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Impact of Molecular Crowding on in vitro Ribozyme Evolution

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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, modulation of chemical interactions, and alteration of structure and activity of water. The excluded volume effects are thought to favor compact molecular states and foster improved native state folding of biopolymers. Moreover, crowding can have varying impacts on reaction rates, by increasing them or decreasing them 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 impact of molecular crowding on the 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 mutagenized populations were evolved for an additional three rounds in dilute buffer, 20% Dextran 6000, or 20% PEG 8000. These populations were sequenced through high throughput sequencing. We find that populations evolved in dilute solutions have the highest levels of activity, which is inhibited by PEG. Populations evolved in PEG are indiscriminant with respect to crowding. Among the most abundant sequences, all have a preference for a particular environment. Populations evolved in dilute solution and in the presence of dextran are largely composed of a shared set of sequences and secondary structures, whereas populations evolved in PEG are dominated by a single ribozyme.