Landscapes in RNA Folding and Evolution

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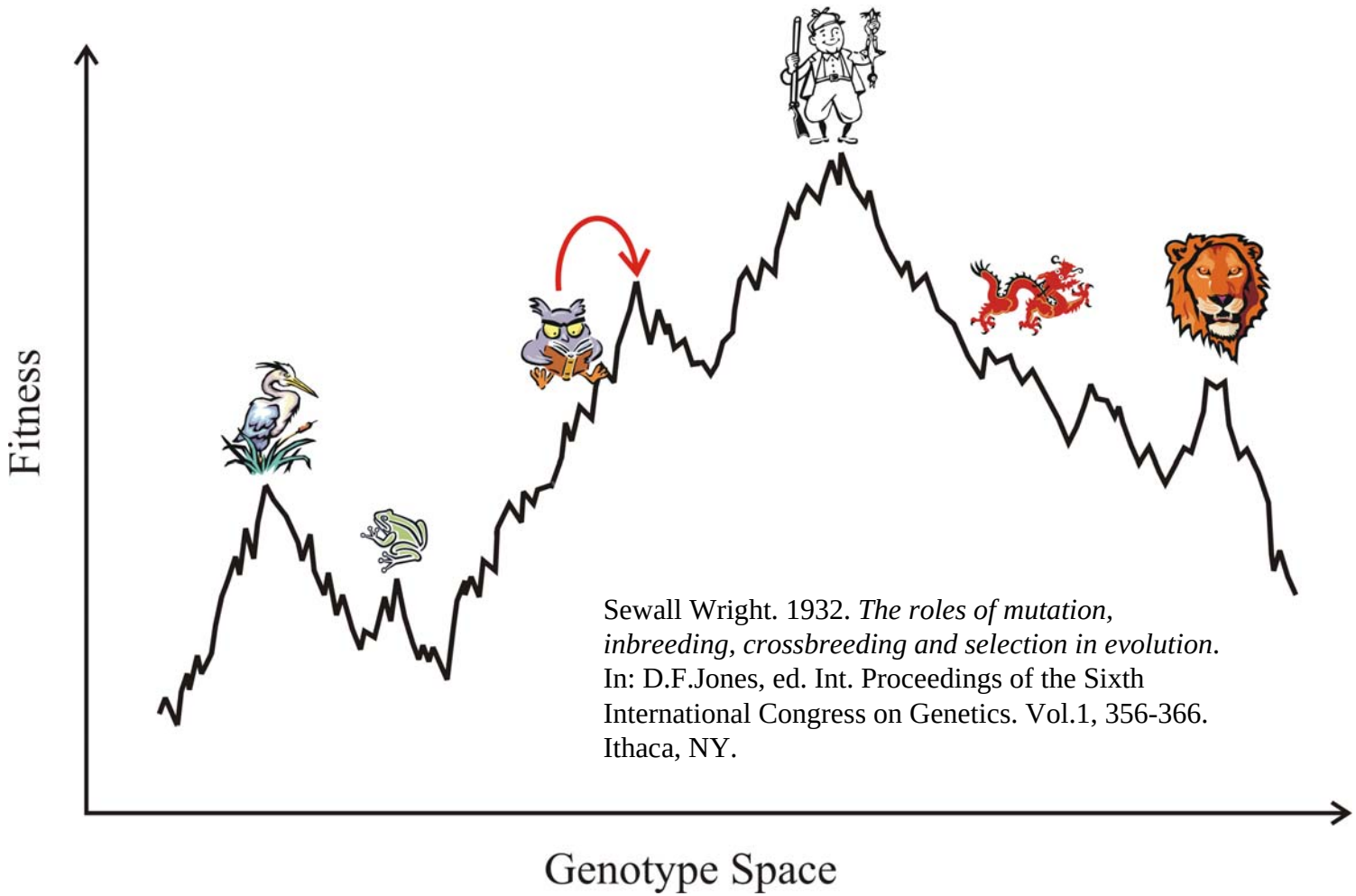
Web-Page for further information:

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



Sewall Wright. 1932. *The roles of mutation, inbreeding, crossbreeding and selection in evolution*. In: D.F.Jones, ed. Int. Proceedings of the Sixth International Congress on Genetics. Vol.1, 356-366. Ithaca, NY.

Sewall Wrights landscape as metaphor for Darwinian evolution

↓

Fitness landscape of evolutionary optimization

Stationary molecular Schrödinger equation

$$\left(-\frac{\hbar^2}{2m_0} \sum_i \nabla_i^2 + V(\vec{r}, \vec{R}) \right) \Psi_k(\vec{r}) = E_k(\vec{R}) \Psi_k(\vec{r})$$

$$\left(-\frac{\hbar^2}{2} \sum_j \frac{1}{M_j} \nabla_j^2 + E_k(\vec{R}) \right) \Xi_m^{(k)}(\vec{R}) = W_m^{(k)} \Xi_m^{(k)}(\vec{R})$$

Quantum mechanical energy (hyper)surface

↓

Free energy surface of macromolecular conformations

(Proteins, nucleic acids, carbohydrates, ...)

Free energy landscapes

Force fields for biopolymers
3D Structure refinement
Protein folding

Fitness landscapes, combinatory maps

Genotype-phenotype mapping
Biopolymer models (RNA, lattice proteins, ...)

Continuous spaces

Landscapes and mappings

Discrete spaces

Energy landscapes

Quantum mechanics of molecules
Chemical reactions
Intermolecular forces

Landscape metaphors

Species formation
Darwinian evolution
Selection models

1. Sequence space and shape space
2. Neutral networks and evolution
3. Conformation space and kinetic folding
4. What kind of analogies are there?
5. How to model evolution of kinetic folding?

1. **Sequence space and shape space**
2. Neutral networks and evolution
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4. What kind of analogies are there?
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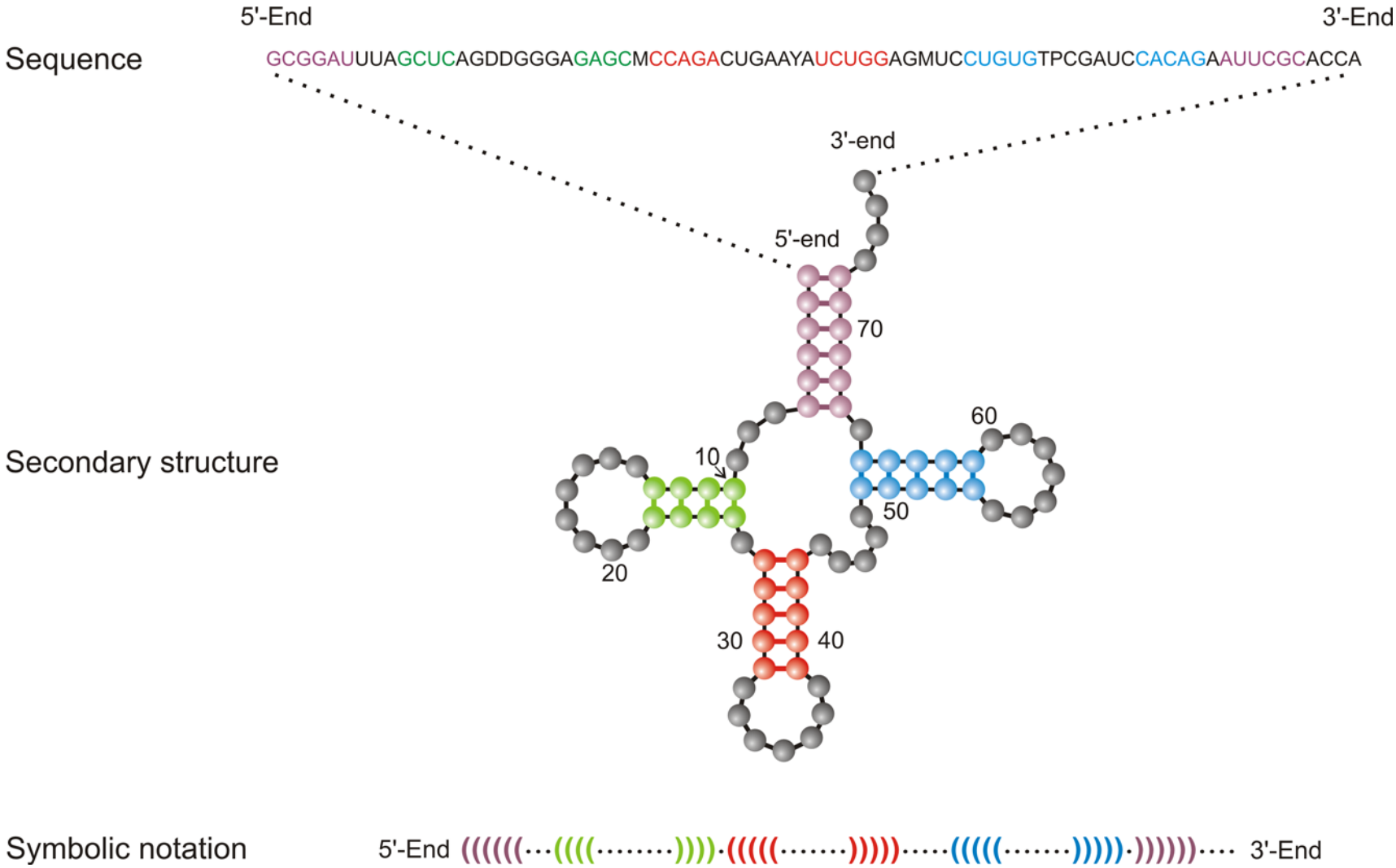
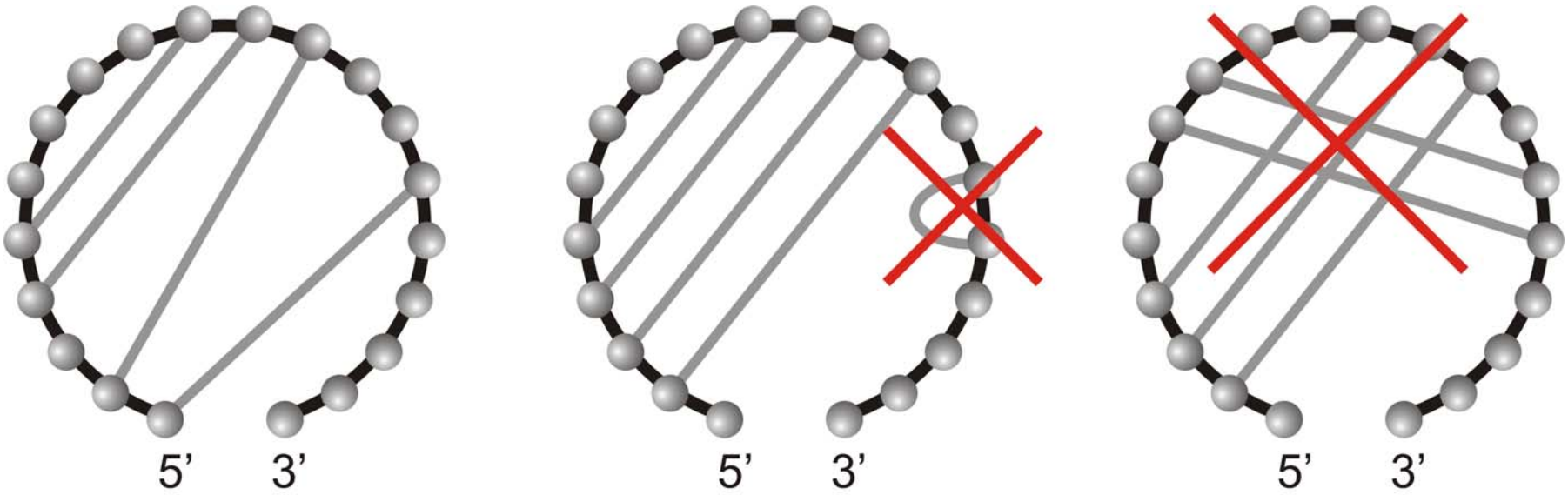


A symbolic notation of RNA secondary structure that is equivalent to the conventional graphs



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

Conventional definition of RNA secondary structures

RNA sequence GUAUCGAAAUACGUAGCGUAUGGGGAUGCUGGACGGUCCCAUCGGUACUCCA

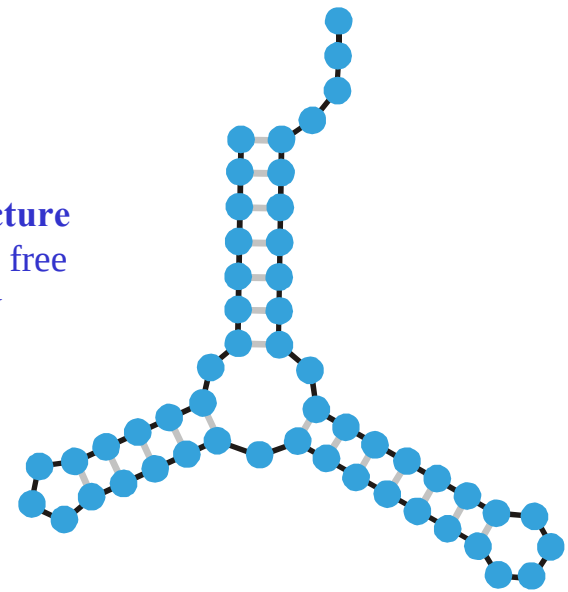
RNA folding:

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

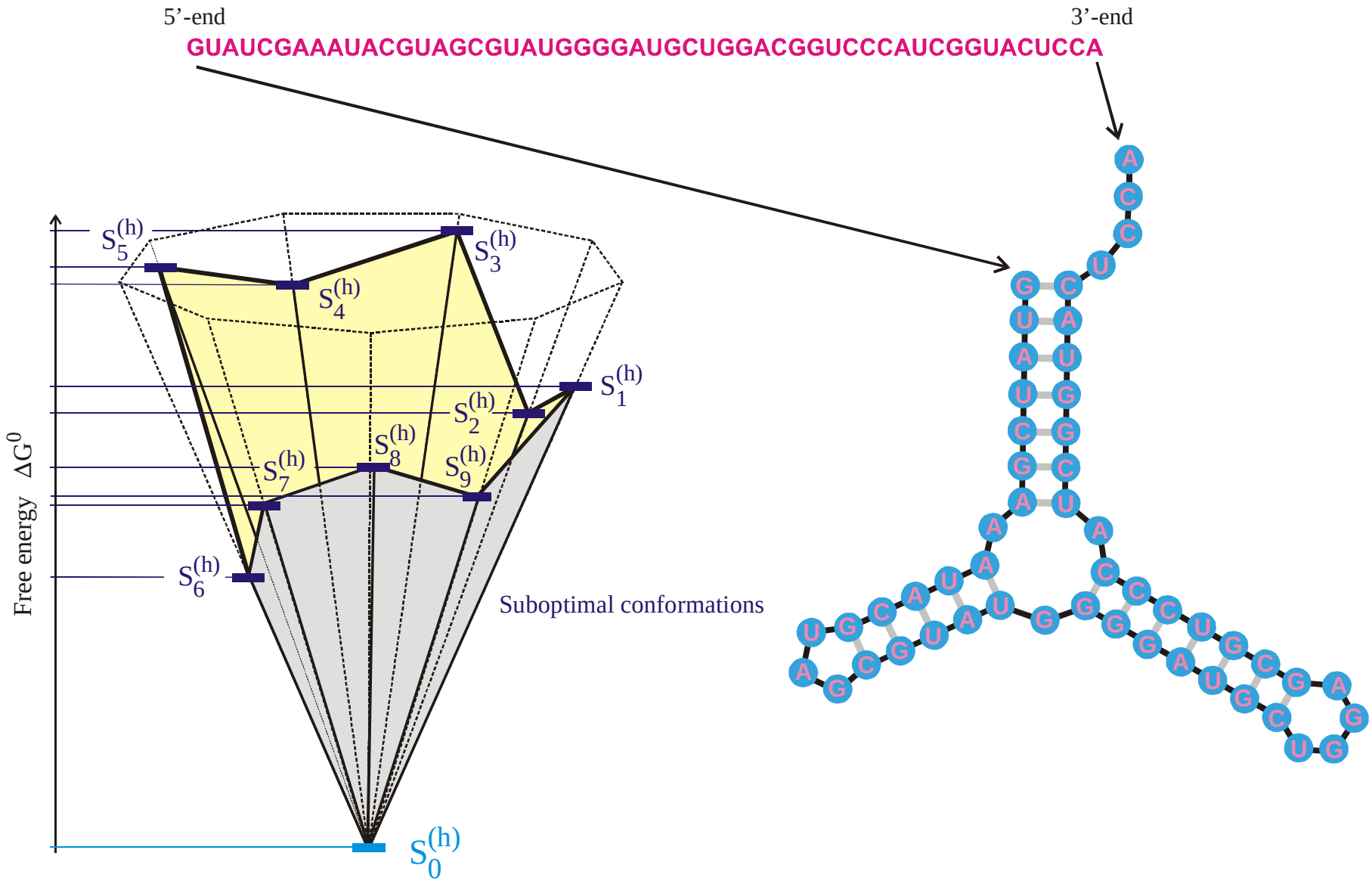
Biophysical chemistry: thermodynamics and kinetics

Empirical parameters

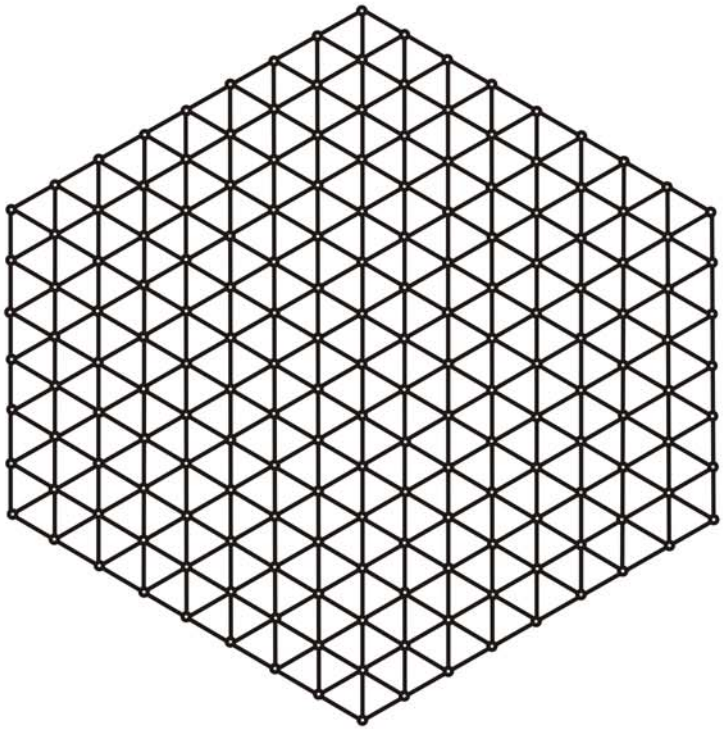
RNA structure of minimal free energy



Sequence, structure, and design

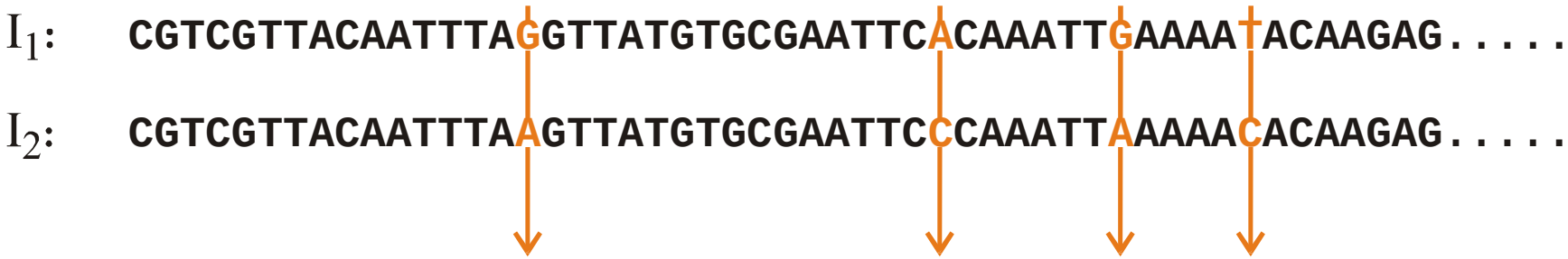


The minimum free energy structures on a discrete space of conformations



Sequence space

Sequence space



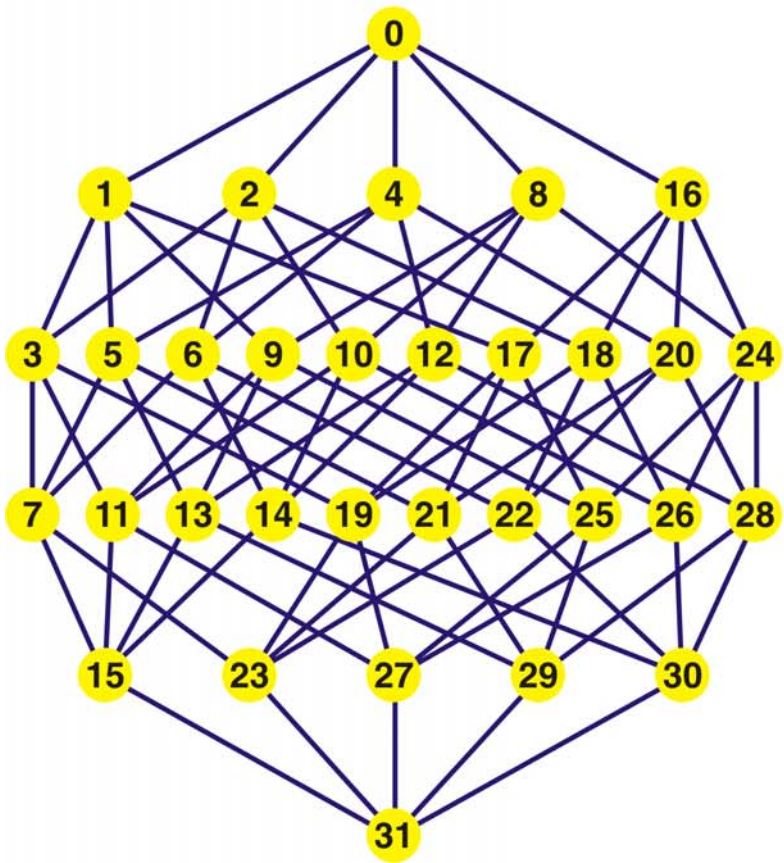
Hamming distance $d_H(I_1,I_2) = 4$

(i) $d_H(I_1,I_1) = 0$

(ii) $d_H(I_1,I_2) = d_H(I_2,I_1)$

(iii) $d_H(I_1,I_3) \leq d_H(I_1,I_2) + d_H(I_2,I_3)$

The Hamming distance between sequences induces a metric in sequence space



Binary sequences can be encoded by their decimal equivalents:

C = 0 and G = 1, for example,

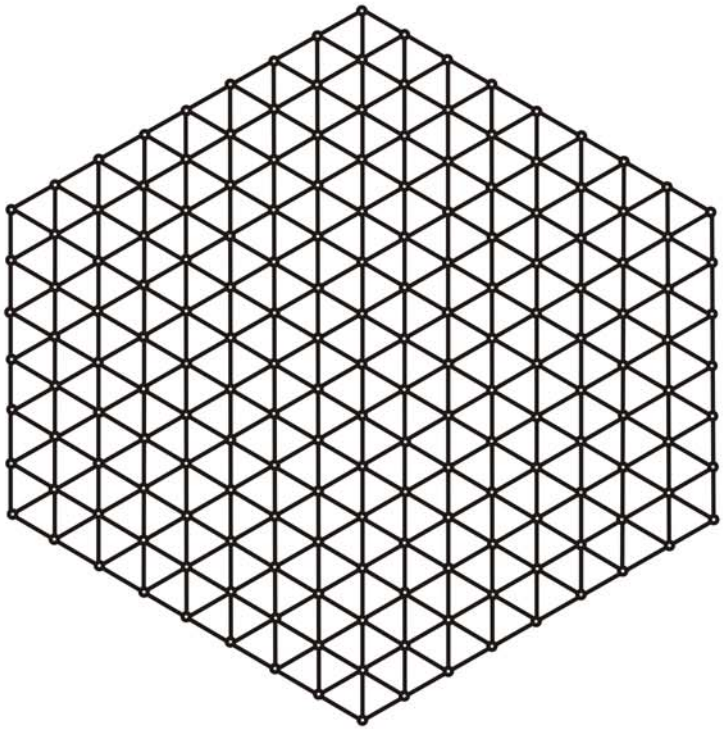
"0" ≡ 00000 = CCCCC,

"14" ≡ 01110 = CGGGC,

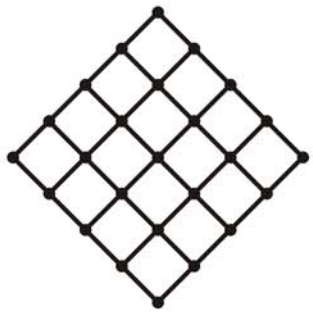
"29" ≡ 11101 = GGGCG, etc.

Every point in sequence space is equivalent

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

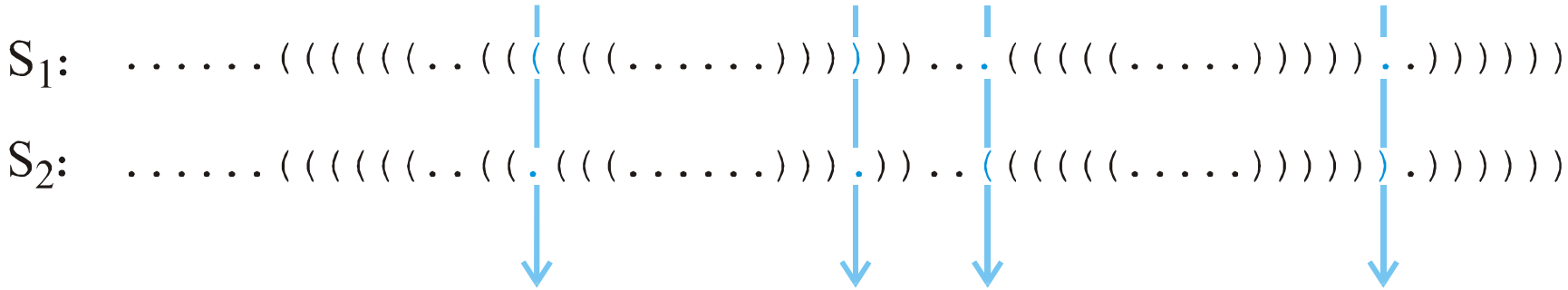


Sequence space



Structure space

Sequence space and structure space



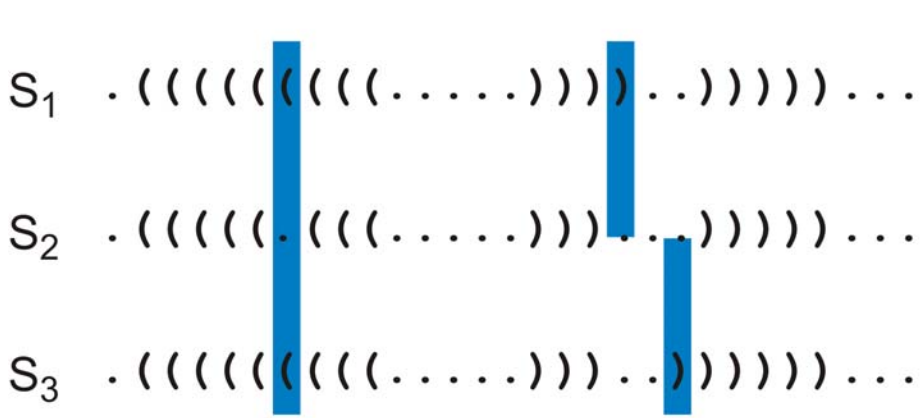
Hamming distance $d_H(S_1,S_2) = 4$

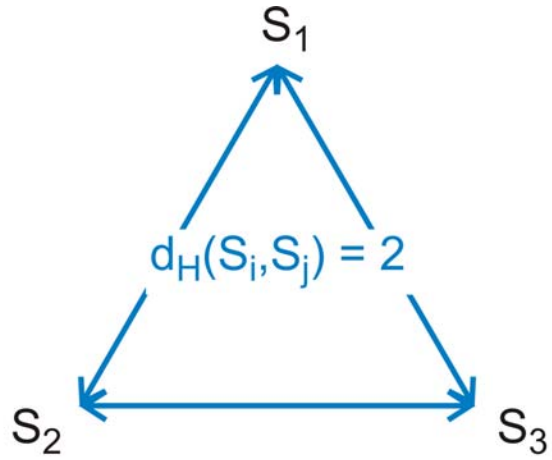
(i) $d_H(S_1,S_1) = 0$

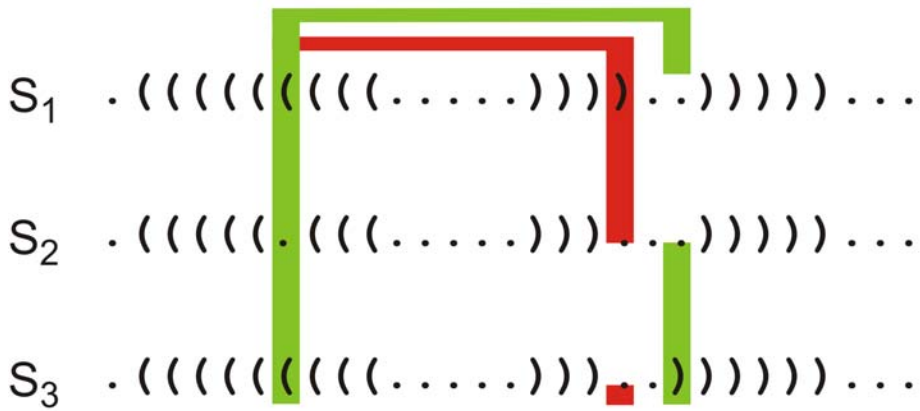
(ii) $d_H(S_1,S_2) = d_H(S_2,S_1)$

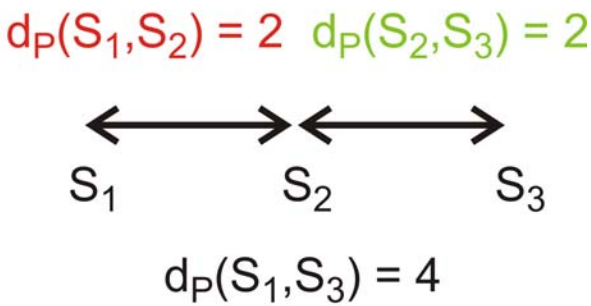
(iii) $d_H(S_1,S_3) \leq d_H(S_1,S_2) + d_H(S_2,S_3)$

The Hamming distance between structures in parentheses notation forms a metric in structure space



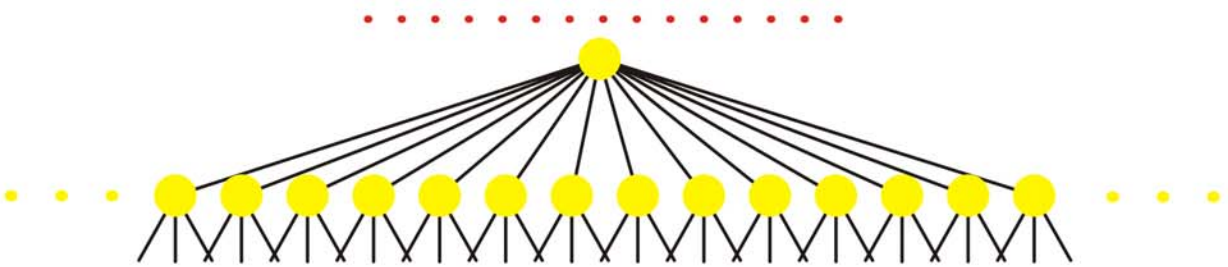






Two measures of distance in shape space:

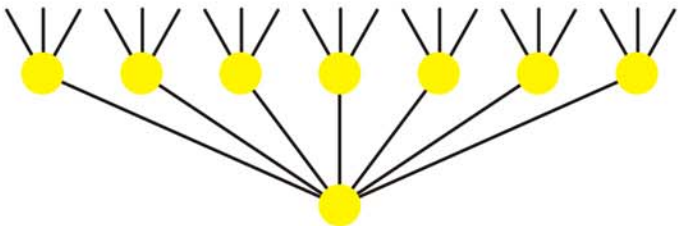
Hamming distance between structures, $d_H(S_i,S_j)$ and base pair distance, $d_P(S_i,S_j)$



open chain

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

66 for n = 15



7 for n = 15

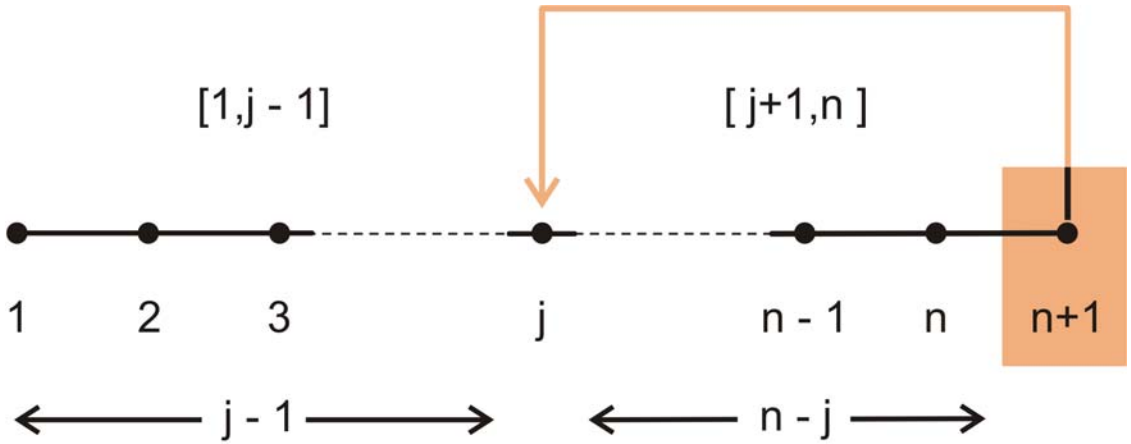
number of edges $\left\lfloor \frac{n-3}{2} \right\rfloor$

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

longest stack

Structures are not equivalent in structure space

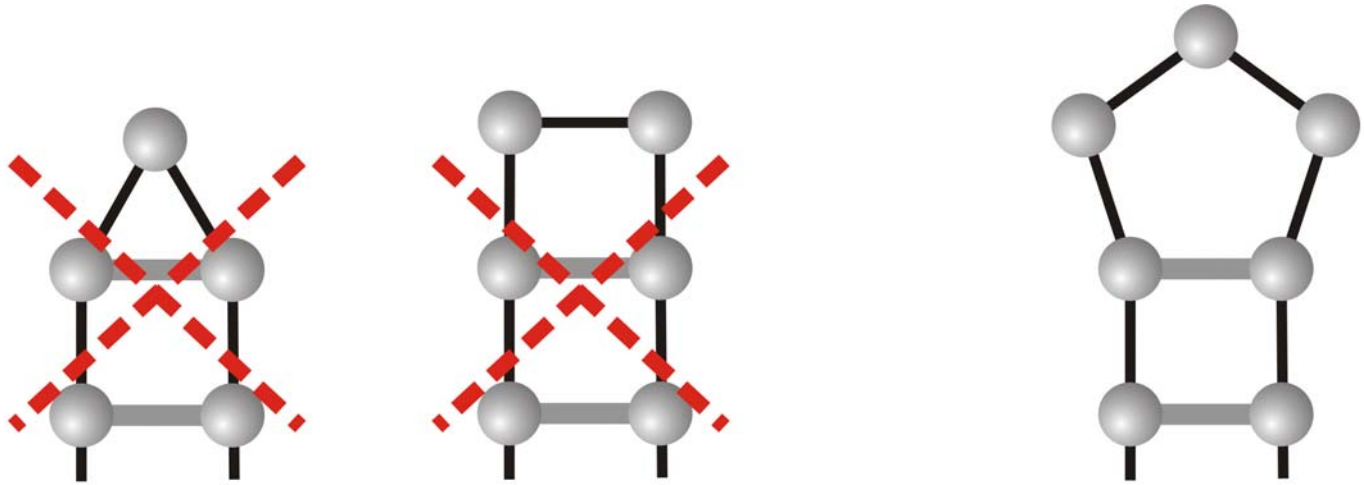
Sketch of structure space



$$S_{n+1} = S_n + \sum_{j=1}^{n-1} S_{j-1} \cdot S_{n-j}$$

Counting the numbers of structures of chain length $n \Rightarrow n+1$

M.S. Waterman, T.F. Smith (1978) *Math.Bioscience* **42**:257-266



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 $\sigma \geq 2$

Size restriction of elements: (i) hairpin loop $n_{\text{loop}} \geq \lambda$

(ii) stack $n_{\text{stack}} \geq \sigma$

$$S_{m+1} = \Xi_{m+1} + \Phi_{m-1}$$

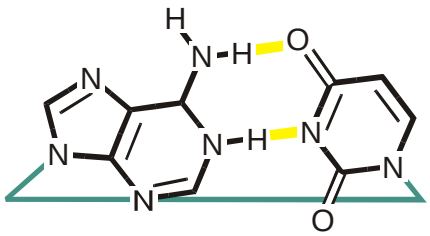
$$\Xi_{m+1} = S_m + \sum_{k=\lambda+2\sigma-2}^{m-2} \Phi_k \cdot S_{m-k+1}$$

$$\Phi_{m+1} = \sum_{k=\sigma-1}^{\lfloor (m-\lambda+1)/2 \rfloor} \Xi_{m-2k+1}$$

$S_n \approx$ # structures of a sequence with chain length n

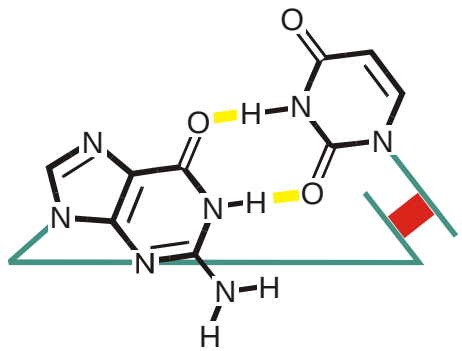
Recursion formula for the number of physically acceptable stable structures

I.L.Hofacker, P.Schuster, P.F. Stadler. 1998. *Discr.Appl.Math*. **89**:177-207

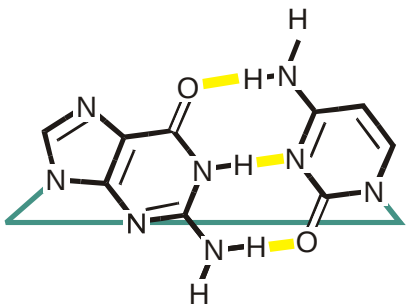


A=U

(U=A)

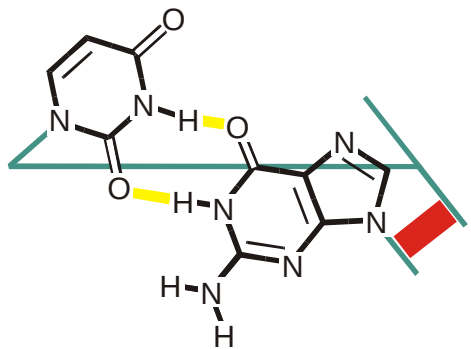


G=U



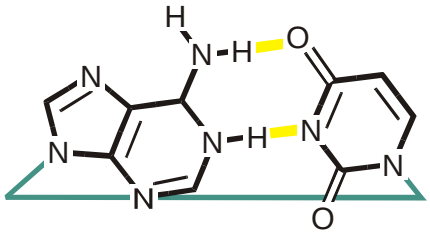
G≡C

(C≡G)



U=G

The six base pairing alphabets built from natural nucleotides **A**, **U**, **G**, and **C**



A=U

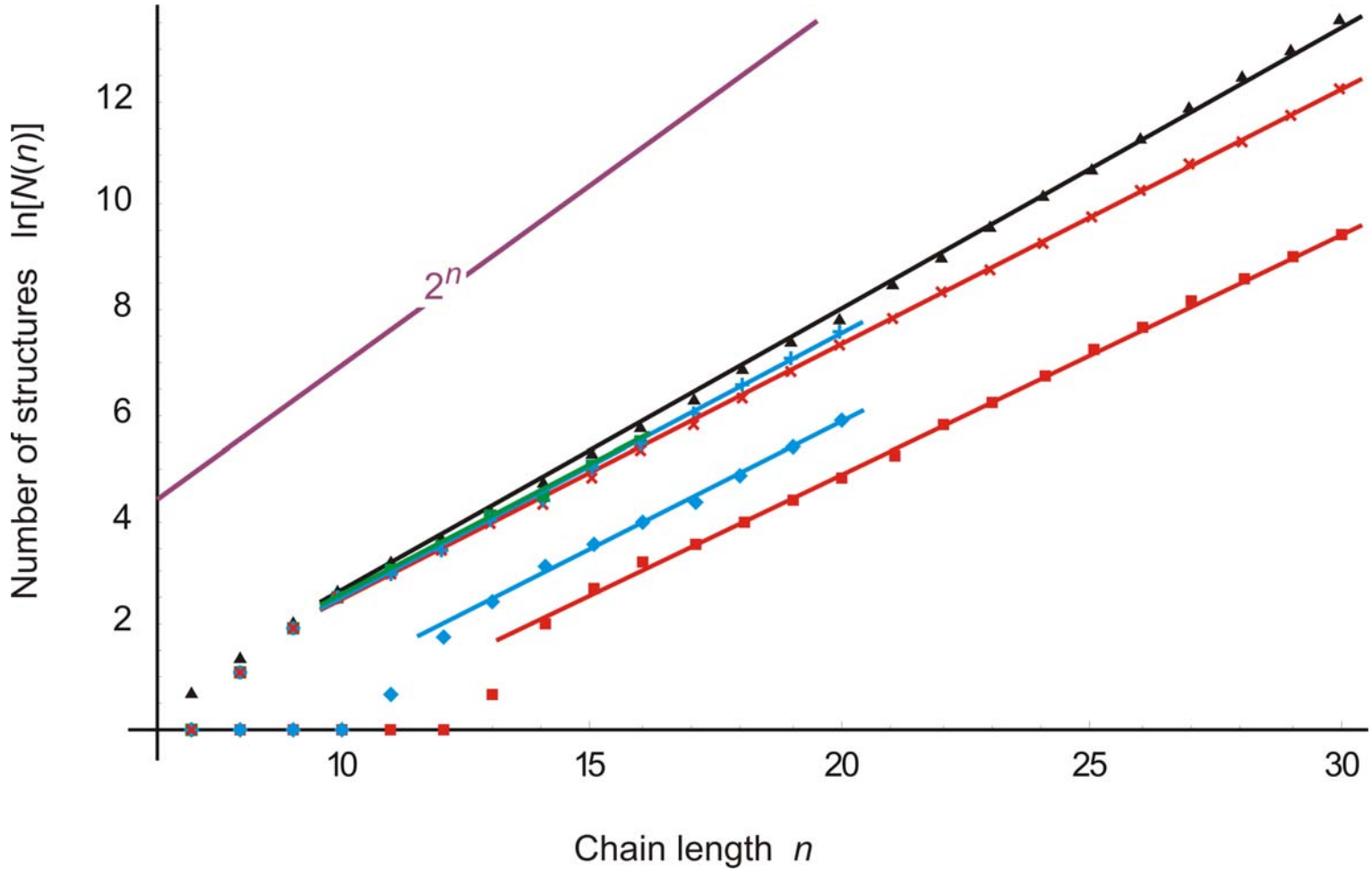
(U=A)

The six base pairing alphabets built from natural nucleotides **A**, **U**, **G**, and **C**

ℓ	Number of Sequences 2^ℓ	4^ℓ	Number of Structures $S_\ell^{(3,2)}$	GC	UGC	AUGC	AUG	AU
7	128	1.64×10^4	2	1	1	1	1	1
8	256	6.55×10^4	4	3	3	3	1	1
9	512	2.62×10^5	8	7	7	7	1	1
10	1 024	1.05×10^6	14	13	13	13	1	1
15	3.28×10^4	1.07×10^9	174	130	145	152	37	15
16	6.55×10^4	4.29×10^9	304	214	245	257	55	25
19	5.24×10^5	2.75×10^{11}	1 587	972	1 235		220	84
20	1.05×10^6	1.10×10^{12}	2 741	1 599	2 112		374	128
29	5.37×10^8	2.88×10^{17}	430 370	132 875				8 690
30	1.07×10^9	1.15×10^{18}	760 983	218 318				13 726

Computed numbers of minimum free energy structures over different alphabets

P. Schuster, *Molecular insights into evolution of phenotypes*. In: J. Crutchfield & P.Schuster, Evolutionary Dynamics. Oxford University Press, New York 2003, pp.163-215.



Cardinalities of RNA sequence spaces and shape spaces:
Many more sequences than structures

Nature **402**, 323-325, 1999

A ribozyme that lacks cytidine

Jeff Rogers & Gerald F. Joyce

Departments of Chemistry and Molecular Biology, and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA

The RNA-world hypothesis proposes that, before the advent of DNA and protein, life was based on RNA, with RNA serving as both the repository of genetic information and the chief agent of catalytic function[1]. An argument against an RNA world is that the components of RNA lack the chemical diversity necessary to sustain life. Unlike proteins, which contain 20 different amino-acid subunits, nucleic acids are composed of only four subunits which have very similar chemical properties. Yet RNA is capable of a broad range of catalytic functions[2–7]. Here we show that even three nucleic-acid subunits are sufficient to provide a substantial increase in the catalytic rate. Starting from a molecule that contained roughly equal proportions of all four nucleosides, we used *in vitro* evolution to obtain an RNA ligase ribozyme that lacks cytidine. This ribozyme folds into a defined structure and has a catalytic rate that is about 10^5-fold faster than the uncatalysed rate of template-directed RNA ligation.

Catalytic activity in the **AUG** alphabet

Nature **420**, 841-844, 2002

A ribozyme composed of only two different nucleotides

John S. Reader & Gerald F. Joyce

Departments of Chemistry and Molecular Biology and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA

RNA molecules are thought to have been prominent in the early history of life on Earth because of their ability both to encode genetic information and to exhibit catalytic function[1]. The modern genetic alphabet relies on two sets of complementary base pairs to store genetic information. However, owing to the chemical instability of cytosine, which readily deaminates to uracil[2], a primitive genetic system composed of the bases A, U, G and C may have been difficult to establish. It has been suggested that the first genetic material instead contained only a single base-pairing unit[3–7]. Here we show that binary informational macromolecules, containing only two different nucleotide subunits, can act as catalysts. *In vitro* evolution was used to obtain ligase ribozymes composed of only 2,6-diaminopurine and uracil nucleotides, which catalyse the template-directed joining of two RNA molecules, one bearing a 5′-triphosphate and the other a 3′-hydroxyl. The active conformation of the fastest isolated ribozyme had a catalytic rate that was about 36,000-fold faster than the uncatalysed rate of reaction. This ribozyme is specific for the formation of biologically relevant 3′,5′-phosphodiester linkages.

Catalytic activity in the **DU** alphabet

1. Sequence space and shape space
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3. Conformation space and kinetic folding
4. What kind of analogies are there?
5. How to model evolution of kinetic folding?

RNA sequence GUAUCGAAAUACGUAGCGUAUGGGGAUGCUGGACGGUCCCAUCGGUACUCCA

RNA folding:

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

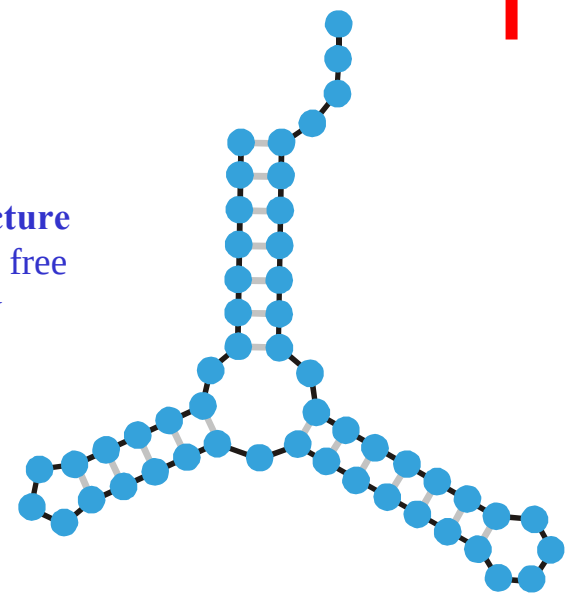
Iterative determination of a sequence for the given secondary structure

Inverse Folding Algorithm

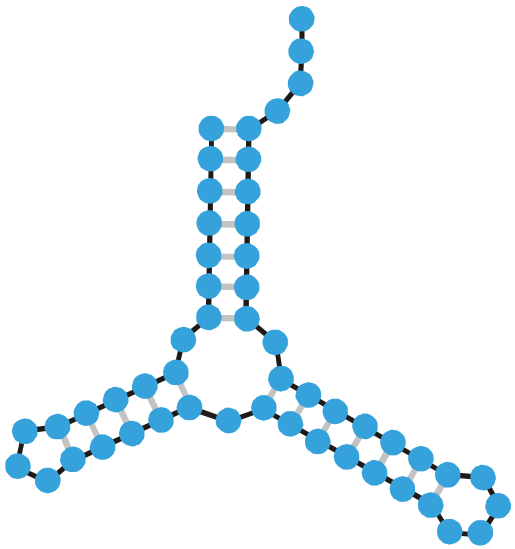
Inverse folding of RNA:

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

RNA structure of minimal free energy



Sequence, structure, and design



Minimum free energy criterion

1st
2nd
3rd trial
4th
5th

UUUAGCCAGCGCGAGUCGUGCGGACGGGGUUAUCUCUGUCGGGCUAGGGCGC

GUGAGCGCGGGGCACAGUUUCUCAAGGAUGUAAGUUUUUGCCGUUUAUCUGG

UUAGCGAGAGAGGAGGCUUCUAGACCCAGCUCUCUGGGUCGUUGCUGAUGCG

CAUUGGUGCUAAUGAUAUUAGGGCUGUAUUCCUGUAUAGCGAUCAGUGUCCG

GUAGGCCCUCUUGACAUAAGAUUUUUCCAAUGGUGGGAGAUGGCCAUUGCAG

Inverse folding

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

Space of genotypes: $\mathcal{I} = \{I_1, I_2, I_3, I_4, \ldots, I_N\}$; Hamming metric

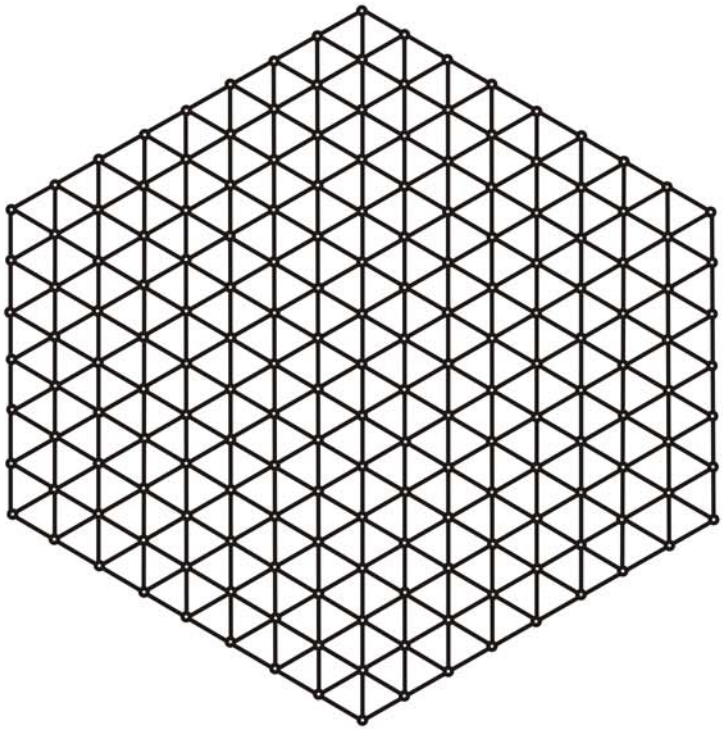
Space of phenotypes: $S = \{S_1, S_2, S_3, S_4, \ldots, S_M\}$; metric (not required)

$$N >> M$$

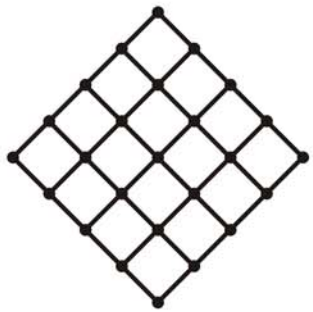
$$\psi(I_j) = S_k$$

$$\mathbf{G}_k = \psi^{-1}(S_k) \cup \{ I_j \mid \psi(I_j) = S_k \}$$

A mapping ψ and its inversion

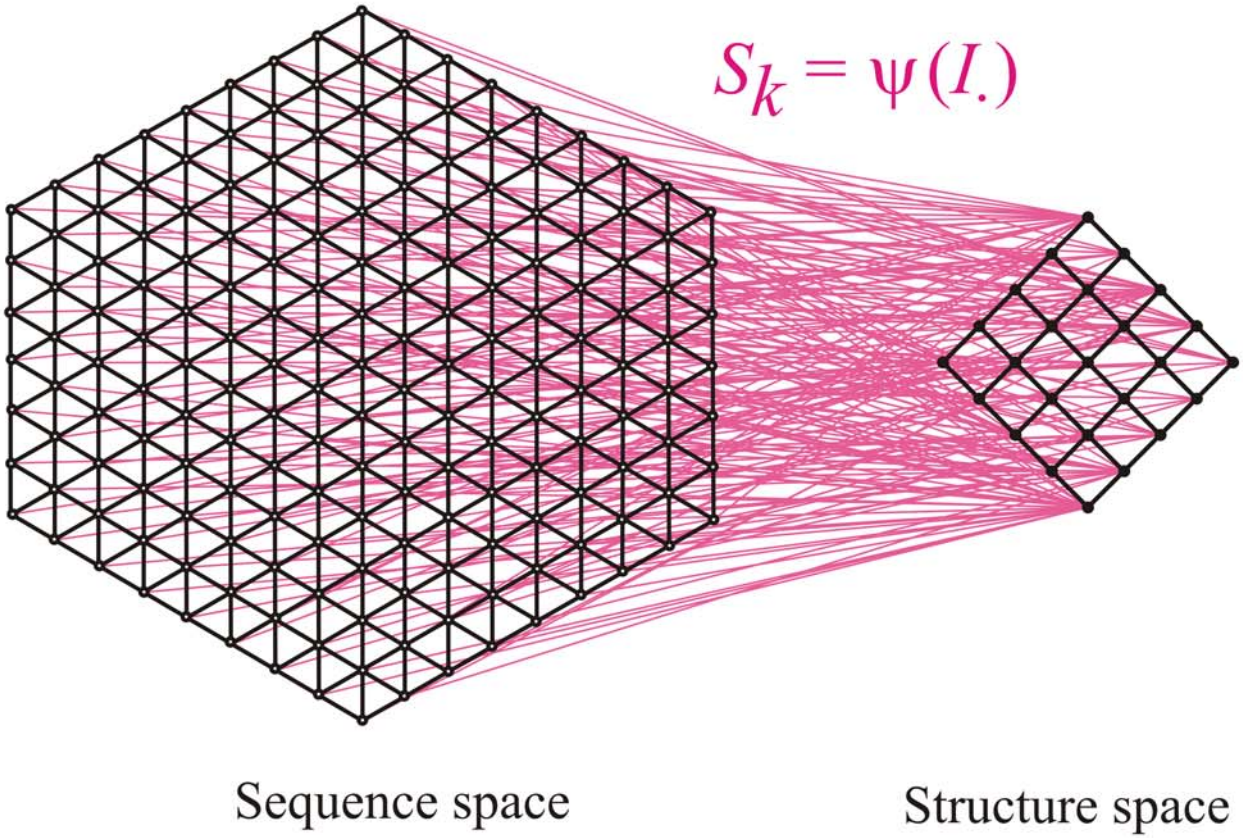


Sequence space

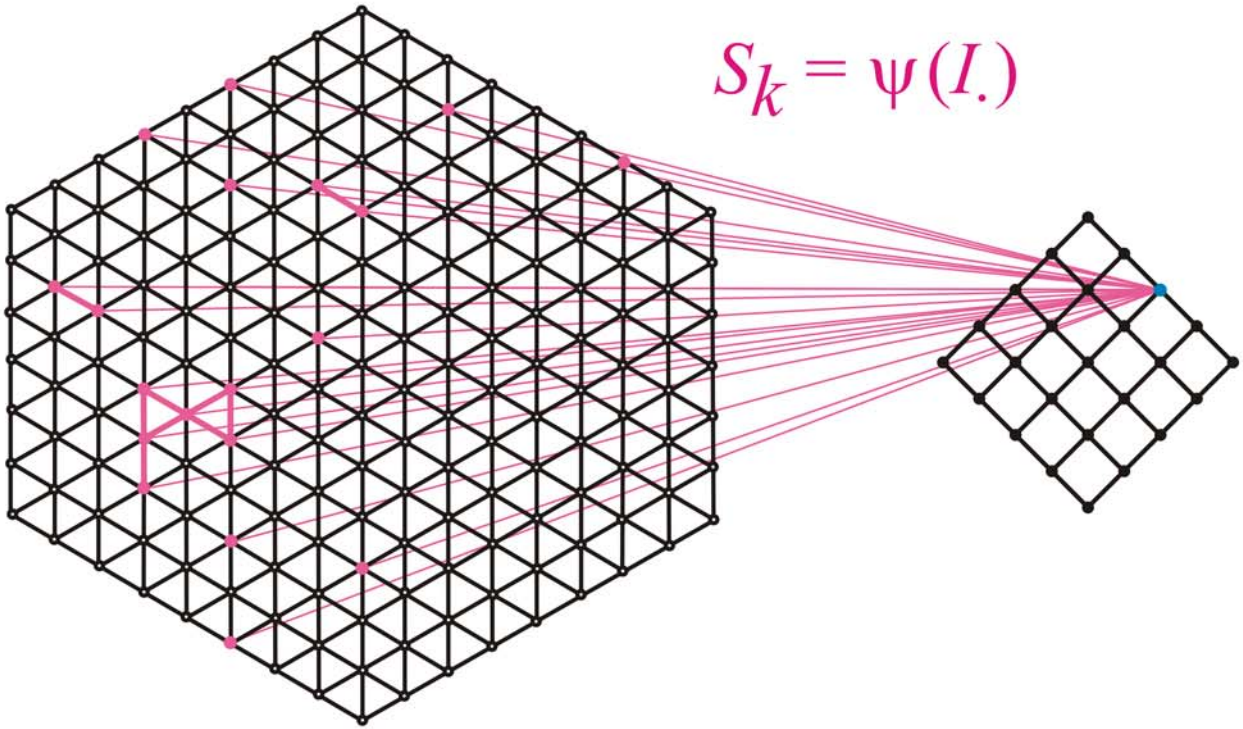


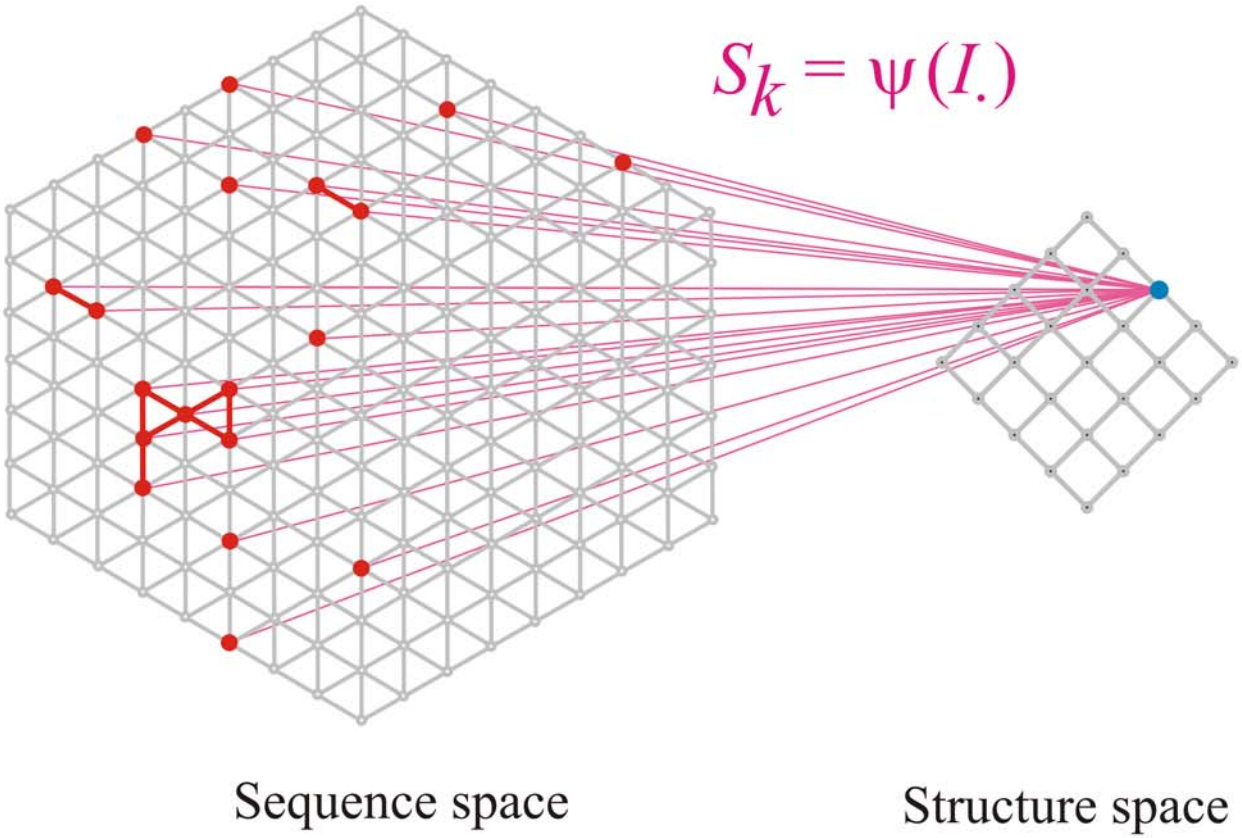
Structure space

Sequence space and structure space

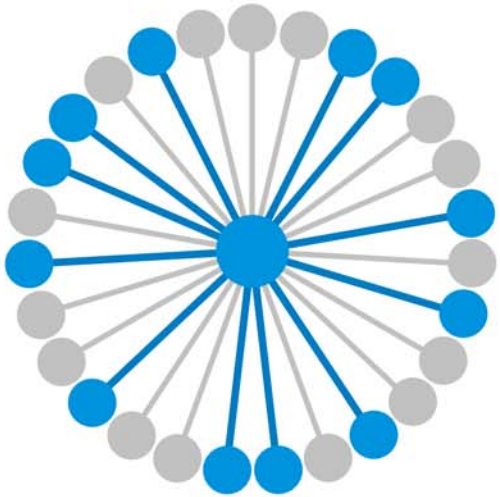


Mapping from sequence space into structure space


$S_k = \psi(I_.)$
Sequence space
Structure space



The pre-image of the structure S_k in sequence space is the **neutral network G_k**



$$G_k = \psi^{-1}(S_k) \doteq \{ I_j \mid \psi(I_j) = S_k \}$$

$$\bar{\lambda}_k = \frac{\sum_{j \in |G_k|} \lambda_j(k)}{|G_k|}$$

$\lambda_j = 12 / 27 = 0.444$

Alphabet size κ :

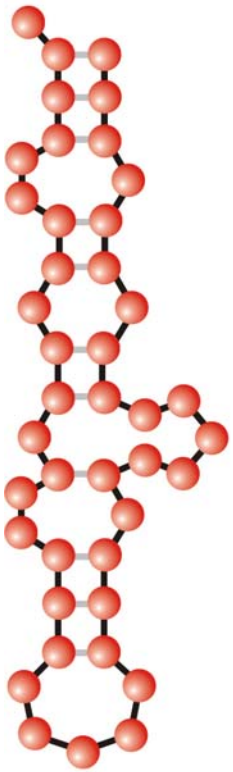
κ	λ_{cr}	
2	0.5	**AU,GC,DU**
3	0.423	**AUG , UGC**
4	0.370	**AUGC**

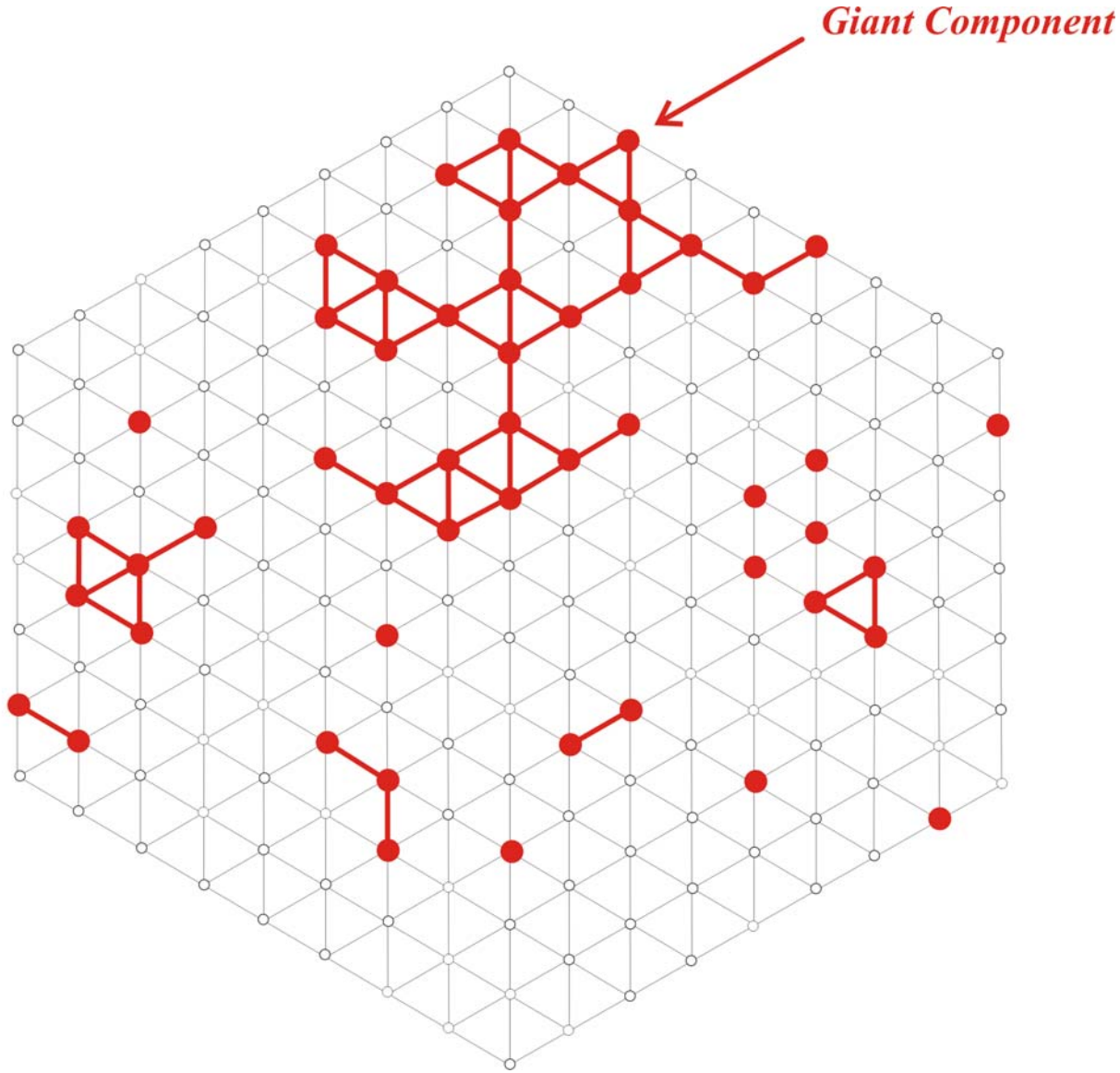
$\bar{\lambda}_k > \lambda_{cr}$ .... network G_k is connected

$\bar{\lambda}_k < \lambda_{cr}$ .... network G_k is **not** connected

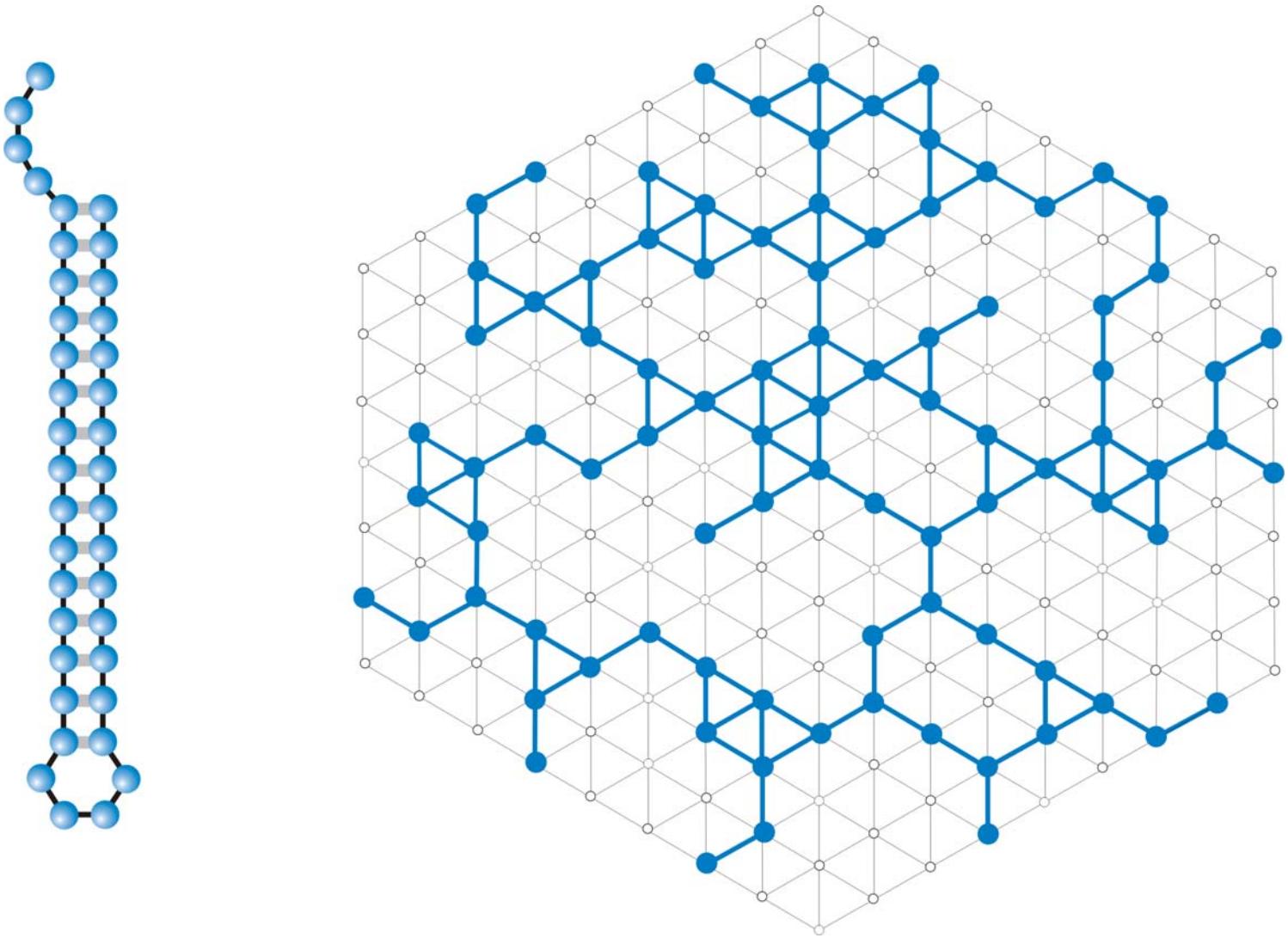
Connectivity threshold: $\lambda_{cr} = 1 - \kappa^{-1/(\kappa-1)}$

Degree of neutrality of neutral networks and the connectivity threshold

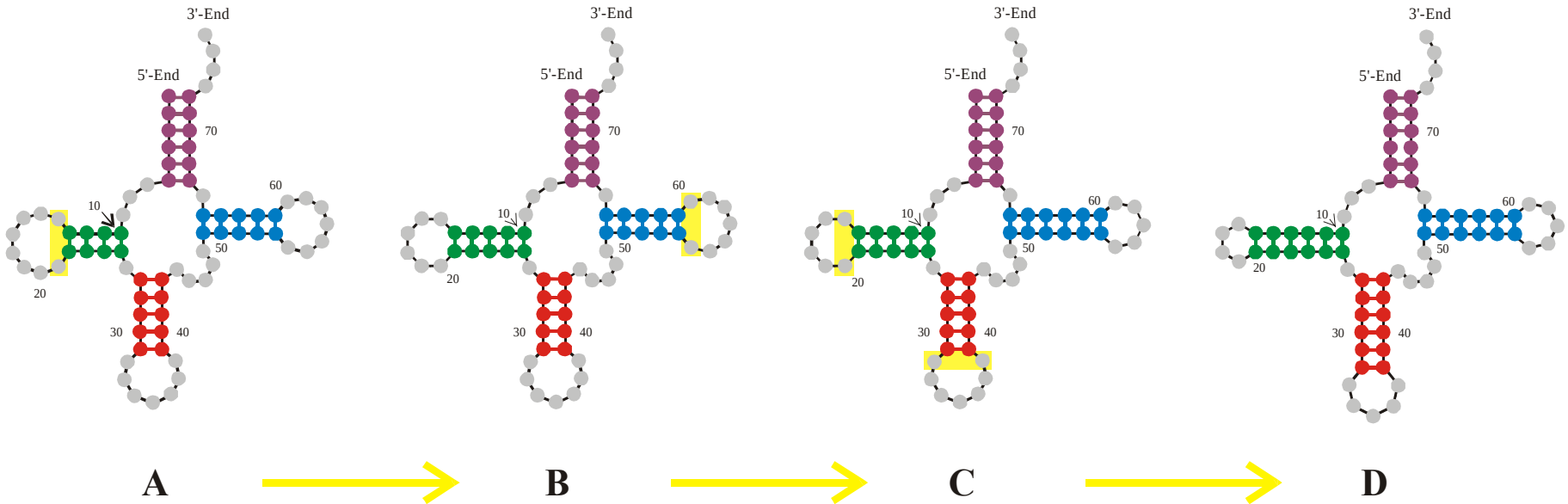




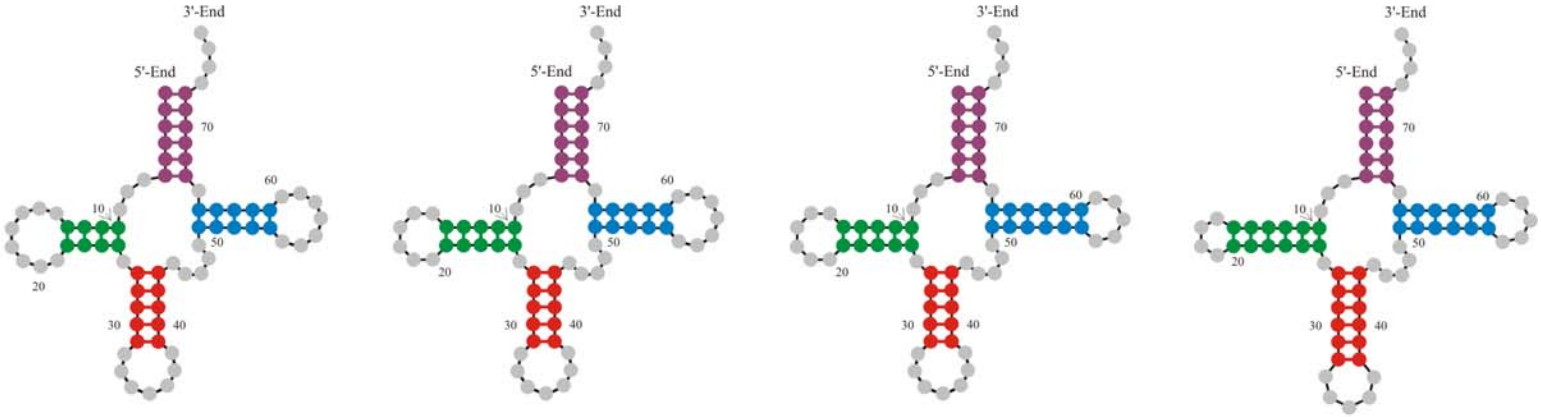
A multi-component neutral network formed by a rare structure: $\lambda < \lambda_{cr}$



A connected neutral network formed by a common structure: $\lambda > \lambda_{cr}$



RNA clover-leaf secondary structures of sequences with chain length n=76



Alphabet	Degree of neutrality $\bar{\lambda}$			
AU	- -	- -	- -	0.073 ± 0.032
AUG	- -	0.217 ± 0.051	0.207 ± 0.055	0.201 ± 0.056
AUGC	0.275 ± 0.064	0.279 ± 0.063	0.289 ± 0.062	0.313 ± 0.058
UGC	0.263 ± 0.071	0.257 ± 0.070	0.251 ± 0.068	0.250 ± 0.064
GC	0.052 ± 0.033	0.057 ± 0.034	0.060 ± 0.033	0.068 ± 0.034

Degree of neutrality of cloverleaf RNA secondary structures over different alphabets

From sequences to shapes and back: a case study in RNA secondary structures

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[3] *Santa Fe Institute, Santa Fe, U.S.A.*

SUMMARY

RNA folding is viewed here as a map assigning secondary structures to sequences. At fixed chain length the number of sequences far exceeds the number of structures. Frequencies of structures are highly non-uniform and follow a generalized form of Zipf's law: we find relatively few common and many rare ones. By using an algorithm for inverse folding, we show that sequences sharing the same structure are distributed randomly over sequence space. All common structures can be accessed from an arbitrary sequence by a number of mutations much smaller than the chain length. The sequence space is percolated by extensive neutral networks connecting nearest neighbours folding into identical structures. Implications for evolutionary adaptation and for applied molecular evolution are evident: finding a particular structure by mutation and selection is much simpler than expected and, even if catalytic activity should turn out to be sparse in the space of RNA structures, it can hardly be missed by evolutionary processes.

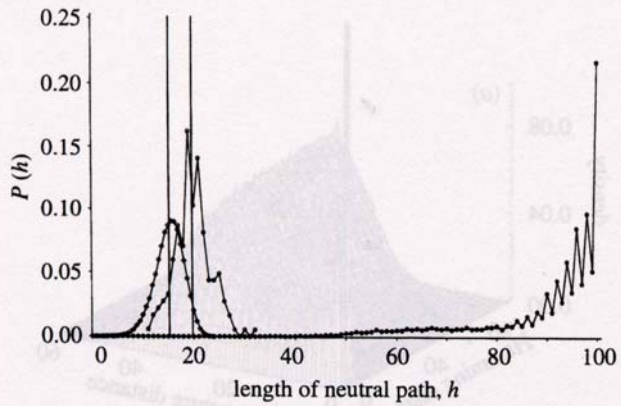

Figure 4. Neutral paths. A neutral path is defined by a series of nearest neighbour sequences that fold into identical structures. Two classes of nearest neighbours are admitted: neighbours of Hamming distance 1, which are obtained by single base exchanges in unpaired stretches of the structure, and neighbours of Hamming distance 2, resulting from base pair exchanges in stacks. Two probability densities of Hamming distances are shown that were obtained by searching for neutral paths in sequence space: (i) an upper bound for the closest approach of trial and target sequences (open circles) obtained as endpoints of neutral paths approaching the target from a random trial sequence (185 targets and 100 trials for each were used); (ii) a lower bound for the closest approach of trial and target sequences (open diamonds) derived from secondary structure statistics (Fontana *et al.* 1993*a*; see this paper, §4); and (iii) longest distances between the reference and the endpoints of monotonously diverging neutral paths (filled circles) (500 reference sequences were used).

Proc. R. Soc. Lond. B (1994) **255**, 279–284
Printed in Great Britain

Reference for postulation and ***in silico*** verification of ***neutral networks***

Evolution of aptamers with a new specificity and new secondary structures from an ATP aptamer

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ABSTRACT

Small changes in target specificity can sometimes be achieved, without changing aptamer structure, through mutation of a few bases. Larger changes in target geometry or chemistry may require more radical changes in an aptamer. In the latter case, it is unknown whether structural and functional solutions can still be found in the region of sequence space close to the original aptamer. To investigate these questions, we designed an in vitro selection experiment aimed at evolving specificity of an ATP aptamer. The ATP aptamer makes contacts with both the nucleobase and the sugar. We used an affinity matrix in which GTP was immobilized through the sugar, thus requiring extensive changes in or loss of sugar contact, as well as changes in recognition of the nucleobase. After just five rounds of selection, the pool was dominated by new aptamers falling into three major classes, each with secondary structures distinct from that of the ATP aptamer. The average sequence identity between the original aptamer and new aptamers is 76%. Most of the mutations appear to play roles either in disrupting the original secondary structure or in forming the new secondary structure or the new recognition loops. Our results show that there are novel structures that recognize a significantly different ligand in the region of sequence space close to the ATP aptamer. These examples of the emergence of novel functions and structures from an RNA molecule with a defined specificity and fold provide a new perspective on the evolutionary flexibility and adaptability of RNA.

Keywords: Aptamer; specificity; fold; selection; RNA evolution

RNA **9**:1456-1463, 2003

Evidence for neutral networks and shape space covering

Evidence for neutral networks and intersection of apatamer functions

J Mol Evol (2003) 57:299–308
DOI: 10.1007/s00239-003-2481-y

JOURNAL OF MOLECULAR EVOLUTION

Evolutionary Landscapes for the Acquisition of New Ligand Recognition by RNA Aptamers

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Received: 15 November 2002 / Accepted: 8 April 2003

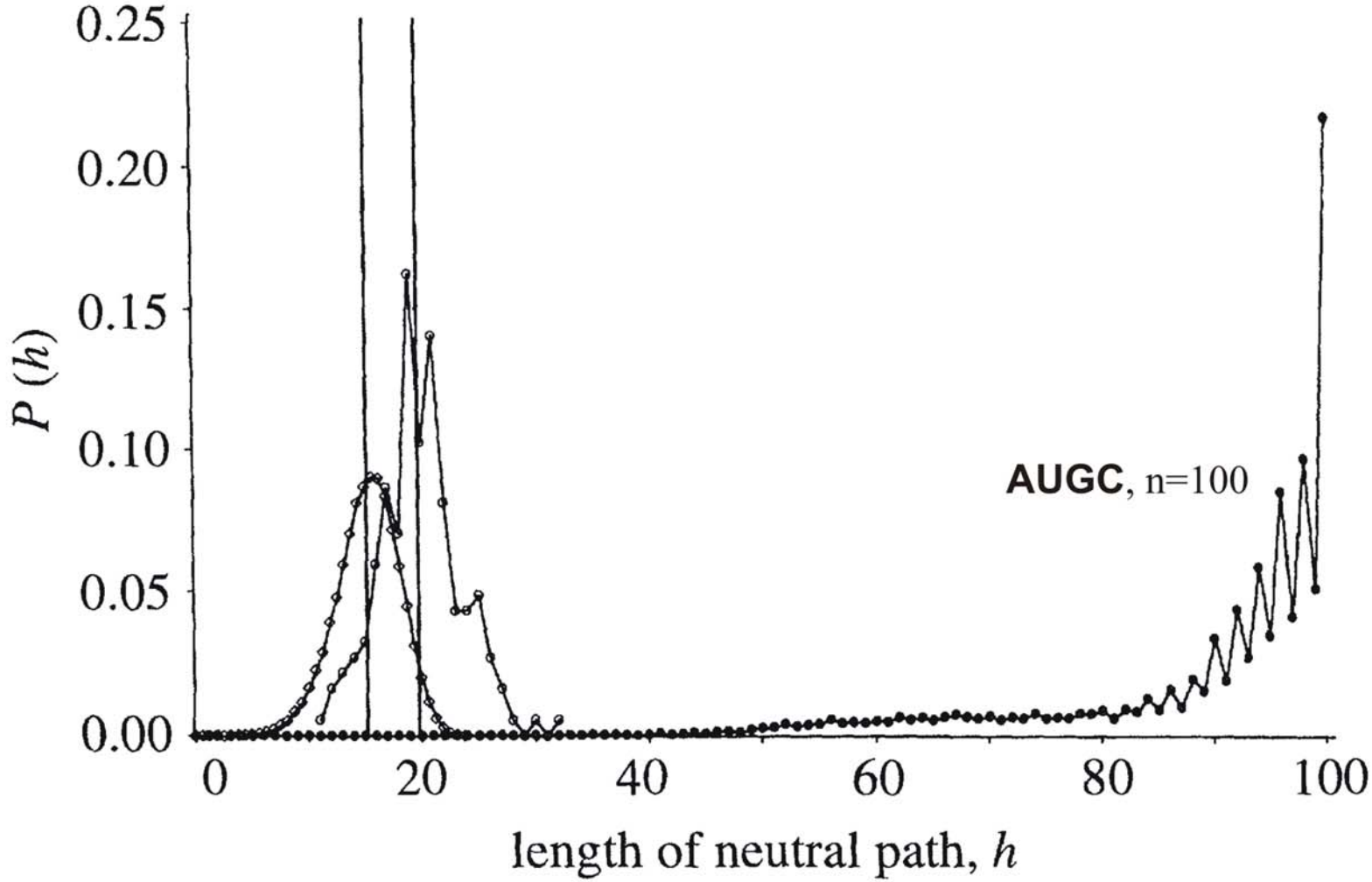
Abstract. The evolution of ligand specificity underlies many important problems in biology, from the appearance of drug resistant pathogens to the re-engineering of substrate specificity in enzymes. In studying biomolecules, however, the contributions of macromolecular sequence to binding specificity can be obscured by other selection pressures critical to bioactivity. Evolution of ligand specificity *in vitro*—unconstrained by confounding biological factors—is addressed here using variants of three flavin-binding RNA aptamers. Mutagenized pools based on the three aptamers were combined and allowed to compete during *in vitro* selection for GMP-binding activity. The sequences of the resulting selection isolates were diverse, even though most were derived from the same flavin-binding parent. Individual GMP aptamers differed from the parental flavin aptamers by 7 to 26 mutations (20 to 57% overall change). Acquisition of GMP recognition coincided with the loss of FAD (flavin-adenine dinucleotide) recognition in all isolates, despite the absence of a counter-selection to remove FAD-binding RNAs. To examine more precisely the proximity of these two activities within a defined sequence space, the complete set of all intermediate sequences between an FAD-binding aptamer and a GMP-binding aptamer were synthesized and assayed for activity. For this set of sequences, we observe a portion of a neutral network for FAD-binding function separated from GMP-binding function by a distance of three mutations. Furthermore, enzymatic probing of these aptamers revealed gross structural remodeling of the RNA coincident with the switch in ligand recognition. The capacity for neutral drift along an FAD-binding network in such close approach to RNAs with GMP-binding activity illustrates the degree of phenotypic buffering available to a set of closely related RNA sequences—defined as the set's functional tolerance for point mutations—and supports neutral evolutionary theory by demonstrating the facility with which a new phenotype becomes accessible as that buffering threshold is crossed.

Key words: Aptamers — RNA structure — Phenotypic buffering — Fitness landscapes — Neutral evolutionary theory — Flavin — GMP

Correspondence to: Donald H. Burke; *email:* dhburke@indiana.edu

Introduction

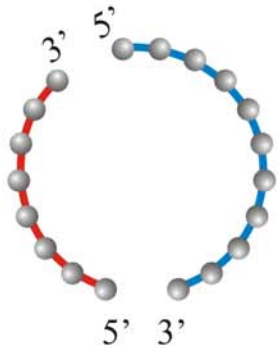
RNA aptamers targeting small molecules serve as useful model systems for the study of the evolution and biophysics of macromolecular binding interactions. Because of their small sizes, the structures of several such complexes have been determined to atomic resolution by NMR spectrometry or X-ray crystallography (reviewed by Herman and Patel 2000). Moreover, aptamers can be subjected to mutational and evolutionary pressures for which survival is based entirely on ligand binding, without the complicating effects of simultaneous selection pressures for bioactivity, thus allowing the relative contributions of each activity to be evaluated separately.



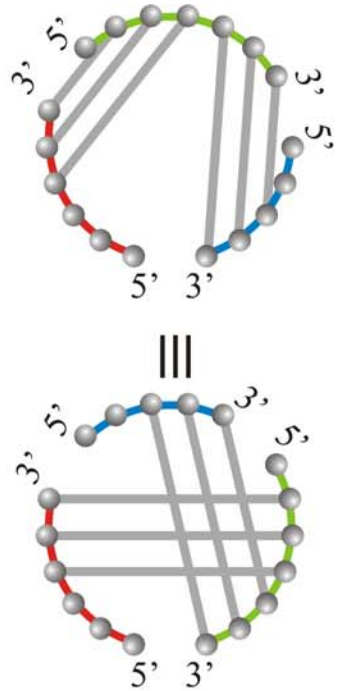
P. Schuster, W. Fontana, P.F. Stadler, I.L. Hofacker. 1994
Proc.Roy.Soc. London **B 255**:279-284.

Circular RNA

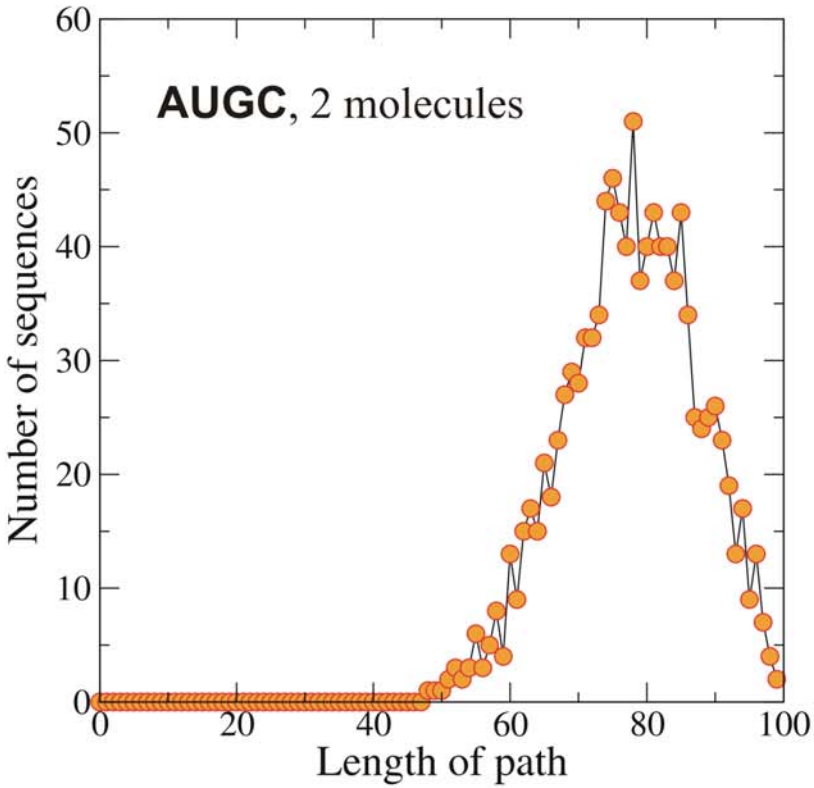
One RNA molecule

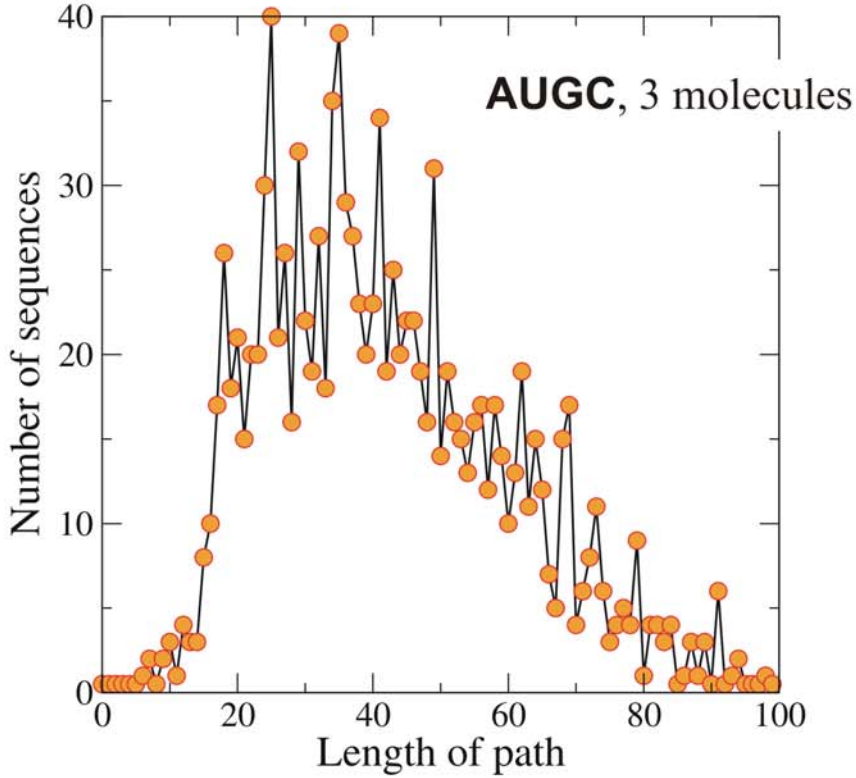


Two RNA molecules



Three RNA molecules





Total chain length: n=100 ; cofolding with one or two fixed sequences

C. Stephan-Otto Attolini, P.F. Stadler, 2005.
Adv.Complex Syst., in press.

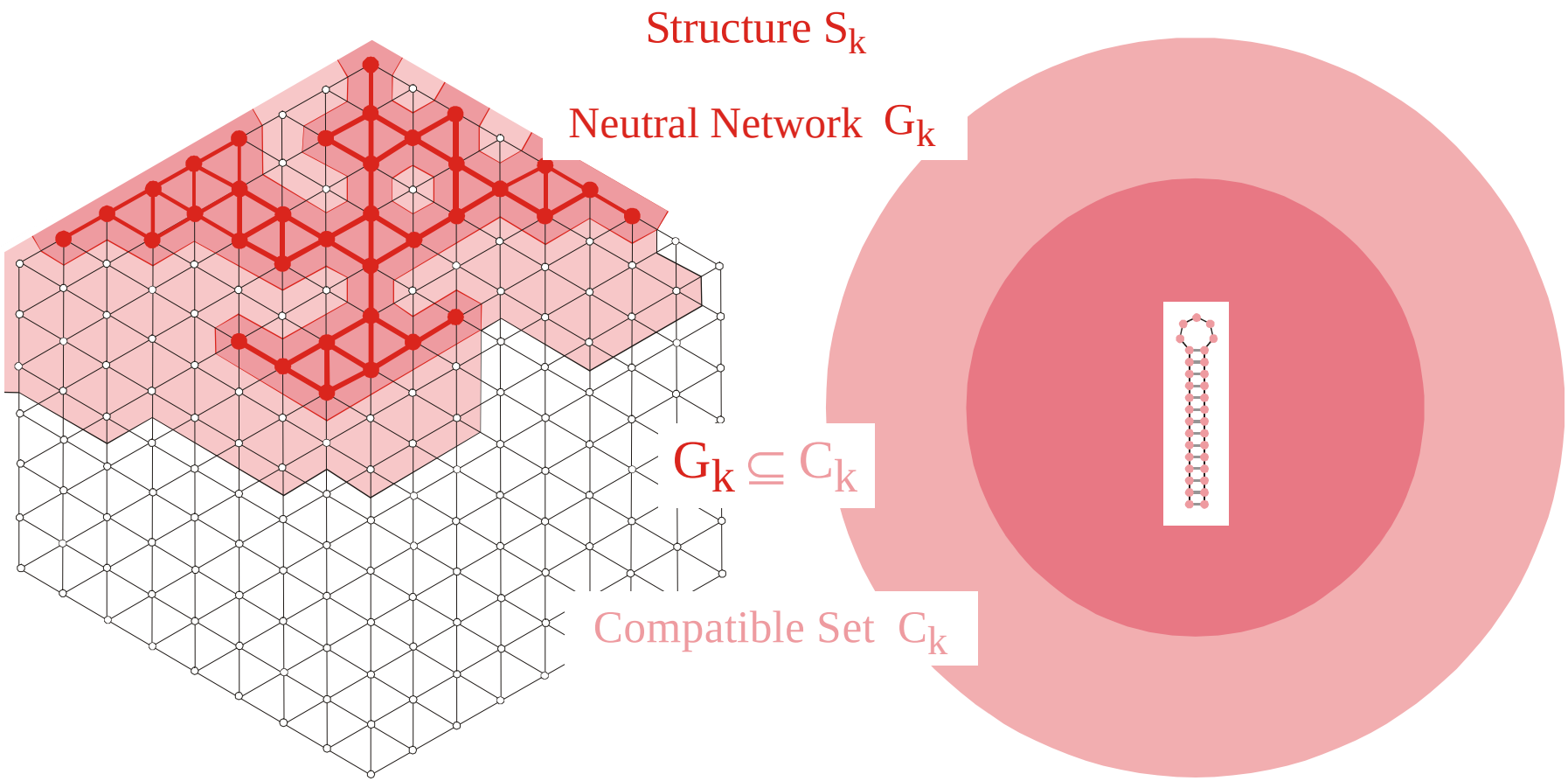
tRNA clover leafs with increasing stack lengths (1→4), n = 76

Alphabet	Clover leaf 1	Clover leaf 2	Clover leaf 3	Clover leaf 4
AU	--	--	--	0.07
AUG	--	0.22	0.21	0.20
AUGC	**0.28**	**0.28**	**0.29**	**0.31**
UGC	0.26	0.26	0.25	0.25
GC	0.05	0.06	0.06	0.07

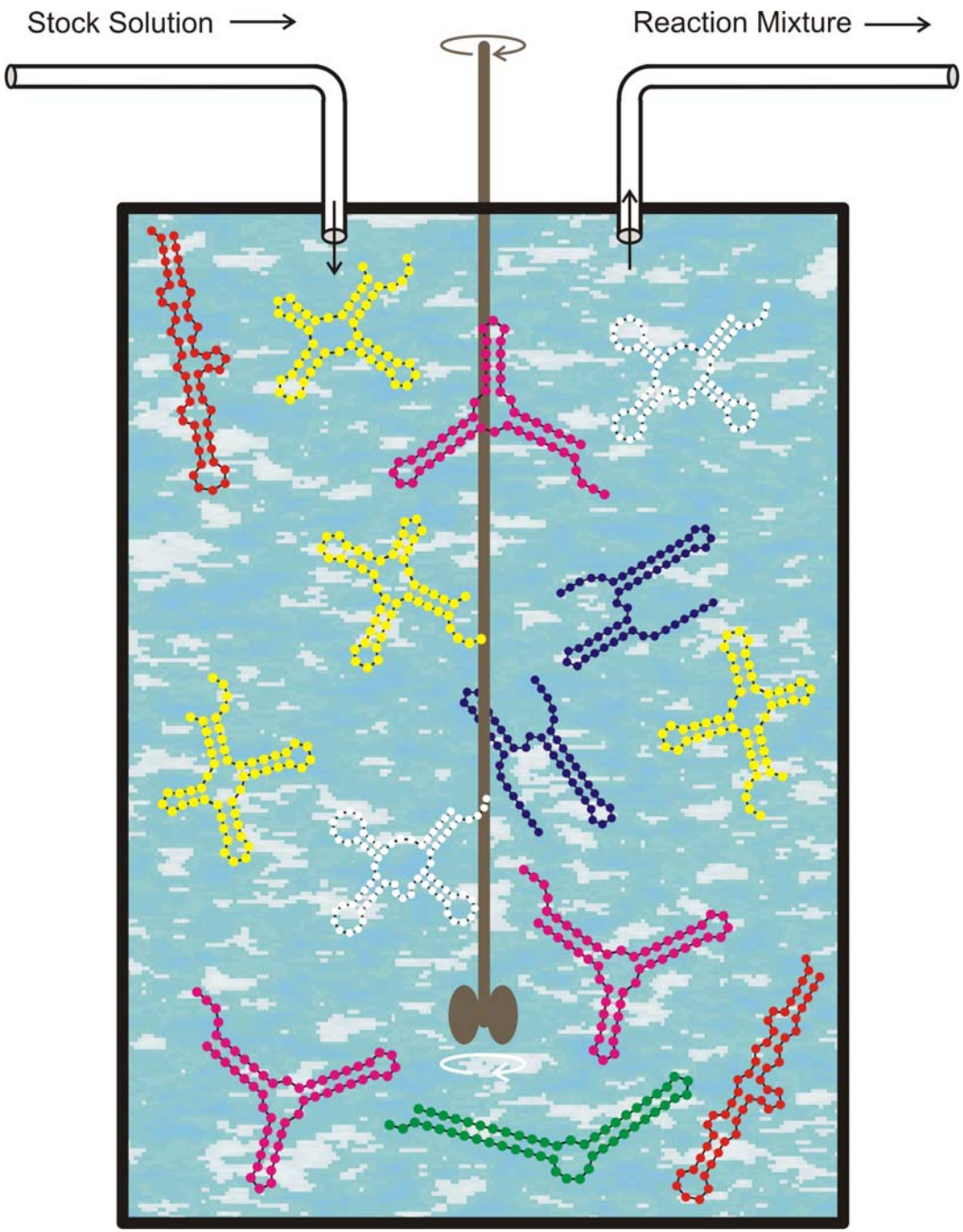
AUGC, n = 100

	Degree of neutrality λ	Mean length of path *h*
Unconstrained fold	0.33	> 95
Cofold with one sequence	0.32	75
Cofold with two sequences	0.18	40

Degree of neutrality and lengths of neutral path



The **compatible set C_k** of a structure **S_k** consists of all sequences which form **S_k** as its minimum free energy structure (the neutral network **G_k**) or one of its suboptimal structures.



Replication rate constant:

$$f_k = \gamma / [\alpha + \Delta d_S^{(k)}]$$

$$\Delta d_S^{(k)} = d_H(S_k, S_\tau)$$

Selection constraint:

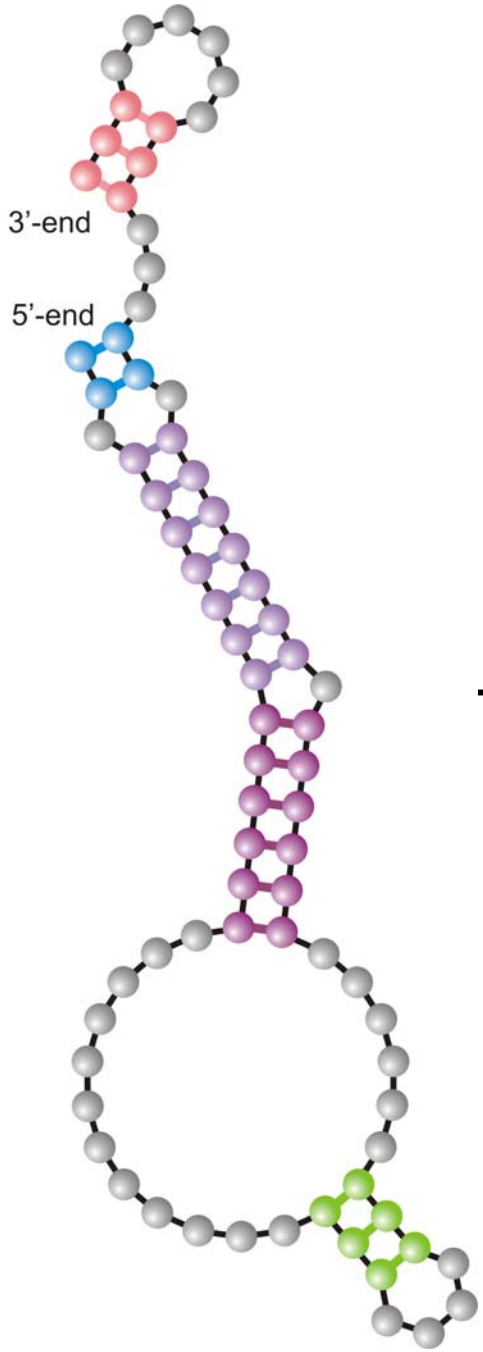
Population size, N = # RNA molecules, is controlled by the flow

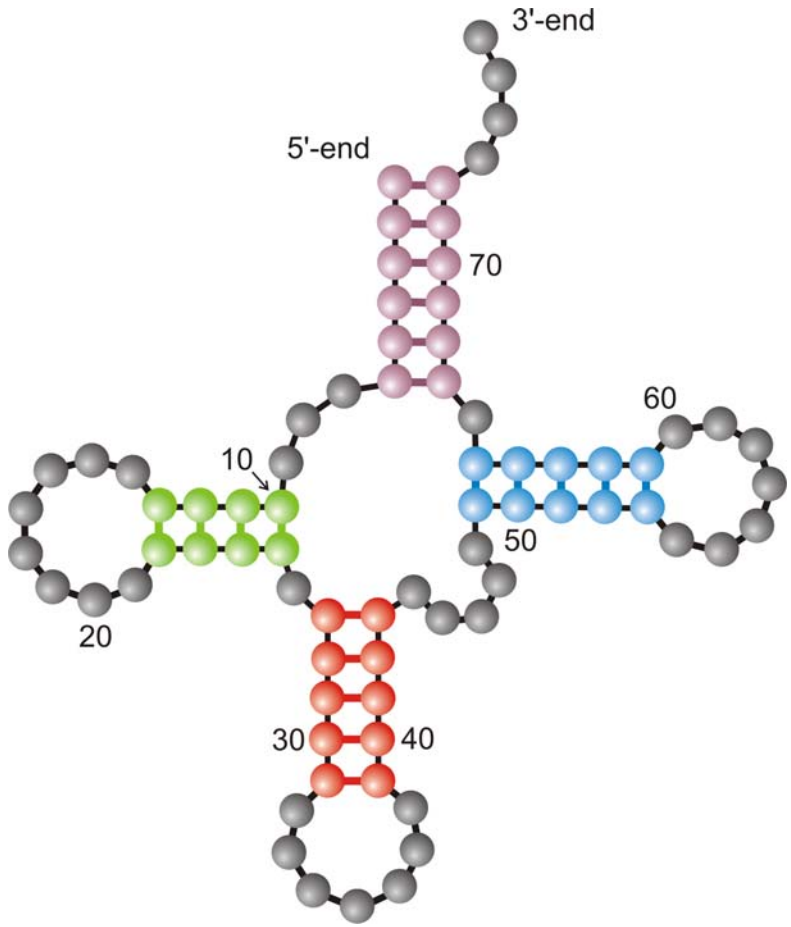
$$N(t) \approx \overline{N} \pm \sqrt{\overline{N}}$$

Mutation rate:

p = 0.001 / site × replication

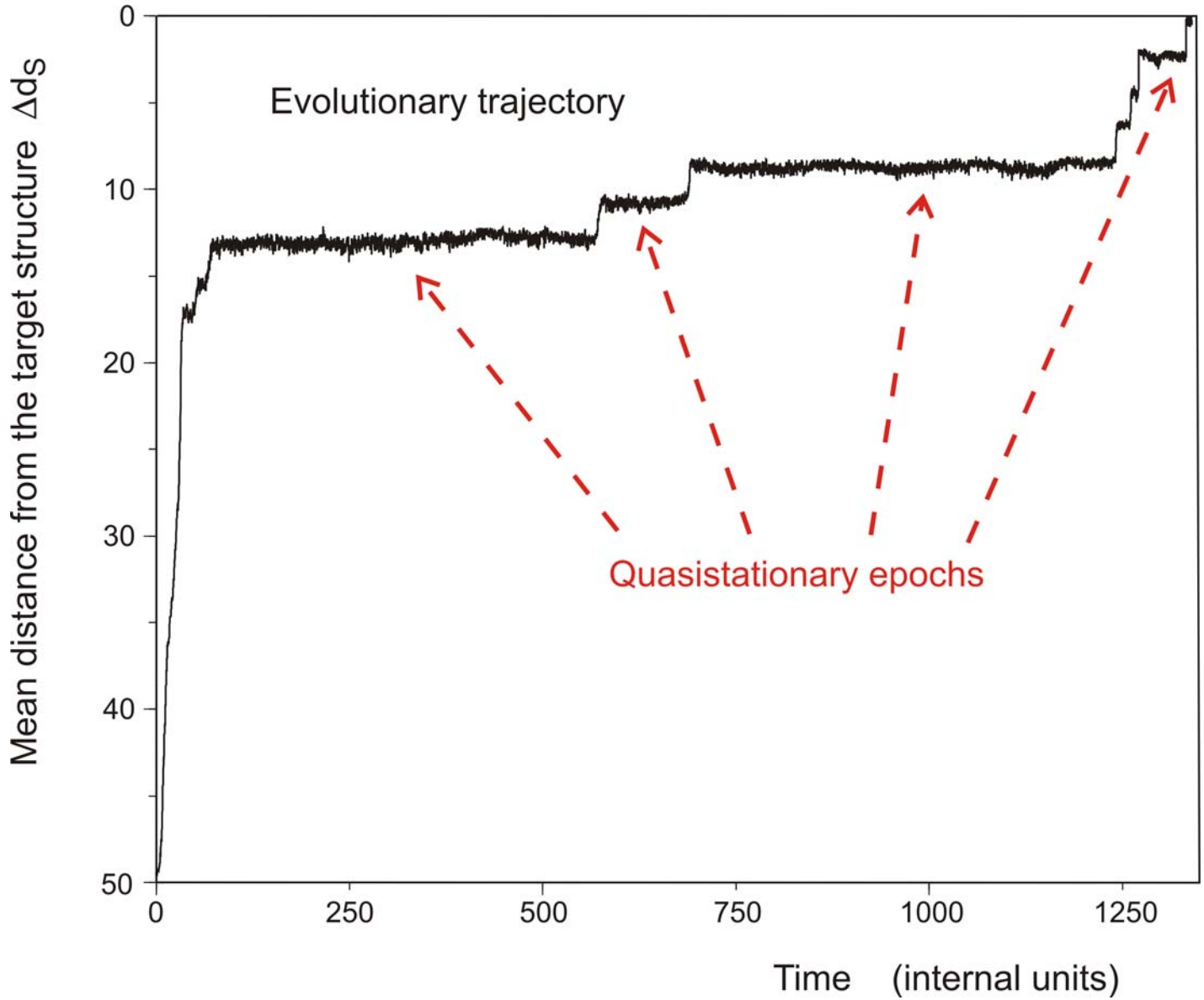
The flowreactor as a device for **studies** of evolution ***in vitro*** and ***in silico***





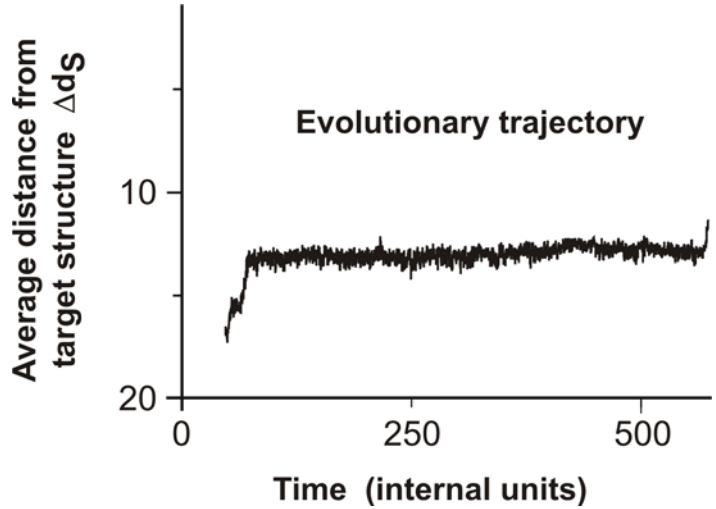
Randomly chosen initial structure

Phenylalanyl-tRNA as target structure



In silico optimization in the flow reactor: Evolutionary Trajectory

28 neutral point mutations during a long quasi-stationary epoch




```
entry   GGUAUGGGCGUUGAAUAGUAGGGUUUAAACCAAUCGGCCAACGAUCUCGUGUGCGCAUUUCAUAUCCCGUACAGAA
8       .((((((((((((.........(((....)))........)))))).....((((.........))))))))))....
exit    GGUAUGGGCGUUGAAUAAUAGGGUUUAAACCAAUCGGCCAACGAUCUCGUGUGCGCAUUUCAUAUCCCAUACAGAA
entry   GGUAUGGGCGUUGAAUAAUAGGGUUUAAACCAAUCGGCCAACGAUCUCGUGUGCGCAUUUCAUAUACCAUACAGAA
9       .((((((.((((.........(((....)))........)))))).....((((.........))))).))))))....
exit    UGGAUGGACGUUGAAUAACAAGGUAUCGACCAAACAACCAACGAGUAAGUGUGUACGCCCCACACACCGUCCCAAG
entry   UGGAUGGACGUUGAAUAACAAGGUAUCGACCAAACAACCAACGAGUAAGUGUGUACGCCCCACACAGCGUCCCAAG
10      .(((((..((((.........(((....)))........)))))).....((((.........))))).)))))....
exit    UGGAUGGACGUUGAAUAACAAGGUAUCGACCAAACAACCAACGAGUAAGUGUGUACGCCCCACACAGCGUCCCAAG
```

Transition inducing point mutations change the molecular structure

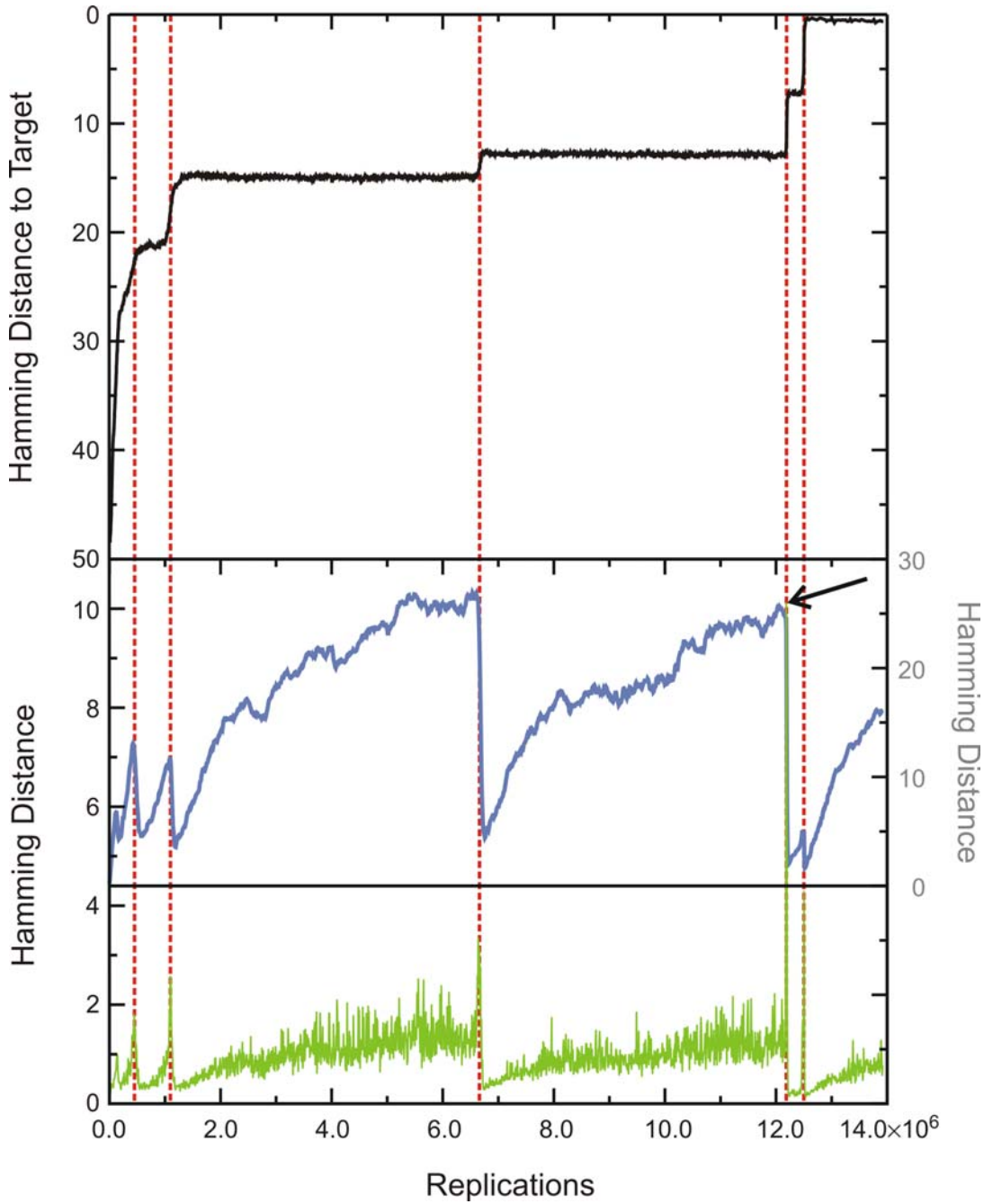
Neutral point mutations leave the molecular structure unchanged

Neutral genotype evolution during phenotypic stasis

Evolutionary trajectory

Spreading of the population on neutral networks

Drift of the population center in sequence space



Alphabet	Runtime	Transitions	Main transitions	No. of runs
AUGC	385.6	22.5	12.6	1017
GUC	448.9	30.5	16.5	611
GC	2188.3	40.0	20.6	107

Mean population size: $N = 3000$; mutation rate: $p = 0.001$

Statistics of trajectories and relay series (mean values of log-normal distributions).

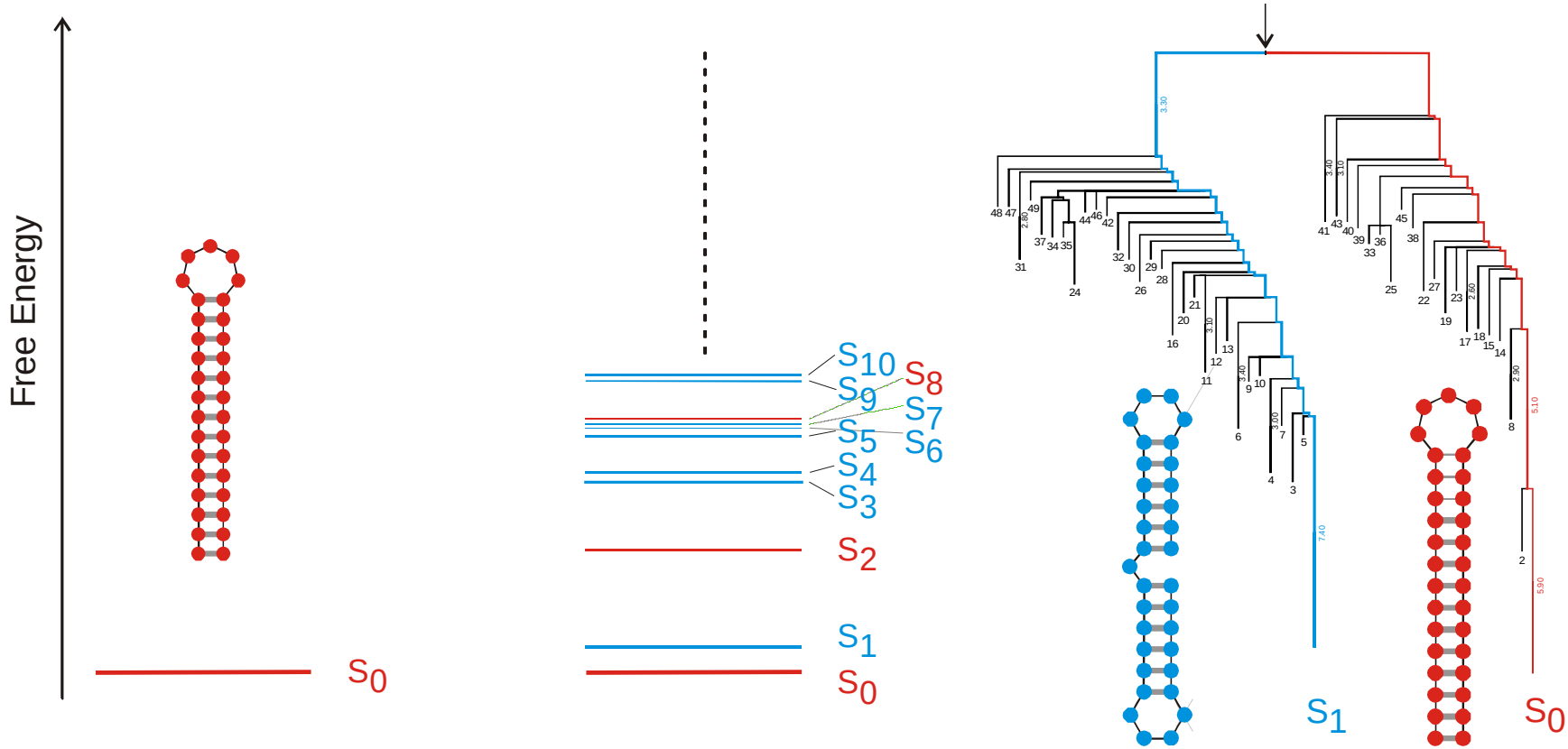
AUGC neutral networks of tRNAs are near the connectivity threshold, **GC** neutral networks are way below.

1. Sequence space and shape space
2. Neutral networks and evolution
3. **Conformation space and kinetic folding**
4. What kind of analogies are there?
5. How to model evolution of kinetic folding?

One sequence - one structure

Many suboptimal structures
Partition function

Metastable structures
Conformational switches



Minimum free energy structure

Suboptimal structures

Kinetic structures

RNA secondary structures derived from a single sequence

Stickiness of sequence I: $p(I) = 2 \sum_{\beta_{ij} \in \mathfrak{B}} p_i(I)\, p_j(I)$

Size restriction of elements: (i) hairpin loop $n_{\text{loop}} \geq \lambda$

(ii) stack $n_{\text{stack}} \geq \sigma$

$$S_{m+1}(p) = \Xi_{m+1}(p) + \Phi_{m-1}(p)$$

$$\Xi_{m+1}(p) = S_m(p) + \sum_{k=\lambda+2\sigma-2}^{m-2} \Phi_k(p) \cdot S_{m-k+1}(p)$$

$$\Phi_{m+1}(p) = p \sum_{k=\sigma-1}^{\lfloor (m-\lambda+1)/2 \rfloor} p^k \cdot \Xi_{m-2k+1}(p)$$

$S_n(p) \approx$ # conformations of a sequence with chain length n and stickiness p

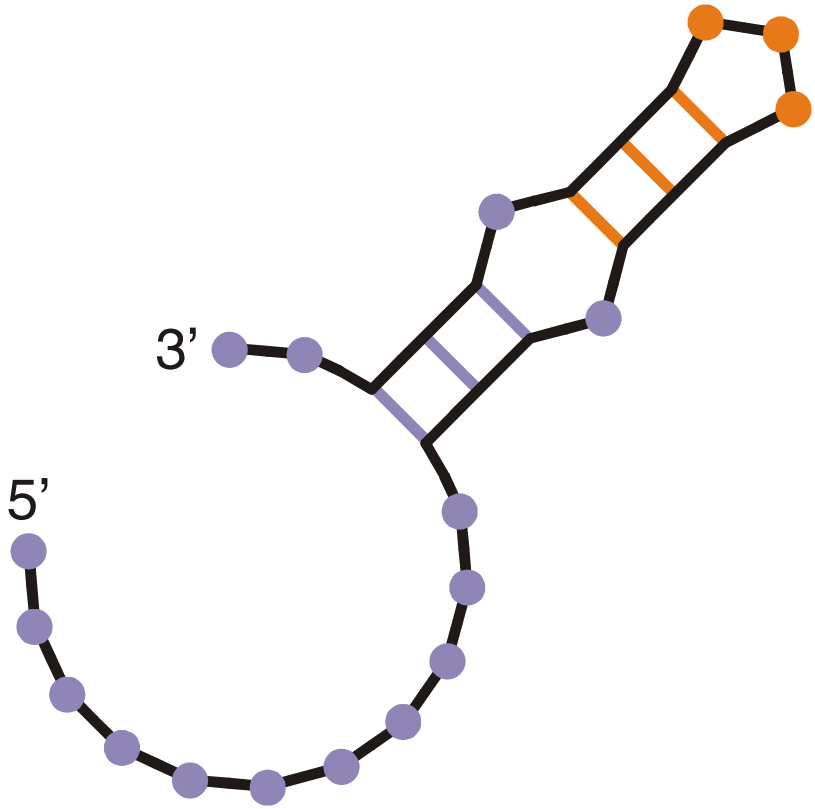
M. Tacker, P.F. Stadler, E.G. Bornberg-Bauer, I.L. Hofacker, P. Schuster. 1996. *Eur.Biophys.J.* **25**:115-130.

Total number of structures including all suboptimal conformations, stable and unstable (with $\Delta G_0 > 0$):

#conformations = **1 416 661**

Estimated number from the stickiness of the sequence:

#conformations ≈ $S_{30}(0.407) = \mathbf{1.17 \times 10^6}$



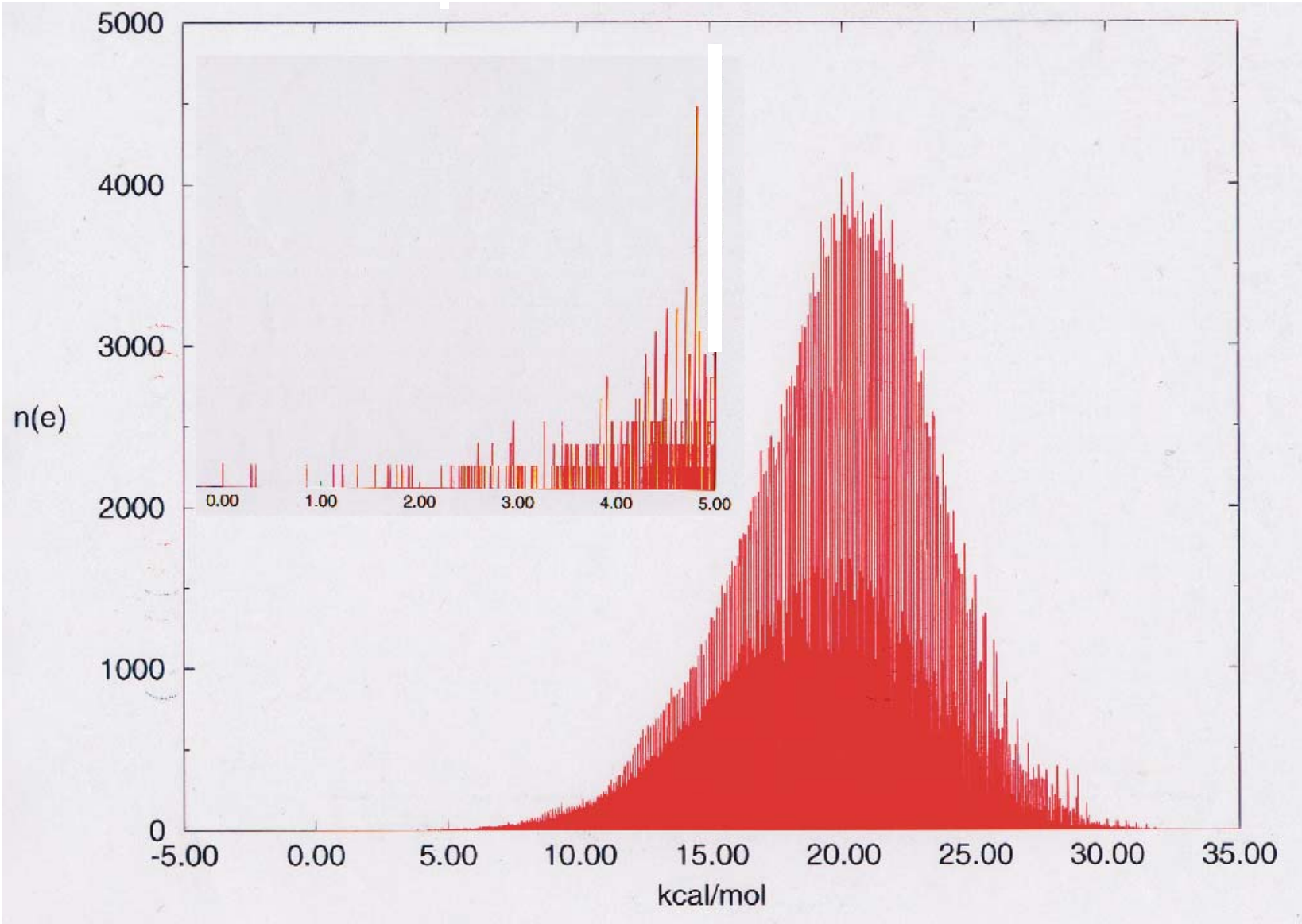
Minimum free energy structure

AAAGGGCACAGGGUGAUUUCAAUAAUUUUA

Sequence

Example of a small RNA molecule: n=30

Jan Cupal, Diploma Thesis



Density of stares of suboptimal structures of the RNA molecule with the sequence:

AAAGGGCACAGGGUGAUUUCAAUAAUUUUA

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:

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

Master equation

$$\frac{dP_k}{dt} = \sum_{i=0}^{m+1}\left(P_{ik}(t) - P_{ki}(t)\right) = \sum_{i=0}^{m+1} k_{ik} P_i - P_k \sum_{i=0}^{m+1} k_{ki}$$

$$k = 0,1,\ldots,m+1$$

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

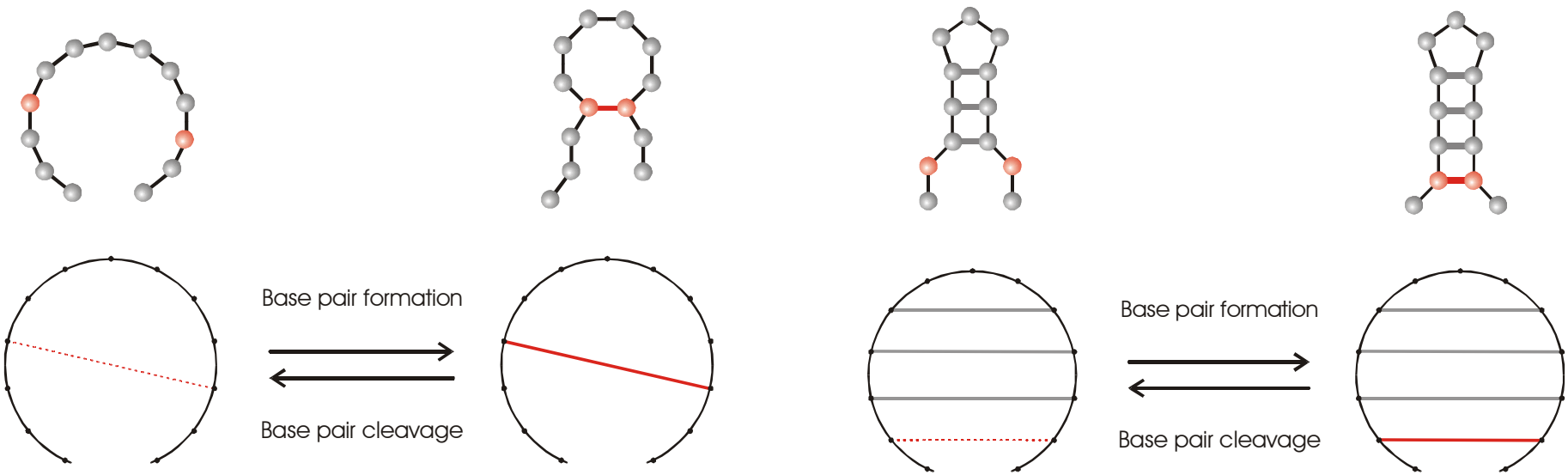
$$P_{ij}(t) = P_i(t)\, k_{ij} = P_i(t) \exp(-\Delta G_{ij}/2RT) / \Sigma_i$$

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

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

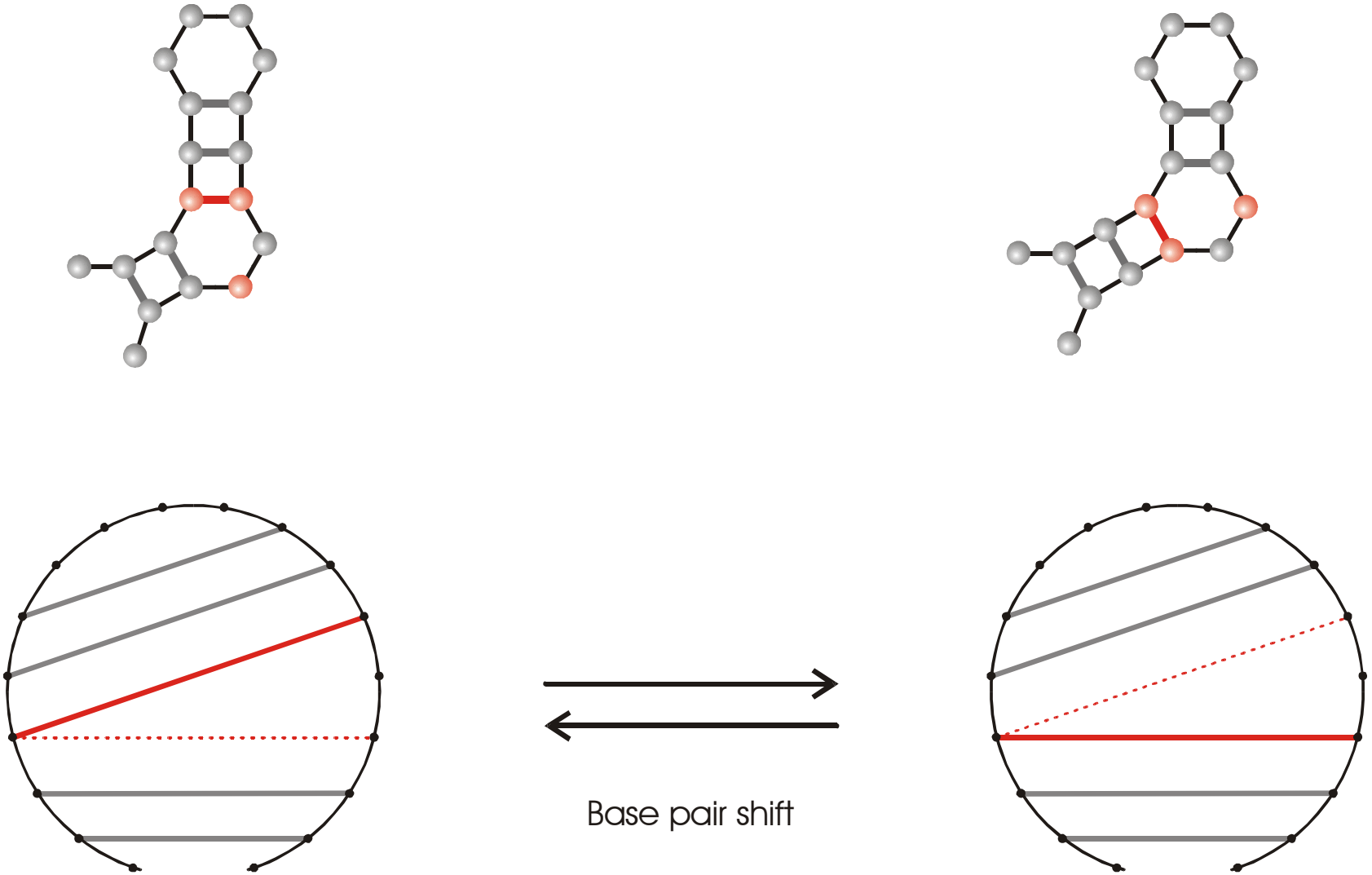
The symmetric rule for transition rate parameters is due to Kawasaki (K. Kawasaki, *Diffusion constants near the critical point for time depen-dent Ising models*. Phys.Rev. **145**:224-230, 1966).

Formulation of kinetic RNA folding as a stochastic process

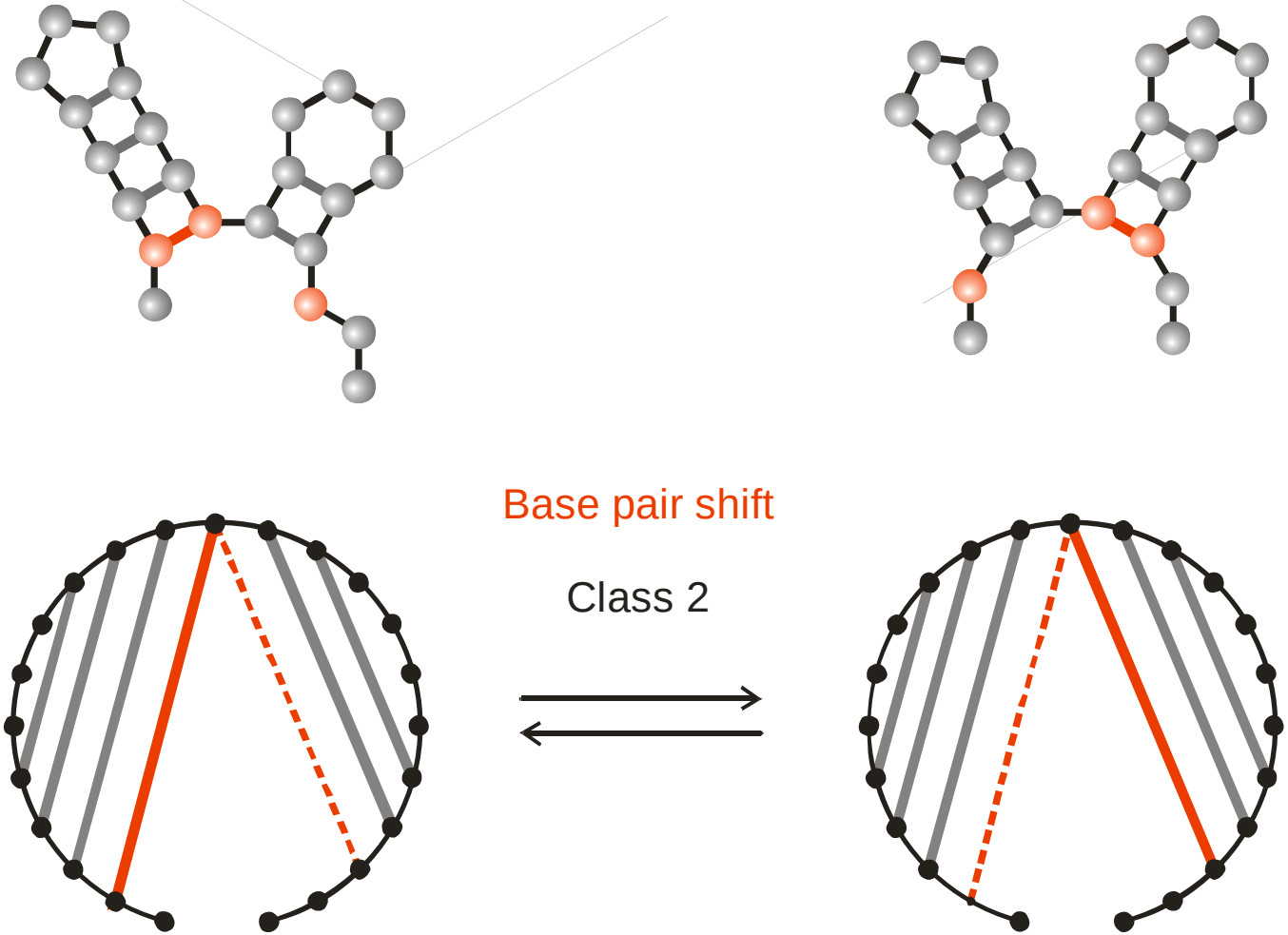


Corresponds to base pair distance: $d_p(S_1,S_2)$

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

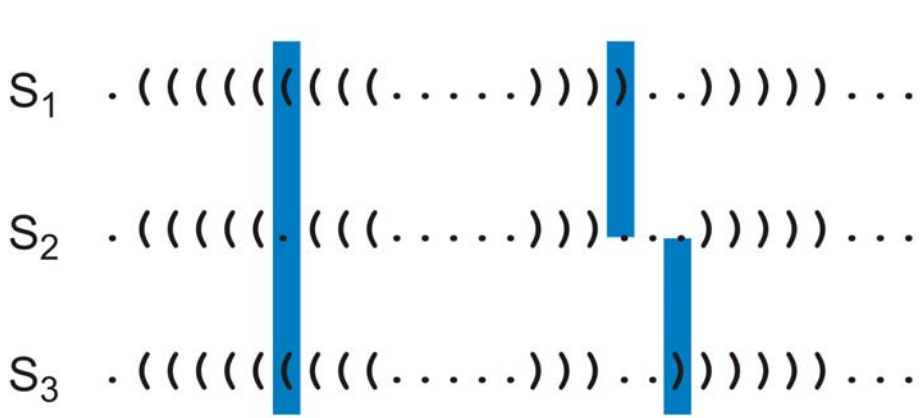


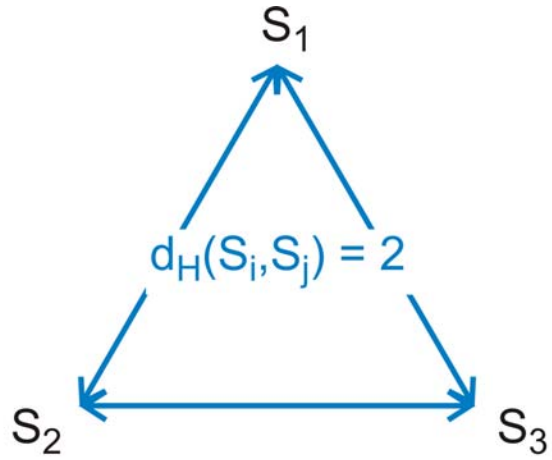
Base pair shift move of class 1: Shift inside internal loops or bulges

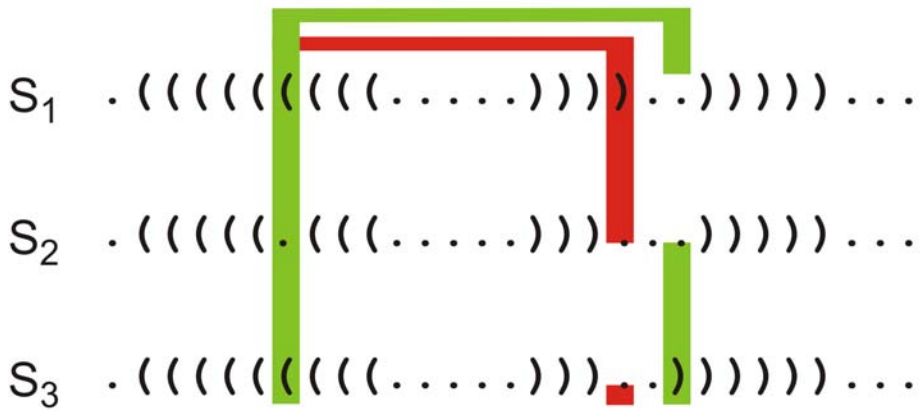


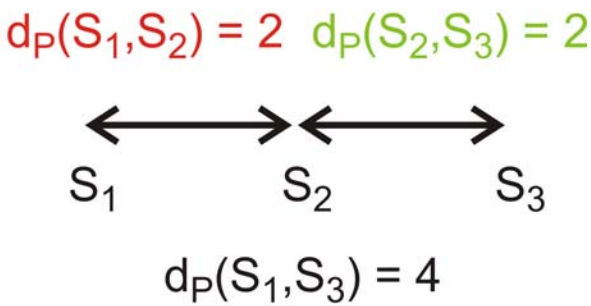
Base pair closure, opening and shift corresponds to Hamming distance: $d_H(S_1,S_2)$

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





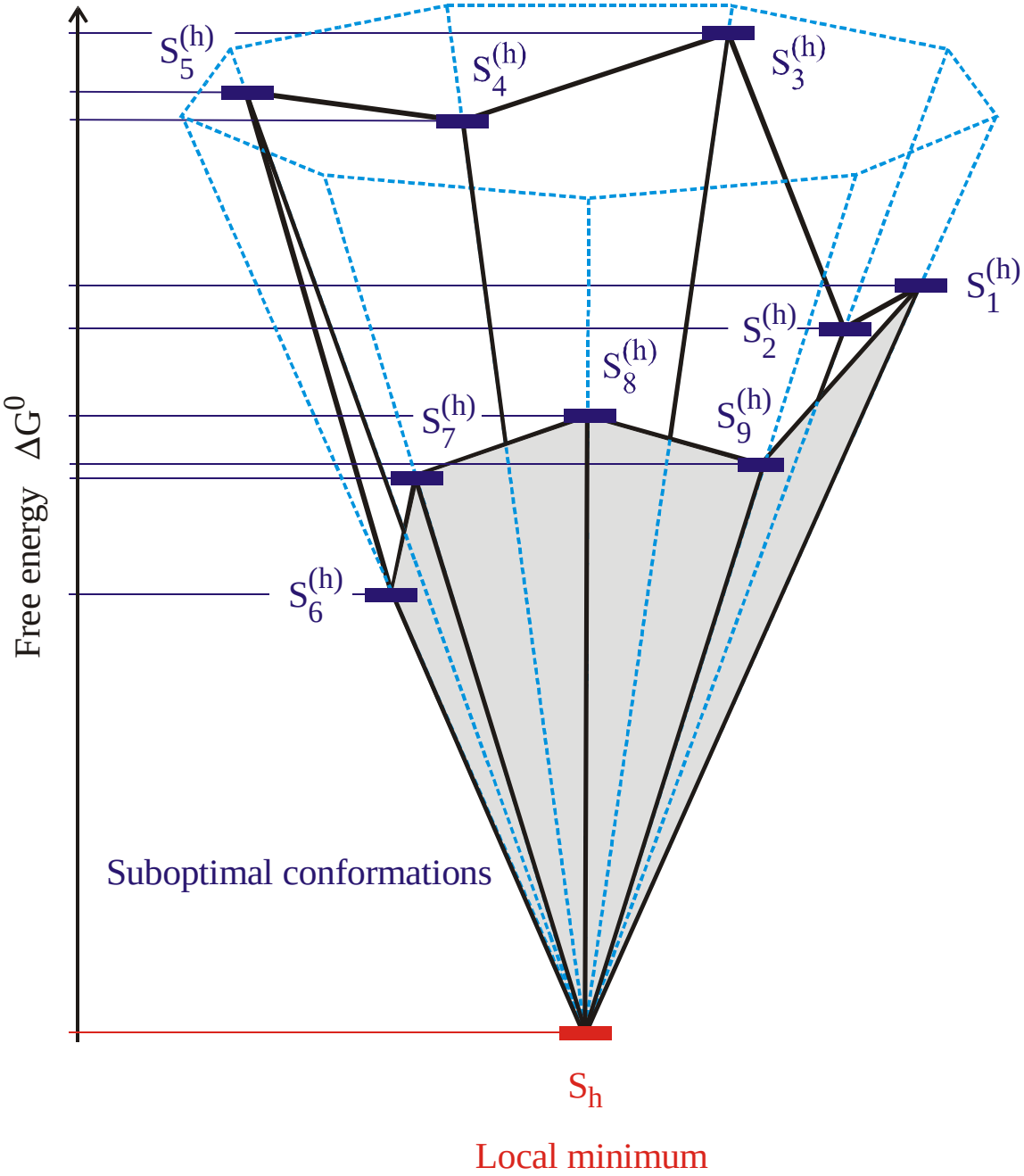


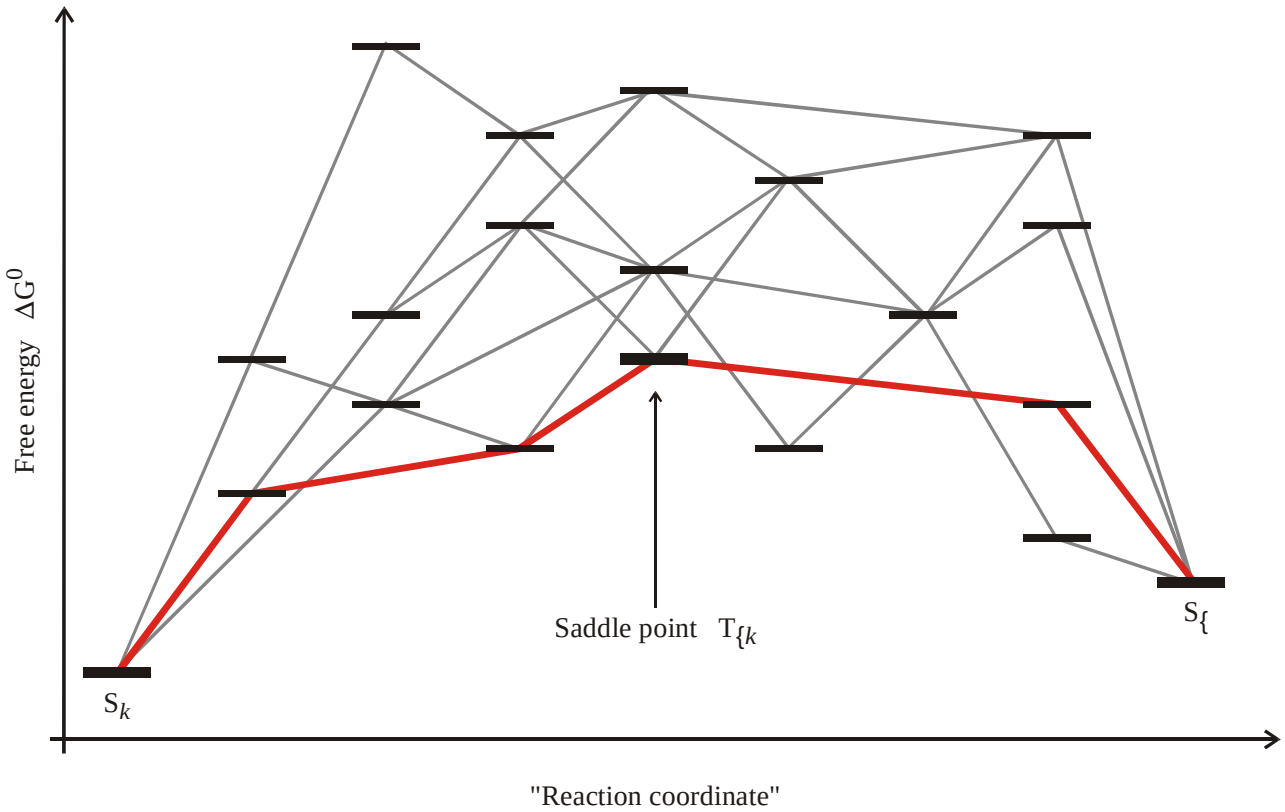


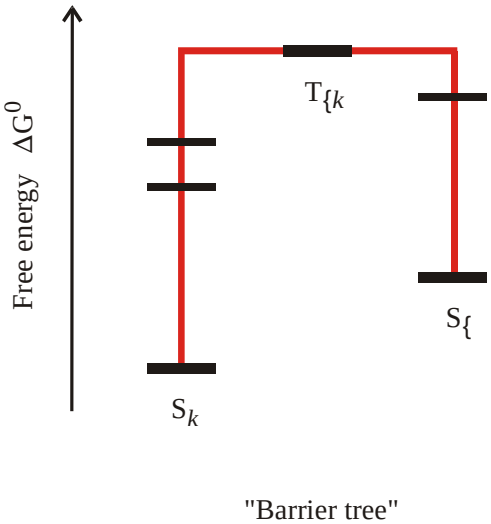
Two measures of distance in shape space:

Hamming distance between structures, $d_H(S_i,S_j)$ and base pair distance, $d_P(S_i,S_j)$

Search for local minima in conformation space

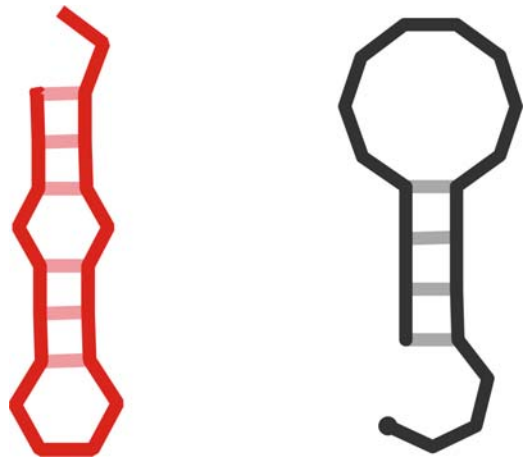






Definition of a ‚barrier tree‘

```
CUGCGGCUUUGGCUCUAGCC
....((((........))))  -4.30
(((.(((....))).)))..  -3.50
(((..((....))..))))..  -3.10
..........(((....)))  -2.80
..(((((....)))...)).  -2.20
....((.........)))  -2.20
((..(((....)))..))..  -2.00
..((.((....)).....)).  -1.60
....(((....)))......  -1.60
.....((........))).  -1.50
.((.(((....))).))...  -1.40
....((((..(...).))))  -1.40
.((..((....))..))...  -1.00
(((.(((....)).))))..  -0.90
(((.((......)).)))..  -0.90
....((((..(....))))) -0.80
.....((....)).......  -0.80
..(.(((....)))).....  -0.60
....(((....)).).....  -0.60
(((..(......)..)))..  -0.50
..(((((....)).)..)).  -0.50
..(.(((....))).)....  -0.40
..((.......))......  -0.30
..........((......))  -0.30
..........((....)).  -0.30
(((.(((....)))).))..  -0.20
....(((.(.......))))  -0.20
....(((..((....)))))  -0.20
..(..((....))..)....  0.00
....................  0.00
.(..(((....)))..)...  0.10
```



M.T. Wolfinger, W.A. Svrcek-Seiler, C. Flamm, I.L. Hofacker, P.F. Stadler. 2004. *J.Phys.A: Math.Gen.* **37**:4731-4741.

```
CUGCGGCUUUGGCUCUAGCC
....((((........))))  -4.30
(((.(((....))).)))..  -3.50
(((..((....))..))))..  -3.10
..........(((....)))  -2.80
..(((((....)))...)).  -2.20
....(((.........)))  -2.20
((..(((....)))..))..  -2.00
..((.((....))....)).  -1.60
....(((....)))......  -1.60
.....(((........))).  -1.50
.((.(((....))).))...  -1.40
....((((..(...).))))  -1.40
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..(((((....)).)..)).  -0.50
..(.(((....))).)....  -0.40
..((.......))......   -0.30
..........((......))  -0.30
..........((....)).   -0.30
(((.(((....)))).))..  -0.20
....(((.(.......))))  -0.20
....(((..((....)))))  -0.20
..(..((....))..)....   0.00
....................   0.00
.(..(((....)))..)...   0.10
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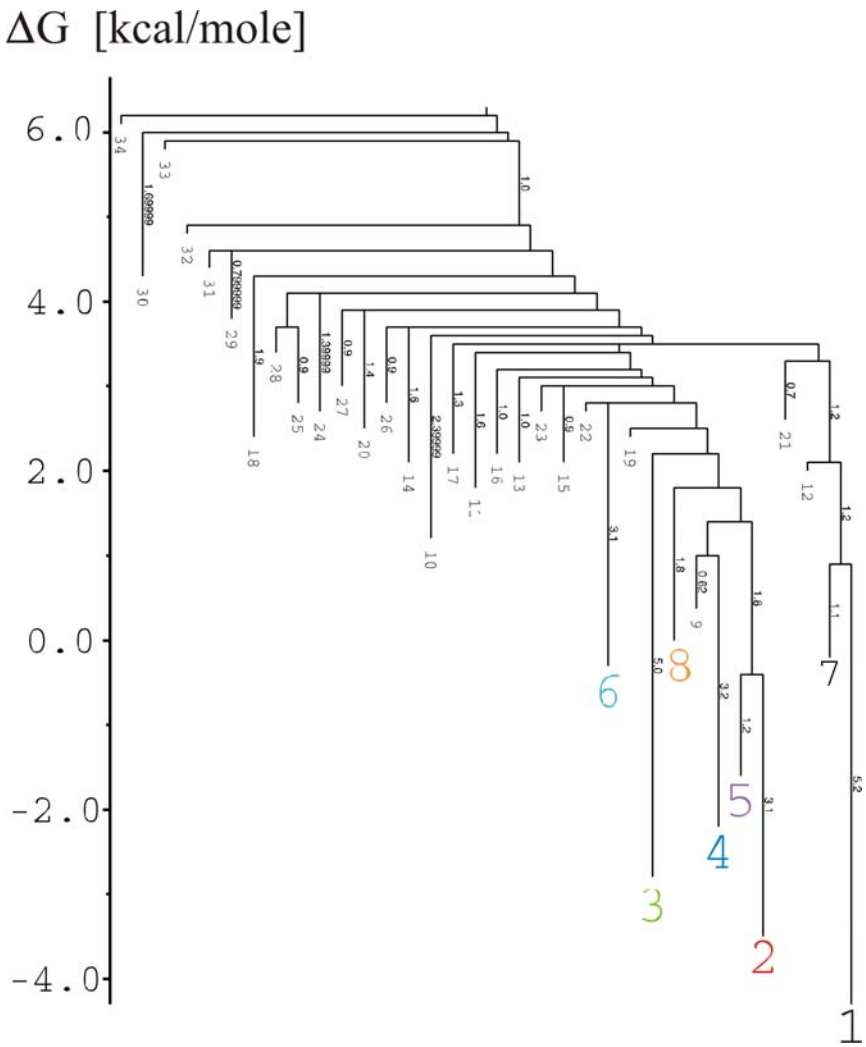
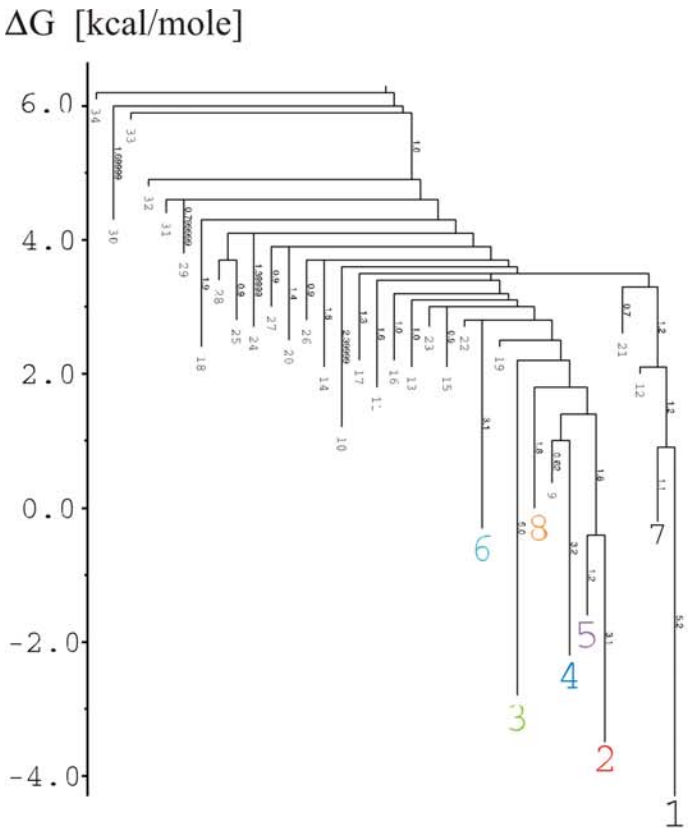
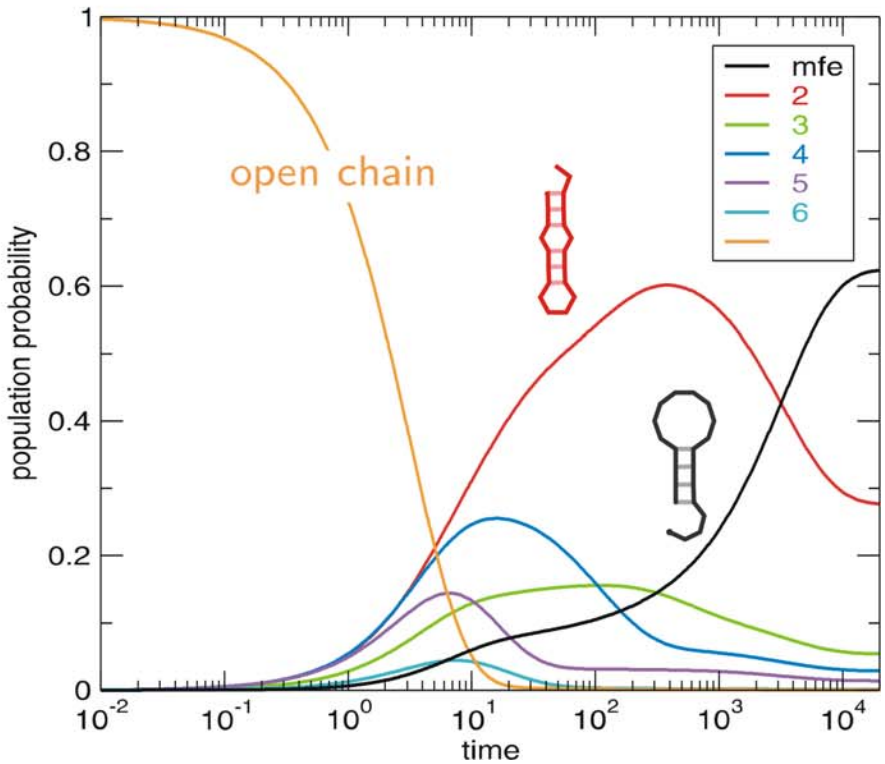


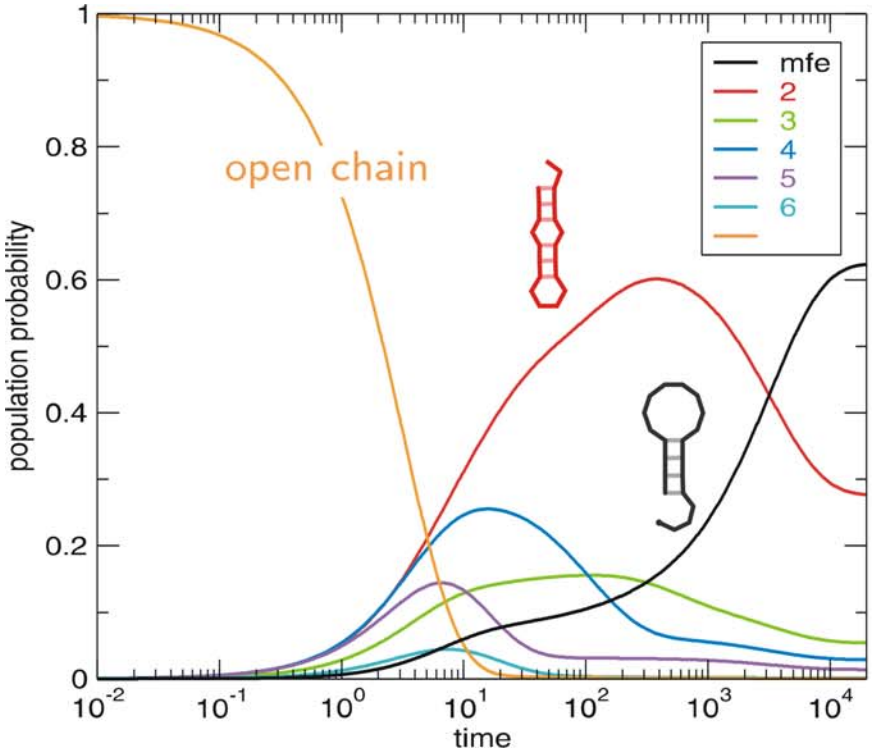
M.T. Wolfinger, W.A. Svrcek-Seiler, C. Flamm, I.L. Hofacker, P.F. Stadler. 2004. *J.Phys.A: Math.Gen.* **37**:4731-4741.



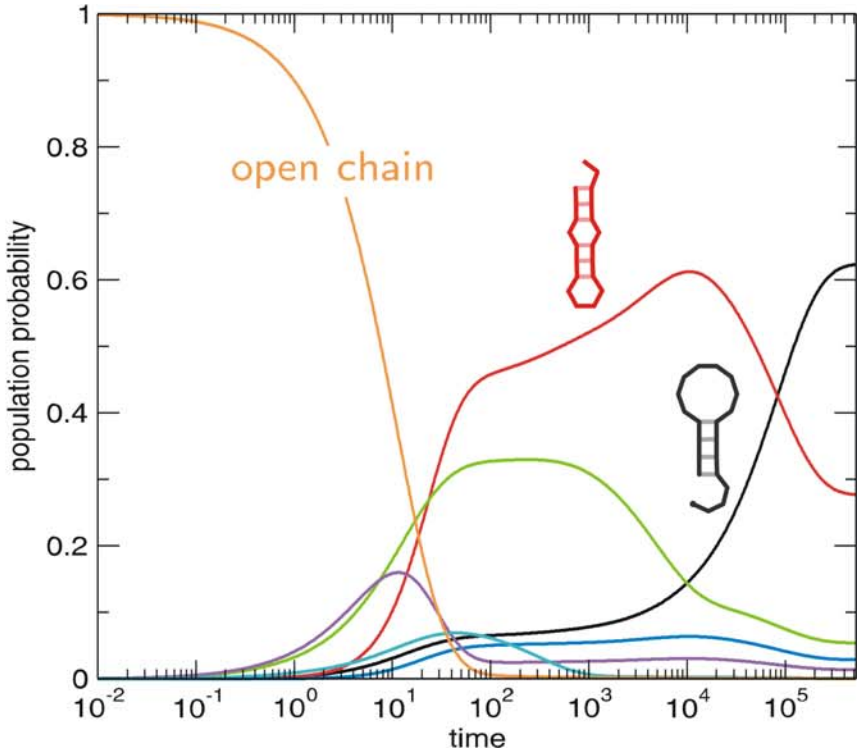


Arrhenius kinetics

M.T. Wolfinger, W.A. Svrcek-Seiler, C. Flamm, I.L. Hofacker, P.F. Stadler. 2004. *J.Phys.A: Math.Gen.* **37**:4731-4741.



Arrhenius kinetic



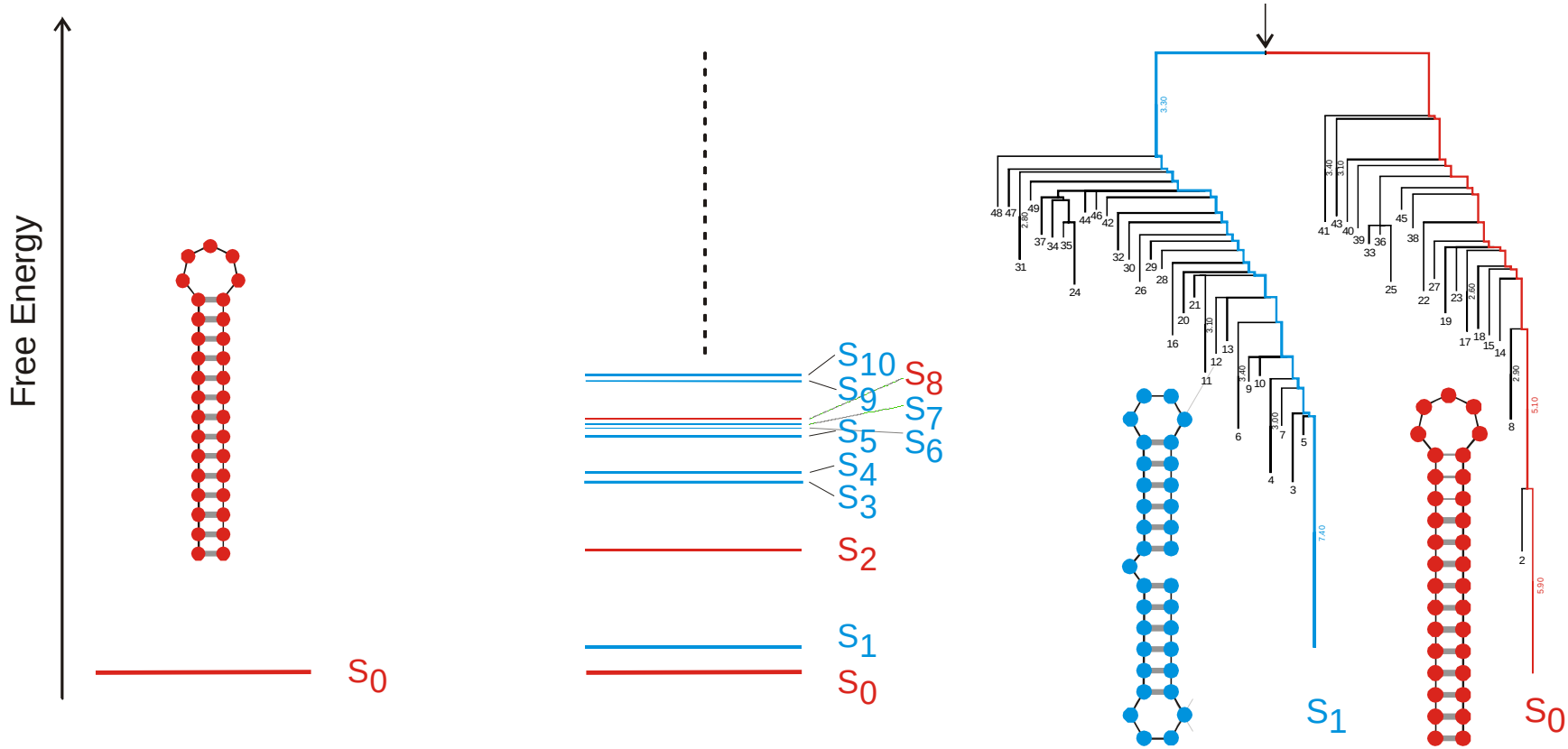
Exact solution of the master equation

M.T. Wolfinger, W.A. Svrcek-Seiler, C. Flamm, I.L. Hofacker, P.F. Stadler. 2004. *J.Phys.A: Math.Gen.* **37**:4731-4741.

One sequence - one structure

Many suboptimal structures
Partition function

Metastable structures
Conformational switches

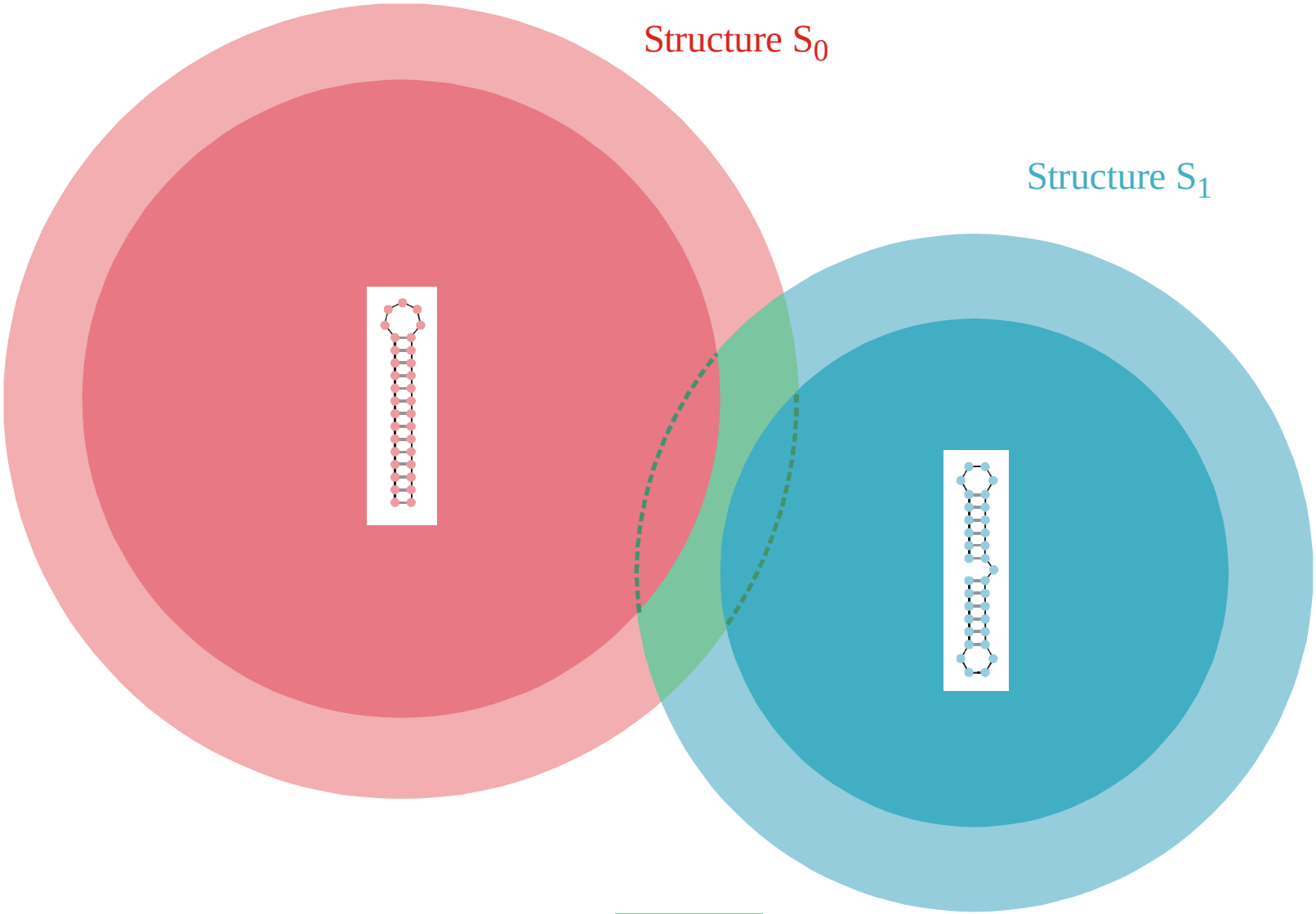


Minimum free energy structure

Suboptimal structures

Kinetic structures

RNA secondary structures derived from a single sequence



Intersection of two compatible sets: $C_0 \cap C_1$

The intersection of two compatible sets is always non empty: $C_0 \cap C_1 \notin \varnothing$

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S0092-8240(96)00089-4

GENERIC PROPERTIES OF COMBINATORY MAPS: NEUTRAL NETWORKS OF RNA SECONDARY STRUCTURES[1]

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Random graph theory is used to model and analyse the relationships between sequences and secondary structures of RNA molecules, which are understood as mappings from sequence space into shape space. These maps are non-invertible since there are always many orders of magnitude more sequences than structures. Sequences folding into identical structures form *neutral networks*. A neutral network is embedded in the set of sequences that are *compatible* with the given structure. Networks are modeled as graphs and constructed by random choice of vertices from the space of compatible sequences. The theory characterizes neutral networks by the mean fraction of neutral neighbors (λ). The networks are connected and percolate sequence space if the fraction of neutral nearest neighbors exceeds a threshold value ($\lambda > \lambda^*$). Below threshold ($\lambda < \lambda^*$), the networks are partitioned into a largest "giant" component and several smaller components. Structures are classified as "common" or "rare" according to the sizes of their pre-images, i.e. according to the fractions of sequences folding into them. The neutral networks of any pair of two different common structures almost touch each other, and, as expressed by the conjecture of *shape space covering* sequences folding into almost all common structures, can be found in a small ball of an arbitrary location in sequence space. The results from random graph theory are compared to data obtained by folding large samples of RNA sequences. Differences are explained in terms of specific features of RNA molecular structures. © *1997 Society for Mathematical Biology*

THEOREM 5. INTERSECTION-THEOREM. *Let* s *and* s′ *be arbitrary secondary structures and* **C**[s], **C**[s′] *their corresponding compatible sequences. Then,*

$$\mathbf{C}[s] \cap \mathbf{C}[s'] \neq \varnothing.$$

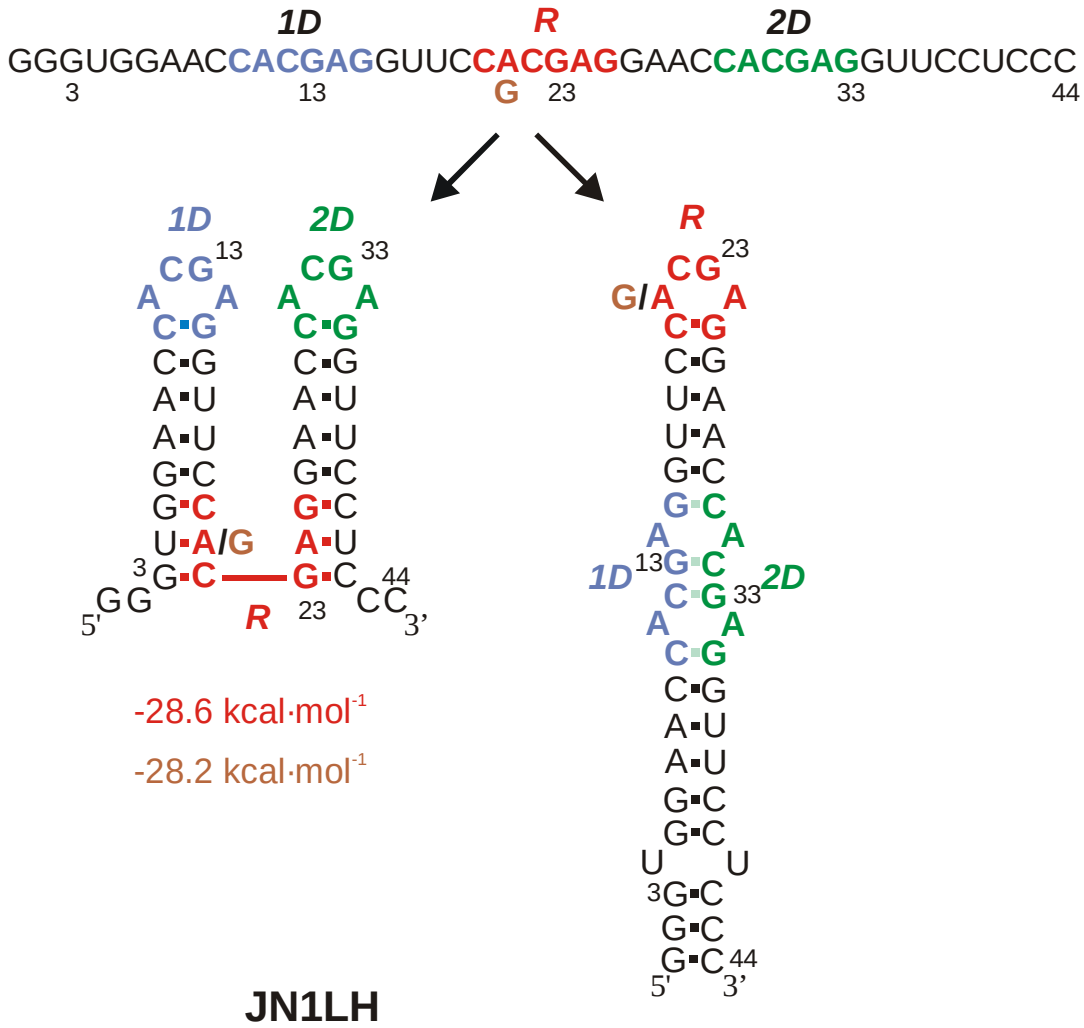
Proof. Suppose that the alphabet admits only the complementary base pair $[XY]$ and we ask for a sequence x compatible to both s and s'. Then $\gamma(s, s') \cong D_m$ operates on the set of all positions $\{x_1, \ldots, x_n\}$. Since we have the operation of a dihedral group, the orbits are either cycles or chains and the cycles have even order. A constraint for the sequence compatible to both structures appears only in the cycles where the choice of bases is not independent. It remains to be shown that there is a valid choice of bases for each cycle, which is obvious since these have even order. Therefore, it suffices to choose an alternating sequence of the pairing partners X and Y. Thus, there are at least two different choices for the first base in the orbit. ■

Remark. A generalization of the statement of theorem 5 to three different structures is false.

Reference for the definition of the intersection and the proof of the **intersection theorem**

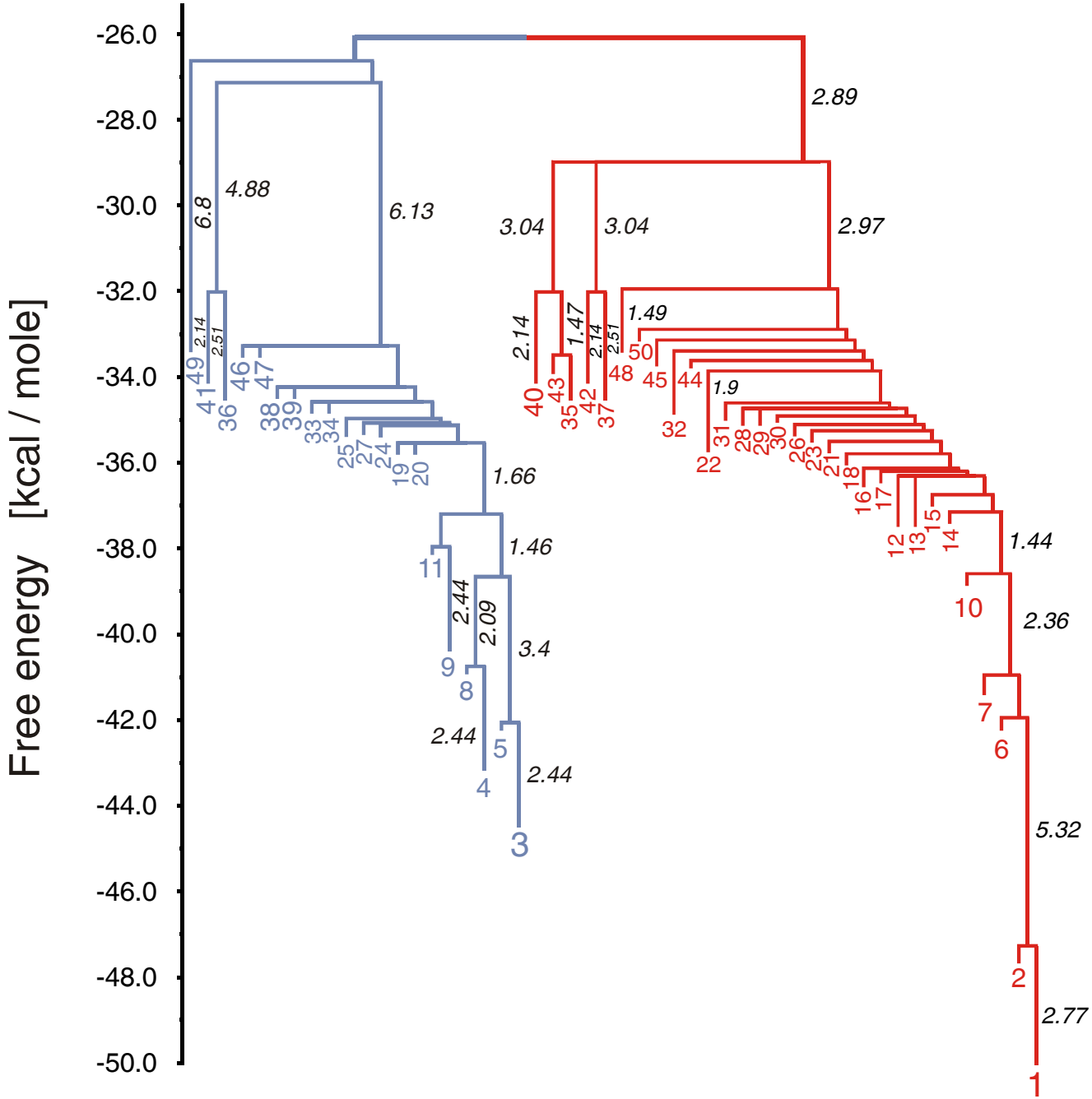
J. H. A. Nagel, C. Flamm, I. L. Hofacker, K. Franke, M. H. de Smit, P. Schuster, and C. W. A. Pleij. ***Structural parameters affecting the kinetic competition of RNA hairpin formation***, *Nucleic Acids Res.*, in press 2005.

J. H. A. Nagel, J. Møller-Jensen, C. Flamm, K. J. Öistämö, J. Besnard, I. L. Hofacker, A. P. Gultyaev, M. H. de Smit, P. Schuster, K. Gerdes and C. W. A. Pleij. ***The refolding mechanism of the metastable structure in the 5'-end of the*** hok ***mRNA of plasmid*** R1, submitted 2005.



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, in press 2005.



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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1. Sequence space and shape space
2. Neutral networks and evolution
3. Conformation space and kinetic folding
4. **What kind of analogies are there?**
5. How to model evolution of kinetic folding?

Kinetic Folding

Conformation space:

Set of **stuctures compatible with** a given **sequence**

Folding trajectory:

Time ordered series of structures

Folding process:

Average of trajectories on the ensemble level

Criterium: **minimizing free energy**

Evolutionary optimization

Compatible set:

Set of **sequences compatible with** a given **structure**

mfe restriction ↓

Neutral network

Genealogy on a neutral network:

Time ordered series of sequences

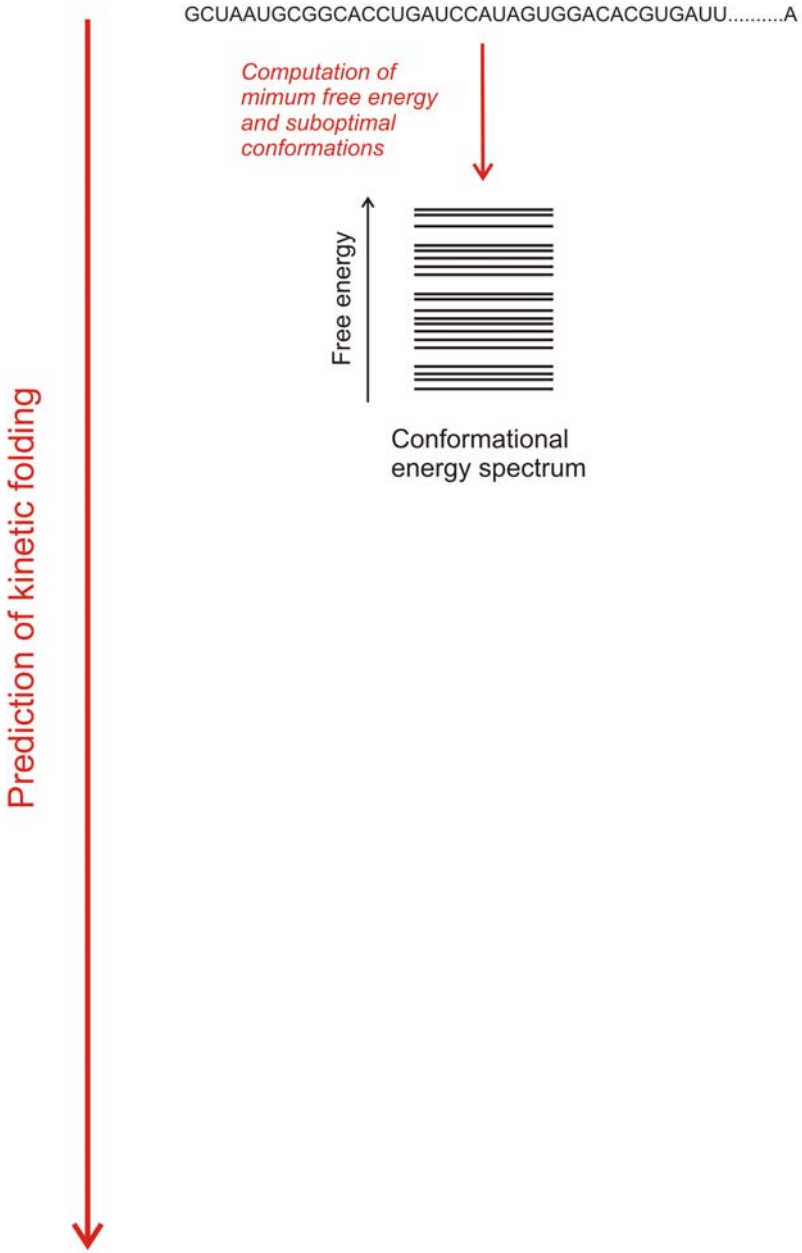
Optimization process:

Average over genealogies on the population level

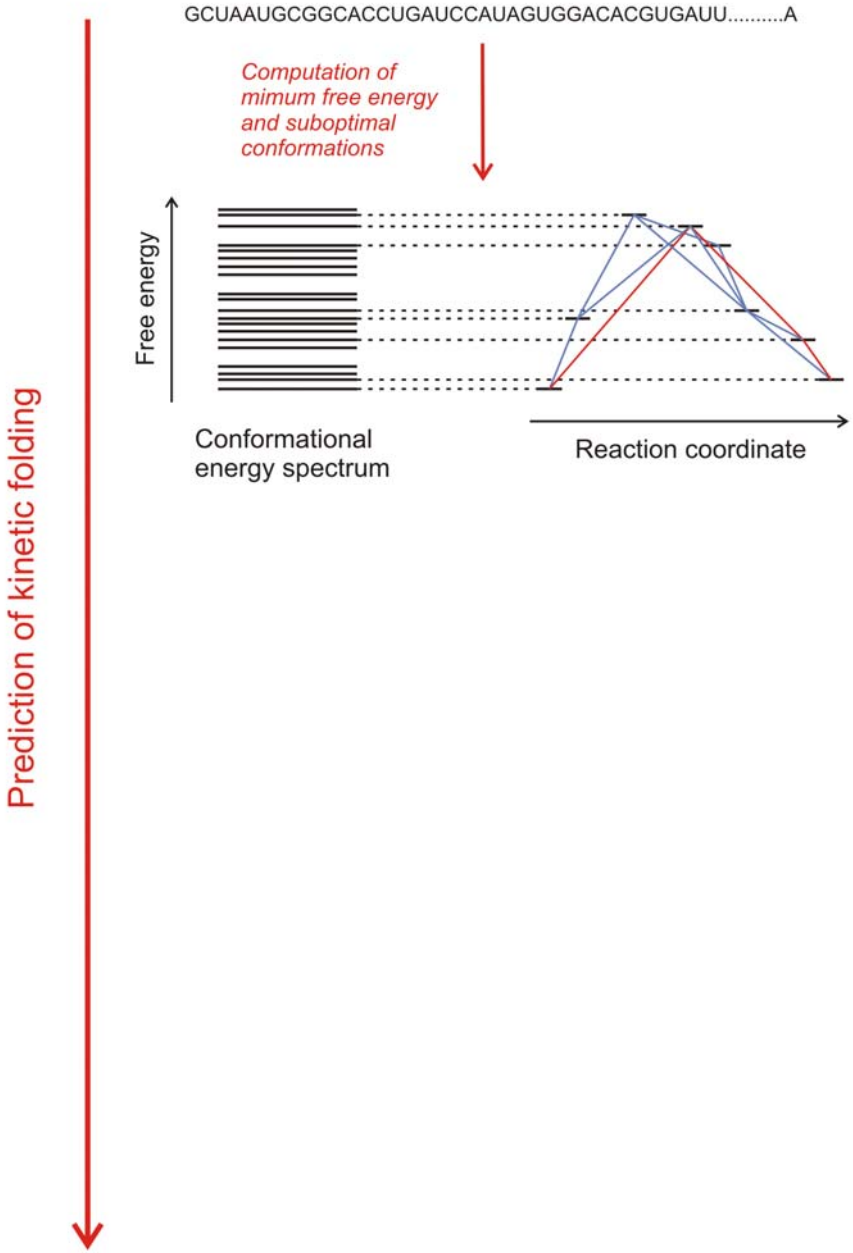
Criterium: **maximizing fitness**

1. Sequence space and shape space
2. Neutral networks and evolution
3. Conformation space and kinetic folding
4. What kind of analogies are there?
5. **How to model evolution of kinetic folding?**

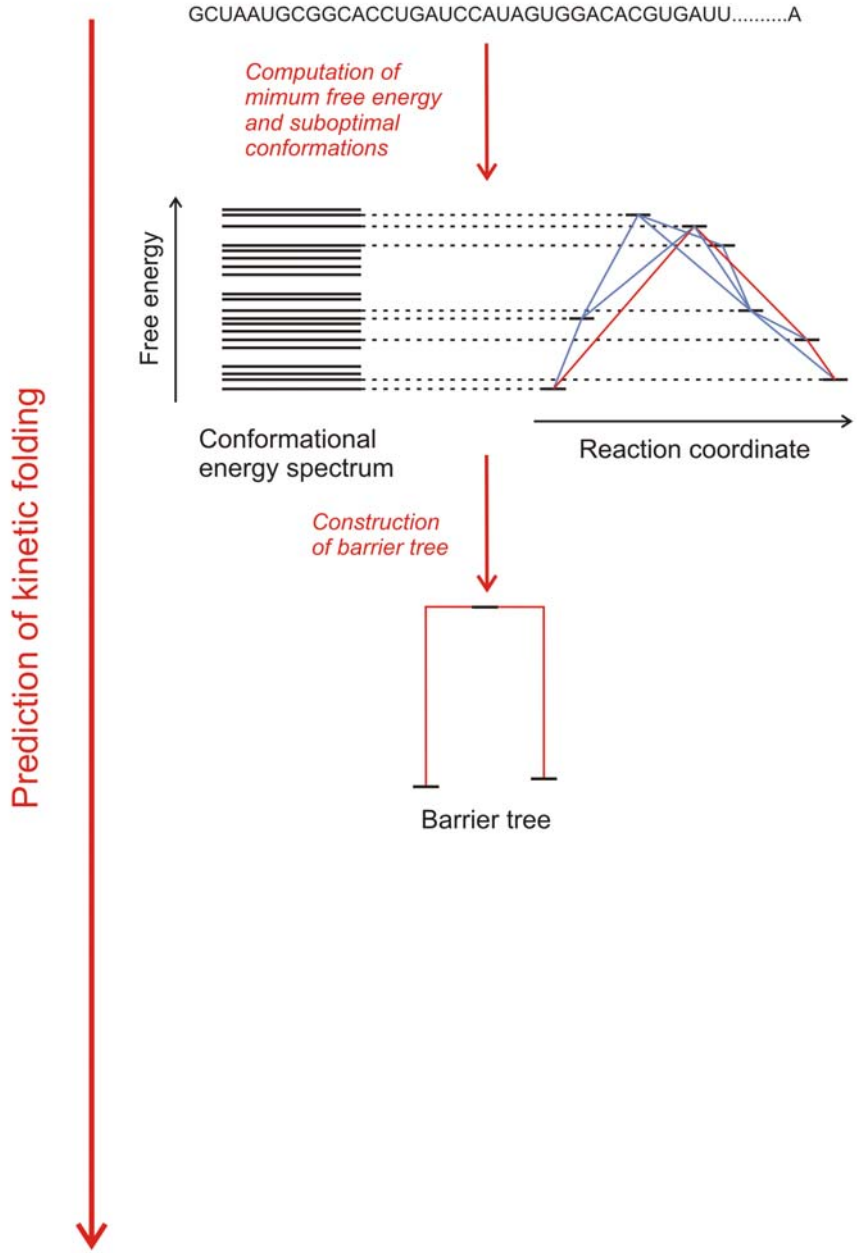
Prediction of RNA kinetic folding of secondary structures based on Arrhenius kinetics



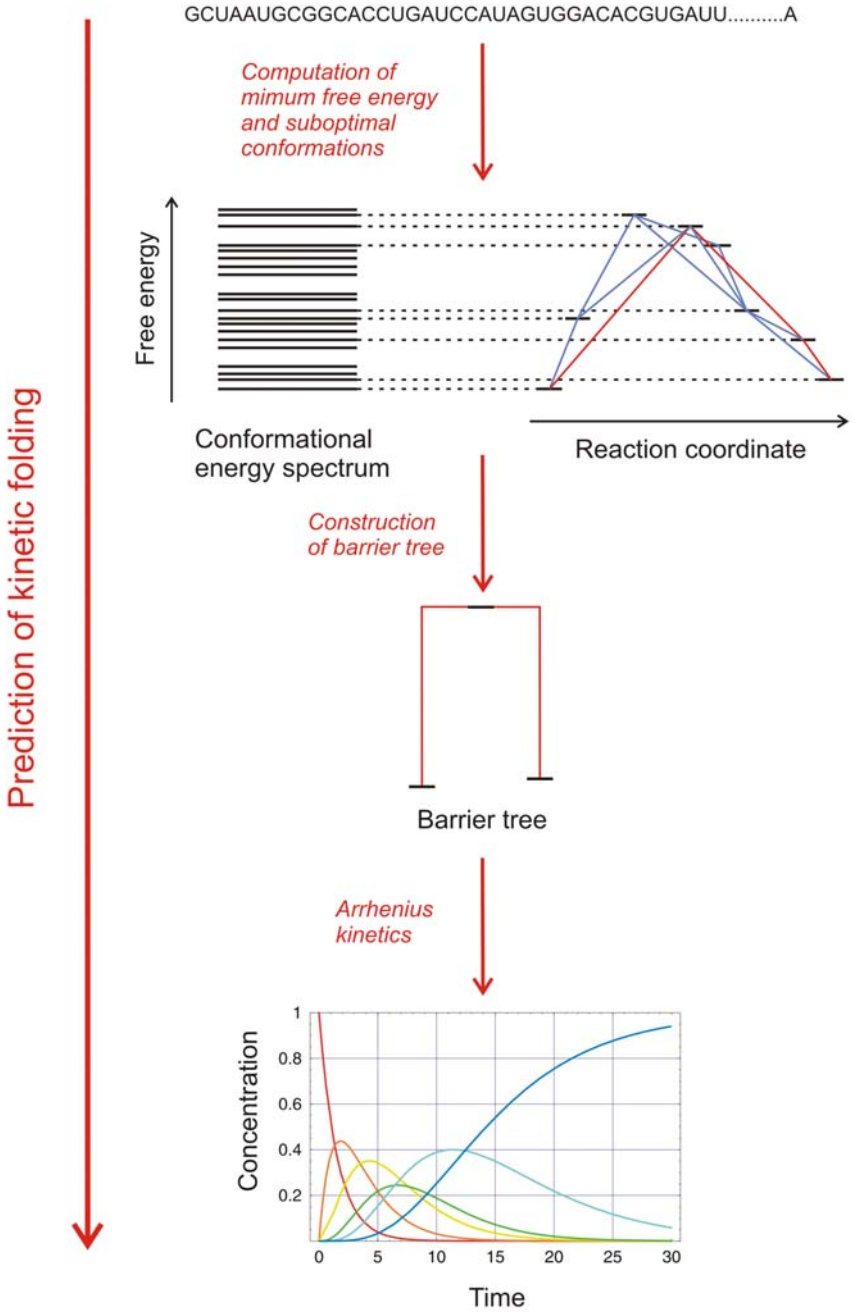
Prediction of RNA kinetic folding of secondary structures based on Arrhenius kinetics



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

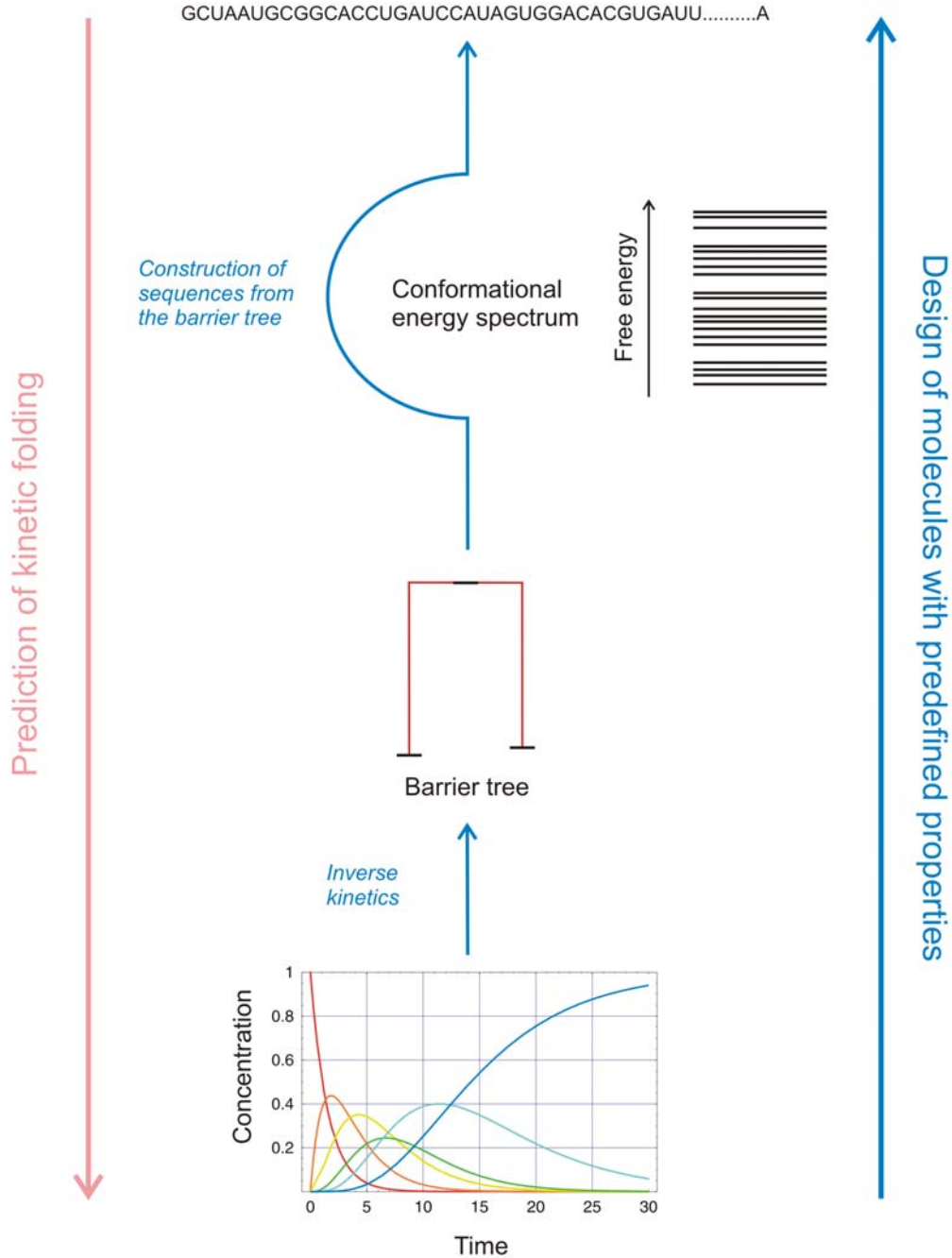


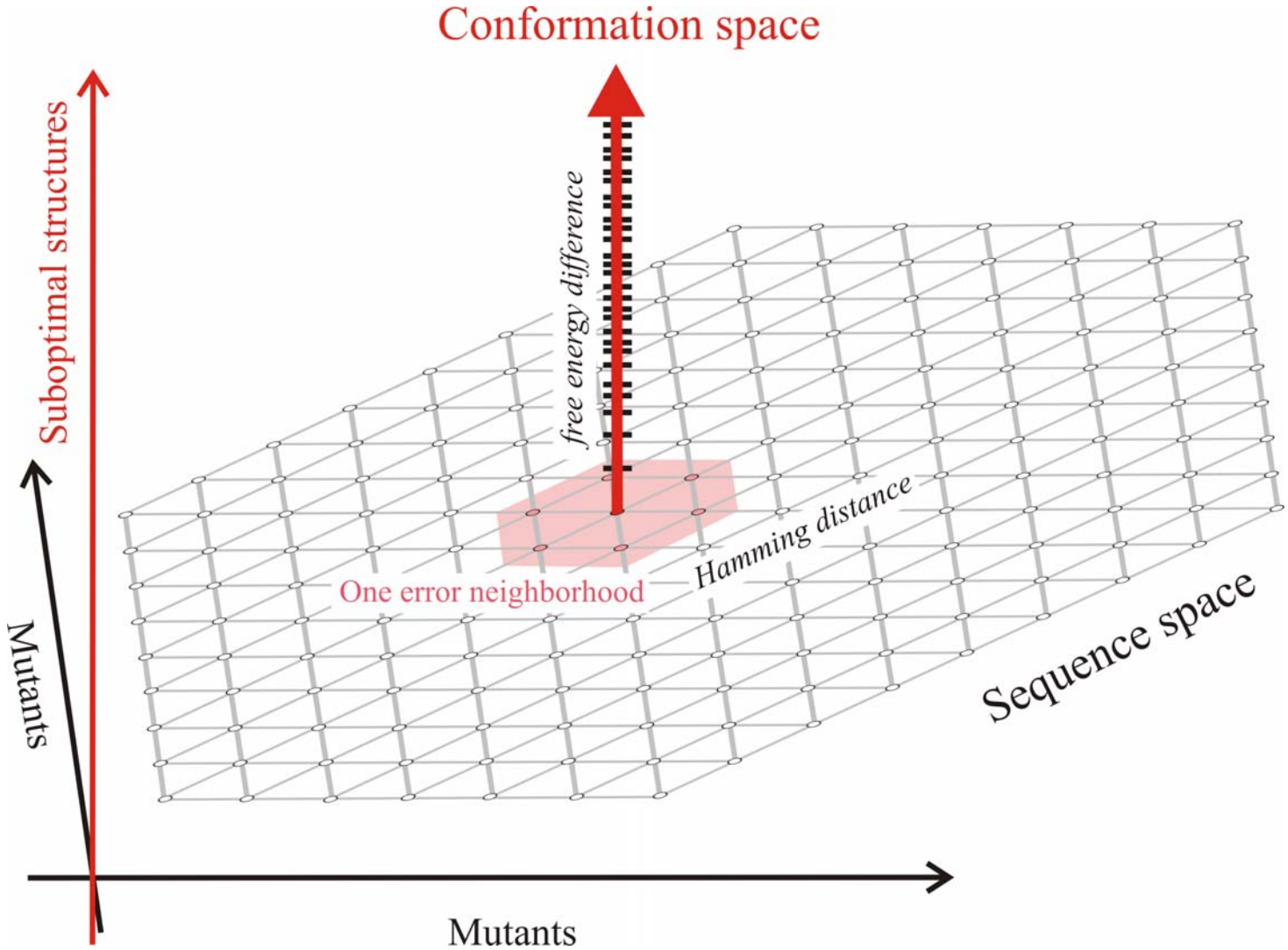
Prediction of RNA kinetic folding of secondary structures based on Arrhenius kinetics



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

Design of RNA molecules with
with predefined folding kinetics





Construction of a combined landscape for folding and evolution

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