Exploring the Mutual Interactions between RNA Enantiomers

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Our research aims **at identification of (possibly non-canonical) modes of interactions between natural ribonucleic acids (D-RNAs) and their mirror-reflected counterparts (L-RNAs)**. We will perform experimental assessment and an *in silico* prediction of possible secondary structural motifs in L-RNA:D-RNA complexes.

We propose to approach this problem both by experimental and theoretical methods. The **calorimetric, spectroscopic and electrophoretic experiments** will allow us to assert the mutual binding. By **computational modeling** we will trace the geometric patterns in complexes of mixed chirality.

At the beginning, we will conduct spectroscopic, calorimetric and electrophoretic experiments. By isothermal titration calorimetry we will measure changes in thermodynamic parameters upon mutual binding of L- and D-oligonucleotides. We will also determine the changes in circular dichroism (CD) spectra upon binding and measure the melting temperatures (T_m) of the products. Our preliminary experiments clearly indicate that the heterochiral $(rA)_{12}$ and $(rU)_{12}$ strands are capable of forming some complexes (although not as much stable as homochiral ones). We measured T_m values for L-$(rA)_{12}$·D-$(rU)_{12}$ and D-$(rA)_{12}$·L-$(rU)_{12}$, obtaining 17.3°C and 18.8°C, respectively. We present CD preliminary data for titration of D-$(rU)_{12}$ into L-$(rA)_{12}$ at 25°C and D-$(rA)_{12}$ into L-$(rU)_{12}$ at 8°C. The addition of D-$(rA)_{12}$ into L-$(rU)_{12}$ at 8°C substantially alters the CD spectrum, which indicates that there was CD-detectable change in the RNA helical structure. Moreover, this phenomenon was not present for heterochiral mixture at room temperature, which remains in agreement with T_m measurements. Finally we will use electrophoretic mobility shift assays to check for complex formation. Obtained data will be interpreted together with simulation results.

To begin the *in silico* investigation of L-RNA:D-RNA interactions, we will predict the most preferred geometries for pairs of enantiomeric nucleotides. For this purpose we will use a variant of docking, where random mutual orientations of single L- and D-nucleotides are ranked according to the Amber energy function. As we proved in preliminary studies, the Amber potential energy function can be applied to both enantiomers of nucleic acids. We performed preliminary docking studies on all pairs of free nucleoside phosphates D-r(A,C,G,U)×L-r(A,C,G,U), obtaining few possible building blocks for heterochiral RNA duplexes.

To complement the aforementioned bottom-up approach, we will run sets of all-atom molecular dynamics (MD) simulations of pairs of short oligonucleotides of mixed chirality. As a control a few MD simulations of duplexes with similar chirality will be also performed. We will vary the lengths and compositions of strands to observe their influence on the mode of binding. We will further analyse the MD trajectories to find the preferred geometric patterns. We have begun with proof–of–concept MD simulations that aimed at proving the applicability of Amber force field to Spiegelmers. Chosen short fragment of L:L RNA (PDB code 1R3O) has been simulated for the period of ca. 100 ns at 300 K, in physiological conditions. We checked the stability of the simulated structure. The average Root Mean Square Deviation (RMSD) of the heavy atoms over 100 ns of production was 1.28 ± 0.25 Å from the initial crystal structure.

The knowledge acquired up to this point should be sufficient to propose a number of pairs of mixed-chirality sequences (each a few nucleotides long) that should display high affinity towards each other. The affinity will be subjected to both experimental and MD verification.

We believe that the ability to design such specific aptamers can substantially contribute to the field of drug development. The objective of this project is, however, not to directly design drugs, but to elucidate the microscopic principles that govern the interplay between L- and D-RNA.

To date, interactions between L- and D-RNAs have been largely overlooked. The mechanism of action of few known L-RNA aptamers remains entirely unexplored [1]. Neither computational studies nor rigorous experimental measurements in this area have been reported. Proposed project is an unprecedented attempt to fill all these gaps. With the microscopic picture of L-RNA:D-RNA interfaces, we might be able to formulate the rules for *design* of L-RNAs with desired affinity toward various D-RNA motifs. In future, such left-handed aptamers might facilitate invention of novel drugs.

References

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