Emergence and evolution of *de novo* proteins enabling bacterial survival

Dajana Domik & Burckhard Seelig

Proteins are ubiquitous, diverse and responsible for catalyzing nearly every chemical reaction in living organisms. However, our understanding of the mechanisms that originally gave rise to the vast diversity of proteins, is very limited.

We aim to investigate how existing proteins can evolve to acquire new functions and a result in the emergence of new proteins. To address these questions we will use directed evolution methodology. We created two different synthetic combinatorial libraries of $>10^8$ polypeptides variants, which will be subjected to an *in vivo* selection procedure to search for enzymatic activities. One library consist of random peptides and the other is based on $(\beta/\alpha)_8$ barrel fold. To select for a range of different enzymatic functions, we will utilize conditionally auxotrophic *E. coli* strains. These strains harbor a single essential gene deletion, which makes them unable to grown on minimal media unless a protein variant from the library, complements the function of the deleted gene.

In preliminary studies, we tested 67 single gene deletion strains against the two libraries and have already identified three candidate proteins that enable bacterial growth, which are involved in amino acid and lipoate metabolism. Currently, studies are underway to illuminate their mechanism of rescue, in more detail.

The proposed work will yield valuable insights for both basic and applied enzyme research. Through laboratory evolution, we expect to support the theory of the possible origin of functional proteins from random polypeptides with *de novo* proteins. Furthermore, we anticipate to provide answers to the long standing question of how the ubiquitous $(\beta/\alpha)_8$ barrel fold can adopt this multitude of functions in nature. Findings will lay the groundwork for the creation of “greener” biocatalytic platforms as artificial enzymes in organic synthesis can reduce unwanted byproducts and the need for toxic solvents.