Towards Comprehensive Fitness Landscapes of Random Peptides Interacting with Protocells

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Early peptides likely consisted of a limited set of primordial amino acids that could have been formed early during abiotic chemical evolution. Many theories have been set forth for the emergence of early amino acids. However, the experimental data bridging these theories to biological functions of early peptides is largely missing. Compartmentalization of primitive biochemical reactions within protocells has been considered an essential step in the emergence of life on the early Earth. We seek to explore how the chemical nature of short peptides consisting of specific sets of primordial amino acids could influence a simple biological function such as cell penetration.

The goals of this project are to identify random peptides made from primordial amino acids that interact with model protocells and to investigate comprehensive fitness landscapes of membrane-interacting peptides. Exploring fitness landscapes in proteins is challenging due to astronomically large number of genotypes. However, if one utilizes reduced alphabets then synthesis of every variant is possible for short peptide sequences. To this end, we have utilized mRNA display technology and constructed several different libraries consisting of 15 randomized positions of 5- or 6-amino acid (aa) alphabets. The primordial amino acid alphabets for this study were selected based on a combination of theories that led to a plausible order of appearance of amino acids in the genetic code. We also carried out a detailed analysis of sequence diversity of known cell penetrating peptides that deliver nucleic acids as this approach could also be extended to discover novel cell penetrating peptides for biomedical applications. We have successfully piloted and optimized the library construction protocol to produce one of our 5 aa alphabet, GAPDV. Currently, we are optimizing the selection step to screen membrane-penetrating peptides against model membrane lipids. Our next steps are to perform selections, deep sequence selected pools and analyze peptide fitness landscapes. Upon identification of novel cell penetrating peptides after successful rounds of selection we will also test these candidates in fatty acid vesicles such as oleic acid/oleate that are more prebiotically relevant.

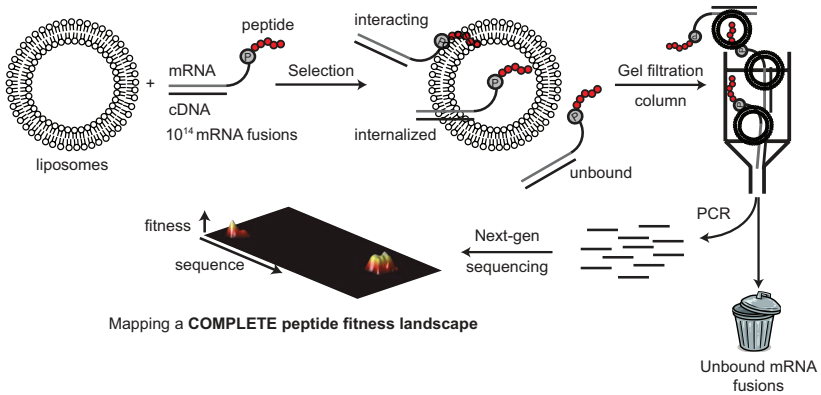

Figure 1. Identification of membrane penetrating peptides by *in vitro* selection