Selection of *de novo* enzymes from a completely randomized sequence library

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How much information is required for a functional protein to form? In order to answer this question we have used an *in vitro* selection technique to isolate enzymes from a large mixture of completely random polypeptides. We are searching for enzymes capable of ligating a 5'-triphosphorylated RNA to the 3'-OH of a second RNA. The utilized library contains a completely randomized core region flanked by sequence elements for *in vitro* transcription and translation as well as tags for protein purification. Our mRNA display technology allows for selection with highly diverse libraries; in this case more than $2x10^{12}$ unique sequences. This high diversity increases the probability of successfully identifying active ligases and allows the potential analysis and comparison of convergently evolved enzymes and their variants.

Promising preliminary results indicate that for the first time an active enzyme has been selected without any scaffold from a completely randomized sequence library. The results of this project will yield insights into the extent of sequence space required for the evolution of new proteins.