

Purification and analysis (*in vitro* & *in silico*) of putative *de novo* 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*, as most are predicted to be short and disordered. Further experimental exploration 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. Genes of interest will be cloned into respective vectors and proteins expressed in *E. coli* in combination with chaperones to enable 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 function, such as tat assay (folding), thermal shift assay (folding), phage display (function) and Circular dichroism (structure). Laboratory work will be computationally supported using AlphaFold2, Gromacs (molecular dynamics simulations), fIDPnn and if successful ancestral sequence reconstruction. International collaborations are possible.

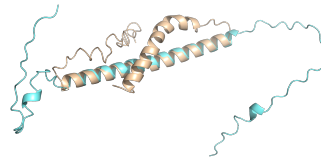


Figure 1: AlphaFold2 prediction of *de novo* protein Goddard (cyan) aligned with its partial structural determination (Lange et al. 2021)

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
- Interest in lab work on DNA and protein level and basic knowledge of PCR, DNA-cloning, protein expression & purification

Methods:

- Structural bioinformatics
- Biochemical characterization of proteins via SDS-PAGE, Western Blot, TSA, tat, CD

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