

**MOLECULAR CROWDING AND EVOLUTION OF LIGASE RIBOZYMES.** Milena Popovic<sup>1,2</sup> and Mark Ditzler<sup>1</sup>, <sup>1</sup>Exobiology Branch, NASA Research Center, Mail Stop 239-4, Moffett Field, CA 94035; <sup>2</sup>Blue Marble Space Institute of Science, Seattle, WA 98145, milena.popovic@nasa.gov, mark.a.ditzler@nasa.gov.

**Abstract:** The cellular environments in which RNA functions in contemporary biology are characterized by extensive macromolecular crowding which is a feature likely shared by protocellular life and by the environments of prebiotic synthesis from which life emerged. Molecular crowding encompasses a complex set of effects such as excluded volume effects through steric hindrance<sup>1</sup>, modulation of chemical interactions<sup>2</sup>, and alteration of structure and activity of water<sup>3-5</sup>. The excluded volume effects are thought to favor compact molecular states<sup>6</sup> and foster improved native state folding of biopolymers<sup>7,8</sup>. Moreover, crowding can have varying impacts on reaction rates<sup>1</sup>, by increasing them or decreasing them<sup>9,10</sup>, depending on the dominant catalytic mechanism. Despite the importance of crowding, this environmental parameter has not been explored through *in vitro* evolution.

We investigated the effects of molecular crowding on evolution of ligase ribozymes. We evolved populations of ligase ribozymes in dilute and crowded buffered solutions. After 5 rounds of evolution, populations were randomly mutagenized. The desired level of mutagenesis was confirmed by a decrease in population activity. The populations were evolved for additional three rounds in buffer, 20% Dextran 6000 and 20% PEG 8000. These populations were sequenced through high throughput sequencing (HTS).

We find that populations evolved in uncrowded solutions have the highest levels of activity, which is inhibited by addition of PEG. PEG-evolved populations are indiscriminant with respect to crowding. Comparison of sequence abundance between populations evolved in buffer and PEG suggests that crowding has a moderate effect on evolution of RNA ligases. Among the most abundant sequences, all have a distinct preference for a particular environment, although none show a difference in abundance larger than two orders of magnitude.

Several proposed secondary structures have been determined, including very short motifs (<20 nt). Buffer and Dextran populations are represented by similar sequences and secondary structures, whereas the PEG-population is dominated by a single ribozyme. We assayed individual ribozyme sequences for activity. The highest levels of activity are observed in buffered solutions, followed by Dextran, with PEG-assays showing the lowest levels of activity. Only one ribo-

zyme (a representative of a short motif) shows increased activity in the presence of PEG. The effects of different crowding agents on evolution of ligase ribozymes will be discussed.

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