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Biology

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Collaborative Research: Sequence Selective Recognition of Double-Stranded Non-Coding RNA via Triplex Forming PNA

Proposed research: Determination of structure and dynamics of PNA-RNA complex. Objective 3 will start with unambiguous identification of the nucleoside spin systems of target hairpins. We will start with HRP3, and later, depending on results in Objectives 2 and 3, will add other hairpin targets, as necessary. To assign the nucleoside resonances, we will synthesize and study by NMR hairpins having stems with re-oriented base pairs. The RNA hairpin that binds the PNA is designed with the purine of the pairs (including the G iminos) on the 5' side of the stem; whereas the pyrimidines (U iminos) are on the 3' side of the stem. We will construct the stem of each new hairpin minimally with one inverted 5'-C-G-3'and one 5'-U-A-3' oriented pair. Less than six constructs would suffice to identify the imino and spin systems. Later we will add HRP11-16 (Figures 8 and 9) to confirm the assignment and expand the structural database of dsRNA targets. Since NMR is non-destructive the same constructs could be used in binding experiments that analyze the importance of the altered base pair pattern in Objectives 1 and 2. Once NMR resonances and spin systems are assigned, the RNA hairpin will be titrated with the PNA. In the first experiments that will be HRP3 and PNA31 (Figure 16); other combinations will be studied depending on results of Objective 1 and 2. Changes in chemical shifts and line broadening will report on binding, and will be compared with those for control sequences that do not form a complex. 3D structures and molecular interactions of the PNA-RNA complexes will be achieved through Molecular Dynamics and Energy Minimization with NMR and base pair constraints. Next, we will complete similar experiments for the DNA versions of the target hairpins. Comparison of PNA-RNA and PNA-DNA structures will provide important insights into the unique RNA selectivity of our PNAs and suggest routes for design of novel chemical modifications and rational optimization of these novel RNA ligands.

Experiments and methods: NMR and Molecular Dynamics and Energy Minimization experiments will be done by Dziyana Hnedzko (graduate student, Rozners' lab) who will continue visiting Prof. Agris at the University of Albany to learn all experiments and take advantage of the 700 MHz cryoprobe equipped instrument.

Meanwhile, we will use the 600 MHz instrument recently acquired at Binghamton with support of NSF (CHE 0922815) to perform experiments that do not require the high sensitivity of a 700 MHz. For example, structure and dynamics of PNA-DNA triplexes can be studied at Binghamton as these complexes can be prepared at much higher concentrations due to low cost of DNA synthesis.