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Three-Dimensional Model for Slipped Loop RNA

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Three-Dimensional Model for Slipped Loop RNA

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Abstract

Earlier a three-dimensional model for a new unusual DNA conformation referred to as Slipped Loop Structure (SLS) has been suggested by us (1). The same type of folding could occur with RNA as well which means that one must use the A-form of the double helix rather than the B-one. The present paper discusses the creation of an all-atom stereochemically sound model for SLS-RNA. This calculated model, while possessing the same folding topology as the SLS-DNA, differs dramatically from the SLS-DNA by an overall folding geometry. It also differs radically from the RNA-pseudoknot and can thus be regarded as a new type of an RNA folding.

Introduction

First indications of the existence of a new DNA folding type were obtained for the double-stranded DNA containing short direct repeats. When negatively supercoiled, these fragments are non-uniformly sensitive to the single-strand specific nuclease S1 (2-4). This might account for a hypothetical conformation with two shifted loops protruding from the opposite strands and partially pairing with each other (Figure 1). It depicts two isomers of the structure. The feasibility of the complementary interaction between these loops (Figure 1) has been previously demonstrated by means of chemical probes (1,5), NMR (6). This folding type with the additional mini-helix between the loops will be referred to as SLS (Slipped Loop Structure).

The SLS folding significantly differs from other known foldings of the DNA such as "cruciform" or "H-form". These structures have no geometrical restrictions on their length. In contrast, the geometrical restrictions do exist for the SLS. The formation of the mini-helix between the shifted loops depends on the interloop distance and 'phasing' (angular orientation) of the loops. In this present paper we deal with RNA-SLS. An example of primary and secondary structure of an RNA-transcript that might fold into SLS is shown in Figure 2.

The tertiary structure of native RNAs was considered to be a combination of only double-helical stems and single stranded loops for a long time. This general belief collapsed after a discovery of the pseudoknot folding type whose presence in several types of native RNAs was shown now as well as its participation in miscellaneous cellular processes (7).

The topologies of the pairing schemes for the "stem and loop" and "pseudoknot" folding types are shown on Figure 3 together with the SLS built from the single-stranded RNA. This figure reveals similarity of the topologies of the SLS and the pseudoknot - their H-bond sets intersect, while those of the stem and loop do not. This allows one to treat the SLS as a special type of the pseudoknot. However, geometries of the classical pseudoknot and the SLS are quite different. The helices

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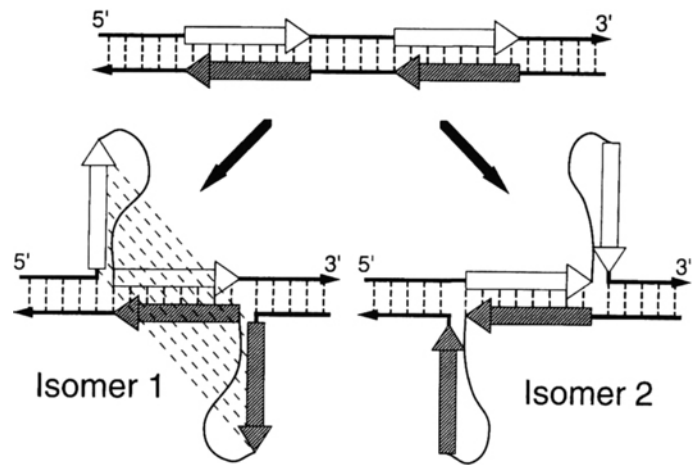
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Figure 1: 2D representation of the DNA-SLS. Both isomers of the DNA-SLS are shown. The Watson-Crick pairing within the additional mini-helice (*sail*) is shown with inclined dashed lines (absent for the 2nd isomer, since such an interaction is sterically impossible). The *corners* connect 5'-ends of the *sail* with 3'-ends of the helices coaxial with *core* (for the 1st isomer). The *shrouds* (thin curves) connect 3'-ends of *sail* with 5'-ends of *core*. The *core* and main helix were assumed to be coaxial and the structure of 3'-end of *core* - 5'-end of coaxial helix connections was assumed to be in the A-helix.



in the ordinary pseudoknot are co-axial, while the axes of the SLS's helices are crossed in space, as it will be shown below.

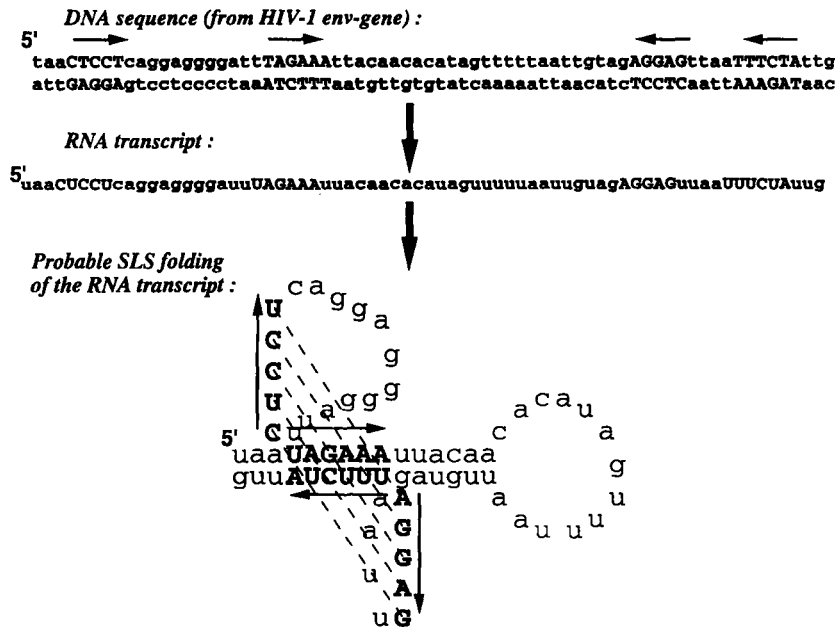
The structural peculiarity of the “pseudoknot” folding was shown (8 and references therein) to be crucial for its activity in ribozymes. Their detailed 3-D structure and its functional role is currently under investigation and several structures of “pseudoknot” containing ribozymes were recently proposed (9,10).

In the present work the stereochemistry of the RNA-SLS is investigated by computational means. The main aim of this study is to demonstrate the feasibility of this structure by creating a stereochemically sound all-atom model.

Materials and Methods

Terminology

“SLS” refers to the folding with shifted loops if the Watson-Crick pairs exist between the complementary regions of these loops. The additional mini-helix will be referred to as *sail* and the main helix, from which these by-loops protrude will be referred to as *core*. Single-stranded segments connecting *sail* to *core* are called *shrouds*, following the marine terminology. The sugar-phosphate chain connecting the 3'-end of the *core* with the 5'-end of the *sail* (for the 1st isomer; vice versa for the 2nd one) is referred to as a *corner*. Figure 4 shows these structural elements of the SLS and 3 axes of pseudosymmetry of this structure - reciprocally perpendicular rotational axes of the 2nd order.



RNA transcript :

**Probable SLS folding
of the RNA transcript :**

Figure 2: The primary structure of DNA yielding an RNA-transcript that may form SLS (Slipped Loop Palindrome - SLP). It is different from common direct repeat that may fold into SLS in the double stranded DNA.

Three-Dimensional Model for Slipped Loop RNA

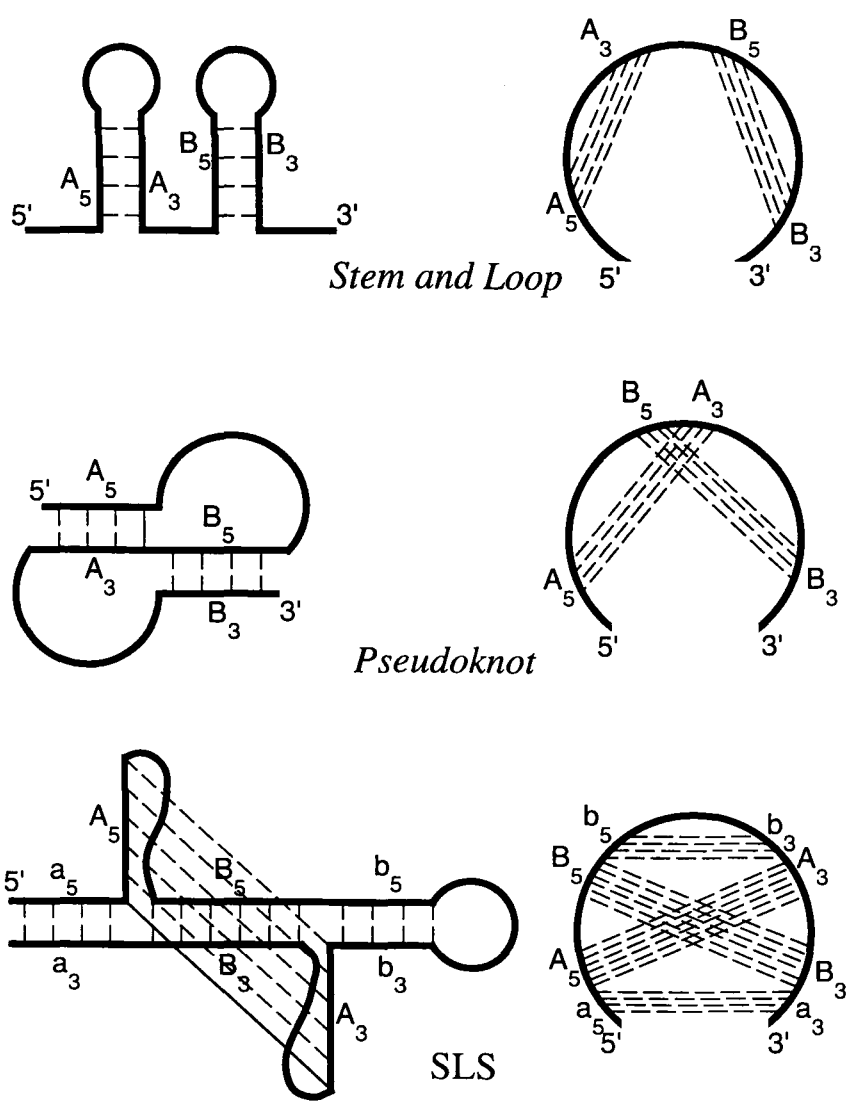


Figure 3: Stem and loop, pseudoknot and SLS in traditional and "wheel-like" representation (the same letters denotes Watson-Crick paired fragments of sequences). The latter clearly shows the identity of topologies for the pseudoknot and the SLS - their sets of H-bonds intersect. Only 1st isomer of SLS is represented - *sail* is built with A₅ and A₃ complementary regions, *core* - with the B₅ and B₃ ones. The IInd isomer would be built *vice versa* : *sail* - B₅ and B₃, *core* - A₅ and A₃.

Formulation of Model for Calculation

The calculations of the SLS were performed for the A-form of DNA and RNA. The isomer I (Figure 1) has both loops directed towards the non-glycosidic (major) groove, while the loops of the isomer II protrude to the side of the glycosidic (minor) groove (Figure 5). One can readily see from Figure 5, that the loops in the isomer II are further spaced than those for the isomer I. Simple measurements verify that an interloop helix can not be formed in the isomer II.

Before modelling of the RNA-SLS the structure of the A-DNA-SLS was calculated, for the following reasons:

1. Though the model for B-DNA-SLS was already built (5), it would be interesting to elucidate how the substitution of the B-family helix with the A-helix will change the geometry of the folding. The RNA-based structure is inappropriate one for this comparison.
2. The A-DNA-SLS is a useful initial conformation for the optimization of the RNA-SLS.

The starting point was a structure with *core* and *sail* consisting of 6 base pairs with two extra base pairs on the sides of the *core*. The two *shrouds* were of the same length - 4 nucleotides. The nucleotide sequence of this structure corresponds to the RNA-SLS-6x6x4 in Figure 6 with thymine instead of uracils.

Particular attention was paid to conserve the symmetry of the structure relatively to

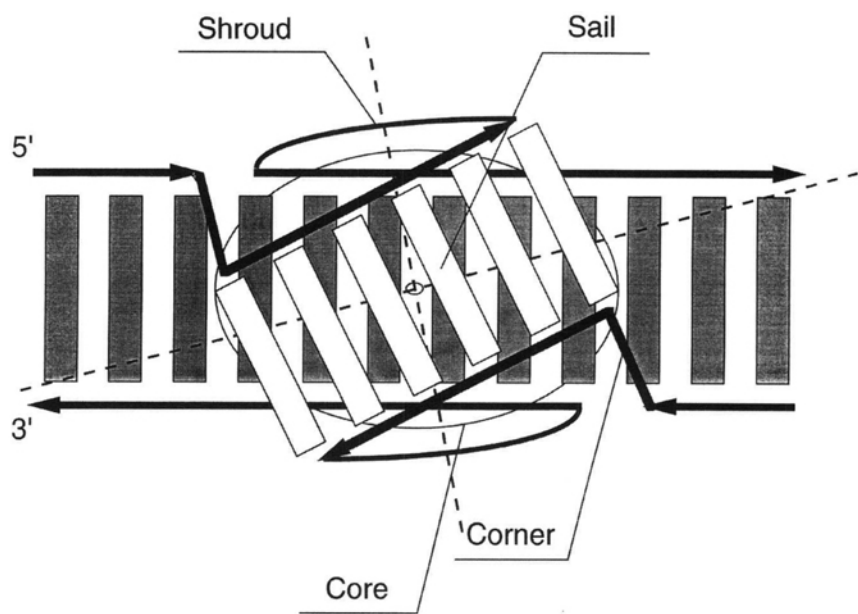


Figure 4: Three axes of the rotational (pseudo)symmetry and structural elements of the SLS. Two dyad axes are in plane of the drawing. The third dyad axis is perpendicular to the plane of the drawing.

the dyad axis (perpendicular to the plain of the Figure 4) during the computations. This halved the number of the variables of the system and ensured, that when an acceptable conformation of one *corner* or *shroud* was found, another would have the same one. For this reason the sequences were chosen symmetrical relatively to the same axis (Figure 6).

Calculations of the Models

The models of A-DNA-SLS and RNA-SLS were constructed using the DNAMiniCarlo program (11 and the references therein) with several modifications that took into account the symmetry of the given structure. It performed a molecular-mechanical calculation in a semi-empirical force field (12) specifically designed for the optimization of the structure of the nucleic acids. These algorithms were particularly appropriate for our task, as they operated with a relatively small (in comparison to the Cartesian coordinates of atoms) set of variables with clear physical meaning. Therefore, the bases were assumed to be rigid and the sugar rings were described by one-parametric model (13). Thus, the only variables of the system were mutual dispositions of the neighboring bases, glycosidic rotations and conformations of the sugar rings, following nomenclature from (14).

The energy consisted of the Van-der-Waals' terms for nonbonded atoms together with the conventional torsional energies and semi-empirical potentials for the distortions of valent bonds and angles. The minimizations were performed by means of a modified method of the conjugate gradients (15) until the conformation of the sugar-phosphate backbone became acceptable.

The sugar-phosphate backbone structure was considered acceptable if two criteria were fulfilled: 1) if covalent bonds and angles¹ (with the exception for the angles C3'-O3'-P and C5'-O5'-P, which can deviate from the standard values, but no more than by 5 deg.) equaled standard values (16), 2) if there were no too close interatomic contacts. The latters were considered as too close, if the distance between them was shorter than the distance between the same atoms common for the the Brookhaven database of protein and nucleic acids structures with better than 2 Å resolution.

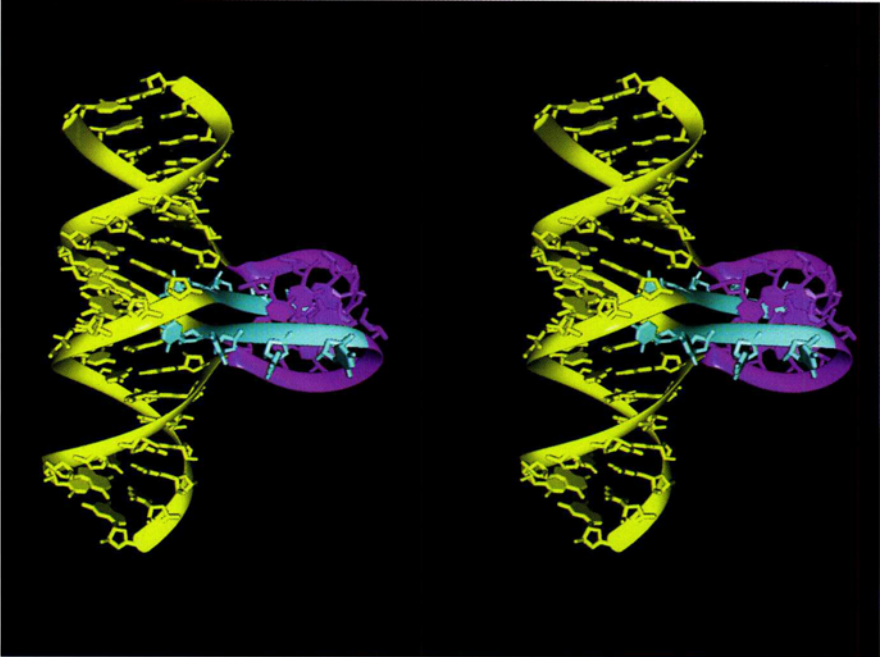
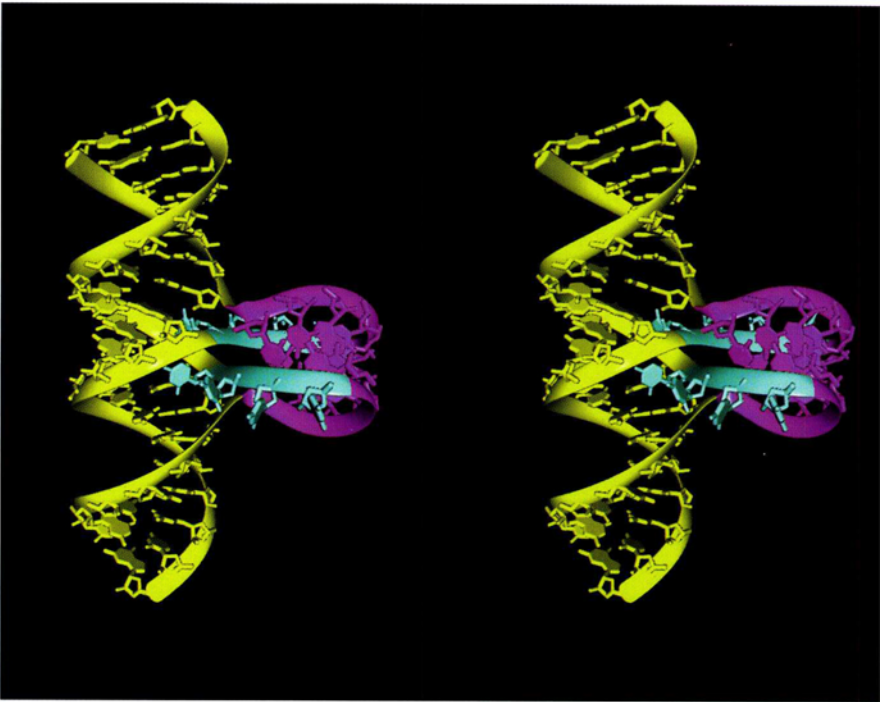
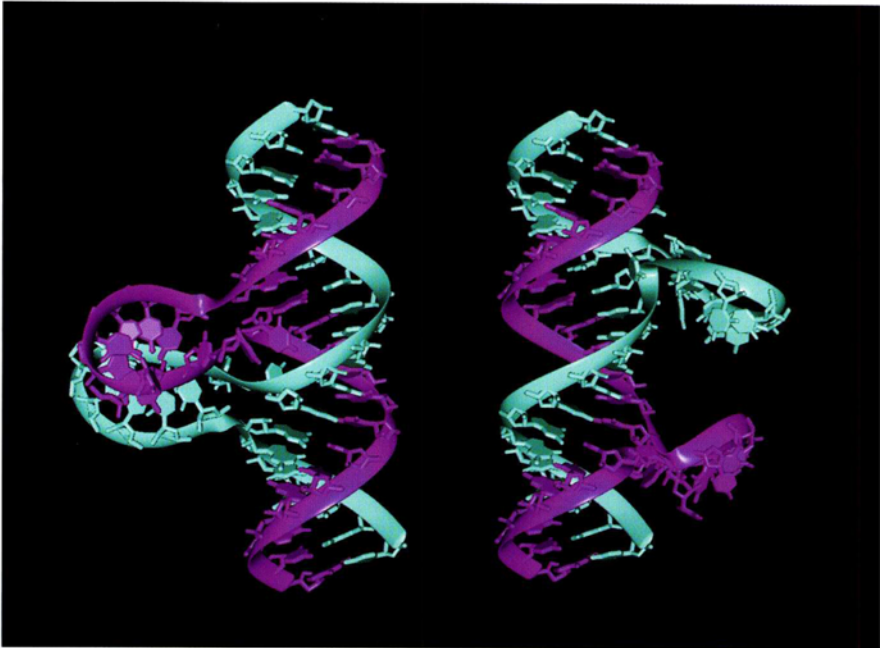
Results

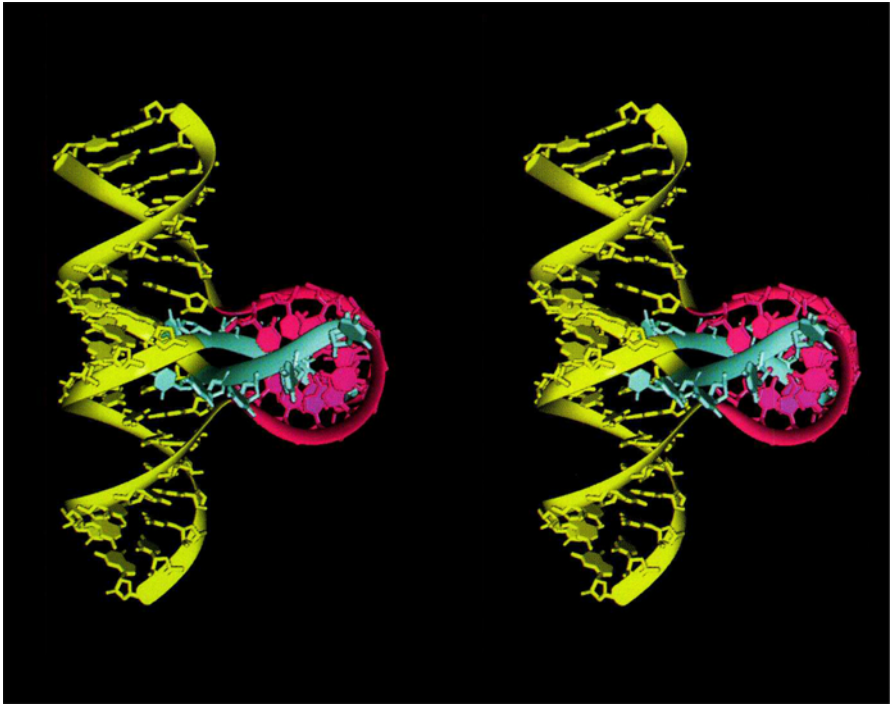
Mutual Orientation of the Sail and the Core. Conformation of the Corner

Initially, the *sail* and *core* were built using helical parameters for A-DNA from (17). Thereupon, the structure of the *corner* was optimized until it became acceptable. At

Figure 5: 3D representation of both isomers of A-form based-SLS. Positions of the loops are shown as if they were protruded from the 1st (left) and 2nd (right) isomers. A long distance between the loops and their mutual orientation in the 2nd isomer prevent them from Watson-Crick pairing.

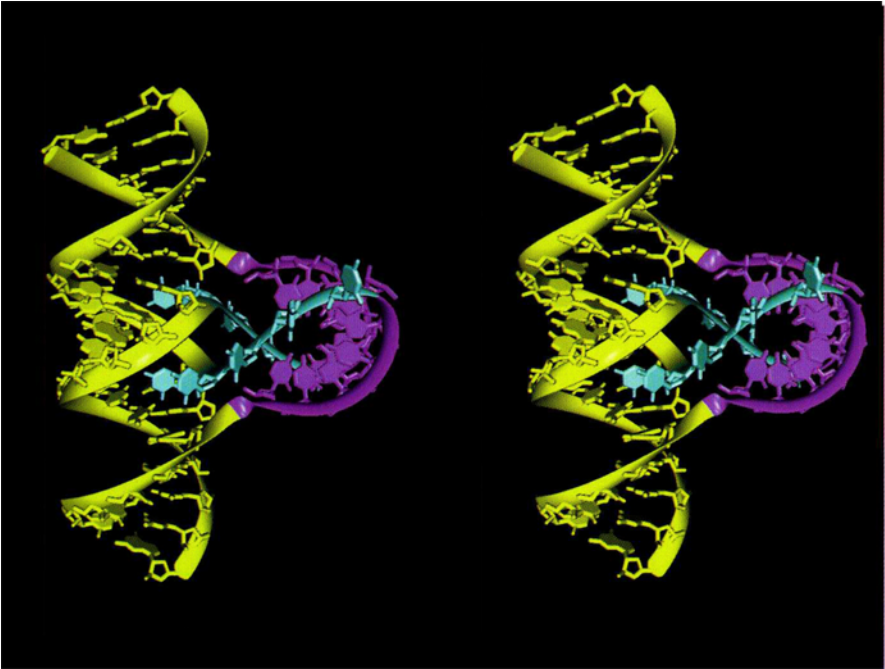
Figure 8: Stereopairs of the RNA-SLS : 6×5×4 - a), 6×6×4 - b), 6×7×5 - c) and 7×6×5 - d). Their main duplexes and *cores* (left vertical helices) are shown in yellow, *sails* (left mini-duplexes) - in magenta and *shrouds* (crossed strands) - in cyan. The structural similarity of these structures with different numbers of base pairs is evident from this picture.





8c

**Three-Dimensional Model for
Slipped Loop RNA**



8d

Three-Dimensional Model for Slipped Loop RNA

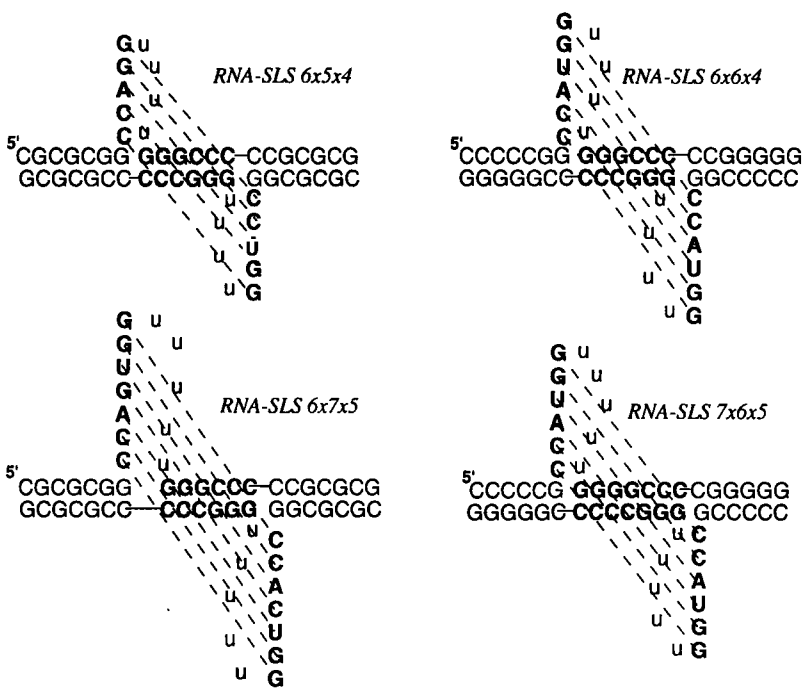


Figure 6: The secondary structures of the computed RNA-SLSes (*core* × *sail* × *shroud*): 6 × 5 × 4, 6 × 6 × 4, 6 × 7 × 5 & 7 × 6 × 5. Only 1st isomers are represented.

this stage the structure of the *shrouds* was not taken into the consideration (as if they were absent). During this process, we varied: 1) the shift of the *sail* versus *core* along common dyad axis and the rotation angle around it, 2) the conformations of sugars and 3) the positions of bases connected by the *corner*. At the beginning these sets of variables were used for minimization separately and sequentially.

Following this, the best structures were optimized using all the variables that were considered to be crucial for their acceptability. Eventually the axes of the helices occurred to be roughly perpendicular. Further optimization of the *shrouds* affected this orientation minimally.

Structure of Shrouds

The calculation of the stereochemically acceptable structure of the *shrouds* proved to be the most difficult stage of the SLS modelling. Optimization of the *shrouds* was divided into the following steps:

- 1. Initial approximations of their structure were built placing their bases far enough from the *core* and the *sail*. The concern was to avoid their collision with the dou-

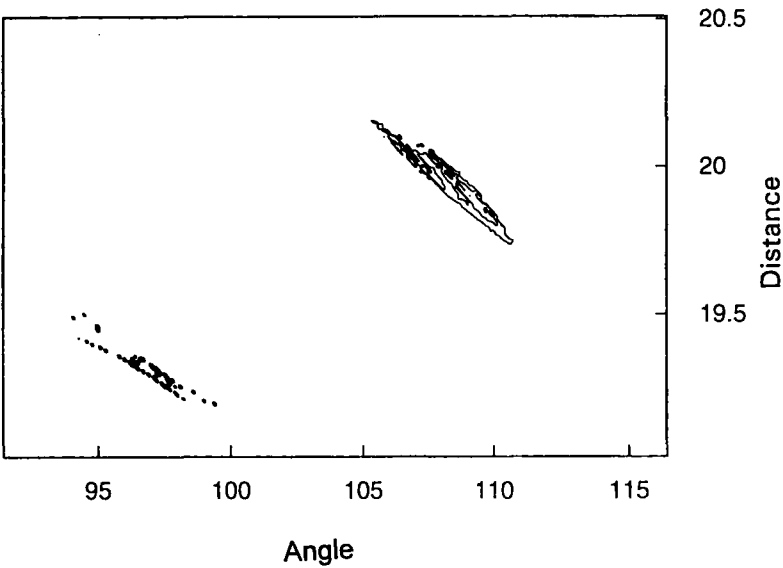


Figure 7: Sterically permitted values of the rotation angle and distance between the axes of *core* and *sail* for RNA-SLS-6x6x4. Their overall area is not more than 1 - 2 deg × Ang.

ble helices and with each other. Hence no attention was paid to the distortions of the backbone connecting them. Consequently, the main contribution to the energy resulted from the penalty for the violations of the backbone.

2. Further minimization optimized mostly the backbone structure by varying the positions of the bases and the conformations of the corresponding sugars. However, the resulting structure possessed the unacceptable (too close) interatomic contacts. Most of these contacts were at the 5'-ends of the *shrouds* where the backbone bent sharply at the *sail* joint, and at the 3'-ends, where the physical space for the last base of the *shroud* was rather tight.

3. To further optimize these fragments their flexibility was increased by including to the set of variables the helical parameters of the basepairs at the ends of the *sail* and at the beginnings of the *core*.

4. Even the best result of the 3rd stage still possessed a few short interatomic distances. The variation of carefully selected parameters can eliminate some of them. Thereupon we either obtained an acceptable structure or returned to one of the above stages to move in another direction of the multidimensional energy space.

All the above stages were performed repeatedly, each time changing the initial values, the set and the order of variables. These changes depended on the structure of the above conformation that we couldn't further improved. The reiterations were unavoidable since the determined methods of optimizations that were applied for this task promptly reach an arbitrary local minimum and do not leave it afterwards.

This procedure was successfully applied to the SLS built on the basis of the A-DNA. The model of the A-DNA-SLS served as the starting point for the optimization of the RNA-SLS structure. The modification involved substitutions of H2' of deoxyriboses with OH2'-group of riboses and methyl groups of thymines with the hydrogens of uracils. This gave rise to new unacceptable interatomic contacts. Further optimizations followed the procedure described above varying several selected parameters, e.g. conformation or glycosidic rotation of the sugars or positions of the bases involved in these clashes. The closest contacts in this structure upon optimization were similar to those found in the DNA-SLS.

The obstacles, met on applying the above trial-and-error method, testified to the high rigidity of the SLS conformation. This stimulated the study of the diversity of the SLS type conformations.

How Flexible is the Mutual Disposition of the Core and Sail?

The first question that was investigated was whether the *core* and the *sail* at given lengths were fixed rigidly relatively to each other. The effects of the *shrouds* on the positions of the helices were neglected. It is safe to assume that the long *shrouds* can connect the *core* and the *sail* of any lengths. Therefore, we studied how the structure of the *corner* depends on the angle and the distance between the axes of the helices. To take into account the flexibility of the *corner*, its structure was optimized for every point of the explored energy surface. Only the variables describing the structure of nucleotides connected by the *corner* were used for this purpose.

The narrow areas shown in Figure 7 contain the values of the above defined angle and the distance for which the sterically acceptable *corner* exists. This highlights the severely restricted internal mobility of the SLS.

How Variable Can be the Lengths of the Core and the Sail?

We investigated whether 6 b.p. is the unique length for double-helical fragments

that allows the SLS folding. If not, then how variable these lengths could be? For this purpose the structures of three other RNA-SLSes (*Core* × *Sail* × *Shroud*): 6×5×4, 6×7×5 and 7×6×5 were computed. We were not able to obtain a stereochemically sound SLS with the core and/or sail consisting of 8 base pairs.

The starting point was the already determined structure of RNA-SLS-6×6×4. Subsequently, one base pair was added to or subtracted from the *core* or the *sail*, and the optimization procedures were carried out as described above. The overall geometric characteristics for these new structures - distance and rotation angle between the axes of *core* and *sail* - are given in Table I.

Table I				
Distance and angle between axes of the helices of the SLS				
	6x5x4	6x6x4	6x7x5	7x6x5
Angle (deg.)	93.2	104.7	78.7	69.2
Distance (Å)	18.1	19.8	21.7	21.7

Figure 8 contains colour-coded stereoviews of all the computed structures. The length of the *shrouds* in the structures with 7 base pairs double helical fragments was increased by 1 to permit the computations of stereochemically sound models. The atomic coordinates of all the computed structures (in PDB format) are available from the authors upon request.

Discussion

It should be noticed that unlike the B-DNA-SLS (5), the formation of A-DNA(RNA)-SLS does not require the unpairing of the bases at the edges of the *sail* for the *shrouds* of 4 nucleotides. This fact testifies that the helices belonging to A-family are preferable for the SLS.

Another important point concerns the conformation of these short *shrouds*. They are almost completely stretched, since all the dihedral angles of the chain are presented in the anti-conformations (dihedral angle in *shrouds* being averaged over all the computed structures equals 191°). Hence, it is clear that for the symmetric structure the four nucleotides' long *shrouds* are the shortest possible.

The conformational peculiarity of the *corner* is the C3'-endo (pseudorotation of the sugar ring (13) = 25°) -> C2'-endo (150°) shift with the sugar at the 3'-end of the main helix (forming the *corner* together with the 5'-end sugar of the *sail*). Thereby, the structure of the *corner* resembles the conformation at the junction of A- and B-form helices.

The most remarkable structural feature of the SLS is the severely fixed mutual orientation of the axes of the *sail* and the *core*. For example, the sterically permitted variations of the angle between them is less than 10 deg. for the RNA-SLS-6×6×4 (Figure 7). In most cases the orientation is close to perpendicular (Table II, the SLSes: 6×6×4, 6×5×4, 6×7×5, and only SLS-7×6×5 deviates noticeably).

This rigidity of the SLS as well as its feasibility for very special patterns of RNA sequence may facilitate recognition of this site by regulatory proteins.

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