Evolutionary Biotechnology

From Experiments to Theory and Back

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Second European Medical and Biological Engineering Conference

Austria Center Vienna, 24.– 28.12.2002

	Generation time	10 000 generations	10^6 generations	10^7 generations
RNA molecules	10 sec 1 min	27.8 h = 1.16 d 6.94 d	115.7 d 1.90 a	3.17 a 19.01 a
Bacteria	20 min 10 h	138.9 d 11.40 a	38.03 a 1 140 a	380 a 11 408 a
Higher multicelluar organisms	10 d 20 a	274 a 200 000 a	27 380 a 2×10^7 a	273 800 a 2×10^8 a

Generation times and evolutionary timescales

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

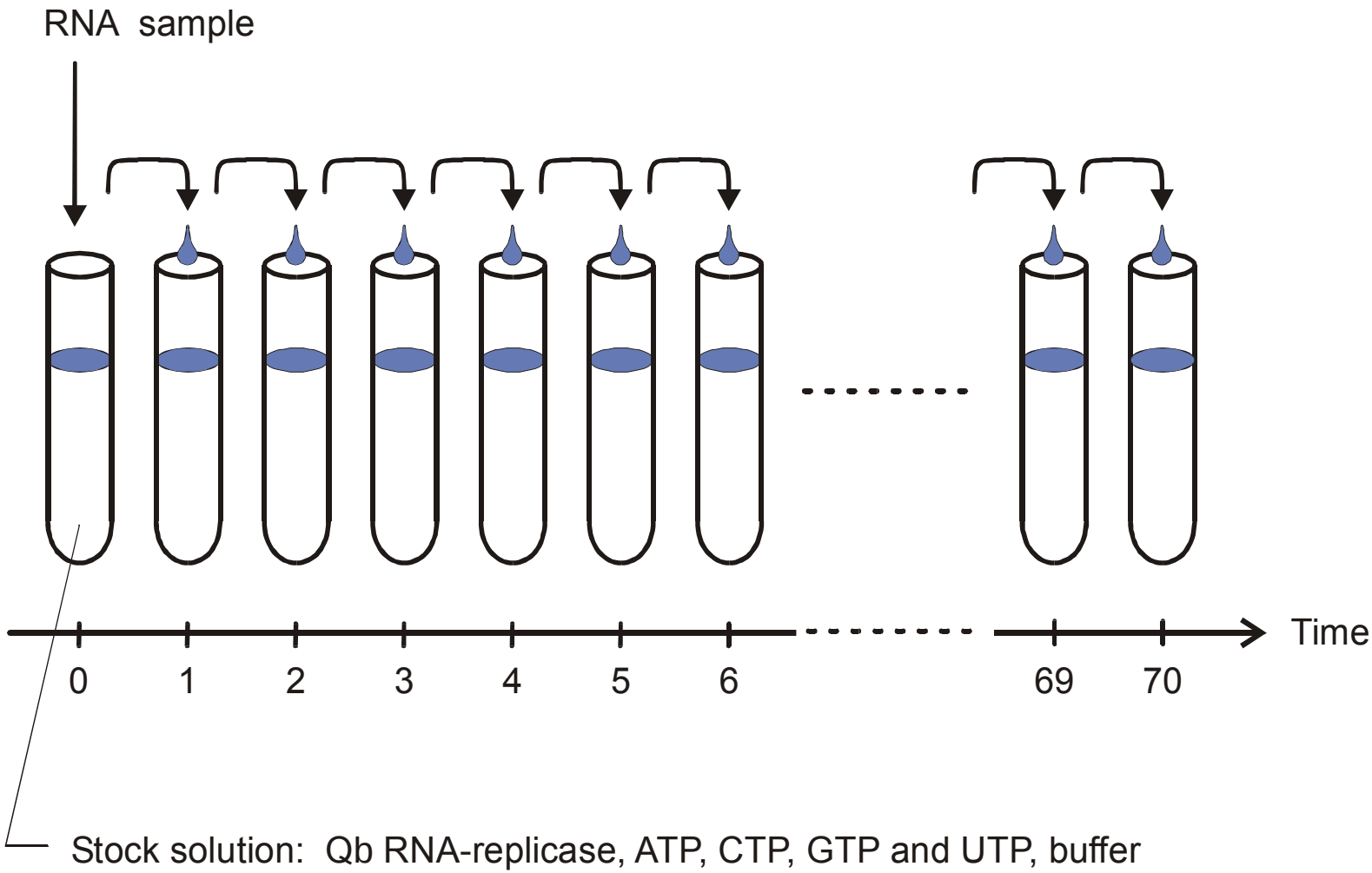
S.Spiegelman, ***An approach to the experimental analysis of precellular evolution***. Quart.Rev.Biophys. **4** (1971), 213-253

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

G.Bauer, H.Otten, J.S.McCaskill, ***Travelling waves of*** **in vitro** ***evolving RNA.*** *Proc.Natl.Acad.Sci.USA* **86** (1989), 7937-7941

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

G.Strunk, T.Ederhof, ***Machines for automated evolution experiments*** **in vitro** ***based on the serial transfer concept***. Biophysical Chemistry **66** (1997), 193-202



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

Reproduction of the original figure of the serial transfer experiment with Qβ RNA

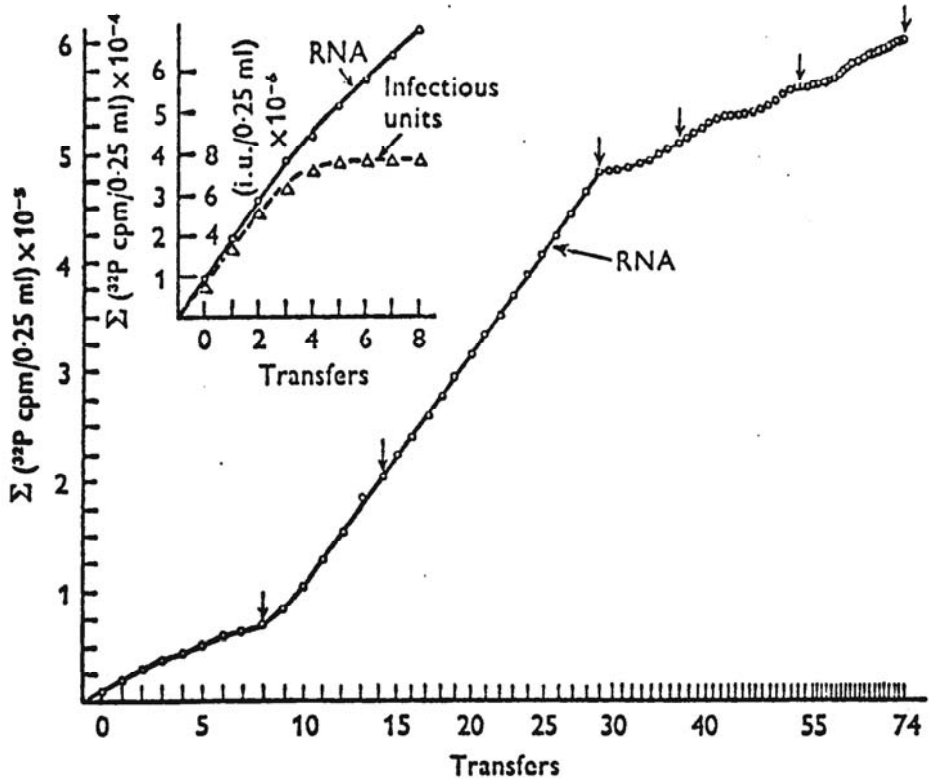
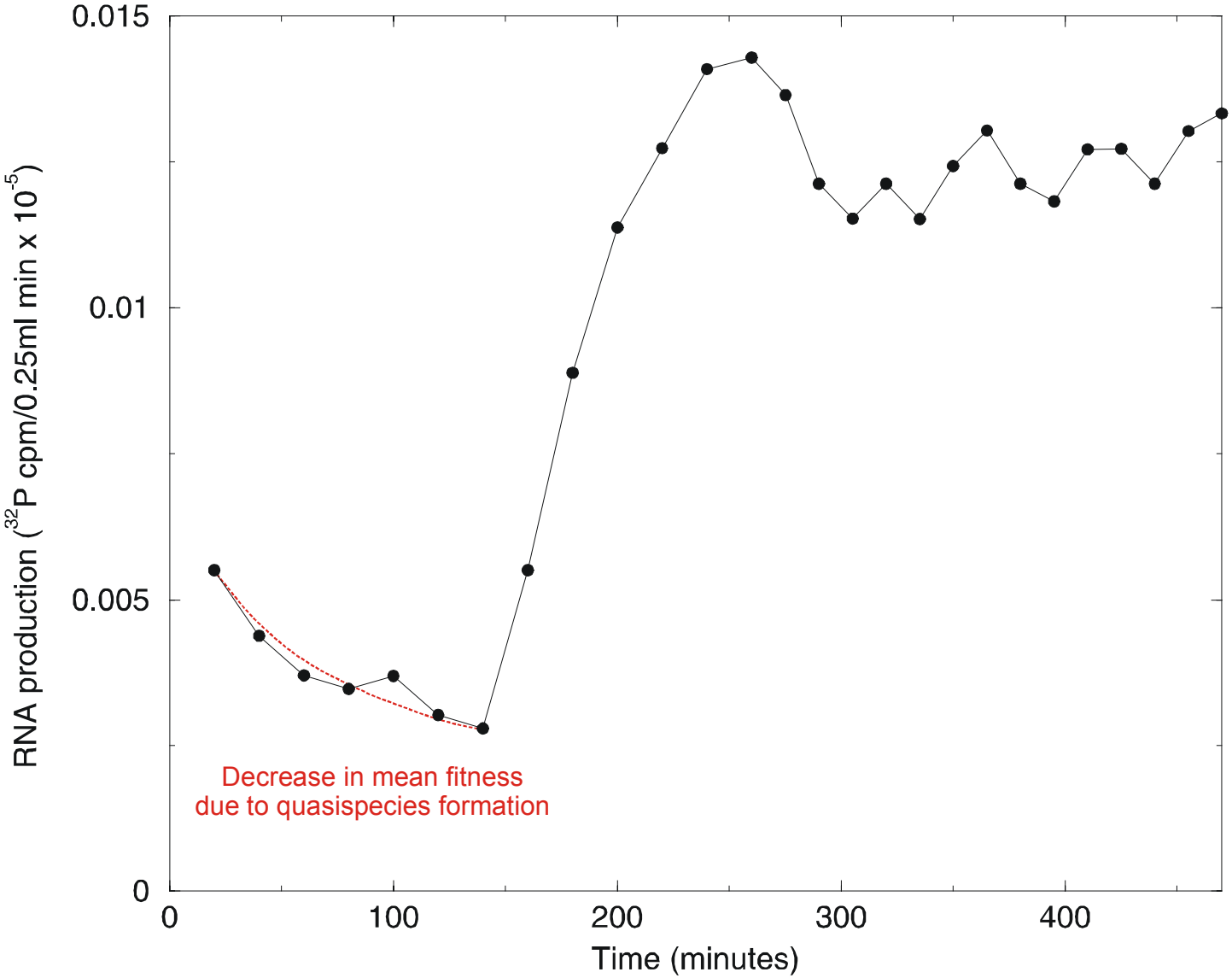


Fig. 9. Serial transfer experiment. Each 0·25 ml standard reaction mixture contained 40 μg of Qβ replicase and ^{32}P-UTP. The first reaction (0 transfer) was initiated by the addition of 0·2 μg ts-1 (temperature-sensitive RNA) and incubated at 35 °C for 20 min, whereupon 0·02 ml was drawn for counting and 0·02 ml was used to prime the second reaction (first transfer), and so on. After the first 13 reactions, the incubation periods were reduced to 15 min (transfers 14–29). Transfers 30–38 were incubated for 10 min. Transfers 39–52 were incubated for 7 min, and transfers 53–74 were incubated for 5 min. The arrows above certain transfers (0, 8, 14, 29, 37, 53, and 73) indicate where 0·001–0·1 ml of product was removed and used to prime reactions for sedimentation analysis on sucrose. The inset examines both infectious and total RNA. The results show that biologically competent RNA ceases to appear after the 4th transfer (Mills *et al.* 1967).

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



The increase in RNA production rate during a serial transfer experiment

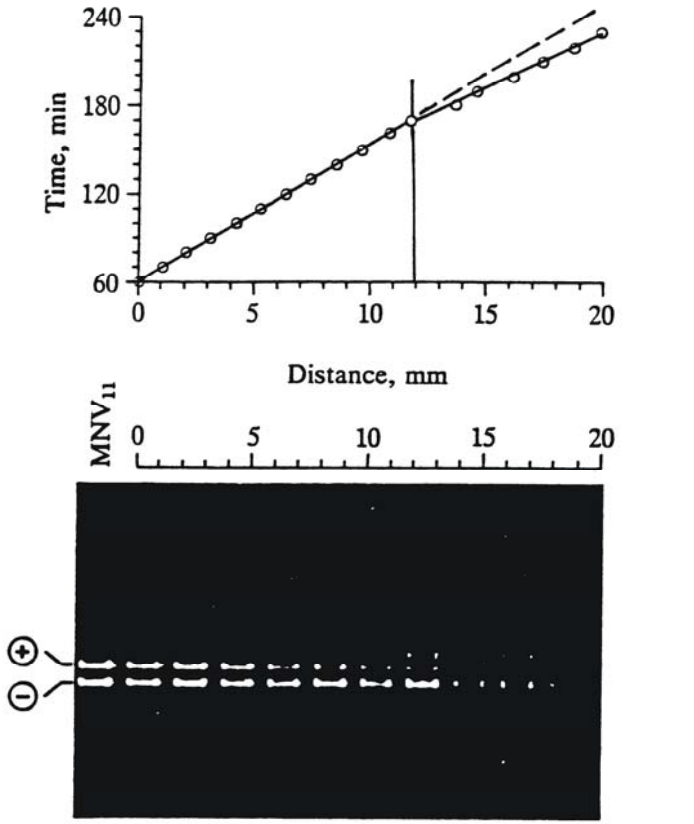


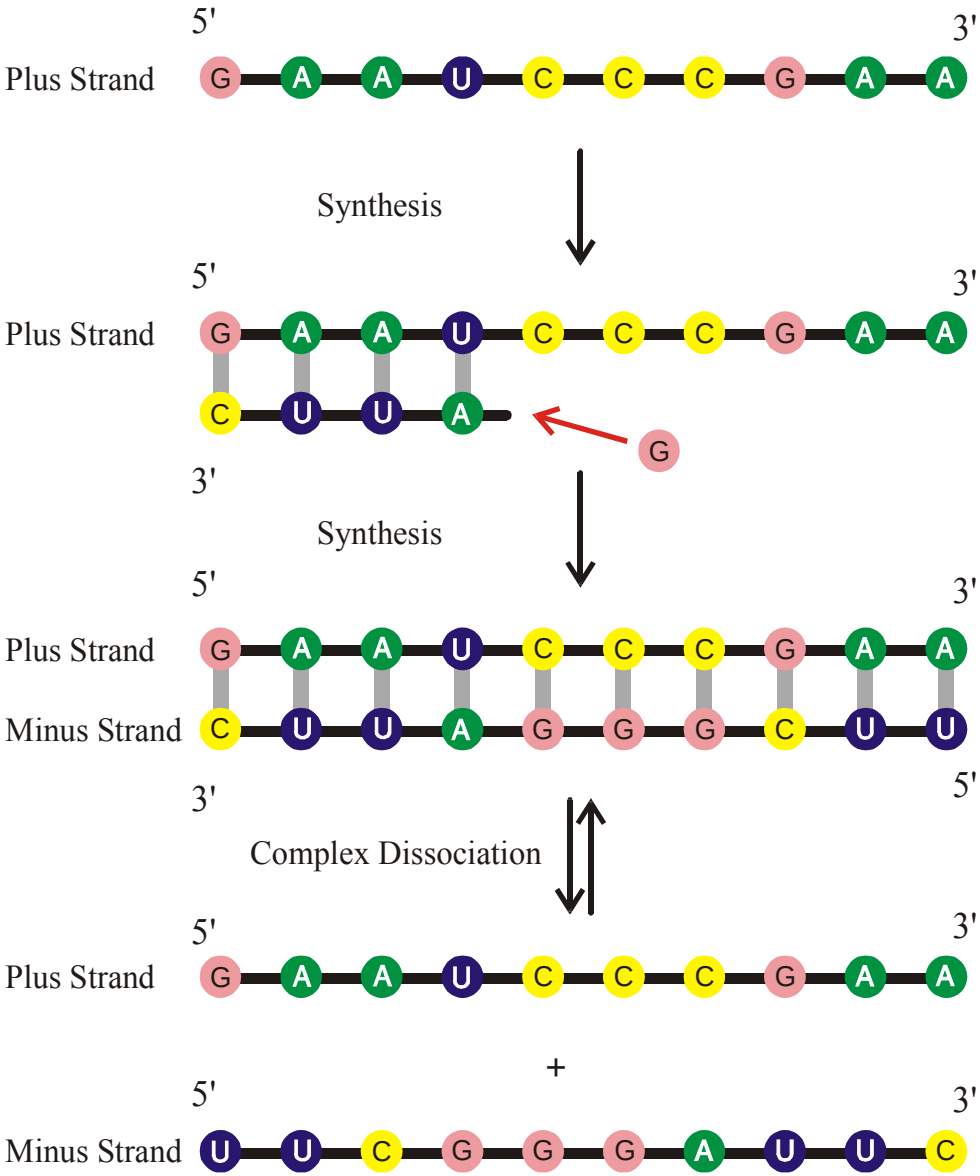
FIG. 3. Evolution of a new quasi-species along the capillary. (*Upper*) Front position measured using setup B. (*Lower*) Gel containing the fractions at 2.5-mm intervals. Regression lines are shown for the periods before and after 170 min. Aliquots (2 μl) of the fractions were withdrawn after 240 min, mixed with 2 μl of loading buffer, boiled for 3 min to melt the double strands, immediately chilled on dry ice, and loaded into the gel slots. The polyacrylamide gel contained 13% (wt/vol) acrylamide and 0.26% *N,N'*-methylenebisacrylamide in running buffer (100 mM Tris borate, pH 8.3). Electrophoresis was for 6 hr at 5 V/cm at 4°C (16). Lane MNV_{11} contains MNV_{11} single strands (plus and minus strands) as reference. The concentration shift to new bands is centered at 12 mm where the velocity changes.

Selection of **Q**b-RNA through replication in a capillary

G.Bauer, H.Otten, J.S. McCaskill,
Proc.Natl.Acad.Sci.USA **90**:4191, 1989

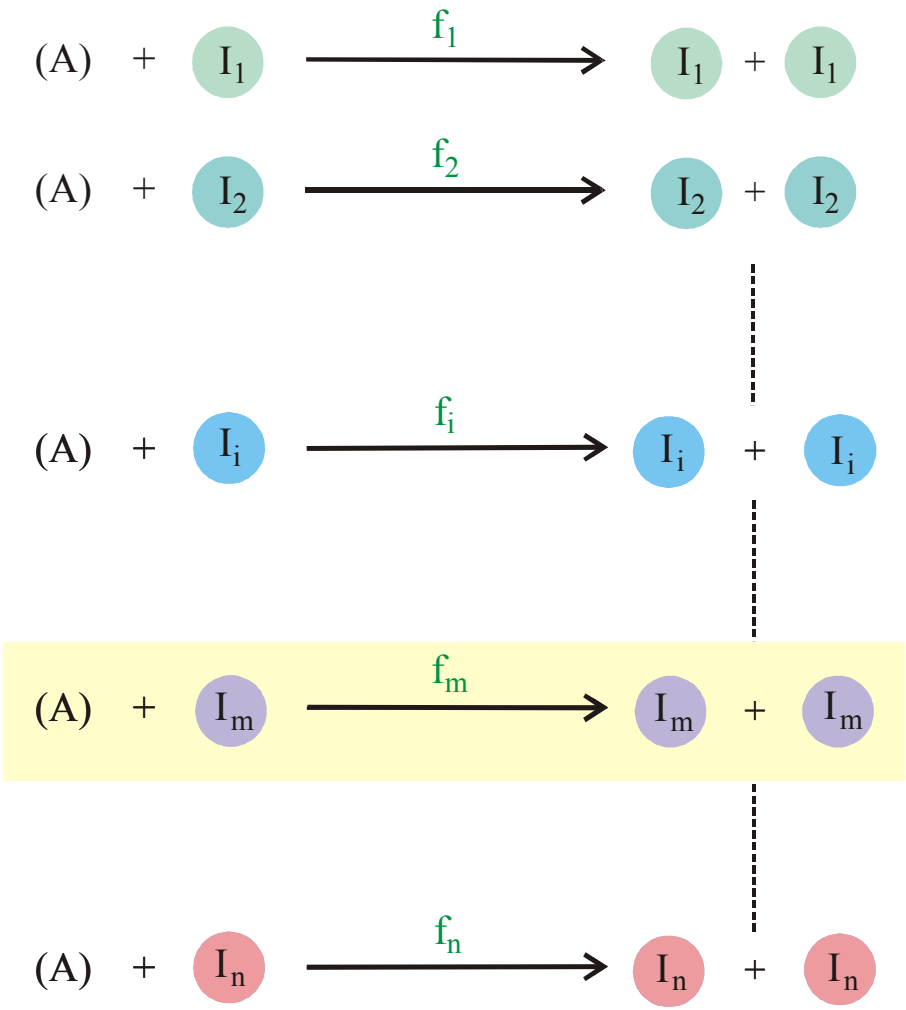
No new principle will declare itself from below a heap of facts.

Sir Peter Medawar, 1985



Complementary replication as the simplest copying mechanism of RNA Complementarity is determined by Watson-Crick base pairs:

G Í C and A=U



$$dx_i / dt = f_i x_i - x_i \Phi = x_i (f_i - \Phi)$$

$\Phi = \Sigma_j f_j x_j$; $\Sigma_j x_j = 1$; i,j =1,2,...,n

$[I_i] = x_i$ Æ 0 ; i =1,2,...,n ;

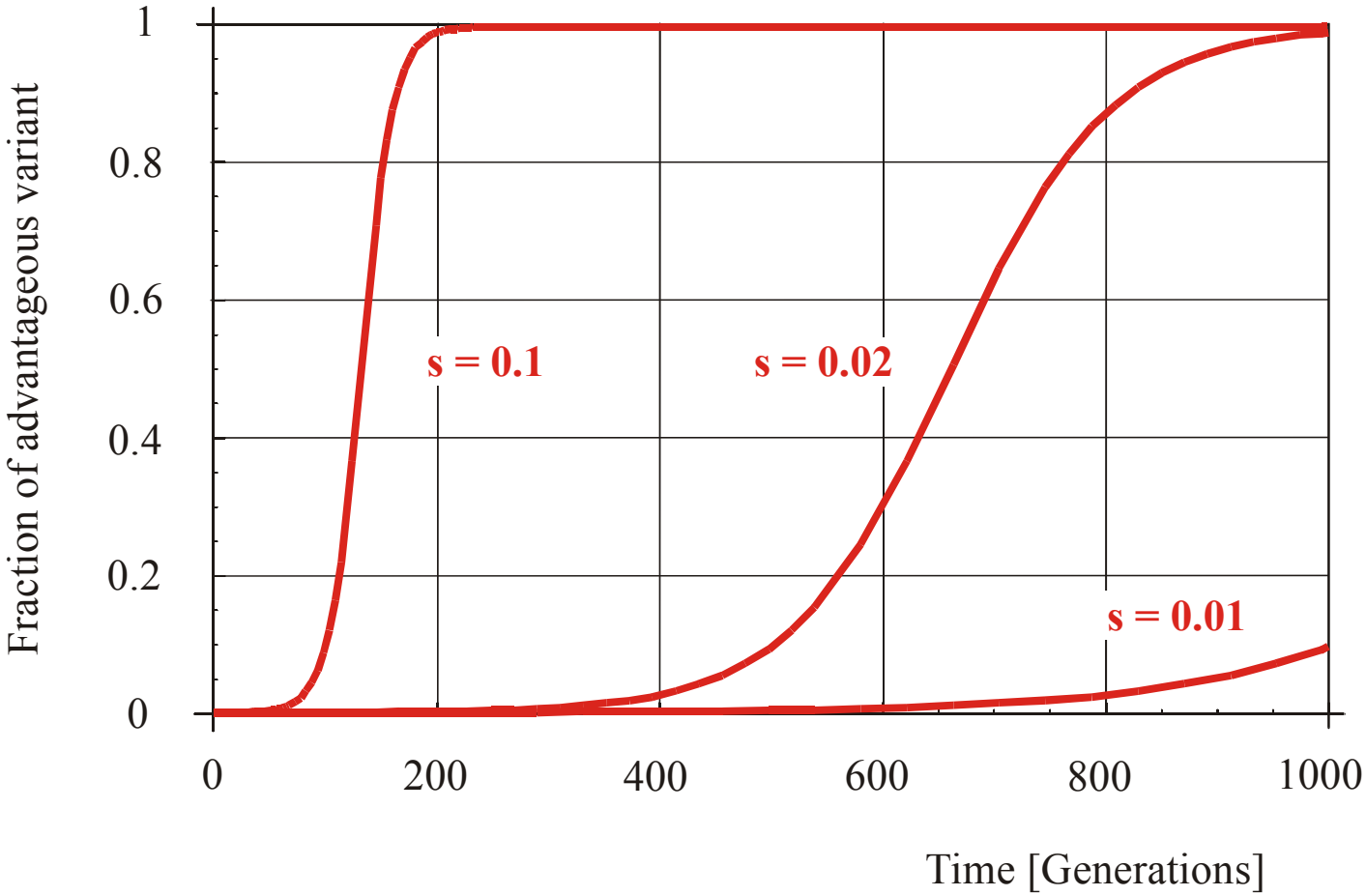
[A] = a = constant

$f_m = \max \{f_j; j=1,2,...,n\}$

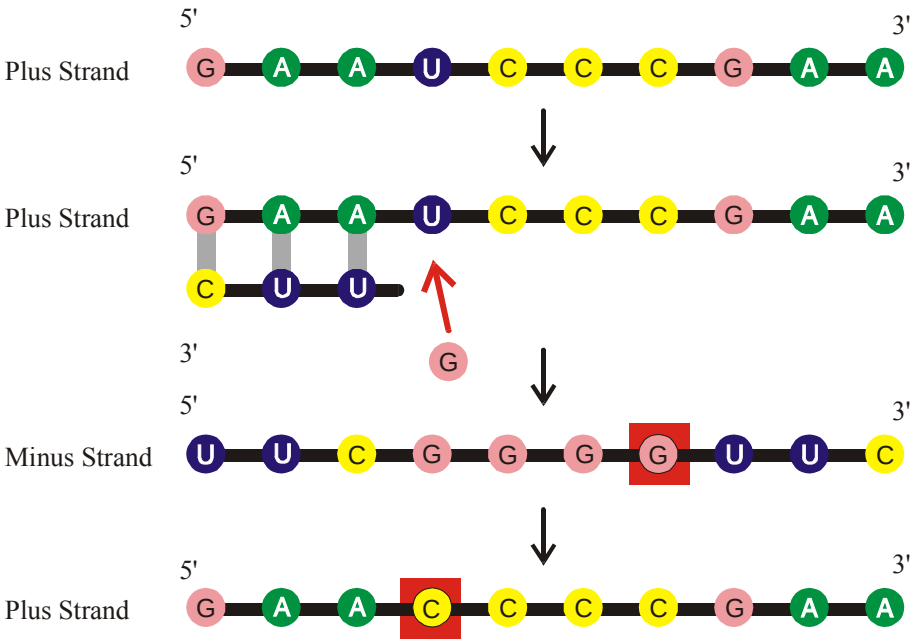
$x_m(t)$ Á 1 for t Á ¸

Reproduction of organisms **or replication** of molecules as the basis of selection

$$\mathbf{s} = (f_2 - f_1) / f_1;\ f_2 > f_1\ ;\ x_1(0) = 1 - 1/N\ ;\ x_2(0) = 1/N$$



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



Point Mutation

GAAUCCCGAA → GAAUCCCGUCCCGAA

Insertion

GAAUCCCGAA → GAAUCCGA

Deletion

Mutations in nucleic acids represent the mechanism of **variation** of **genotypes**.

Theory of molecular evolution

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

C.J.Thompson, J.L.McBride, ***On Eigen's theory of the self-organization of matter and the evolution of biological macromolecules***. Math. Biosci. **21** (1974), 127-142

B.L.Jones, R.H.Enns, S.S.Rangnekar, ***On the theory of selection of coupled macromolecular systems.*** Bull.Math.Biol. **38** (1976), 15-28

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

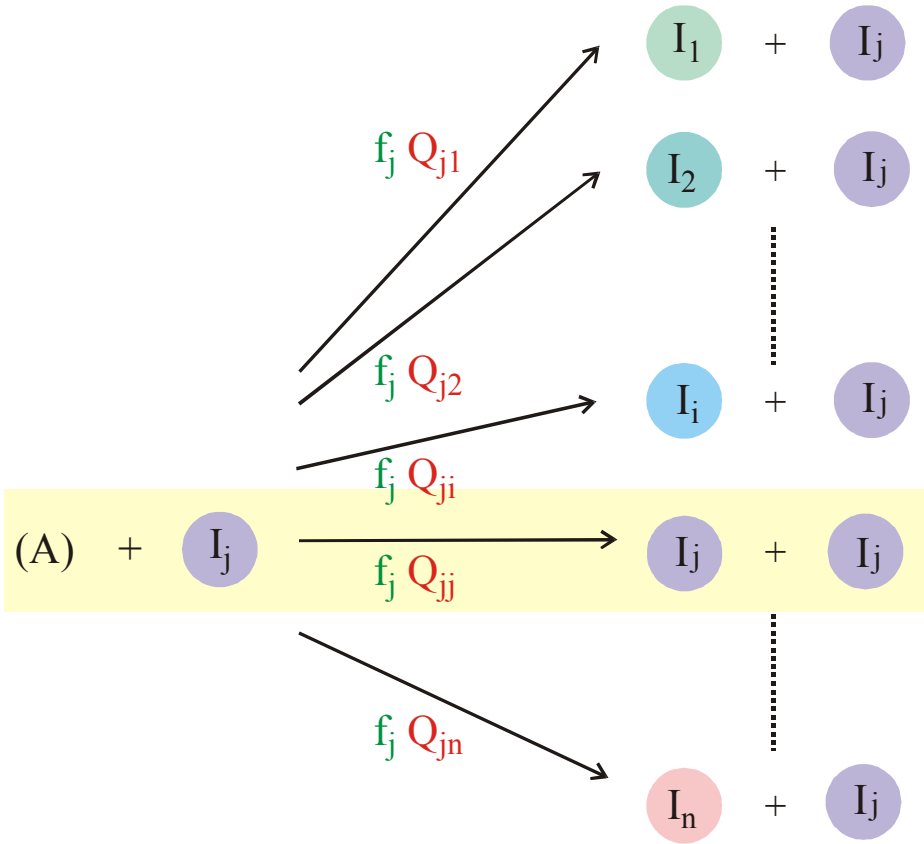
M.Eigen, P.Schuster, ***The hypercycle. A principle of natural self-organization. Part C: The realistic hypercycle***. Naturwissenschaften **65** (1978), 341-369

J.Swetina, P.Schuster, ***Self-replication with errors - A model for polynucleotide replication.*** Biophys.Chem. **16** (1982), 329-345

J.S.McCaskill, ***A localization threshold for macromolecular quasispecies from continuously distributed replication rates***. J.Chem.Phys. **80** (1984), 5194-5202

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



$$dx_i / dt = \Sigma_j f_j Q_{ji} x_j - x_i \Phi$$

$$\Phi = \Sigma_j f_j x_i ; \quad \Sigma_j x_j = 1 ; \quad \Sigma_i Q_{ij} = 1$$

$[I_i] = x_i \neq 0 ; \quad i = 1,2,...,n ;$

$[A] = a = \text{constant}$

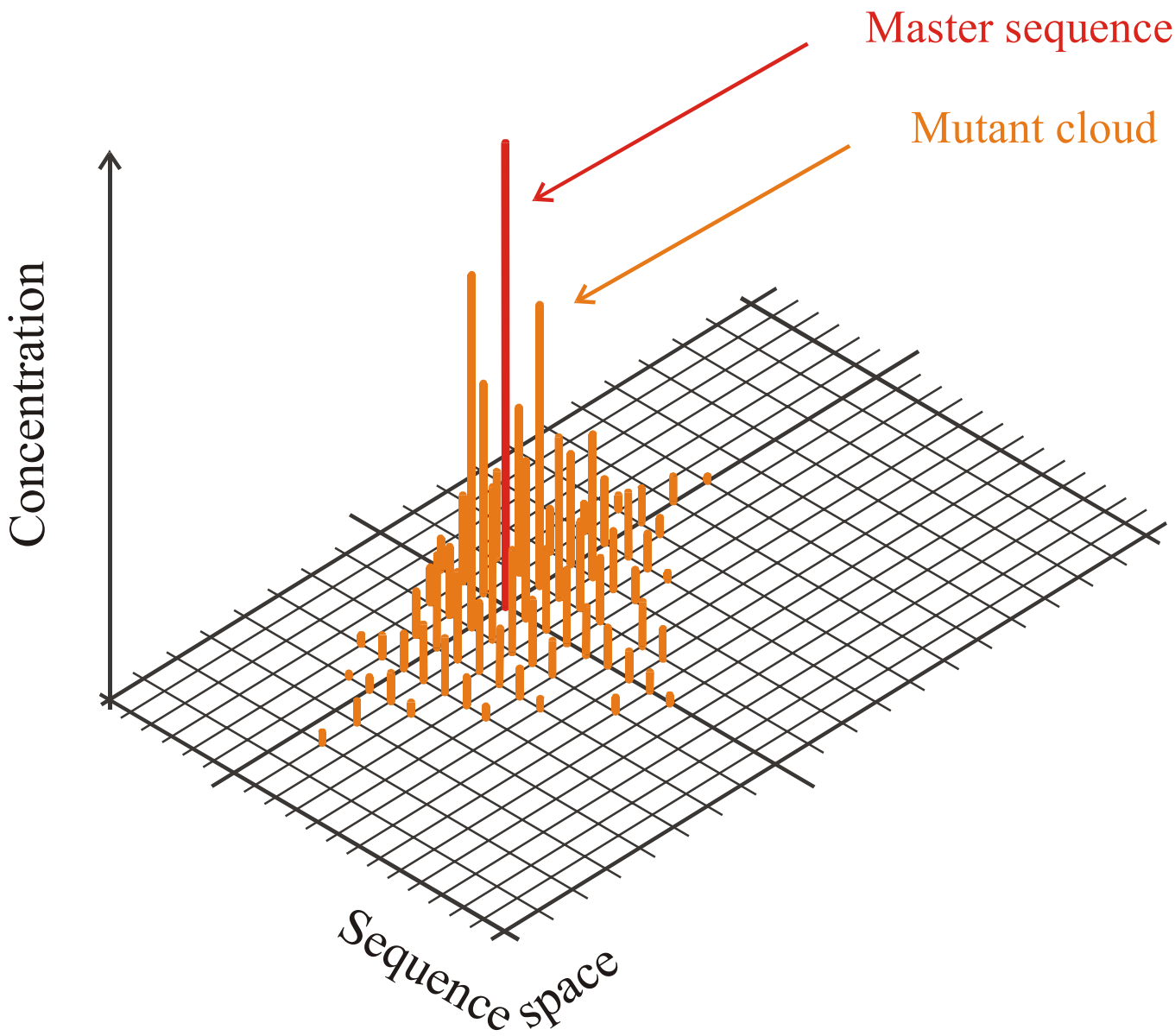
$$Q_{ij} = (1-p)^{\ell - d(i,j)} p^{d(i,j)}$$

p ..... Error rate per digit

ℓ ..... Chain length of the polynucleotide

d(i,j) .... Hamming distance between I_i and I_j

Chemical kinetics of replication and mutation as parallel reactions



The molecular quasispecies in sequence space

Theory of genotype – phenotype mapping

P. Schuster, W.Fontana, P.F.Stadler, I.L.Hofacker, ***From sequences to shapes and back: A case study in RNA secondary structures***. Proc.Roy.Soc.London **B 255** (1994), 279-284

W.Grüner, R.Giegerich, D.Strothmann, C.Reidys, I.L.Hofacker, P.Schuster, ***Analysis of RNA sequence structure maps by exhaustive enumeration. I. Neutral networks***. Mh.Chem. **127** (1996), 355-374

W.Grüner, R.Giegerich, D.Strothmann, C.Reidys, I.L.Hofacker, P.Schuster, ***Analysis of RNA sequence structure maps by exhaustive enumeration. II. Structure of neutral networks and shape space covering***. Mh.Chem. **127** (1996), 375-389

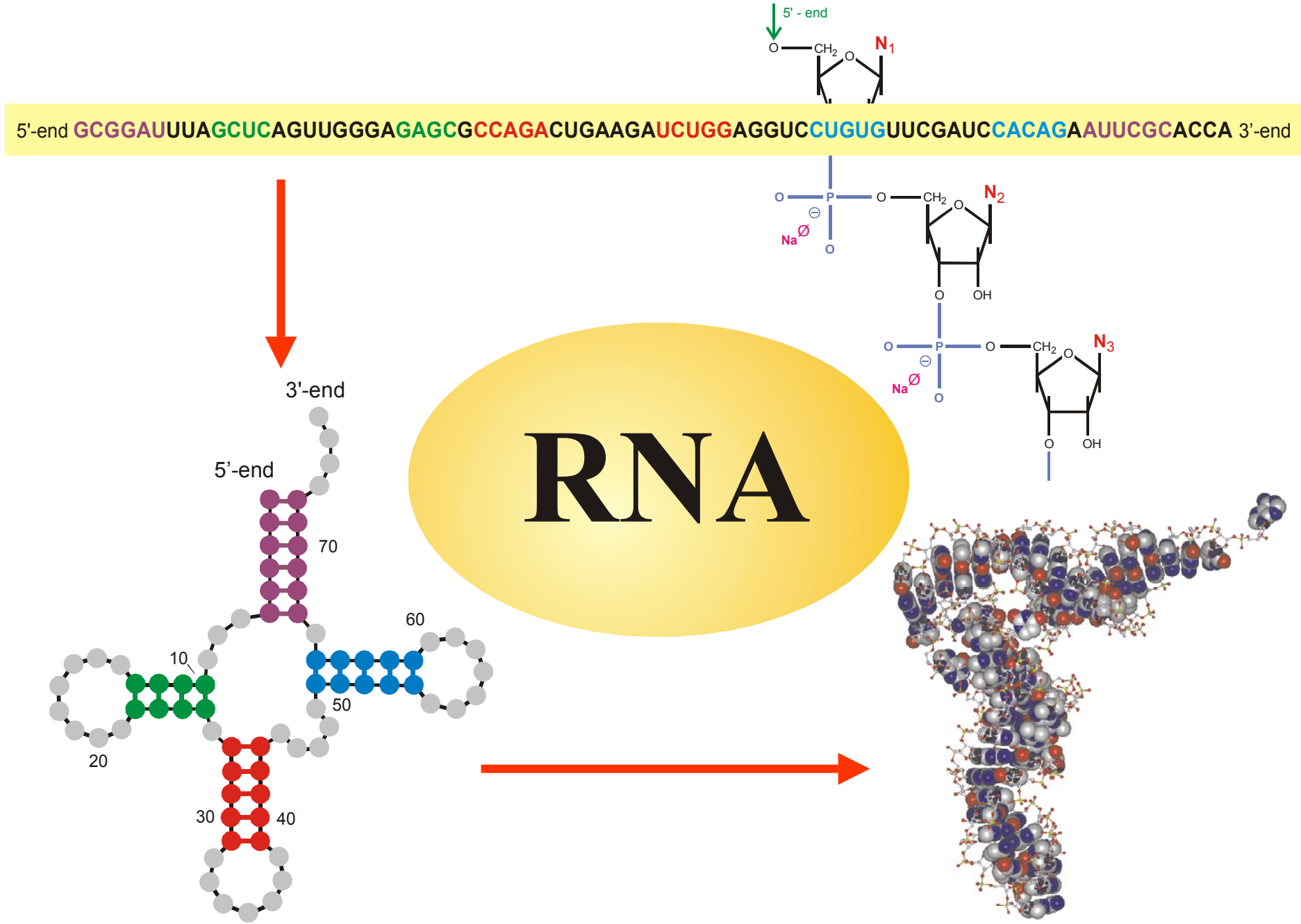
C.M.Reidys, P.F.Stadler, P.Schuster, ***Generic properties of combinatory maps***. Bull.Math.Biol. **59** (1997), 339-397

I.L.Hofacker, P. Schuster, P.F.Stadler, ***Combinatorics of RNA secondary structures***. Discr.Appl.Math. **89** (1998), 177-207

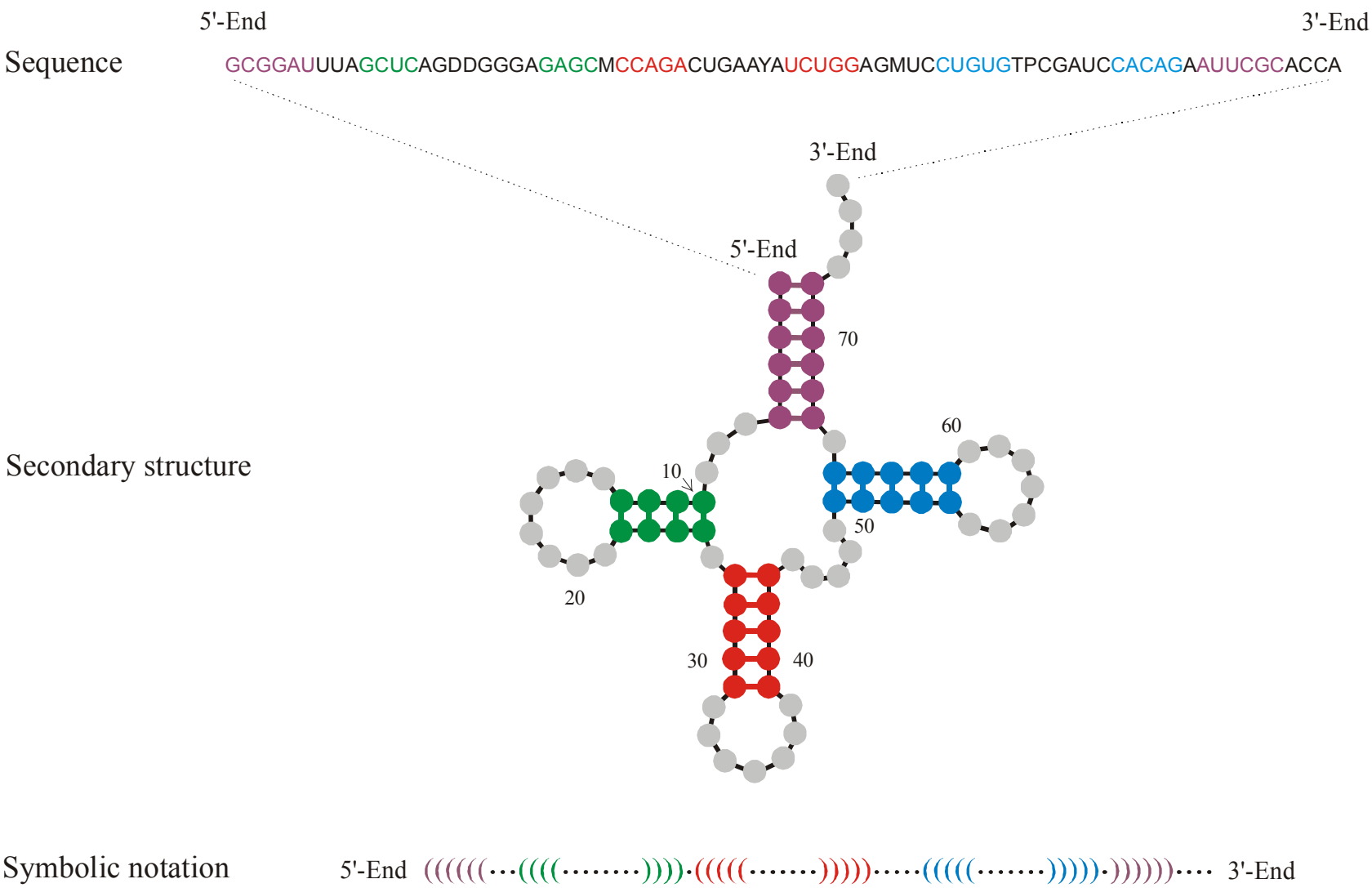
C.M.Reidys, P.F.Stadler, ***Combinatory landscapes***. SIAM Review **44** (2002), 3-54

Genotype-phenotype relations are highly complex and only the most simple cases can be studied. One example is the folding of RNA sequences into RNA structures represented in course-grained form as secondary structures.

The RNA genotype-phenotype relation is understood as a mapping from the space of RNA sequences into a space of RNA structures.



Definition of RNA structure



The **RNA secondary structure** is a listing of **GC**, **AU**, and **GU** base pairs. It is understood in contrast to the full 3D- or **tertiary structure** at the resolution of atomic coordinates. RNA secondary structures are biologically relevant. They are, for example, conserved in evolution.

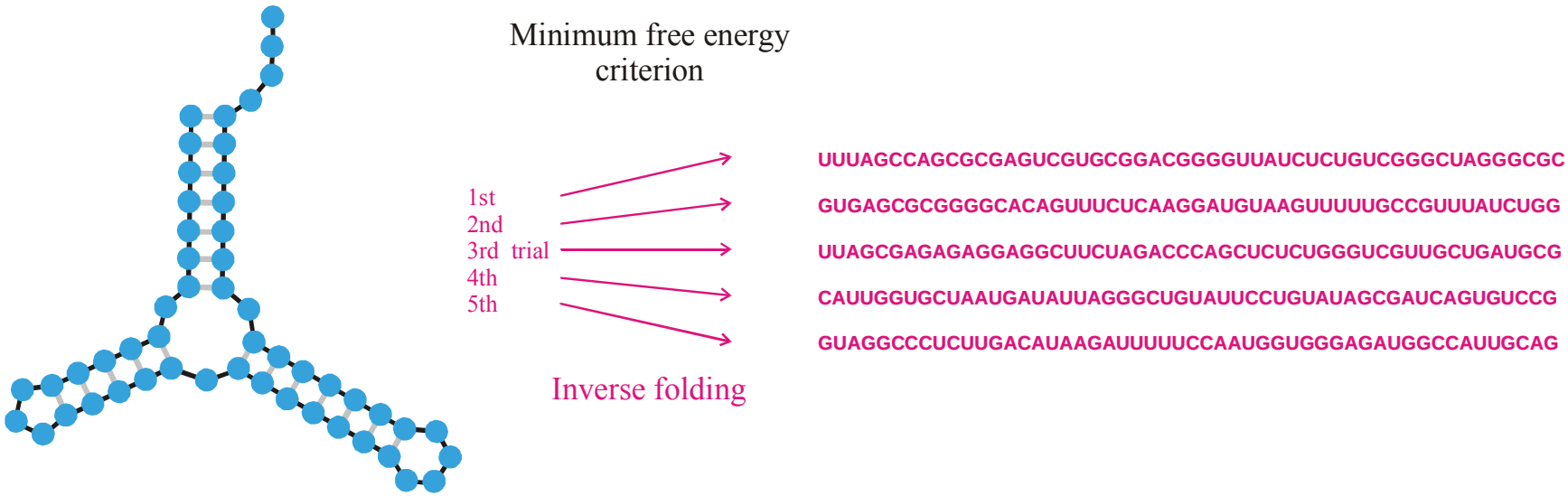
RNA Minimum Free Energy Structures

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)



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

Criterion of
Minimum Free Energy

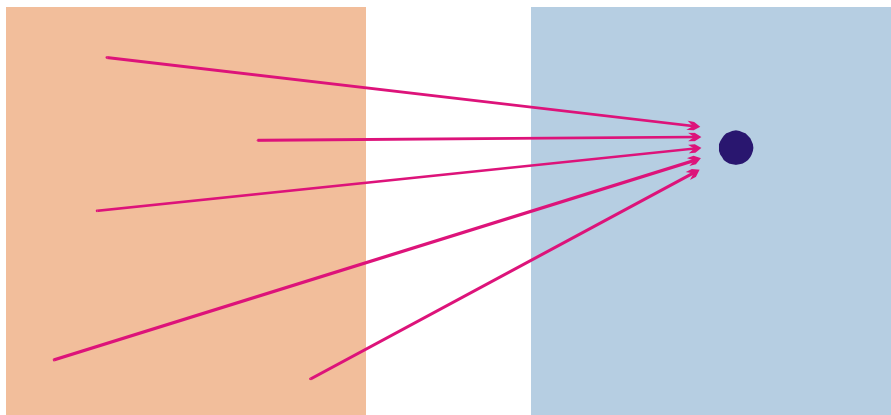
UUUAGCCAGCGCGAGUCGUGCGGACGGGGUUAUCUCUGUCGGGCUAGGGCGC

GUGAGCGCGGGGCACAGUUUCUCAAGGAUGUAAGUUUUUGCCGUUUAUCUGG

UUAGCGAGAGAGGAGGCUUCUAGACCCAGCUCUCUGGGUCGUUGCUGAUGCG

CAUUGGUGCUAAUGAUAUUAGGGCUGUAUUCCUGUAUAGCGAUCAGUGUCCG

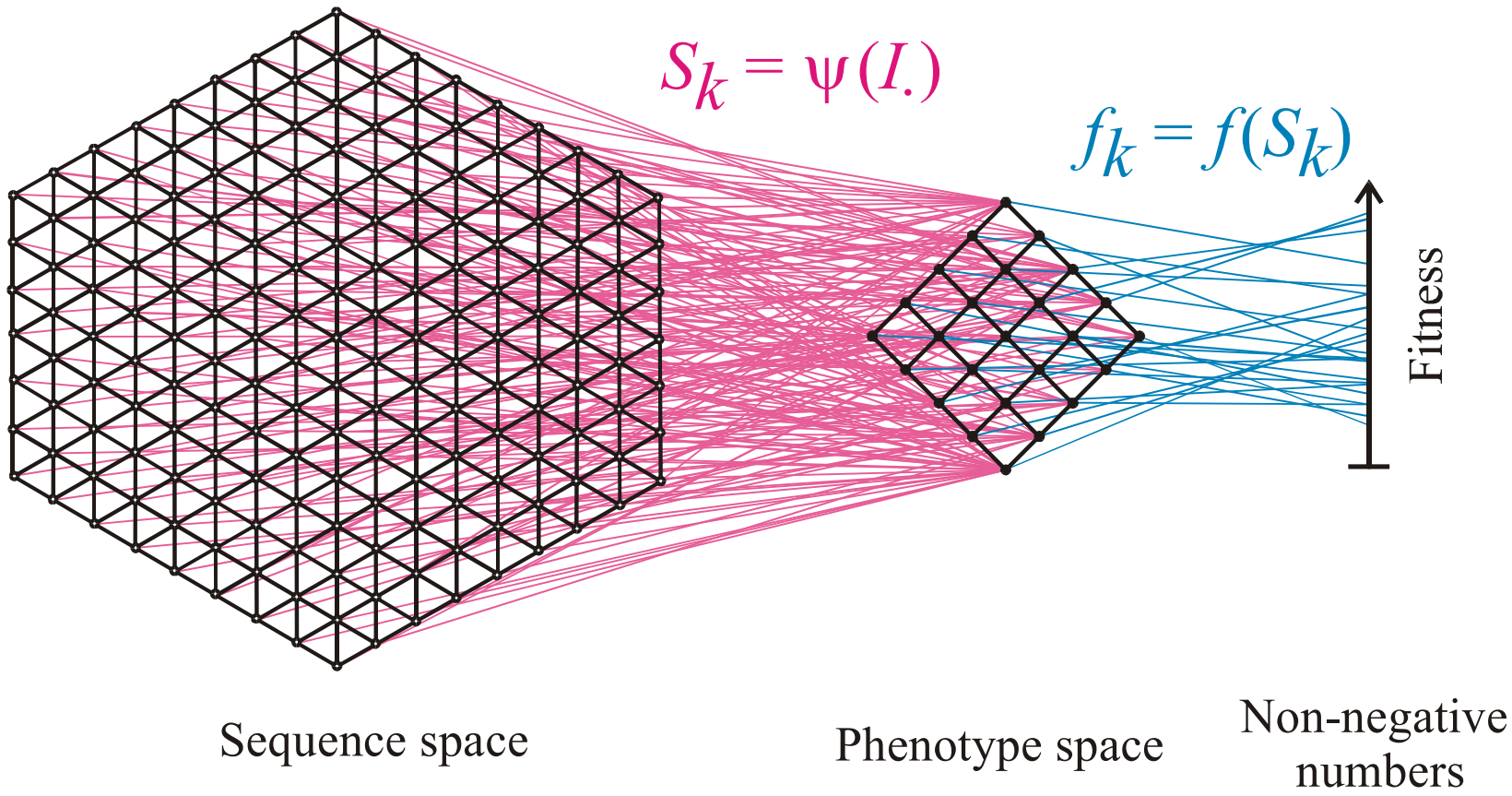
GUAGGCCCUCUUGACAUAAGAUUUUUCCAAUGGUGGGAGAUGGCCAUUGCAG



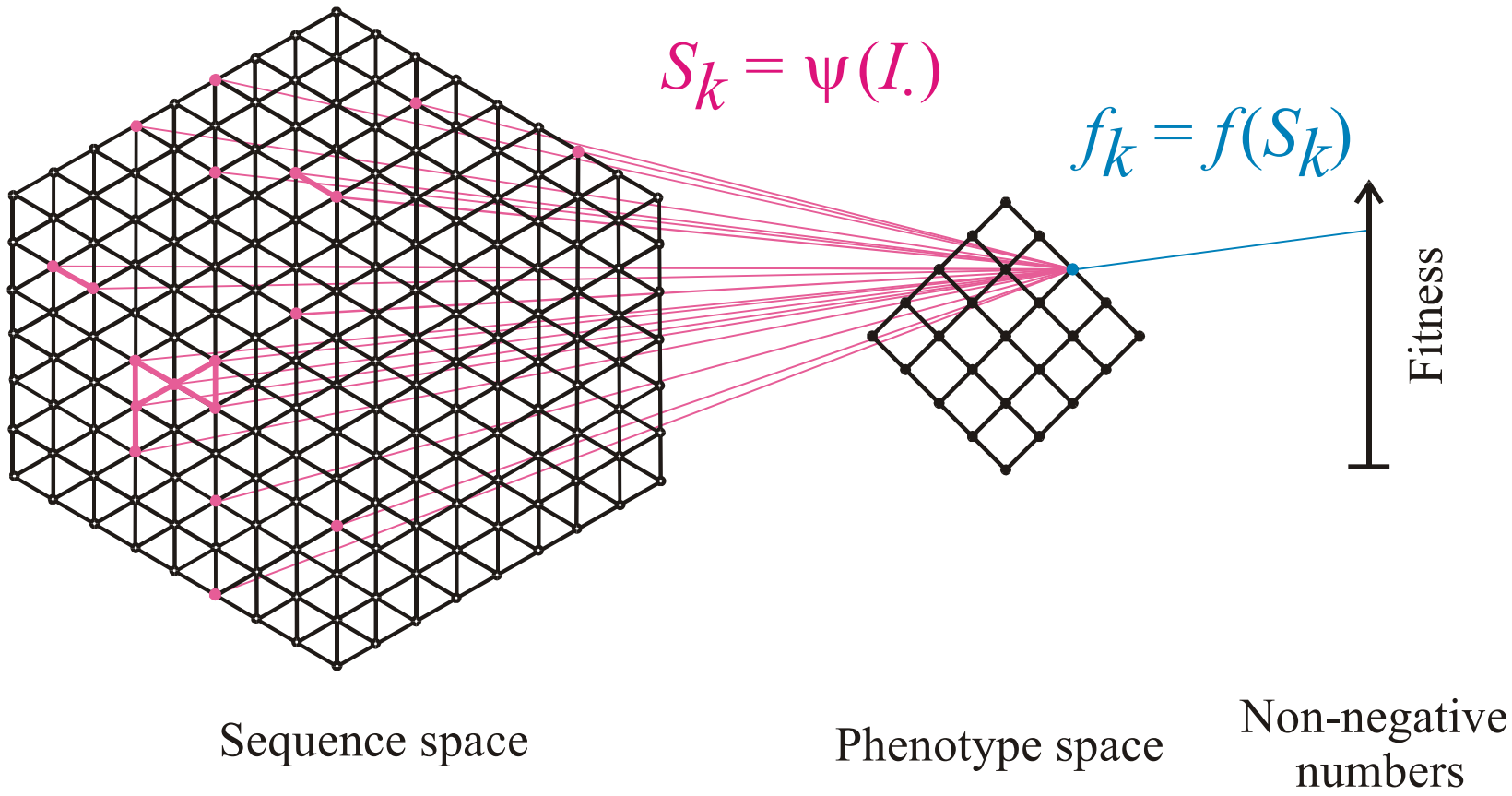
The **RNA model** considers RNA sequences as genotypes and simplified RNA structures, called secondary structures, as phenotypes.

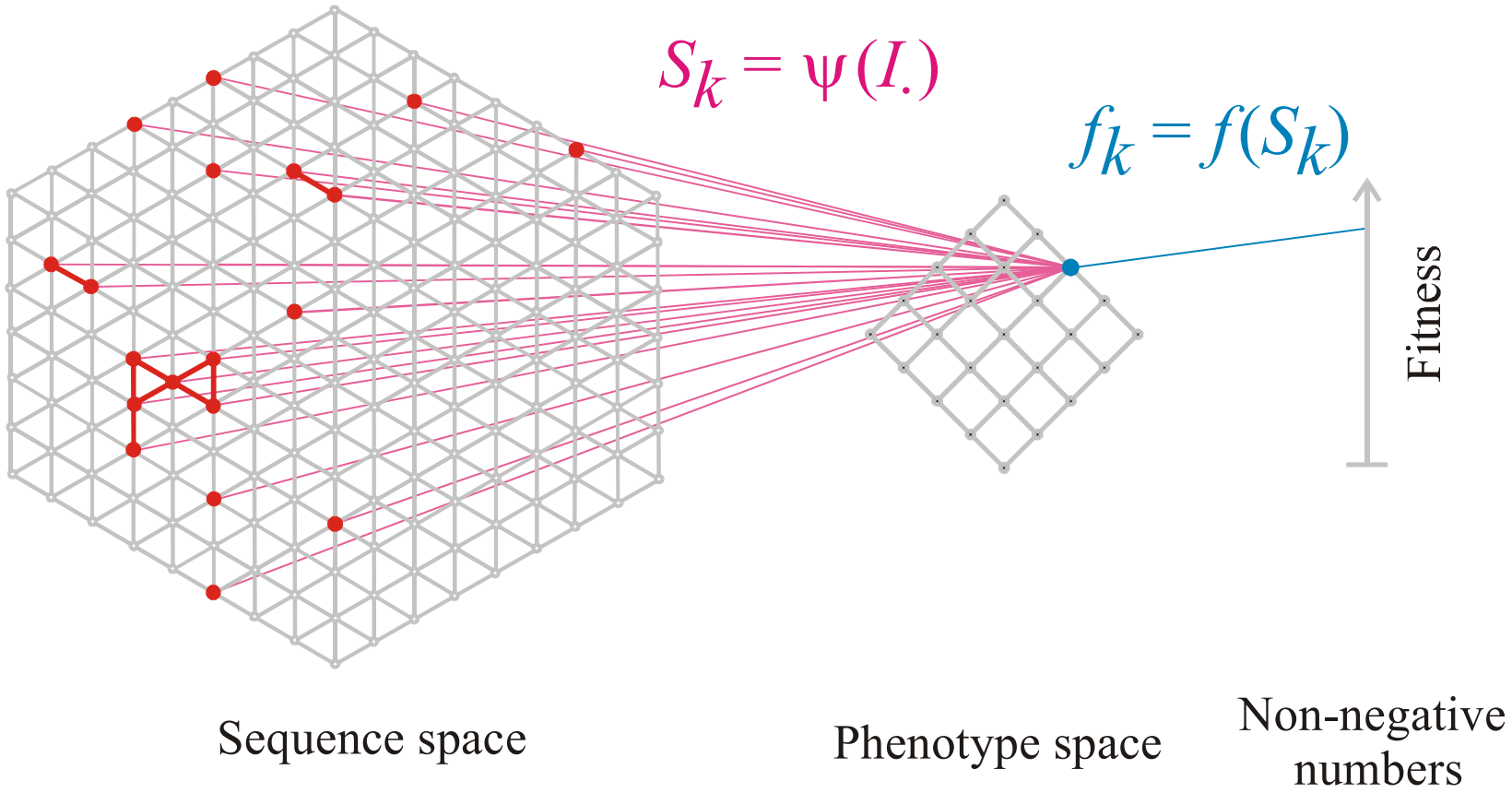
The **mapping** from genotypes into phenotypes is many-to-one. Hence, it is redundant and not invertible.

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.

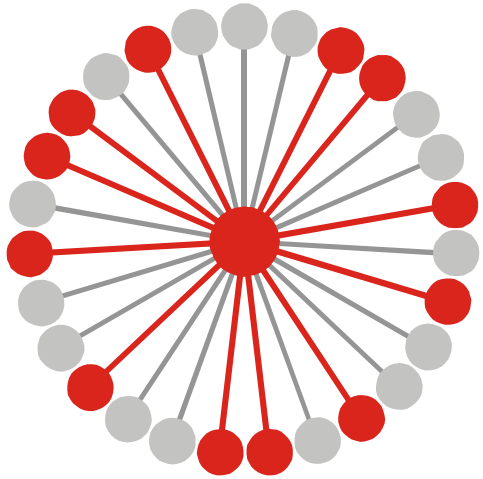


Mapping from sequence space into phenotype space and into fitness values


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



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



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

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

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

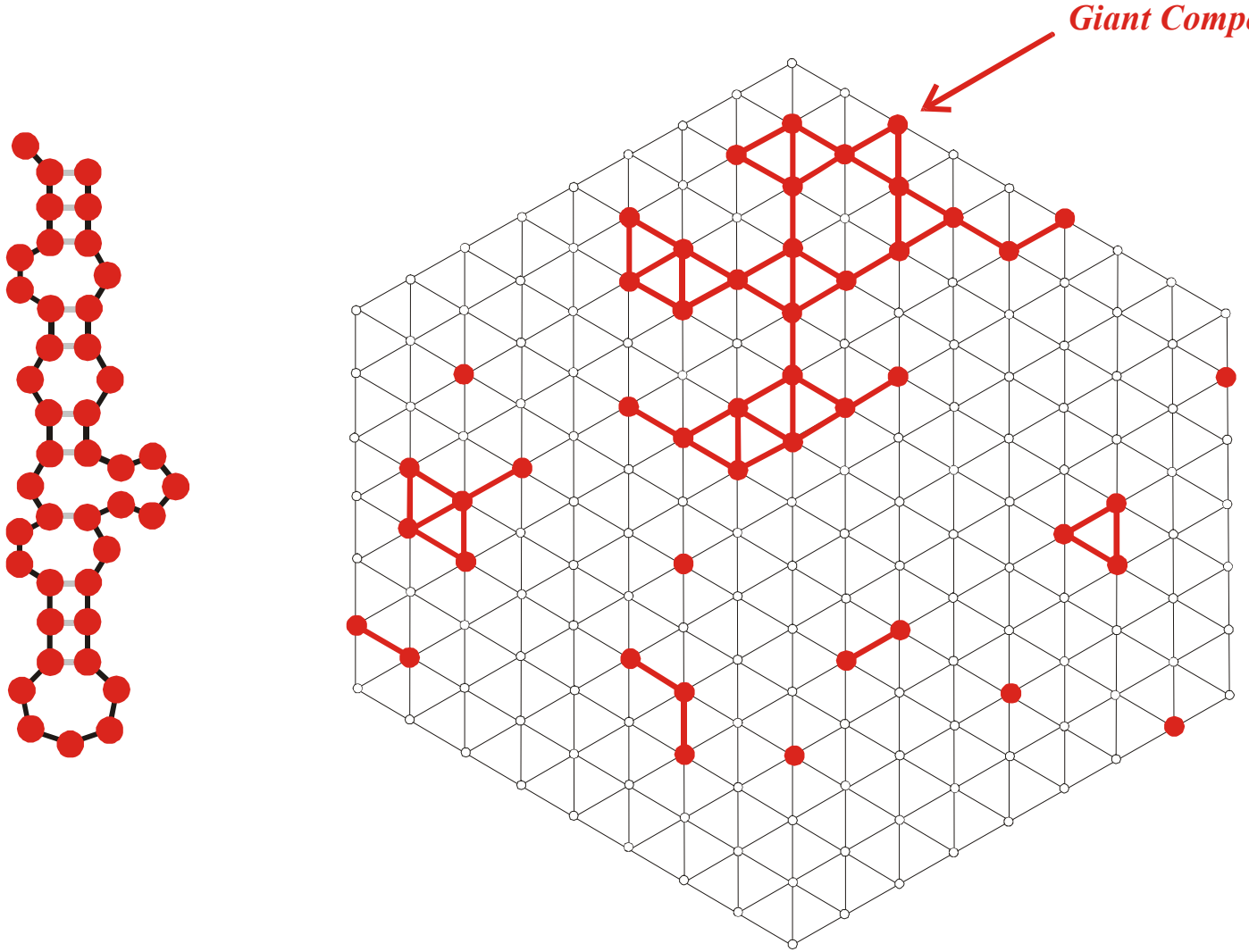
Alphabet size k : **AUGC** ñ k = 4

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

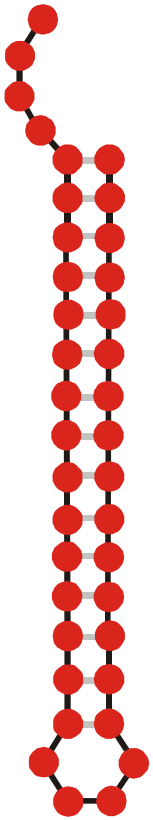
$\bar{\lambda}_k < \lambda_{cr}$ . . . . network G_k is **not** connected

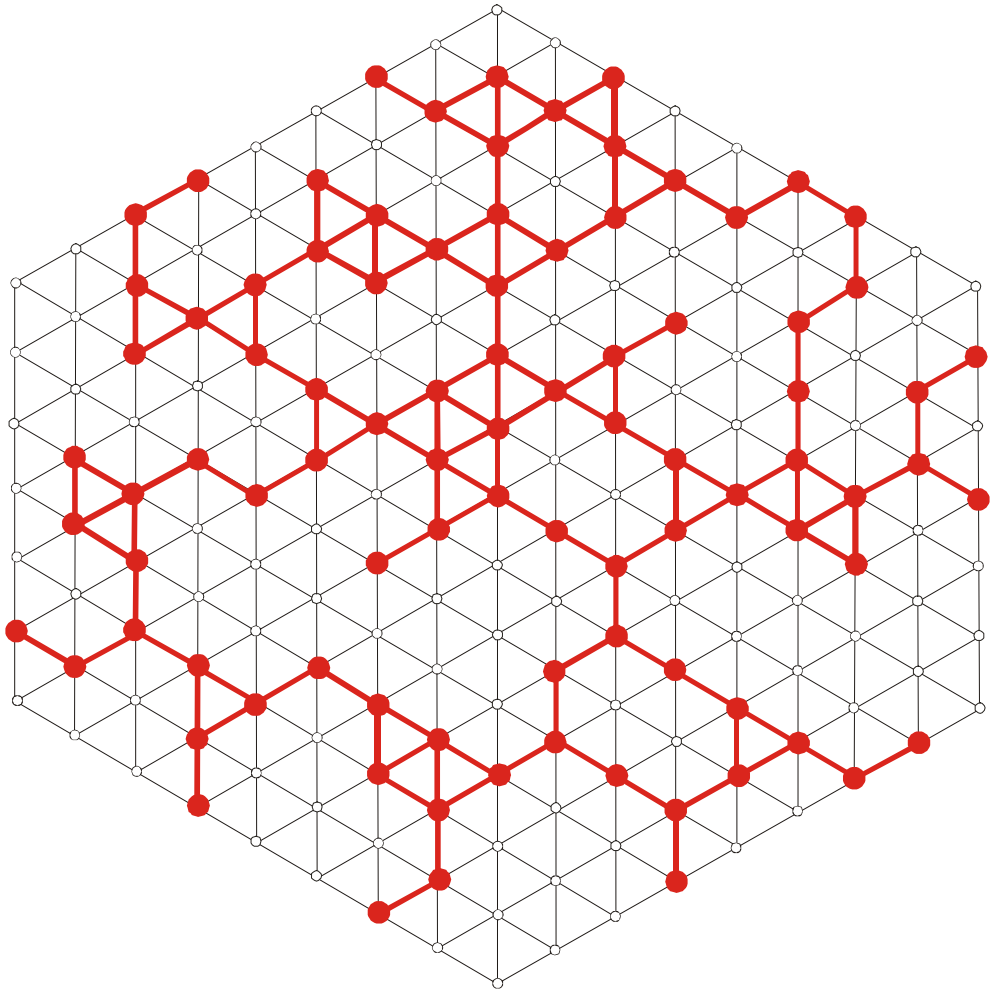
k	I_{cr}
2	0.5
3	0.4226
4	0.3700

Mean degree of neutrality and connectivity of **neutral networks**

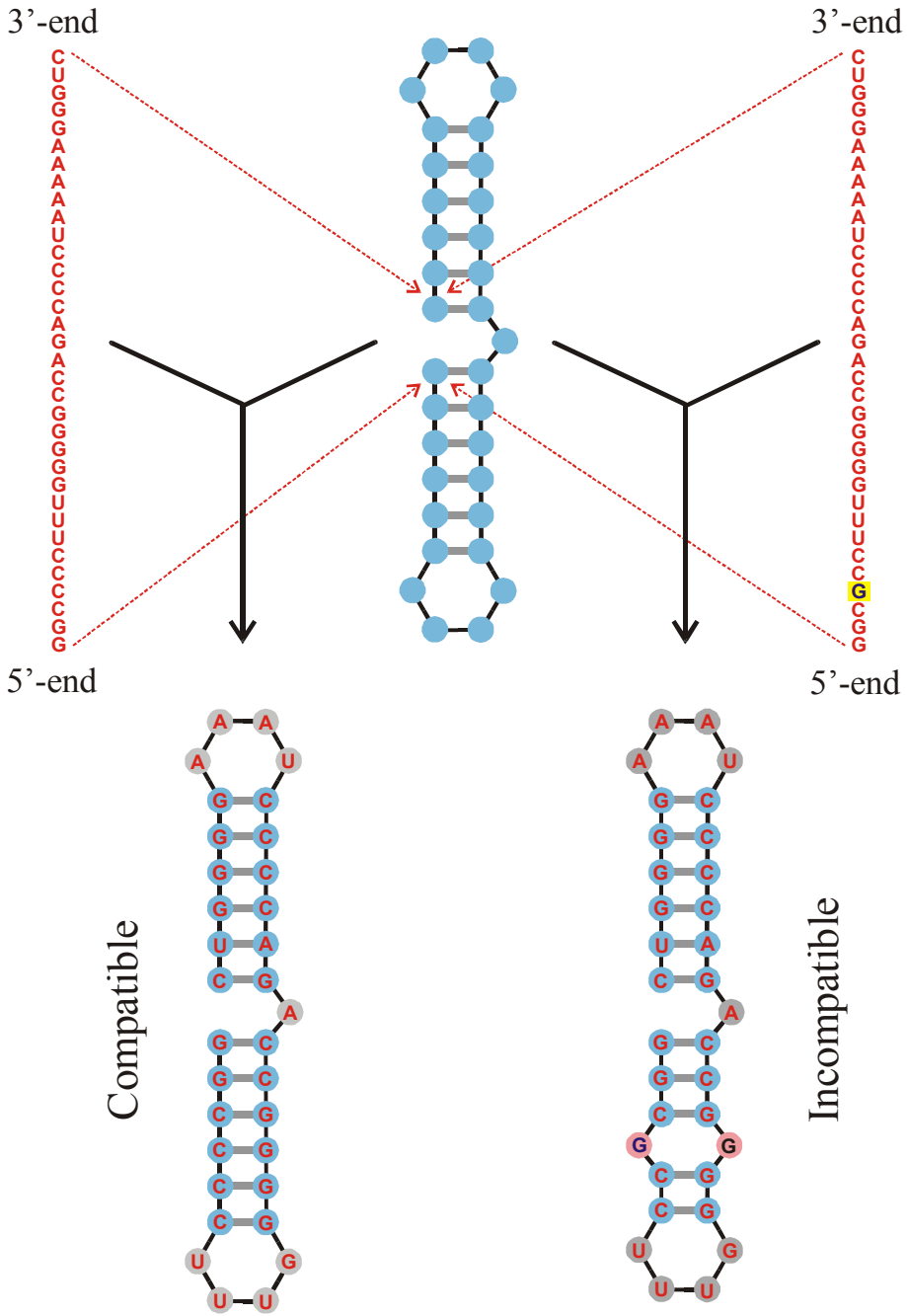


A multi-component neutral network





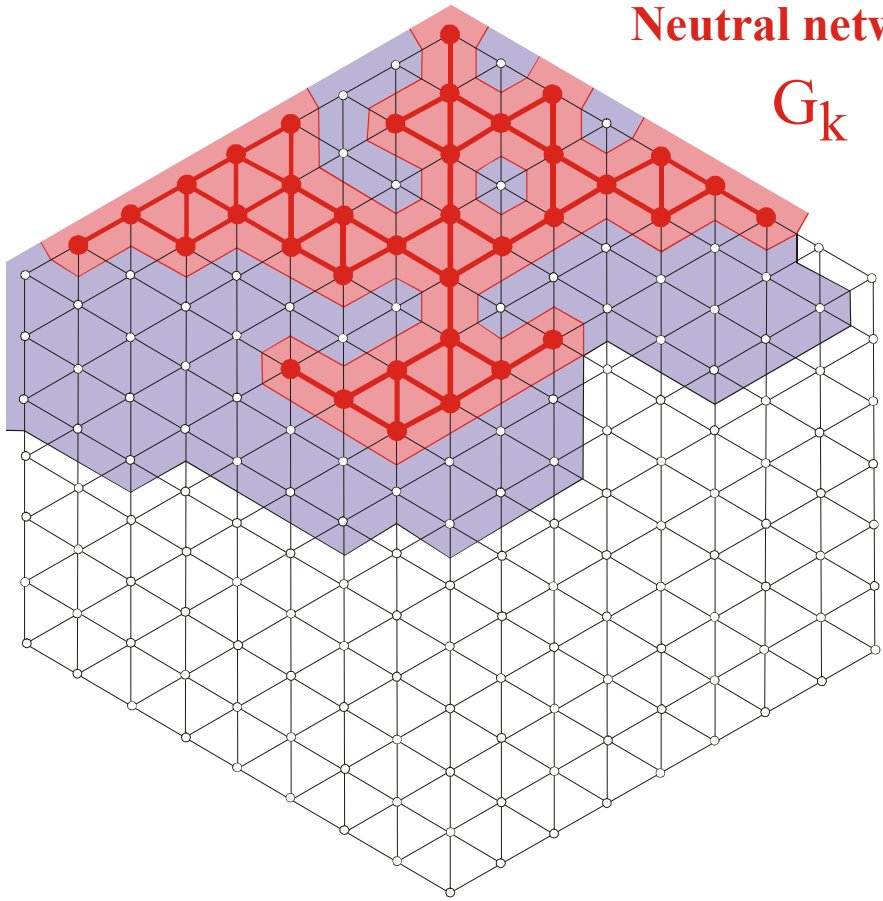
A connected neutral network



Compatibility of sequences with structures

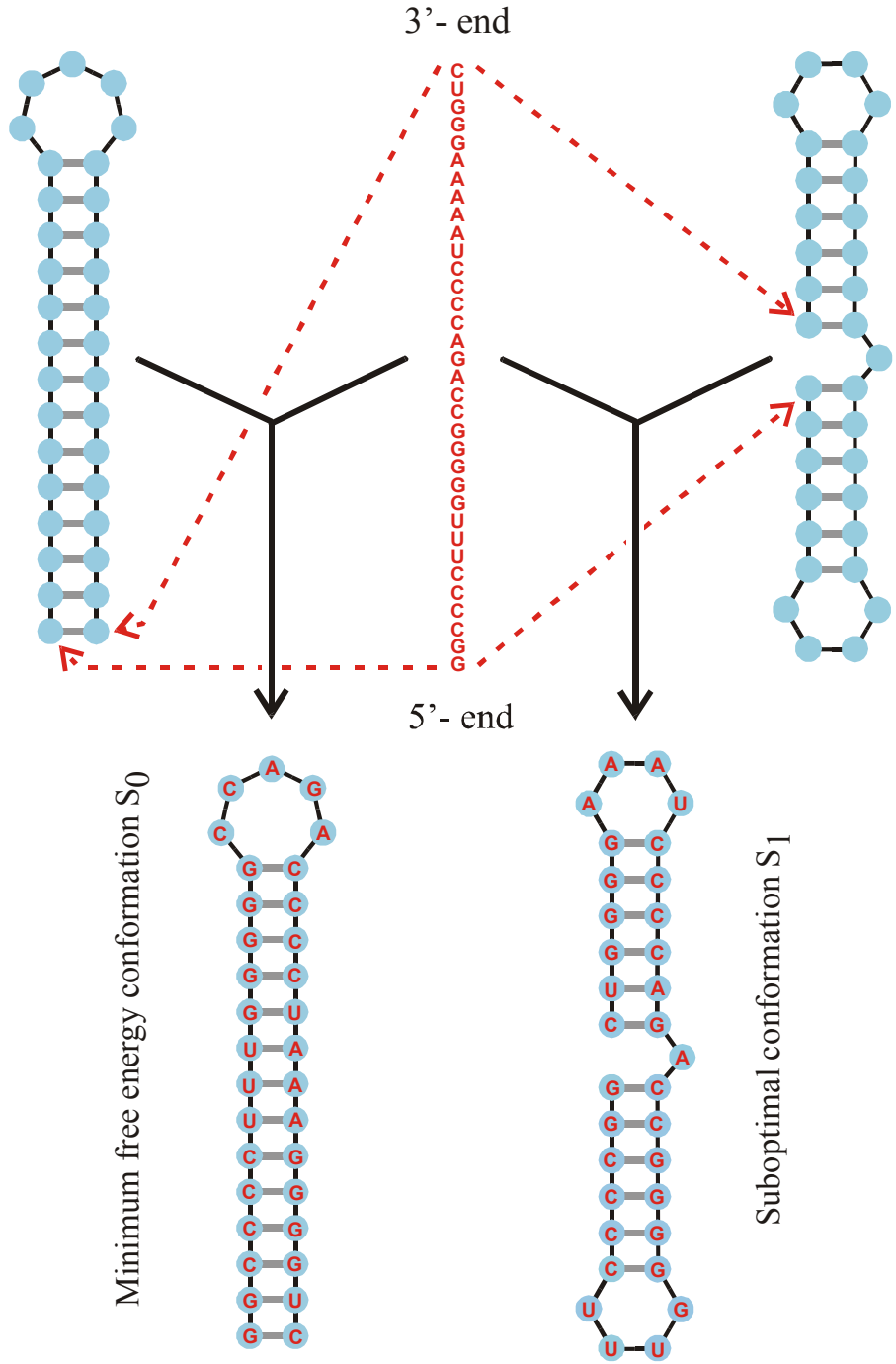
A sequence is compatible with its minimum free energy structure and all its suboptimal structures.

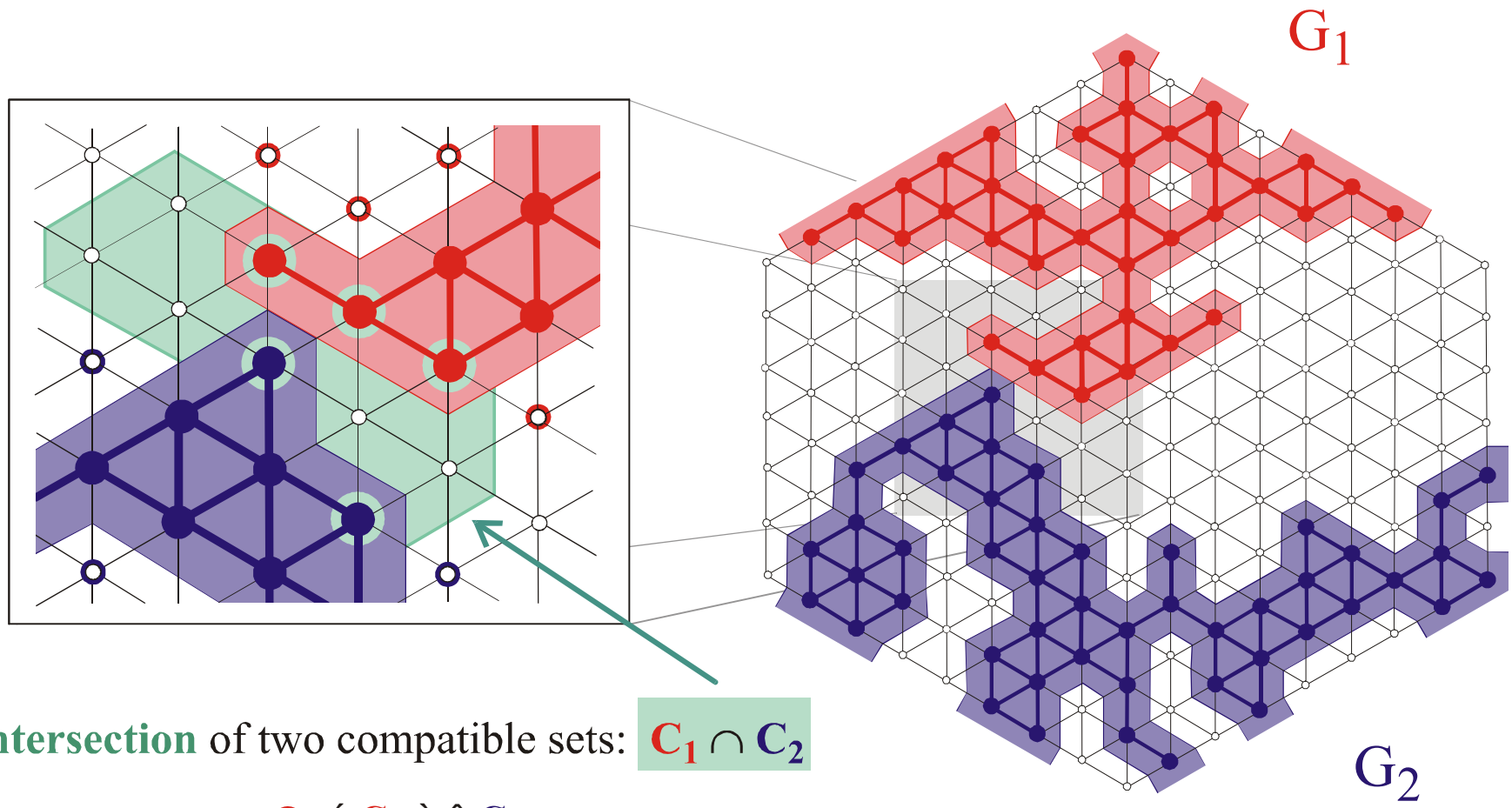
G_k à C_k



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

A sequence at the **intersection** of two neutral networks is compatible with both structures





Intersection of two compatible sets: $C_1 \cap C_2$

: á C_1 ì â C_2

: â C_1 ì á C_2

The intersection of two compatible sets is always non empty: C_1 Ú C_2 â Ù

Bulletin of Mathematical Biology, Vol. 59, No. 2, pp. 339–397, 1997
Elsevier Science Inc.

0092-8240/97 $17.00 + 0.00

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***

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

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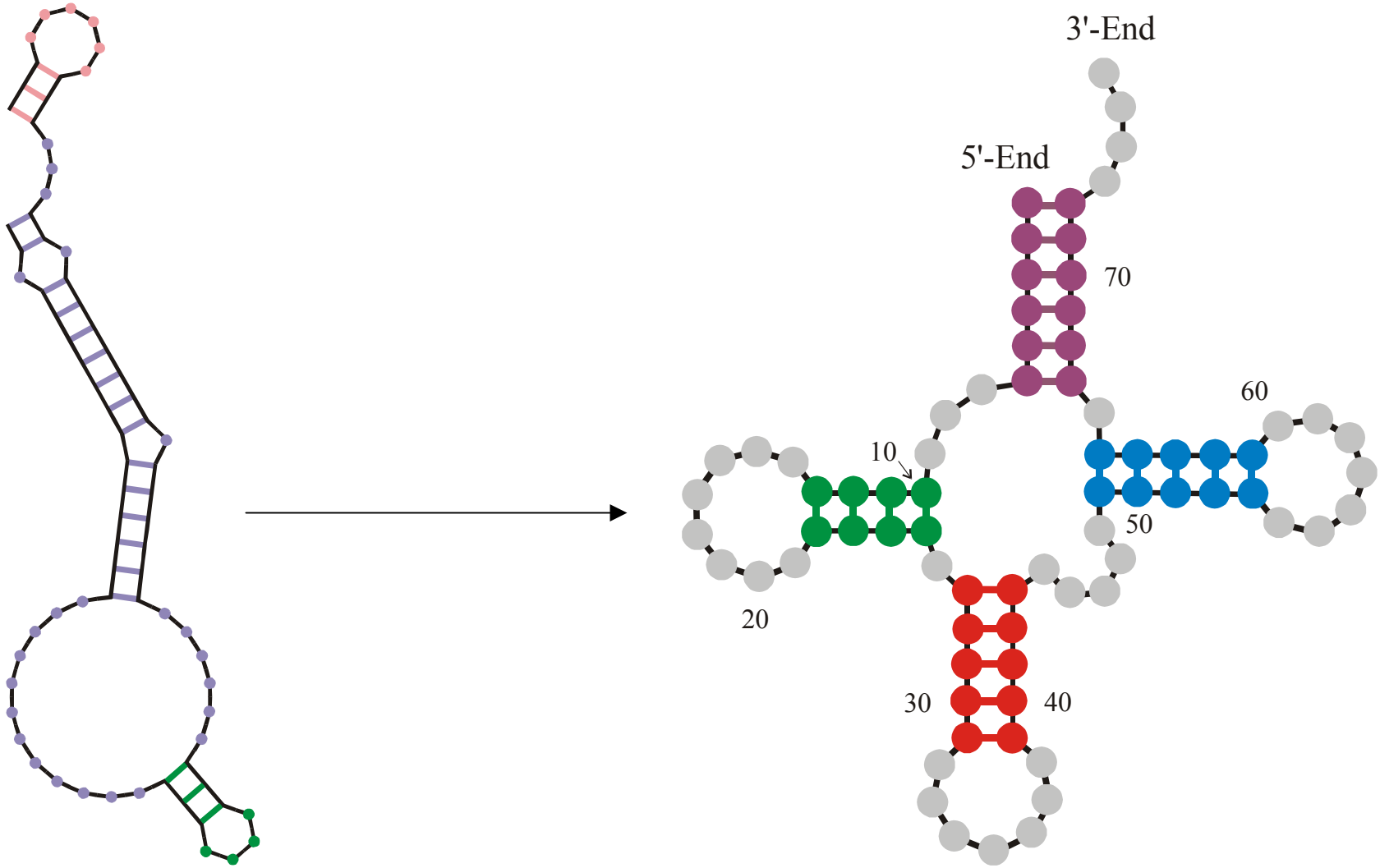
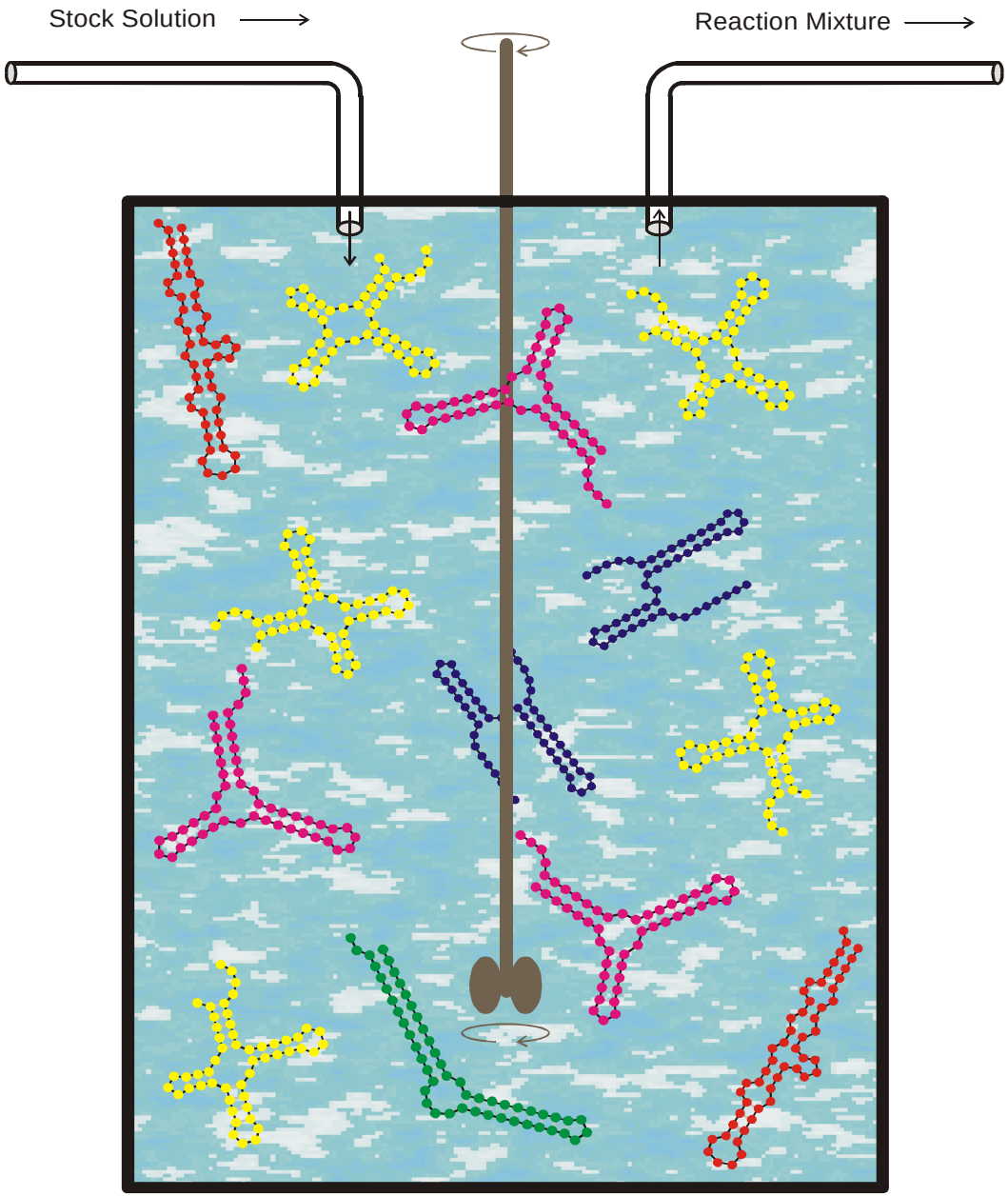
B.M.R.Stadler, P.F.Stadler, G.P.Wagner, W.Fontana, ***The topology of the possible: Formal spaces underlying patterns of evolutionary change.*** J.Theor.Biol. **213** (2001), 241-274



Randomly chosen
initial structure

Phenylalanyl-tRNA as
target structure

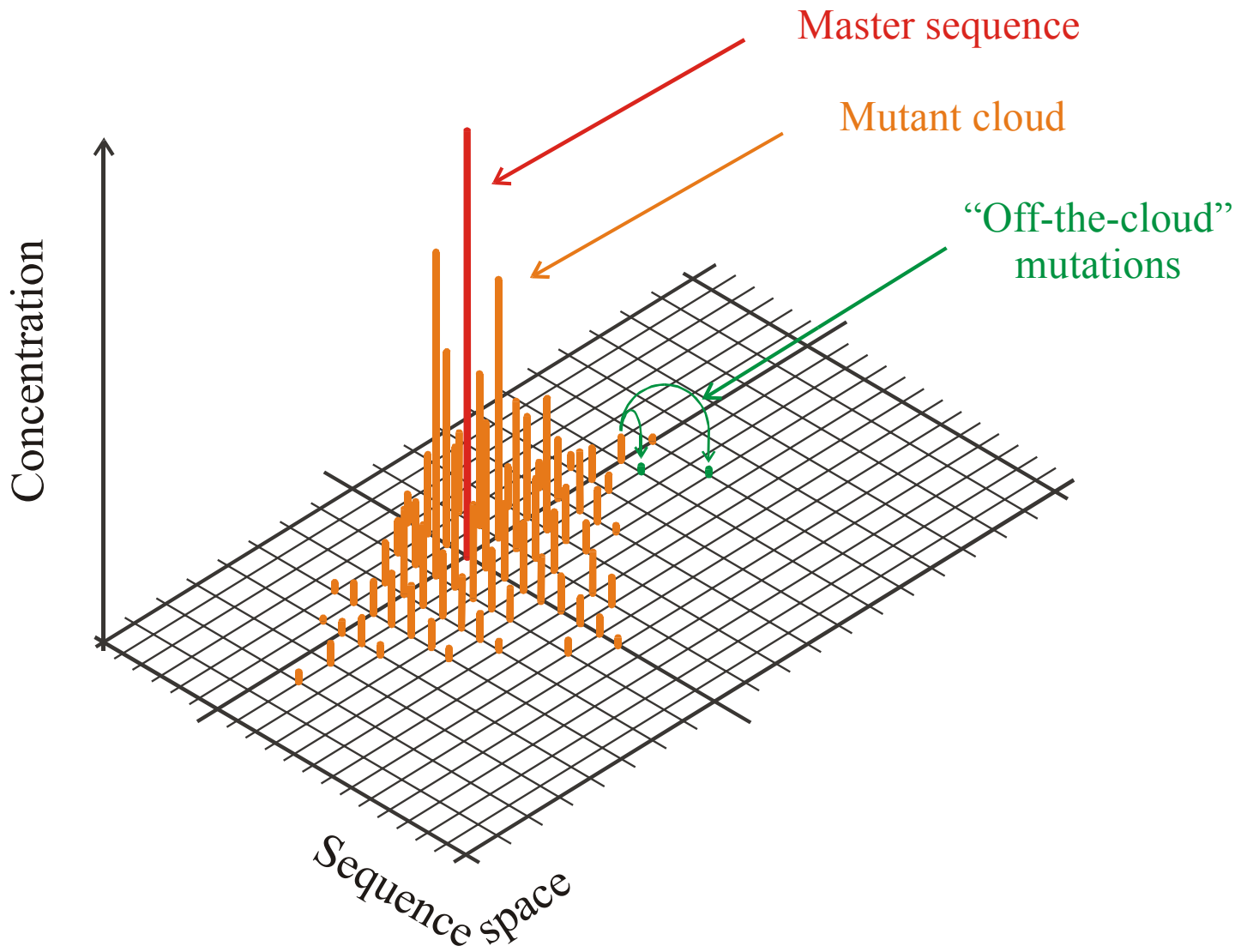


Fitness function:

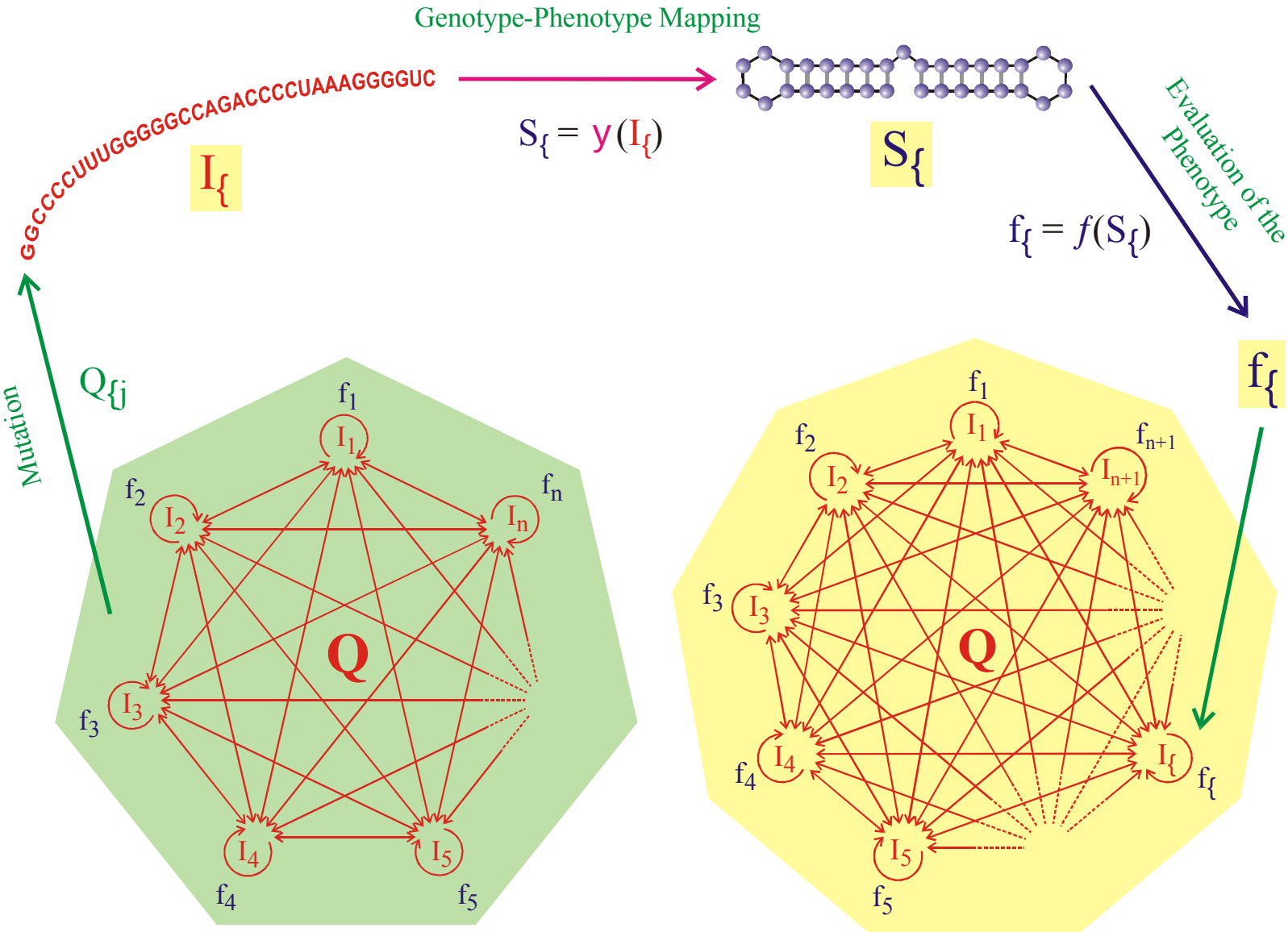
$f_k = g / [a + Dd_S^{(k)}]$

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

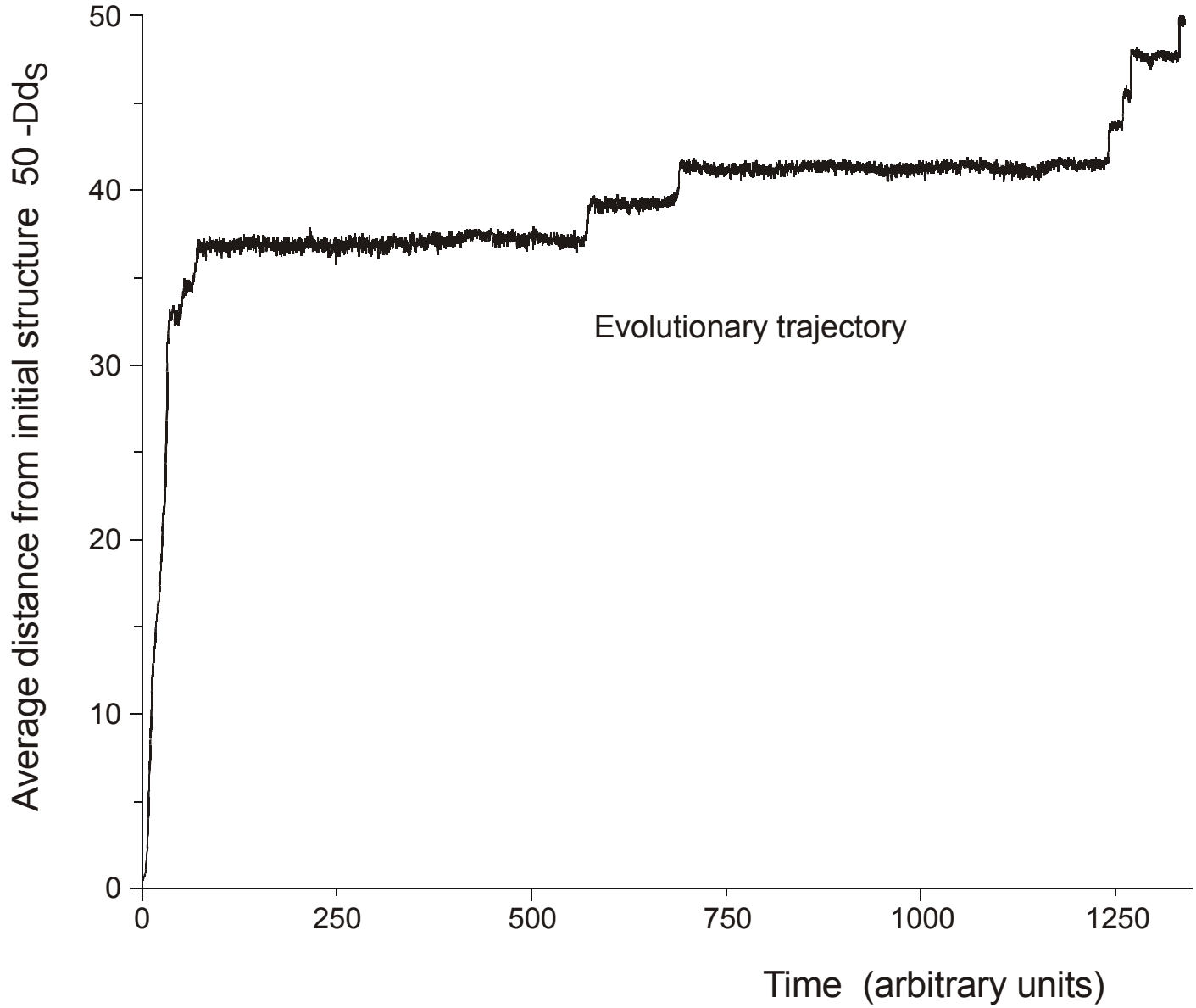
The flowreactor as a device for studies of evolution ***in vitro*** and ***in silico***



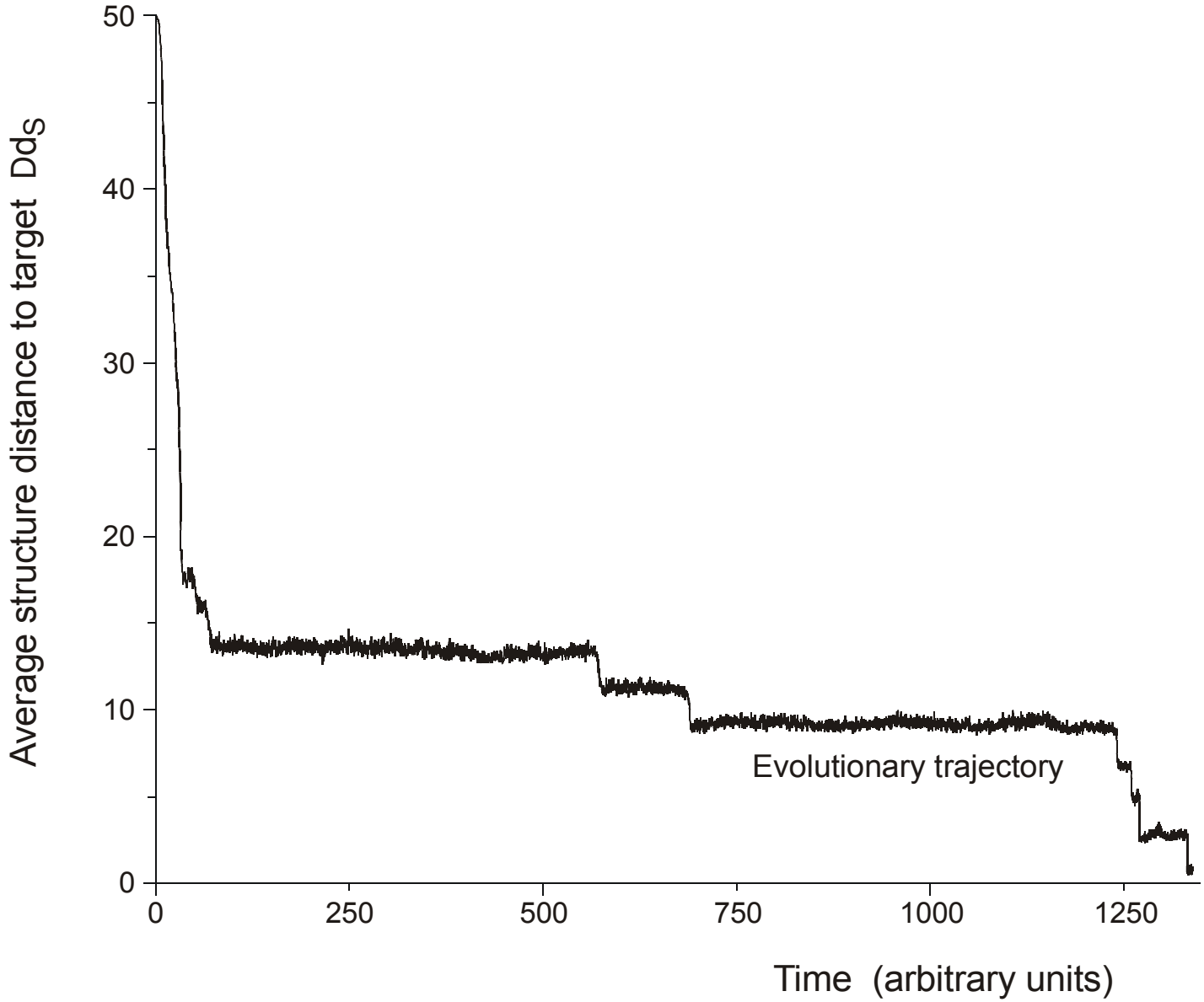
The molecular quasispecies in sequence space



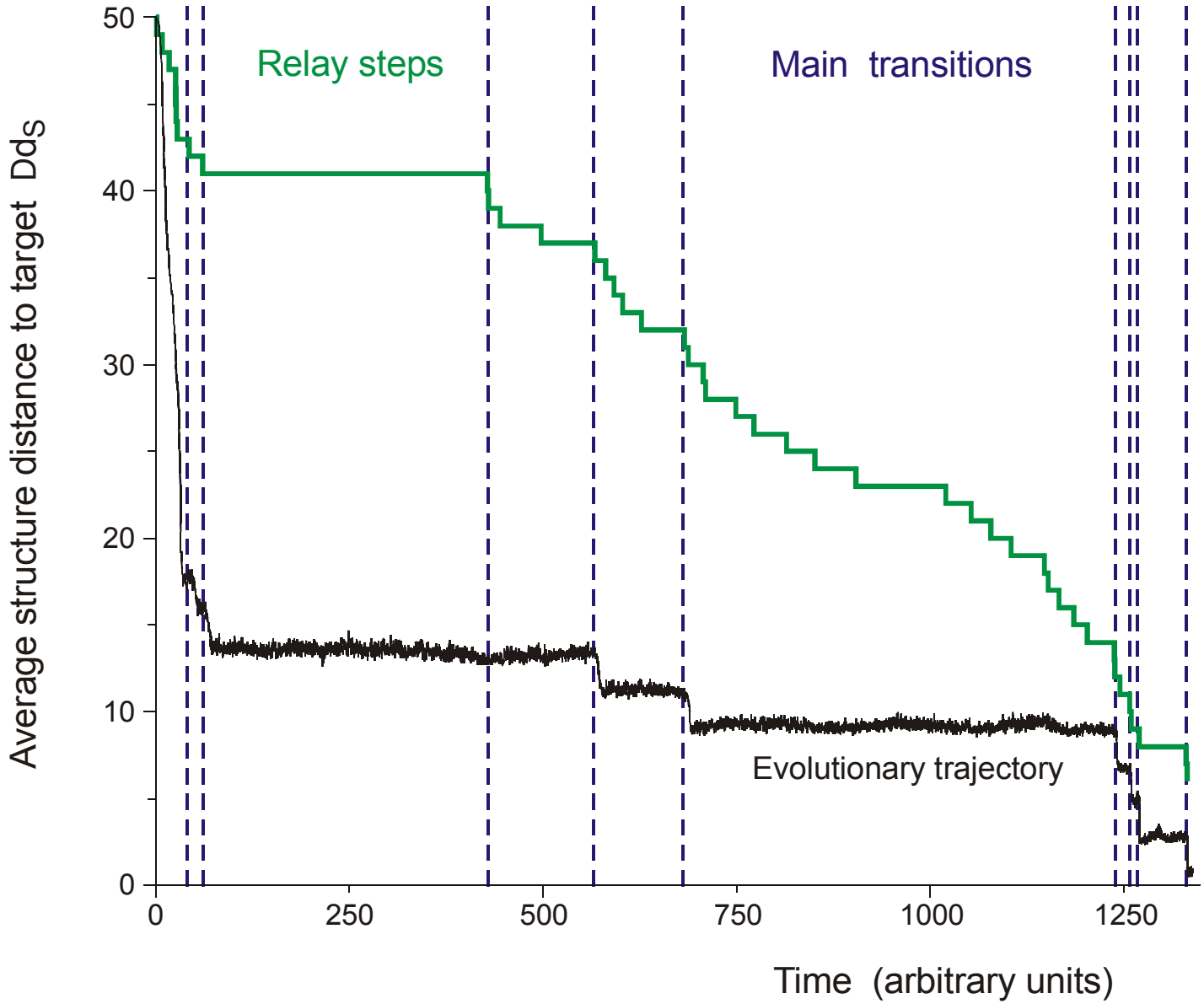
Evolutionary dynamics
including molecular phenotypes



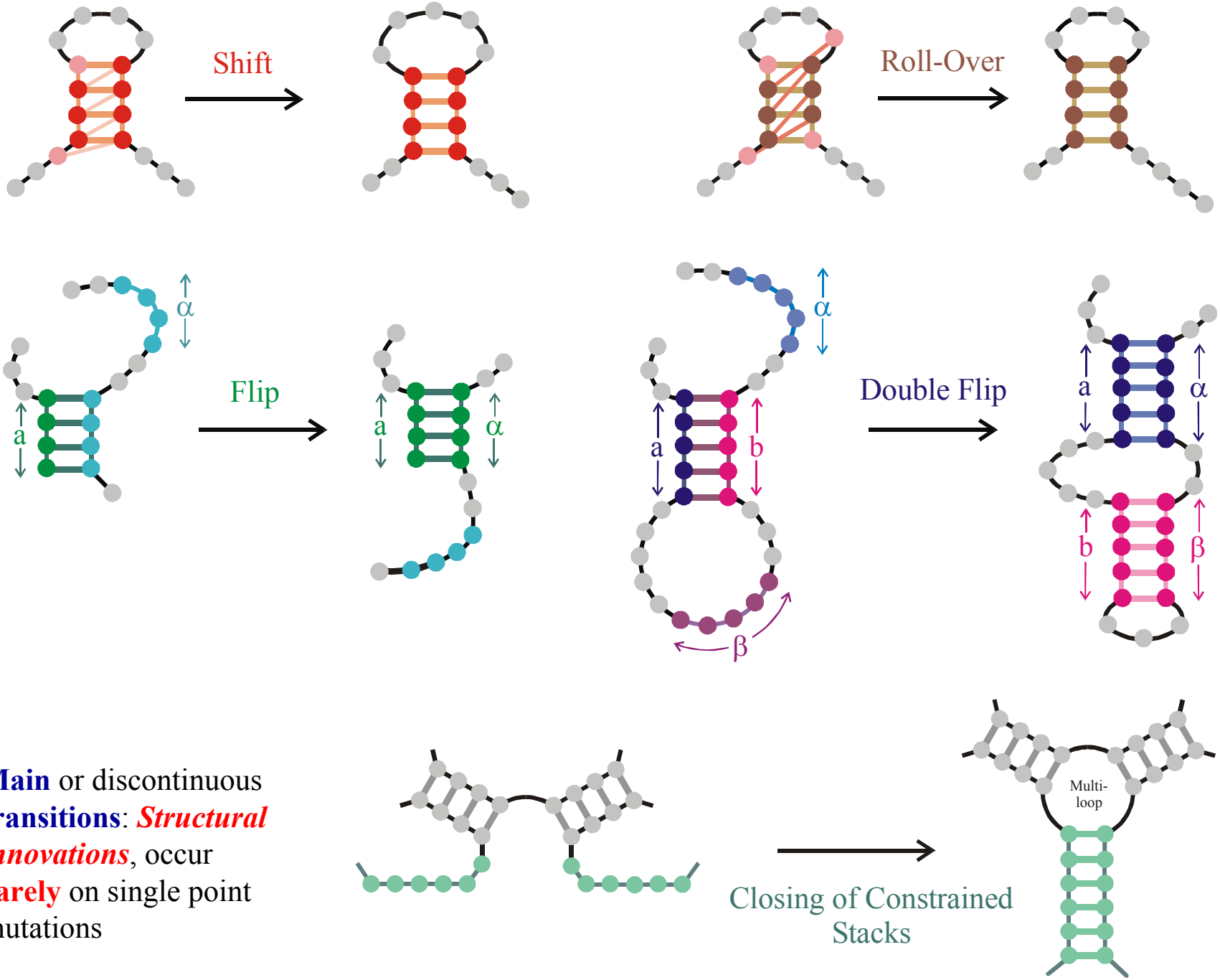
In silico optimization in the flow reactor: Trajectory (**biologists‘ view**)

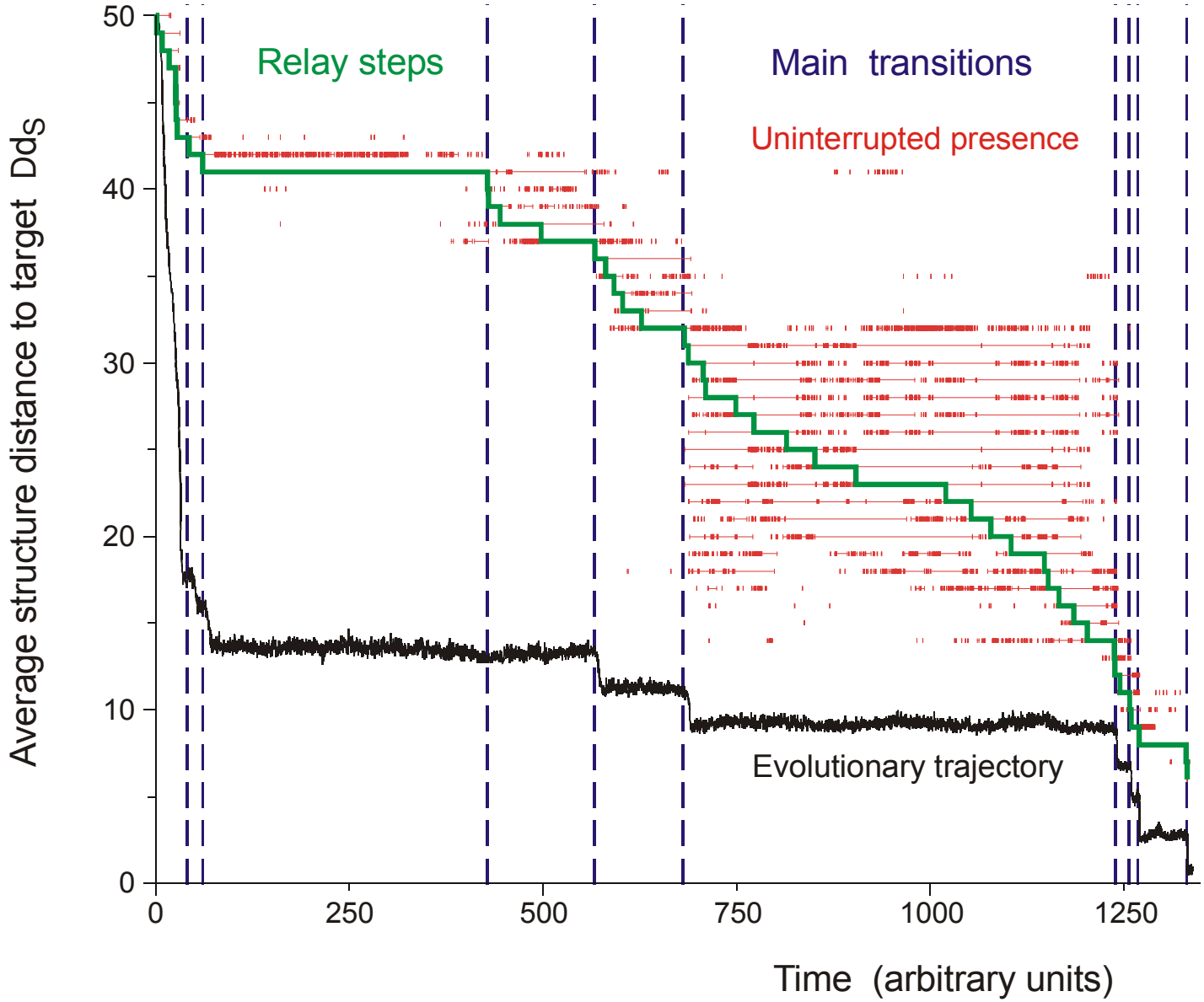


In silico optimization in the flow reactor: Trajectory (**physicists‘ view**)

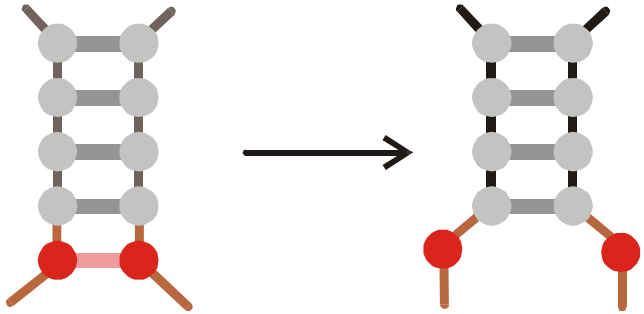


In silico optimization in the flow reactor: Main transitions

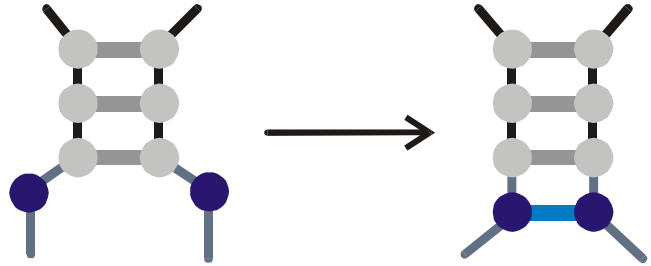

Shift
Roll-Over
α
a
Flip
α
a
α
a
b
β
Double Flip
a
α
b
β
Main or discontinuous transitions: Structural innovations, occur rarely on single point mutations
Multi-loop
Closing of Constrained Stacks



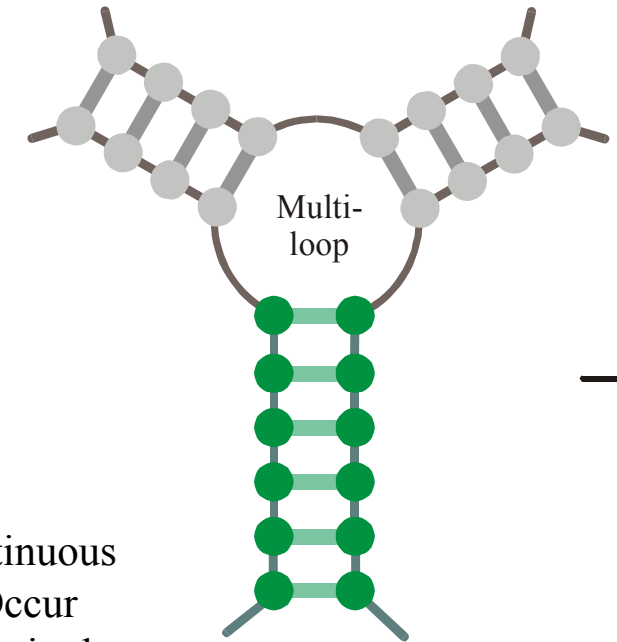
In silico optimization in the flow reactor

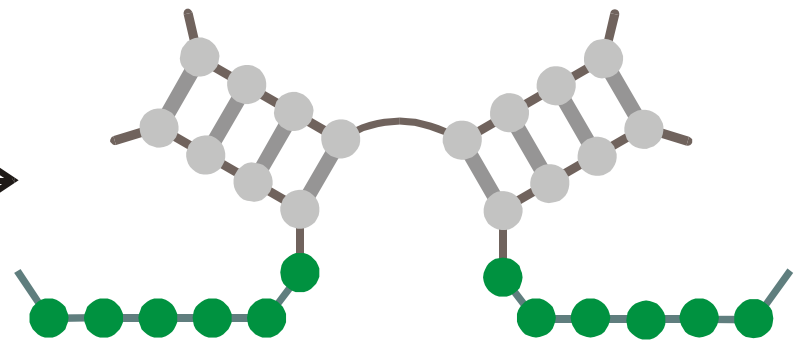


Shortening of Stacks



Elongation of Stacks





Opening of Constrained Stacks

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

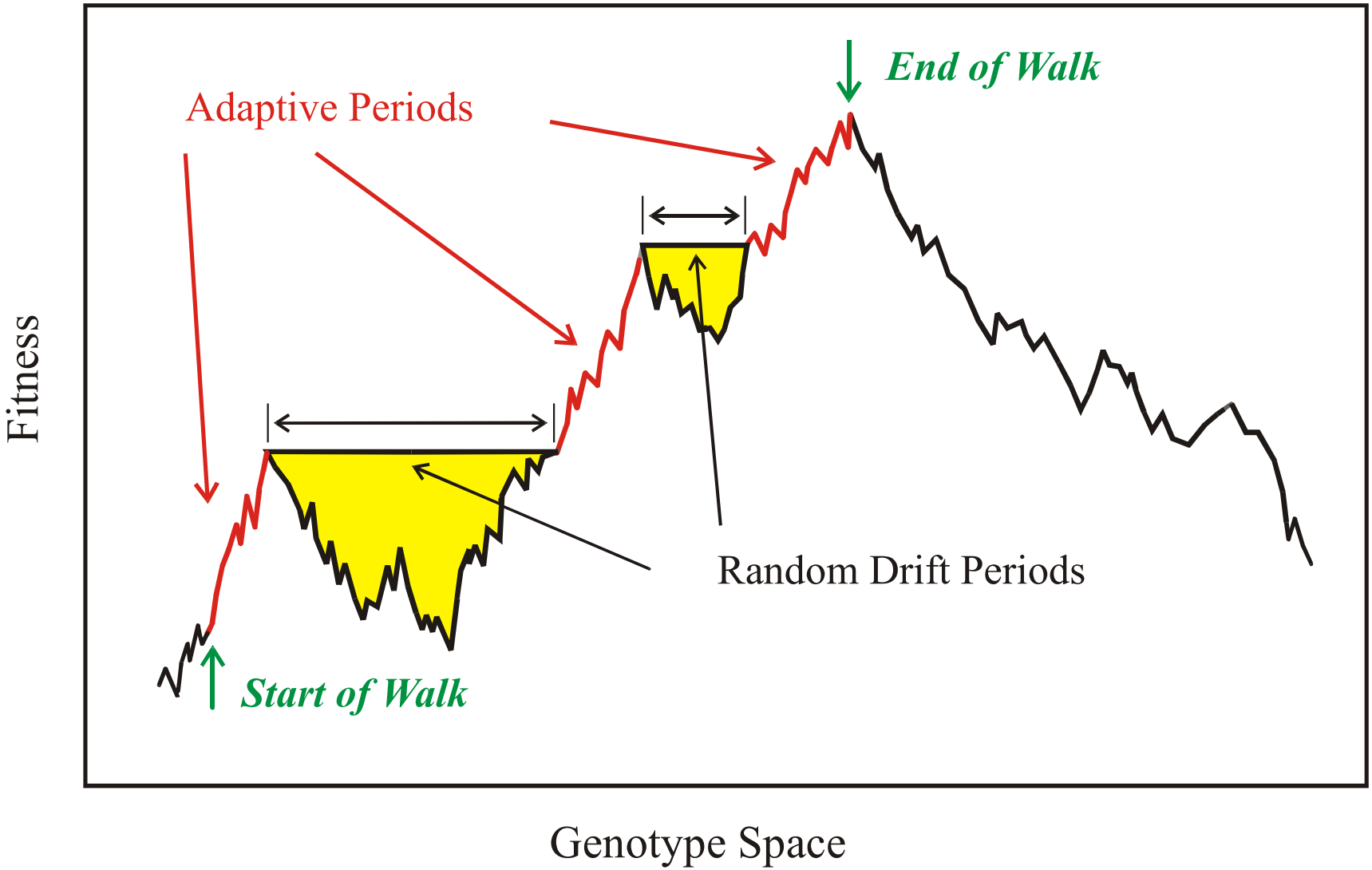
Statistics of evolutionary trajectories

Population size N	Number of replications $< n_{rep} >$	Number of transitions $< n_{tr} >$	Number of main transitions $< n_{dtr} >$
1 000	$(5.5 \pm [6.9,3.1]) \times 10^7$	$92.7 \pm [80.3,43.0]$	$8.8 \pm [2.4,1.9]$
2 000	$(6.0 \pm [11.1,3.9]) \times 10^7$	$55.7 \pm [30.7,19.8]$	$8.9 \pm [2.8,2.1]$
3 000	$(6.6 \pm [21.0,5.0]) \times 10^7$	$44.2 \pm [25.9,16.3]$	$8.1 \pm [2.3,1.8]$
10 000	$(1.2 \pm [1.3,0.6]) \times 10^8$	$35.9 \pm [10.3,8.0]$	$10.3 \pm [2.6,2.1]$
20 000	$(1.5 \pm [1.4,0.7]) \times 10^8$	$28.8 \pm [5.8,4.8]$	$9.0 \pm [2.8,2.2]$
30 000	$(2.2 \pm [3.1,1.3]) \times 10^8$	$29.8 \pm [7.3,5.9]$	$8.7 \pm [2.4,1.9]$
100 000	$(3 \pm [2,1]) \times 10^8$	$24 \pm [6,5]$	9 ± 2

The number of **main transitions** or evolutionary innovations is constant.

„...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

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

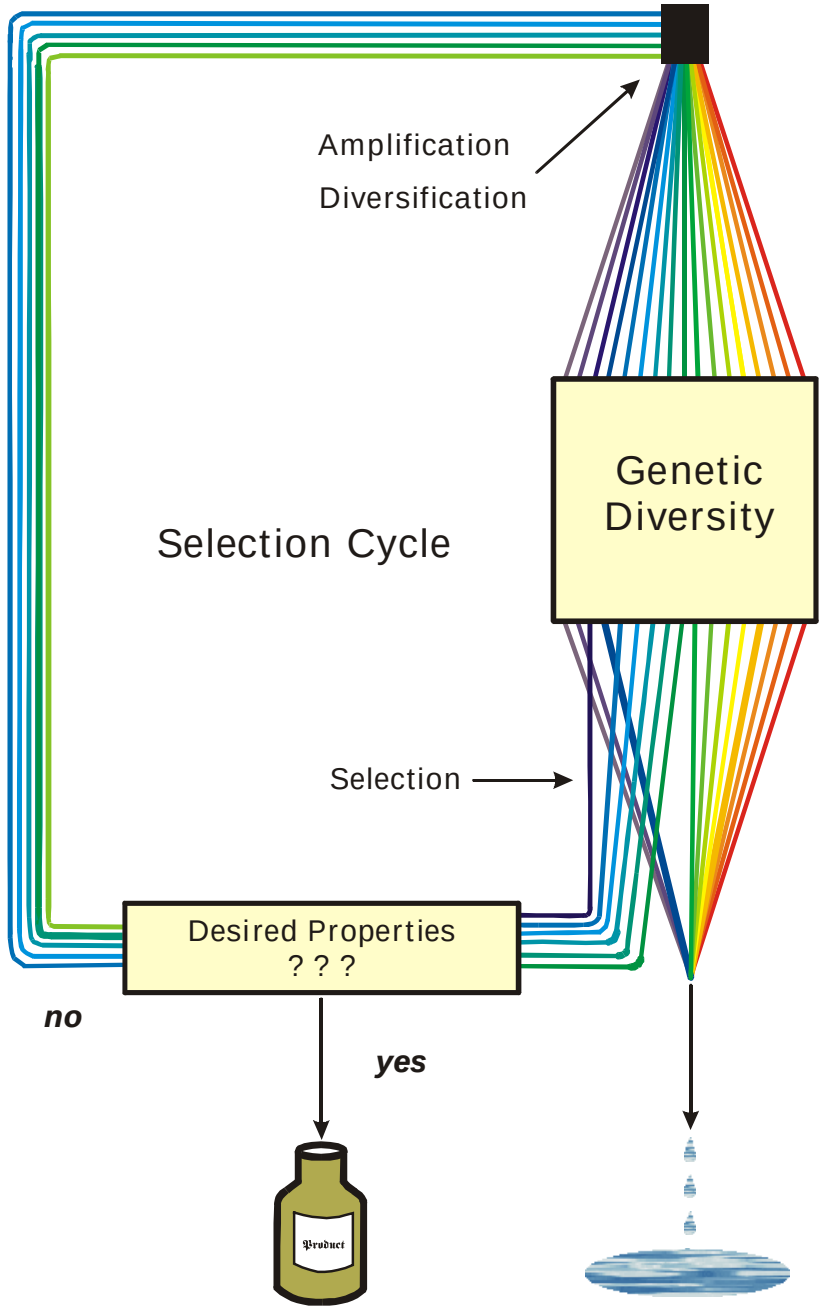
D.P.Bartel, J.W.Szostak, ***Isolation of new ribozymes from a large pool of random sequences***. Science **261** (1993), 1411-1418

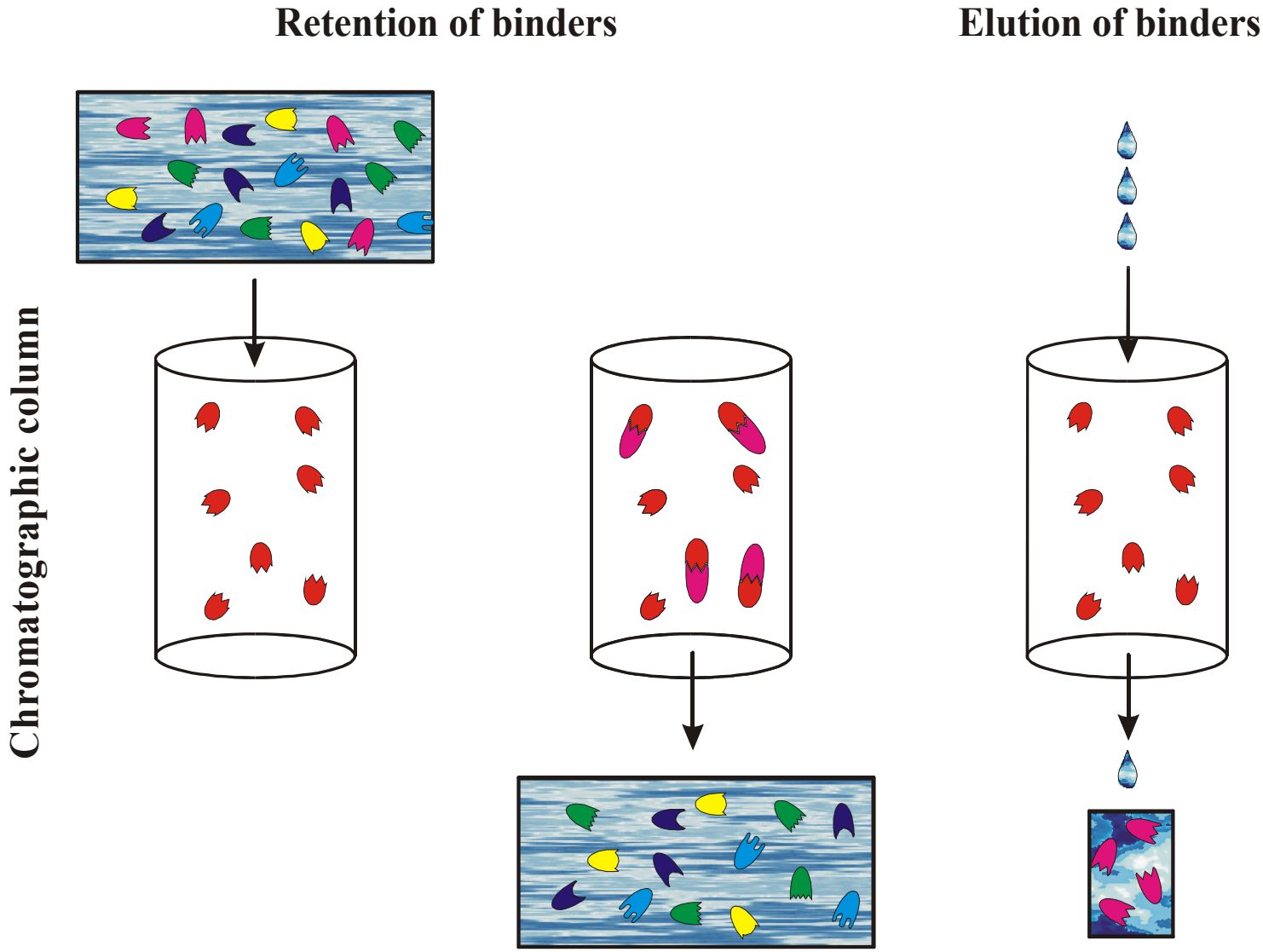
R.D.Jenison, S.C.Gill, A.Pardi, B.Poliski, ***High-resolution molecular discrimination by RNA***. Science **263** (1994), 1425-1429

Y.Wang, R.R.Rando, ***Specific binding of aminoglycoside antibiotics to RNA***. Chemistry & Biology **2** (1995), 281-290

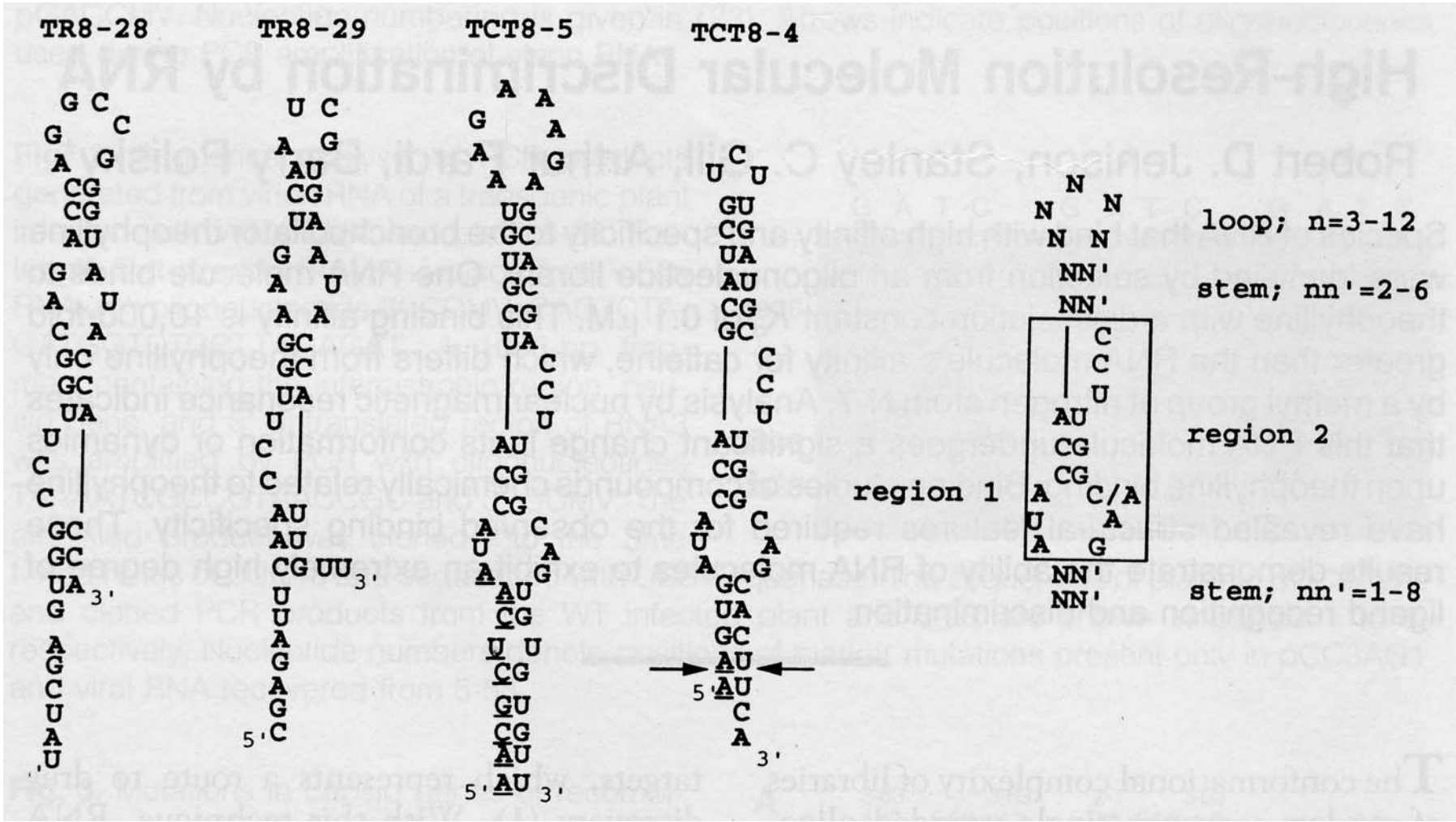
L.Jiang, A.K.Suri, R.Fiala, D.J.Patel, ***Saccharide-RNA recognition in an aminoglycoside antibiotic-RNA aptamer complex***. Chemistry & Biology **4** (1997), 35-50

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



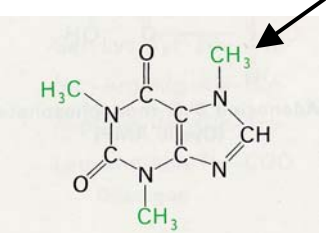


The SELEX technique for the evolutionary design of ***aptamers***

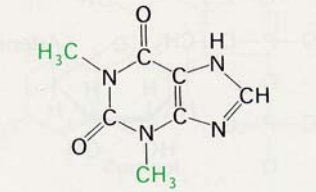


Secondary structures of aptamers binding theophyllin, caffeine, and related compounds

additional methyl group



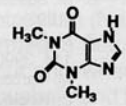
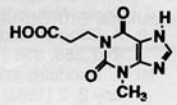
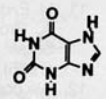
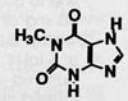
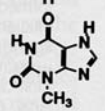
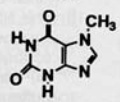
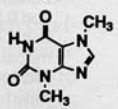
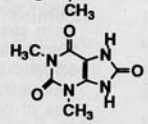
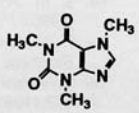
Caffeine
(1,3,7-Trimethylxanthine)



Theophylline
(1,3-Dimethylxanthine)

Dissociation constants and specificity of theophylline, caffeine, and related derivatives of uric acid for binding to a discriminating aptamer TCT8-4

Table 1. Competition binding analysis with TCT8-4 RNA. The chemical structures are shown for a series of derivatives used in competitive binding experiments with TCT8-4 RNA (Fig. 2) (*20*). The right column represents the affinity of the competitor relative to theophylline, $K_d(c)/K_d(t)$, where $K_d(c)$ is the individual competitor dissociation constant and $K_d(t)$ is the competitive dissociation constant of theophylline. Certain data (denoted by >) are minimum values that were limited by the solubility of the competitor. Each experiment was carried out in duplicate. The average error is shown.

Compound	Structure	$K_d(c)$ (μM)	$K_d(c)/K_d(t)$
Theophylline		0.32 ± 0.13	1
CP-theophylline		0.93 ± 0.20	2.9
Xanthine		8.5 ± 0.40	27
1-Methylxanthine		9.0 ± 0.30	28
3-Methylxanthine		2.0 ± 0.7	6.3
7-Methylxanthine		> 500	>1500
3,7-Dimethylxanthine		> 500	> 1500
1,3-Dimethyluric acid		> 1000	>3100
Hypoxanthine		49 ± 10	153
Caffeine		3500 ± 1500	10,900

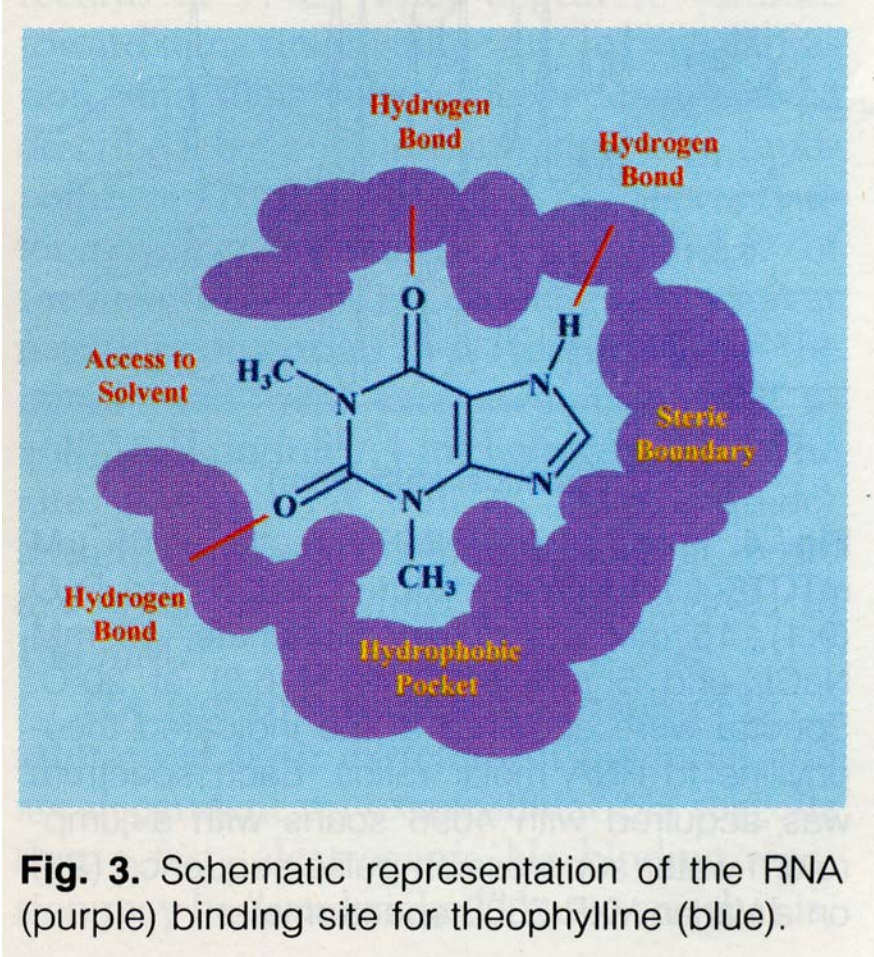
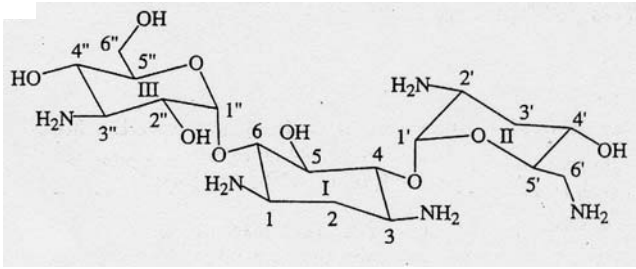


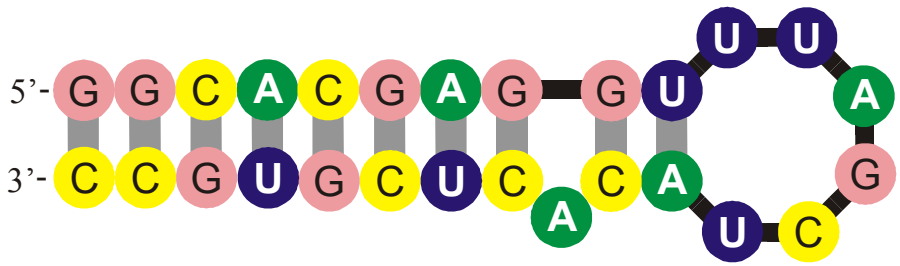
Fig. 3. Schematic representation of the RNA (purple) binding site for theophylline (blue).

Schematic drawing of the aptamer binding site for the theophylline molecule



tobramycin

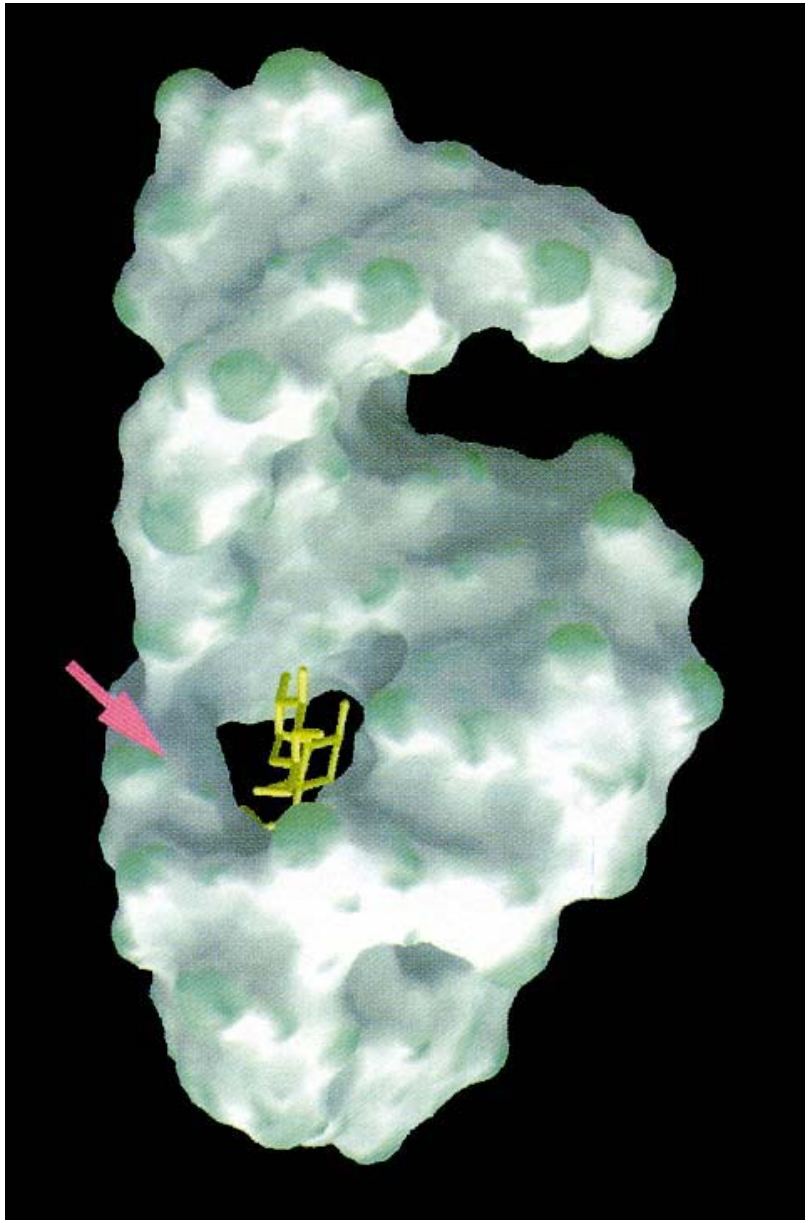
5'-GGCACGAGGUUUAGCUACACUCGUGCC-3'



RNA aptamer

Formation of secondary structure of the tobramycin binding RNA aptamer

L. Jiang, A. K. Suri, R. Fiala, D. J. Patel, ***Saccharide-RNA recognition in an aminoglycoside antibiotic-RNA aptamer complex.*** 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)

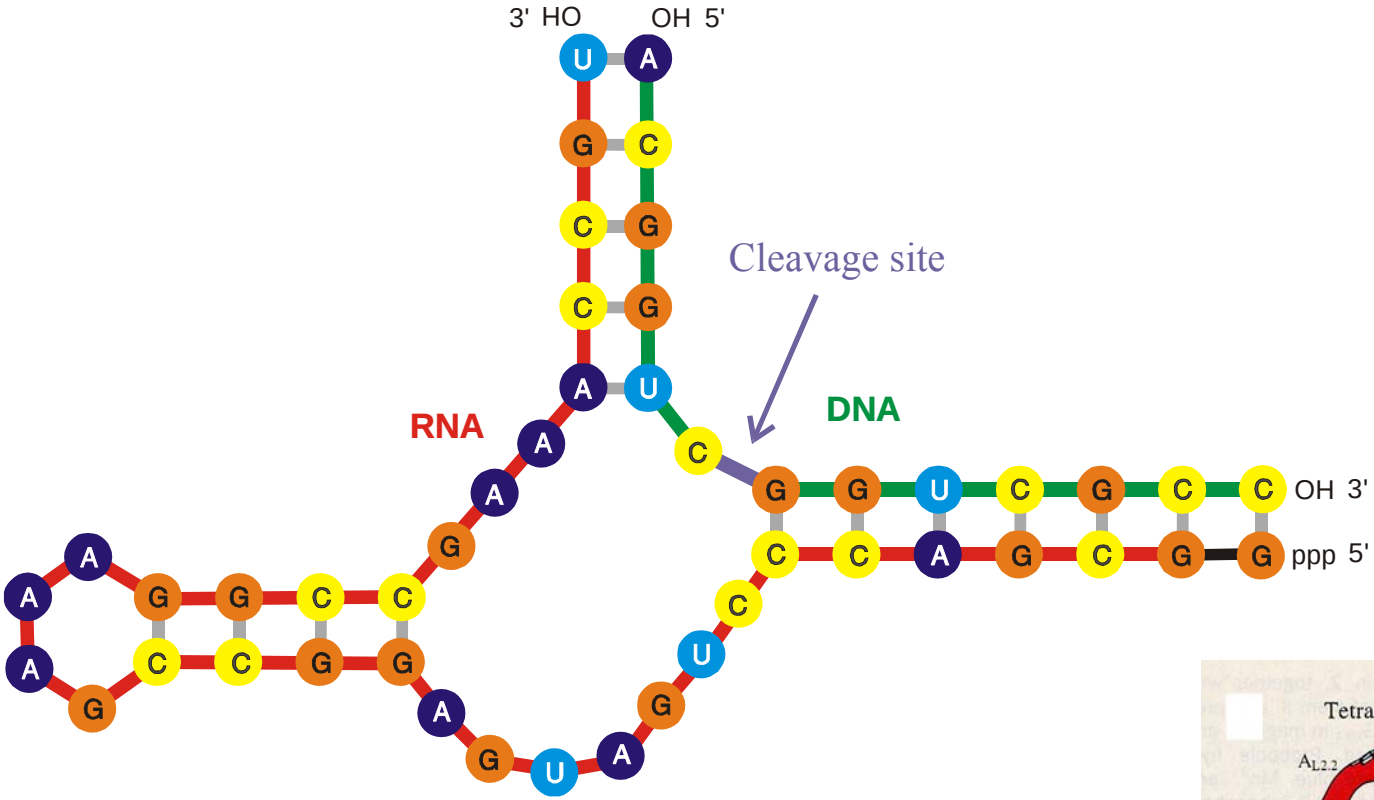
Hammerhead ribozyme – The smallest RNA based catalyst

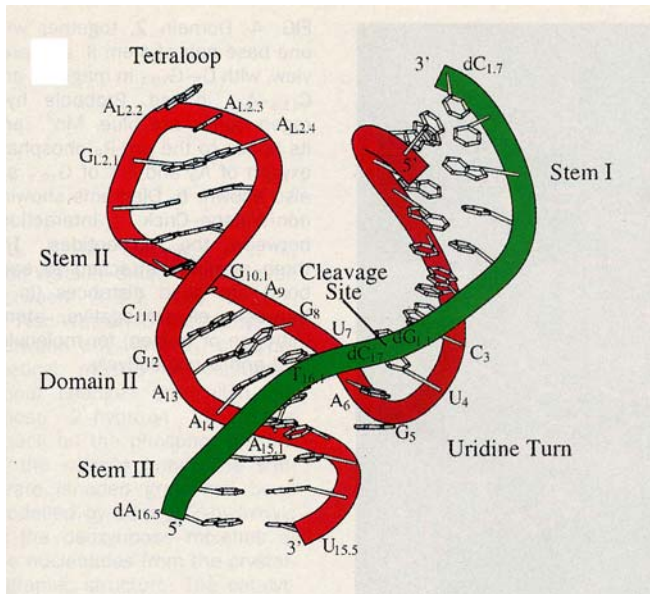
H.W.Pley, K.M.Flaherty, D.B.McKay, ***Three dimensional structure of a hammerhead ribozyme***. Nature **372** (1994), 68-74

W.G.Scott, J.T.Finch, A.Klug, ***The crystal structures of an all-RNA hammerhead ribozyme: A proposed mechanism for RNA catalytic cleavage***. Cell **81** (1995), 991-1002

J.E.Wedekind, D.B.McKay, ***Crystallographic structures of the hammerhead ribozyme: Relationship to ribozyme folding and catalysis***. Annu.Rev.Biophys.Biomol.Struct. 27 (1998), 475-502

G.E.Soukup, R.R.Breaker, ***Design of allosteric hammerhead ribozymes activated by ligand-induced structure stabilization***. Structure **7** (1999), 783-791



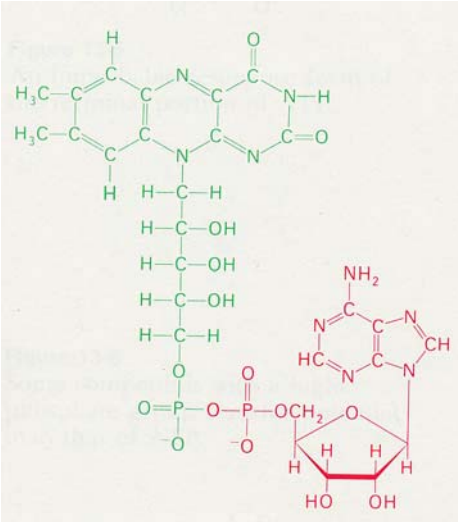


Hammerhead ribozyme: The smallest known catalytically active RNA molecule

Allosteric effectors:

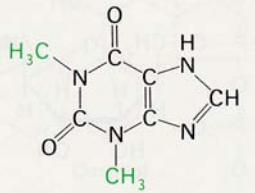
FMN = flavine mononucleotide

H10 – H12



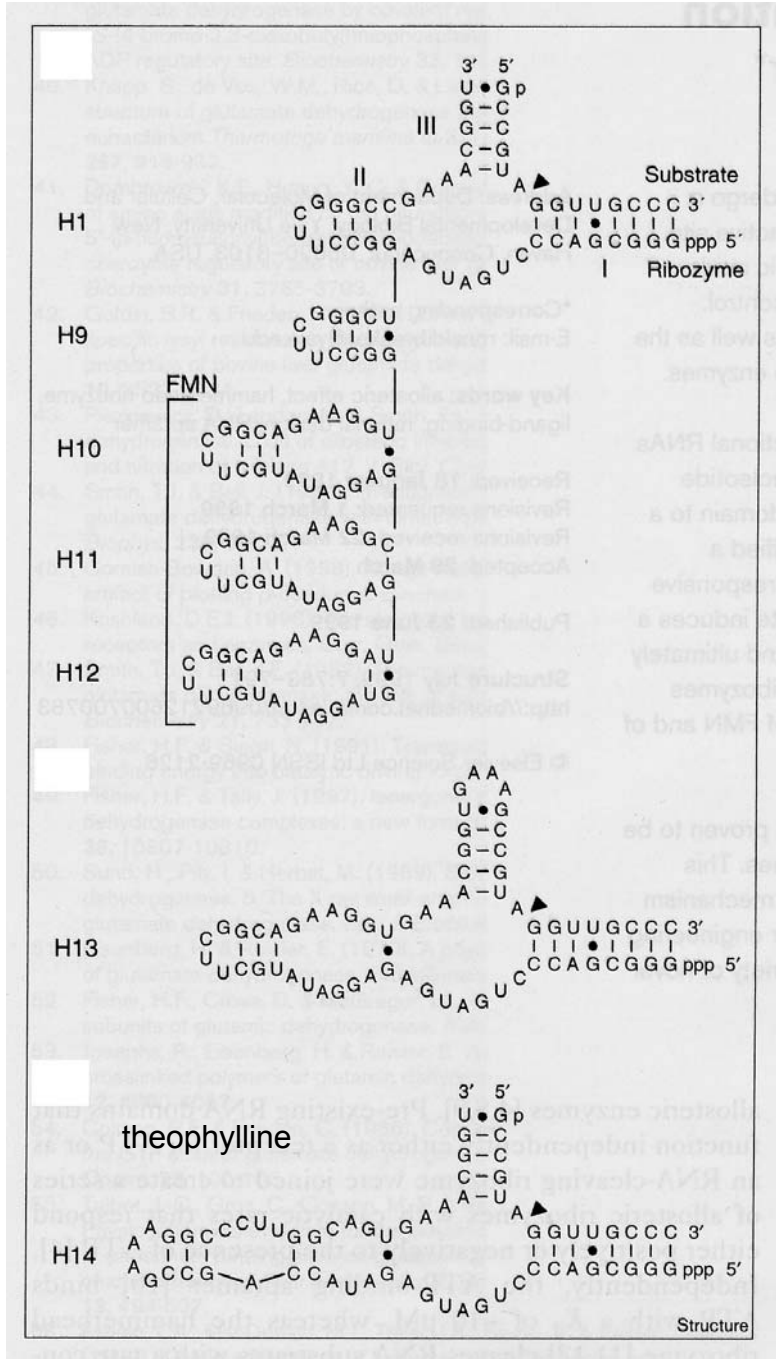
theophylline

H14



Self-splicing allosteric ribozyme

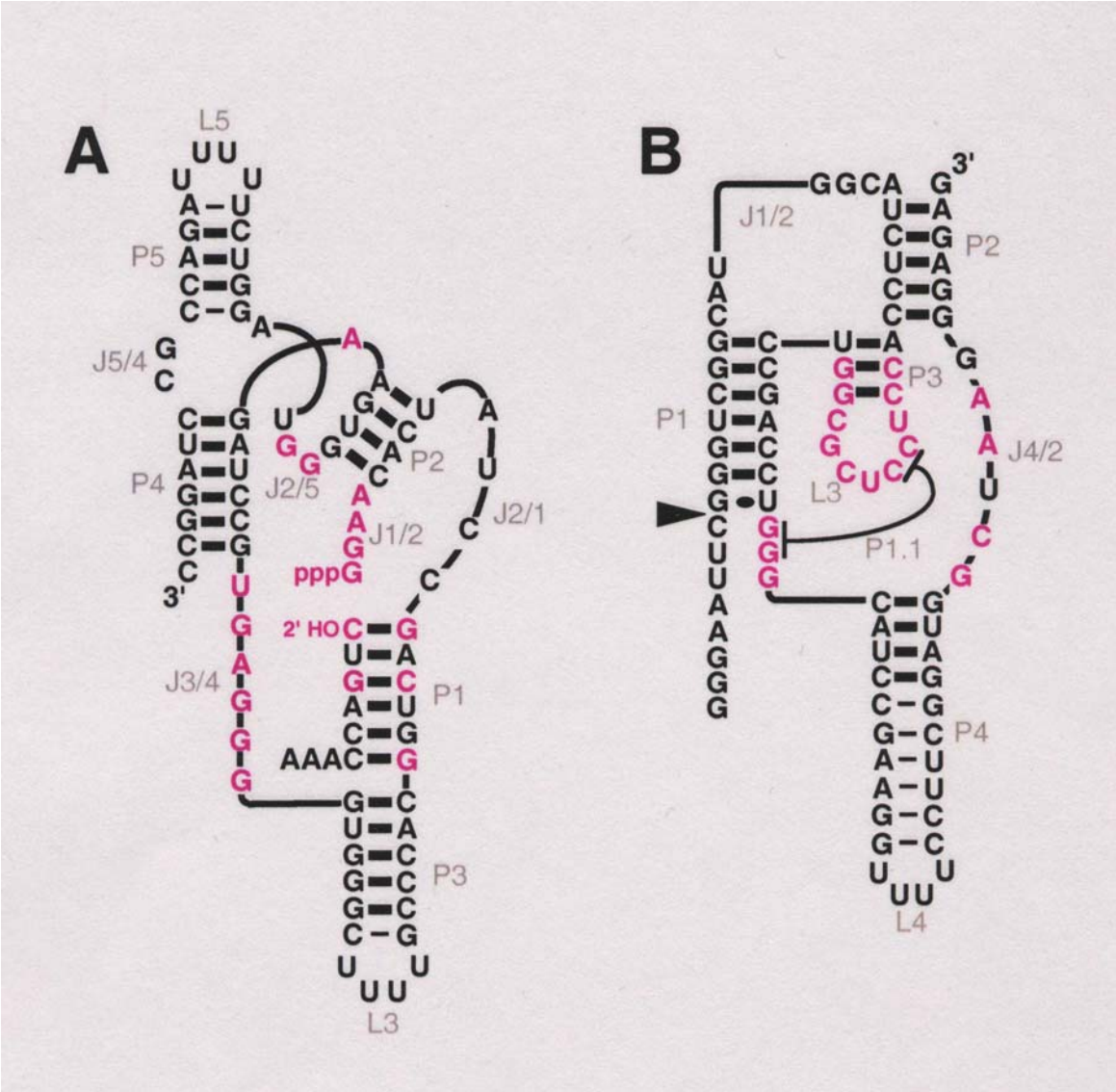
H13



Hammerhead ribozymes with allosteric effectors

A ribozyme switch

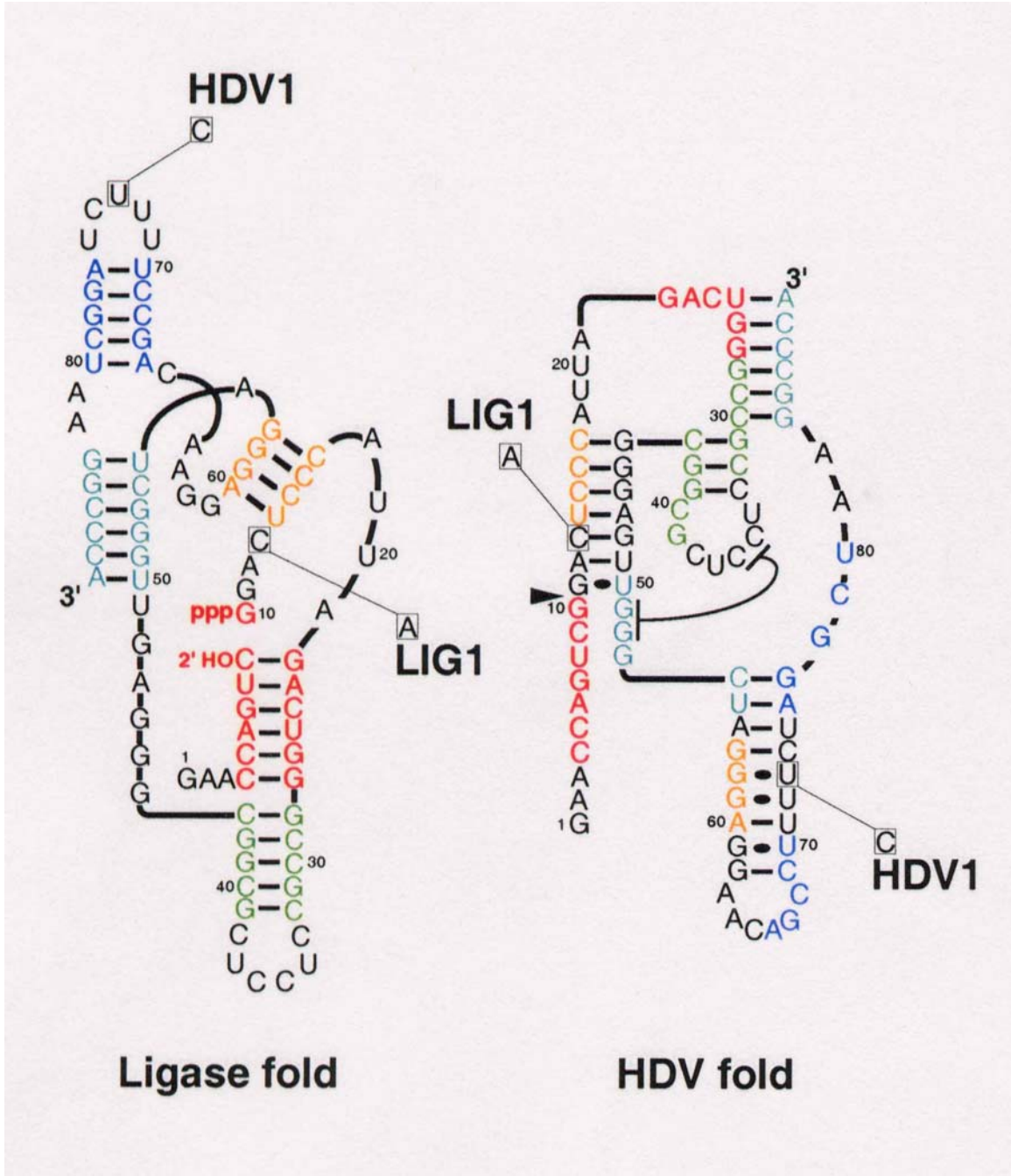
E.A.Schultes, D.B.Bartel, ***One sequence, two ribozymes: Implication for the emergence of new ribozyme folds***. Science **289** (2000), 448-452



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

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
Elsevier Science Inc.

0092-8240/97 $17.00 + 0.00

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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D-07708 Jena, Germany

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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*

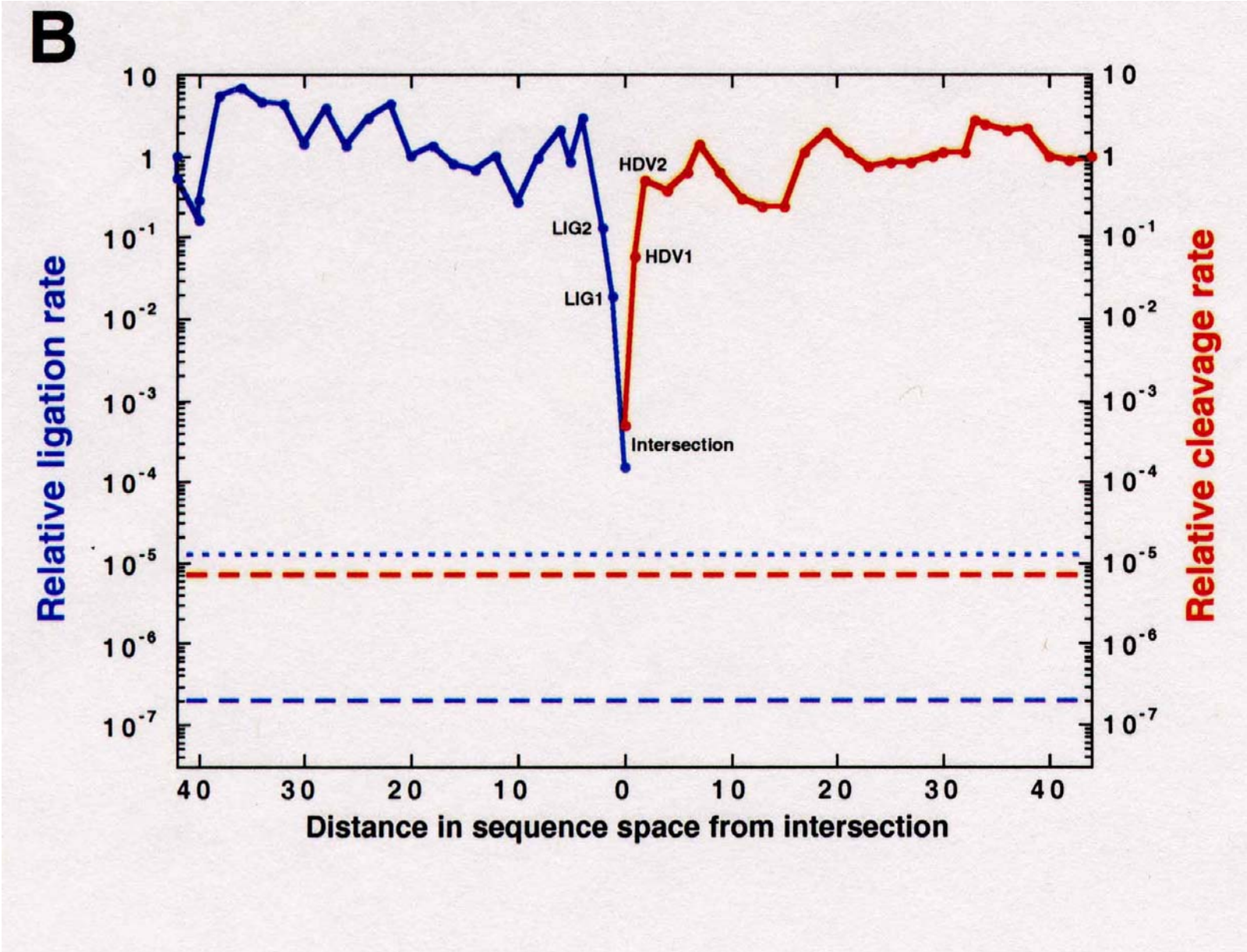
THEOREM 5. INTERSECTION-THEOREM. *Let* s *and* s′ *be arbitrary secondary structures and* **C**[s], **C**[s′] *their corresponding compatible sequences. Then,*

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

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***



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

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.

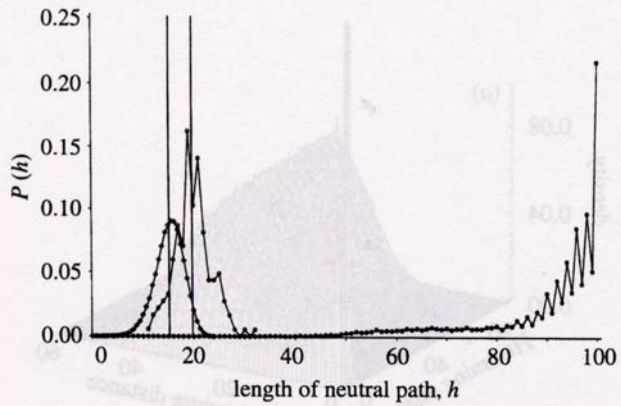

Figure 4. Neutral paths. A neutral path is defined by a series of nearest neighbour sequences that fold into identical structures. Two classes of nearest neighbours are admitted: neighbours of Hamming distance 1, which are obtained by single base exchanges in unpaired stretches of the structure, and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

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

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

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