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Improving mRNA-display for *in vitro* RNA-protein co-evolution

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Introduction: Biopolymers such as RNA, proteins and their complexes known as ribonucleoproteins (RNPs) play crucial roles in modern biology, sustaining the evolvability and chemical reactions for life. While the origin and evolution of these biopolymers remain largely elusive, recent advances in synthetic biology have allowed for the synthesis, screening, and evolution of RNA and protein polymers in an *in vitro* setting. Here, mRNA-display represents a powerful and high-throughput approach to probe large sequence spaces for screening functional peptides by the attachment of translated nascent peptide chains to the 3′-termini of their mRNA by employing puromycin (aminoacyl tRNA analog) as the linker. Combined with downstream high-throughput sequencing and recurring amplification/selection, mRNA-display allows for the mapping of trajectories of polymer evolution under specific selective pressures.

Currently, I am optimizing the mRNA-display method towards efficient sampling of primitive functional RNPs from random sets of sequences. One promising strategy for expanding the available sequence spaces is to improve the efficiency of puromycin's ability to enter the A site of the ribosome. Enhancing the efficiency of this key process will allow a greater number of mRNA-peptide conjugates to be synthesized expanding the potential to evolve complex catalytic functions. Considerably many factors can affect puromycin chemistry, i.e., linker design, chemical modification, temperature and pH. I will perform iterative analyses to optimize the covalent linkage of puromycin to the translated nascent peptide, by increasing affinity to the ribosomal A site, as well as covalent reactivity, by mRNA-display protocol optimization and chemical modification of puromycin itself. Expansion of the available sequence space in mRNA- display will not only contribute to understanding the origin of RNA-protein co-evolution, but could also have benefits for biotechnology and medicine. I look forward to discuss the latest results during the meeting.