Molekularer Einblick in die Evolution von Phänotypen

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Computergestützte Analyse evolutionärer Optimierungsprozesse in komplexen Systemen

Blankensee, 25.05.2002

Darwinian principle

Reproduction efficiency expressed by fitness of **phenotypes**.

Variation of **genotypes** through imperfect copying and recombination.

Selection of **phenotypes** based on differences in fitness.

Additional requirements

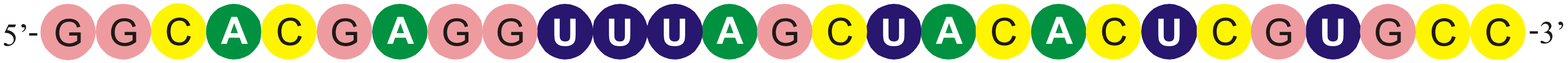
Large reservoirs of genotypes and sufficiently rich repertoires of phenotypes.

Proper mapping of genotypes into phenotypes.

The **genotypes** or **genomes** of individuals and species, being reproductively related ensembles of individuals, are DNA or RNA sequences. They are changing from generation to generation through mutation and recombination.

Genotypes unfold into **phenotypes** or organisms, which are the targets of the evolutionary selection process.

Point mutations are single nucleotide exchanges. The **Hamming distance** of two sequences is the minimal number of single nucleotide exchanges that mutually converts the two sequence into each other.


5’- G G C A C G A G G U U U A G C U A C A C U C G U G C C -3’

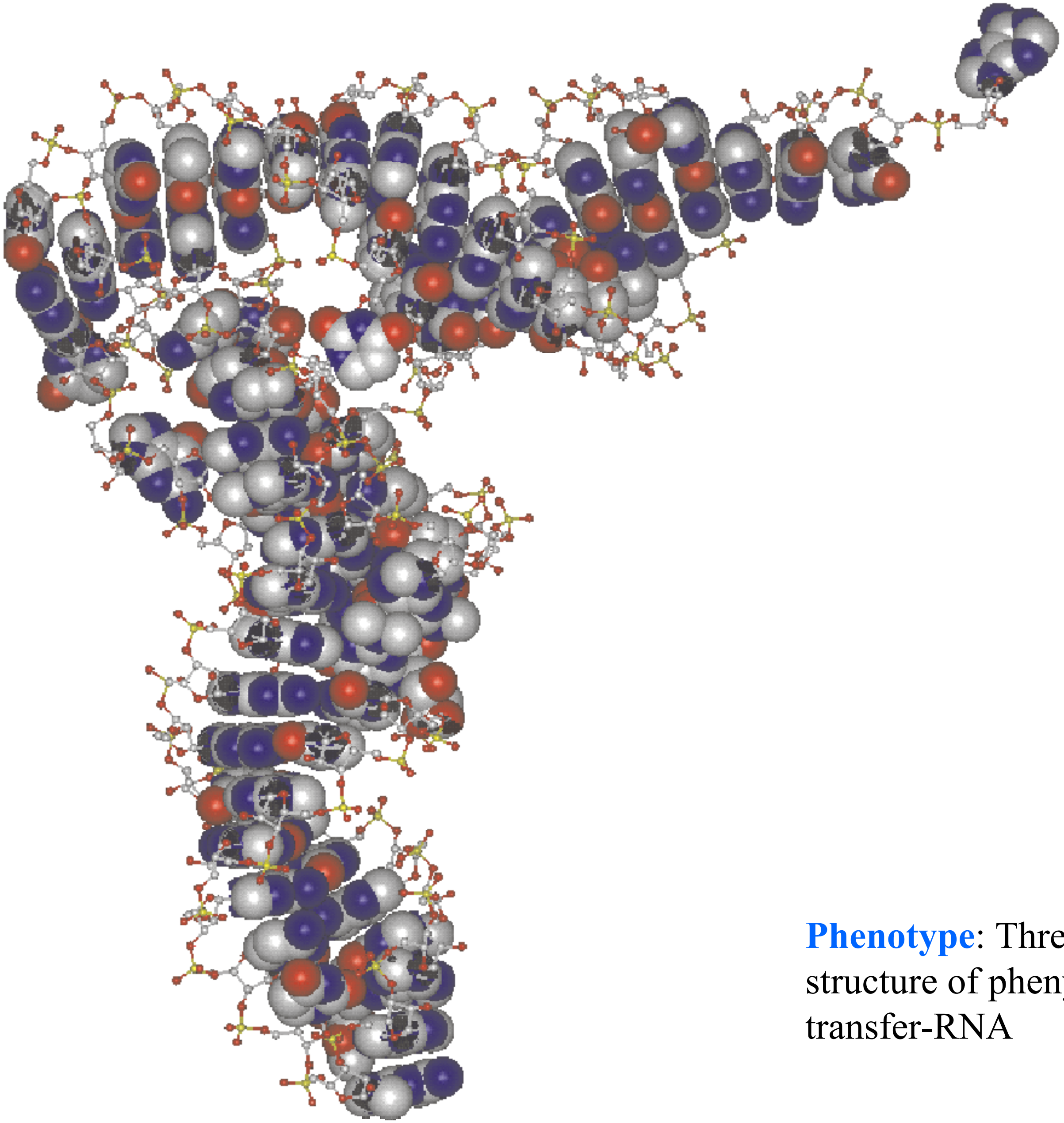
A = adenylate

C = cytidylate

U = uridylate

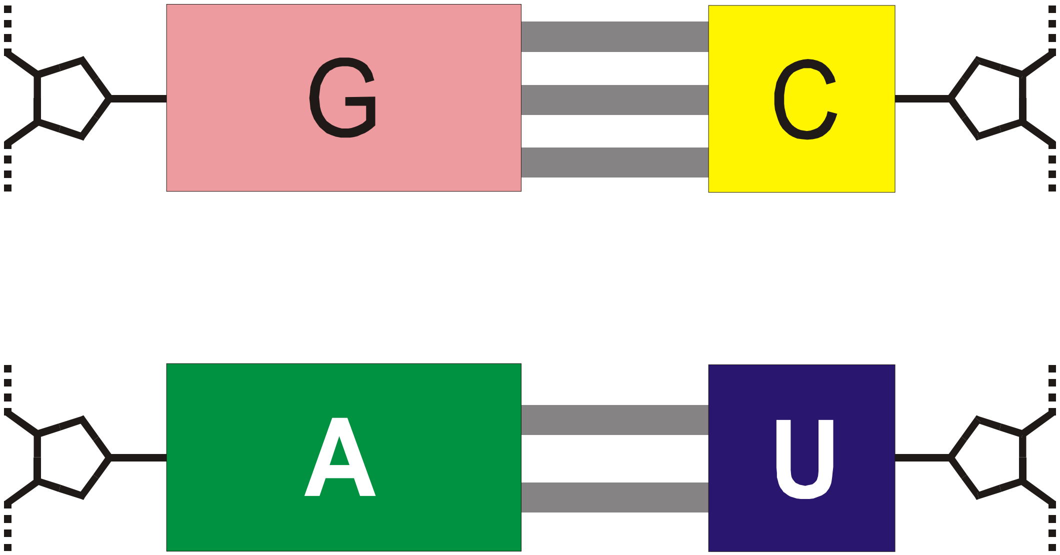
G = guanylate

Genotype: The sequence of an RNA molecule consisting of monomers chosen from four classes.

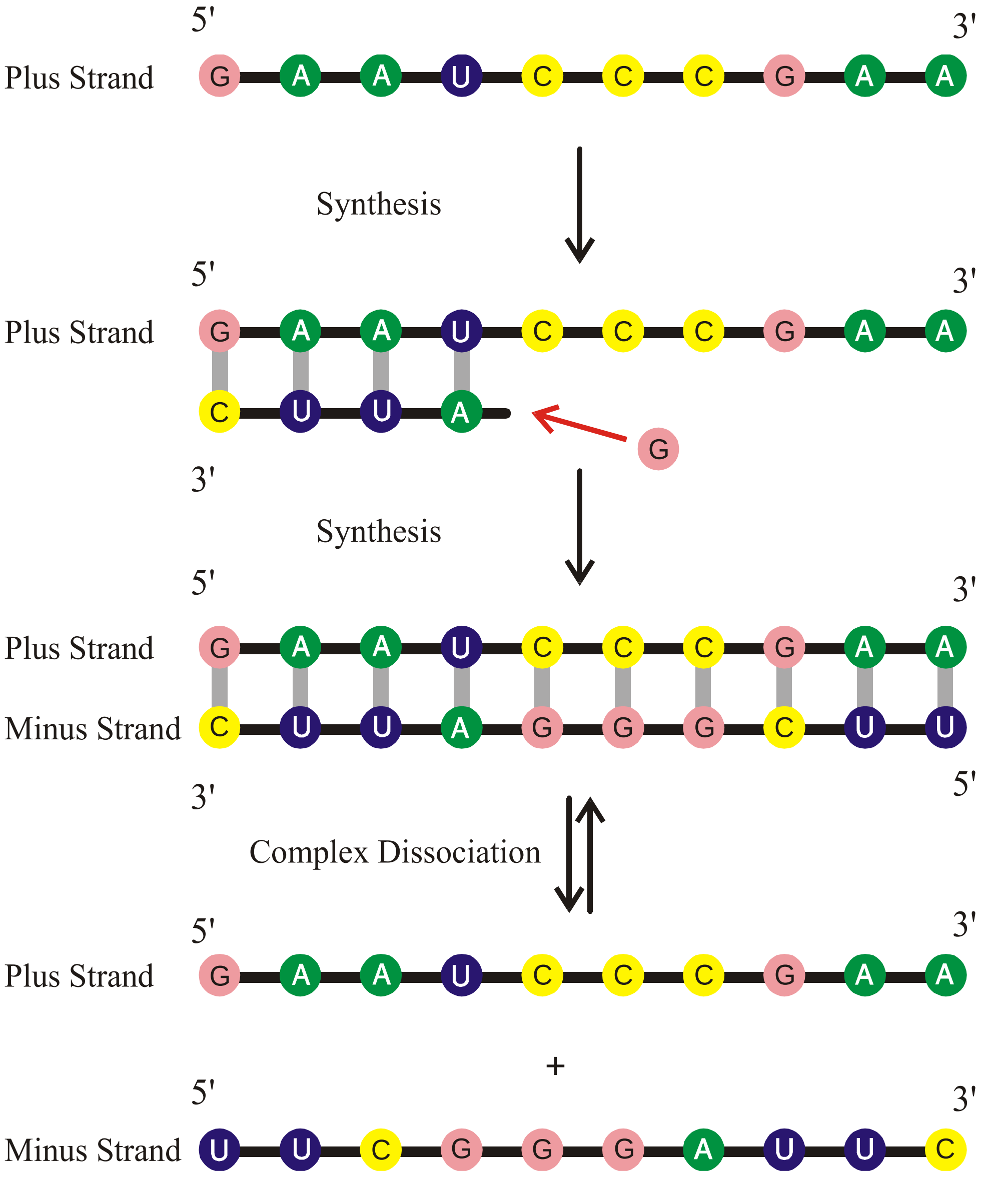


Phenotype: Three-dimensional structure of phenylalanyl transfer-RNA

Hydrogen bonds



Hydrogen bonding between nucleotide bases is the principle of template action of RNA and DNA.



Complementary replication as the simplest copying mechanism of RNA

$$dx_j / dt = \Sigma_i k_i x_i - x_j \Phi$$

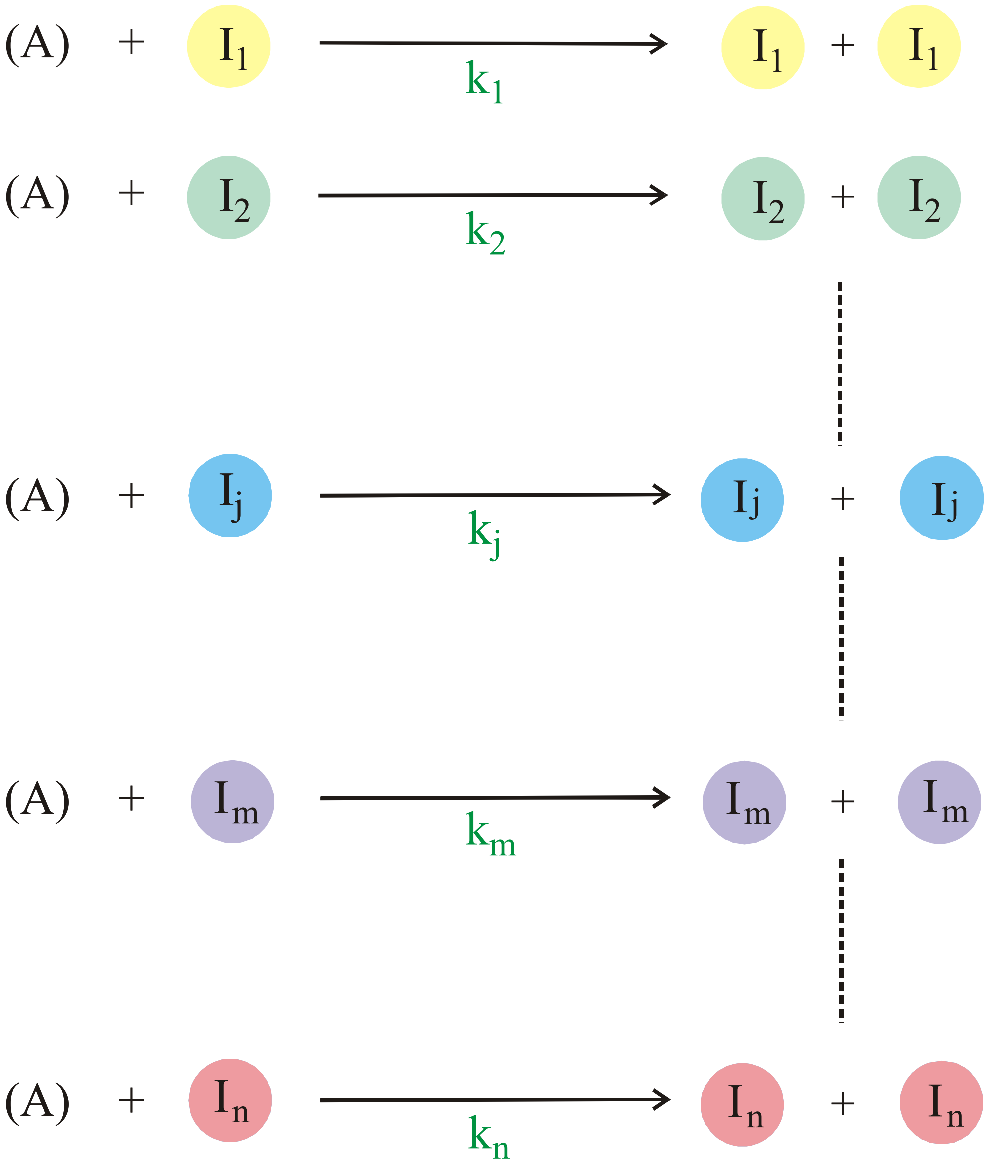
$$\Phi = \Sigma_i k_i x_i \; ; \quad \Sigma_i x_i = 1$$

$[A] = a =$ constant

$k_m = \max \{k_j ; j=1,2,...,n\}$

$x_m(t)$ Á 1 for t Á ¸

$s = (k_{m+1}-k_m)/k_m$



$$(A) + I_1 \xrightarrow[k_1]{} I_1 + I_1$$

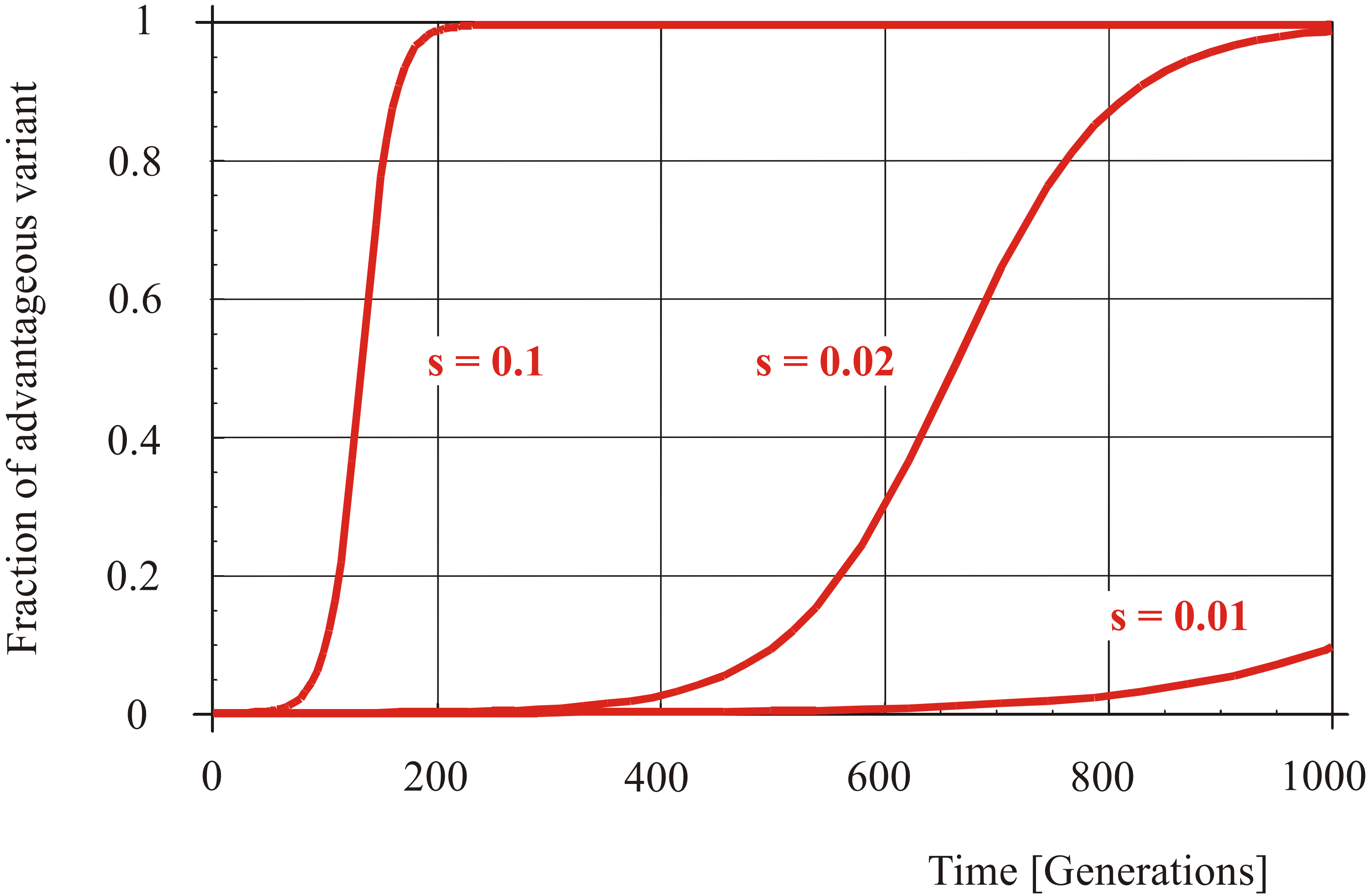
$$(A) + I_2 \xrightarrow[k_2]{} I_2 + I_2$$

$$(A) + I_j \xrightarrow[k_j]{} I_j + I_j$$

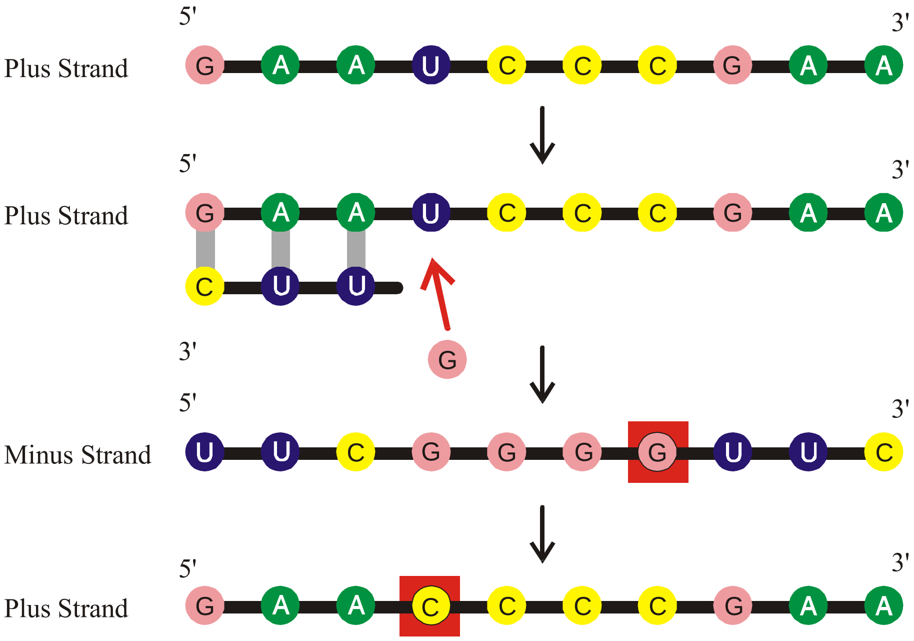
$$(A) + I_m \xrightarrow[k_m]{} I_m + I_m$$

$$(A) + I_n \xrightarrow[k_n]{} I_n + I_n$$

Selection of the „fittest“ or fastest replicating species



Selection of advantageous mutants in populations of N = 10 000 individuals



Point Mutation

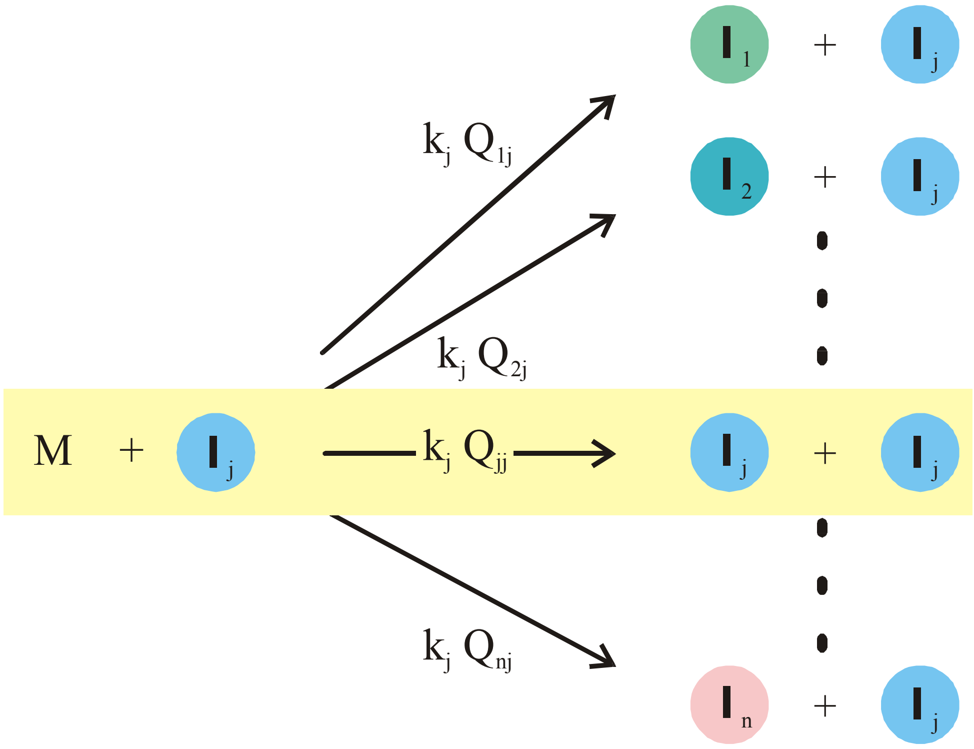
GAAUCCCGAA → GAAUCCCGUCCCGAA

Insertion

GAAUCCCGAA → GAAUCCA

Deletion

Mutations represent the mechanism of variation in nucleic acids.



$\mathbf{I}_1$ + $\mathbf{I}_j$
$k_j\,Q_{1j}$
$\mathbf{I}_2$ + $\mathbf{I}_j$
$k_j\,Q_{2j}$
M + $\mathbf{I}_j$
$k_j\,Q_{jj}$
$\mathbf{I}_j$ + $\mathbf{I}_j$
$k_j\,Q_{nj}$
$\mathbf{I}_n$ + $\mathbf{I}_j$

$$\Sigma_i\, Q_{ij} = 1$$

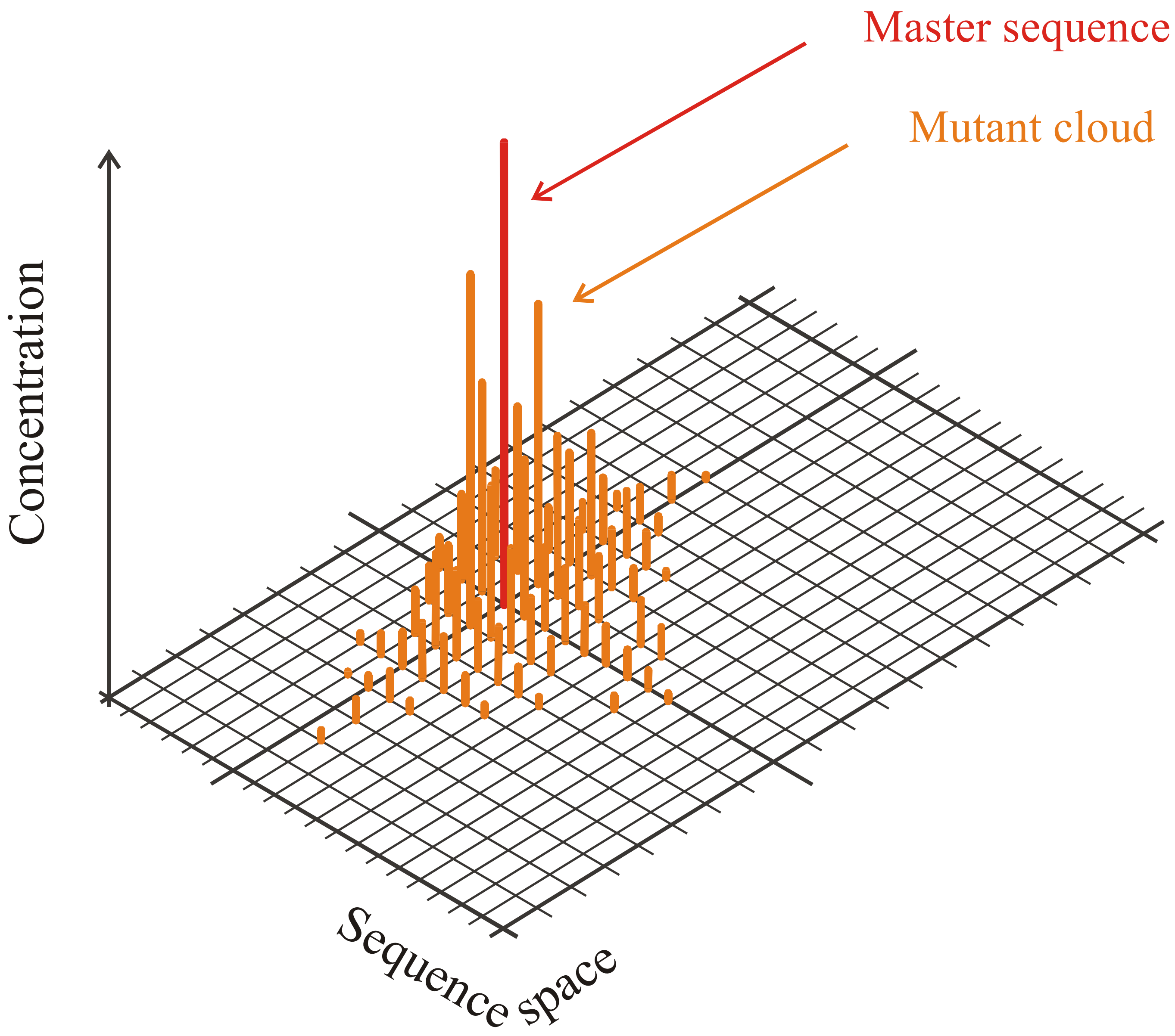
$$Q_{ij} = (1-p)^{n-d(i,j)}\, p^{d(i,j)} \;; \quad p \text{ ...... error rate per digit}$$

d(i,j) ...... Hamming distance between $\mathbf{I}_i$ and $\mathbf{I}_j$

$$dx_j / dt = \Sigma_i\, k_i Q_{ji} x_i - x_j\, \Phi$$

$$\Phi = \Sigma_i\, k_i\, x_i \;; \quad \Sigma_i\, x_i = 1$$

Chemical kinetics of replication
and mutation as parallel reactions



The molecular quasispecies in sequence space

Theory of molecular evolution

M.Eigen, ***Self-organization of matter and the evolution of biological macromolecules***. Naturwissenschaften **58** (1971), 465-526

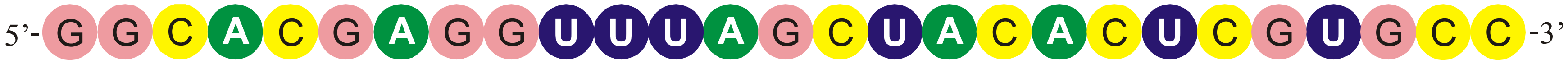
M.Eigen, P.Schuster, ***The hypercycle. A principle of natural self-organization. Part A: Emergence of the hypercycle***. Naturwissenschaften **58** (1977), 465-526

M.Eigen, P.Schuster, ***The hypercycle. A principle of natural self-organization. Part B: The abstract hypercycle***. Naturwissenschaften **65** (1978), 7-41

M.Eigen, P.Schuster, ***The hypercycle. A principle of natural self-organization. Part C: The realistic hypercycle***. Naturwissenschaften **65** (1978), 341-369

M.Eigen, J.McCaskill, P.Schuster, ***The molecular quasispecies***. Adv.Chem.Phys. **75** (1989), 149-263

C. Reidys, C.Forst, P.Schuster, ***Replication and mutation on neutral networks***. Bull.Math.Biol. **63** (2001), 57-94



4^{27} = 1.801 Ç 10^{16} possible different sequences

Combinatorial diversity of sequences: $N = 4^{\{}$

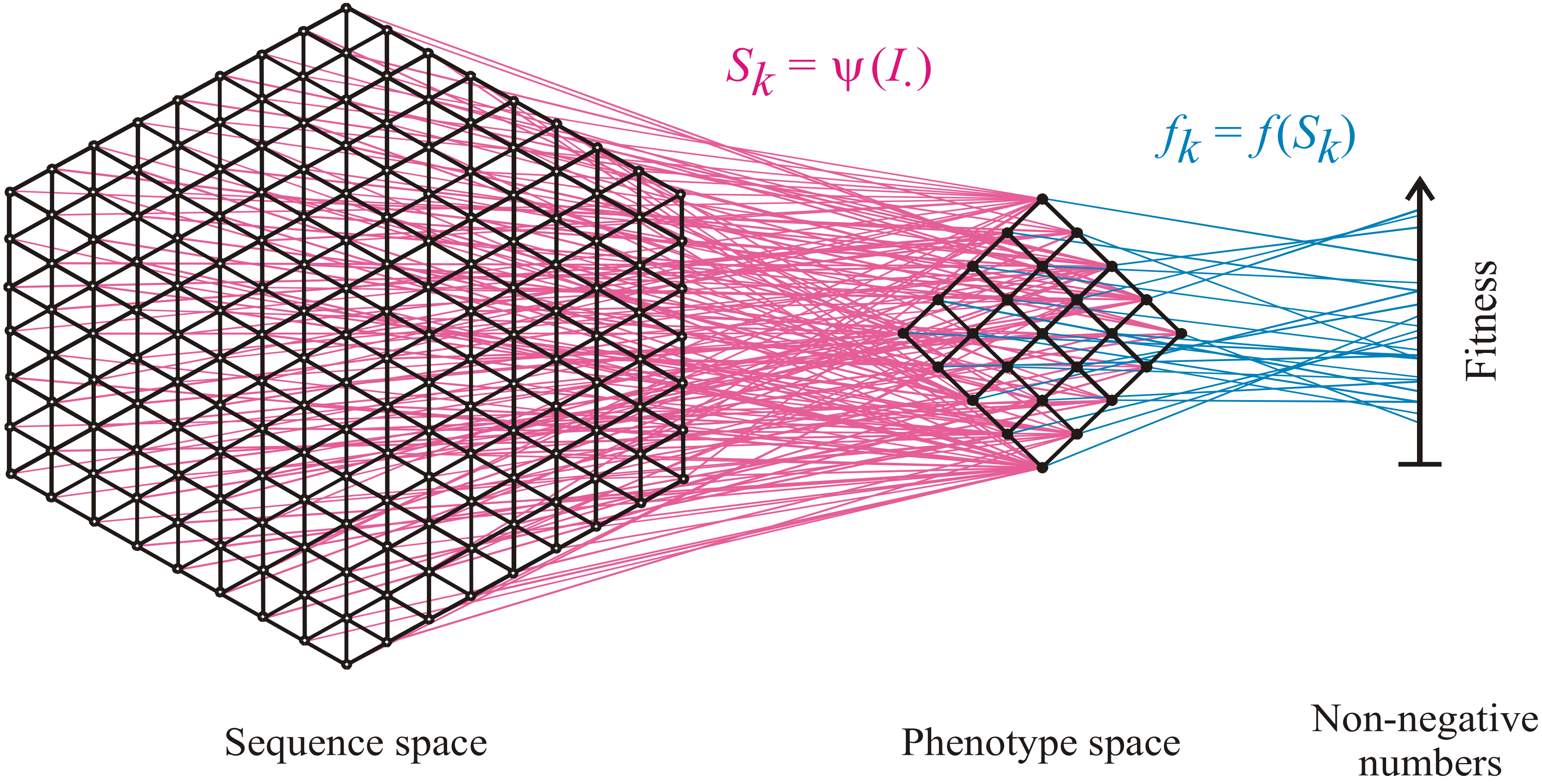
A = adenylate

U = uridylate

C = cytidylate

G = guanylate

Combinatorial diversity of heteropolymers illustrated by means of an RNA aptamer that binds to the antibiotic tobramycin



Mapping from sequence space into phenotype space and into fitness values

The **RNA model** considers RNA sequences as genotypes and simplified RNA structures, called secondary structures, as phenotypes.

The **mapping** from genotypes into phenotypes is many-to-one. Hence, it is redundant and not invertible.

Genotypes, i.e. RNA sequences, which are mapped onto the same phenotype, i.e. the same RNA secondary structure, form **neutral networks**. Neutral networks are represented by graphs in sequence space.

RNA Secondary Structures and their Properties

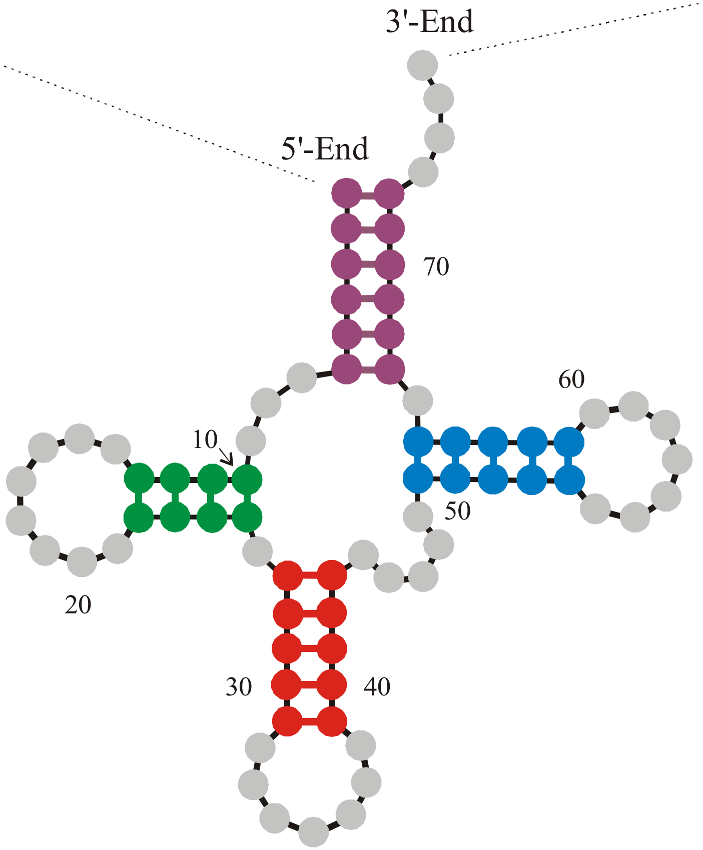
RNA secondary structures are listings of Watson-Crick and GU wobble base pairs, which are free of knots and pseudokots. Secondary structures are folding intermediates in the formation of full three-dimensional structures.

D.Thirumalai, N.Lee, S.A.Woodson, and D.K.Klimov.
Annu.Rev.Phys.Chem. **52**:751-762 (2001)

5'-End 3'-End

Sequence GCGGAUUUAGCUCAGDDGGGAGAGCMCCAGACUGAAYAUCUGGAGMUCCUGUGTPCGAUCCACAGAAUUCGCACCA

Secondary Structure



Symbolic Notation 5'-End ((((((···((((········))))·(((((·······)))))·····(((((·······)))))·))))))···· 3'-End

Definition and formation of the secondary structure of phenylalanyl-tRNA

RNA Minimum Free Energy Structures

Efficient algorithms based on dynamical programming are available for computation of secondary structures for given sequences. Inverse folding algorithms compute sequences for given secondary structures.

M.Zuker and P.Stiegler. *Nucleic Acids Res*. **9**:133-148 (1981)

Vienna RNA Package: http:www.tbi.univie.ac.at (includes inverse folding, suboptimal structures, kinetic folding, etc.)

I.L.Hofacker, W. Fontana, P.F.Stadler, L.S.Bonhoeffer, M.Tacker, and P. Schuster. *Mh.Chem*. **125**:167-188 (1994)

Criterion of
Minimum Free Energy

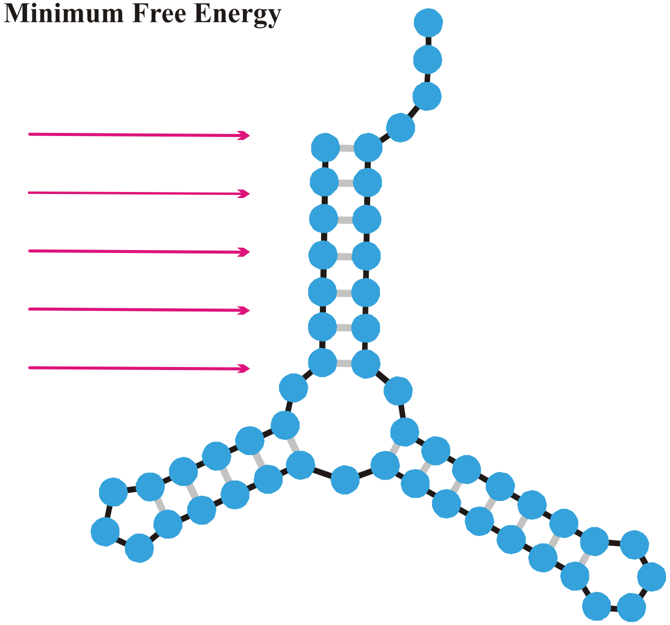
UUUAGCCAGCGCGAGUCGUGCGGACGGGGUUAUCUCUGUCGGGCUAGGGCGC

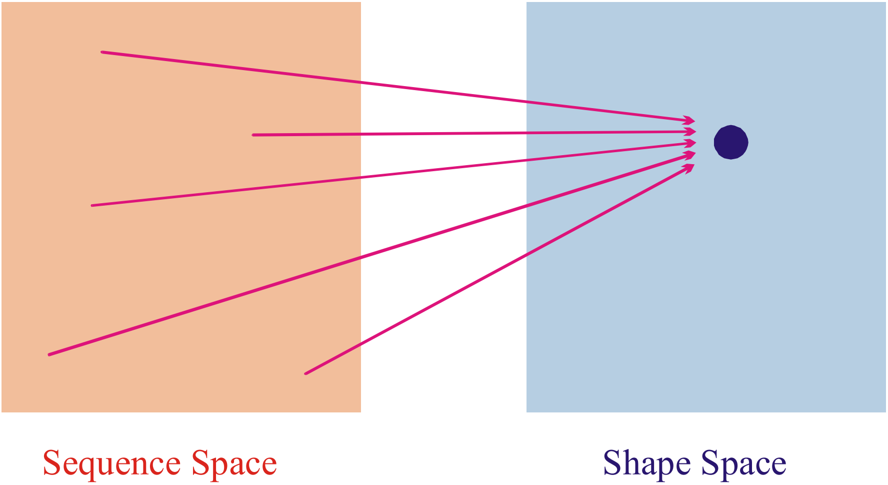
GUGAGCGCGGGGCACAGUUUCUCAAGGAUGUAAGUUUUUGCCGUUUAUCUGG

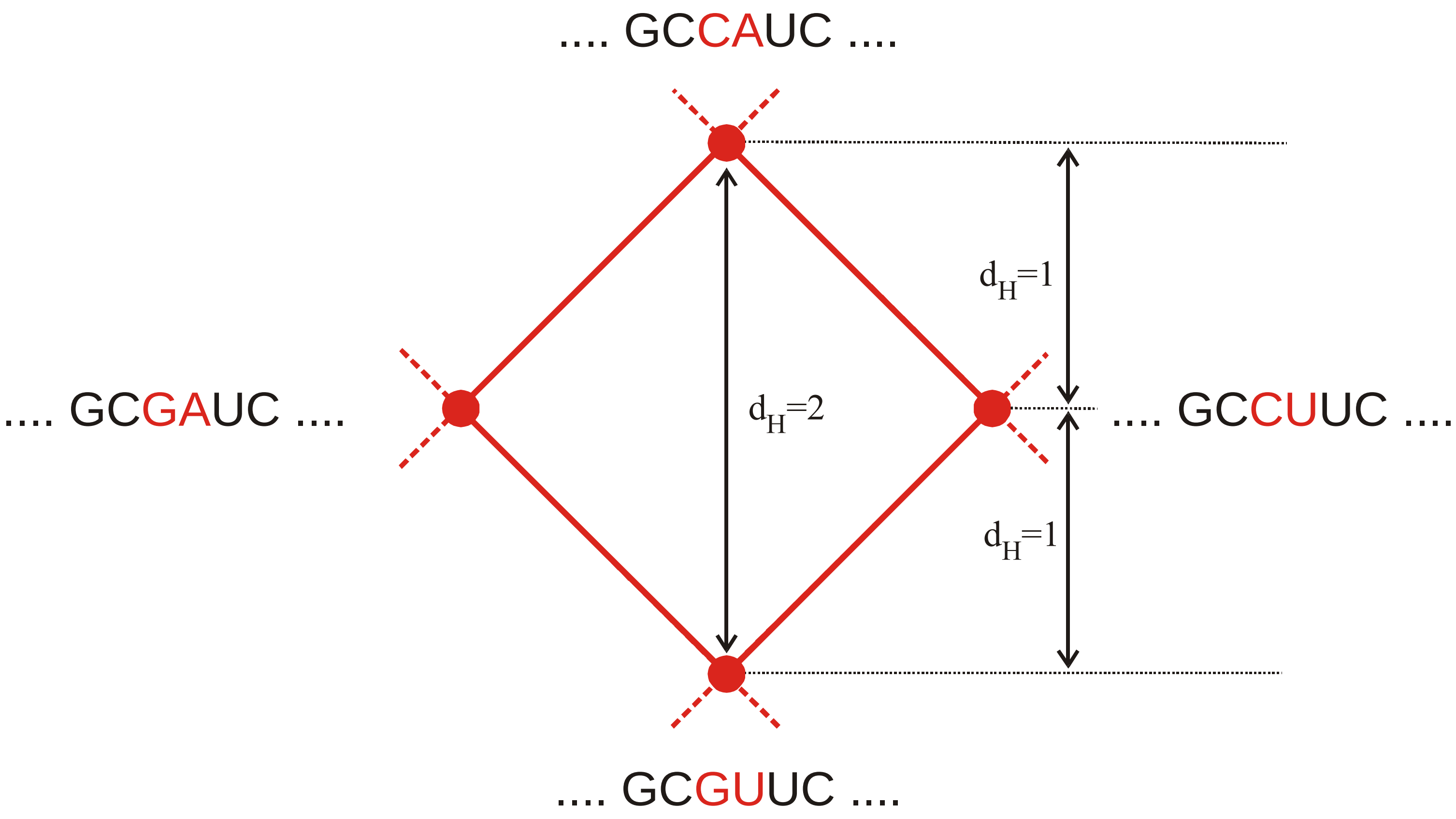
UUAGCGAGAGAGGAGGCUUCUAGACCCAGCUCUCUGGGUCGUUGCUGAUGCG

CAUUGGUGCUAAUGAUAUUAGGGCUGUAUUCCUGUAUAGCGAUCAGUGUCCG

GUAGGCCCUCUUGACAUAAGAUUUUUCCAAUGGUGGGAGAUGGCCAUUGCAG







.... GCCAUC ....

.... GCGAUC ....

d_H=2

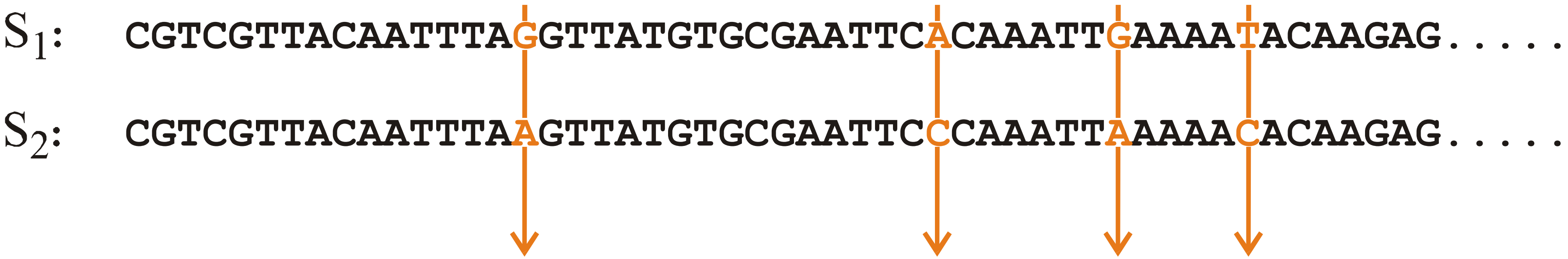
d_H=1

.... GCCUUC ....

d_H=1

.... GCGUUC ....

Point mutations as moves in sequence space



S_1: CGTCGTTACAATTTAGGTTATGTGCGAATTCACAAATTGAAAATACAAGAG.....

S_2: CGTCGTTACAATTTAAGTTATGTGCGAATTCCCAAATTAAAAACACAAGAG.....

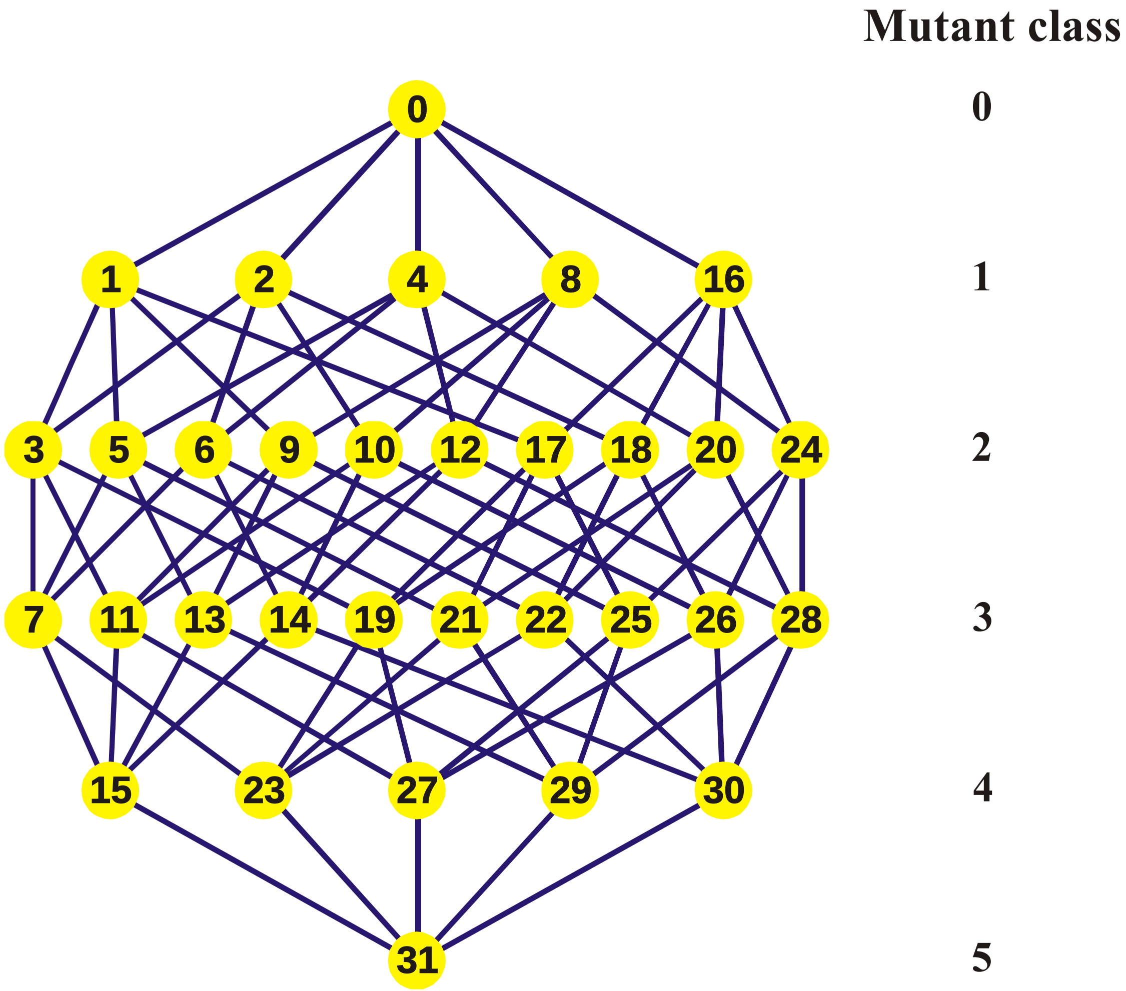
Hamming distance $d_H(S_1,S_2) = 4$

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

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

(iii) $d_H(S_1,S_3)$ ¶ $d_H(S_1,S_2) + d_H(S_2,S_3)$

The Hamming distance induces a metric in sequence space



Binary sequences are encoded by their decimal equivalents:

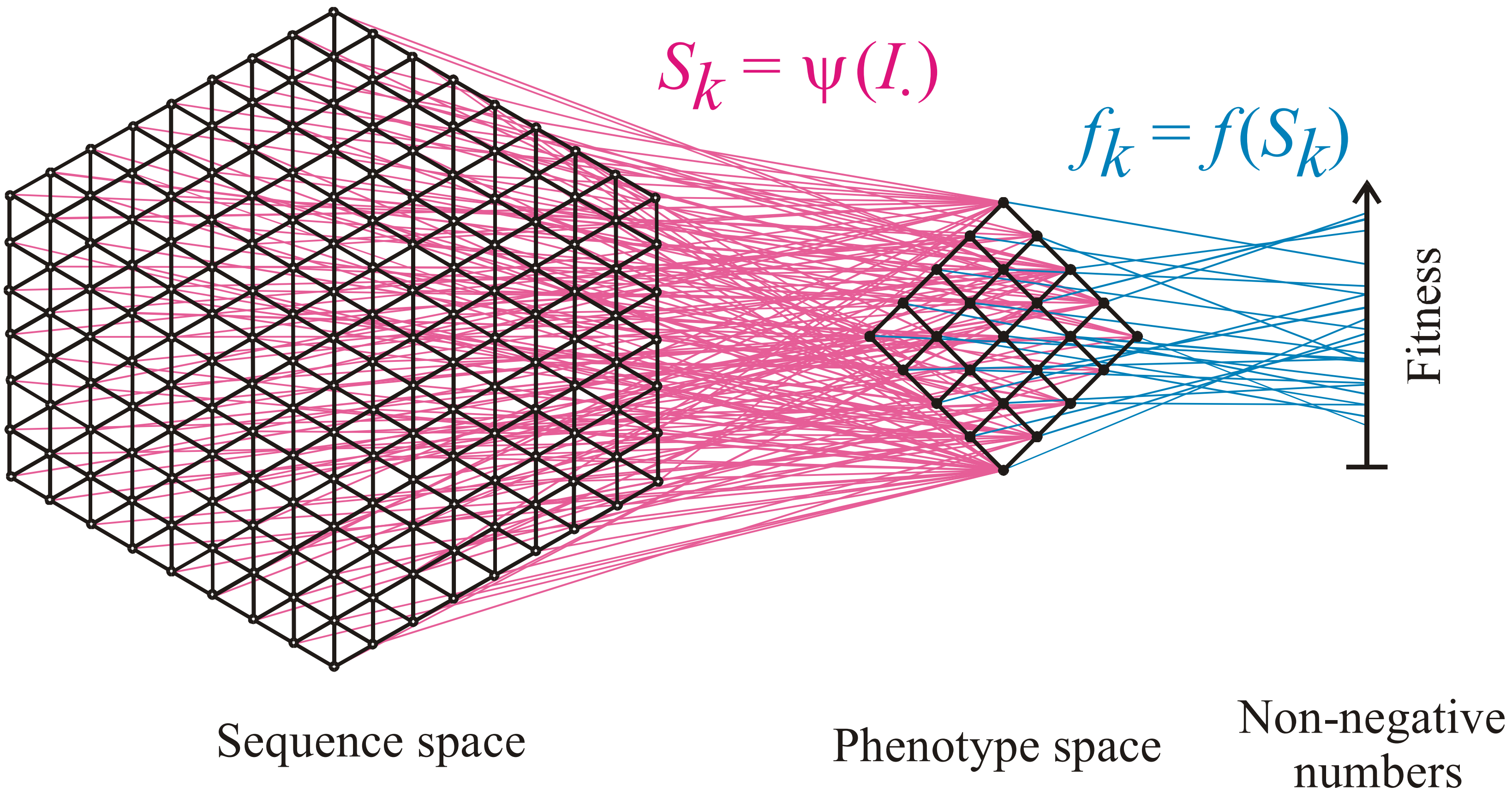
C = 0 and G = 1, for example,

"0" ≡ 00000 = CCCCC,

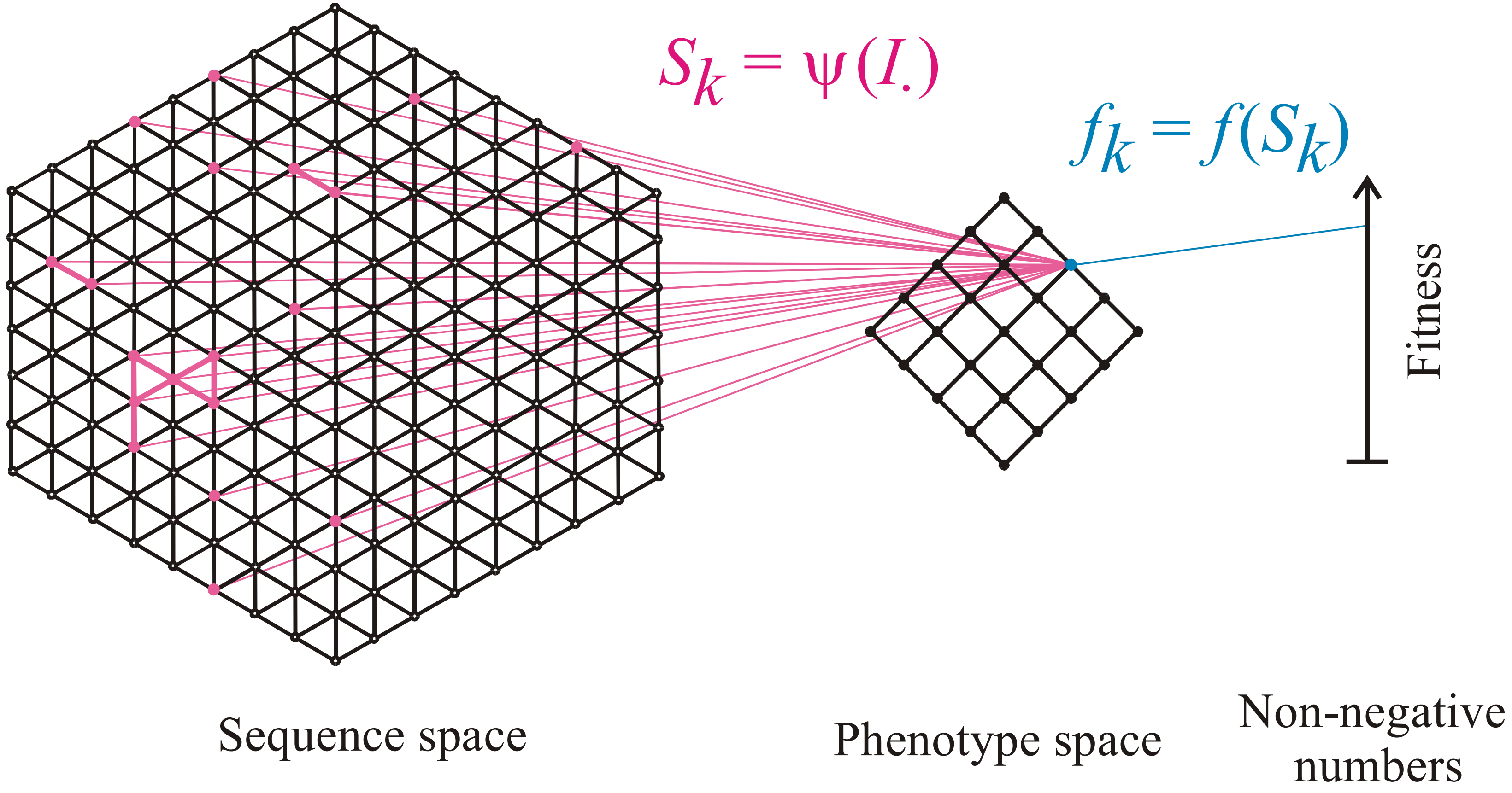
"14" ≡ 01110 = CGGGC,

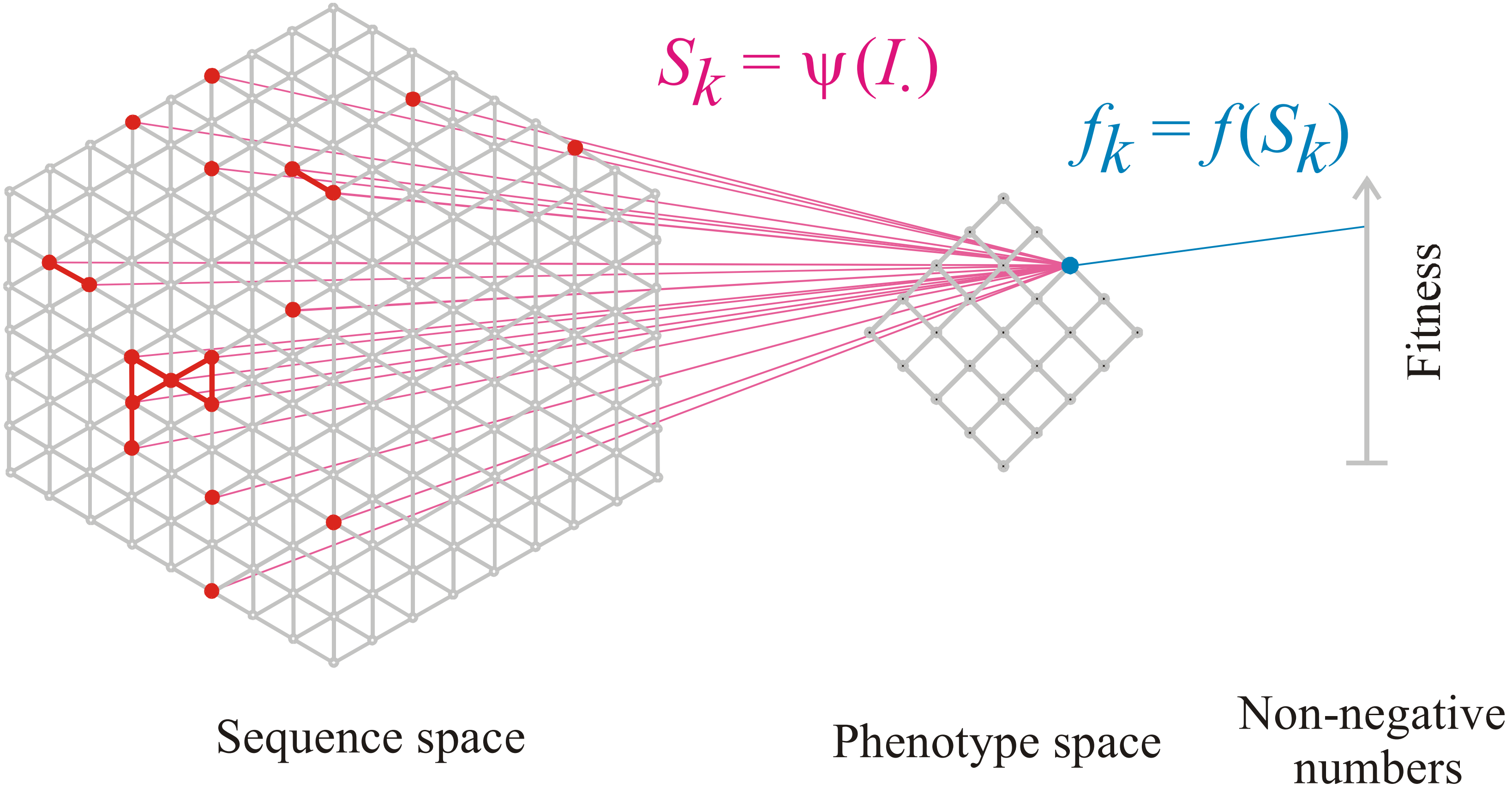
"29" ≡ 11101 = GGGCG, etc.

Sequence space of binary sequences of chain lenght n=5



Mapping from sequence space into phenotype space and into fitness values


$S_k = \psi(I_.)$
$f_k = f(S_k)$
Fitness
Sequence space
Phenotype space
Non-negative numbers

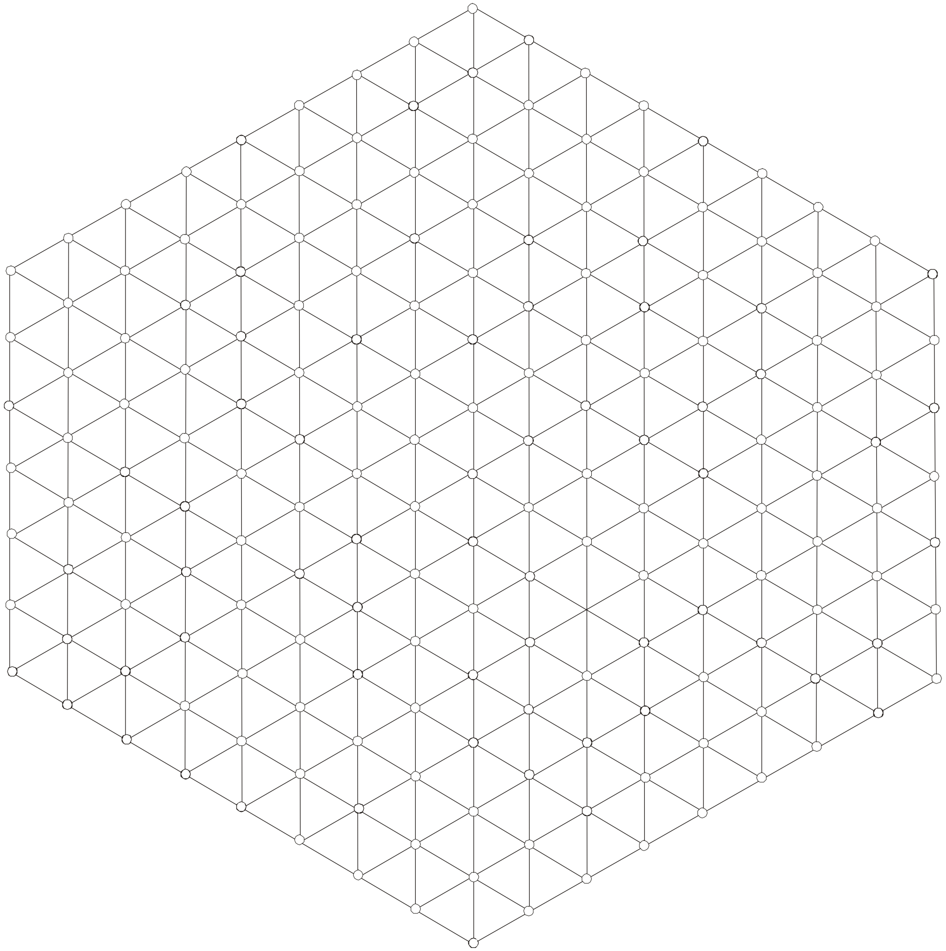

$S_k = \psi(I_.)$
$f_k = f(S_k)$
Fitness
Sequence space
Phenotype space
Non-negative numbers

Neutral networks of small RNA molecules can be computed by exhaustive folding of complete sequence spaces, i.e. all RNA sequences of a given chain length. This number, **$N=4^n$** , becomes very large with increasing length, and is prohibitive for numerical computations.

Neutral networks can be modelled by **random graphs** in sequence space. In this approach, nodes are inserted randomly into sequence space until the size of the pre-image, i.e. the number of neutral sequences, matches the neutral network to be studied.

Step 00

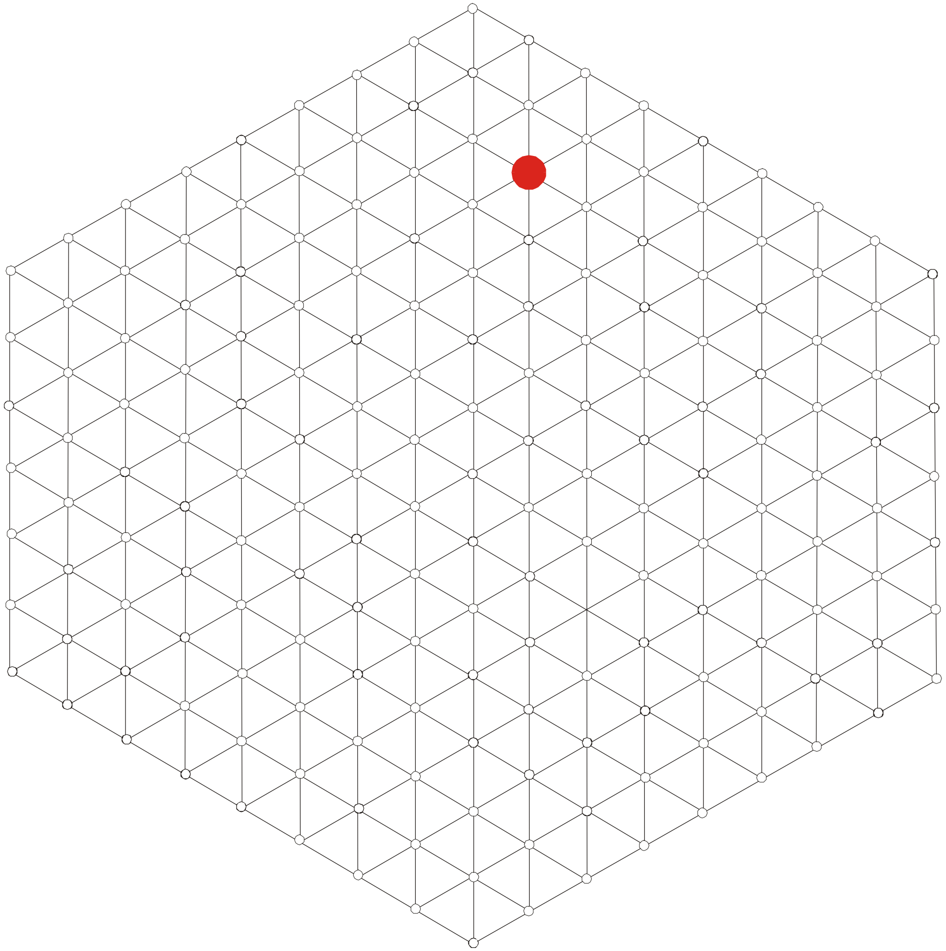
Sketch of sequence space



Random graph approach to neutral networks

Step 01

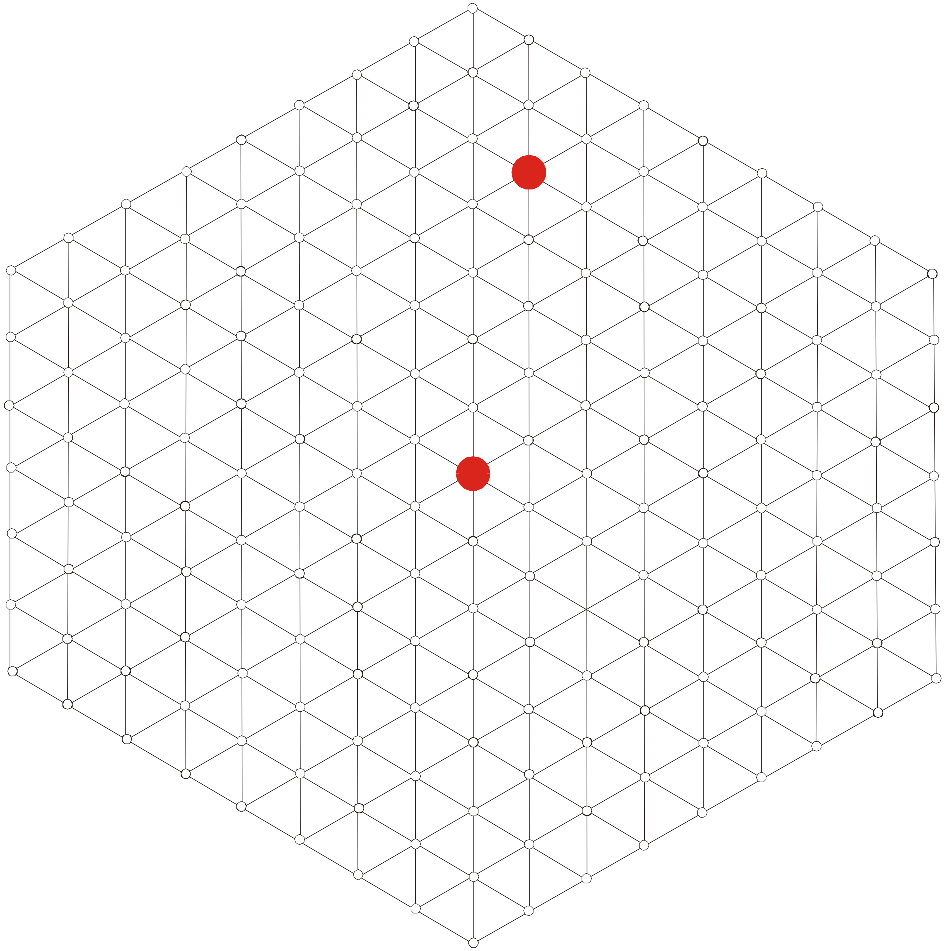
Sketch of sequence space



Random graph approach to neutral networks

Step 02

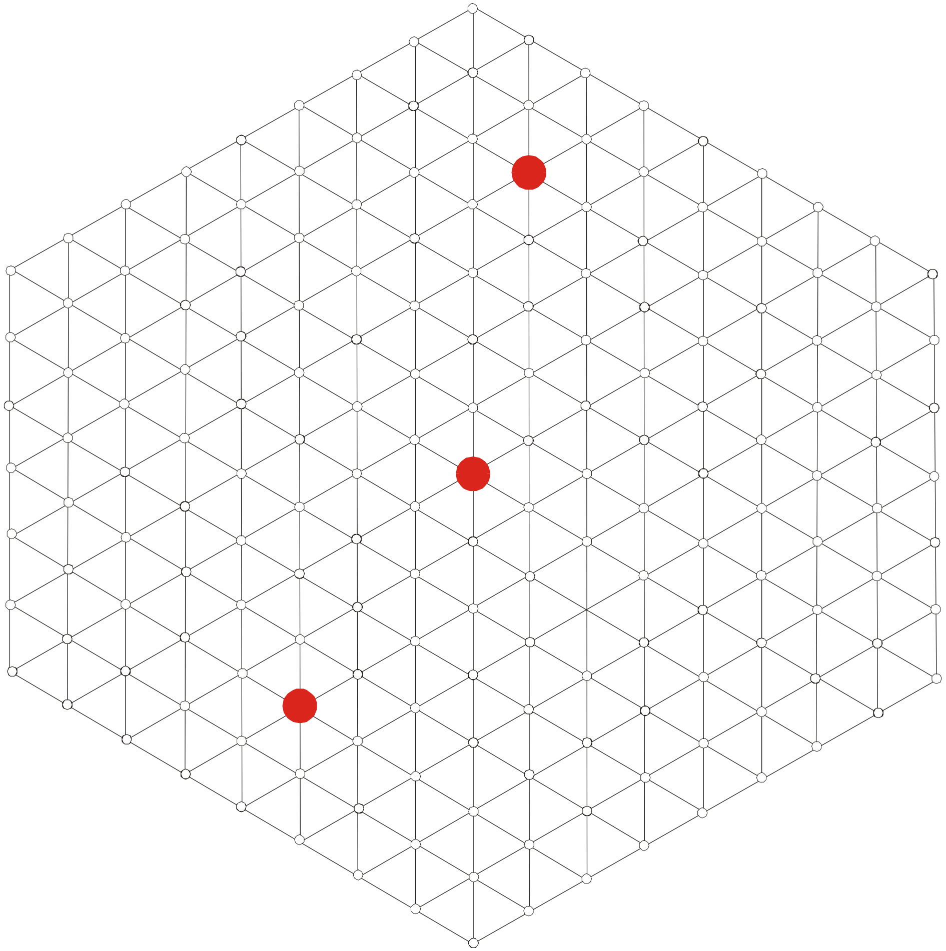
Sketch of sequence space



Random graph approach to neutral networks

Step 03

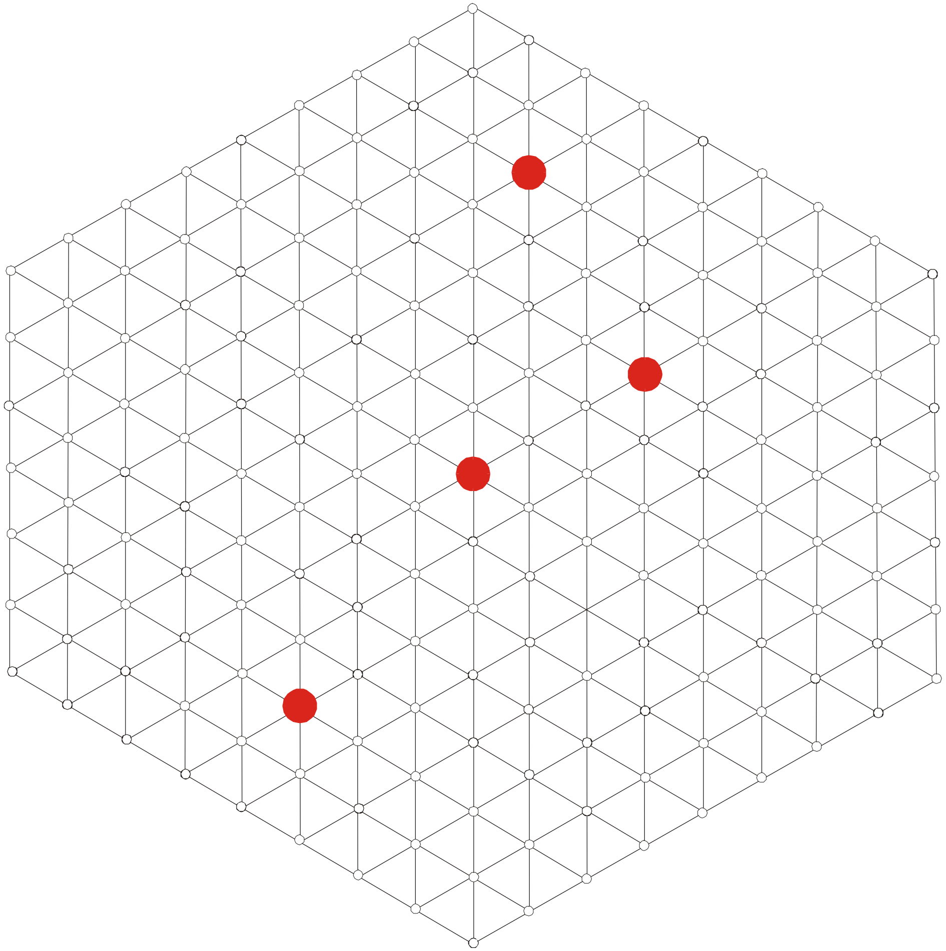
Sketch of sequence space



Random graph approach to neutral networks

Step 04

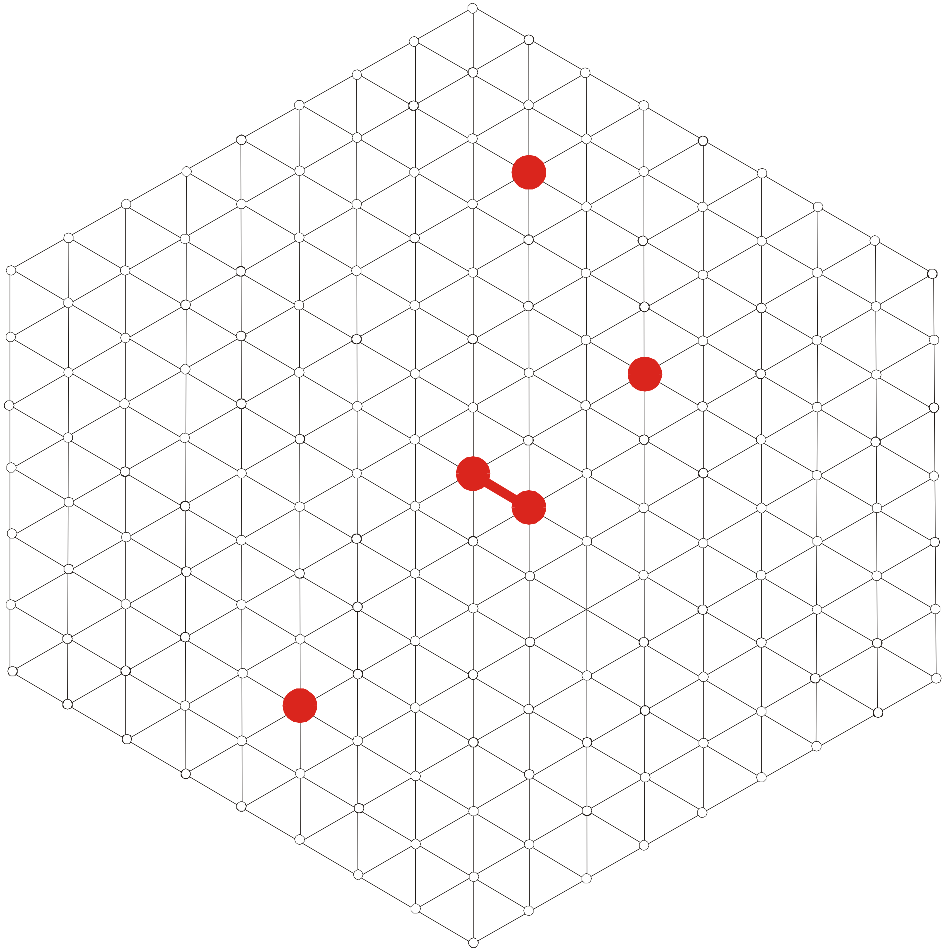
Sketch of sequence space



Random graph approach to neutral networks

Step 05

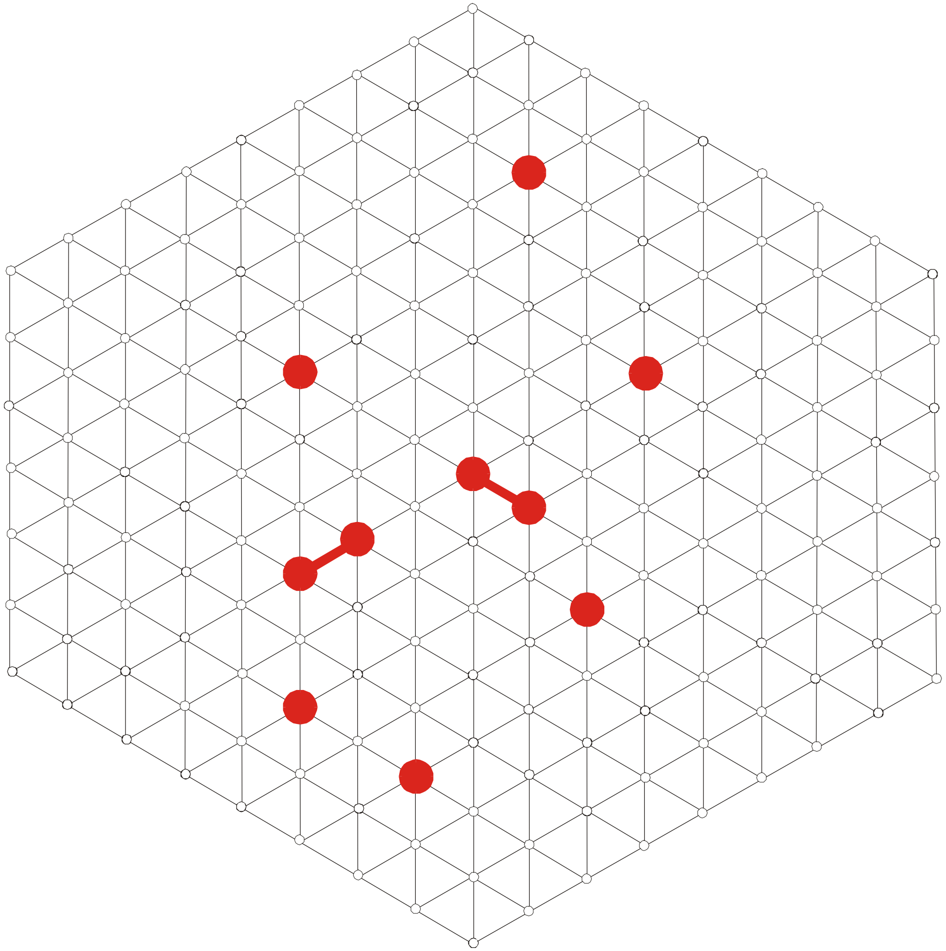
Sketch of sequence space



Random graph approach to neutral networks

Step 10

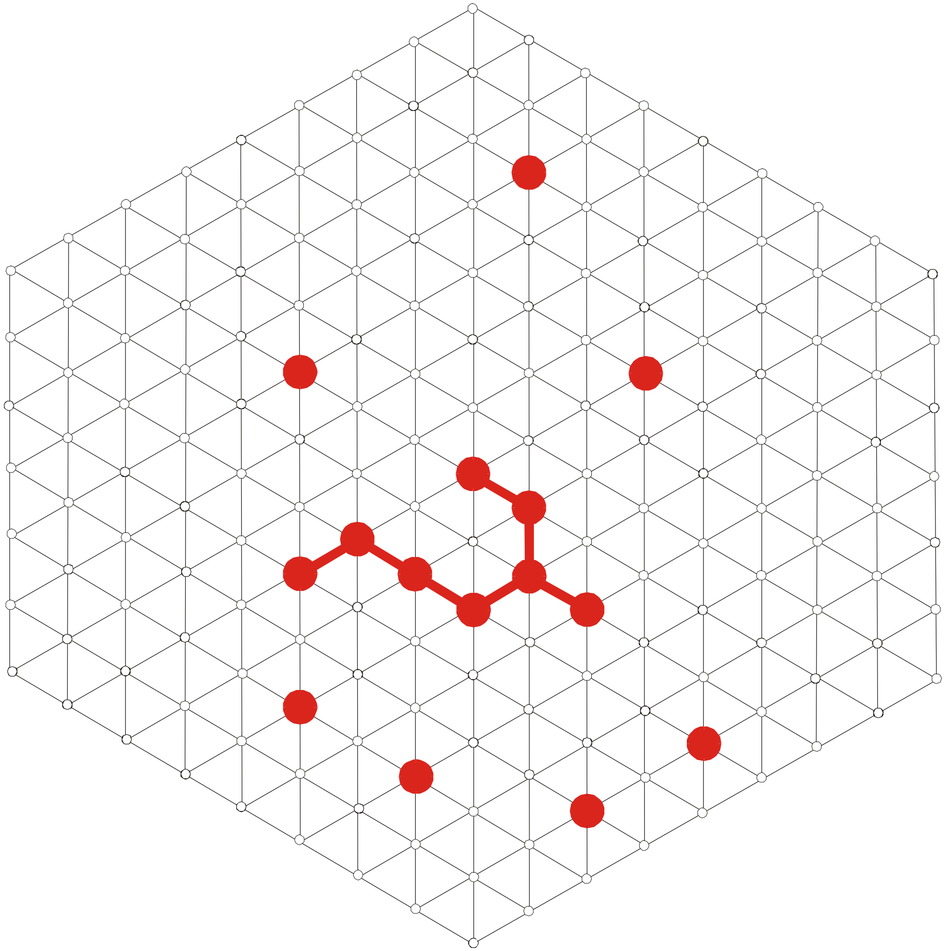
Sketch of sequence space



Random graph approach to neutral networks

Step 15

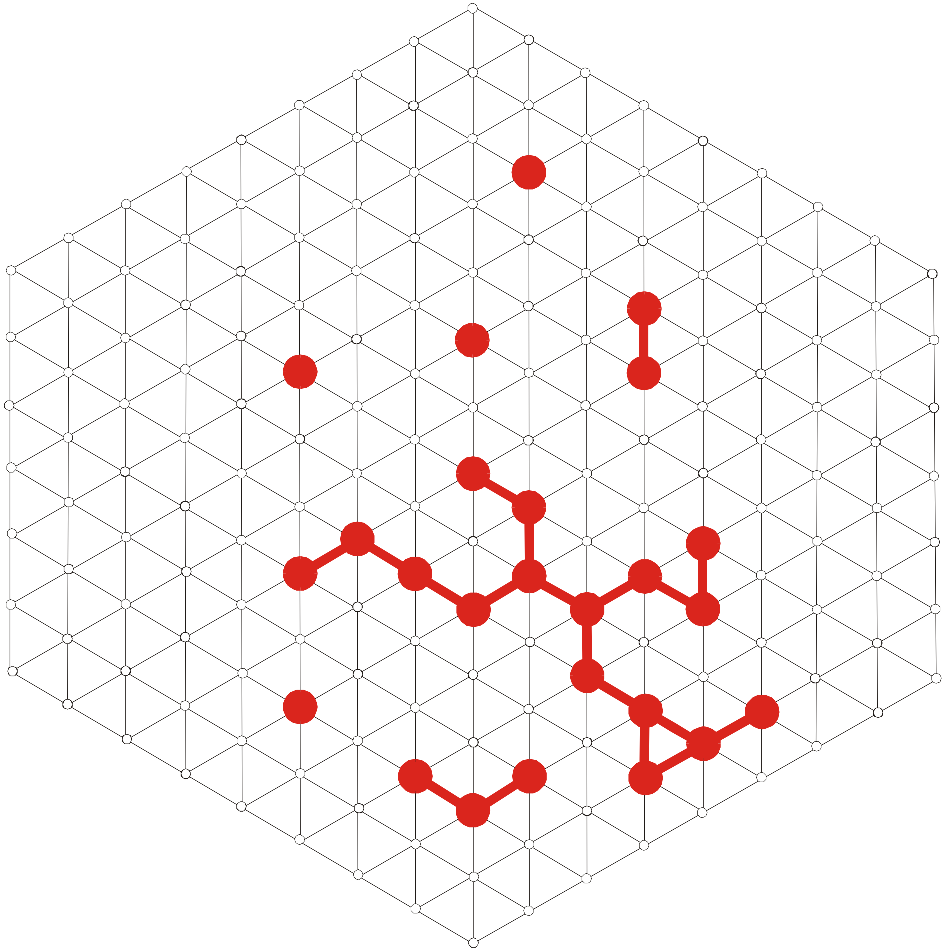
Sketch of sequence space



Random graph approach to neutral networks

Step 25

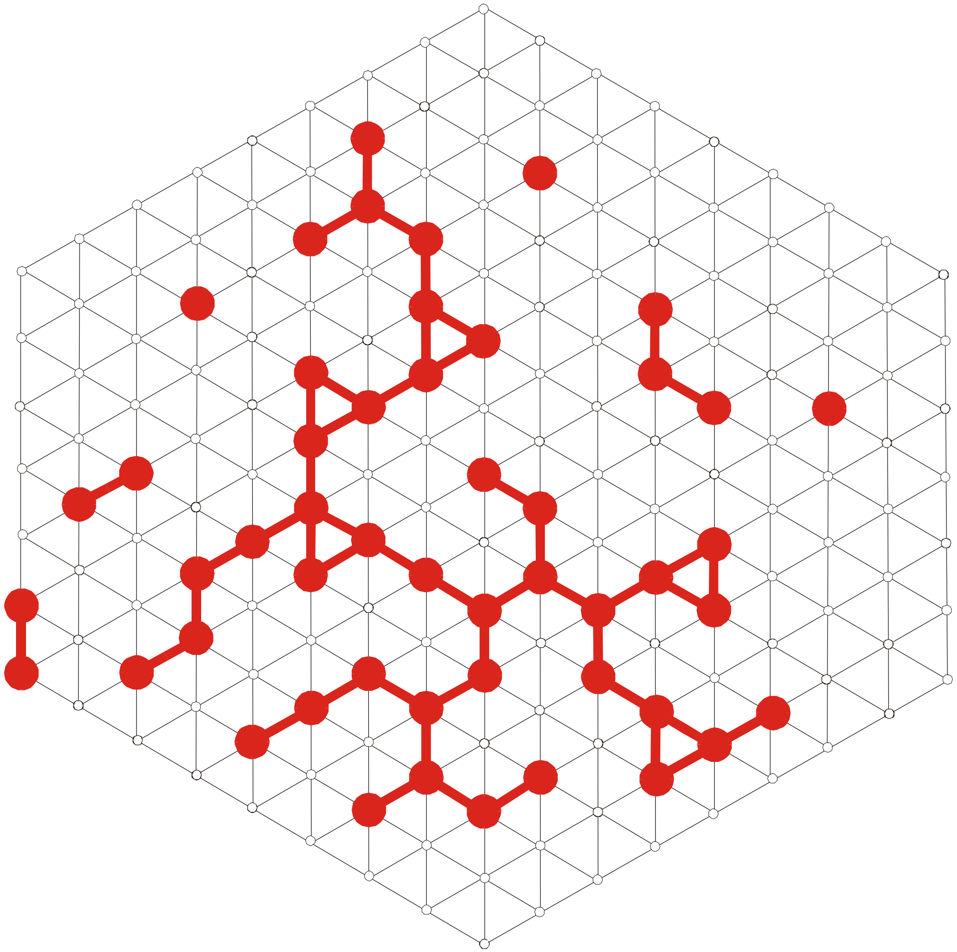
Sketch of sequence space



Random graph approach to neutral networks

Step 50

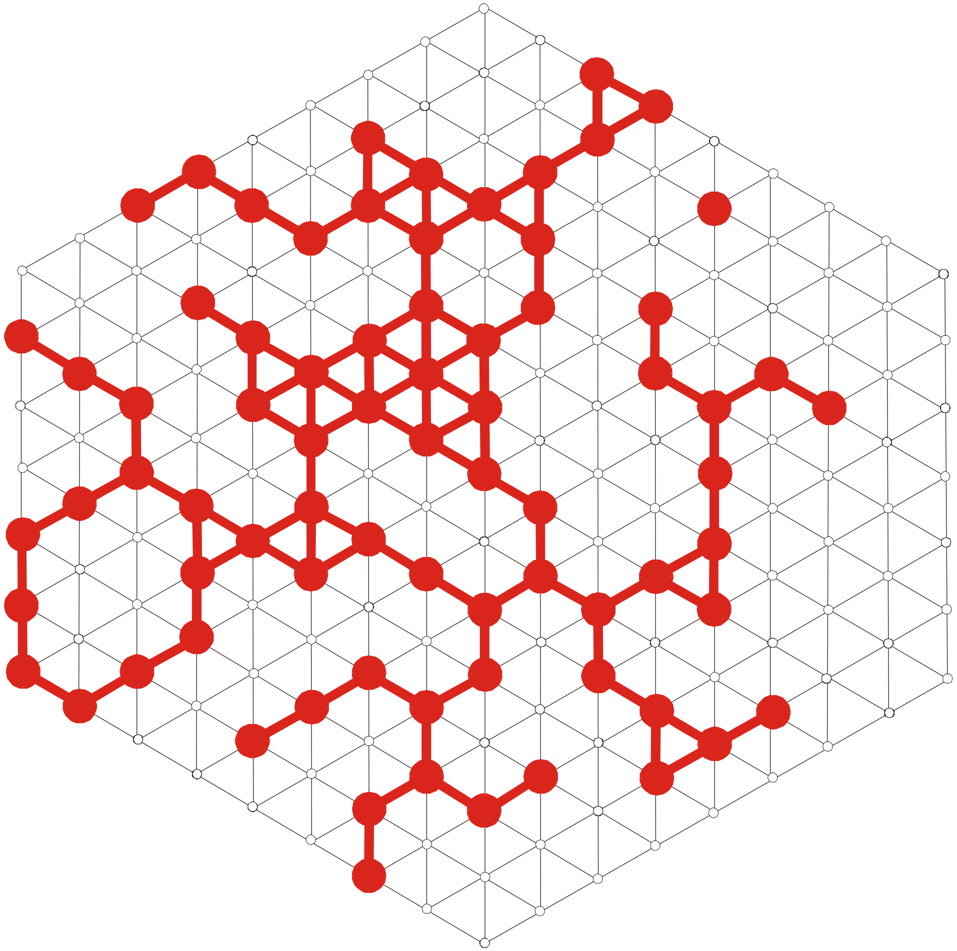
Sketch of sequence space



Random graph approach to neutral networks

Step 75

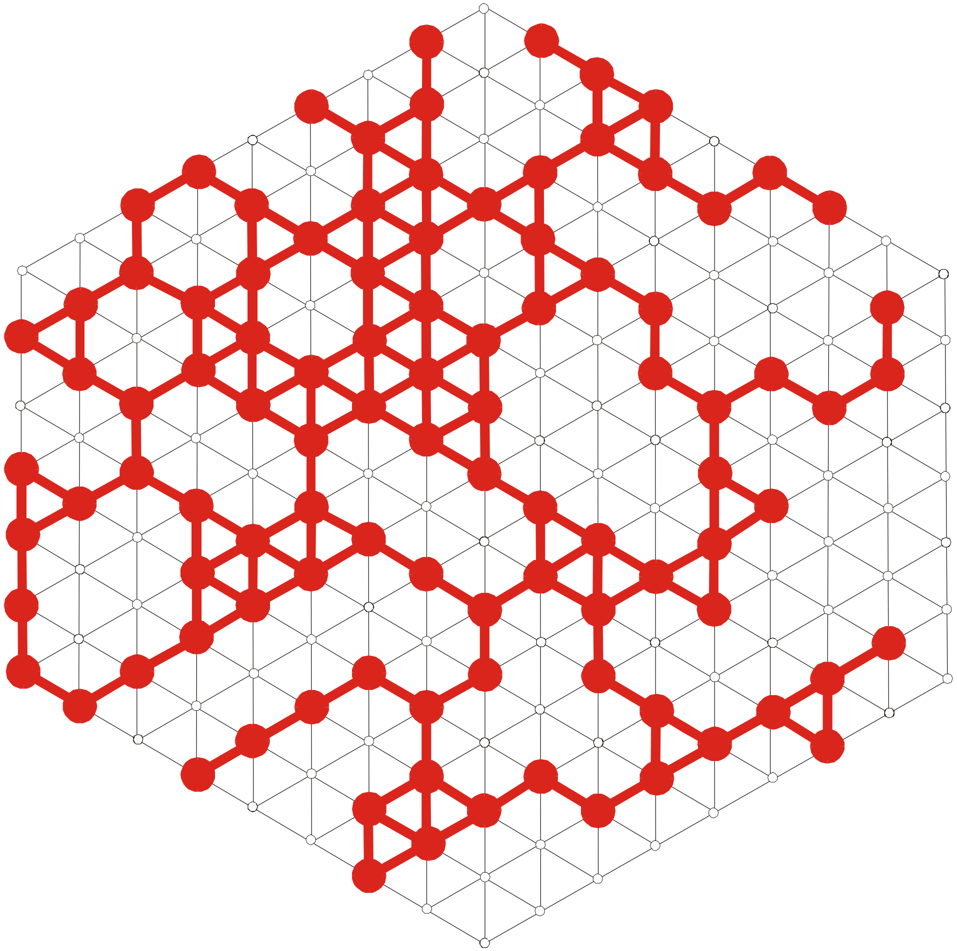
Sketch of sequence space



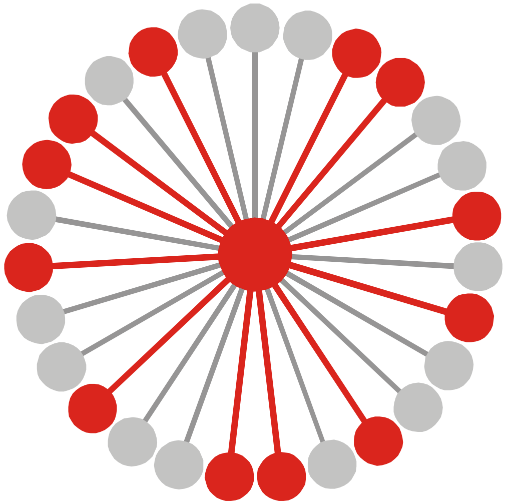
Random graph approach to neutral networks

Step 100

Sketch of sequence space



Random graph approach to neutral networks



$$G_k = y^{-1}(S_k)\ \mathrm{U} \{ I_j \mid y(I_j) = S_k \}$$

$$\lambda_j = 12 / 27 , \qquad \bar{\lambda}_k = \frac{\underset{j \in |G_k|}{ø} \ l_j(k)}{|G_k|}$$

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

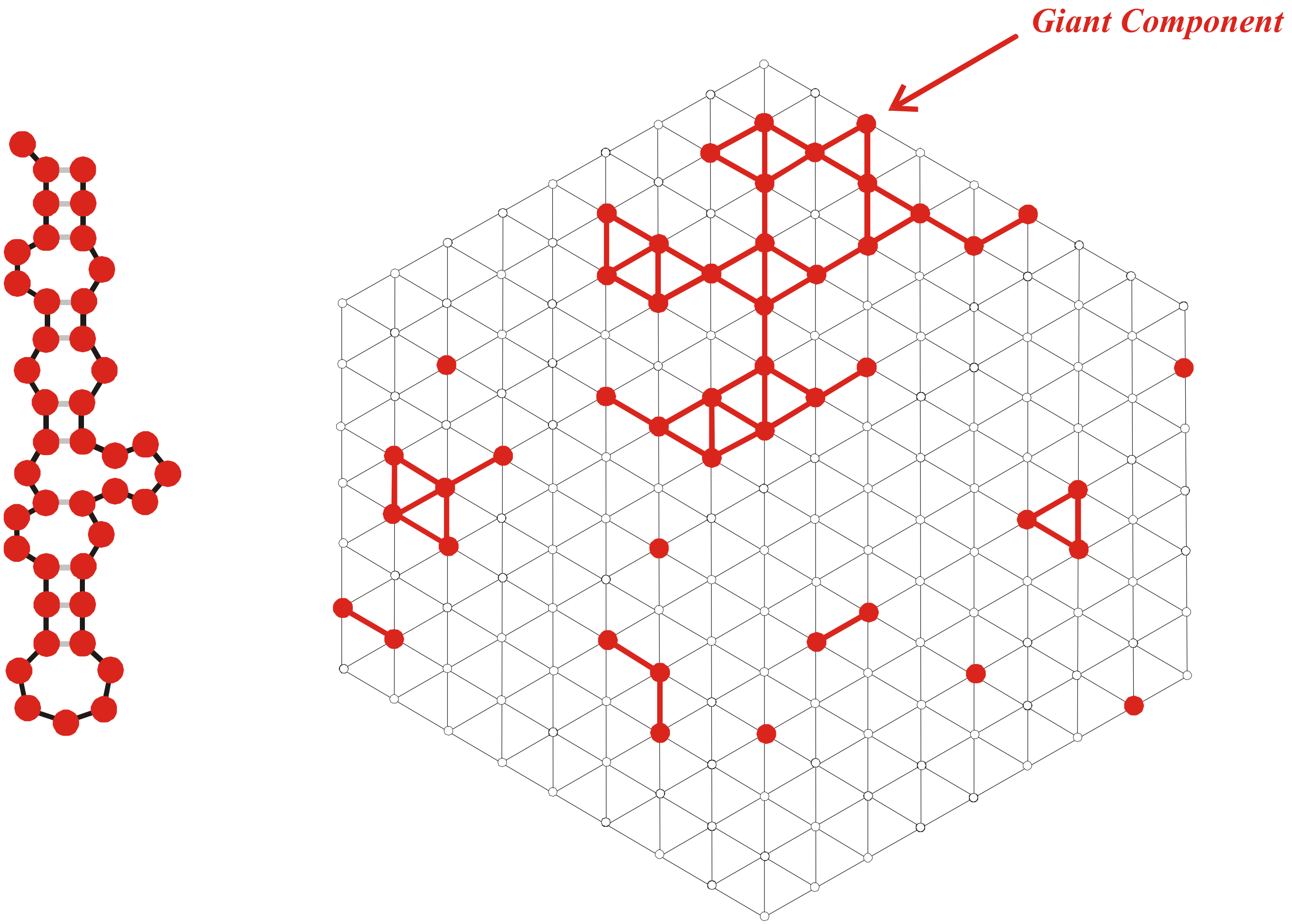
Alphabet size k : **AUGC** ñ k = 4

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

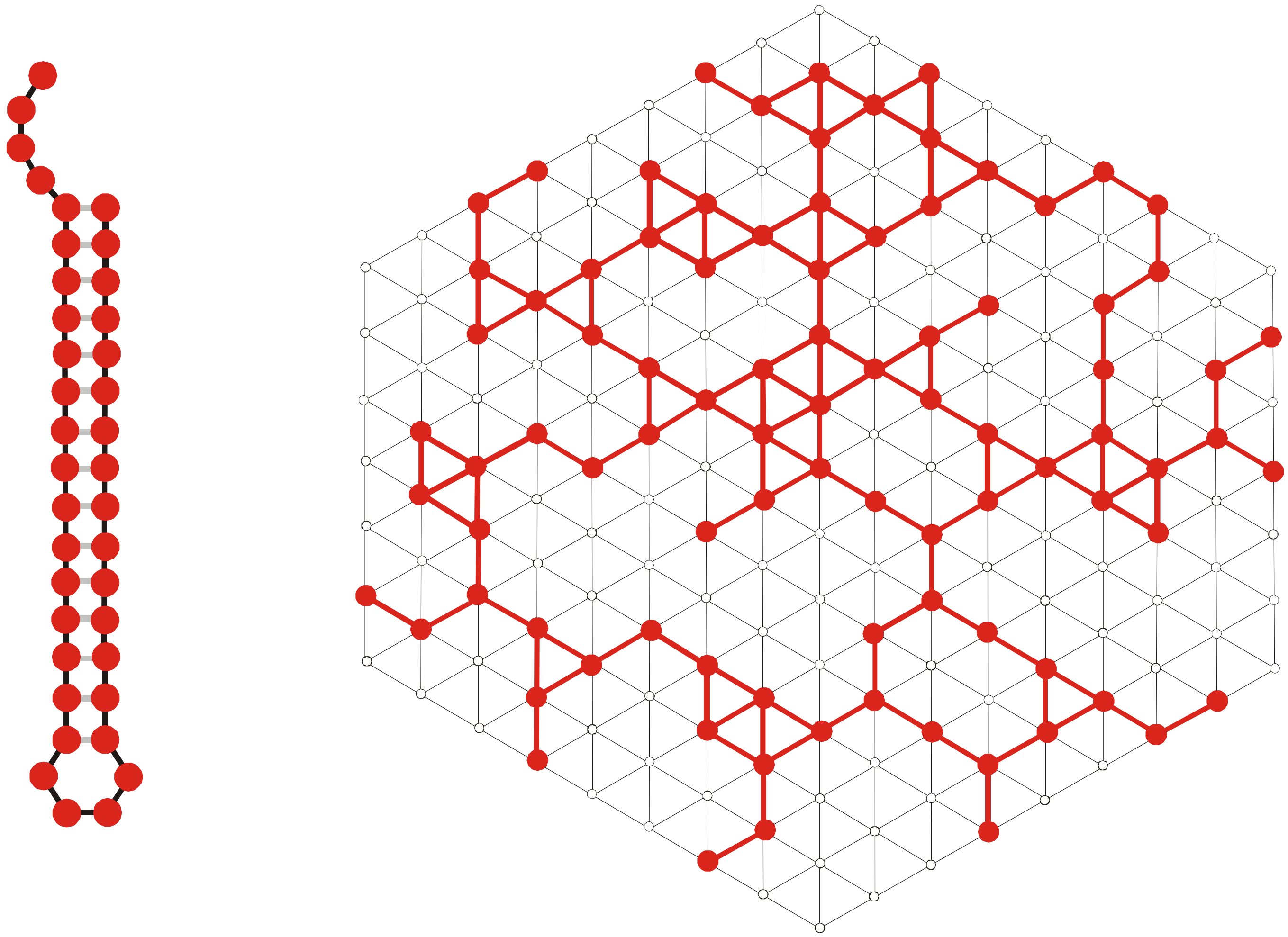
$\bar{\lambda}_k < \lambda_{cr}$ . . . . network **G**$_k$ is **not** connected

k	l_{cr}
2	0.5
3	0.4226
4	0.3700

Mean degree of neutrality and connectivity of neutral networks



A multi-component neutral network



A connected neutral network

Optimization of RNA molecules *in silico*

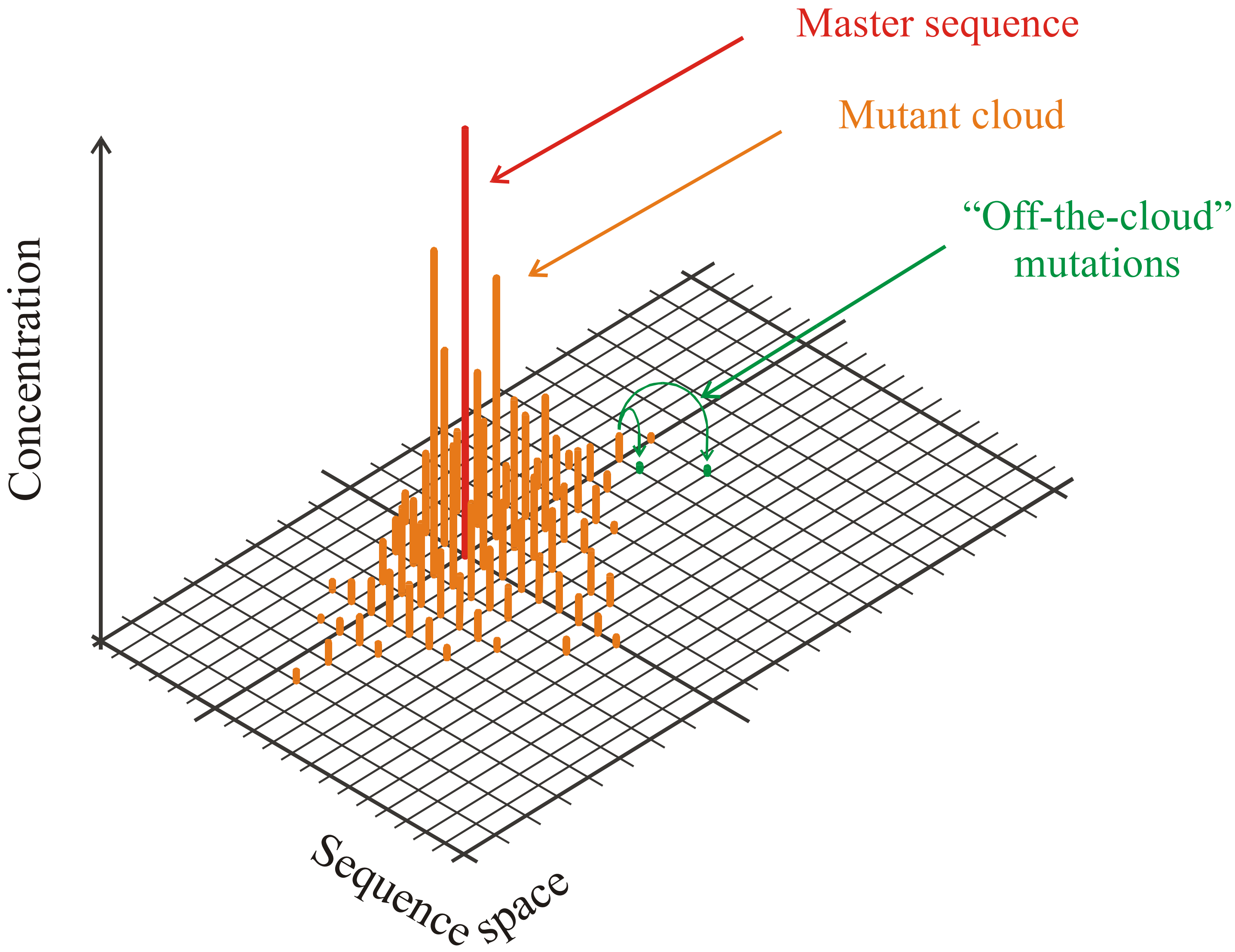
W.Fontana, P.Schuster, ***A computer model of evolutionary optimization***. Biophysical Chemistry **26** (1987), 123-147

W.Fontana, W.Schnabl, P.Schuster, ***Physical aspects of evolutionary optimization and adaptation***. Phys.Rev.A **40** (1989), 3301-3321

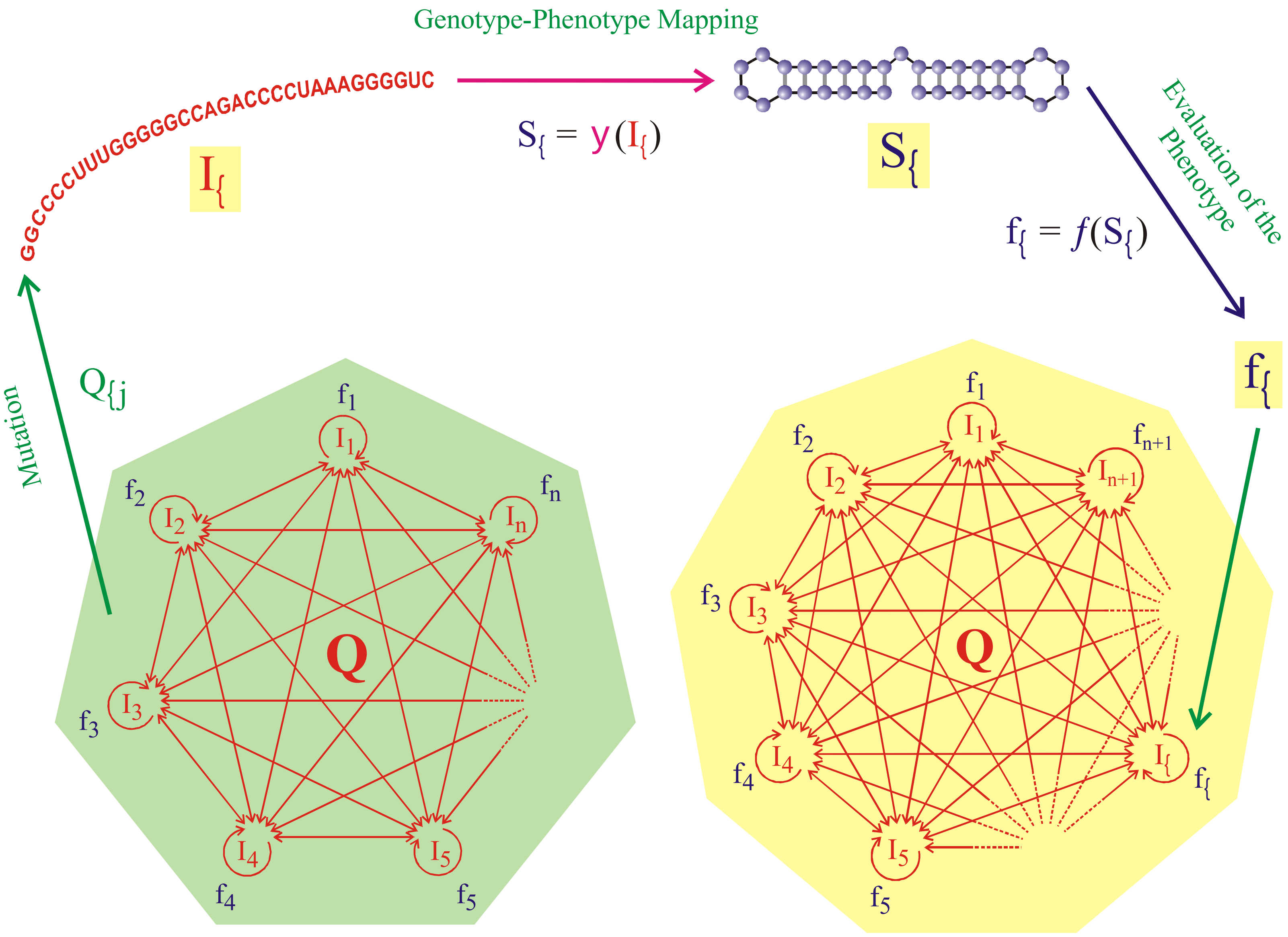
M.A.Huynen, W.Fontana, P.F.Stadler, ***Smoothness within ruggedness. The role of neutrality in adaptation***. Proc.Natl.Acad.Sci.USA **93** (1996), 397-401

W.Fontana, P.Schuster, ***Continuity in evolution. On the nature of transitions***. Science **280** (1998), 1451-1455

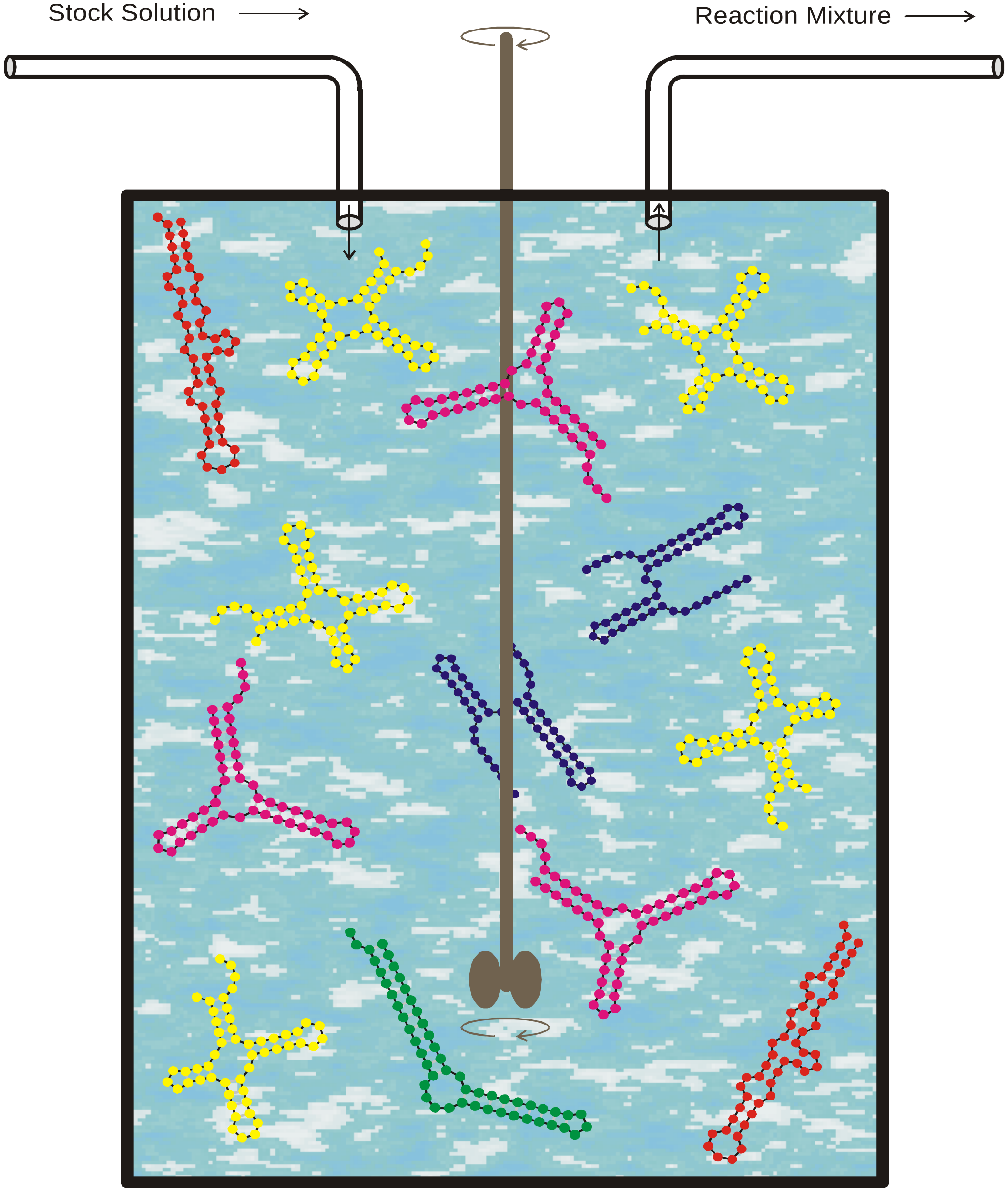
W.Fontana, P.Schuster, ***Shaping space. The possible and the attainable in RNA genotype-phenotype mapping***. J.Theor.Biol. **194** (1998), 491-515



The molecular quasispecies
in sequence space


Genotype-Phenotype Mapping
GGCCCCUUUGGGGGCCAGACCCCUAAAGGGGUC
I{
S{ = y(I{)
S{
Evaluation of the Phenotype
f{ = f(S{)
f{
Mutation
Q{j
Q
f1
I1
f2
I2
fn
In
f3
I3
f4
I4
f5
I5
fn+1
In+1
I{
f{

Evolutionary dynamics
including molecular phenotypes

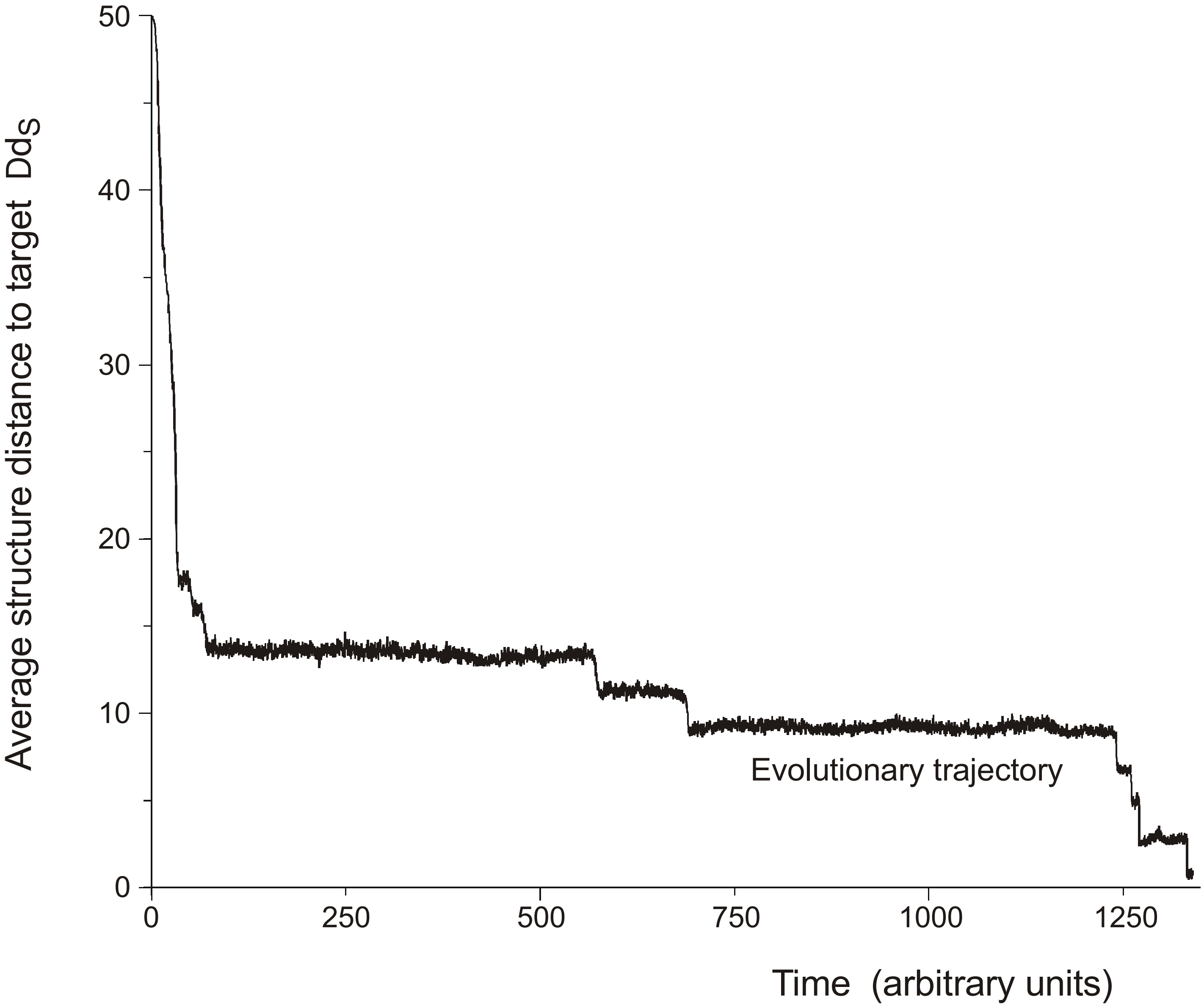


Fitness function:

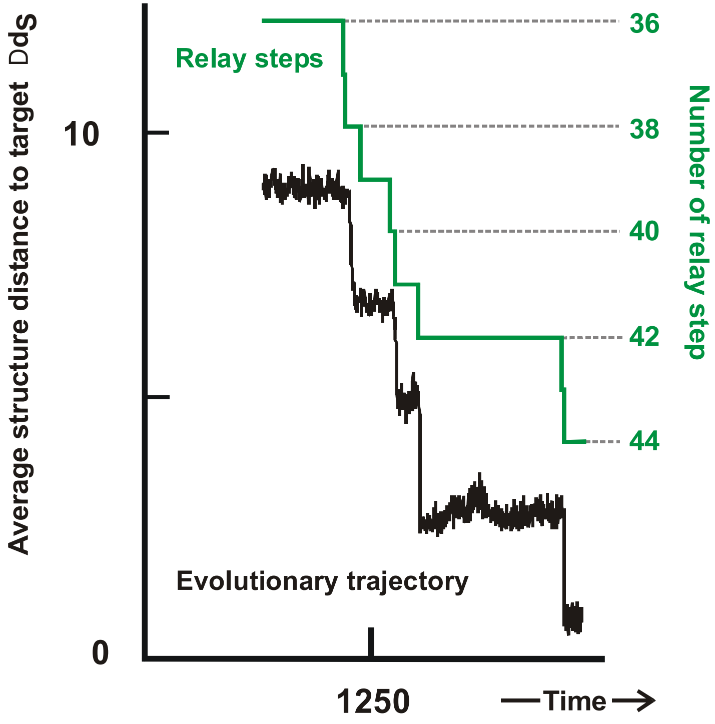
$$f_k = g / [a + Dd_S^{(k)}]$$

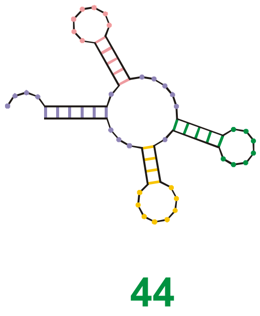
$$Dd_S^{(k)} = d^s(I_k, I_t)$$

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

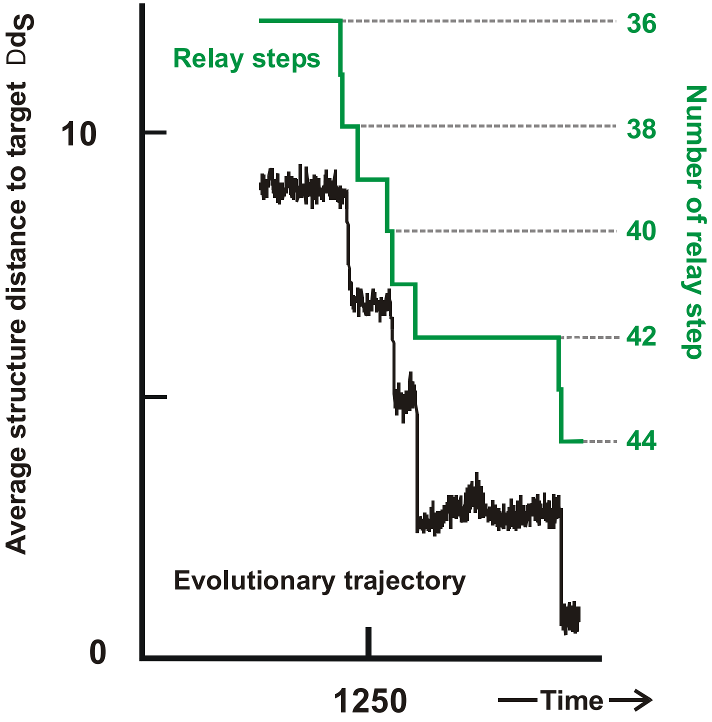


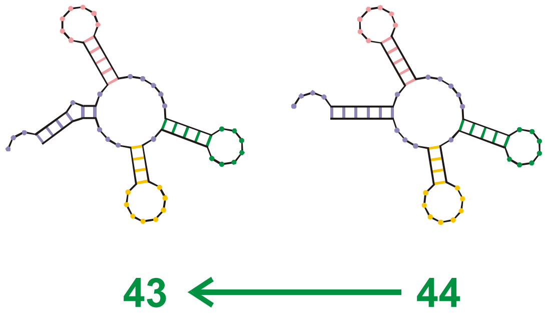
In silico optimization in the flow reactor: Trajectory



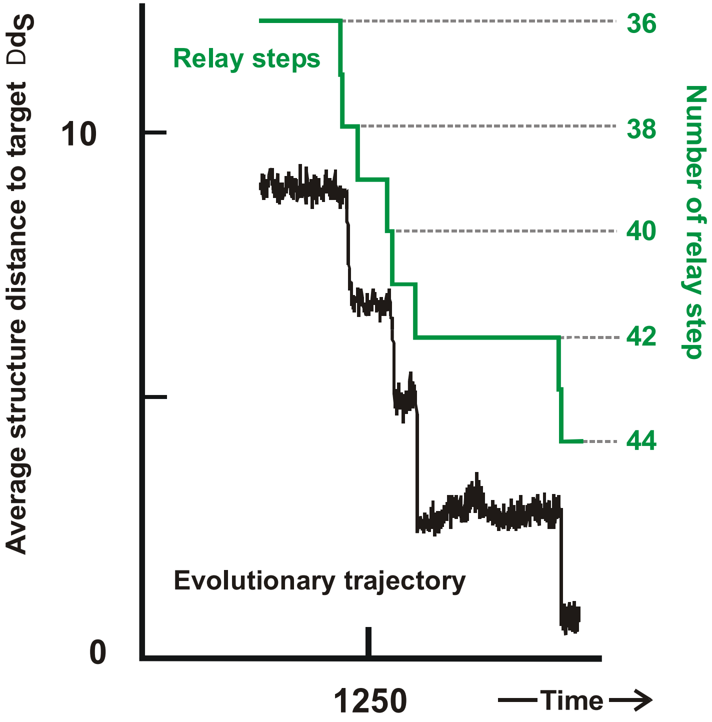


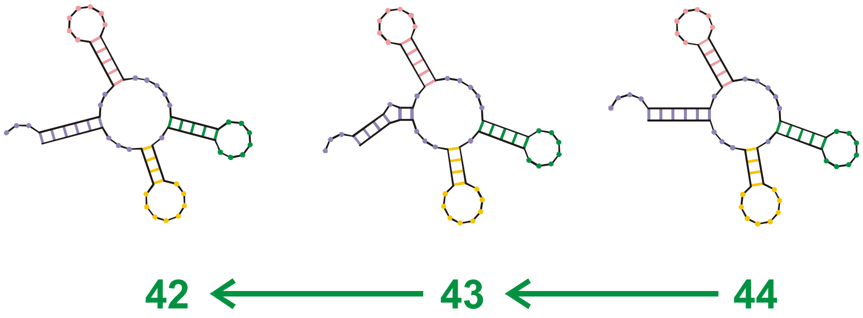
Endconformation of optimization



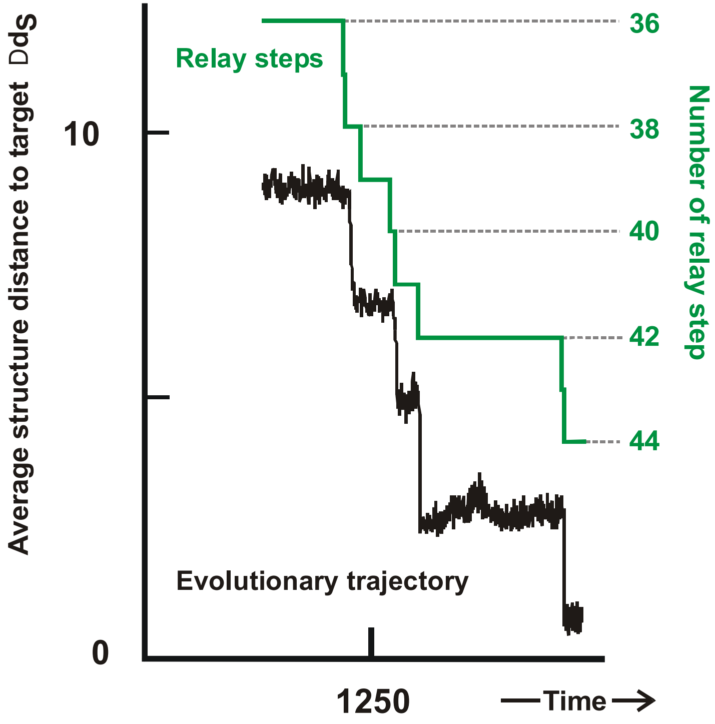


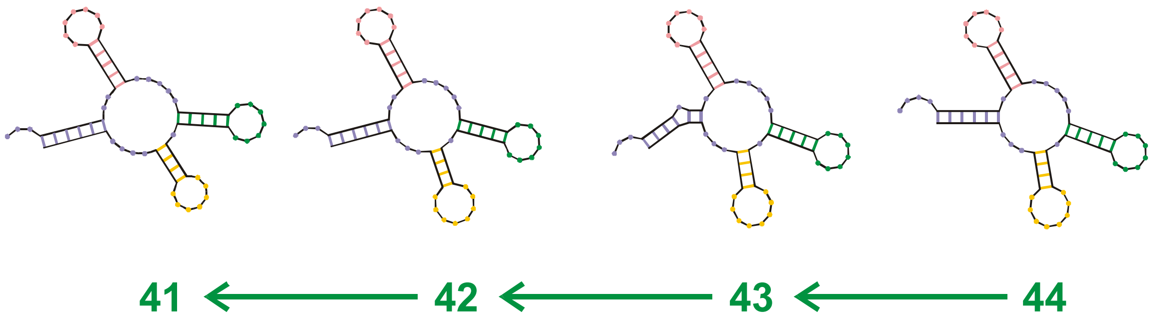
Reconstruction of the last step 43 Á 44



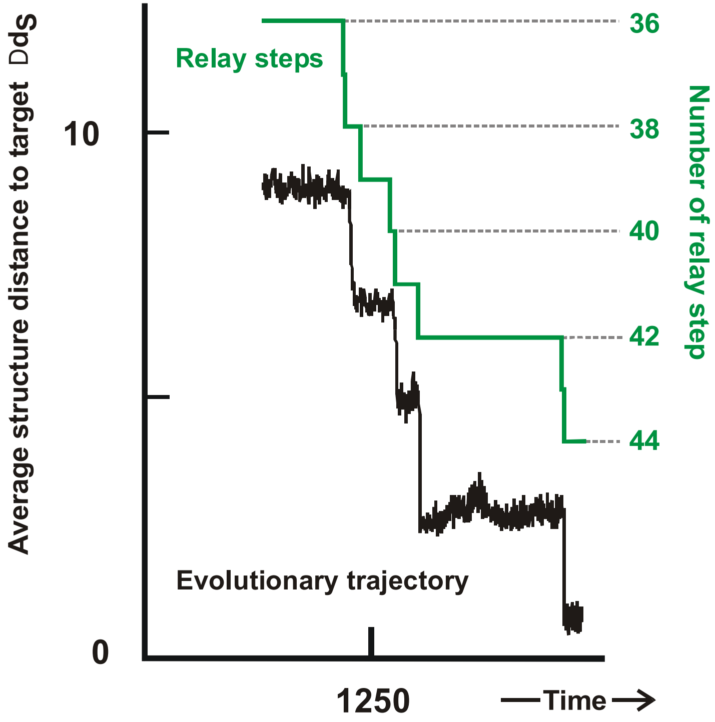


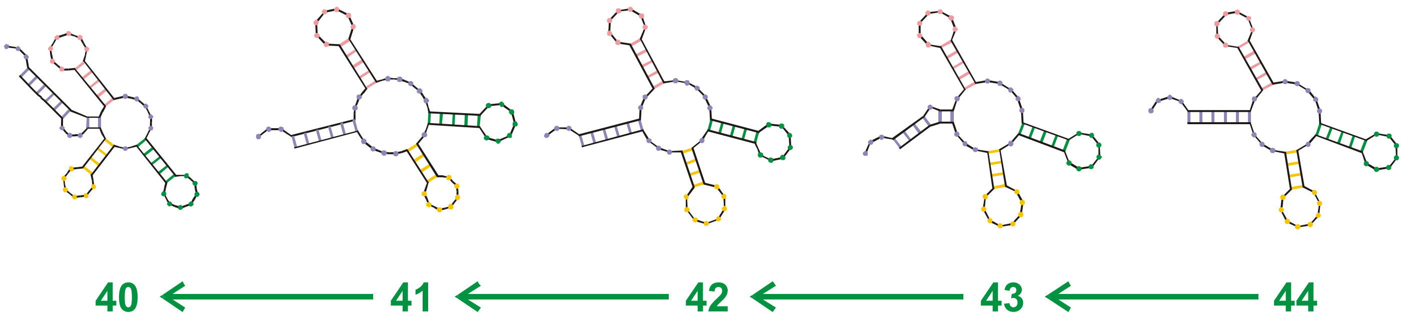
Reconstruction of last-but-one step 42 Á 43 (Á 44)



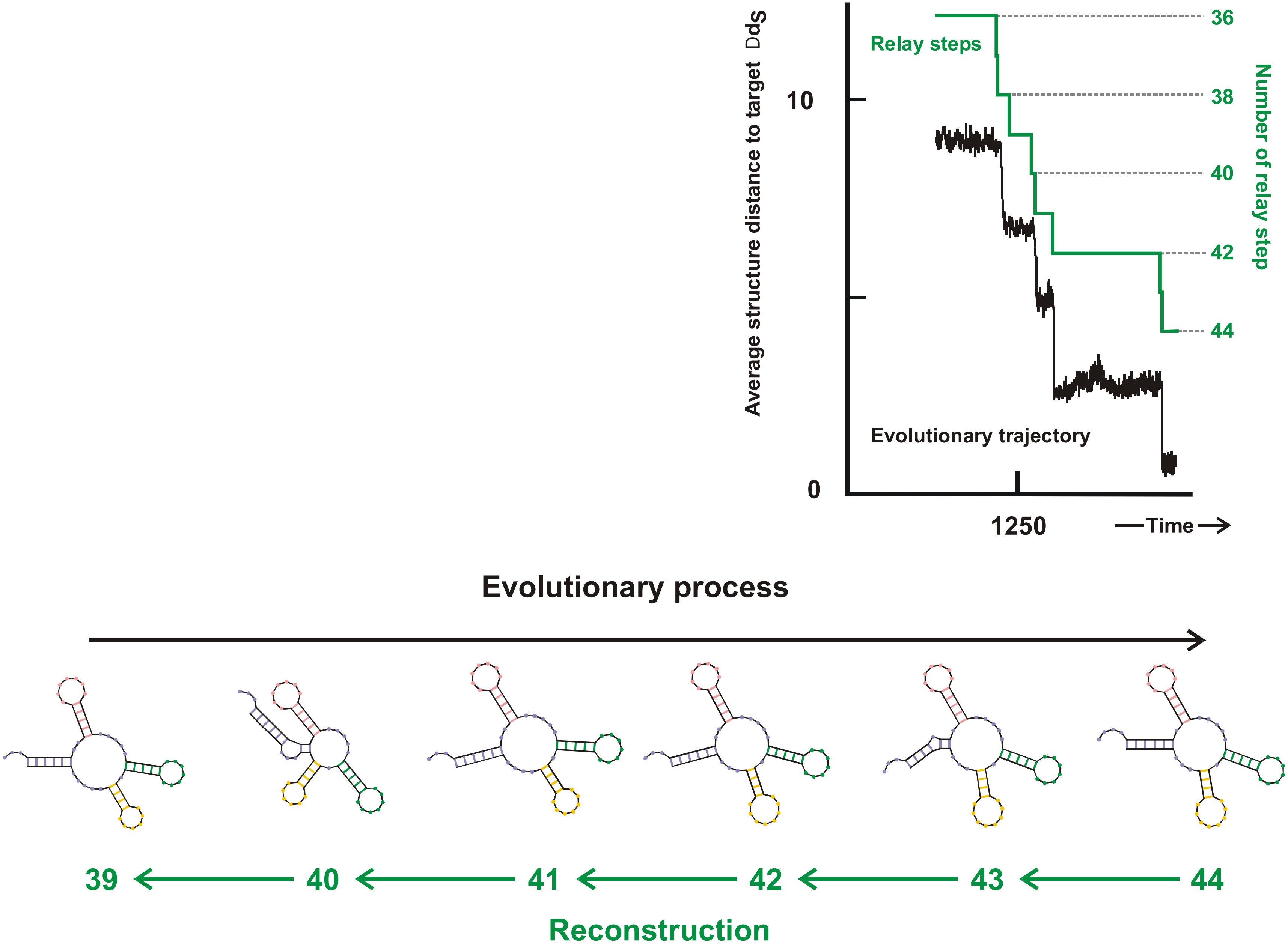


Reconstruction of step 41 Á 42 (Á 43 Á 44)

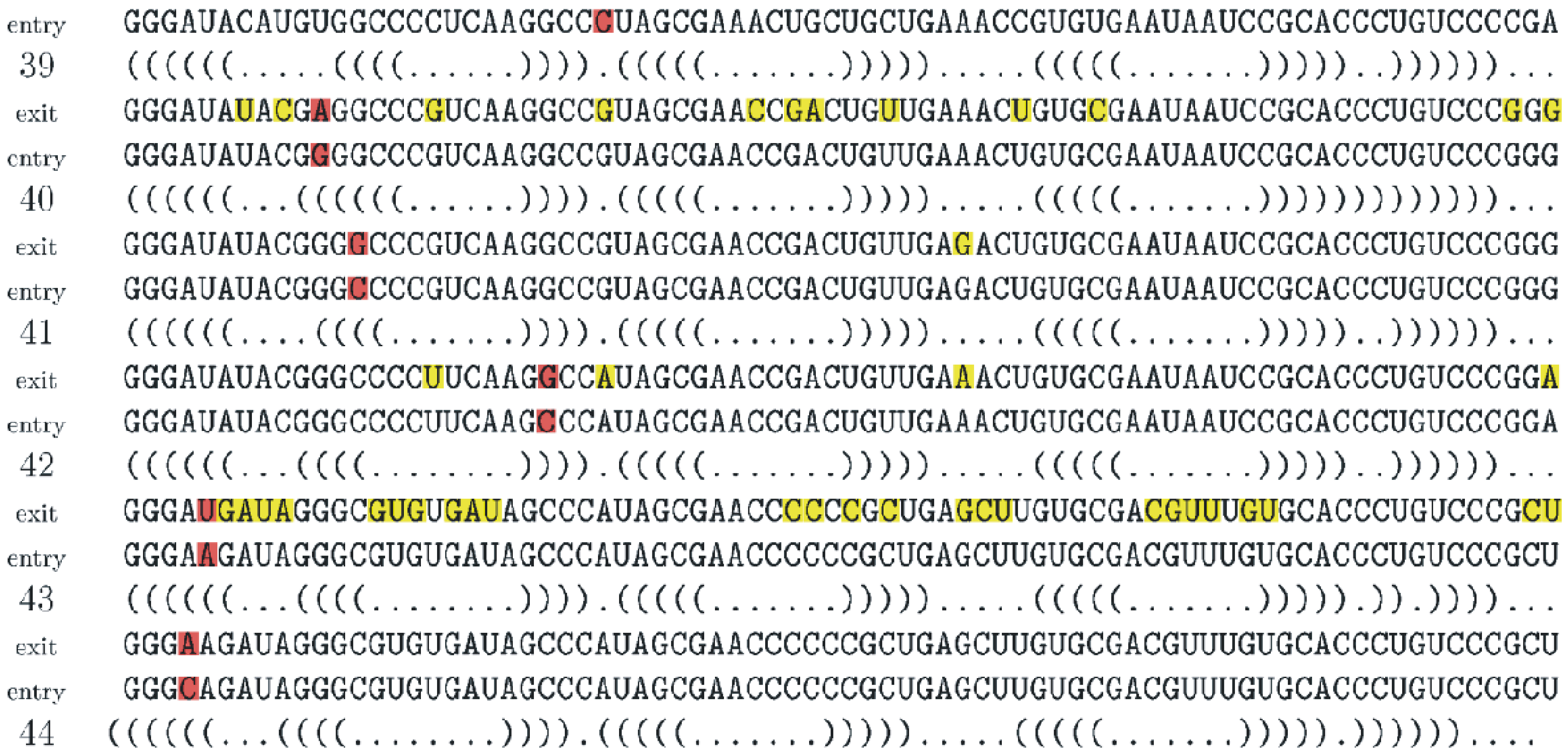




Reconstruction of step 40 Á 41 (Á 42 Á 43 Á 44)



Reconstruction of the relay series

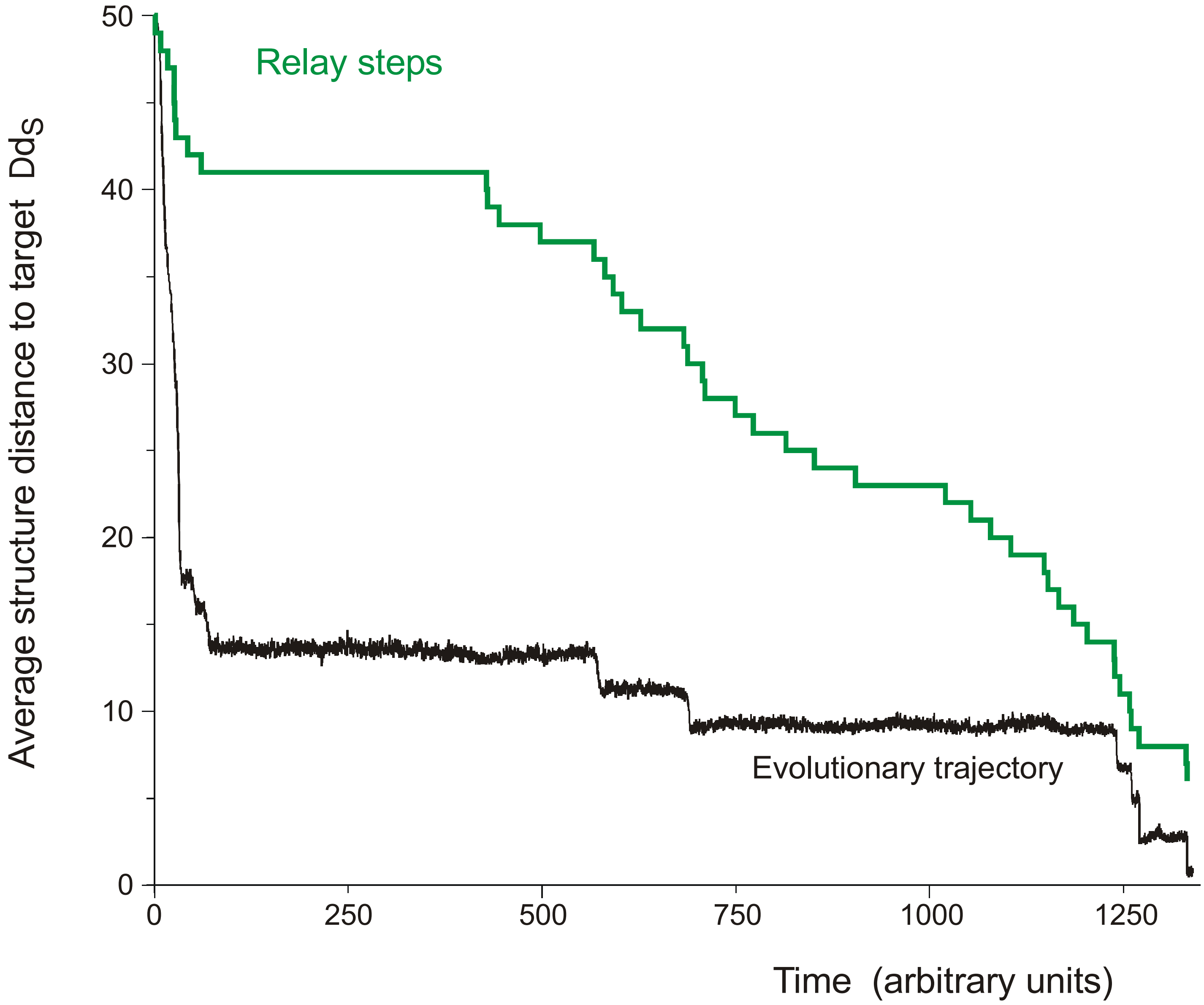


```
entry   GGGAUACAUGUGGCCCCUCAAGGCCCUAGCGAAACUGCUGCUGAAACCGUGUGAAUAAUCCGCACCCUGUCCCCGA
39      ((((((.....((((......)))).(((((.......))))).....(((((.......)))))..))))))...
exit    GGGAUAUACGAGGCCCGUCAAGGCCGUAGCGAACCGACUGUUGAAACUGUGCGAAUAAUCCGCACCCUGUCCCGGG
entry   GGGAUAUACGGGGCCCGUCAAGGCCGUAGCGAACCGACUGUUGAAACUGUGCGAAUAAUCCGCACCCUGUCCCGGG
40      ((((((...((((((......)))).(((((.......))))).....(((((.......)))))))))))))...
exit    GGGAUAUACGGGGCCCGUCAAGGCCGUAGCGAACCGACUGUUGAGACUGUGCGAAUAAUCCGCACCCUGUCCCGGG
entry   GGGAUAUACGGGCCCCGUCAAGGCCGUAGCGAACCGACUGUUGAGACUGUGCGAAUAAUCCGCACCCUGUCCCGGG
41      ((((((....((((.......)))).(((((.......))))).....(((((.......)))))..))))))...
exit    GGGAUAUACGGGCCCCUUCAAGGCCAUAGCGAACCGACUGUUGAAACUGUGCGAAUAAUCCGCACCCUGUCCCGGA
entry   GGGAUAUACGGGCCCCUUCAAGCCCAUAGCGAACCGACUGUUGAAACUGUGCGAAUAAUCCGCACCCUGUCCCGGA
42      ((((((...((((........)))).(((((.......))))).....(((((.......)))))..))))))...
exit    GGGAUGAUAGGGCGUGUGAUAGCCCAUAGCGAACCCCCCGCUGAGCUUGUGCGACGUUUGUGCACCCUGUCCCGCU
entry   GGGAAGAUAGGGCGUGUGAUAGCCCAUAGCGAACCCCCCGCUGAGCUUGUGCGACGUUUGUGCACCCUGUCCCGCU
43      ((((((...((((........)))).(((((.......))))).....(((((.......))))).)).))))...
exit    GGGAAGAUAGGGCGUGUGAUAGCCCAUAGCGAACCCCCCGCUGAGCUUGUGCGACGUUUGUGCACCCUGUCCCGCU
entry   GGGCAGAUAGGGCGUGUGAUAGCCCAUAGCGAACCCCCCGCUGAGCUUGUGCGACGUUUGUGCACCCUGUCCCGCU
44      ((((((...((((........)))).(((((.......))))).....(((((.......))))).))))))....
```

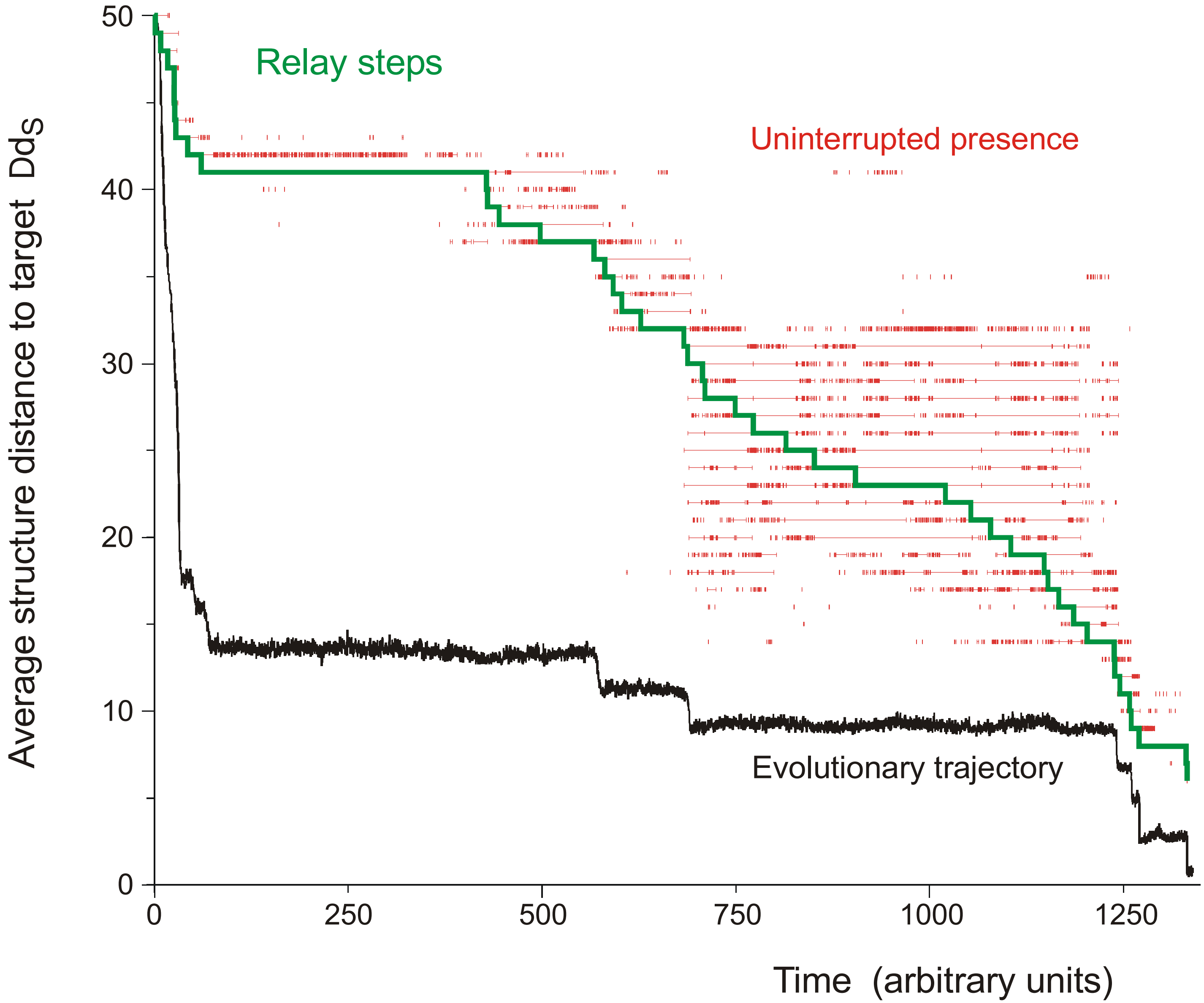
Transition inducing point mutations

Neutral point mutations

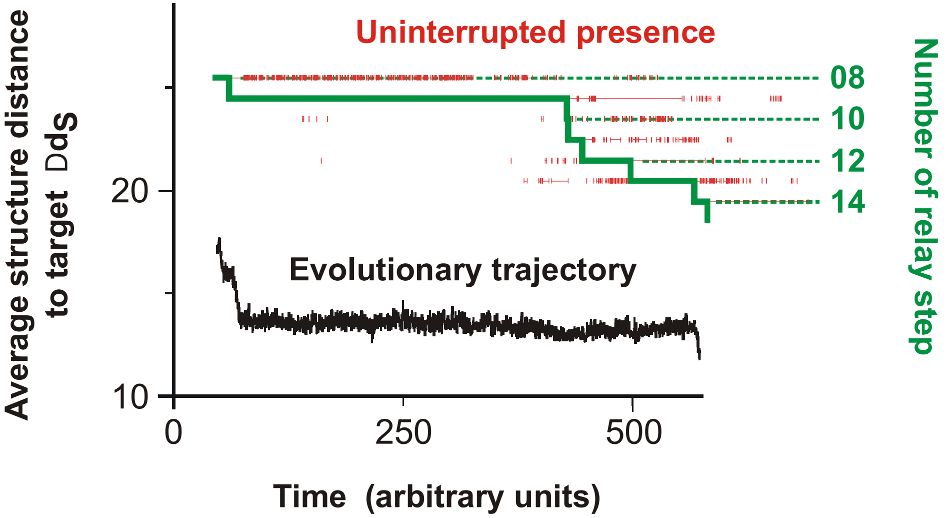
Change in RNA sequences during the final five relay steps 39 Á 44

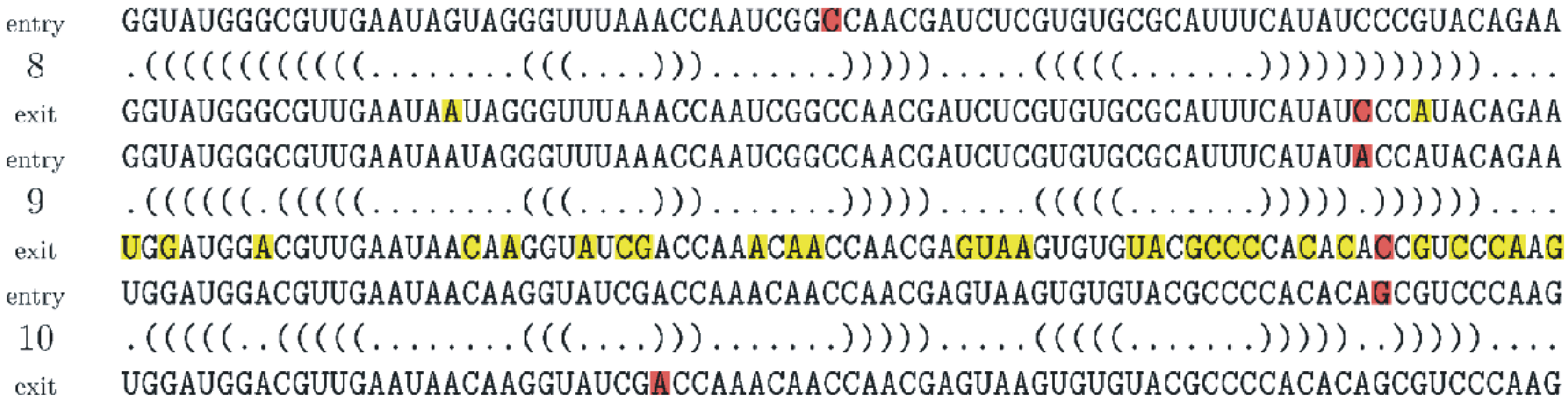


In silico optimization in the flow reactor: Trajectory and relay steps



In silico optimization in the flow reactor: Uninterrupted presence



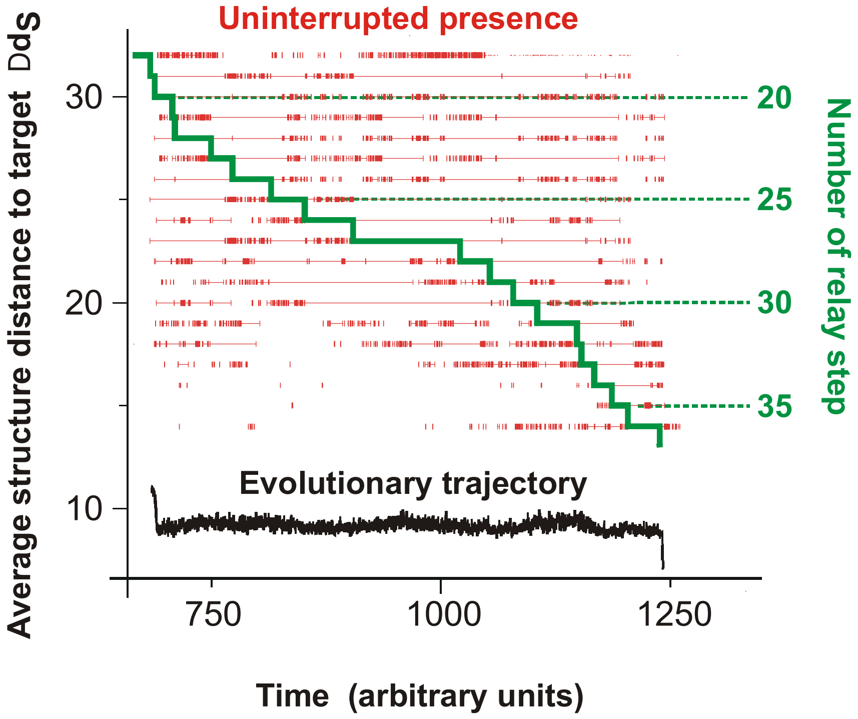


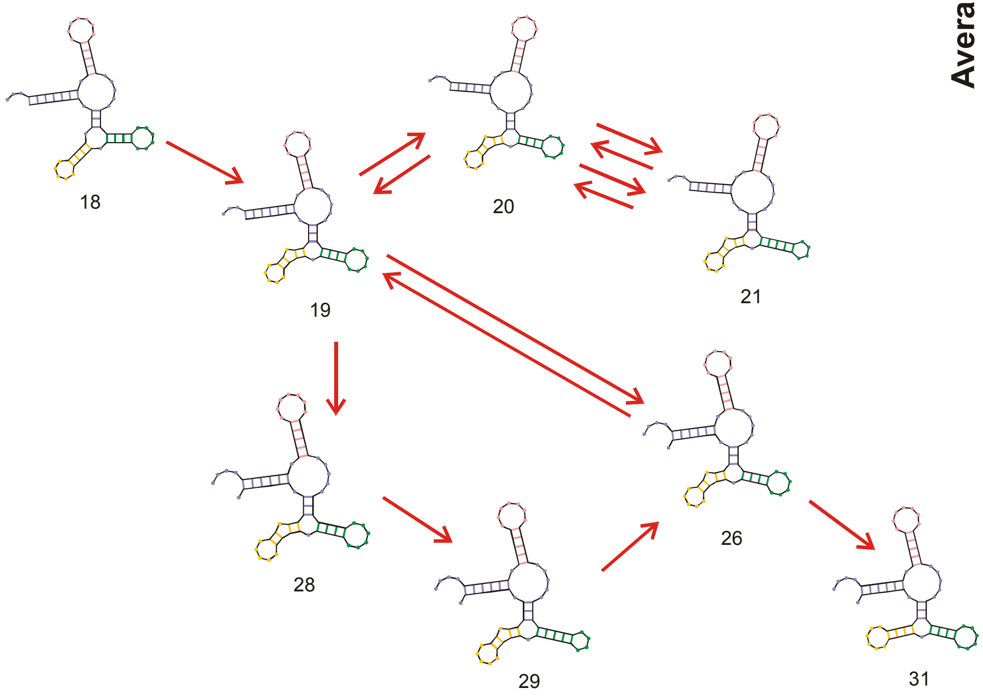
```
entry  GGUAUGGGCGUUGAAUAGUAGGGUUUAAACCAAUCGGCCAACGAUCUCGUGUGCGCAUUUCAUAUCCCGUACAGAA
  8    .((((((((((((........(((....))).......))))).....(((((.......))))))))))))....
exit   GGUAUGGGCGUUGAAUAAUAGGGUUUAAACCAAUCGGCCAACGAUCUCGUGUGCGCAUUUCAUAUCCCAUACAGAA
entry  GGUAUGGGCGUUGAAUAAUAGGGUUUAAACCAAUCGGCCAACGAUCUCGUGUGCGCAUUUCAUAUACCAUACAGAA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

Transition inducing point mutations

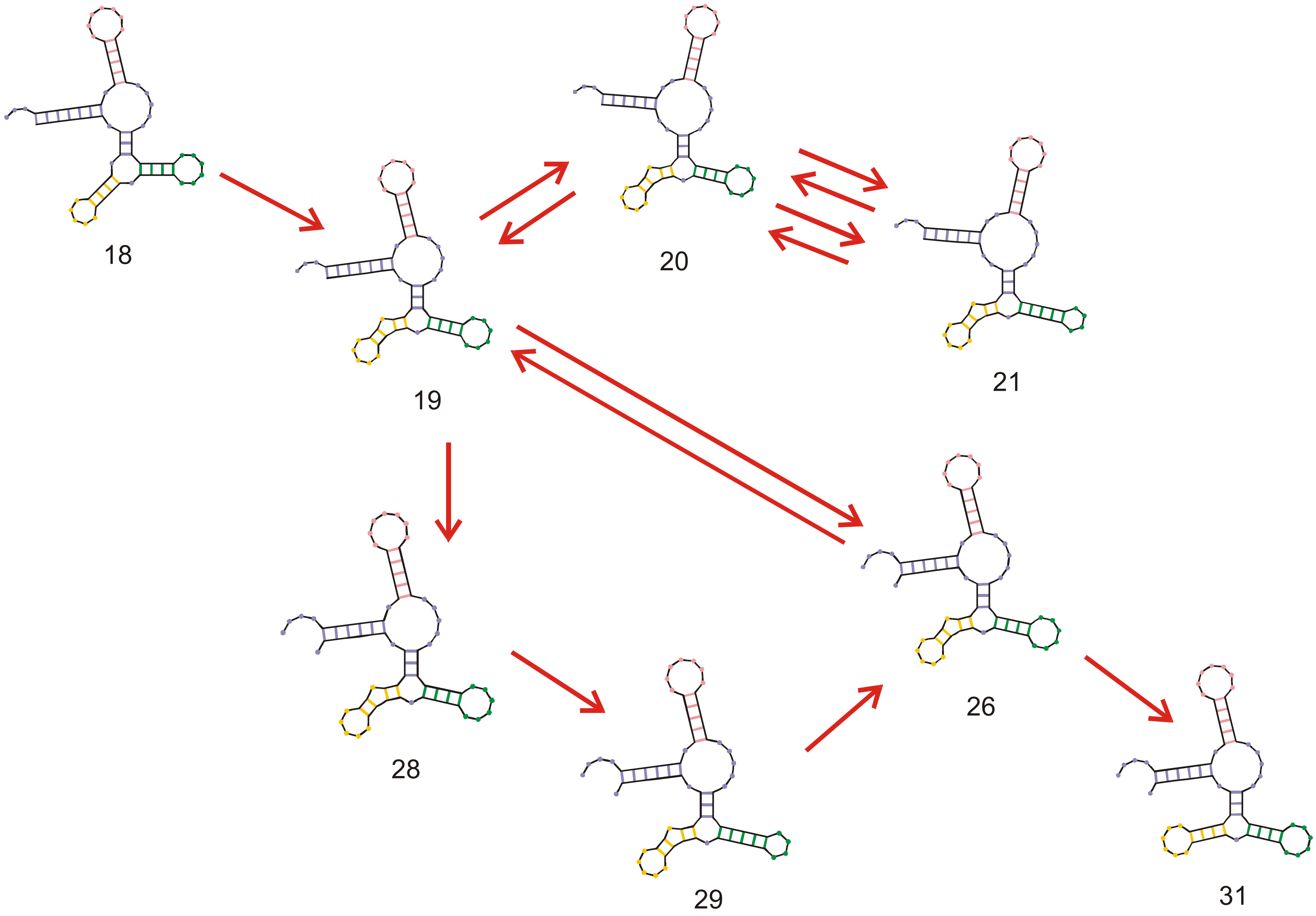
Neutral point mutations

Neutral genotype evolution during phenotypic stasis

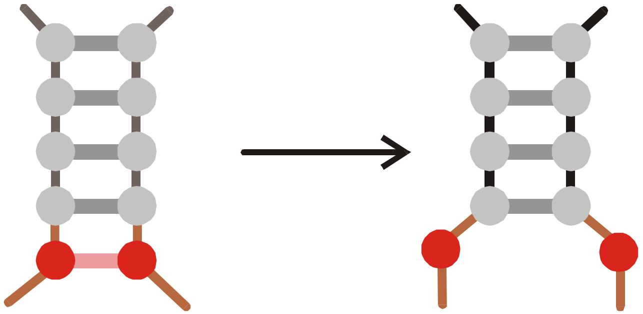




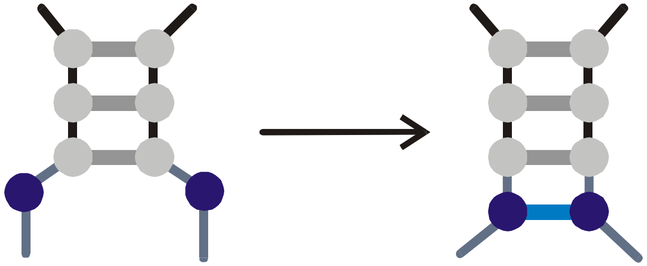
A random sequence of **minor** or continuous **transitions** in the relay series



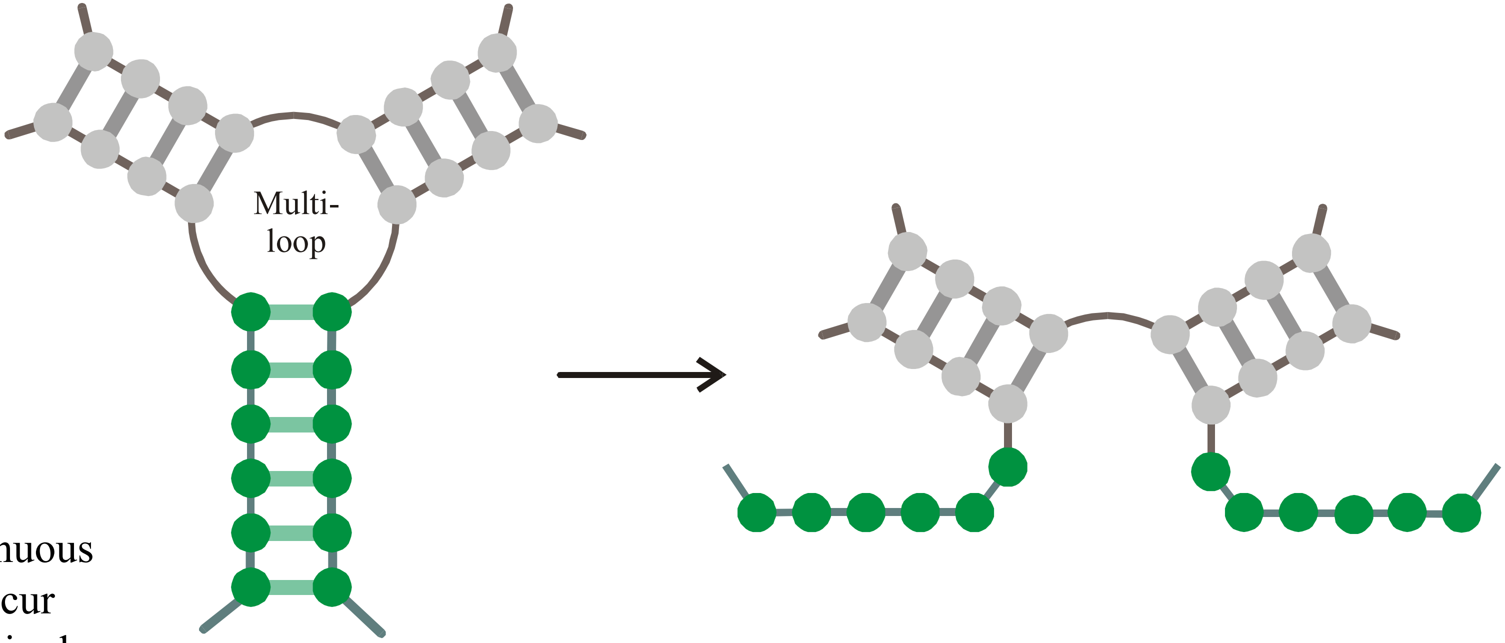
A random sequence of **minor** or continuous **transitions** in the relay series



Shortening of Stacks

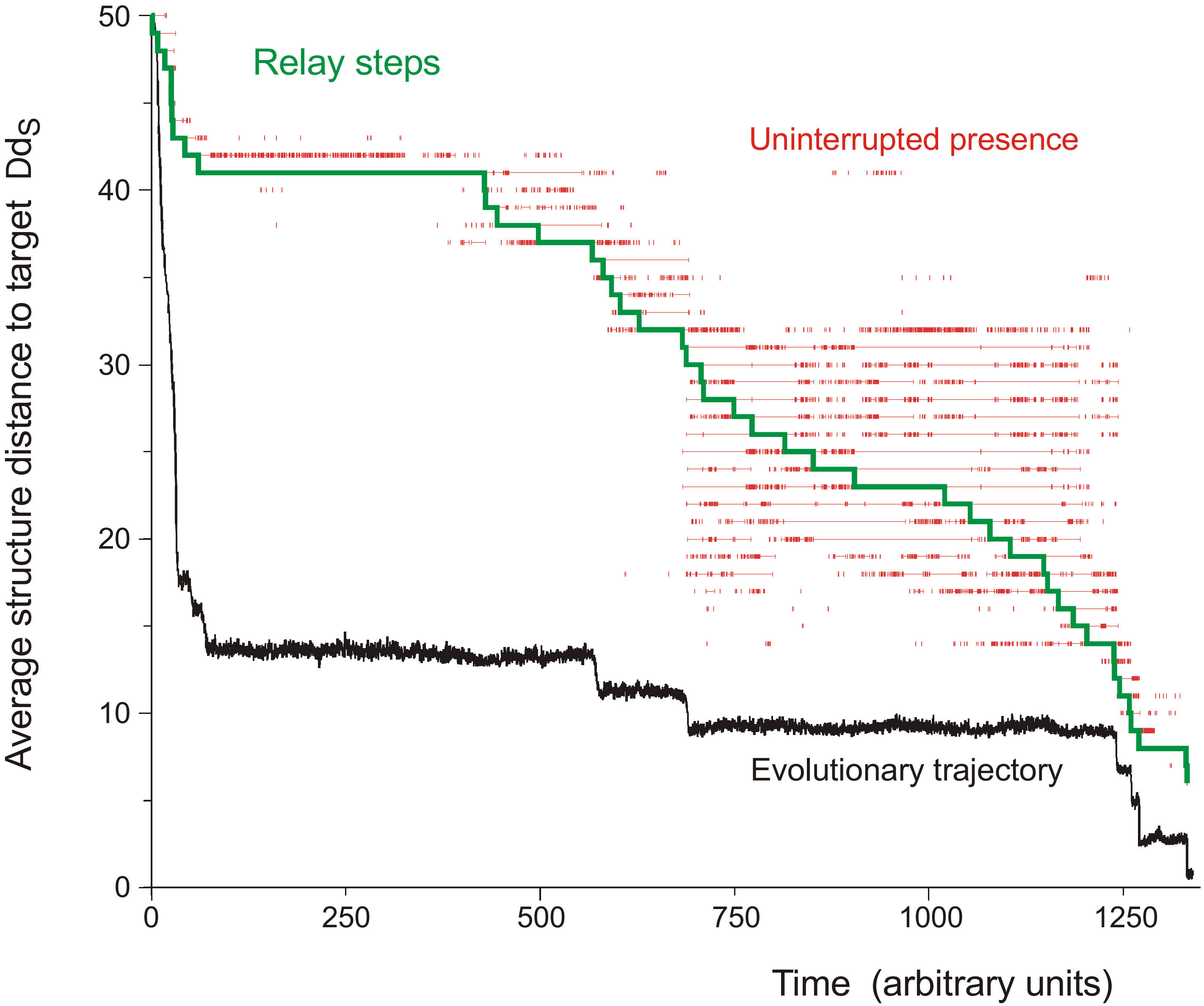


Elongation of Stacks

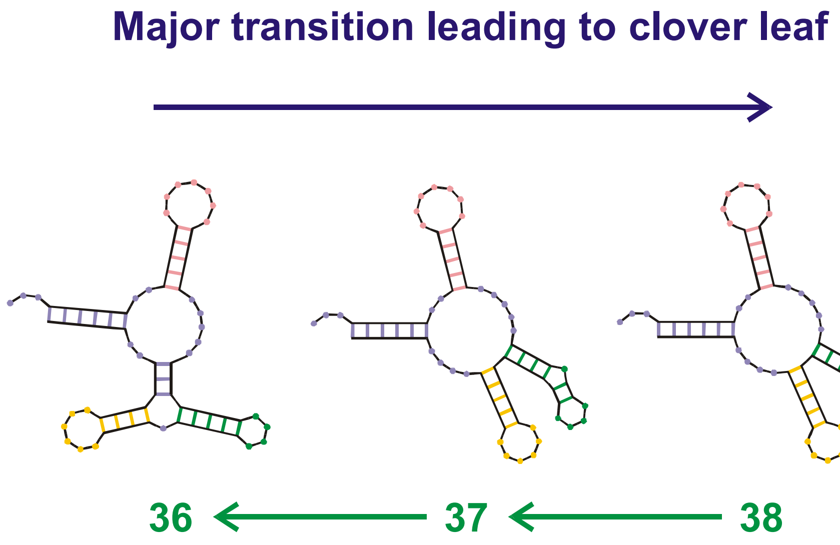


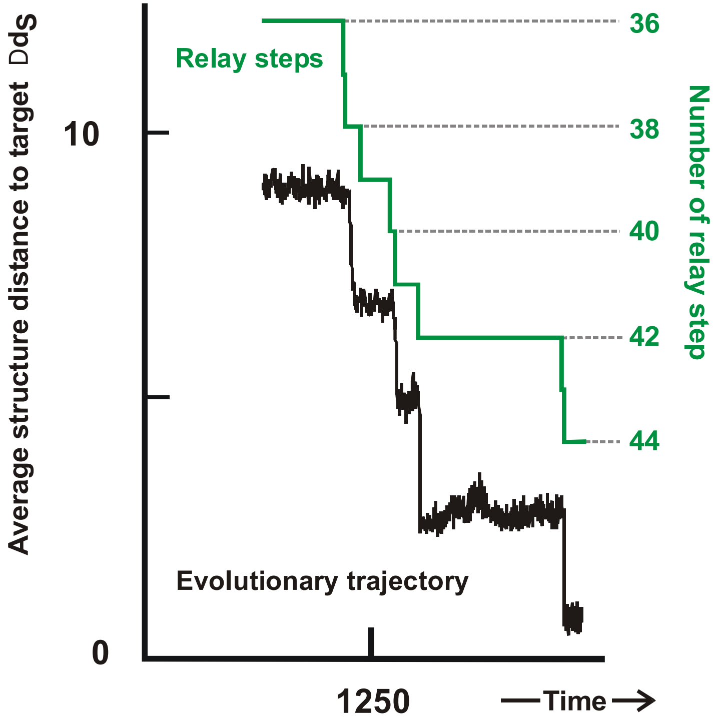
Opening of Constrained Stacks

Minor or continuous **transitions**: Occur **frequently** on single point mutations

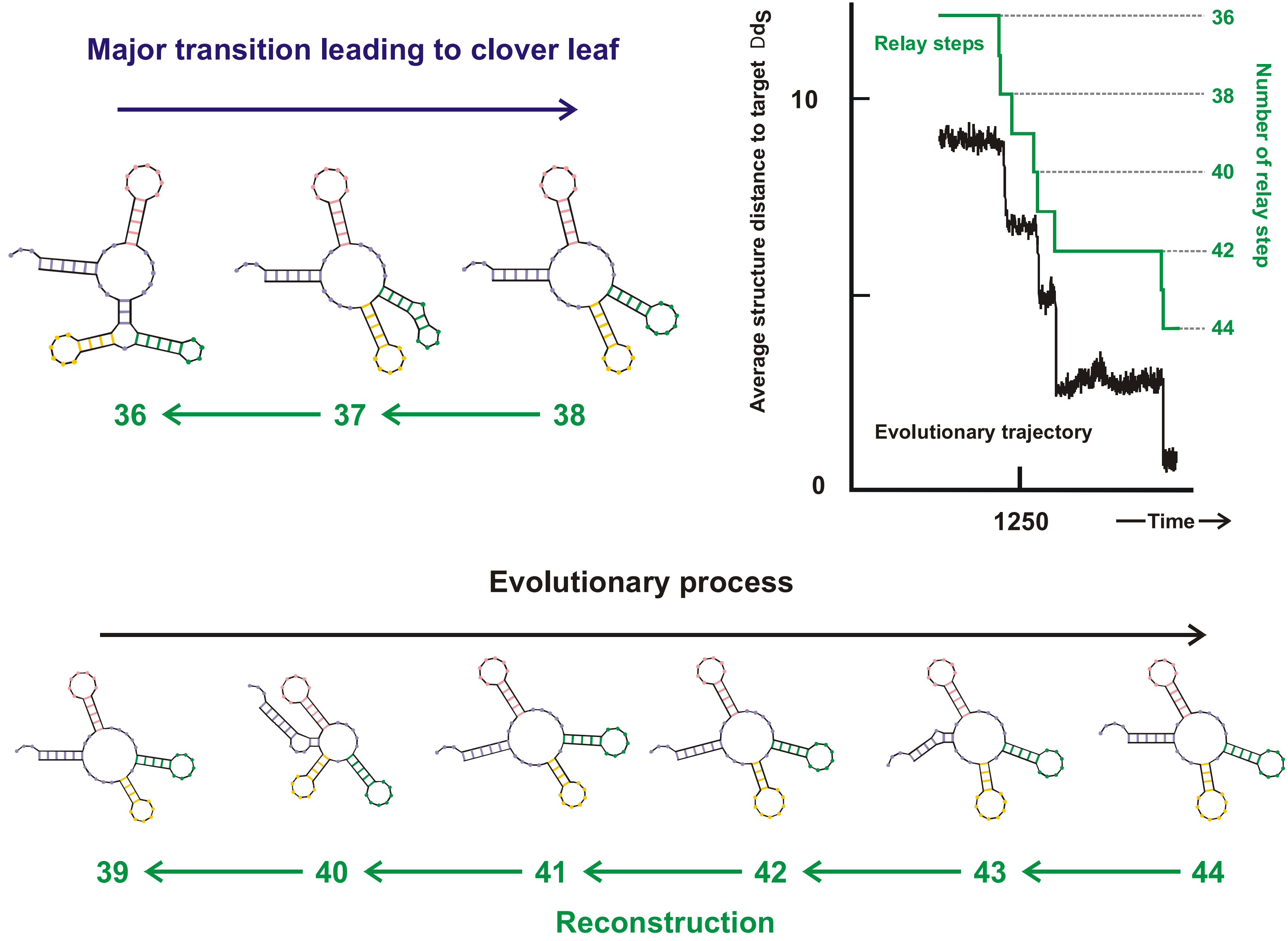


In silico optimization in the flow reactor: **Uninterrupted presence**

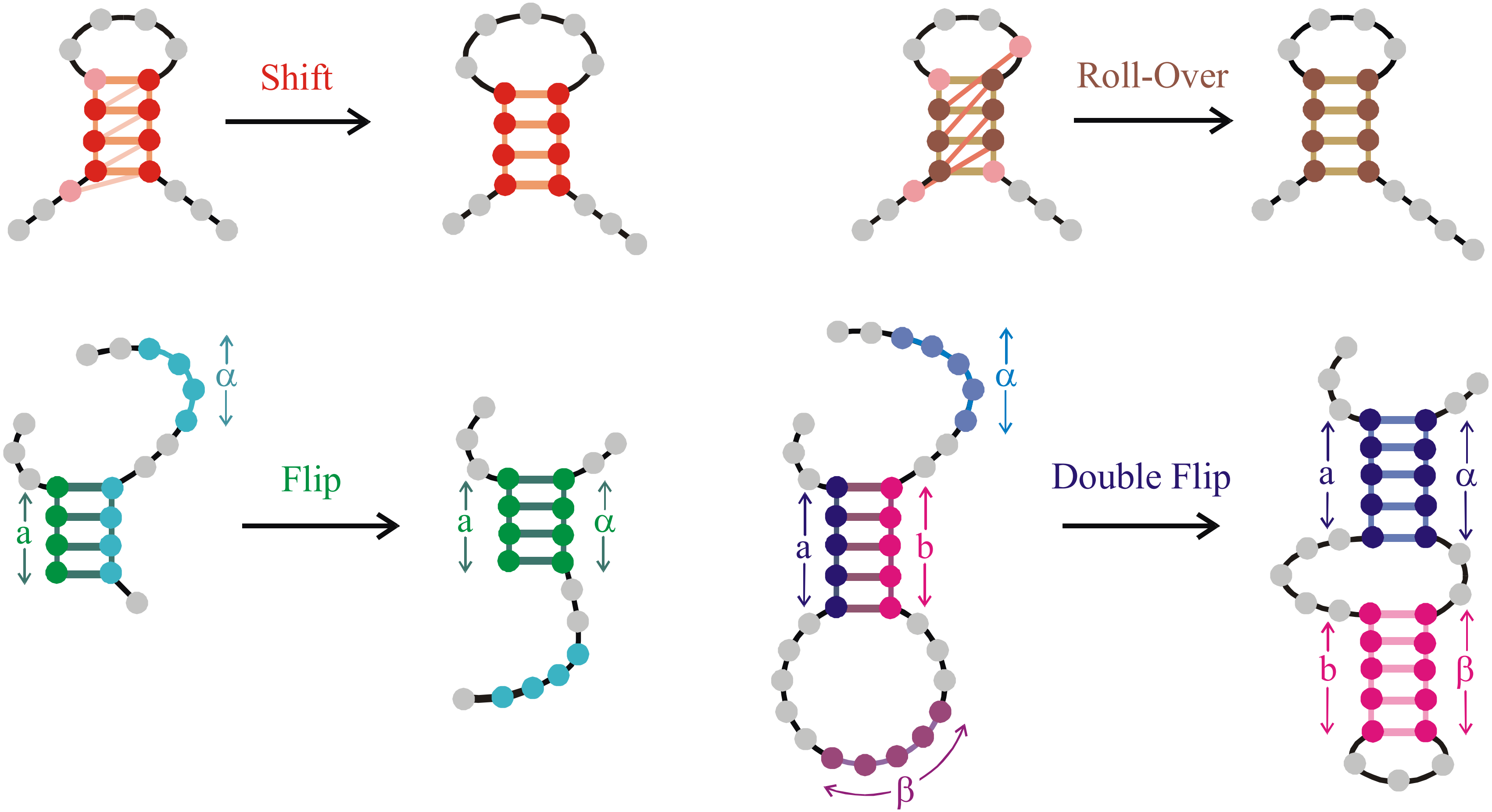




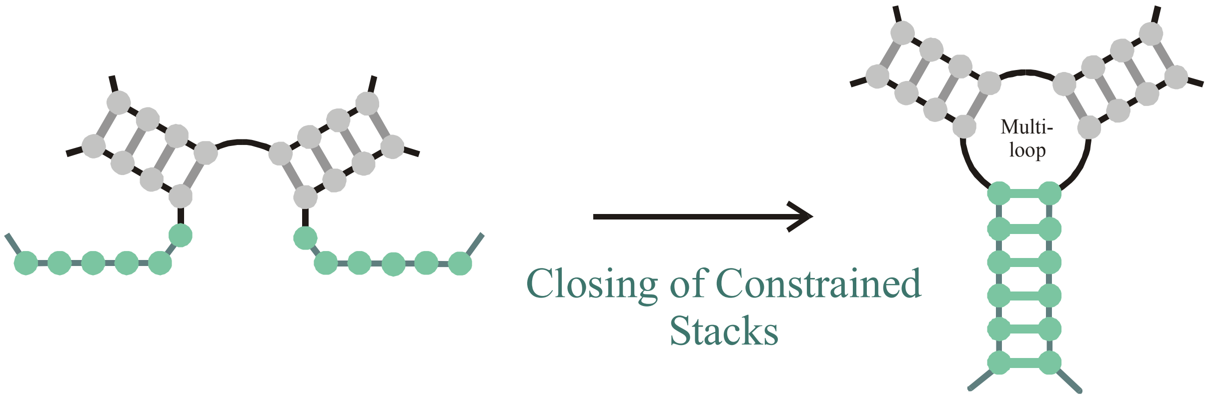
Reconstruction of a **major transitions** 36 Á 37 (Á 38)

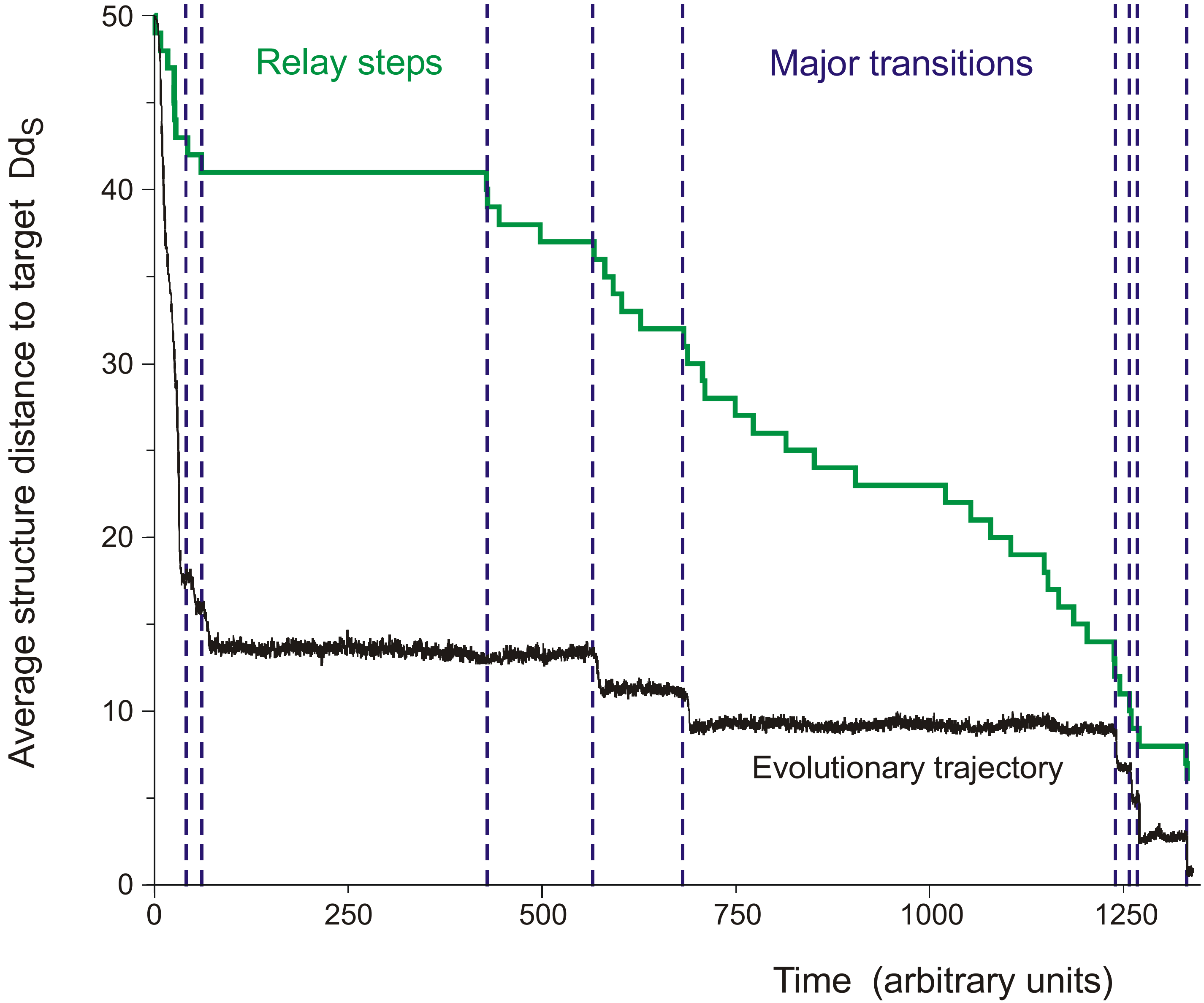


Final reconstruction 36 Á 44

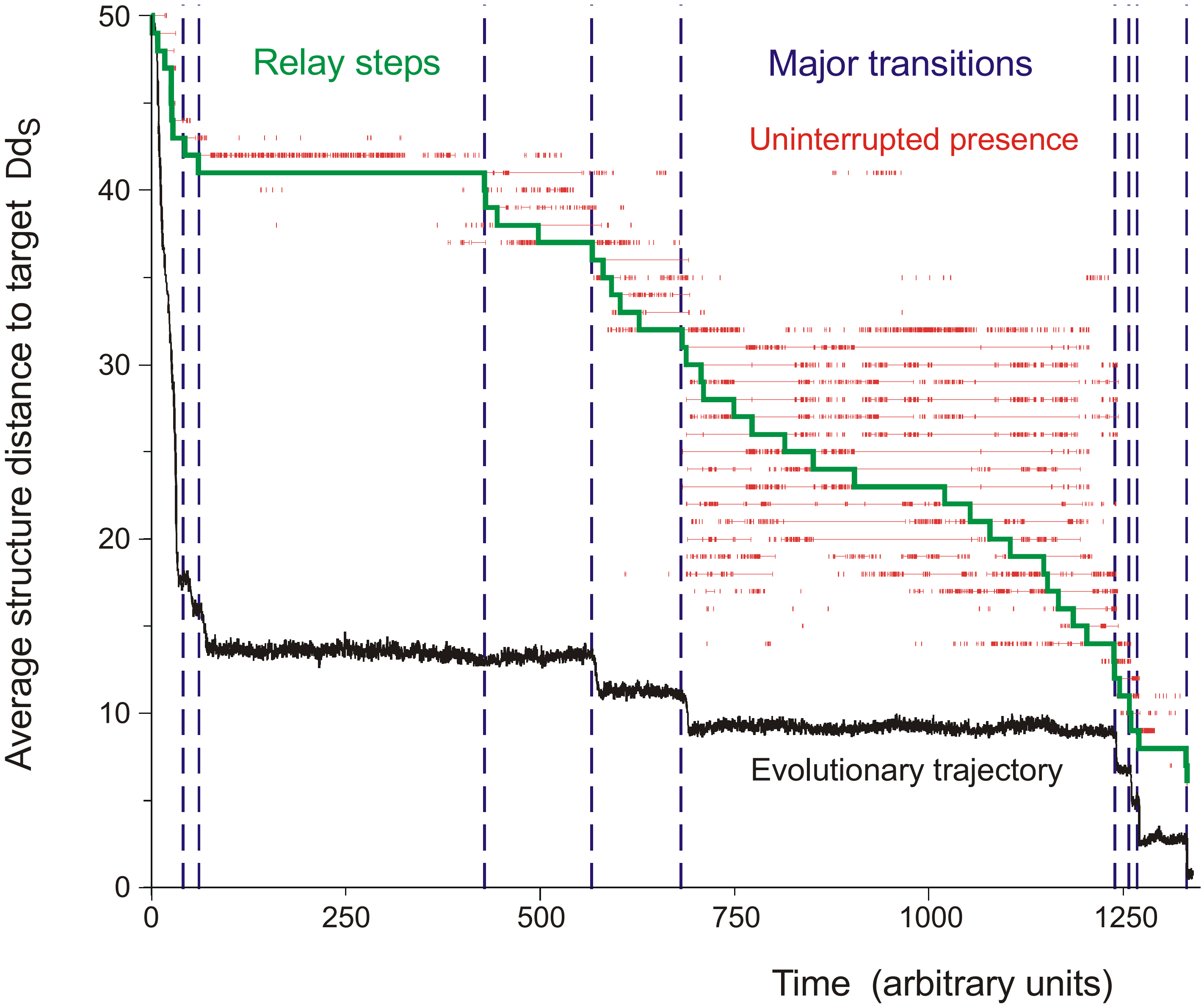


Major or discontinuous **transitions**: ***Structural innovations***, occur **rarely** on single point mutations

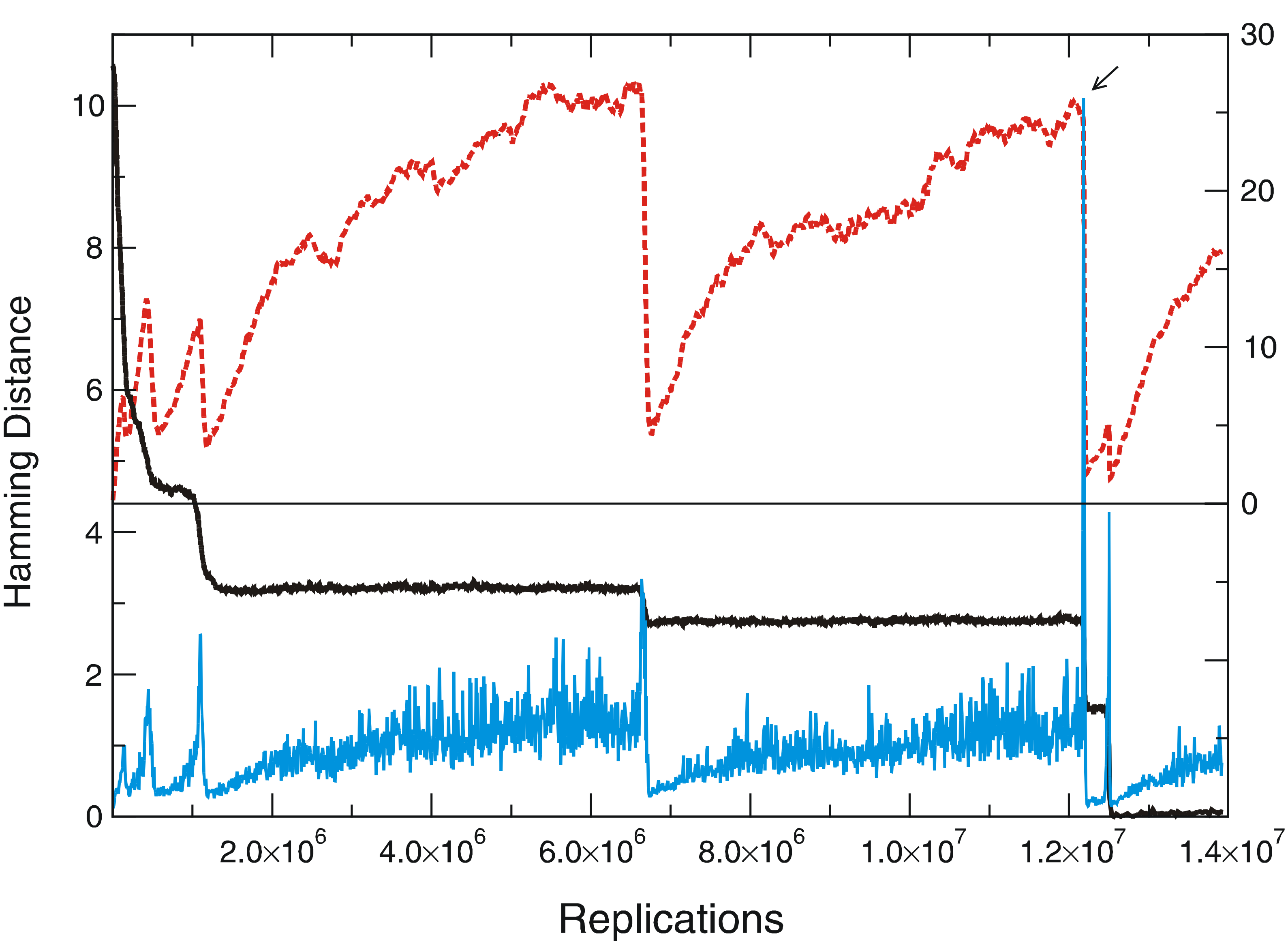




In silico optimization in the flow reactor: **Major transitions**



In silico optimization in the flow reactor



Variation in genotype space during optimization of phenotypes

Statistics of evolutionary trajectories

Population Size N	Number of Replications $< n_{\text{rep}} >$	Number of Transitions $< n_{\text{tr}} >$	Number of Major Transitions $< n_{\text{dtr}} >$	Epochal Phase $< d_{\tau}^{s}(t_{\text{ep}}) >$
1 000	$(5.5 \pm [6.9, 3.1]) \times 10^7$	$92.7 \pm [80.3, 43.0]$	$8.8 \pm [2.4, 1.9]$	$23.7 \pm [5.0, 4.1]$
2 000	$(6.0 \pm [11.1, 3.9]) \times 10^7$	$55.7 \pm [30.7, 19.8]$	$8.9 \pm [2.8, 2.1]$	$22.2 \pm [5.1, 4.2]$
3 000	$(6.6 \pm [21.0, 5.0]) \times 10^7$	$44.2 \pm [25.9, 16.3]$	$8.1 \pm [2.3, 1.8]$	$20.9 \pm [2.4, 2.2]$
10 000	$(1.2 \pm [1.3, 0.6]) \times 10^8$	$35.9 \pm [10.3, 8.0]$	$10.3 \pm [2.6, 2.1]$	$18.4 \pm [2.3, 2.1]$
20 000	$(1.5 \pm [1.4, 0.7]) \times 10^8$	$28.8 \pm [5.8, 4.8]$	$9.0 \pm [2.8, 2.2]$	$17.5 \pm [2.5, 2.2]$
30 000	$(2.2 \pm [3.1, 1.3]) \times 10^8$	$29.8 \pm [7.3, 5.9]$	$8.7 \pm [2.4, 1.9]$	$16.7 \pm [2.0, 1.8]$
100 000	$(3 \pm [2, 1]) \times 10^8$	$24 \pm [6, 5]$	9 ± 2	17 ± 1

Main results of computer simulations of molecular evolution

- No trajectory was reproducible in detail. Sequences of target structures were different. Nevertheless solutions of comparable or the same quality are almost always achieved.

- Transitions between molecular phenotypes represented by RNA structures can be classified with respect to the induced structural changes. Highly probable **minor transitions** are opposed by **major transitions** with low probability of occurrence.

- **Major transitions** represent important **innovations** in the course of evolution.

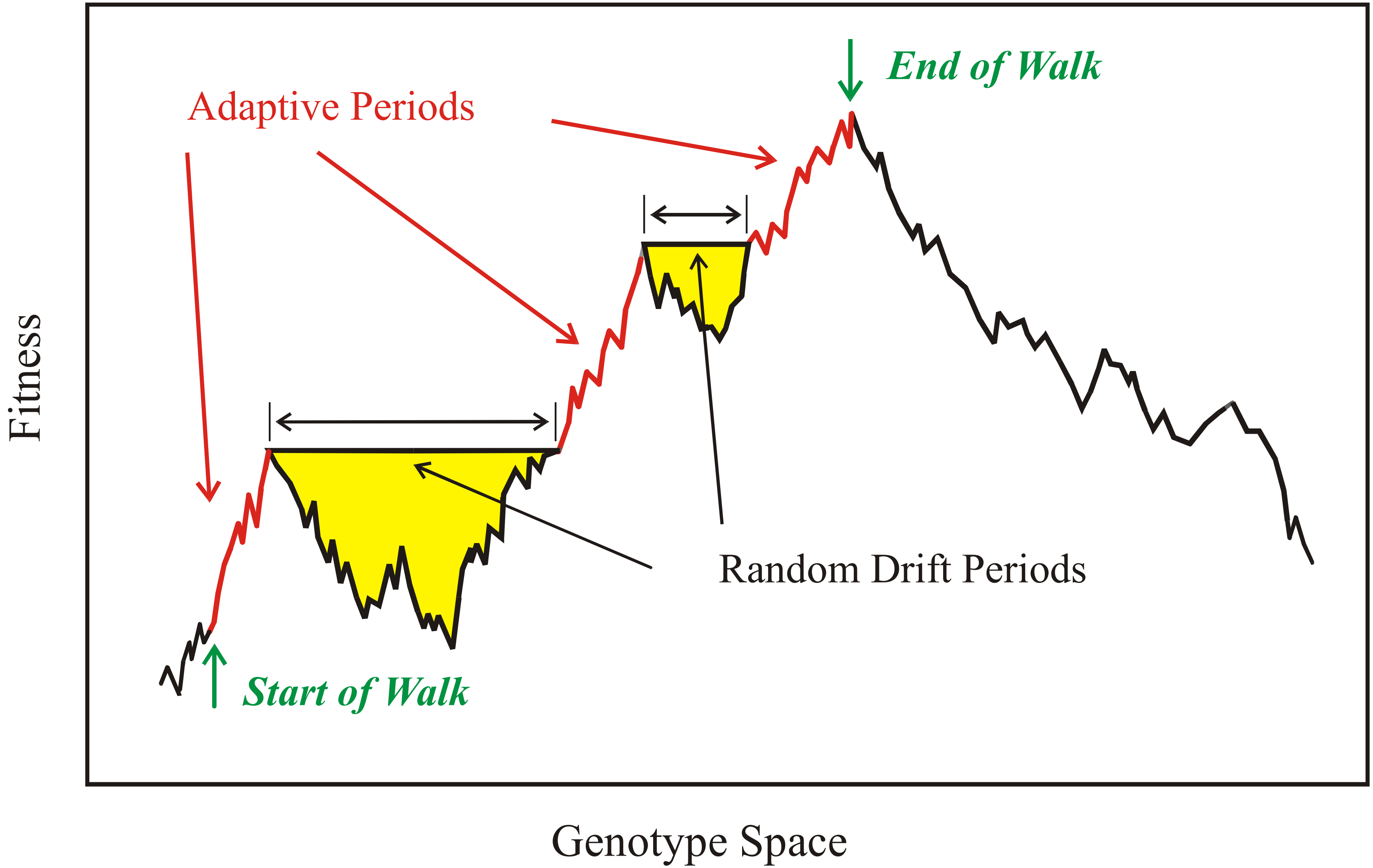
- The number of **minor transitions** decreases with increasing population size.

- The number of **major transitions** or evolutionary innovations is approximately constant for given start and stop structures.

- Not all structures are accessible through evolution in the flow reactor. An example is the tRNA clover leaf for GC-only sequences.

„...Variations neither useful not injurious would not be affected by natural selection, and would be left either a fluctuating element, as perhaps we see in certain polymorphic species, or would ultimately become fixed, owing to the nature of the organism and the nature of the conditions. ...“

Charles Darwin, Origin of species (1859)



Evolution in genotype space sketched as a non-descending walk in a fitness landscape

Coworkers

Walter Fontana, Santa Fe Institute, NM

Christian Reidys, Christian Forst, Los Alamos National Laboratory, NM

Peter F. Stadler, Universität Wien, AT
Ivo L. Hofacker
Christoph Flamm

Bärbel Stadler, Andreas Wernitznig, Universität Wien, AT
Michael Kospach, Ulrike Mückstein, Stefanie Widder, Stefan Wuchty
Jan Cupal, Kurt Grünberger, Andreas Svrček-Seiler

Ulrike Göbel, Institut für Molekulare Biotechnologie, Jena, GE
Walter Grüner, Stefan Kopp, Jaqueline Weber

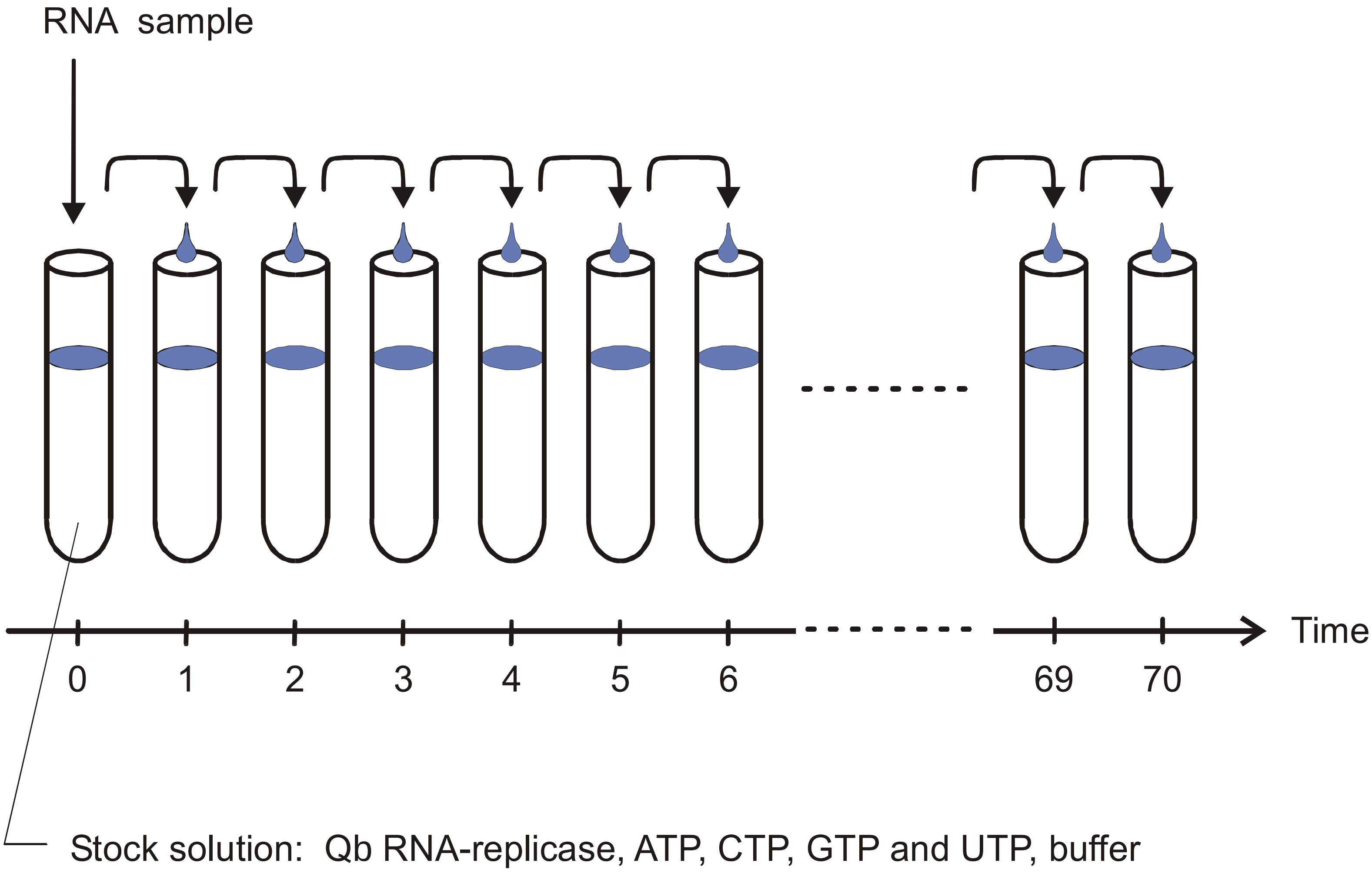
Evolution of RNA molecules based on Qβ phage

D.R.Mills, R,L,Peterson, S.Spiegelman, ***An extracellular Darwinian experiment with a self-duplicating nucleic acid molecule***. Proc.Natl.Acad.Sci.USA **58** (1967), 217-224

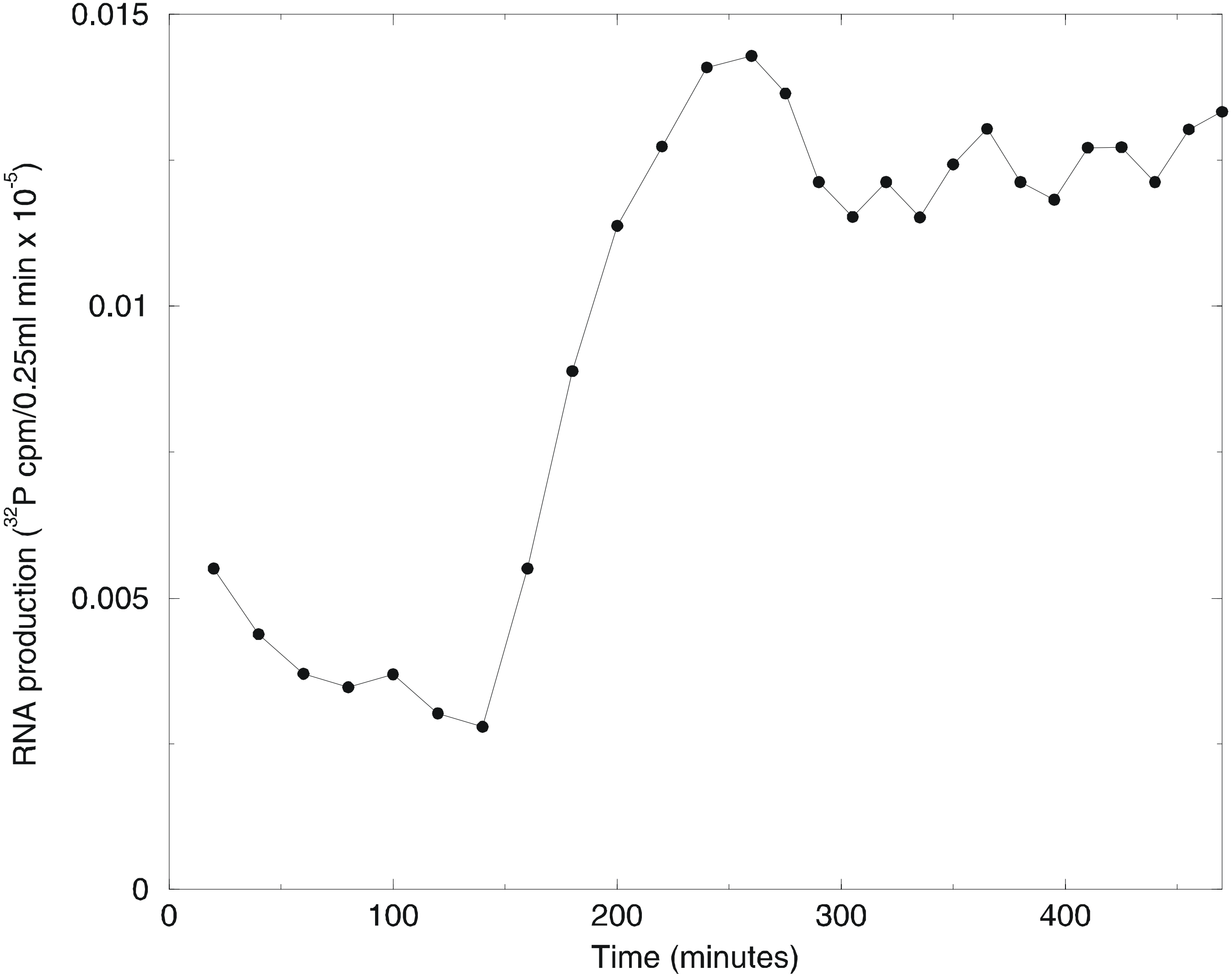
S.Spiegelman, ***An approach to the experimental analysis of precellular evolution***. Quart.Rev.Biophys. **4** (1971), 213-253

C.K.Biebricher, ***Darwinian selection of self-replicating RNA molecules***. Evolutionary Biology **16** (1983), 1-52

C.K.Biebricher, W.C. Gardiner, ***Molecular evolution of RNA* in vitro**. Biophysical Chemistry **66** (1997), 179-192


RNA sample
Time
0
1
2
3
4
5
6
69
70
Stock solution: Qb RNA-replicase, ATP, CTP, GTP and UTP, buffer

The serial transfer technique applied to RNA evolution ***in vitro***



The increase in RNA production rate during a serial transfer experiment

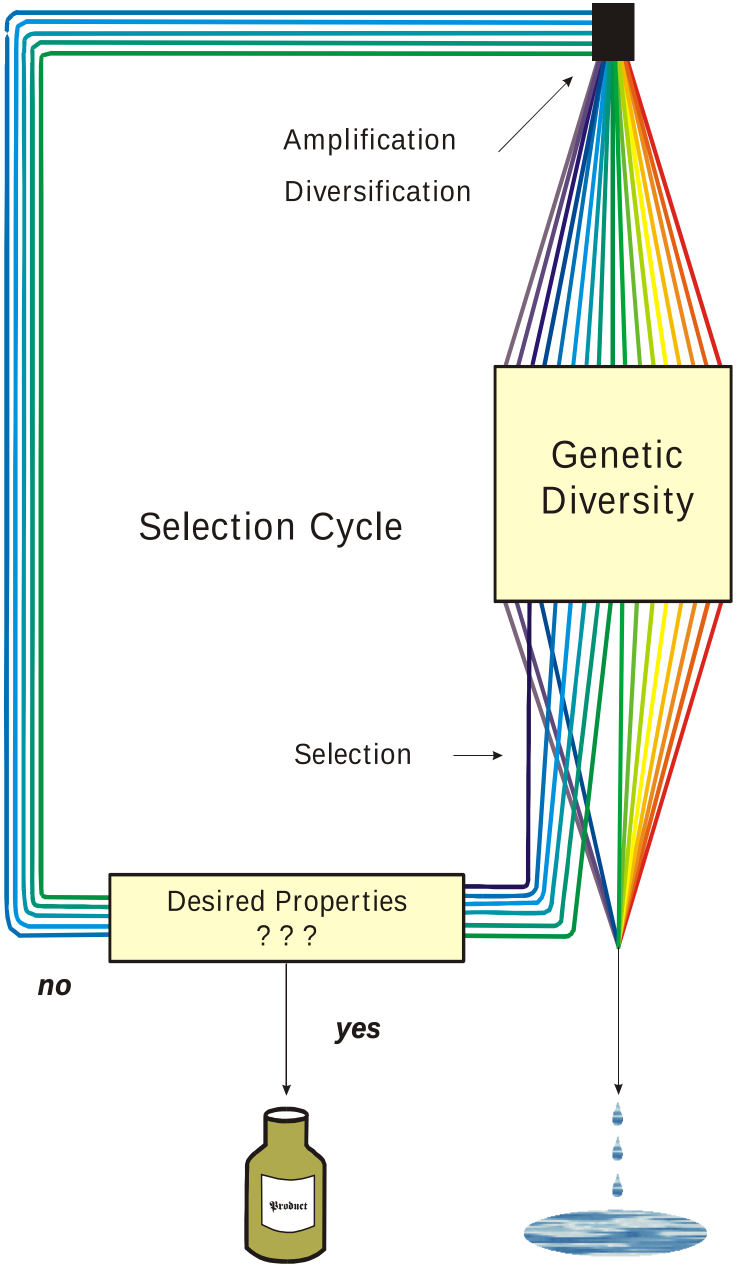
Evolutionary design of RNA molecules

D.B.Bartel, J.W.Szostak, **In vitro** ***selection of RNA molecules that bind specific ligands***. Nature **346** (1990), 818-822

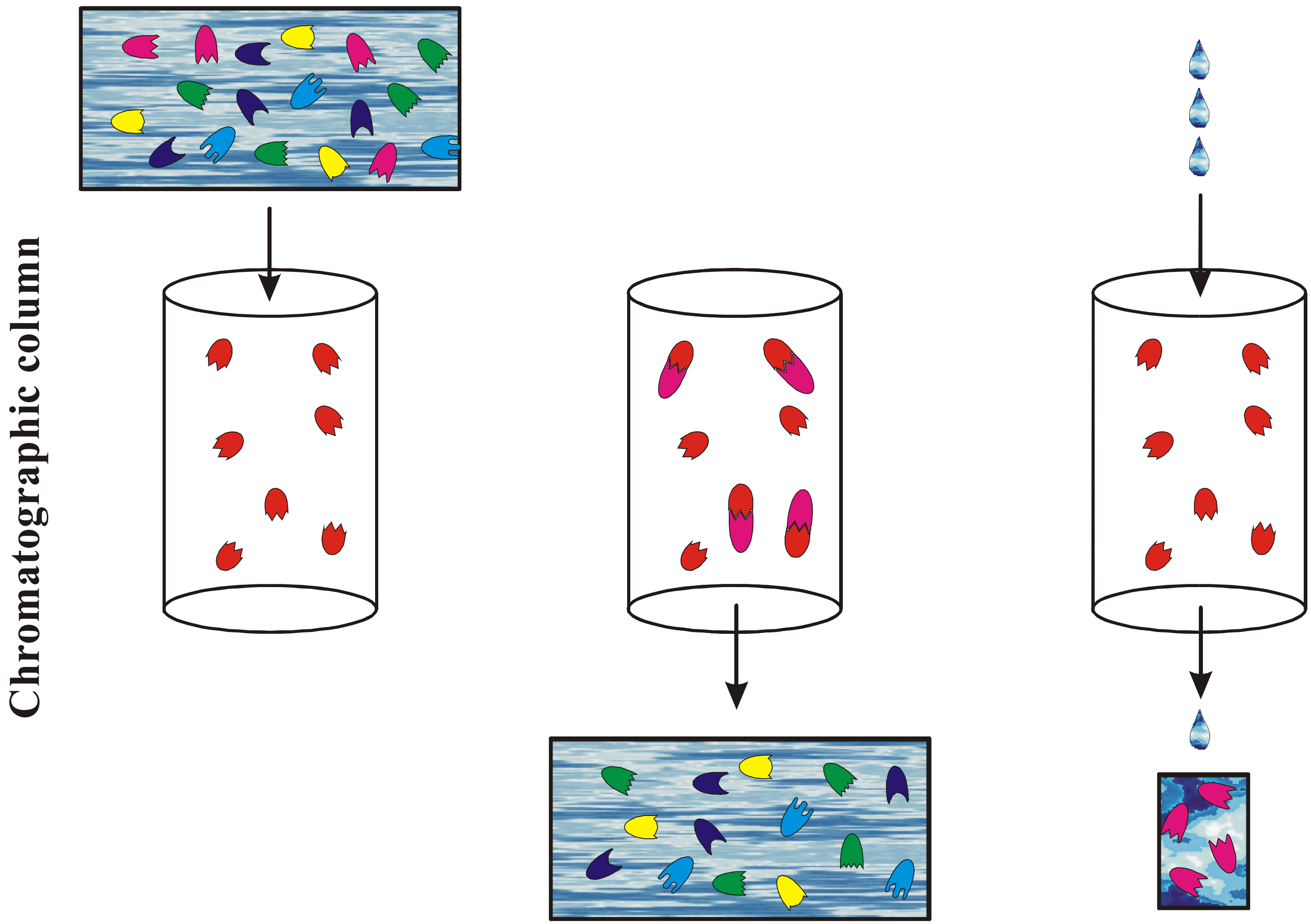
C.Tuerk, L.Gold, **SELEX** - ***Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage*** **T4** ***DNA polymerase***. Science **249** (1990), 505-510

D.P.Bartel, J.W.Szostak, ***Isolation of new ribozymes from a large pool of random sequences***. Science **261** (1993), 1411-1418

R.D.Jenison, S.C.Gill, A.Pardi, B.Poliski, ***High-resolution molecular discrimination by RNA***. Science **263** (1994), 1425-1429



Selection cycle used in applied molecular evolution to design molecules with predefined properties

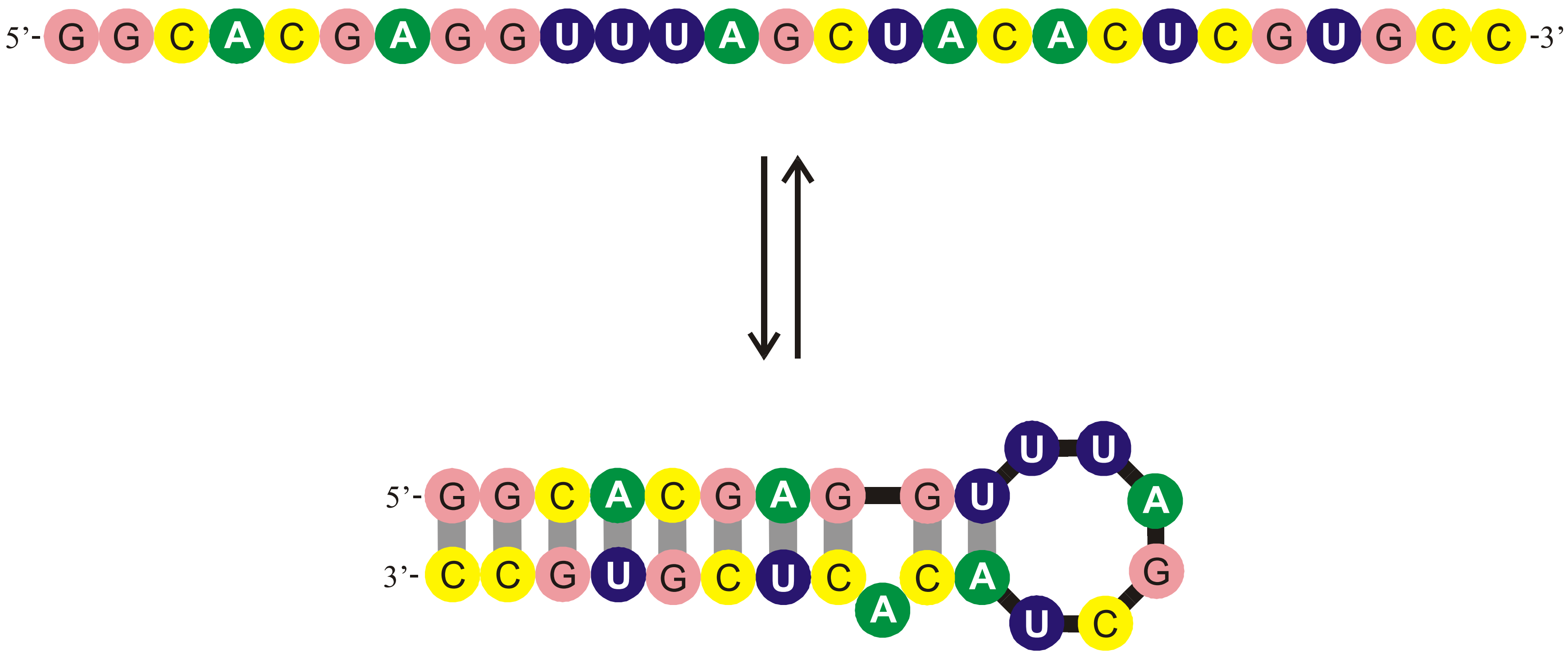


Retention of binders

Elution of binders

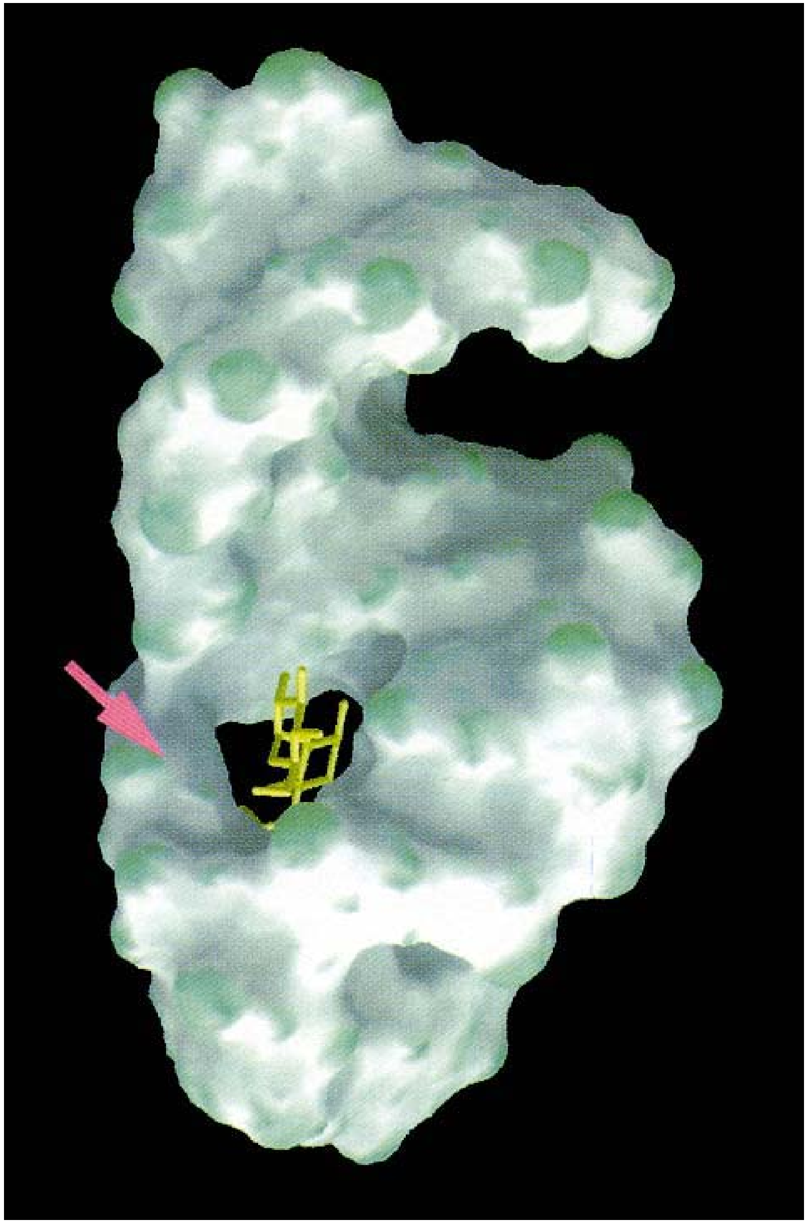
Chromatographic column

The SELEX technique for the evolutionary design of ***aptamers***


5’- G G C A C G A G G U U U A G C U A C A C U C G U G C C -3’
5’- G G C A C G A G G U
U U A G C U
3’- C C G U G C U C A C A

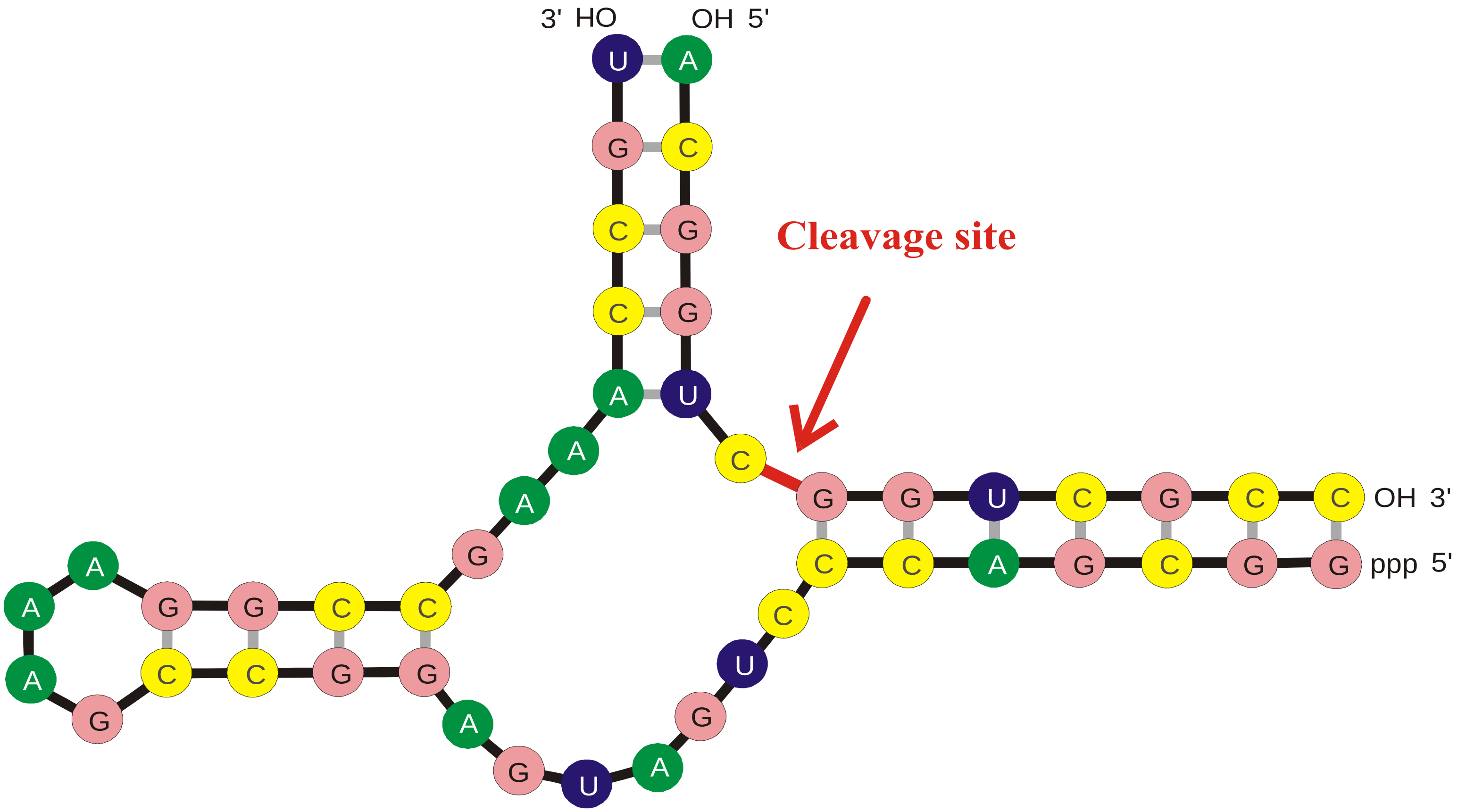
Formation of secondary structure of the tobramycin binding RNA aptamer

L. Jiang, A. K. Suri, R. Fiala, D. J. Patel, Chemistry & Biology **4**:35-50 (1997)



The three-dimensional structure of the tobramycin aptamer complex

L. Jiang, A. K. Suri, R. Fiala, D. J. Patel, Chemistry & Biology **4**:35-50 (1997)

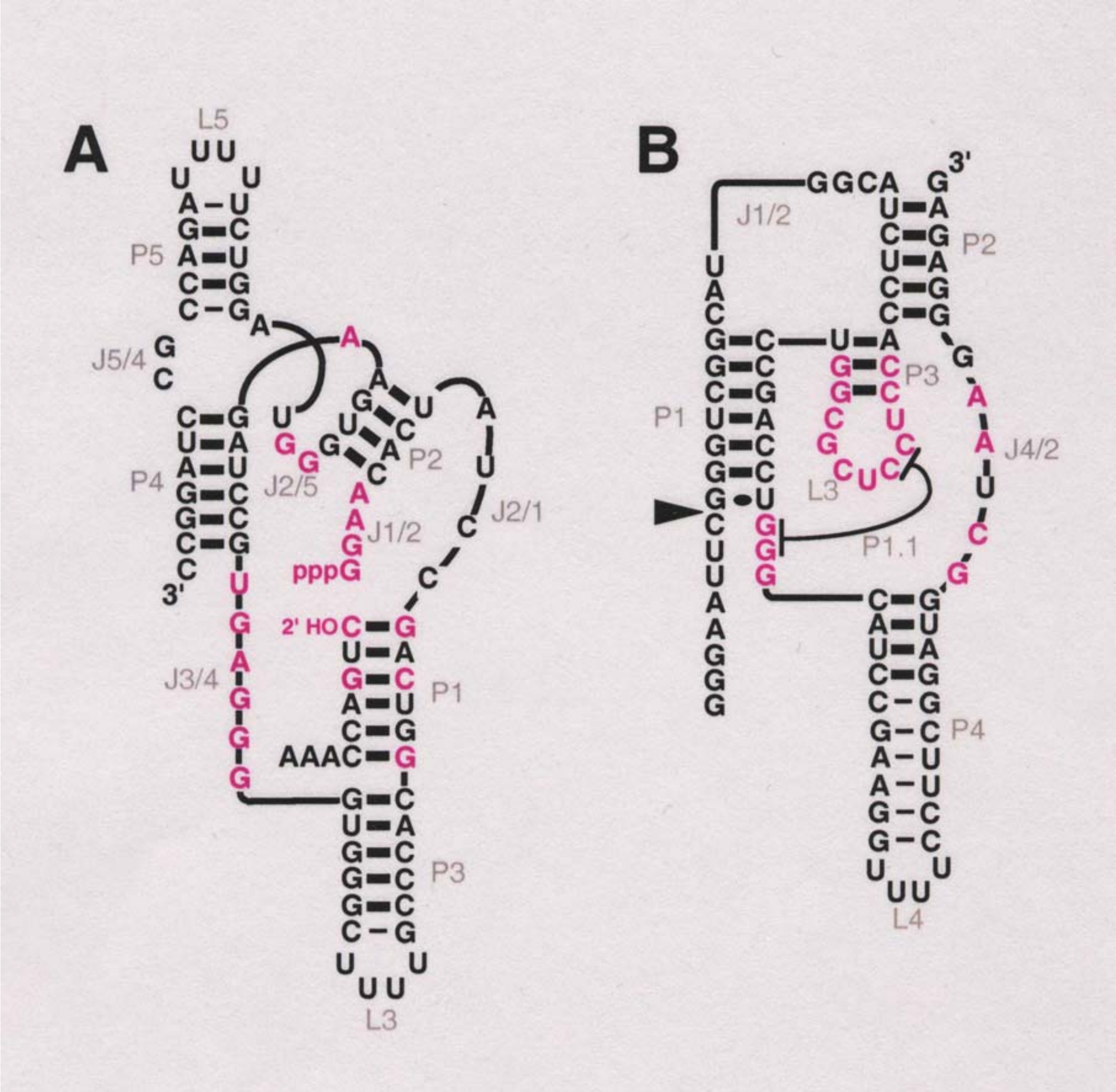


The "hammerhead" ribozyme

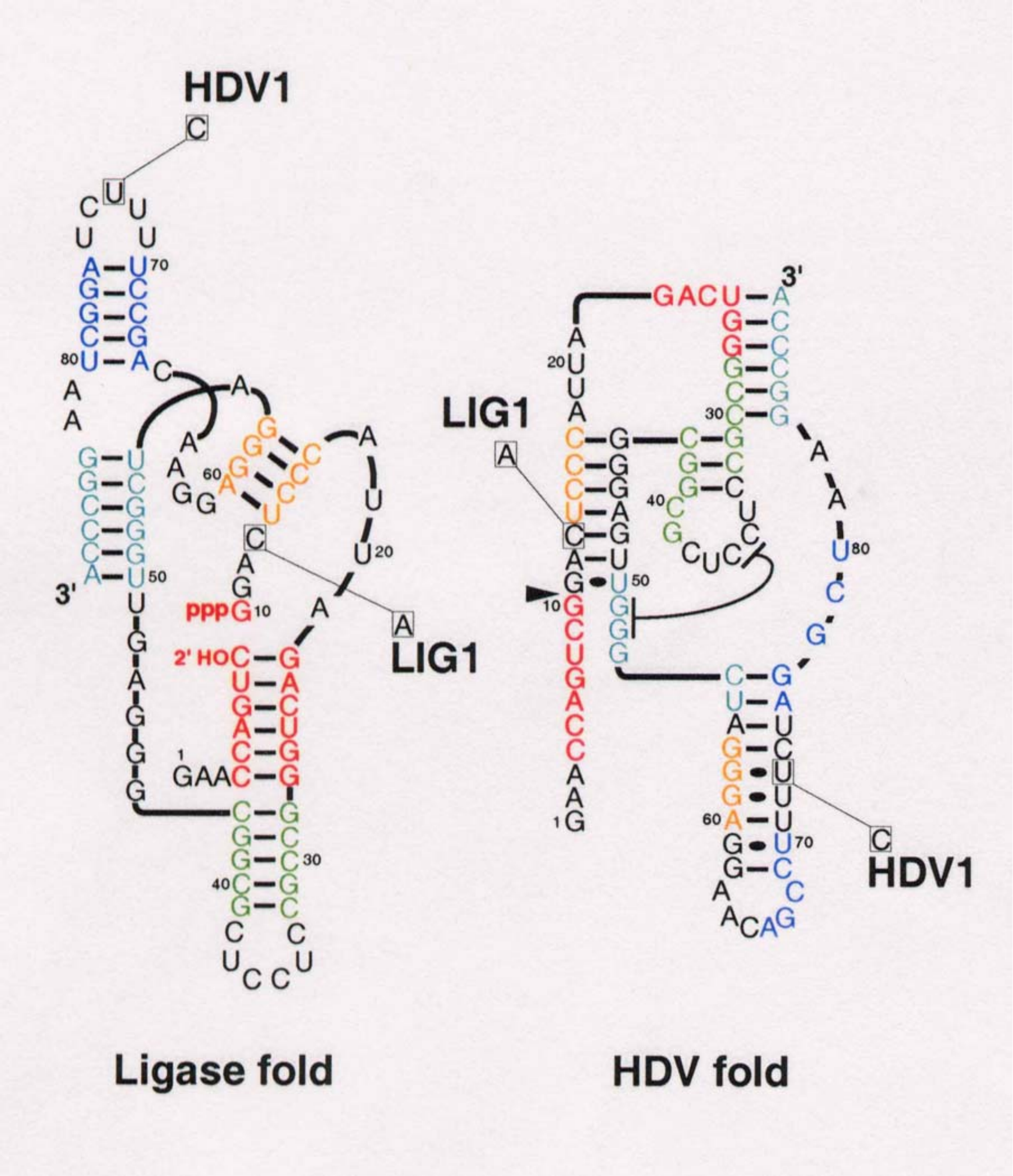
The smallest known
catalytically active
RNA molecule

A ribozyme switch

E.A.Schultes, D.B.Bartel, ***One sequence, two ribozymes: Implication for the emergence of new ribozyme folds***. Science **289** (2000), 448-452



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

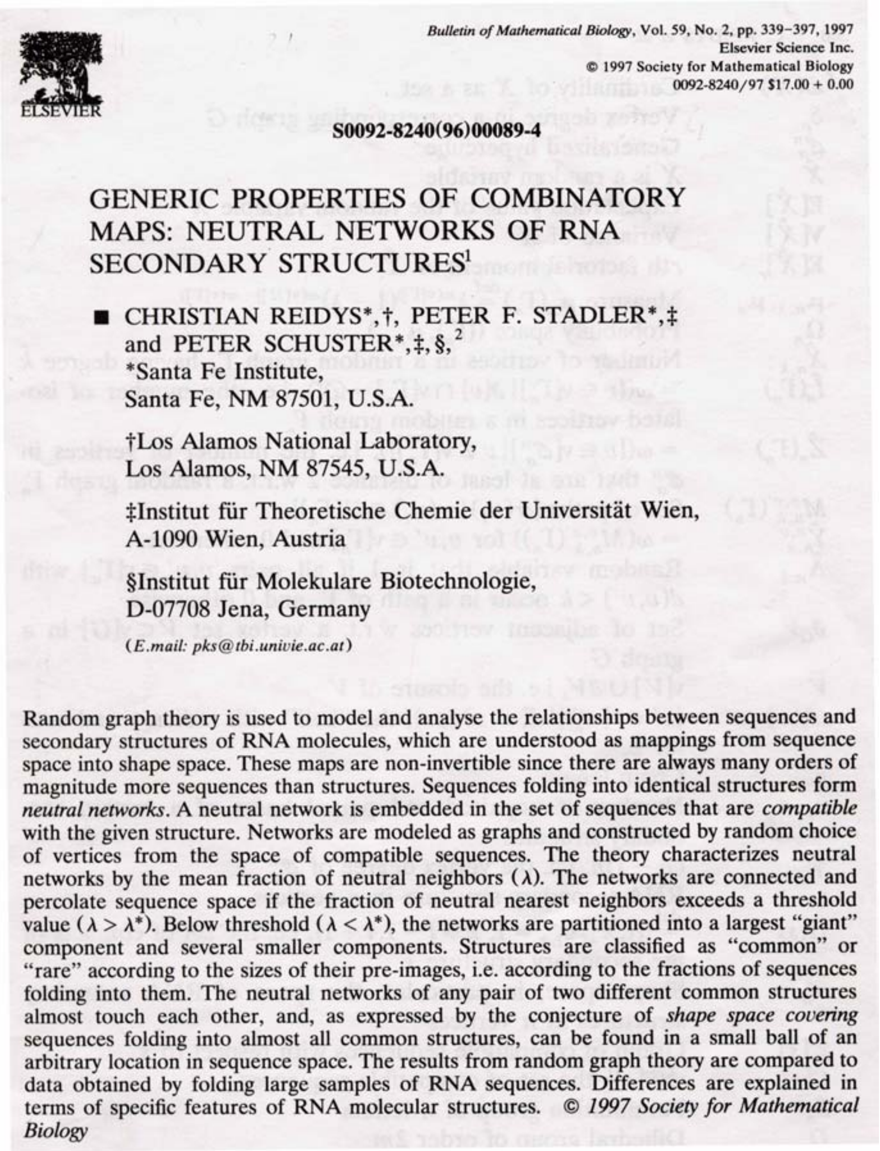


Ligase fold

HDV fold

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

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



Bulletin of Mathematical Biology, Vol. 59, No. 2, pp. 339–397, 1997
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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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‡Institut für Theoretische Chemie der Universität Wien,
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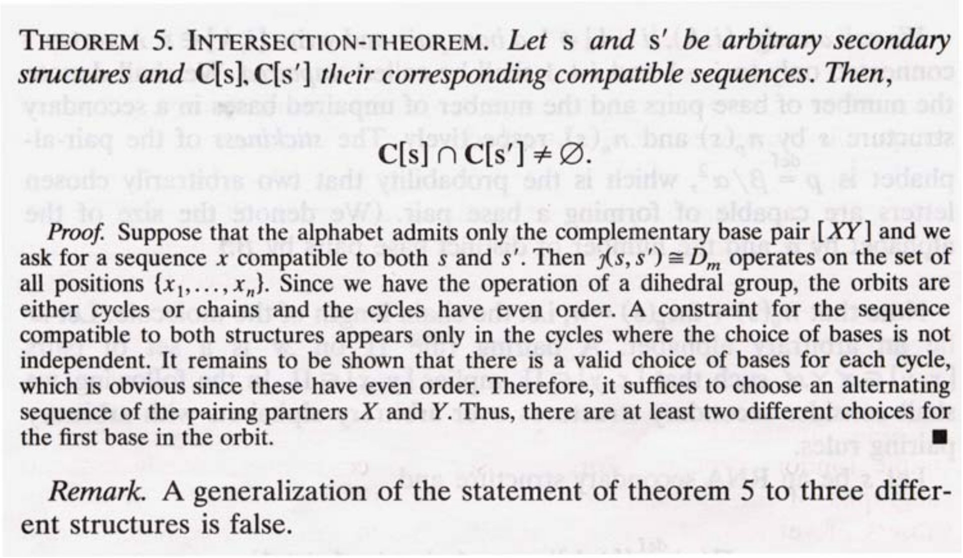
Random graph theory is used to model and analyse the relationships between sequences and secondary structures of RNA molecules, which are understood as mappings from sequence space into shape space. These maps are non-invertible since there are always many orders of magnitude more sequences than structures. 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*



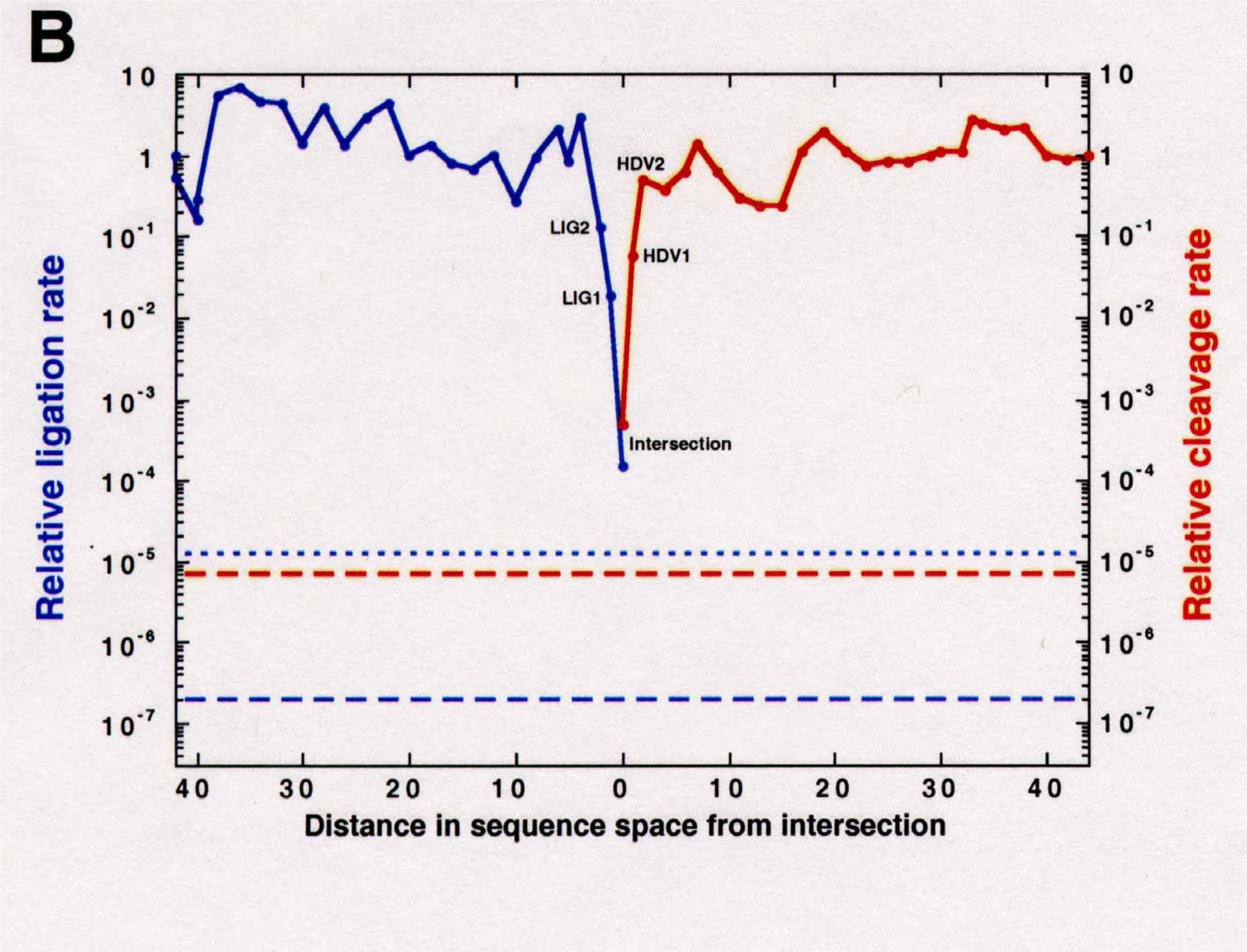
THEOREM 5. INTERSECTION-THEOREM. *Let* s *and* s′ *be arbitrary secondary structures and* C[s], C[s′] *their corresponding compatible sequences. Then,*

$$\mathrm{C[s]} \cap \mathrm{C[s']} \neq \emptyset.$$

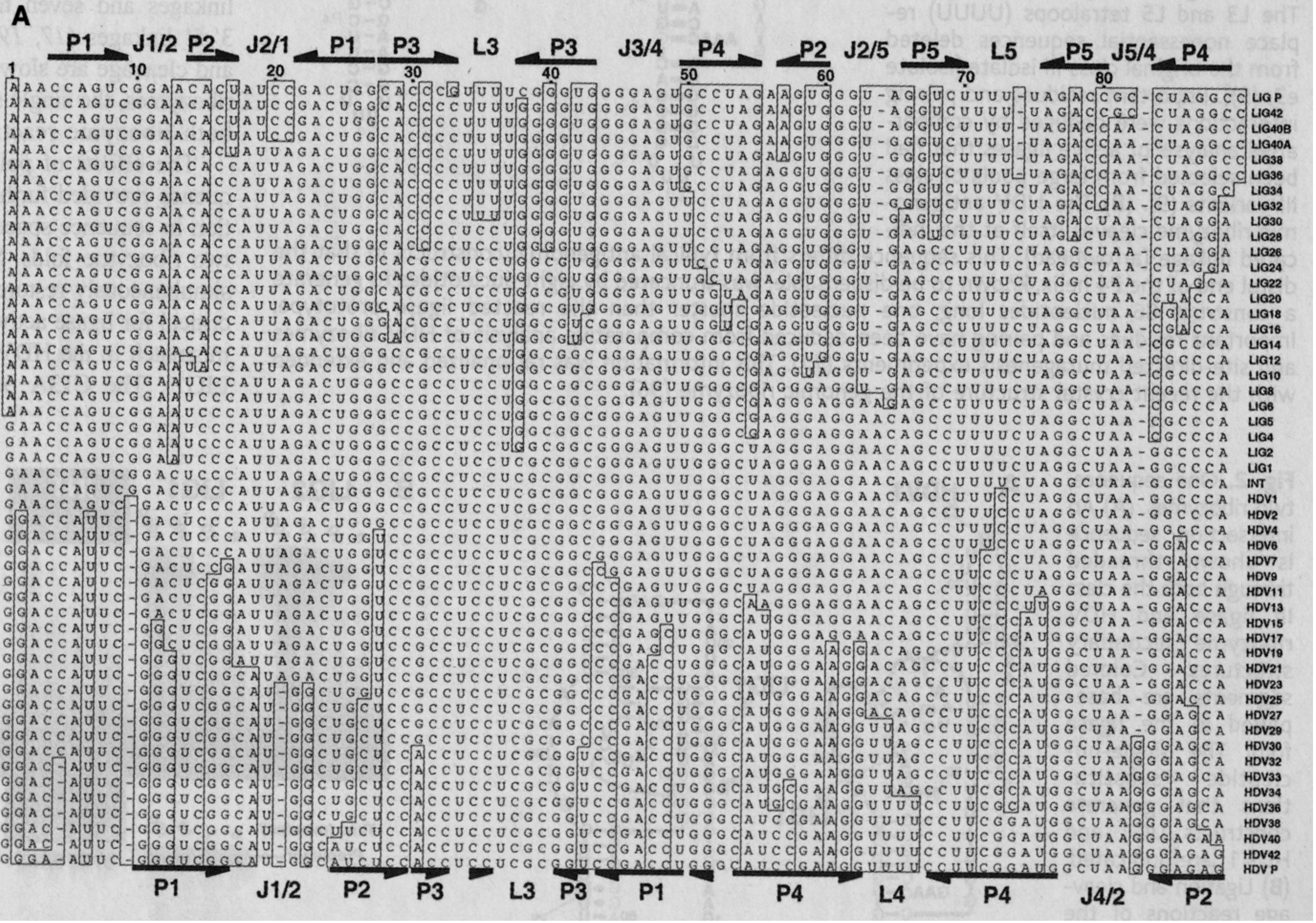
Proof. Suppose that the alphabet admits only the complementary base pair $[XY]$ and we ask for a sequence x compatible to both s and s'. Then $\jmath(s, s') \cong D_m$ operates on the set of all positions $\{x_1, \ldots, x_n\}$. Since we have the operation of a dihedral group, the orbits are either cycles 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***



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



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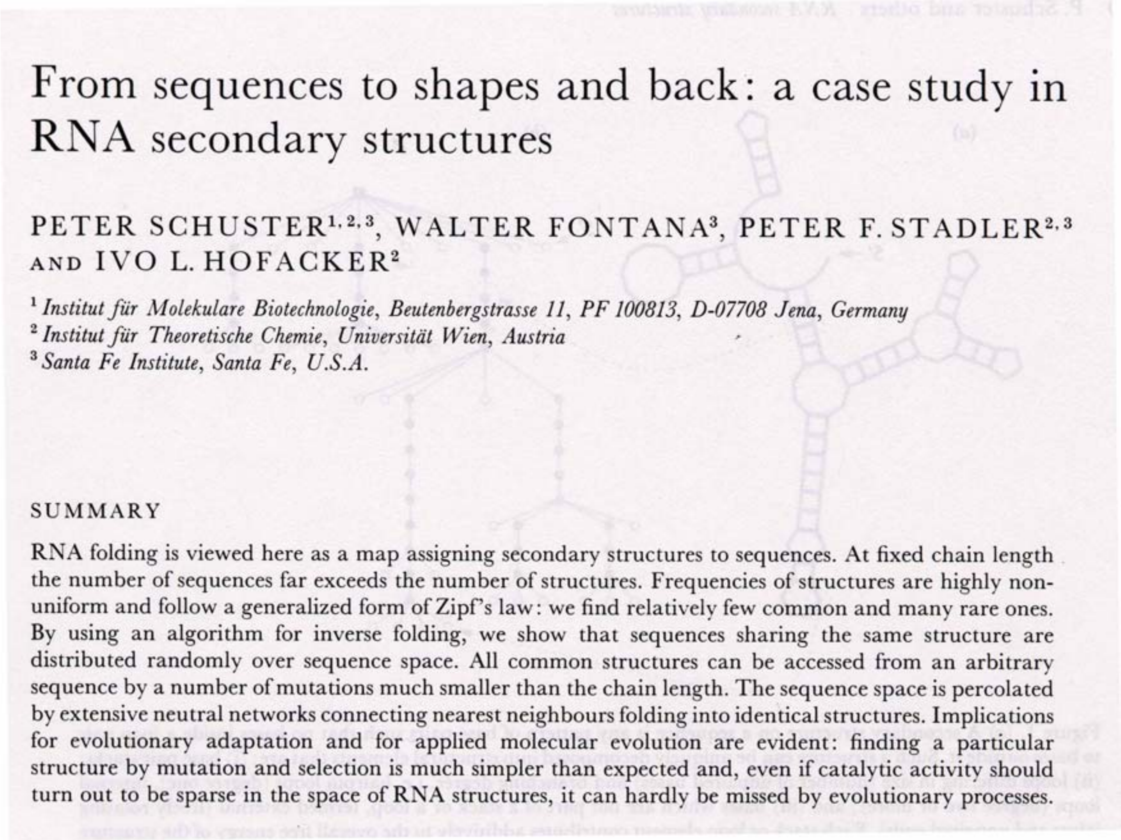
Sequence of mutants from the intersection to both reference ribozymes



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

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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.

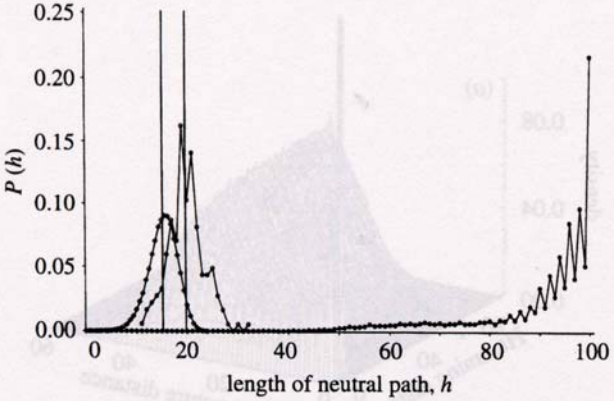

Figure 4. Neutral paths. A neutral path is defined by a series of nearest neighbour sequences that fold into identical structures. Two classes of nearest neighbours are admitted: neighbours of Hamming distance 1, which are obtained by single base exchanges in unpaired stretches of the structure, and 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).

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

No new principle will declare itself from below a heap of facts.

Sir Peter Medawar, 1985