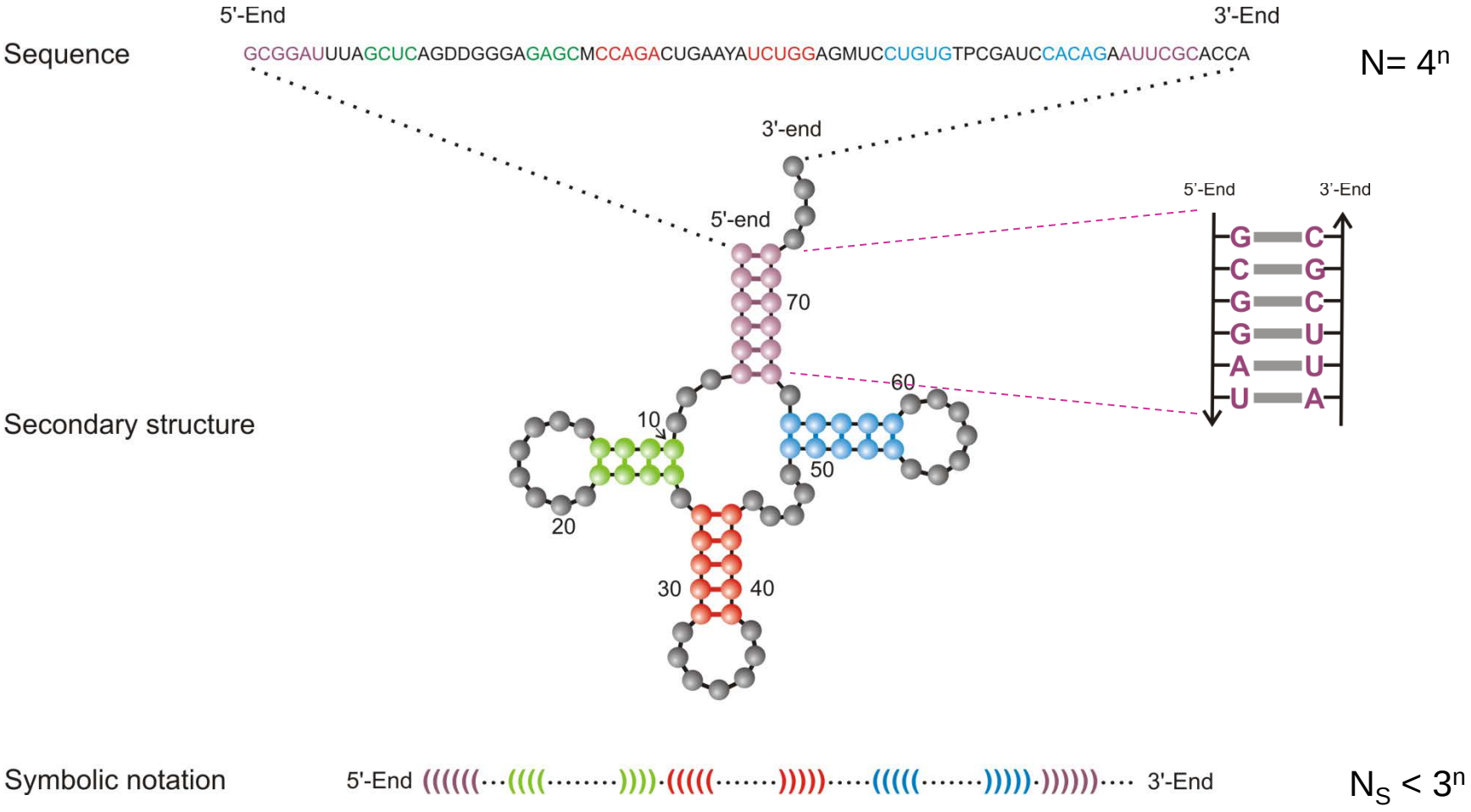


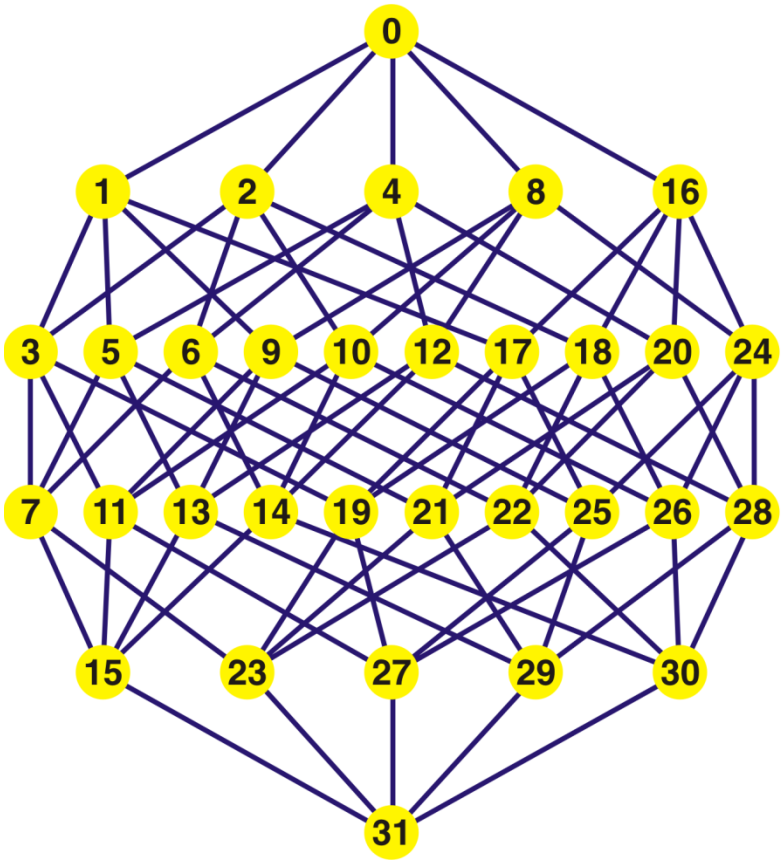
The **inverse folding algorithm** searches for sequences that form a given RNA secondary structure under the minimum free energy criterion.



Criterion: Minimum free energy (mfe)

Rules: _ (_) _ ∈ {**AU**,**CG**,**GC**,**GU**,**UA**,**UG**}

A symbolic notation of RNA secondary structure that is equivalent to the conventional graphs



Binary sequences can be encoded by their decimal equivalents:

C = 0 and **G = 1**, for example,

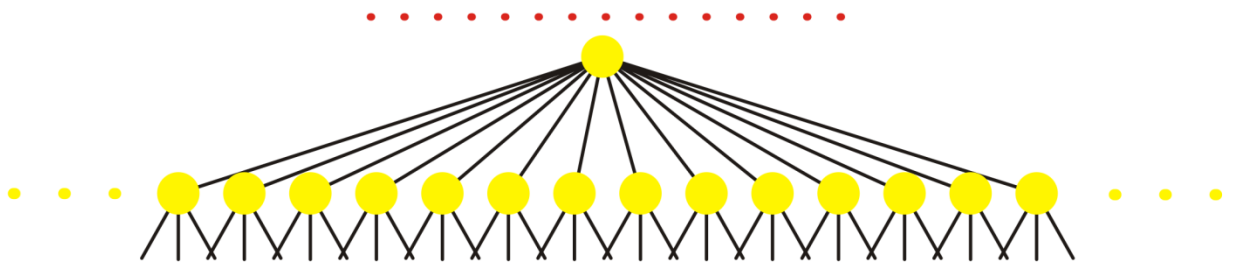
"0" ≡ 00000 = CCCCC,

"14" ≡ 01110 = CGGGC,

"29" ≡ 11101 = GGGCG, etc.

Every point in sequence space is equivalent

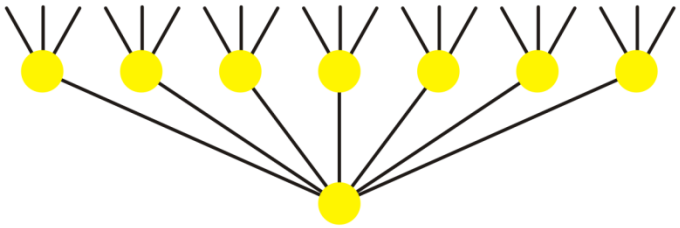
Sequence space of binary sequences with chain length $n = 5$



open chain

number of edges $\frac{n\,(n-7)}{2}+6$

66 for n = 15



((((((. . .))))))

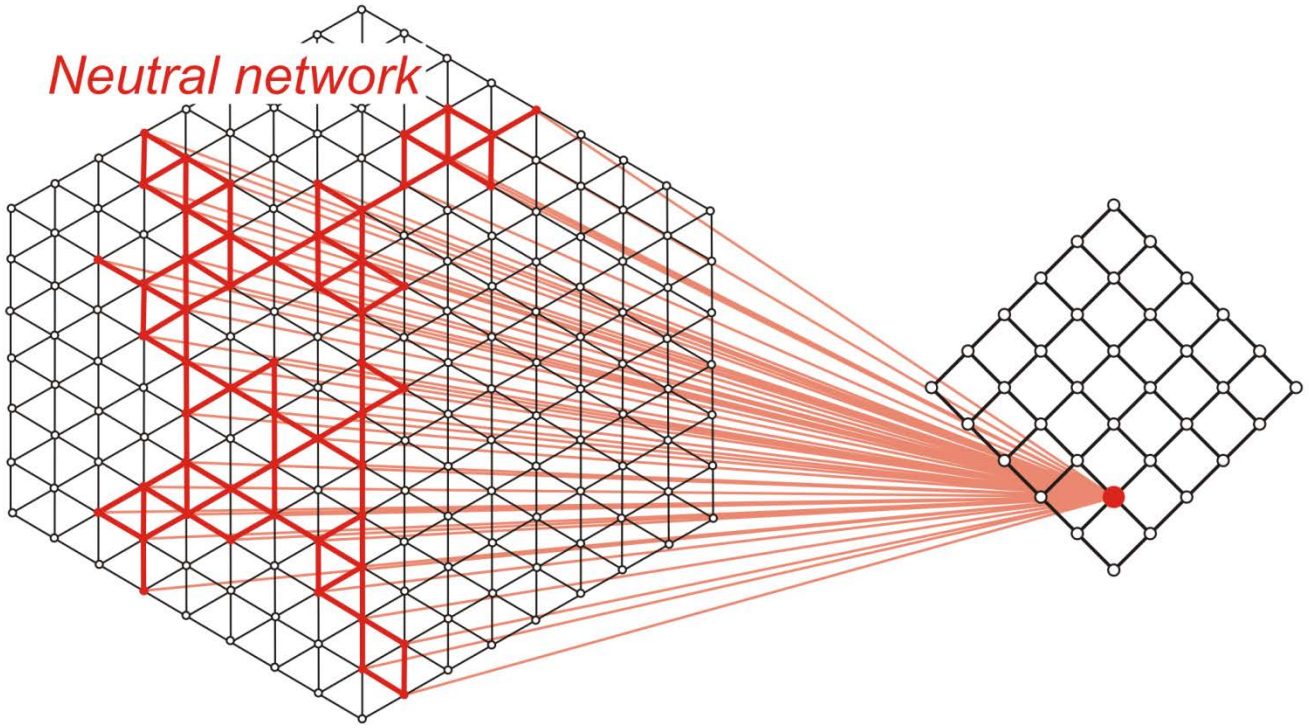
7 for n = 15

number of edges $\left\lfloor \frac{n-3}{2} \right\rfloor$

longest stack

Structures are not equivalent in structure space

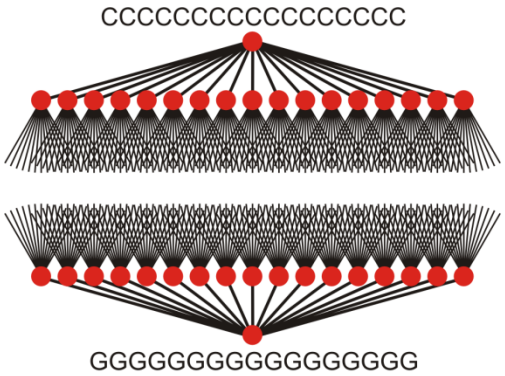
Sketch of structure space



Sequence space

Structure space

many genotypes ⇒ one phenotype



sequence space

Q

genotype → phenotype → selection

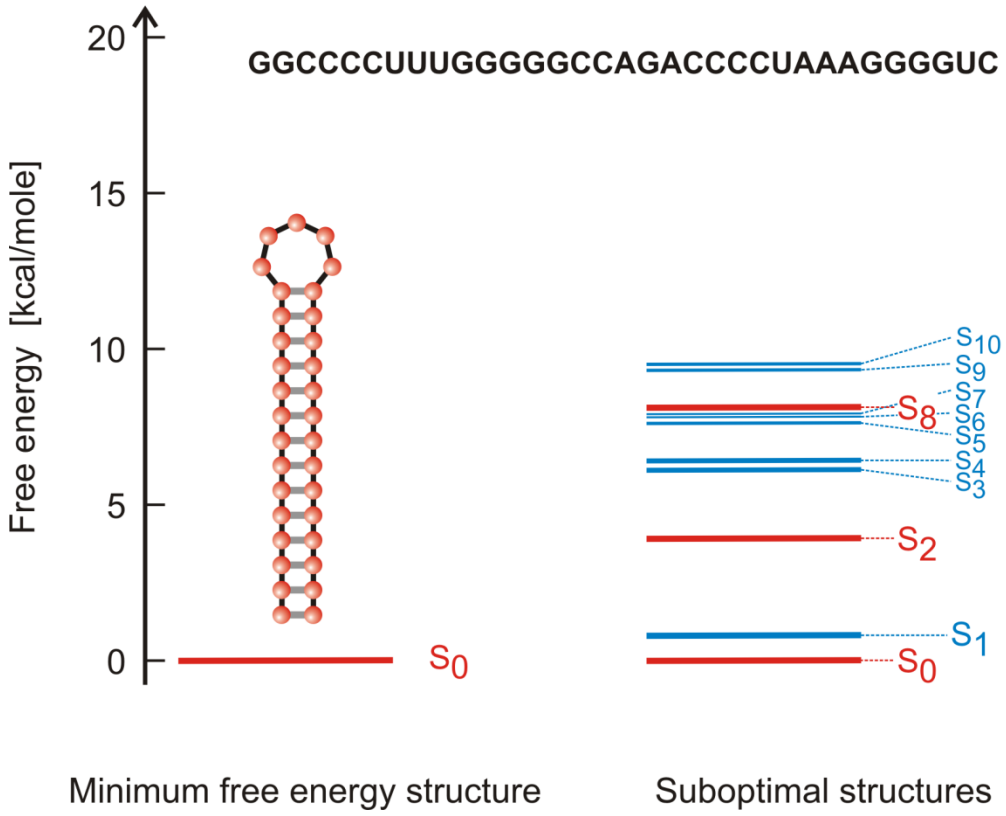
Evolution as a global phenomenon in genotype space

1. One RNA sequence – one structure
2. Many RNA sequences – one structure
3. **One RNA sequence – many structures**
4. RNA switches

Computation of suboptimal secondary structures

Michael Zuker.
On finding all suboptimal foldings of an RNA molecule.
Science **244** (1989), 48-52

Stefan Wuchty, Walter Fontana, Ivo L. Hofacker, Peter Schuster.
Complete suboptimal folding of RNA and the stability of secondary structures.
Biopolymers **49** (1999), 145-165



Interconversion of suboptimal structures

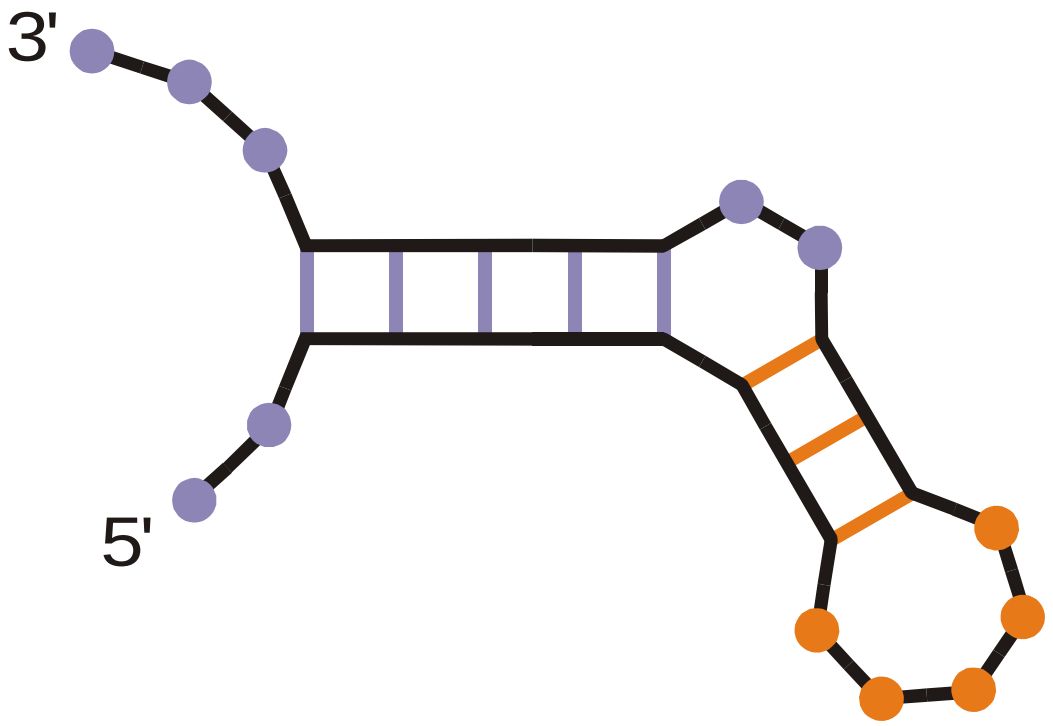
base pair probability $$p_{ij}(X,T) = \sum_k \gamma_k(T) a_{ij}(S_k) \quad \text{with} \quad \gamma_k(T) = g_k \, e^{-(\varepsilon_k - \varepsilon_0)/kT} / Q(T)$$

$$Q(T) = \sum_k \gamma_k(T)$$

Base pair probability derived from the partition function $Q(T)$

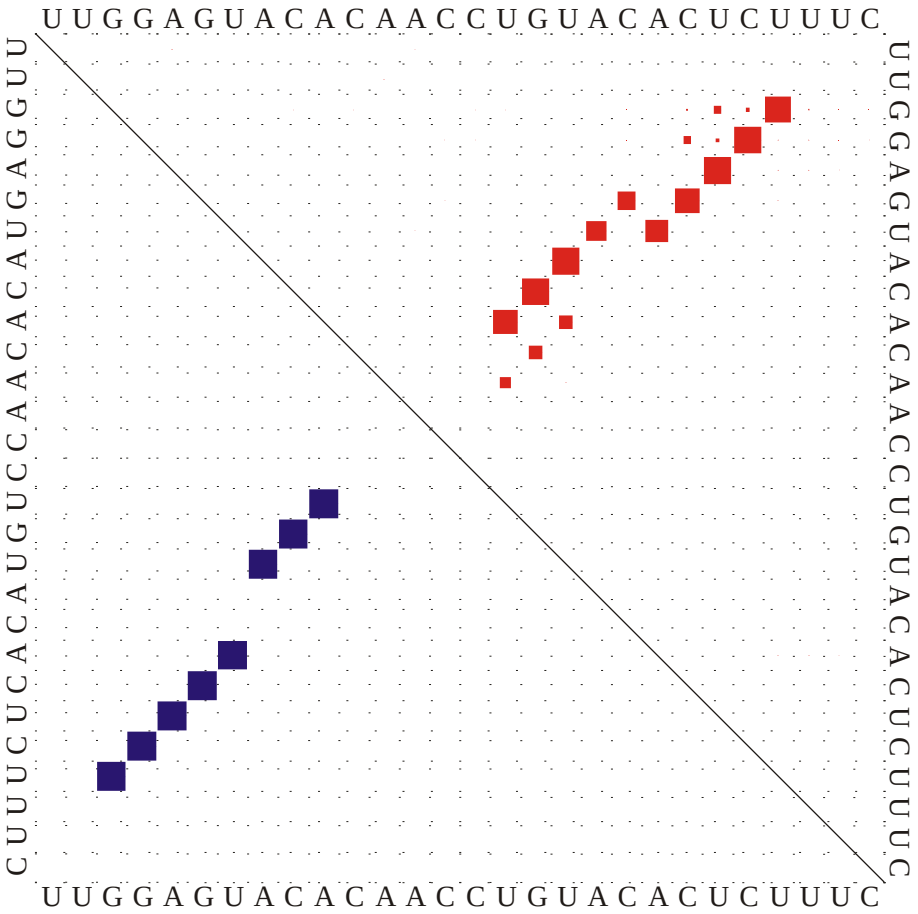
John S. McCaskill. The equilibrium partition function and base pair binding probabilities for RNA secondary structures.
Biopolymers **29**:1105-1119, 1990.

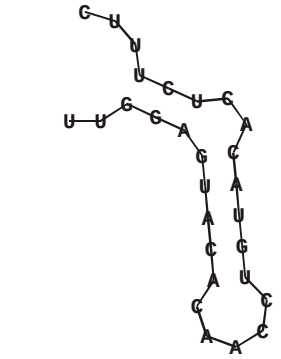
Example of a small RNA molecule with two low-lying suboptimal conformations which contribute substantially to the partition function



UUGGAGUACACAACCUGUACACUCUUUC

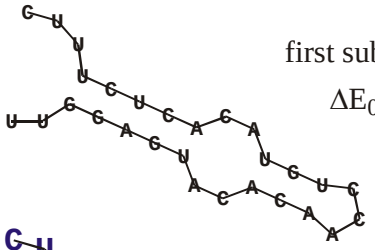
Example of a small RNA molecule: n=28





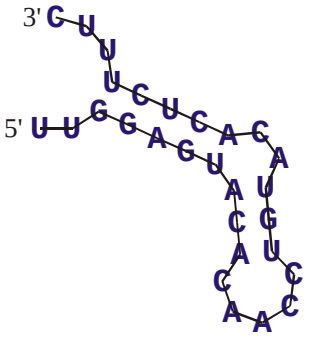
second suboptimal configuration

$\Delta E_{0\rightarrow 2}$ = 0.55 kcal / mole



first suboptimal configuration

$\Delta E_{0\rightarrow 1}$ = 0.50 kcal / mole



minimum free energy configuration

ΔG_0 = - 5.39 kcal / mole

„Dot plot“ of the minimum free energy structure (**lower triangle**) and the partition function (**upper triangle**) of a small RNA molecule (n=28) with low energy suboptimal configurations

Kinetic folding of RNA secondary structures

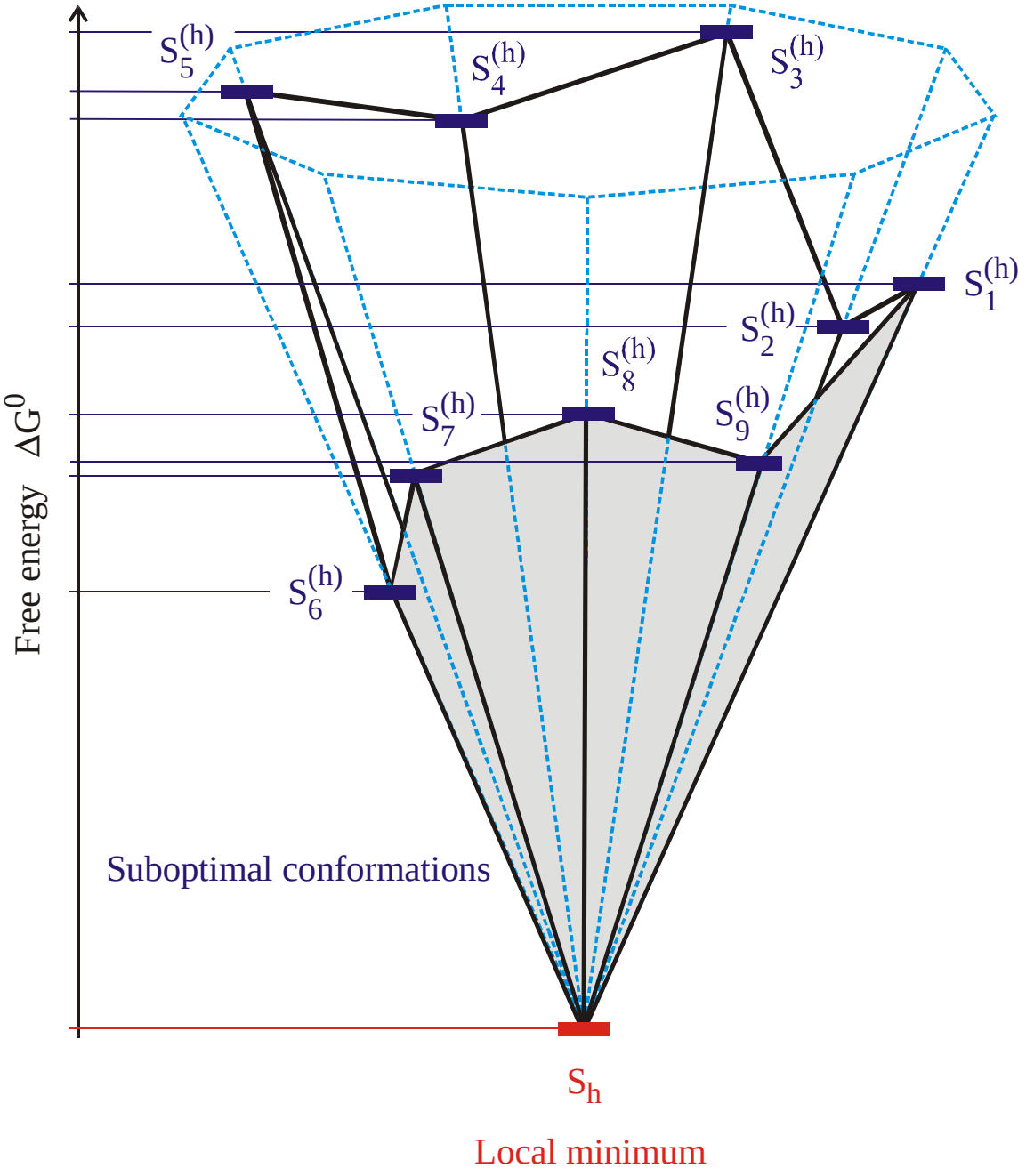
Christoph Flamm, Walter Fontana, Ivo. L. Hofacker, Peter Schuster. 2000.
RNA folding kinetics at elementary step resolution. *RNA*. **6**:325-338.

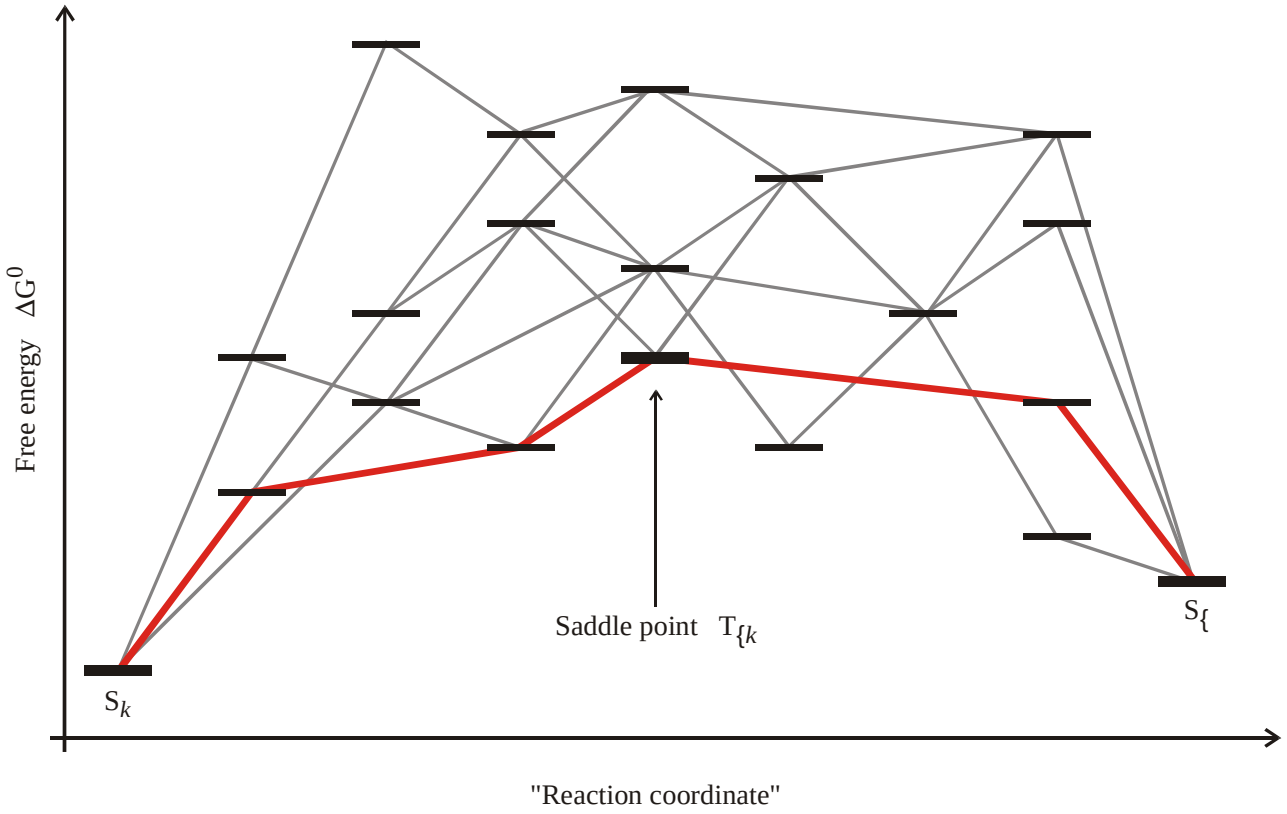
Christoph Flamm, Ivo. L. Hofacker, Sebastian Maurer-Stroh, Peter F. Stadler, Martin Zehl. 2001.
Design of multistable RNA molecules. *RNA*. **7**:254-265

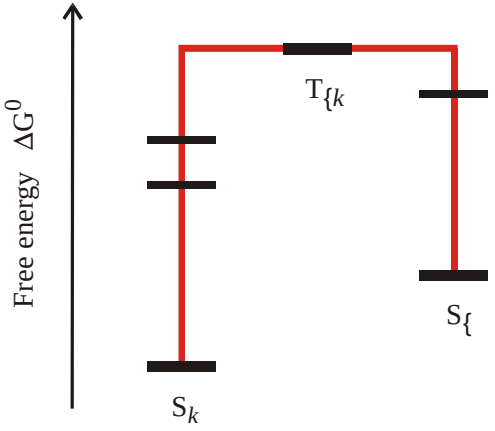
Christoph Flamm, Ivo. L. Hofacker, Peter F. Stadler, Michael T. Wolfinger. 2002.
Barrier trees of degenerate landscapes. *Z.Phys.Chem*. **216**:155-173.

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

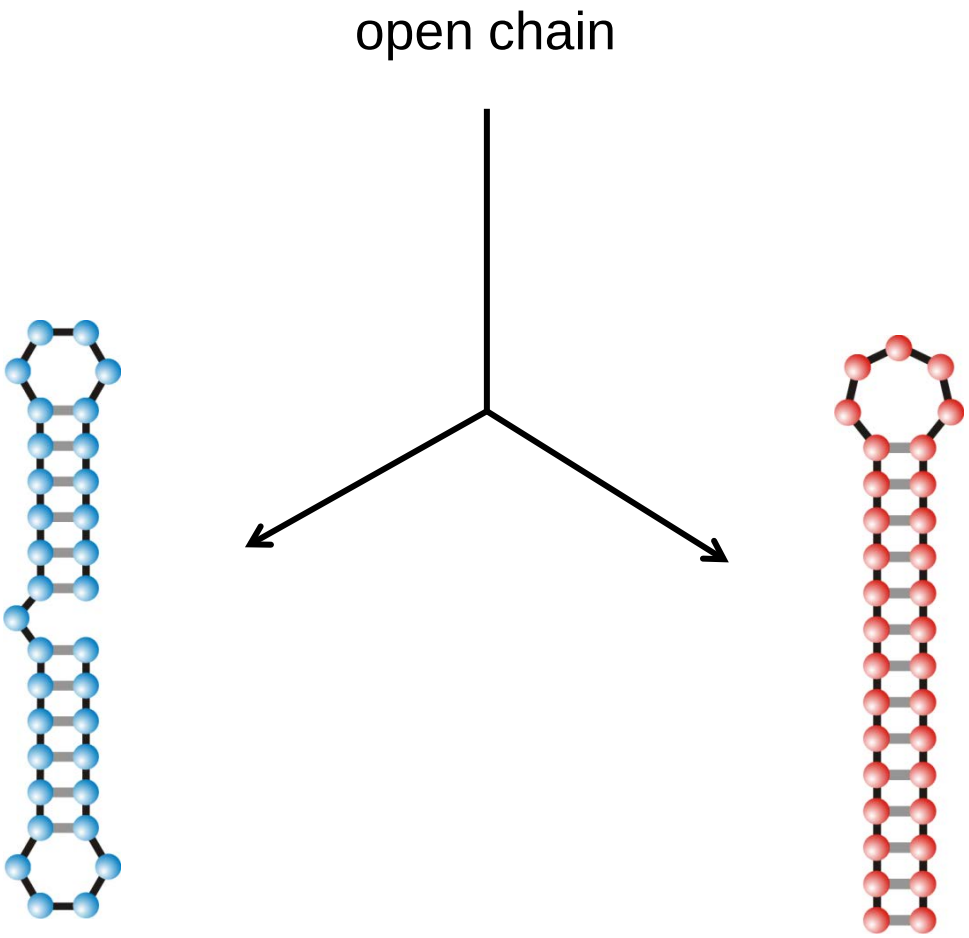
Search for local minima in conformation space



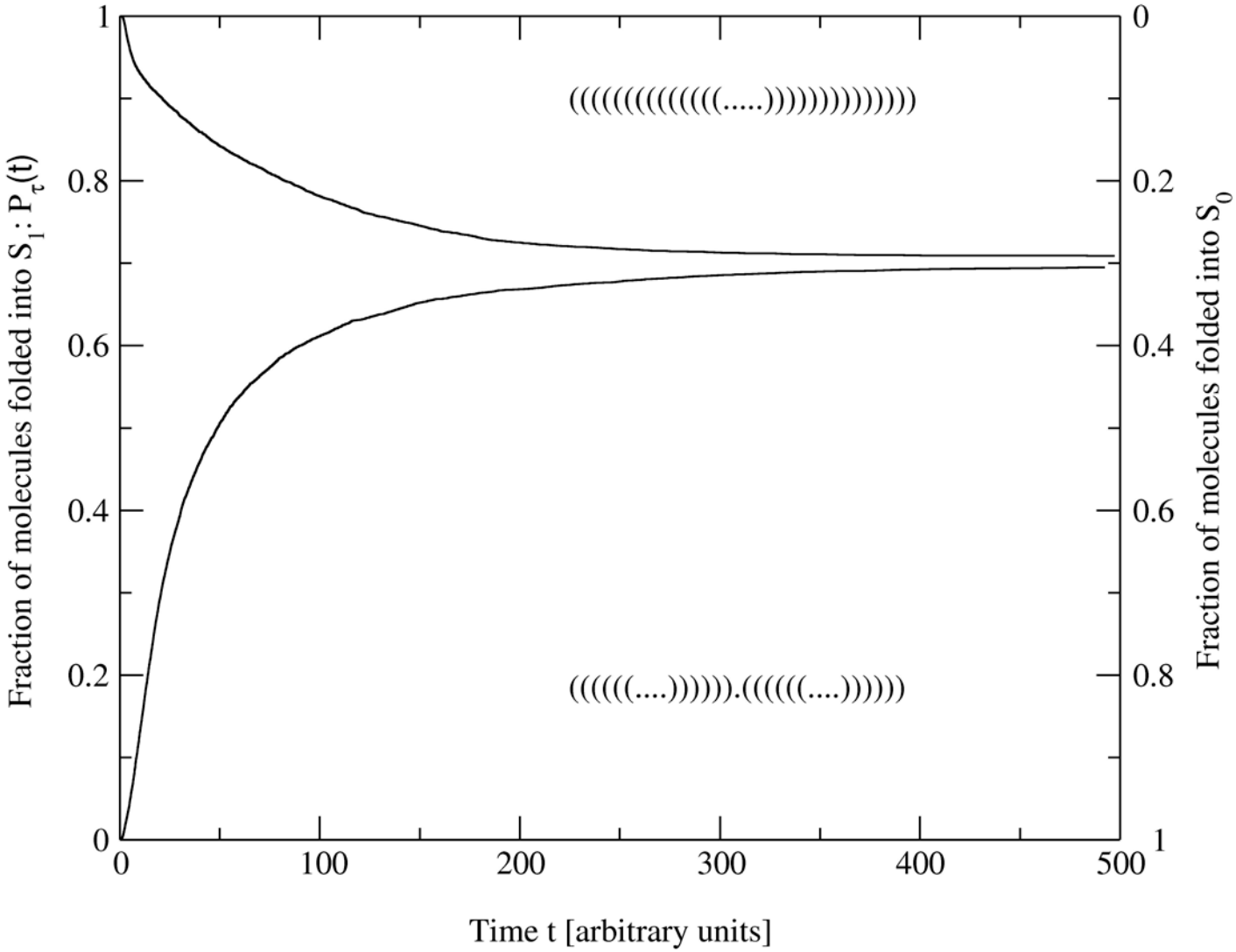




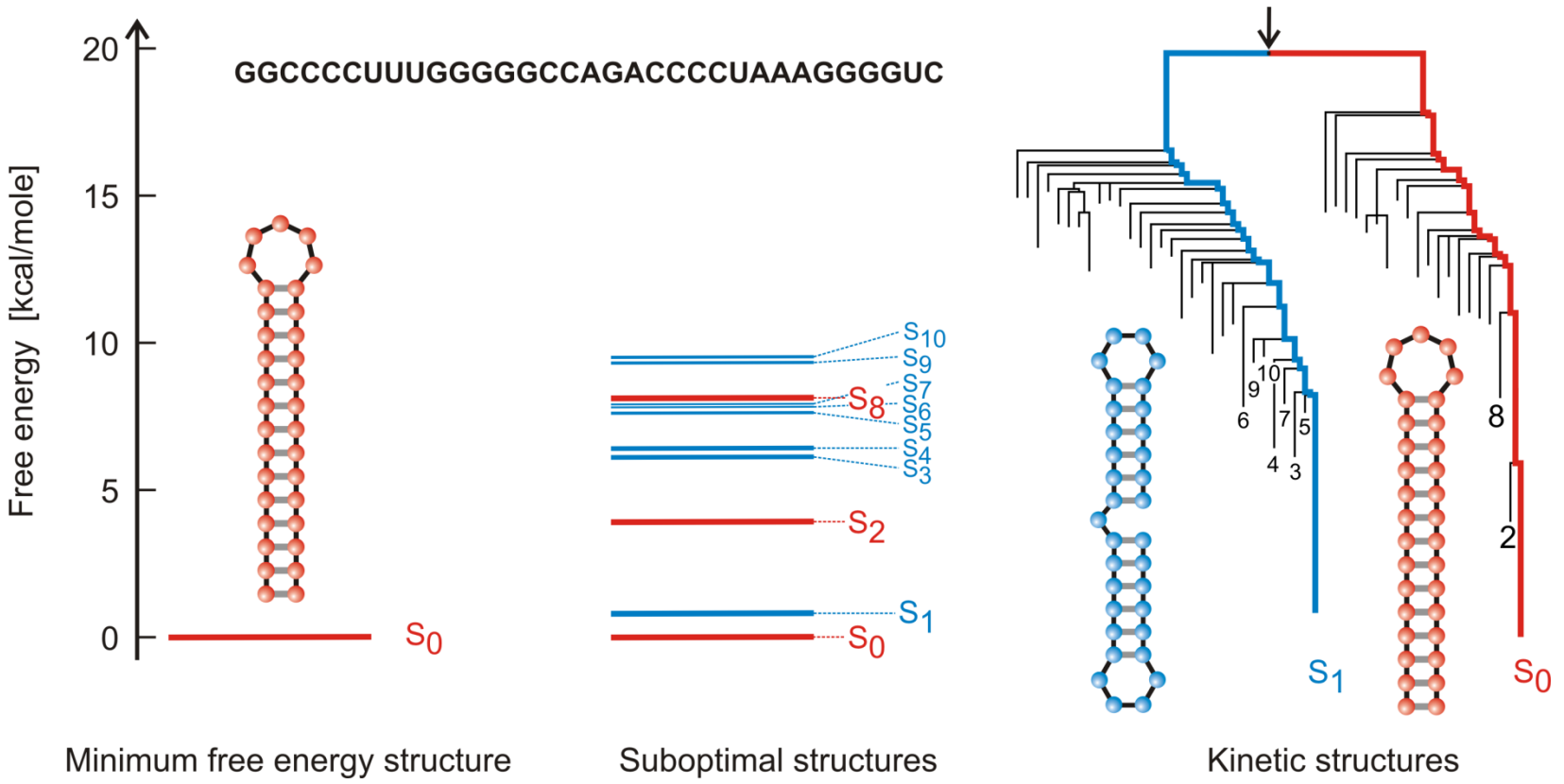
Definition of a ‚barrier tree‘



A nucleic acid molecule folding in two dominant conformations



Folding dynamics of the sequence **GGCCCCUUUGGGGGCCAGACCCCUAAAAAGGGUC**



Interconversion of suboptimal structures

RNA inverse folding and structure design

Vienna RNA Package

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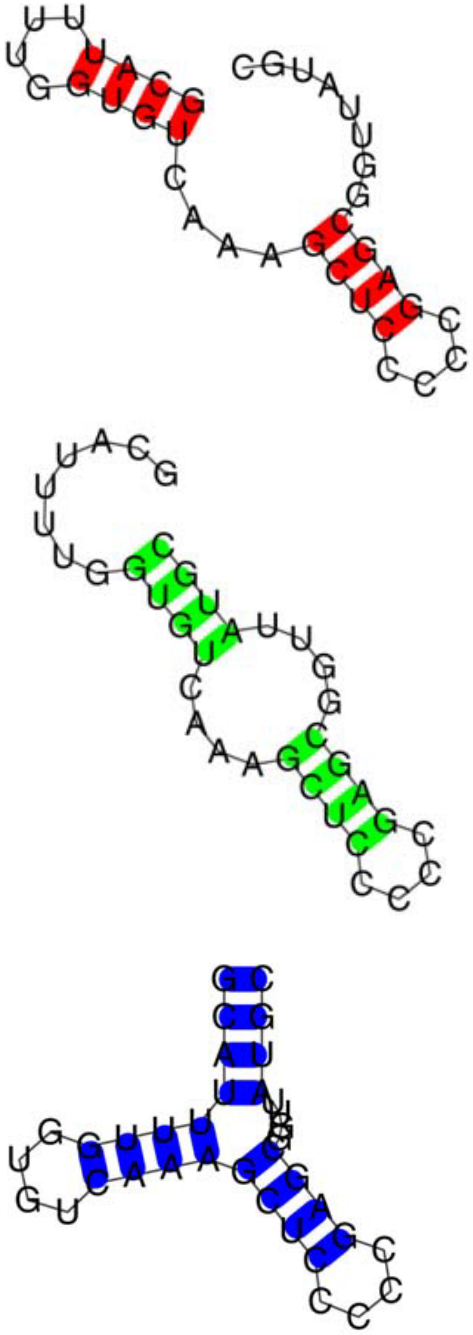
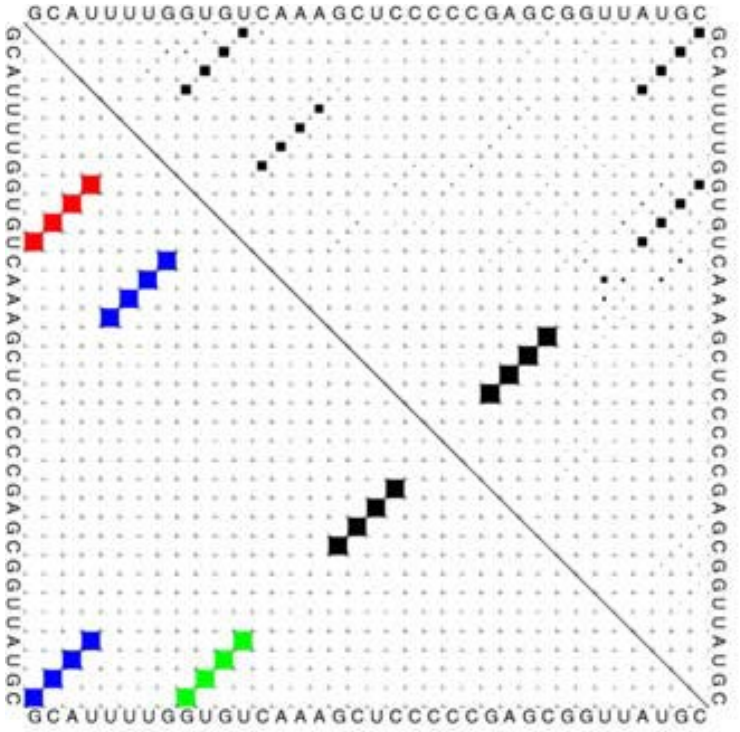
Peter Schuster. 2006.
Prediction of RNA secondary structures: From theory to models and real molecules.
Reports on Progress in Physics **69**:1419-1477.

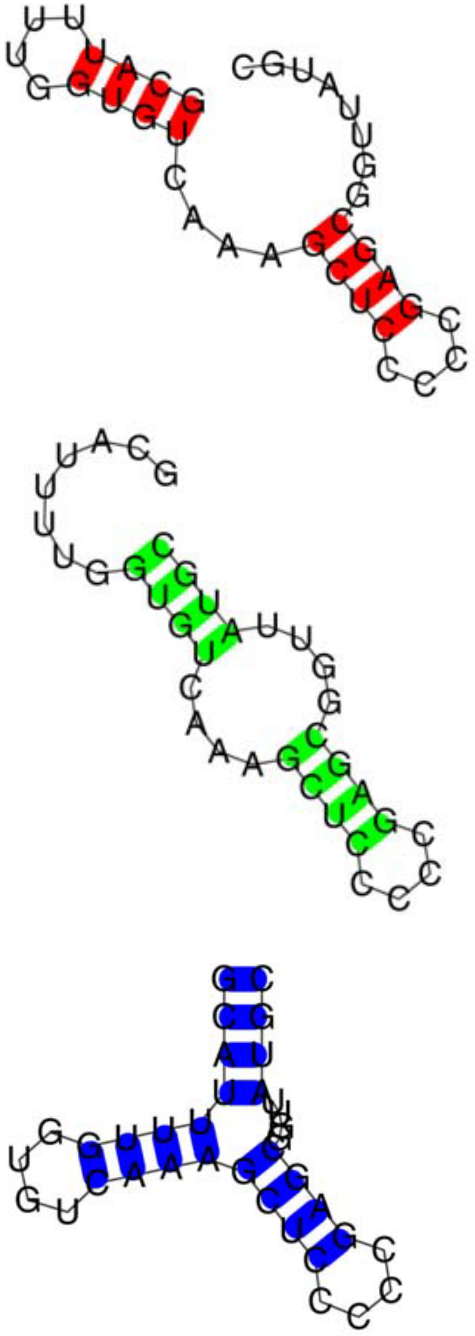
Ronny Lorenz, Stephan H. Bernhart, Christian Höner zu Siederissen, Hakim Tafer, Christoph Flamm,
Peter F. Stadler, Ivo L. Hofacker. 2011.
Vienna RNA Package 2.0. *Algorithms for Molecular Bioology* **6**:e26.

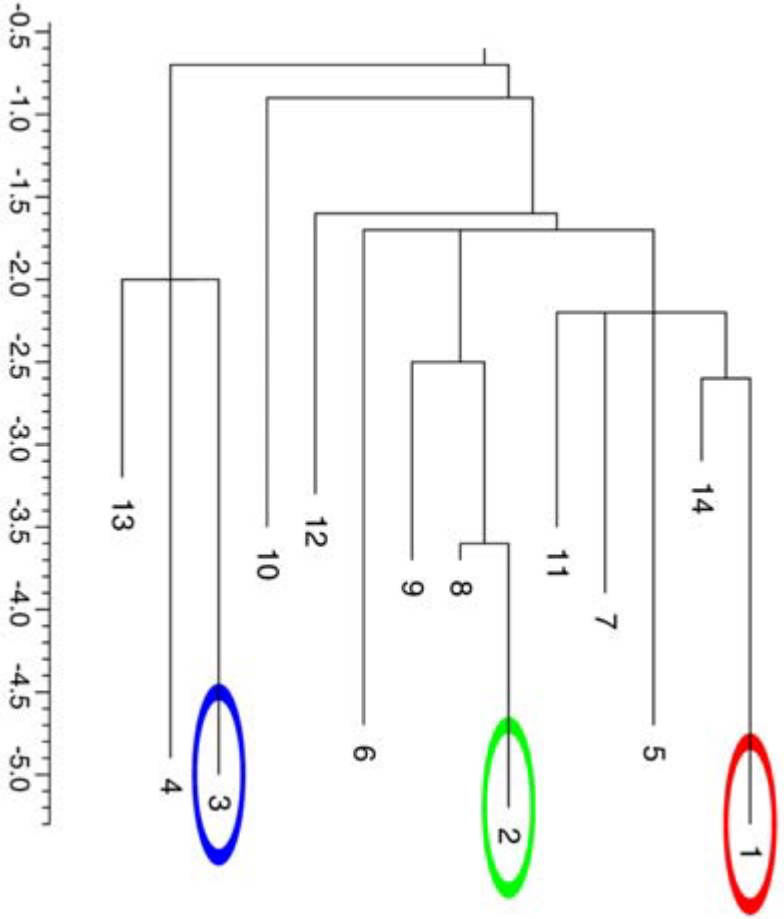
Christian Höner zu Siederissen, Stefan Hammer, Ingrid Abfalter, Ivo L. Hofacker, Christoph Flamm,
Peter F. Stadler. 2013.
Computational design of RNAs with complex energy landscapes. *Biopolymers* **99**:1124-1136.





Christian Höner zu Siederissen, Stefan Hammer, Ingrid Abfalter, Ivo L. Hofacker, Christoph Flamm , Peter F. Stadler. 2013. Computational design of RNAs with complex energy landscapes. Biopolymers 99:1124-1136.





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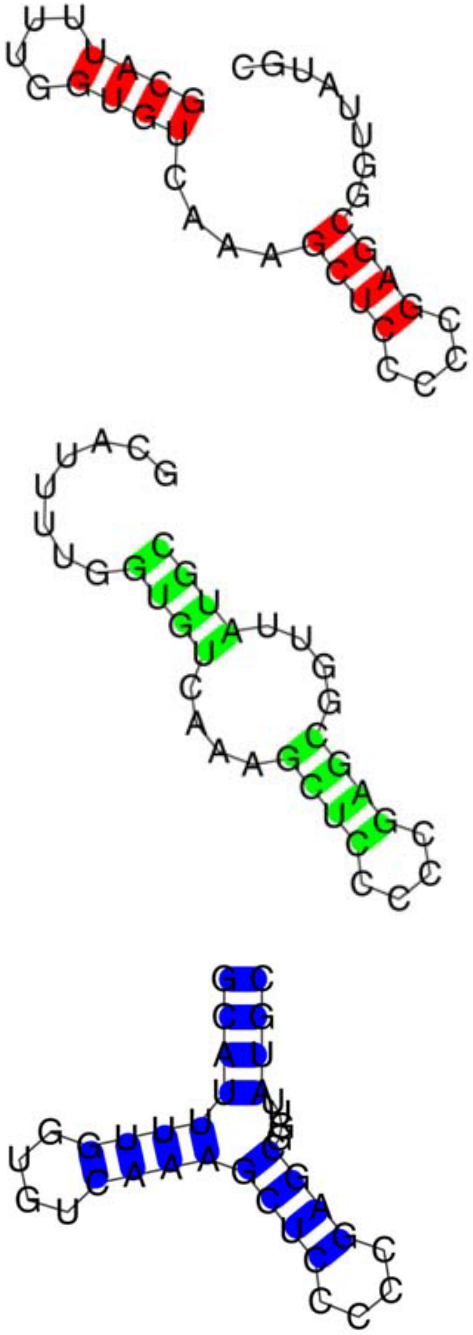

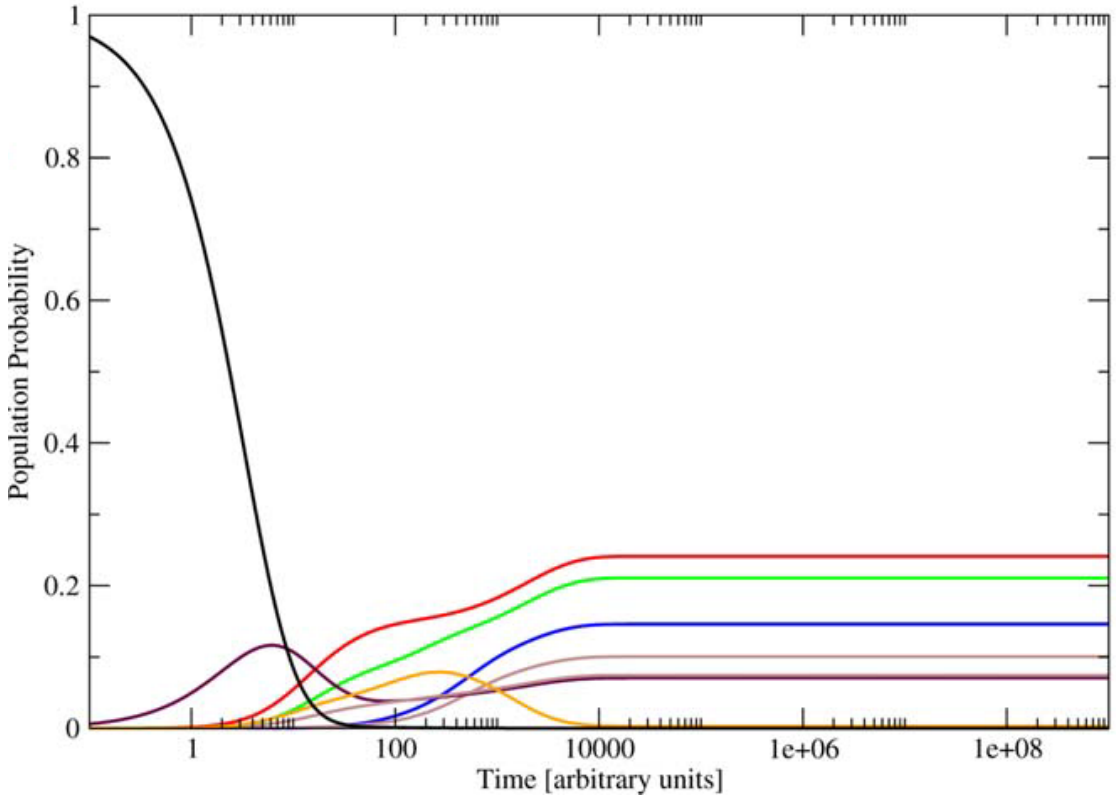

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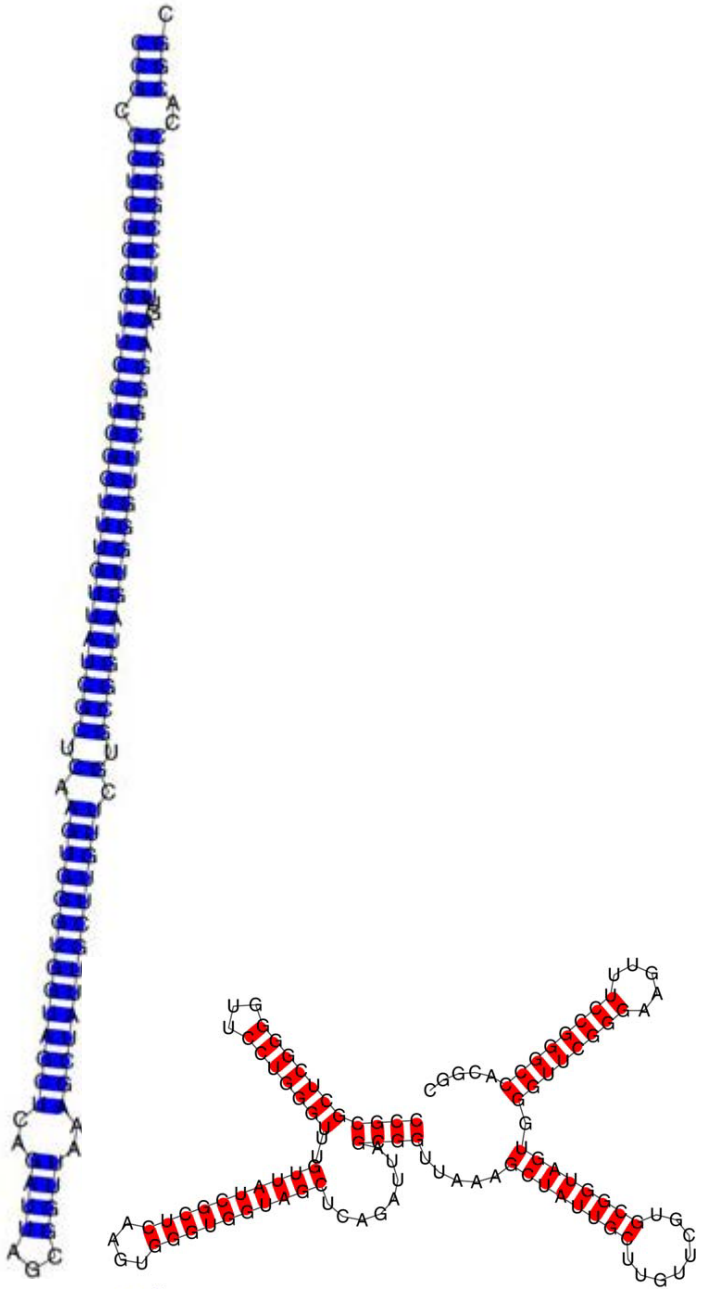

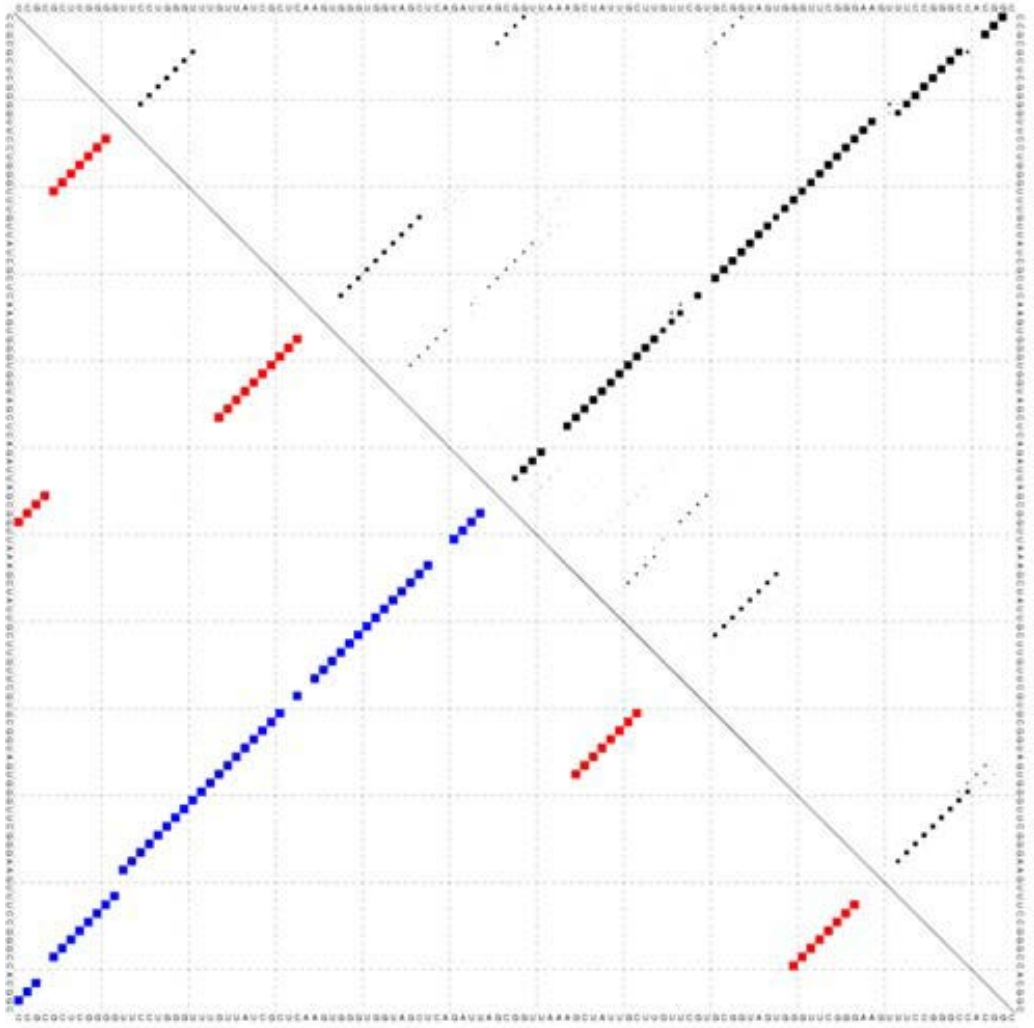

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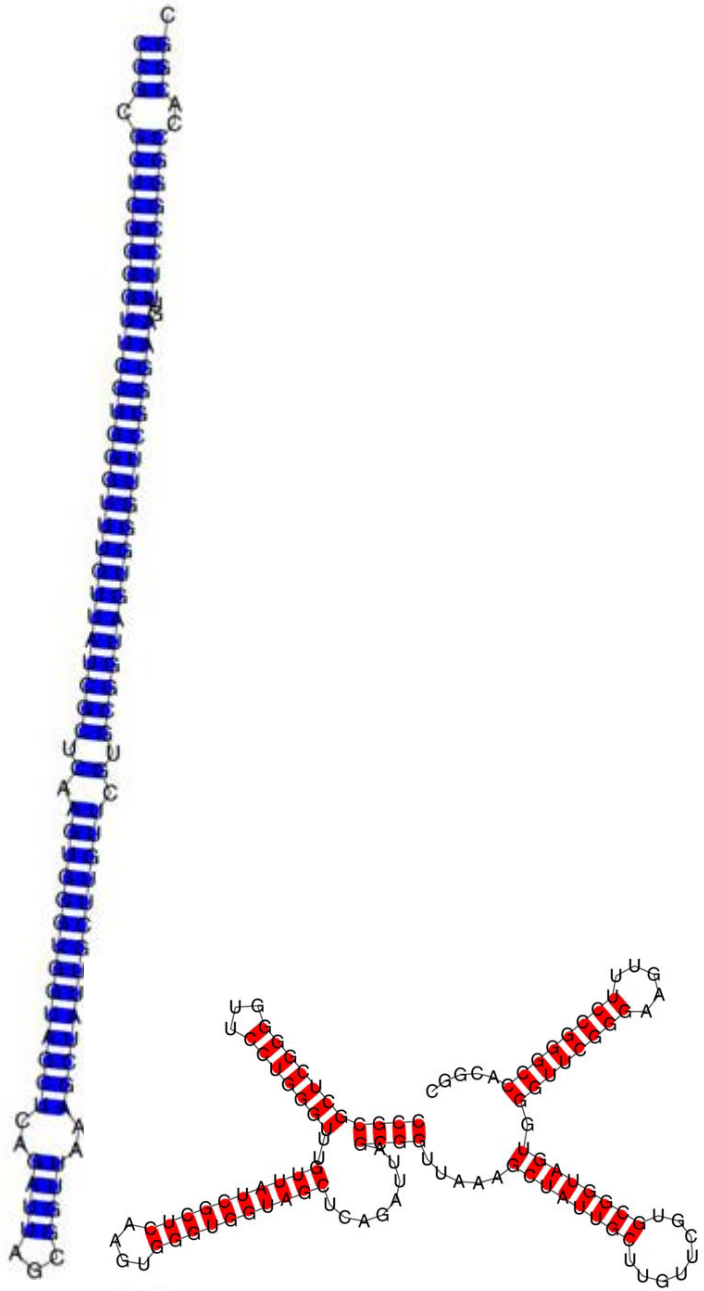

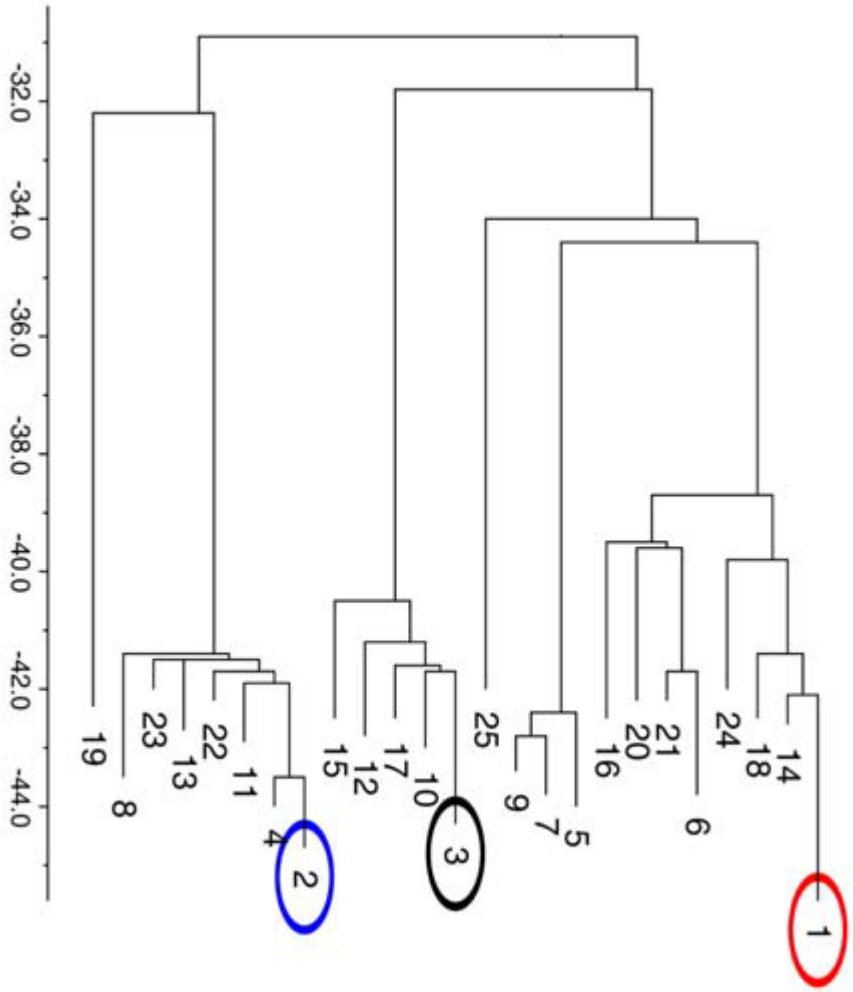

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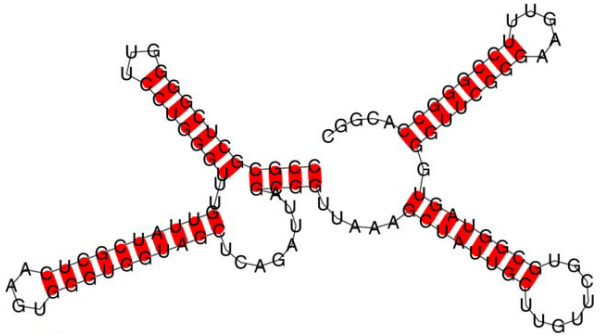

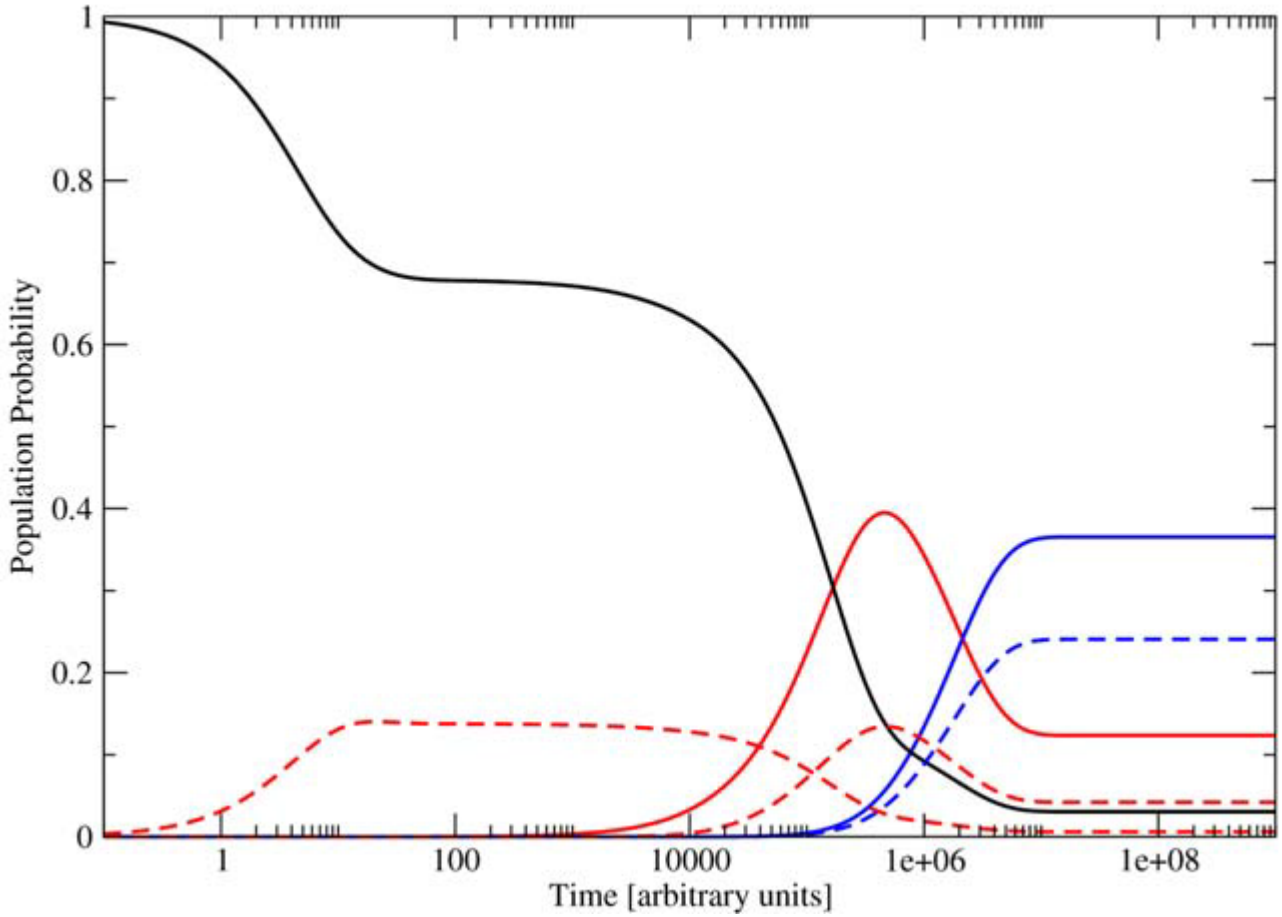

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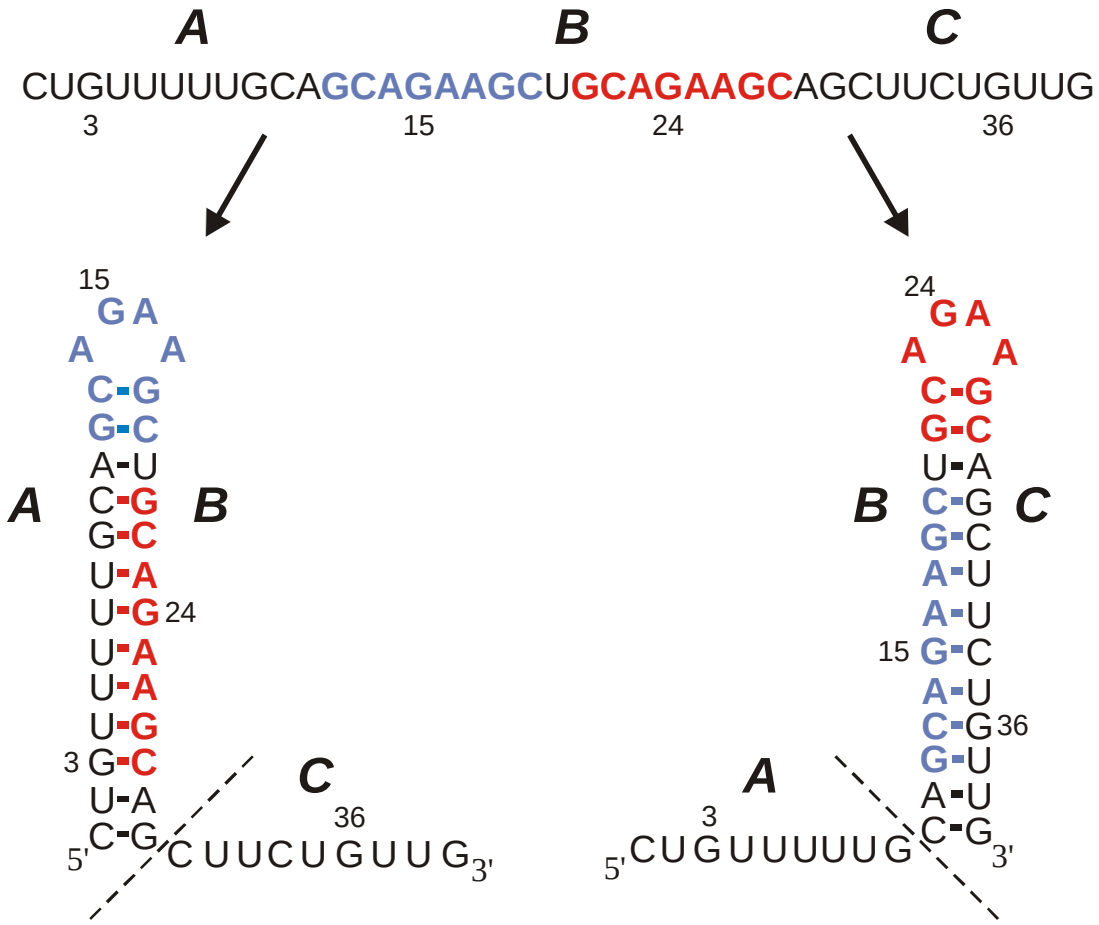
1. One RNA sequence – one structure
2. Many RNA sequences – one structure
3. One RNA sequence – many structures
4. **RNA switches**

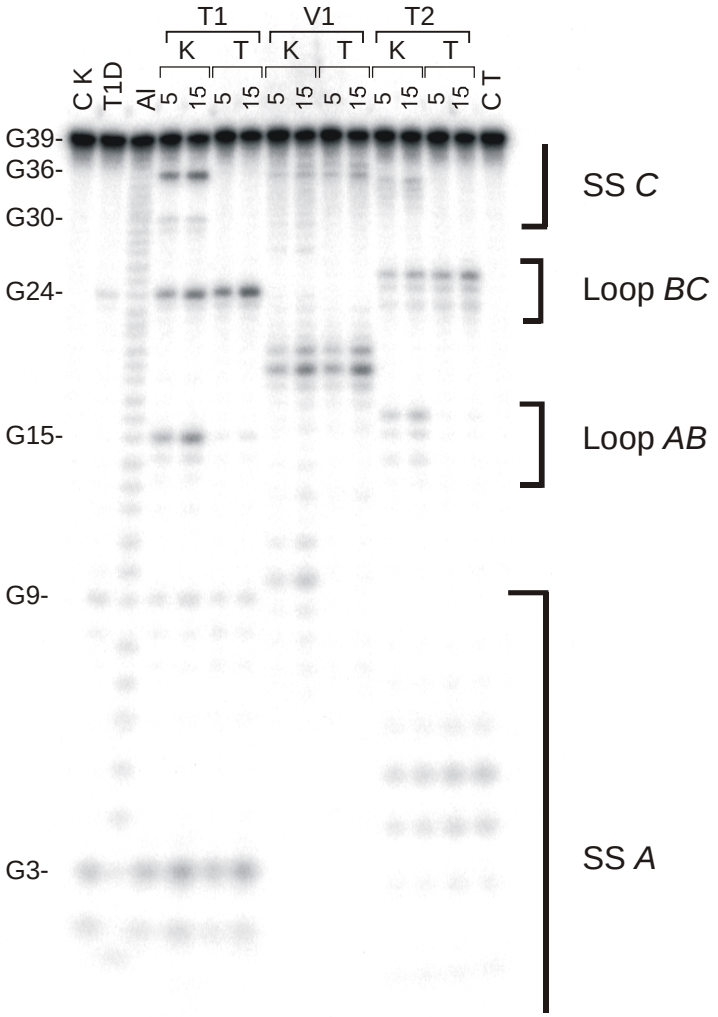


Synthetic RNA switches

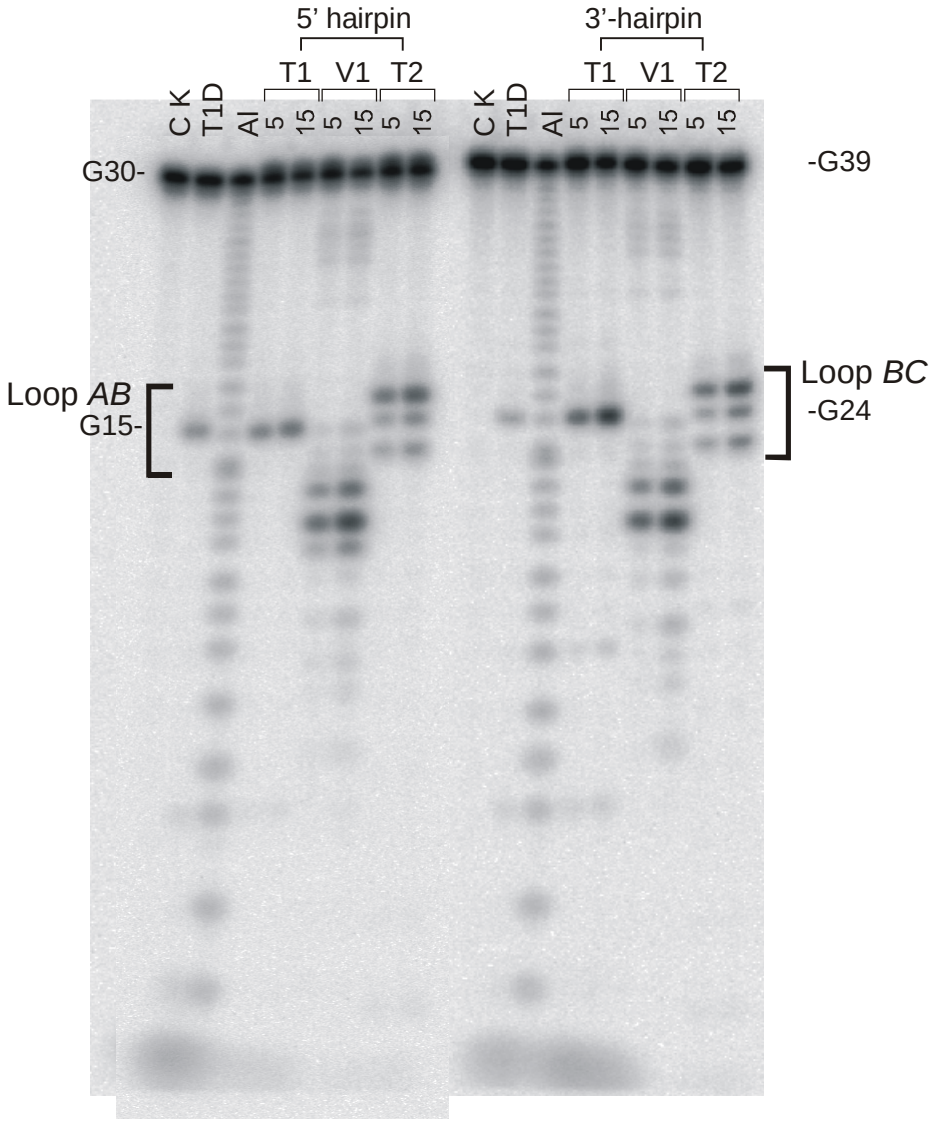
JN2C

J.H.A. Nagel, C. Flamm, I.L. Hofacker, K. Franke, M.H. de Smit, P. Schuster, and C.W.A. Pleij.

Structural parameters affecting the kinetic competition of RNA hairpin formation. *Nucleic Acids Res.* **34**:3568-3576 (2006)

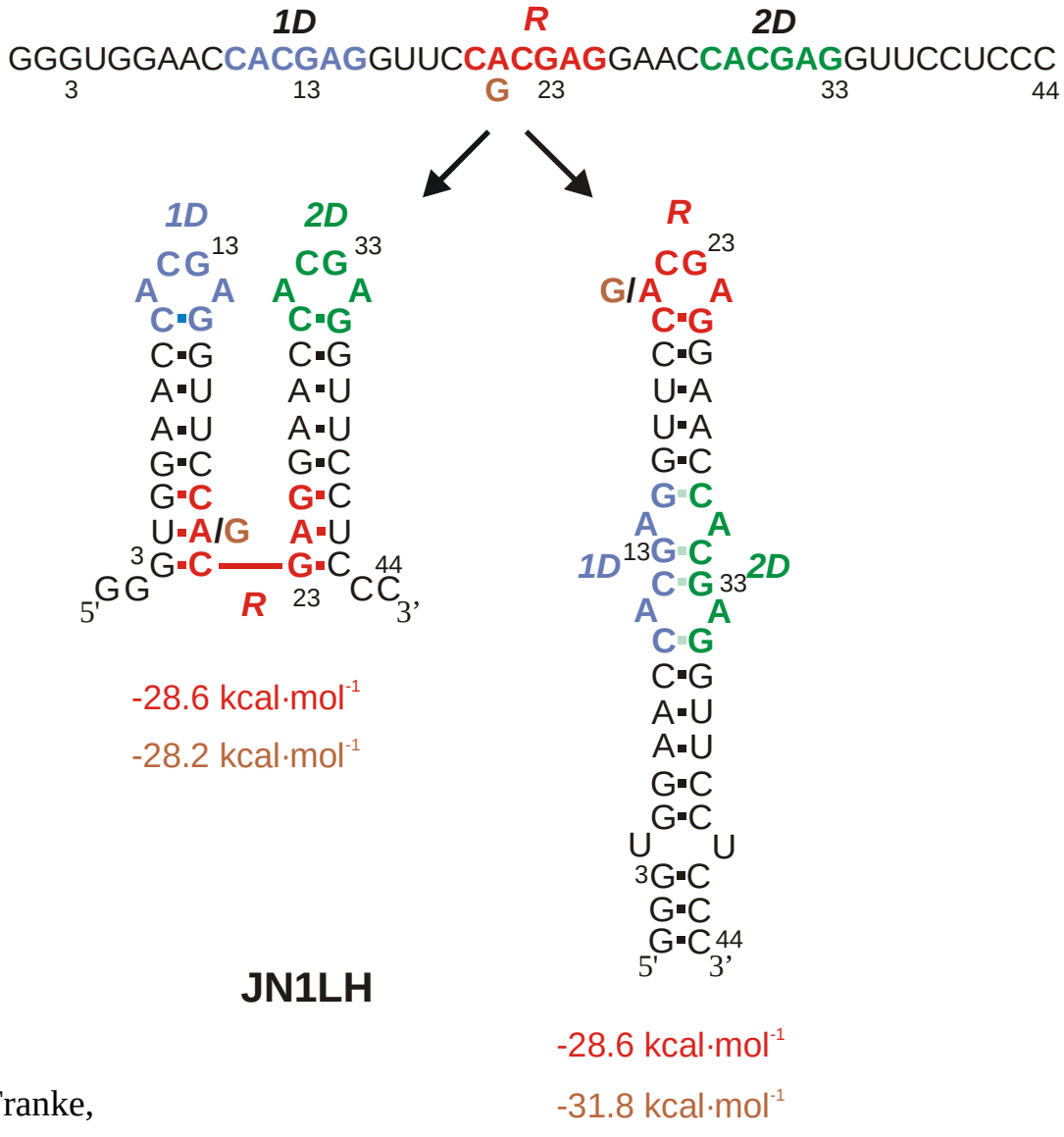


JN2C



JN2C small fragments

Synthetic RNA switches



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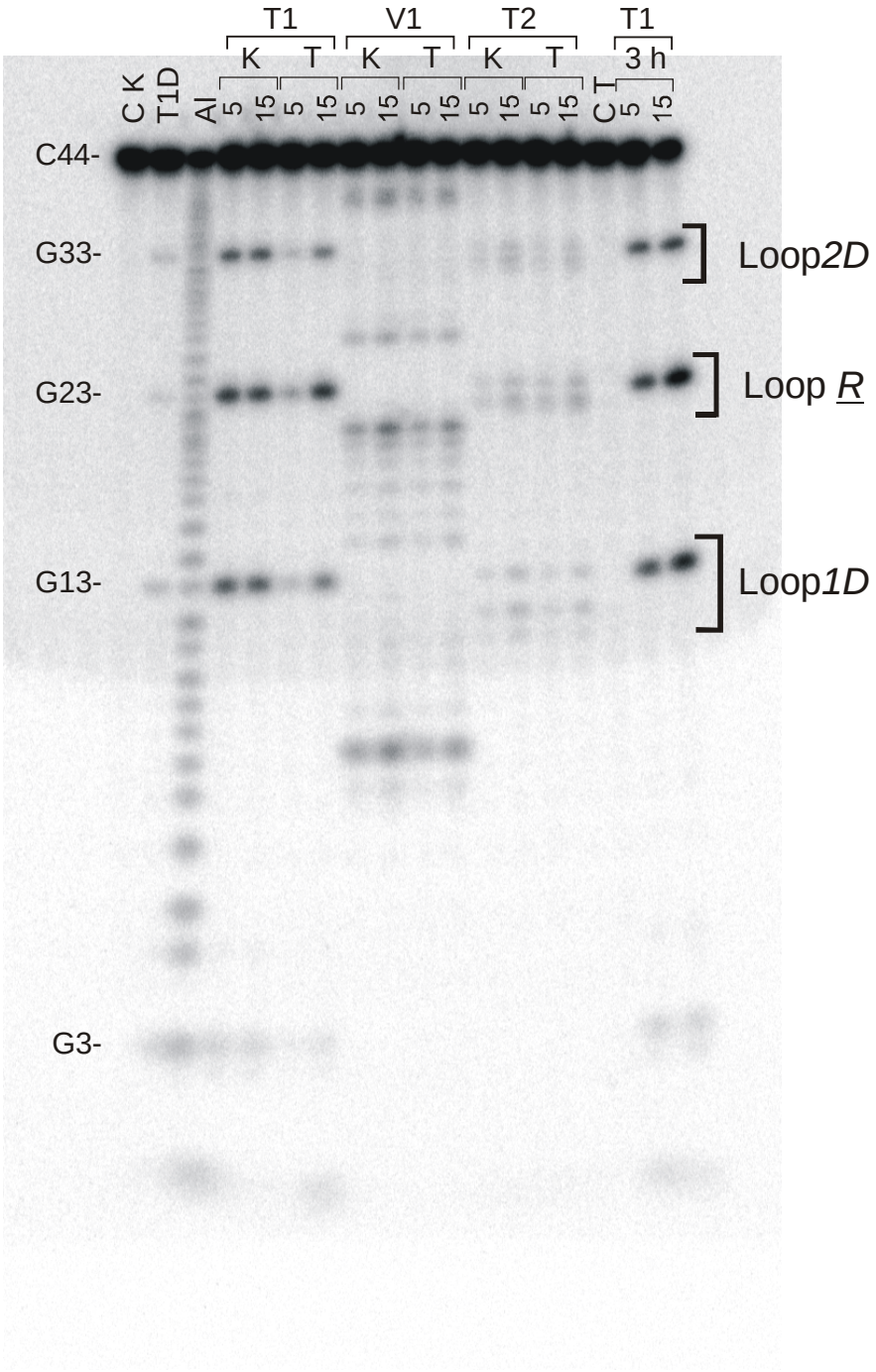
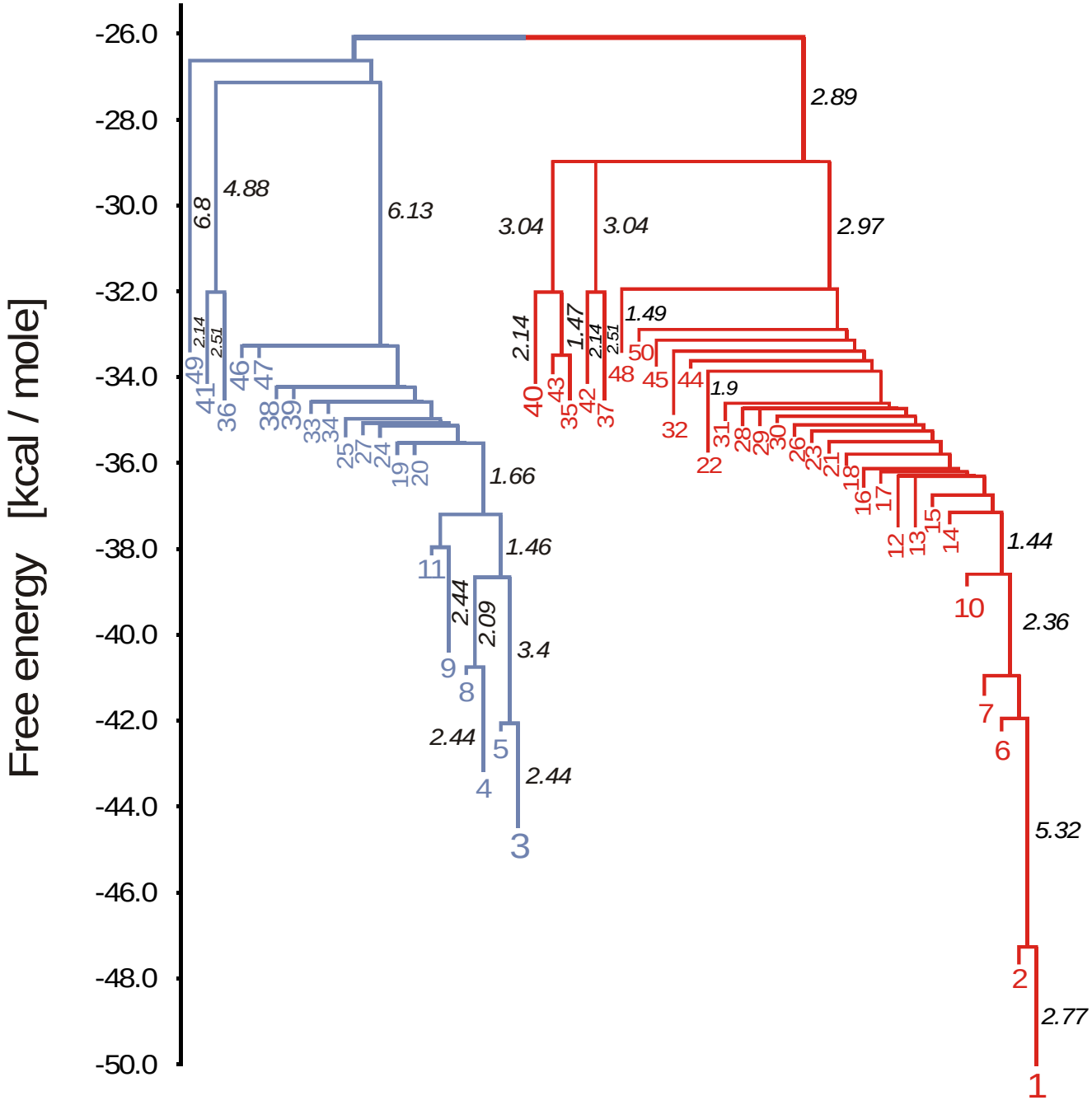


J1LH sequencing gels



J1LH barrier tree

A ribozyme switch

E.A.Schultes, D.B.Bartel, Science **289** (2000), 448-452

minus the background levels observed in the HSP in the control (Sar1-GDP–containing) incubation that prevents COPII vesicle formation. In the microsome control, the level of p115-SNARE associations was less than 0.1%.
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50. GST-SNARE proteins were expressed in bacteria and purified on glutathione-Sepharose beads using standard methods. Immobilized GST-SNARE protein (0.5 μM) was incubated with rat liver cytosol (20 mg) or purified recombinant p115 (0.5 μM) in 1 ml of NS buffer containing 1% BSA for 2 hours at 4°C with rotation. Beads were briefly spun (3000 rpm for 10 s) and sequentially washed three times with NS buffer and three times with NS buffer supplemented with 150 mM NaCl. Bound proteins were eluted three times in 50 μl of 50 mM tris-HCl (pH 8.5), 50 mM reduced glutathione, 150 mM NaCl, and 0.1% Triton X-100 for 15 min at 4°C with intermittent mixing, and elutes were pooled. Proteins were precipitated by MeOH/CH_3Cl and separated by SDS–polyacrylamide gel electrophoresis (PAGE) followed by immunoblotting using p115 mAb 13F12.
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69. We thank G. Waters for p115 cDNA and p115 mAbs; G. Warren for p97 and p47 antibodies; R. Scheller for rbet1, membrin, and sec22 cDNAs; H. Plutner for excellent technical assistance; and P. Tan for help during the initial phase of this work. Supported by NIH grants GM 33301 and GM42336 and National Cancer Institute grant CA58689 (W.E.B.), a NIH National Research Service Award (B.D.M.), and a Wellcome Trust International Traveling Fellowship (B.B.A.).

20 March 2000; accepted 22 May 2000

One Sequence, Two Ribozymes: Implications for the Emergence of New Ribozyme Folds

Erik A. Schultes and David P. Bartel*

We describe a single RNA sequence that can assume either of two ribozyme folds and catalyze the two respective reactions. The two ribozyme folds share no evolutionary history and are completely different, with no base pairs (and probably no hydrogen bonds) in common. Minor variants of this sequence are highly active for one or the other reaction, and can be accessed from prototype ribozymes through a series of neutral mutations. Thus, in the course of evolution, new RNA folds could arise from preexisting folds, without the need to carry inactive intermediate sequences. This raises the possibility that biological RNAs having no structural or functional similarity might share a common ancestry. Furthermore, functional and structural divergence might, in some cases, precede rather than follow gene duplication.

Related protein or RNA sequences with the same folded conformation can often perform very different biochemical functions, indicating that new biochemical functions can arise from preexisting folds. But what evolutionary mechanisms give rise to sequences with new macromolecular folds? When considering the origin of new folds, it is useful to picture, among all sequence possibilities, the distribution of sequences with a particular fold and function. This distribution can range very far in sequence space (*1*). For example, only seven nucleotides are strictly conserved among the group I self-splicing introns, yet secondary (and presumably tertiary) structure within the core of the ribozyme is preserved (*2*). Because these disparate isolates have the same fold and function, it is thought that they descended from a common ancestor through a series of mutational variants that were each functional. Hence, sequence heterogeneity among divergent isolates implies the existence of paths through sequence space that have allowed neutral drift from the ancestral sequence to each isolate. The set of all possible neutral paths composes a "neutral network," connecting in sequence space those widely dispersed sequences sharing a particular fold and activity, such that any sequence on the network can potentially access very distant sequences by neutral mutations (*3–5*).

Theoretical analyses using algorithms for predicting RNA secondary structure have suggested that different neutral networks are interwoven and can approach each other very closely (*3*, *5–8*). Of particular interest is whether ribozyme neutral networks approach each other so closely that they intersect. If so, a single sequence would be capable of folding into two different conformations, would have two different catalytic activities, and could access by neutral drift every sequence on both networks. With intersecting networks, RNAs with novel structures and activities could arise from previously existing ribozymes, without the need to carry nonfunctional sequences as evolutionary intermediates. Here, we explore the proximity of neutral networks experimentally, at the level of RNA function. We describe a close apposition of the neutral networks for the hepatitis delta virus (HDV) self-cleaving ribozyme and the class III self-ligating ribozyme.

In choosing the two ribozymes for this investigation, an important criterion was that they share no evolutionary history that might confound the evolutionary interpretations of our results. Choosing at least one artificial ribozyme ensured independent evolutionary histories. The class III ligase is a synthetic ribozyme isolated previously from a pool of random RNA sequences (*9*). It joins an oligonucleotide substrate to its 5′ terminus. The prototype ligase sequence (Fig. 1A) is a shortened version of the most active class III variant isolated after 10 cycles of in vitro selection and evolution. This minimal construct retains the activity of the full-length isolate (*10*). The HDV ribozyme carries out the site-specific self-cleavage reactions needed during the life cycle of HDV, a satellite virus of hepatitis B with a circular, single-stranded RNA genome (*11*). The prototype HDV construct for our study (Fig. 1B) is a shortened version of the antigenomic HDV ribozyme (*12*), which undergoes self-cleavage at a rate similar to that reported for other antigenomic constructs (*13*, *14*).

The prototype class III and HDV ribozymes have no more than the 25% sequence identity expected by chance and no fortuitous structural similarities that might favor an intersection of their two neutral networks. Nevertheless, sequences can be designed that simultaneously satisfy the base-pairing requirements

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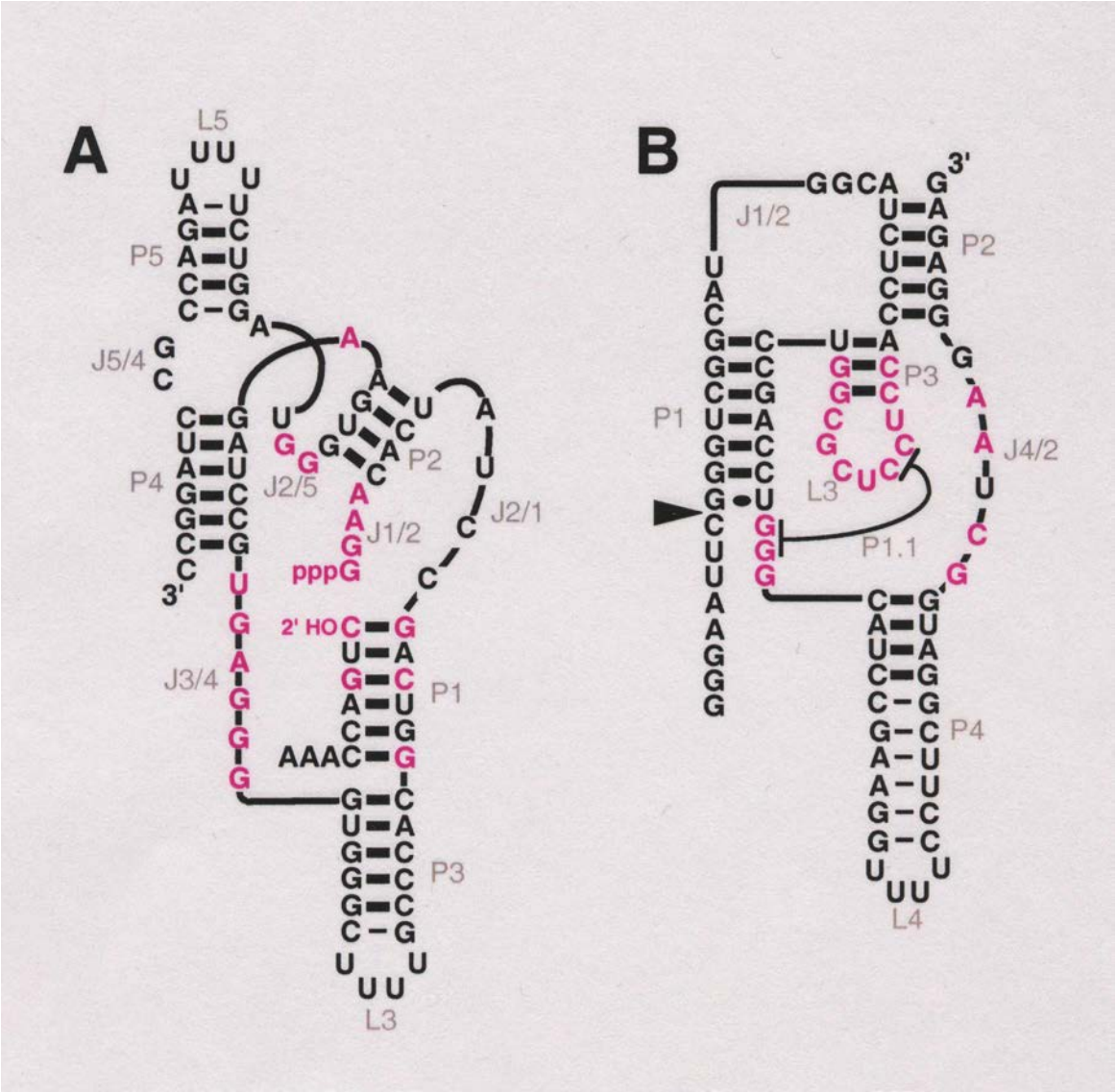
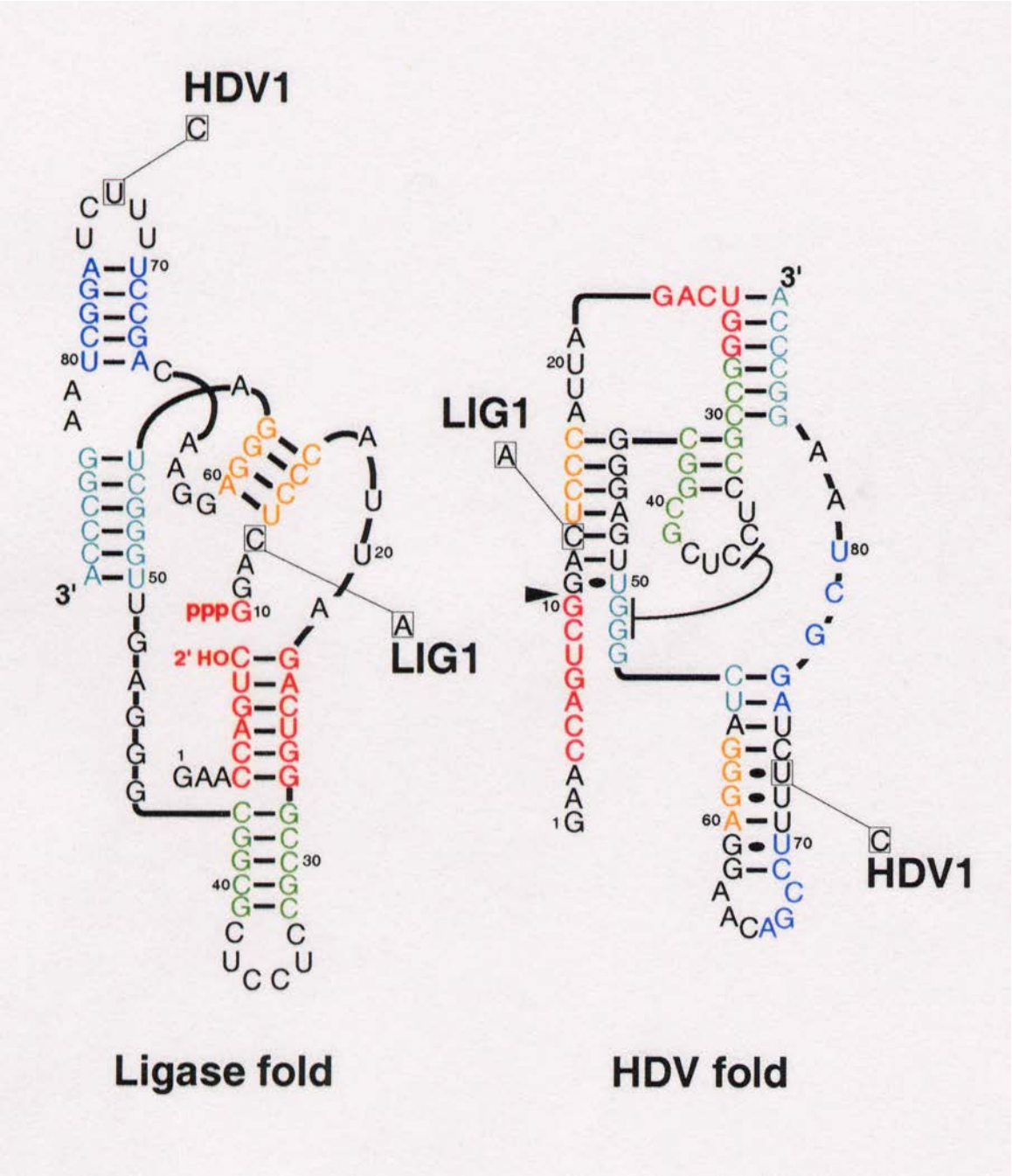
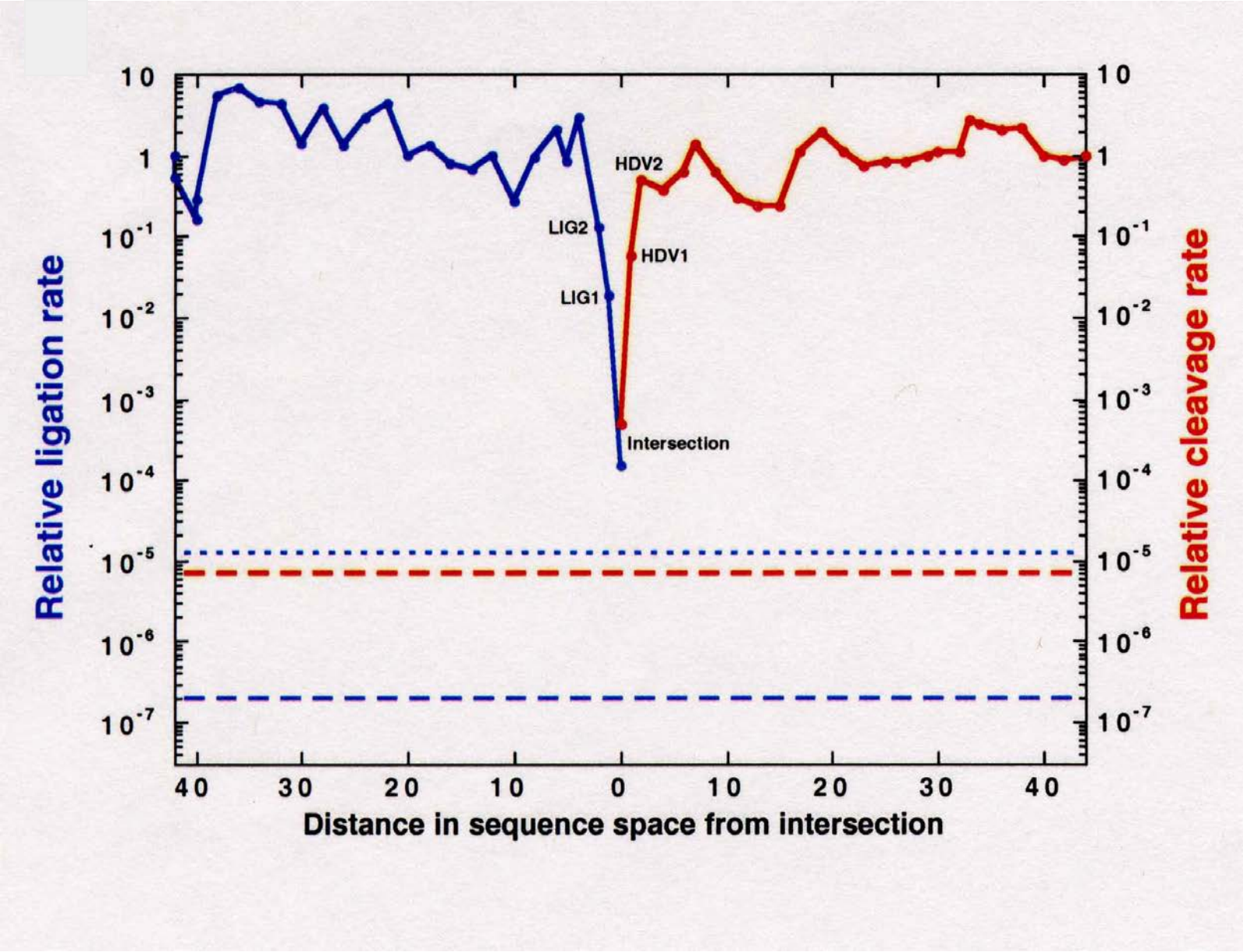


Two ribozymes of chain lengths n = 88 nucleotides: An artificial ligase (**A**) and a natural cleavage ribozyme of hepatitis-δ-virus (**B**)

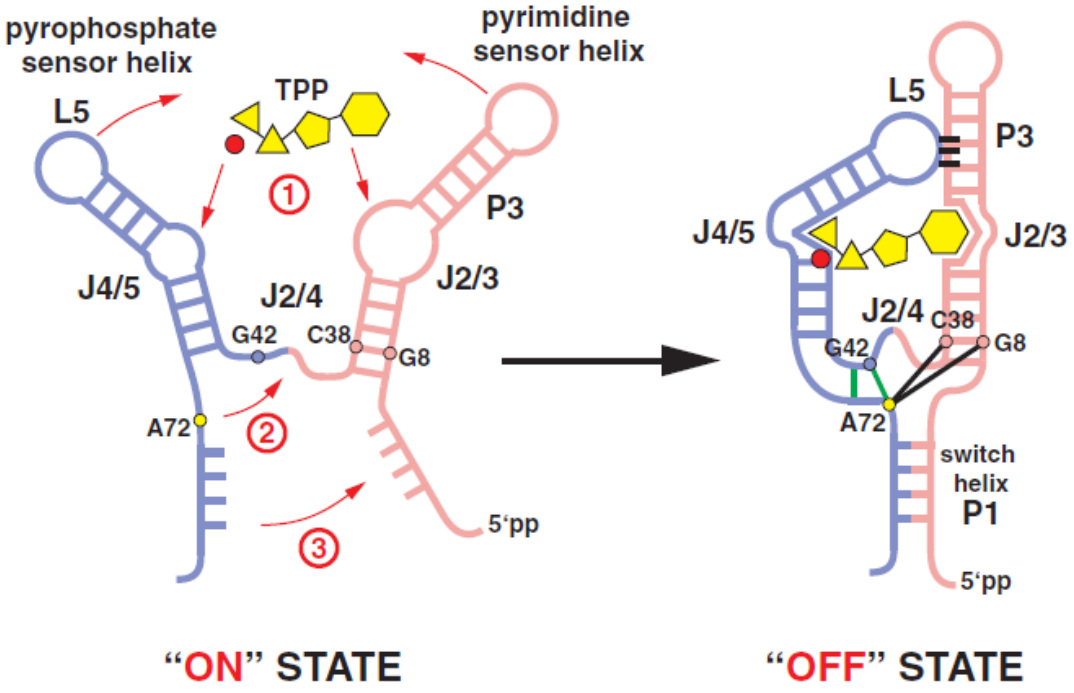
The sequence at the ***intersection***:

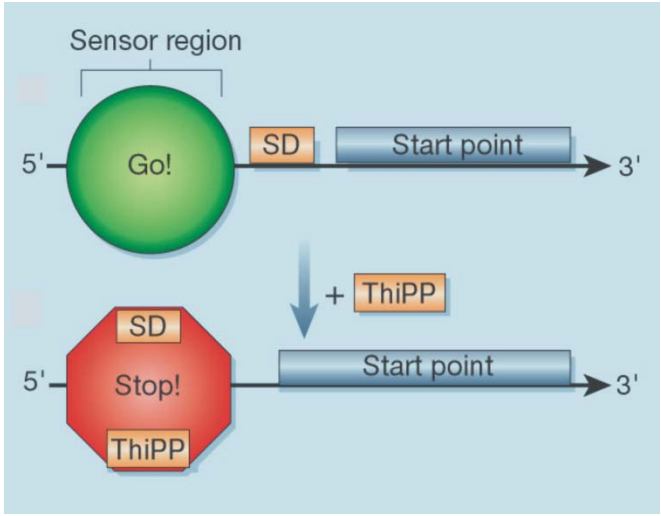
An RNA molecules which is 88 nucleotides long and can form both structures





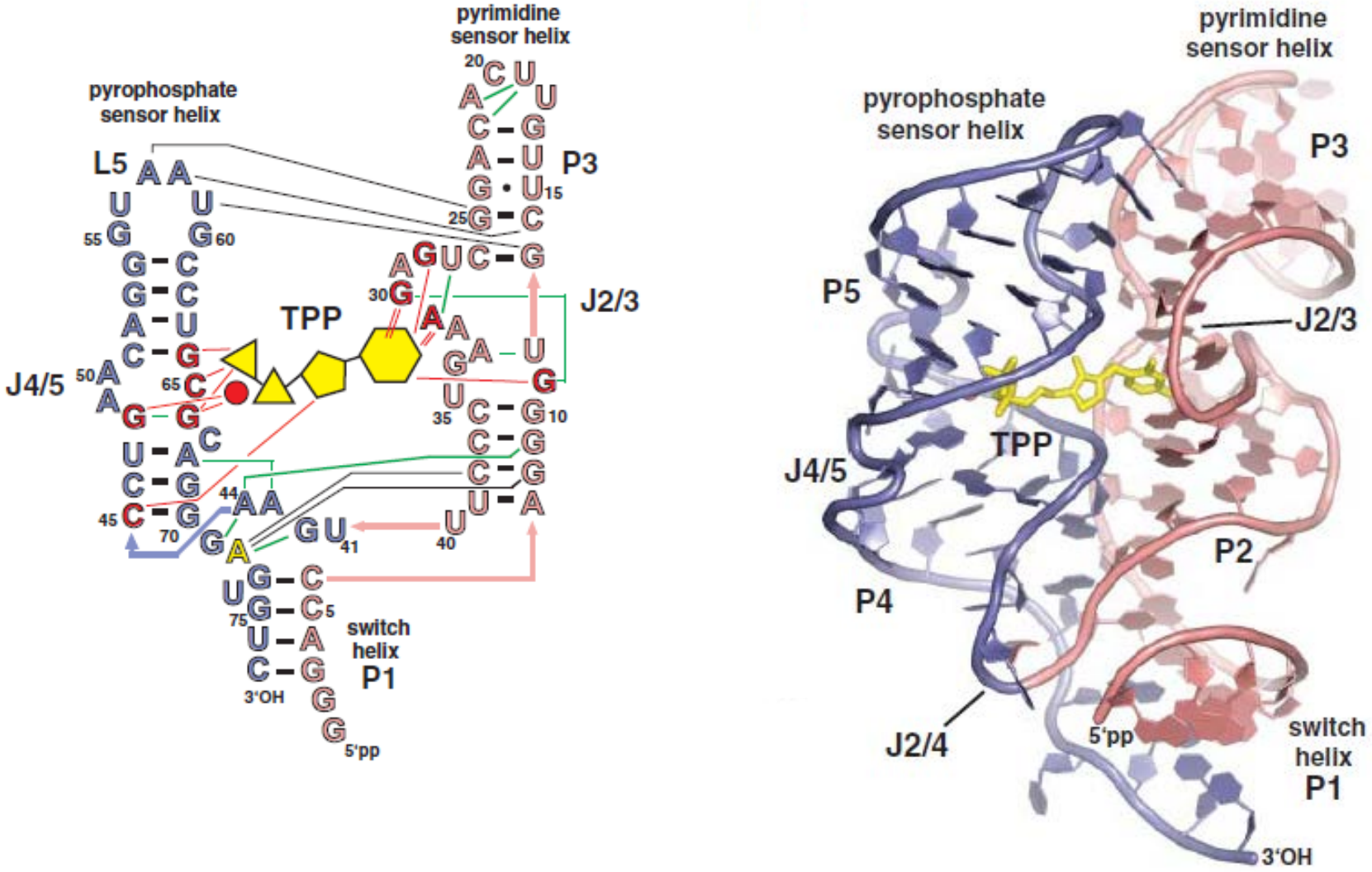
Two neutral walks through sequence space with conservation of structure and catalytic activity





The thiamine-pyrophosphate riboswitch

S. Thore, M. Leibundgut, N. Ban.
Science **312**:1208-1211, 2006.



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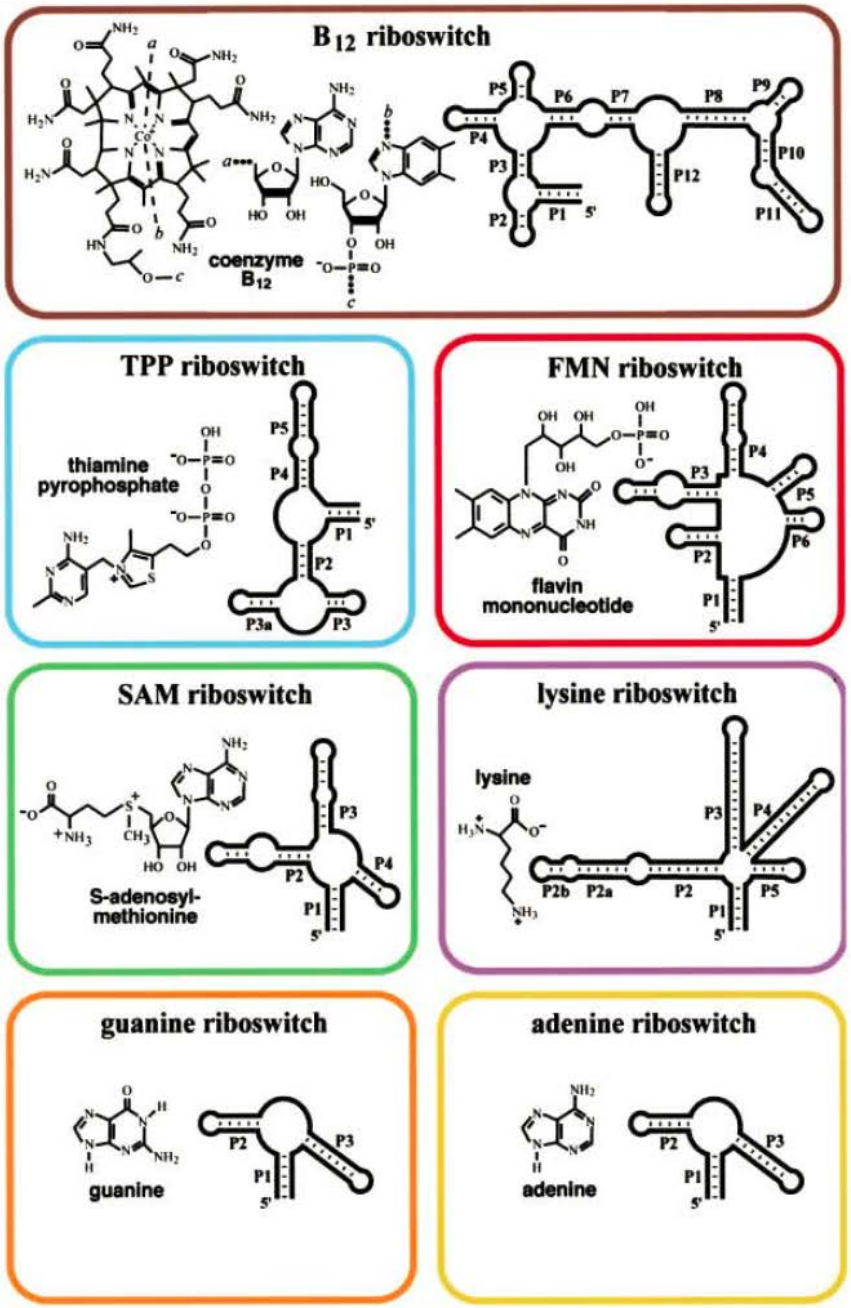

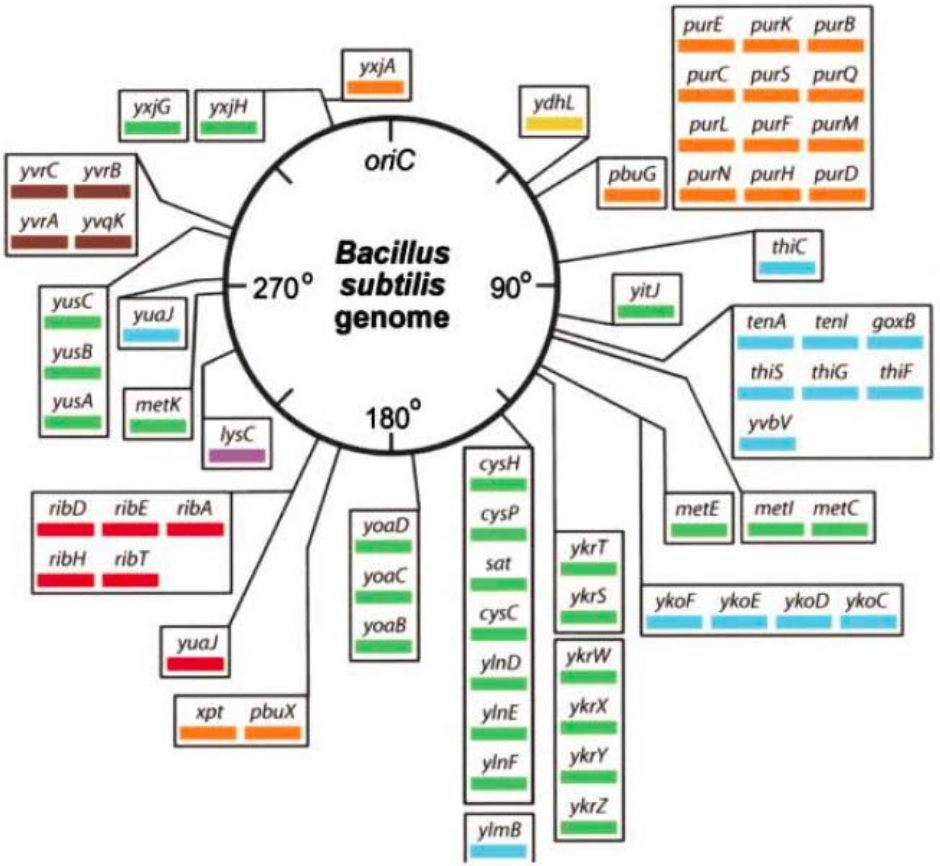

M. Mandal, B. Boese, J.E. Barrick, W.C. Winkler, R.R, Breaker. Cell 113:577-586 (2003)

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