Plausibility of an RNA World

„Never say Never“

Peter Schuster

Institut für Theoretische Chemie, Universität Wien, Austria
and
The Santa Fe Institute, Santa Fe, New Mexico, USA

Open Questions on the Origin of Life

San Sebastian - Donostia, 20.– 23.05.2009

Web-Page for further information:

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

Never say „never“

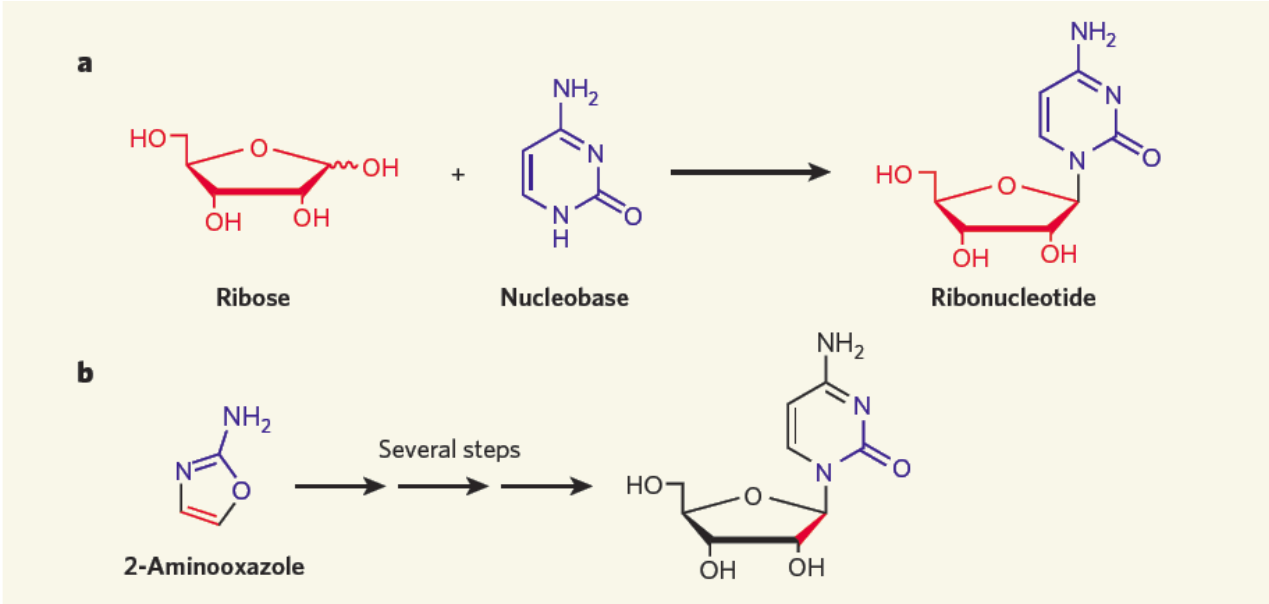


Figure 1 | Theories of prebiotic syntheses of pyrimidine ribonucleotides. The idea that RNA might have formed spontaneously on early Earth has inspired a search for feasible prebiotic syntheses of ribonucleotides, the building blocks of RNA. **a**, The traditional view is that the ribose sugar and nucleobase components of ribonucleotides formed separately, and then combined. But no plausible reactions have been found in which the two components could have joined together. **b**, Powner *et al.*[2] show that a single 2-aminooxazole intermediate could have contributed atoms to both the sugar and nucleobase portions of pyrimidine ribonucleotides, so that components did not have to form separately. For a more detailed overview of the pathways depicted here, see Figure 1 on page 239.

Jack W. Szostak. Systems chemistry on early Earth. Nature 459:171-172, 2009

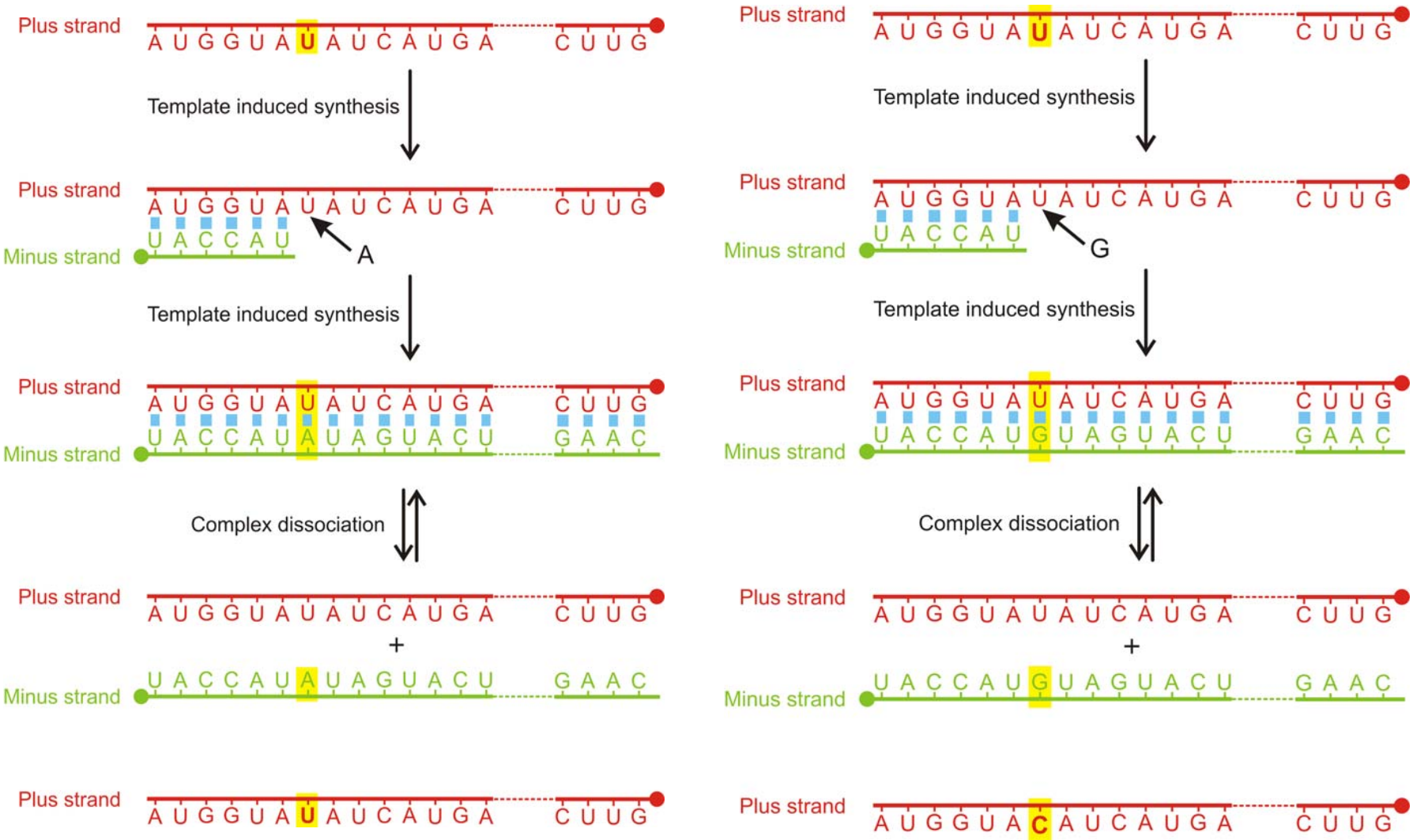
Matthew W. Powner, Béatrice Gerland, John D. Sutherland. *Nature* 459:239-242, 2009

Why RNA and not protein?

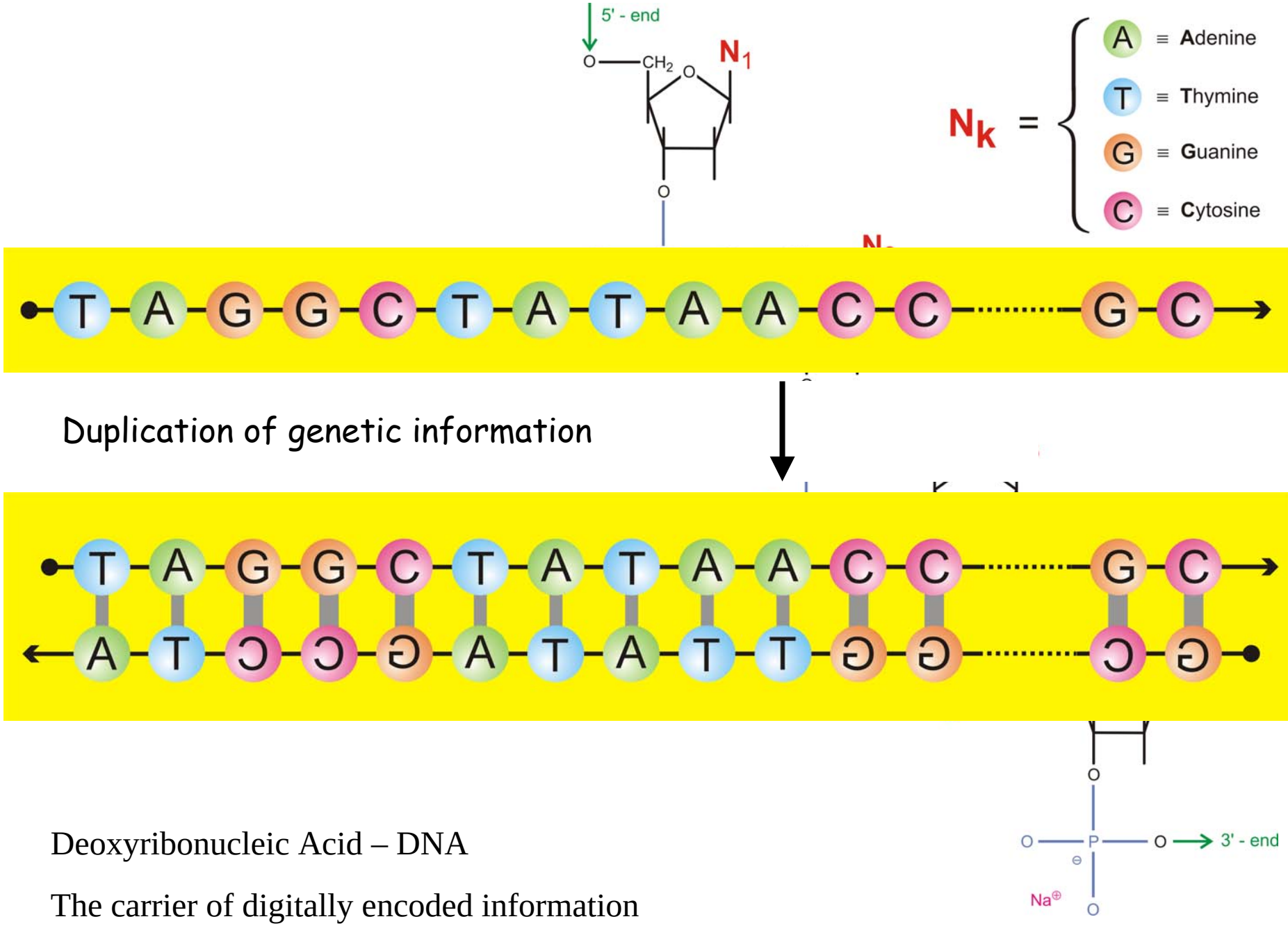
(i) Almost all RNA molecules are soluble in water

(ii) Almost all RNA molecules form structures and hence may have functions

(iii) All RNA molecules can act as templates for replication and hence all mutants of RNA molecules are suitable as templates



Replication and mutation are parallel chemical reactions.



Deoxyribonucleic Acid – DNA

The carrier of digitally encoded information

Why RNA and not DNA?

(i) DNA is more stable against degradation than RNA but much more difficult to synthesize

(ii) Evidence for the historical sequence of events: RNA is in the center of present day cellular metabolism whereas DNA is at the periphery

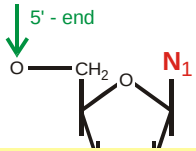
(iii) Single stranded RNA is richer in structure, because base pair stacking is stronger in RNA than in DNA

RNA structures as candidates for function

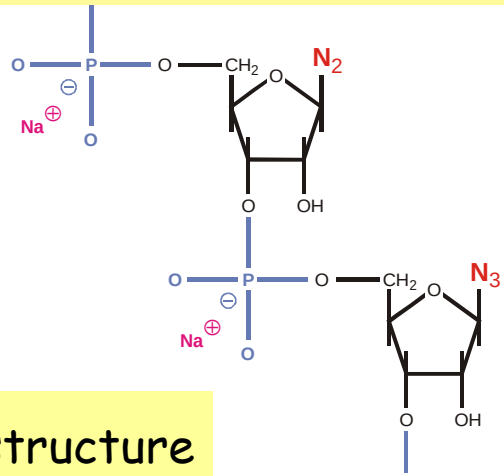
(i) Minimum free energy structures

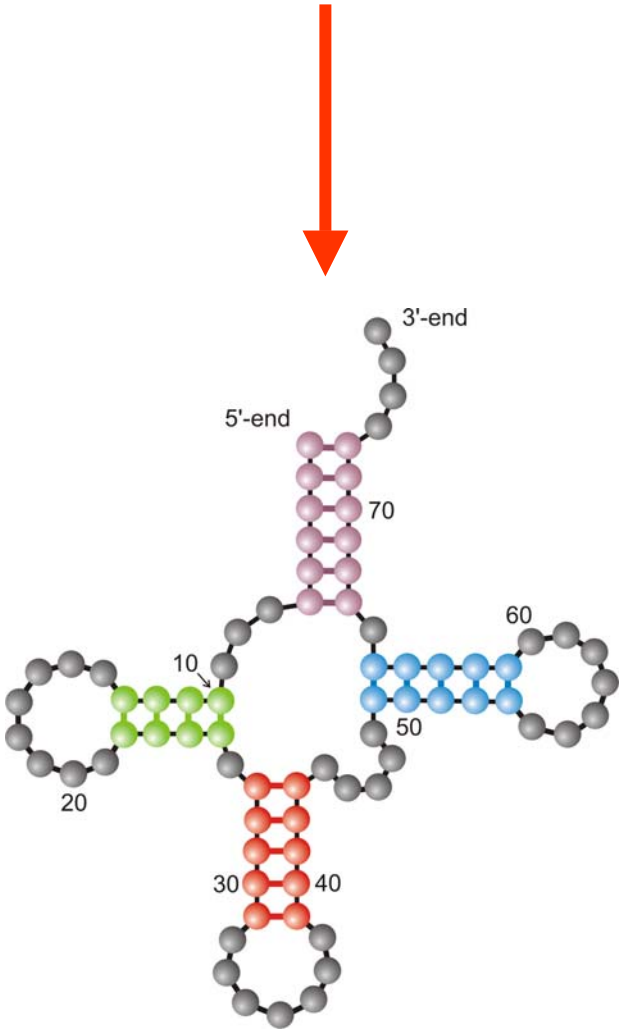
(ii) Suboptimal structures

(iii) Kinetic structures

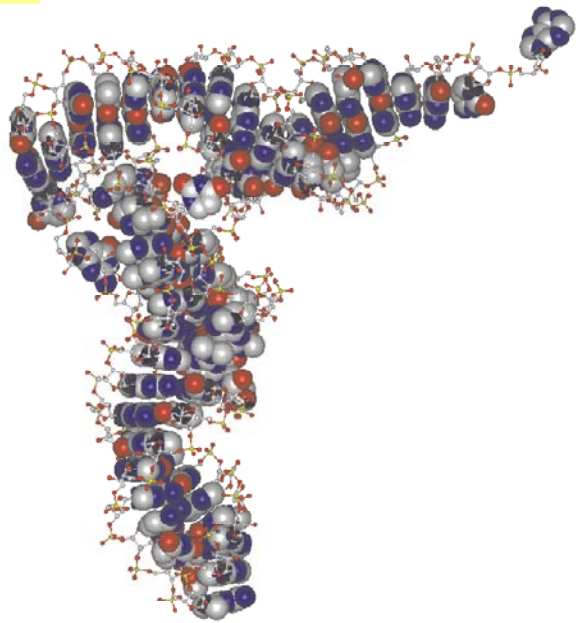


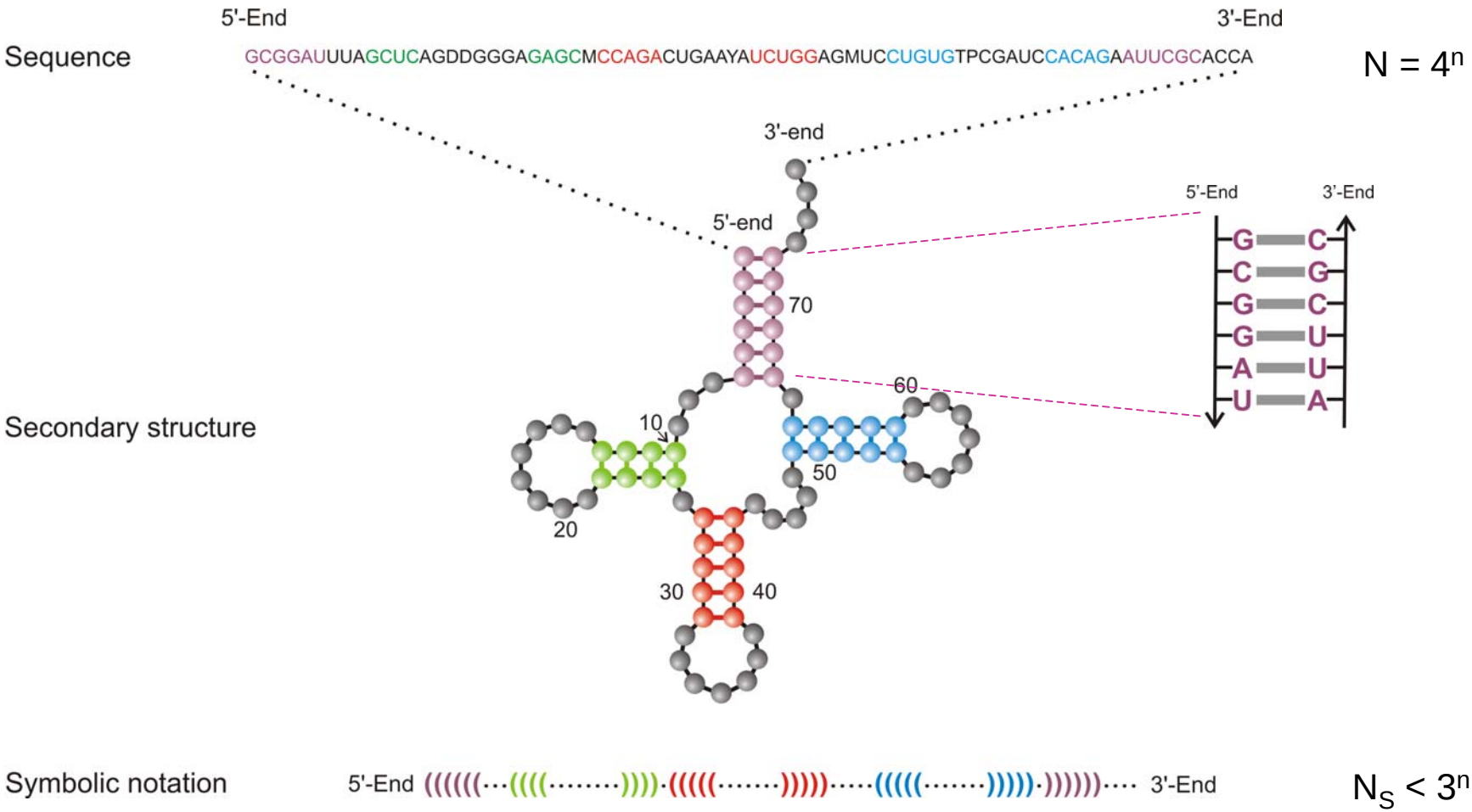
5'-end GCGGAUUUAGCUCAGUUGGGAGAGCGCCAGACUGAAGAUCUGGAGGUCCUGUGUUCGAUCCACAGAAUUCGCACCA 3'-end





Definition of RNA structure

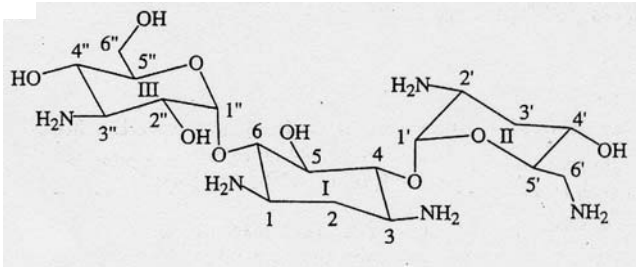




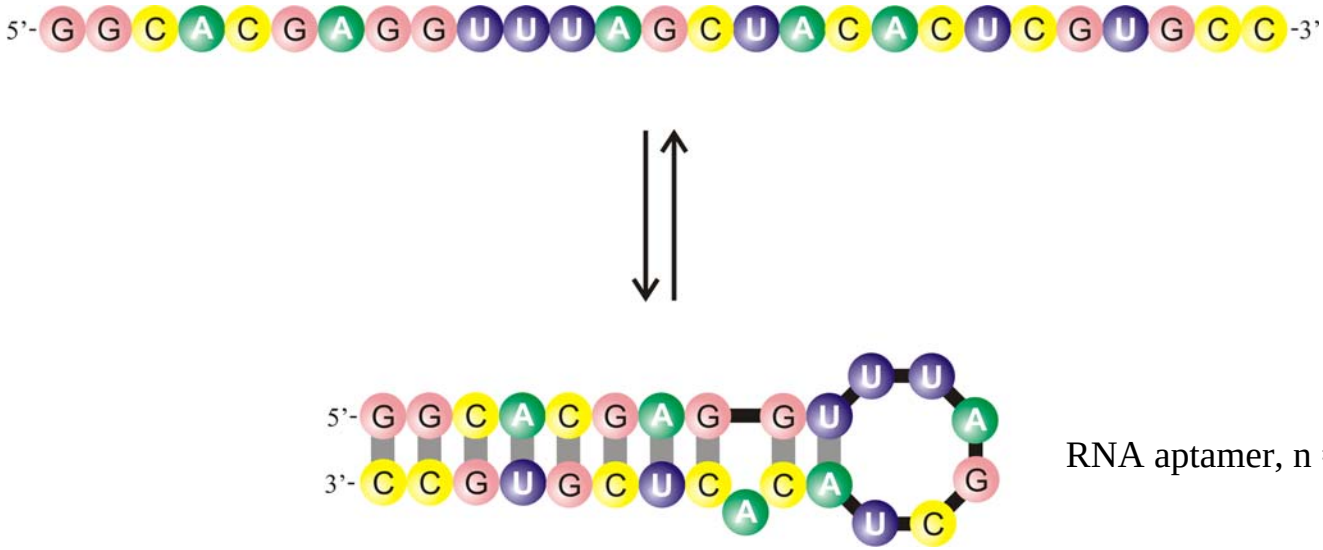
Criterion: Minimum free energy (mfe)

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

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



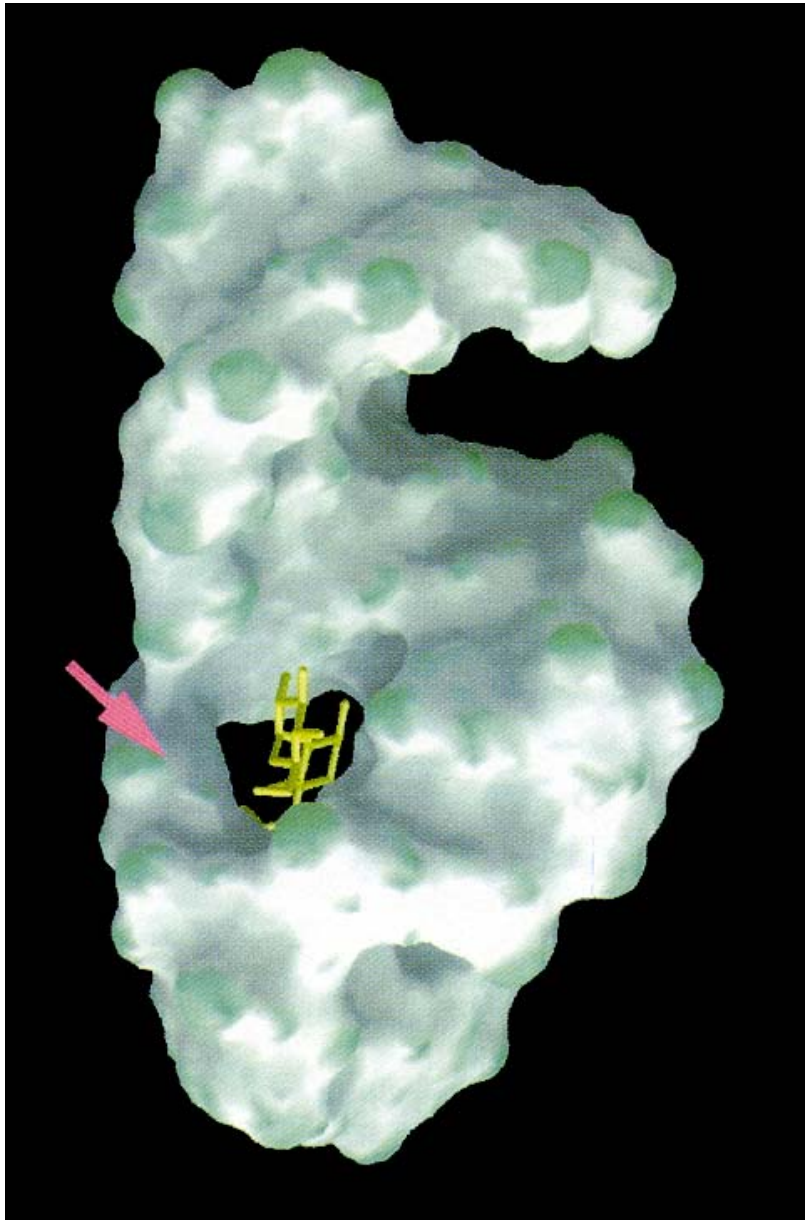
tobramycin



RNA aptamer, n = 27

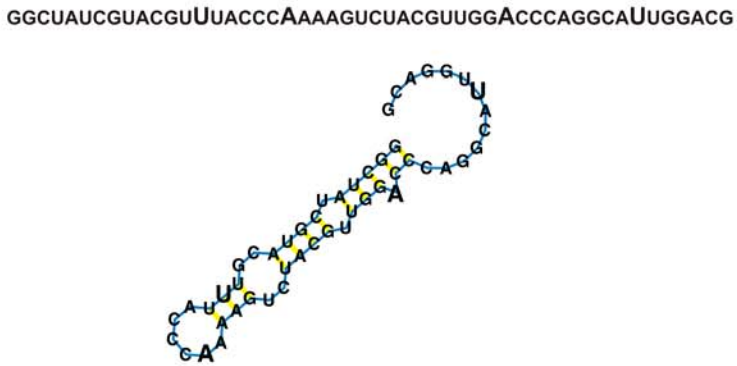
Formation of secondary structure of the tobramycin binding RNA aptamer with **K_D = 9 nM**

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

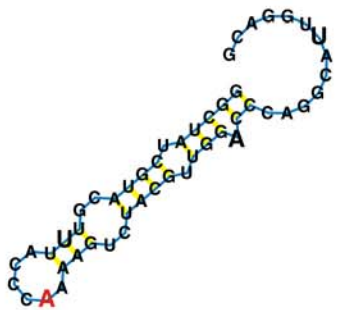
L. Jiang, A. K. Suri, R. Fiala, D. J. Patel, Chemistry & Biology **4**:35-50 (1997)



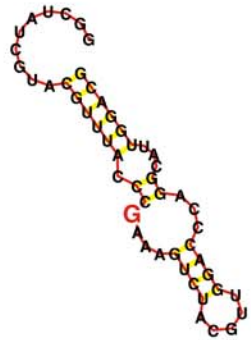
One error neighborhood – Surrounding of an RNA molecule of chain length n=50 in sequence and shape space

GGCUAUCGUACGUUUACCCGAAAGUCUACGUUGGACCCAGGCAUUGGACG

GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG

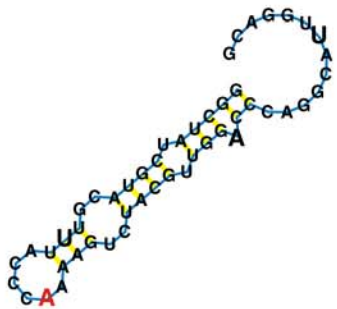


One error neighborhood – Surrounding of an RNA molecule of chain length n=50 in sequence and shape space

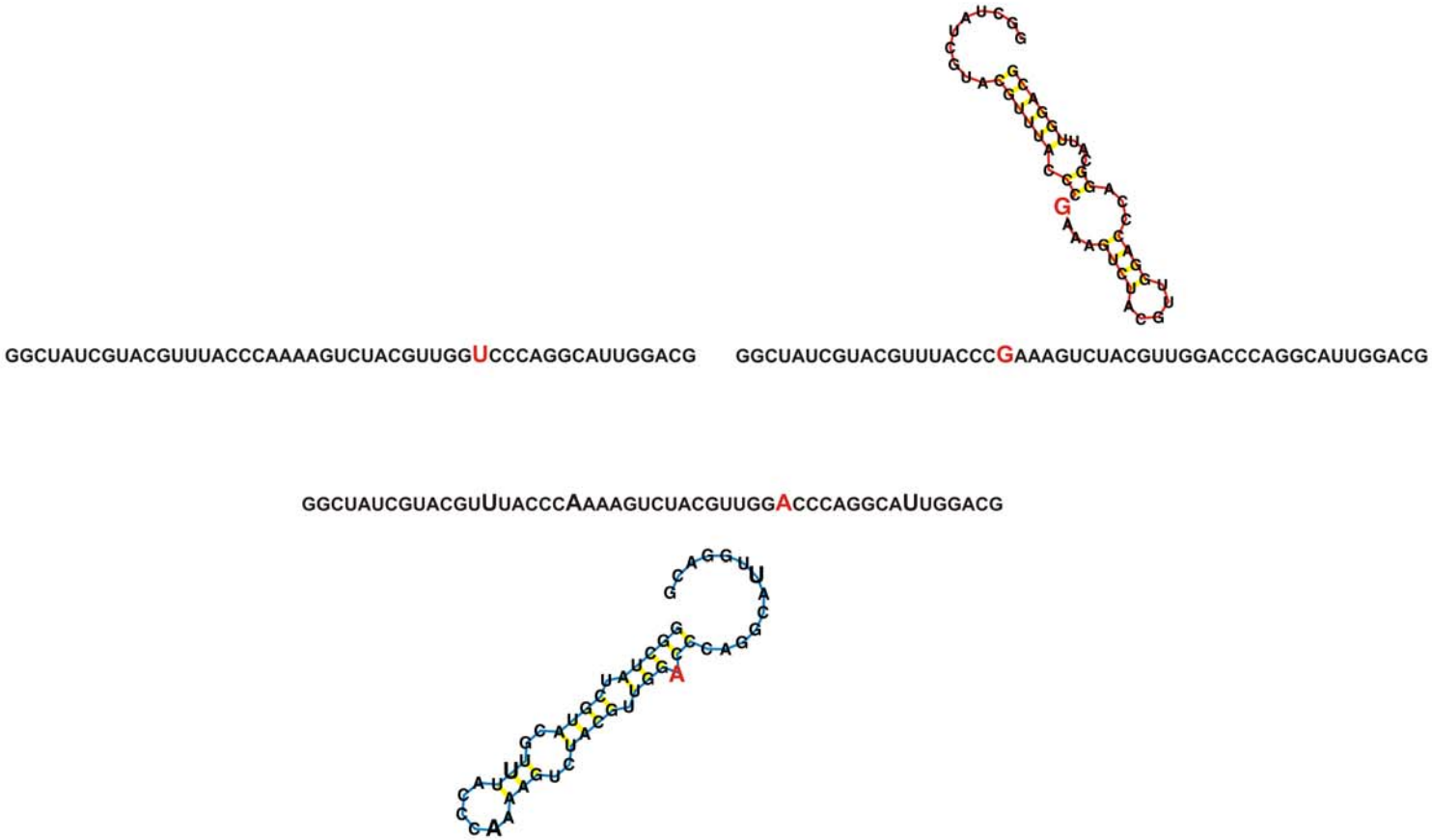


GGCUAUCGUACGUUUACCCGAAAGUCUACGUUGGACCCAGGCAUUGGACG

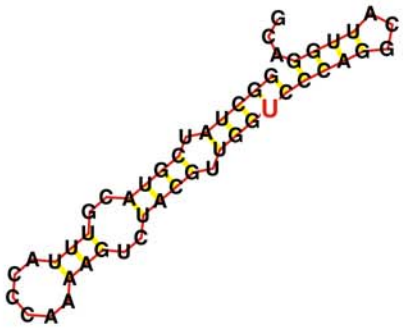
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG



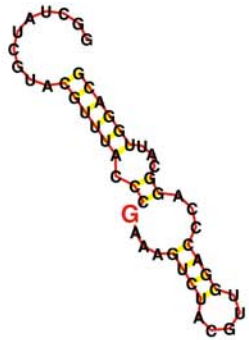
One error neighborhood – Surrounding of an RNA molecule of chain length n=50 in sequence and shape space



One error neighborhood – Surrounding of an RNA molecule of chain length n=50 in sequence and shape space

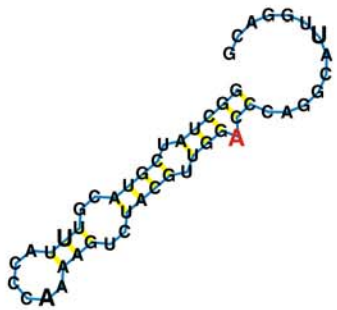


GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGUCCCAGGCAUUGGACG

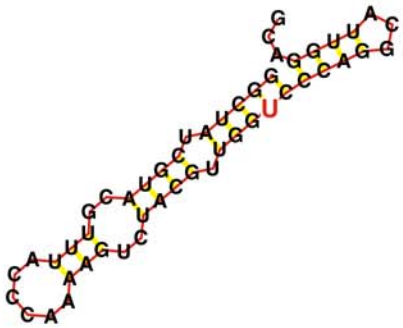


GGCUAUCGUACGUUUACCCGAAAGUCUACGUUGGACCCAGGCAUUGGACG

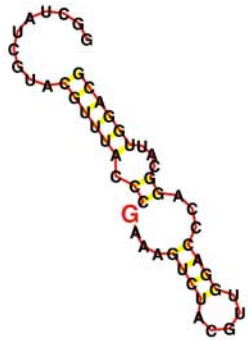
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG



One error neighborhood – Surrounding of an RNA molecule of chain length n=50 in sequence and shape space



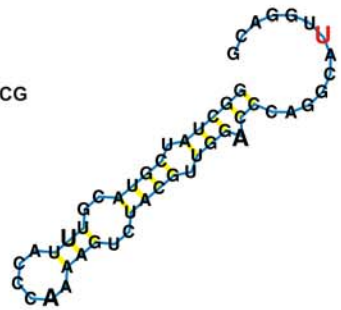
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGUCCCAGGCAUUGGACG



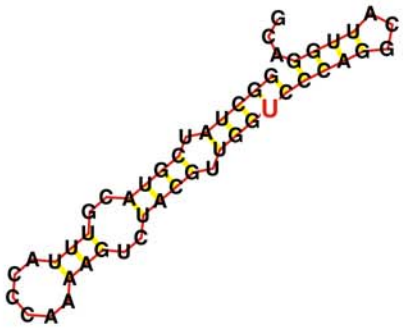
GGCUAUCGUACGUUUACCCGAAAGUCUACGUUGGACCCAGGCAUUGGACG

GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG

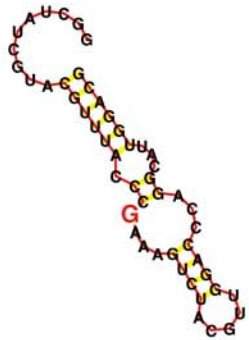
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCACUGGACG



One error neighborhood – Surrounding of an RNA molecule of chain length n=50 in sequence and shape space



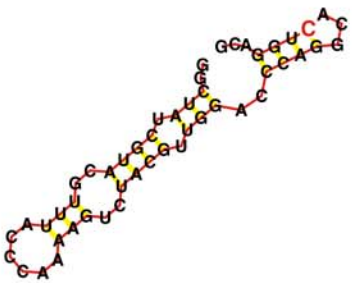
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGUCCCAGGCAUUGGACG

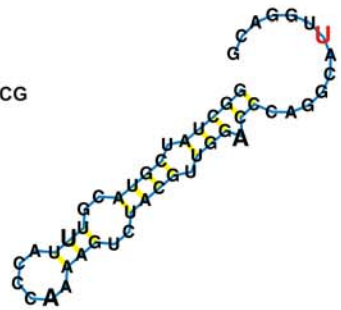


GGCUAUCGUACGUUUACCCGAAAGUCUACGUUGGACCCAGGCAUUGGACG

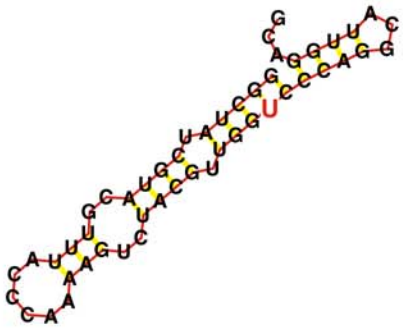
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG

GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCACUGGACG

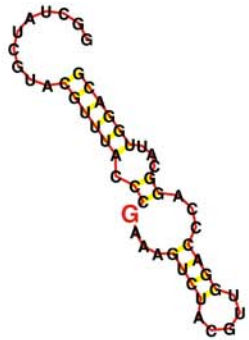




One error neighborhood – Surrounding of an RNA molecule of chain length n=50 in sequence and shape space



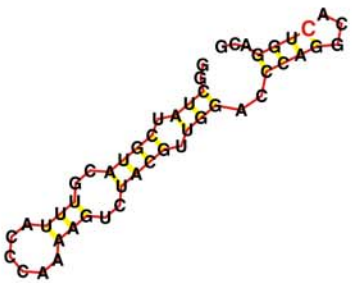
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGUCCCAGGCAUUGGACG

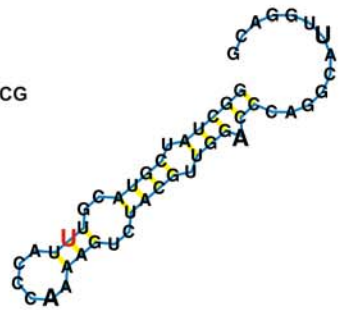


GGCUAUCGUACGUUUACCCGAAAGUCUACGUUGGACCCAGGCAUUGGACG

GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG

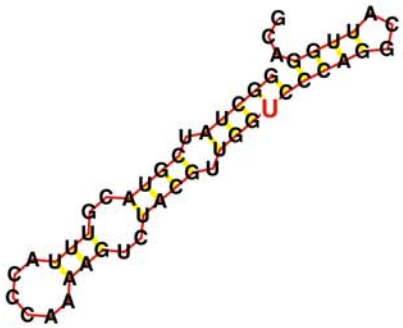
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCACUGGACG



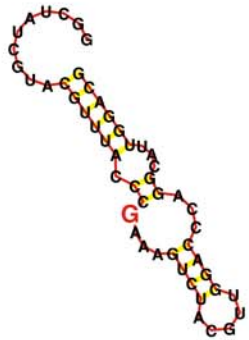


GGCUAUCGUACGUGUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG

One error neighborhood – Surrounding of an RNA molecule of chain length n=50 in sequence and shape space

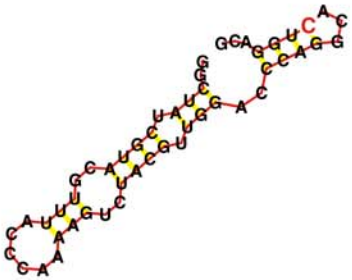


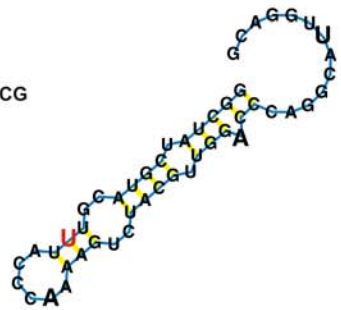
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGUCCCAGGCAUUGGACG



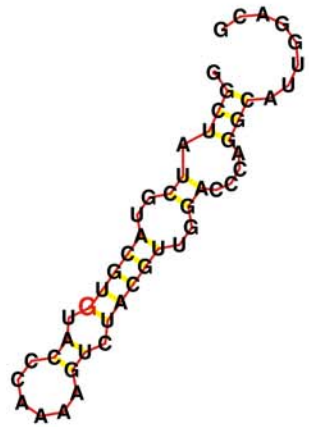
GGCUAUCGUACGUUUACCCGAAAGUCUACGUUGGACCCAGGCAUUGGACG

GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCACUGGACG





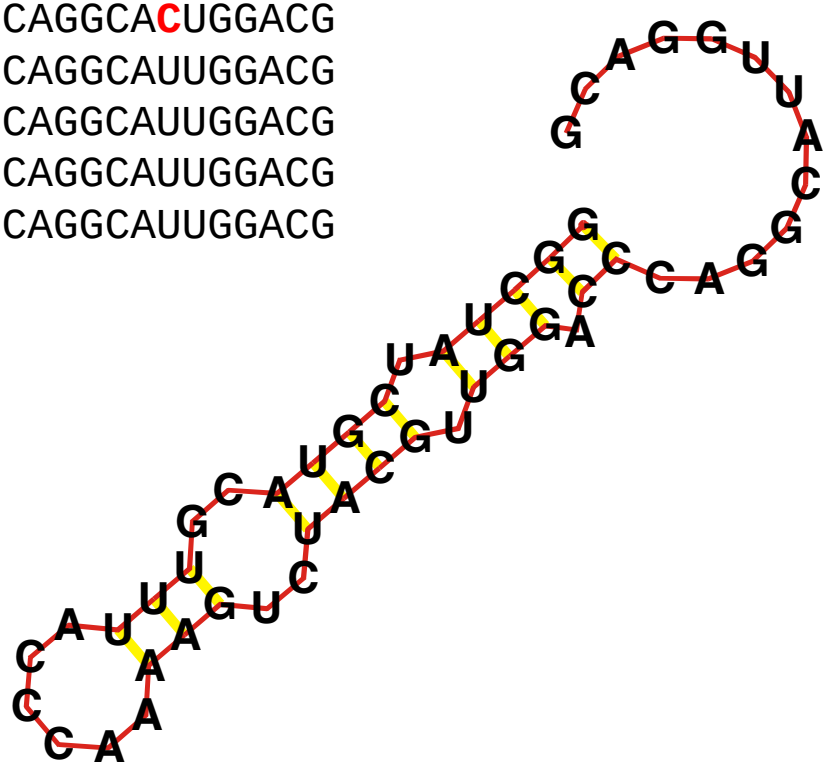
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG



GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG

One error neighborhood – Surrounding of an RNA molecule of chain length n=50 in sequence and shape space

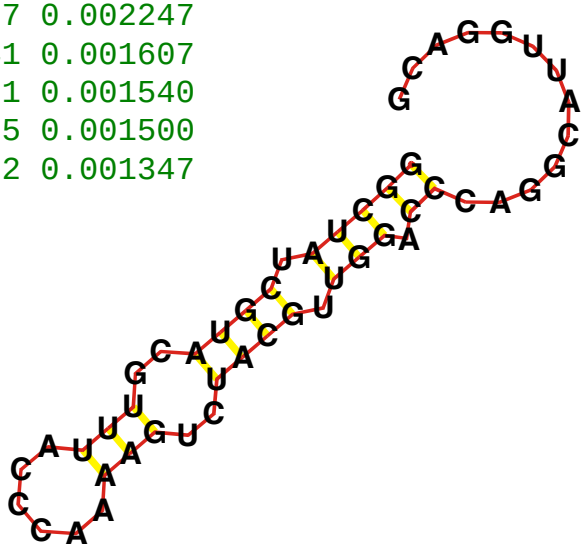
GGCUAUCGUA**U**GUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUU**A**GACG
GGCUAUCGUACGUUUAC**U**CAAAAGUCUACGUUGGACCCAGGCAUUGGACG
GGCUAUCGUACG**C**UUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG
GGC**C**AUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG
GGCUAUCGUACGU**G**UACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG
GGCUA**A**CGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCC**U**GGCAUUGGACG
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCA**C**UGGACG
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGG**U**CCCAGGCAUUGGACG
GGCUA**G**CGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG
GGCUAUCGUACGUUUACCC**G**AAAGUCUACGUUGGACCCAGGCAUUGGACG
GGCUAUCGUACGUUUACCCAAAAG**C**CUACGUUGGACCCAGGCAUUGGACG



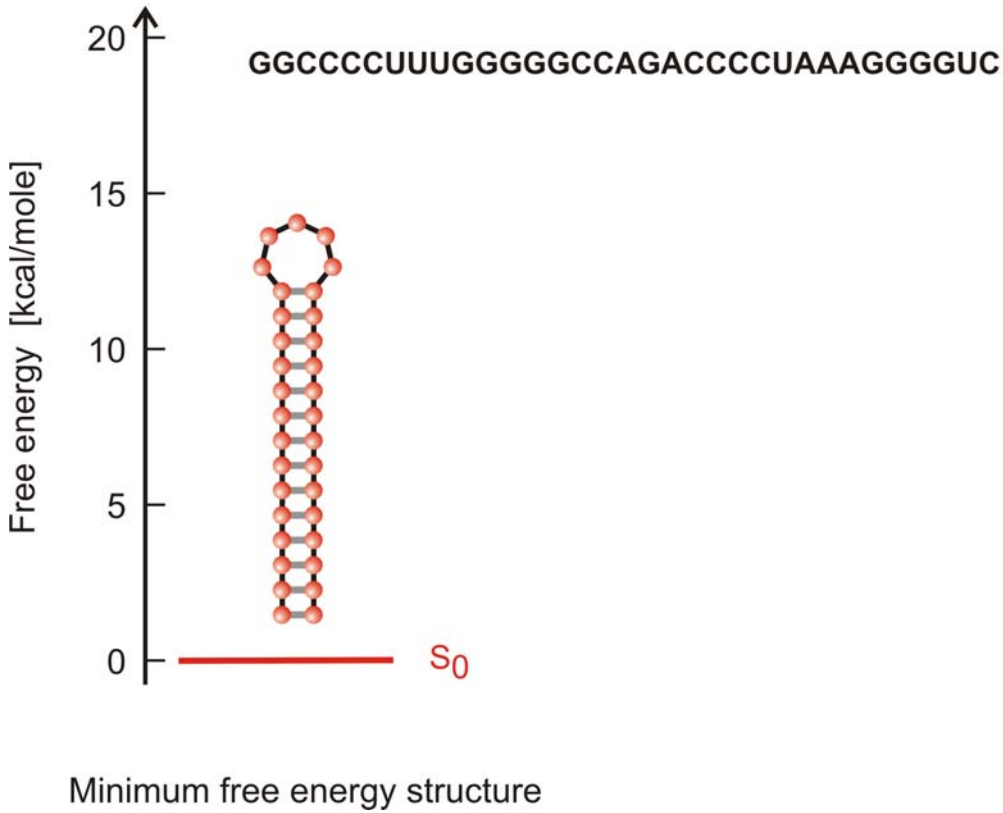
One error neighborhood – Surrounding of an RNA molecule of chain length n=50 in sequence and shape space

	Number	Mean Value	Variance	Std.Dev.
Total Hamming Distance:	150000	11.647973	23.140715	4.810480
Nonzero Hamming Distance:	99875	16.949991	30.757651	5.545958
Degree of Neutrality:	50125	**0.334167**	0.006961	**0.083434**
Number of Structures:	**1000**	**52.31**	85.30	**9.24**

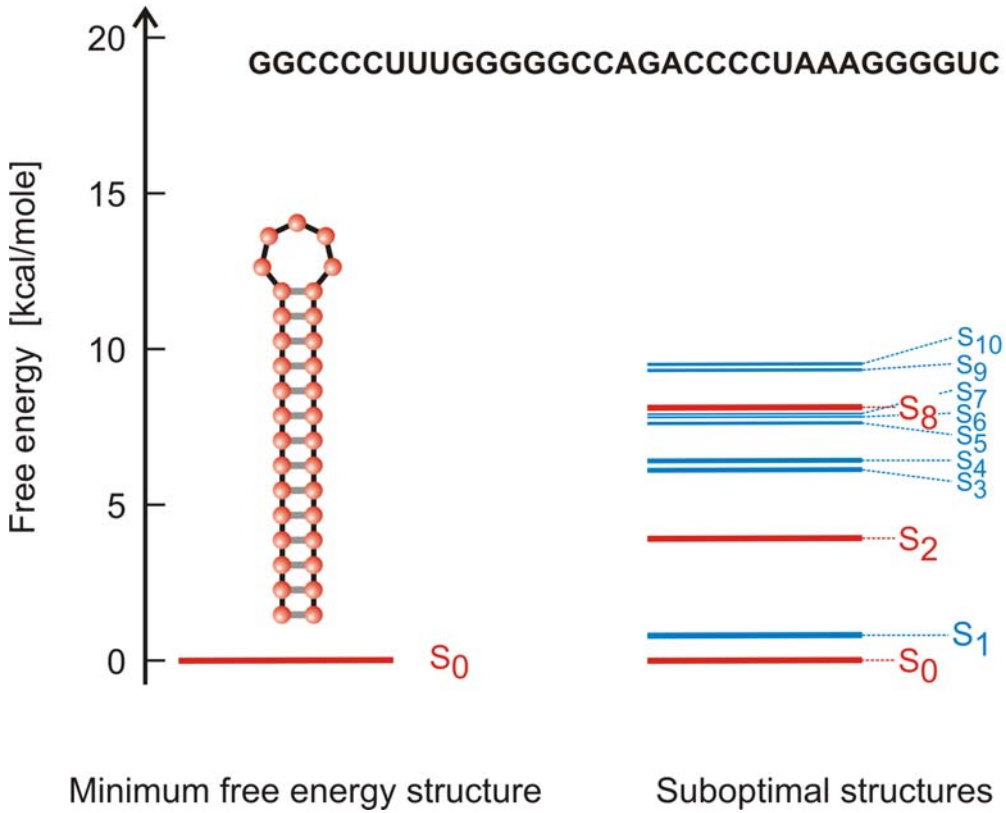
1	(((((.((((..(((.....))))..)))))..))).).....	**50125**	**0.334167**
2	..(((.((((..(((.....))))..)))))..))).....	**2856**	**0.019040**
3	((((((((((..(((.....))))..)))))))).)).....	**2799**	**0.018660**
4	(((((.((((..((((....))))..)))))..))).)).....	**2417**	**0.016113**
5	(((((.((((.((((.....))))).)))))..))).)).....	**2265**	**0.015100**
6	(((((.(((((.(((.....))).)))))..))).)).....	**2233**	**0.014887**
7	(((((..(((..(((.....)))..)))..)))..)).....	1442	0.009613
8	(((((.((((..((.......))..)))))..))).)).....	1081	0.007207
9	((((..((((..(((.....)))..)))))..)).)).....	1025	0.006833
10	(((((.((((..(((.....)))..)))))..))))).....	1003	0.006687
11	.((((.((((..(((.....)))..)))))..)))).....	963	0.006420
12	(((((.(((...(((.....)))...)))..))).)).....	860	0.005733
13	(((((.((((..(((.....)))..)))))..)).))).....	800	0.005333
14	(((((.((((...((.....))...)))))..))).)).....	548	0.003653
15	(((((.((((..............))))..))).)).....	362	0.002413
16	((.((.((((..(((.....)))..)))))..))..)).....	337	0.002247
17	(.(((.((((..(((.....)))..)))))..))).).....	241	0.001607
18	(((((.(((((((((.....)))))))))..))).)).....	231	0.001540
19	((((..((((..(((.....)))..)))))...)))).....	225	0.001500
20	((....((((..(((.....)))..))))).....)).....	202	0.001347



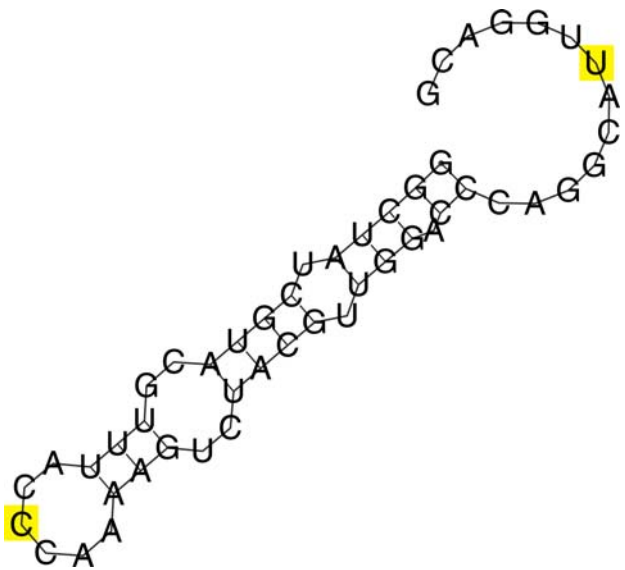
Shadow – Surrounding of an RNA structure in shape space:
AUGC alphabet, chain length n=50



Extension of the notion of structure



Extension of the notion of structure



GGCUAUCGUACGUUUAC**C**CAAAAGUCUACGUUGGACCCAGGCA**U**UGGACG

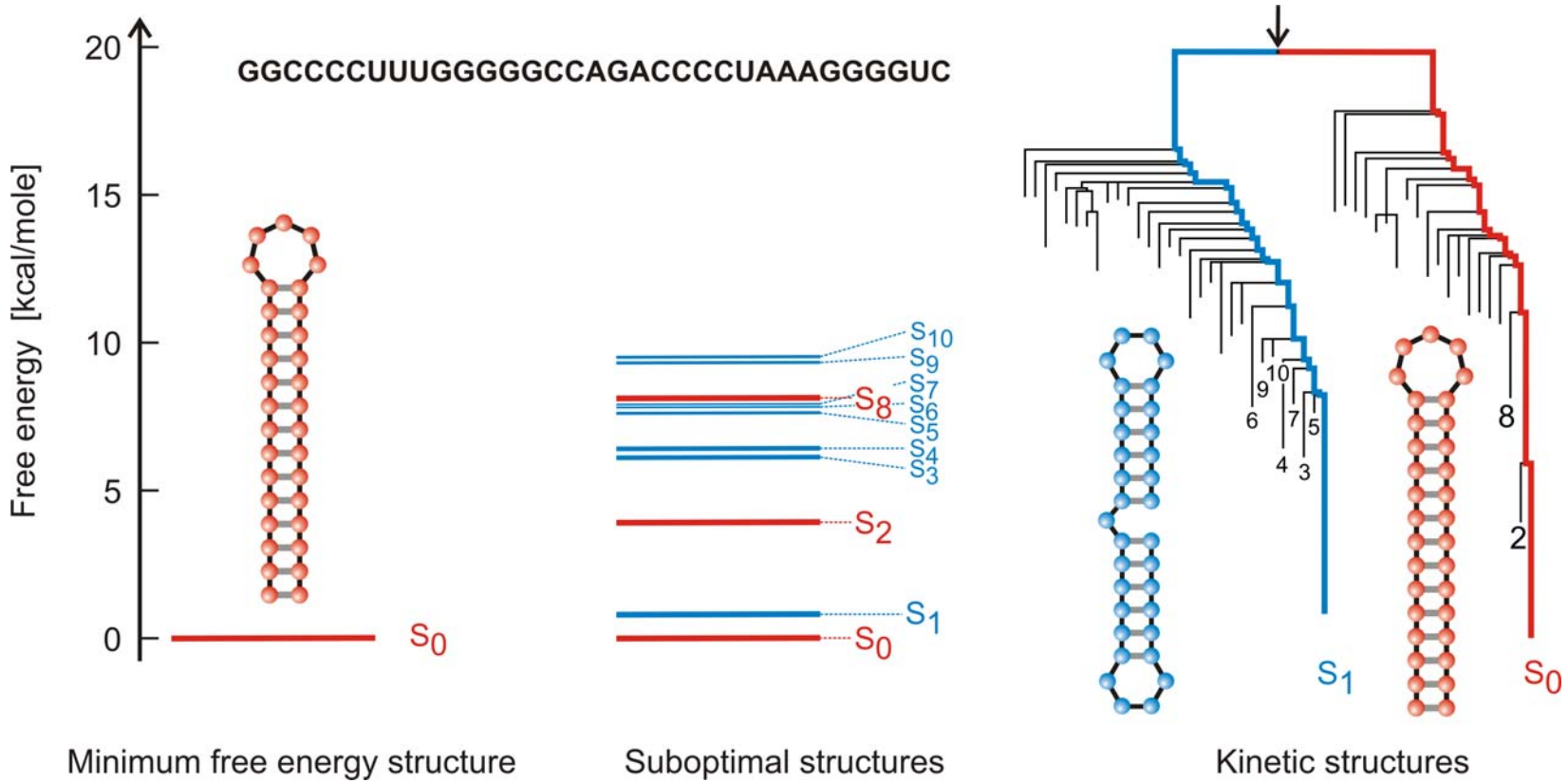
```
(((((.((((..(((......))).))))).))).)).............  -7.30
..........((((((.((....((((.....))))...)).)).))))))  -6.70
..........((((((.((....(((((...)))))...)).)).))))))  -6.60
..(((.((((..(((......))).))))).))).((((...)))).....  -6.10
(((((.((((..(((......))).))))).))).)).(........).  -6.00
(((((.((((..((........))..))))).))).)).............  -6.00
.(((.((..((((..((......)).))))..)).....))).........  -6.00
```

GGCUAUCGUACGUUUAC**A**CAAAAGUCUACGUUGGACCCAGGCAUUGGACG

```
(((((.((((..(((......))).))))).))).)).............  -7.30
.(((.((..((((..((......)).))))..)).....))).........  -6.50
.(((.....((((..((......)).))))((....)))).........  -6.30
..(((.((((..(((......))).))))).))).((((...)))).....  -6.10
(((((.((((..(((......))).))))).))).)).(........).  -6.00
(((((.((((..((........))..))))).))).)).............  -6.00
.(((...((((((..((......)).))))...)).....))).........  -6.00
```

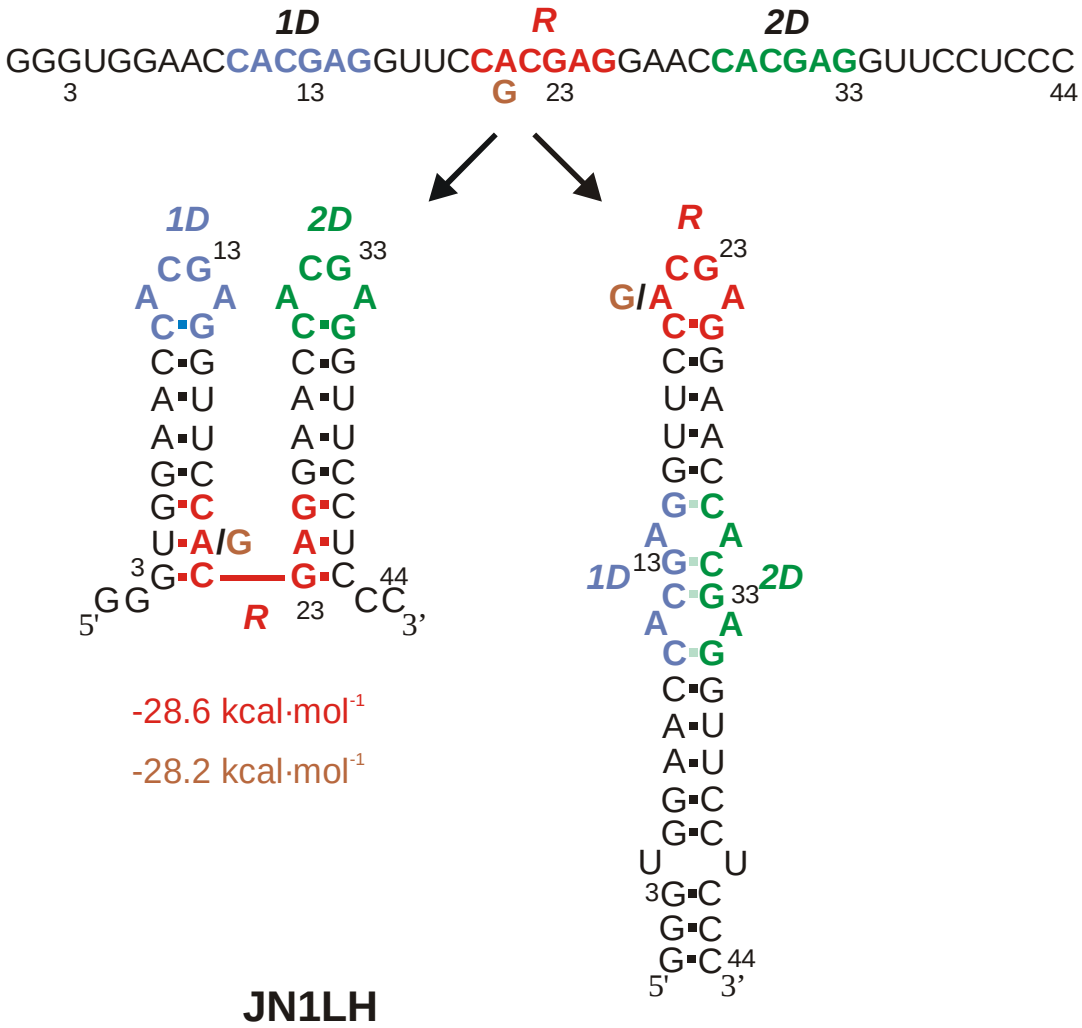
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCA**A**UGGACG

```
(((((.((((..(((......))).))))).))).)).............  -7.30
..(((.((((..(((......))).))))).))).(((.....)))....  -7.20
..........((((((.((....((((.....))))...)).)).))))))  -6.70
..........((((((.((....(((((...)))))...)).)).))))))  -6.60
(((((.((((..(((......))).))))).))).)).(((.....)))....  -6.50
(.(((.((((..(((......))).))))).))).)(((.....)))....  -6.30
.((((.((((..(((......))).))))).))).)(((.....)))....  -6.30
.....(((.((((..((......)).)))))))..(((.....)))....  -6.30
(.(((.((((..(((......))).))))).))).)..(((.....))).).  -6.10
.....((..((((..((......)).))))..)).(((.....)))....  -6.10
......(((.((((...((....((((.....))))...)).)))).))).  -6.10
(((((.((((..(((......))).))))).))).)).(........).  -6.00
(((((.((((..((........))..))))).))).)).............  -6.00
.(((.((..((((..((......)).))))..)).....))).........  -6.00
......(((.((((...((....(((((...)))))...)).)))).))).  -6.00
```



Extension of the notion of structure

An RNA switch



JN1LH

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

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

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%.
46. C. M. Carr, E. Grote, M. Munson, F. M. Hughson, P. J. Novick, *J. Cell Biol.* **146**, 333 (1999).
47. C. Ungermann, B. J. Nichols, H. R. Pelham, W. Wickner, *J. Cell Biol.* **140**, 61 (1998).
48. E. Grote and P. J. Novick, *Mol. Biol. Cell* **10**, 4149 (1999).
49. P. Uetz *et al.*, *Nature* **403**, 623 (2000).
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.
51. V. Rybin *et al.*, *Nature* **383**, 266 (1996).
52. K. G. Hardwick and H. R. Pelham, *J. Cell Biol.* **119**, 513 (1992).
53. A. P. Newman, M. E. Groesch, S. Ferro-Novick, *EMBO J.* **11**, 3609 (1992).
54. A. Spang and R. Schekman, *J. Cell Biol.* **143**, 589 (1998).
55. M. F. Rexach, M. Latterich, R. W. Schekman, *J. Cell Biol.* **126**, 1133 (1994).
56. A. Mayer and W. Wickner, *J. Cell Biol.* **136**, 307 (1997).
57. M. D. Turner, H. Plutner, W. E. Balch, *J. Biol. Chem.* **272**, 13479 (1997).
58. A. Price, D. Seals, W. Wickner, C. Ungermann, *J. Cell Biol.* **148**, 1231 (2000).
59. X. Cao and C. Barlowe, *J. Cell Biol.* **149**, 55 (2000).
60. G. G. Tall, H. Hama, D. B. DeWald, B. F. Horazdovsky, *Mol. Biol. Cell* **10**, 1873 (1999).
61. C. G. Burd, M. Peterson, C. R. Cowles, S. D. Emr, *Mol. Biol. Cell* **8**, 1089 (1997).
62. M. R. Peterson, C. G. Burd, S. D. Emr, *Curr. Biol.* **9**, 159 (1999).
63. M. G. Waters, D. O. Clary, J. E. Rothman, *J. Cell Biol.* **118**, 1015 (1992).
64. D. M. Walter, K. S. Paul, M. G. Waters, *J. Biol. Chem.* **273**, 29565 (1998).
65. N. Hui *et al.*, *Mol. Biol. Cell* **8**, 1777 (1997).
66. T. E. Kreis, *EMBO J.* **5**, 931 (1986).
67. H. Plutner, H. W. Davidson, J. Saraste, W. E. Balch, *J. Cell Biol.* **119**, 1097 (1992).
68. D. S. Nelson *et al.*, *J. Cell Biol.* **143**, 319 (1998).
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*).

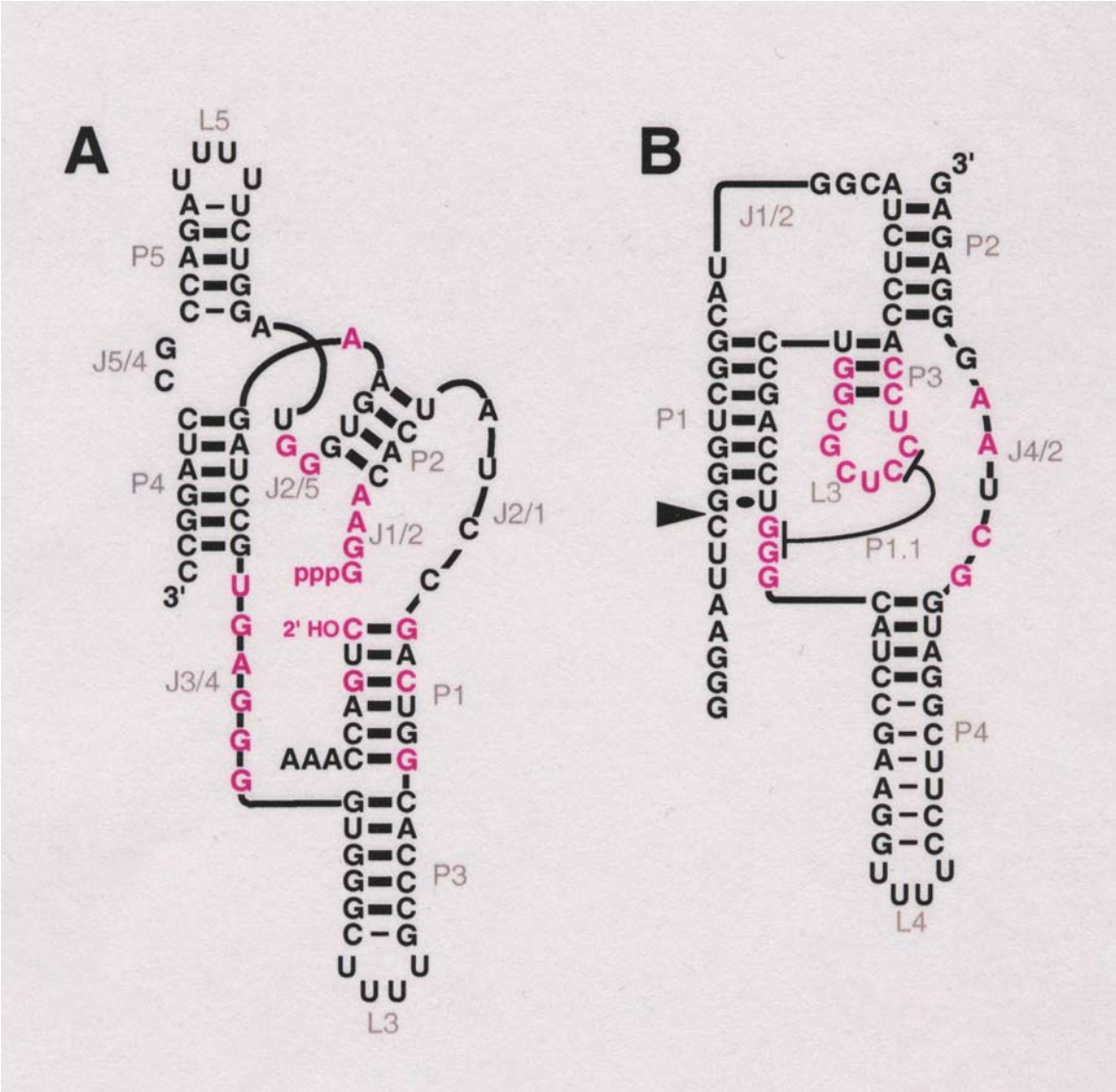
Theoretical analyses using algorithms for predicting RNA secondary structure have suggested that different neutral networks are interwoven and can approach each other very closely (*3*, *5–8*). Of particular interest is whether ribozyme neutral networks approach each other so closely that they intersect. 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

Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, MA 02142, USA.

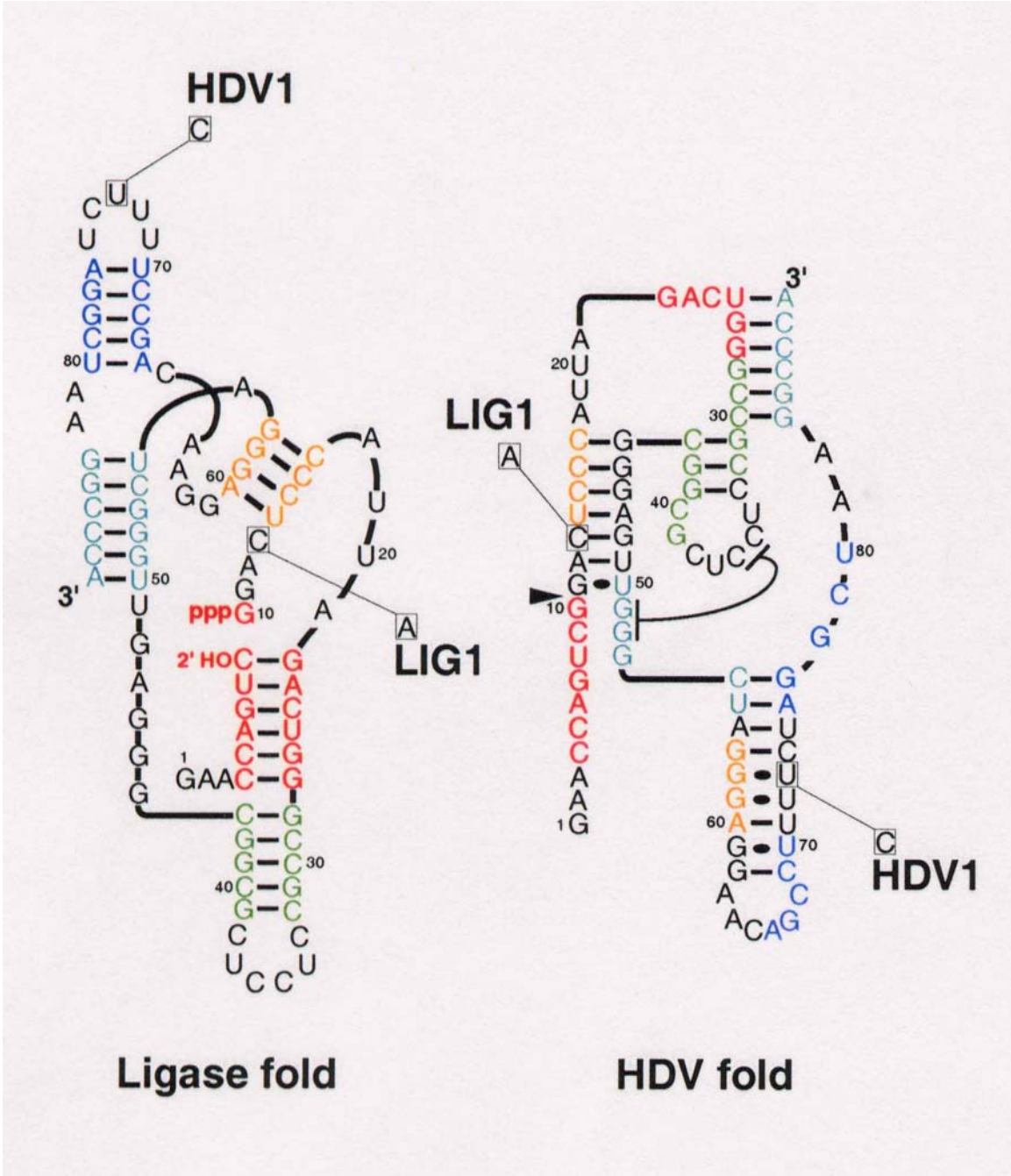
*To whom correspondence should be addressed. E-mail: dbartel@wi.mit.edu

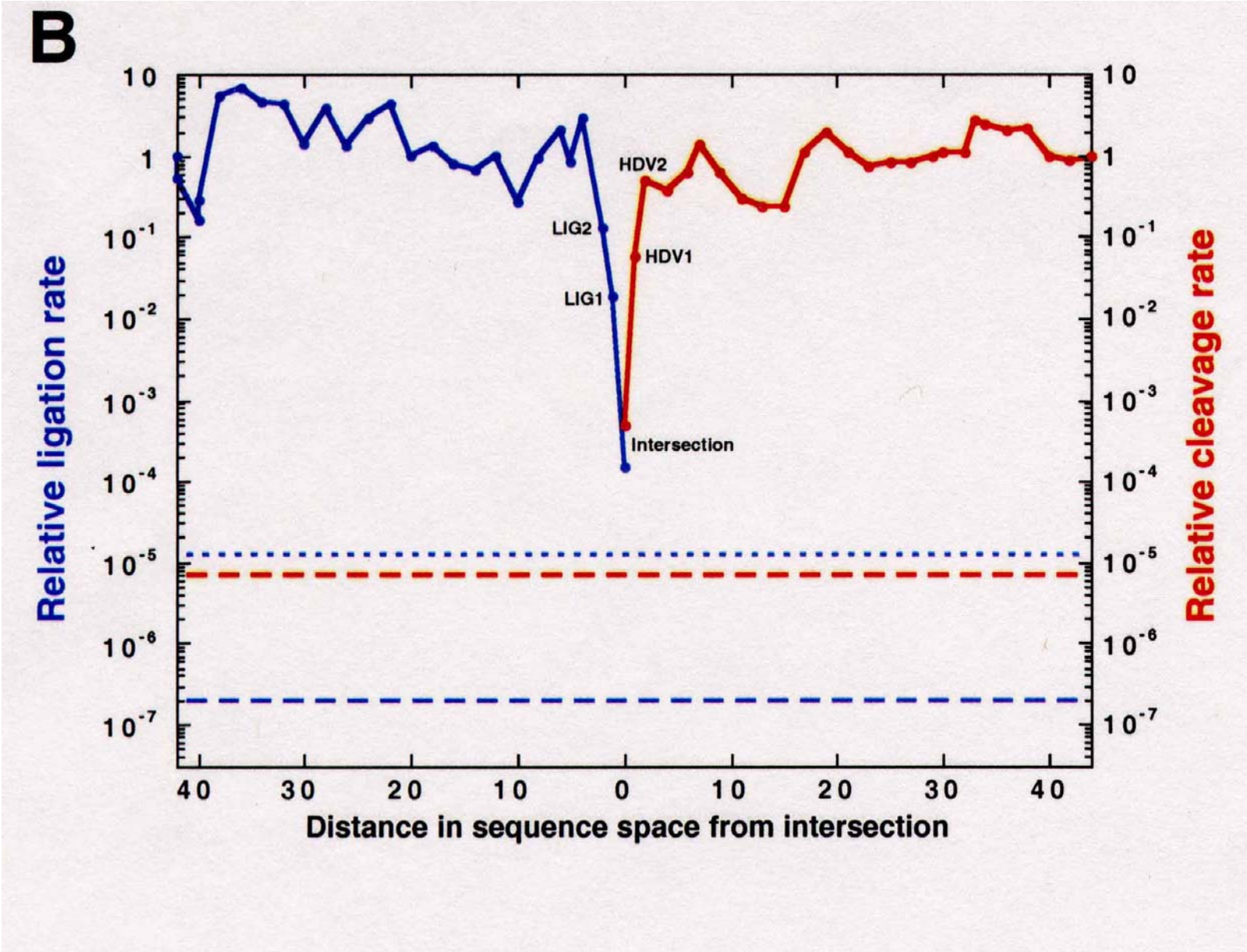


Two ribozymes of chain lengths n = 88 nucleotides: An artificial ligase (**A**) and a natural cleavage ribozyme of hepatitis-δ-virus (**B**)

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

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





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

Web-Page for further information:

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