Tolerance of Sugar Backbone Heterogeneity by Protein-Binding RNA Aptamers. M. Benslimane[1] , K. K. Alam[2], and D. H. Burke[1 2 3]. [1]University of Missouri – Genetics Area Program (mb3c8@mail.missouri.edu), [2]University of Missouri – Biochemistry Department (khalidkalam@mail.missouri.edu), [3] University of Missouri – Molecular Microbiology and Immunology Department (burkedh@missouri.edu).

Abstract:
Sugars other than ribose may have played a role in a prebiotic world, allowing for the coexistence of alternative nucleic acid polymers. In theory, such biopolymers would form a catalytic landscape prior to the evolution of life on Earth. Previous work suggests that a wide variety of potential sugar backbones would have been possible in a prebiotic environment **[1]**; furthermore, the sugars would have been present in mixtures **[2]**, supporting the notion of one or multiple heterogeneous polymers capable of catalyzing distinct and overlapping reactions. However, the implications of ribose derivatives on the evolution of nucleic acid function are not well characterized. Here, we apply evolutionary pressure on a pre-enriched RNA aptamer population to identify the subset that are capable of tolerating a change in sugar backbone composition and to explore the effect of the sugar derivatives on specificity, function, and stability.

RNA aptamers selected to bind to a viral target of interest fold into structures capable of binding with high affinity and specificity, in particular, aptamers that bind HIV-1 reverse transcriptase (RT) inhibit the enzyme's function **[3]**. The first stage of the process used *in vitro* evolution to select for binding of unmodified RNA aptamers to the viral target. In the second stage, the enriched RNA aptamer library was independently re-transcribed with different pyrimidine monomers to generate 2'-Fluoro, 2'-O-Methyl, and 2'-Amino RNA derivatives. A reselection for binding to the same target was performed with the modified aptamer populations to select for those that retained binding affinity. High-Throughput Sequencing (HTS) and FASTAptamer, a Perl-based toolkit for primary sequence analysis of combinatorial libraries **[4]**, were subsequently used to identify enriched and depleted sequences in the latter populations. Functional assays established that a fraction of the enriched aptamer populations with alternative sugar backbones are still capable of binding and inhibiting the viral enzyme of interest. Furthermore, the selected 2'-modified aptamers constitute a set of polymers with likely different molecular stability and folding resulting from changes in helical conformation **[5]** and sugar pucker**,** this in turn allows for variation as well as overlap in their function. In summary, RNA populations display a robust ability to tolerate polymer heterogeneity, an essential feature of the evolution of life on a prebiotic chemical platform.

References:
[1] Eschenmoser, A. (1994). Orig. Life Evol. Biosph. *24*, 389–423. **[2]** Shapiro R. (1988) Orig. Life Evol. Biosph. *18*, 71–85. **[3]** Ditzler M. A. et al. (2011) *Nucleic Acids Res. 39 (18): 8237-8247.* **[4]** Alam, K. K. et al. (2015). *Molecular Therapy Nucleic Acids 4*, e230 **[5]** Pinheiro, V. B. and Holliger, P. (2012). *Curr. Opin. Chem. Biol. 16*, 245-252.