

In silico selection and directed evolution of bioactive *de novo* emerged proteins

Background: Over the past decade, evidence has accumulated that new protein coding genes can emerge *de novo* from previously non-coding DNA. Most studies have focused on large scale computational predictions of *de novo* protein coding genes across a wide range of organisms. In contrast, experimental data concerning the folding and function of *de novo* proteins is scarce. This might be due to difficulties in handling *de novo* proteins *in vitro* and detecting functions *in vivo*. Further experimental exploration of the activities of *de novo* proteins will both, shed new light on molecular evolution, and enable the development of new techniques in protein engineering for biotechnological applications.

Objectives: In this project we aim to express, purify and analyse different putative *de novo* proteins that were computationally predicted to have enzymatic activities. Genes of interest will be cloned into respective vectors and proteins expressed in *E. coli*. Prior, proteins will be designed to allow stable and soluble expression. Proteins will be purified using Affinity chromatography and first analysis will be conducted using SDS PAGE and Western Blots. Further experimental characterization will be performed to test folding and stability e. g. with circular dichroism spectroscopy. Appropriate enzymatic test will be performed followed by directed evolution experiments. Laboratory work will be computational supported using AlphaFold (structure prediction), Gromacs (MD simulation), Pross2 (stability optimization) and if possible ancestral sequence reconstruction. Internship with international collaborators possible and encouraged.

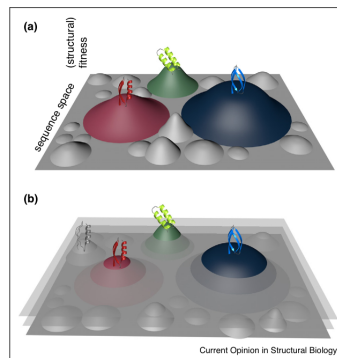


Figure 1: Emergence of novel proteins from within the sequence space (Bornberg-Bauer et al. 2021)

Requirements:

- Experience in bash scripting, good command of unix command line
- Interest in evolution at the level of individual proteins and in protein structure & folding, biotechnology, protein design
- Interest in lab work on DNA and protein level and basic knowledge of PCR, DNA-cloning, protein expression & purification

Methods:

- Structural bioinformatics, protein design
- Biochemical characterization of proteins via SDS-PAGE, CD, enzymatic assays, directed evolution

Supervision:

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Selected Literature:

1 Lars A. Eicholt, Margaux Aubel, Katrin Berk, Erich Bornberg-Bauer, Andreas Lange

Heterologous expression of naturally evolved putative *de novo* proteins with chaperones

Protein Science, 2022

2 Erich Bornberg-Bauer, Klara Hlouchova, and Andreas Lange

Structure and function of naturally evolved *de novo* proteins

COSB, 2021

3 Andreas Lange, Prajal H Patel, Brennen Heames, Adam M Damry, Thorsten Saenger, Colin J Jackson, Geoffrey D Findlay, Erich Bornberg-Bauer

Structural and functional characterization of a putative *de novo* gene in *Drosophila*

Nat. Comms, 2021