

Expression, purification and analysis 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 from *Homo sapiens* and *Drosophila melanogaster*. 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 structure determination (structure). Laboratory work will be computationally supported using AlphaFold2, Gromacs (molecular dynamics simulations) and other structure and disorder predictors (IUPred2a, Porter 5.0). International collaborations are possible.

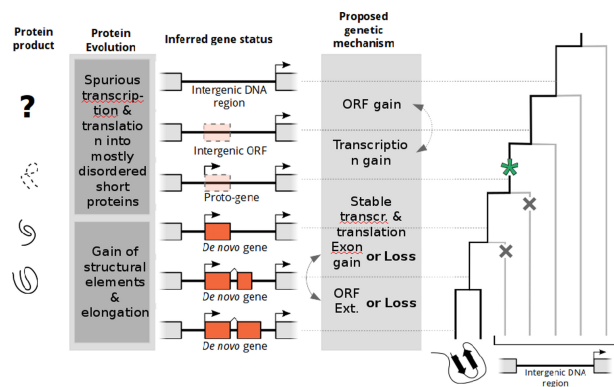


Figure 1: Mechanisms of *de novo* gene emergence from non-coding DNA

Requirements:

- Interest in evolution at the level of individual proteins
- Interest in lab work on DNA and protein level and basic knowledge of PCR, DNA-cloning, protein expression & purification

Methods:

- Molecular biochemistry & expression and purification of proteins
- Biochemical characterization of the proteins via SDS-PAGE & Western Blot

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Selected Literature:

- 1 Lars A. Eicholt, Margaux Aubel, Katrin Berk, Erich Bornberg-Bauer, Andreas Lange
Chaperones facilitate heterologous expression of naturally evolved putative de novo proteins, *bioRxiv*, 2022
- 2 Keeling, DM., Garza, P., Nartey, CM., Carvunis, AR.
Philosophy of Biology: The meanings of 'function' in biology and the problematic case of de novo gene emergence
elife, 2019
- 3 Erich Bornberg-Bauer, Klara Hlouchova, and Andreas Lange
Structure and function of naturally evolved de novo proteins
COSB, 2021
- 4 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