

# Role of phase separation in the activity of *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. While *de novo* proteins have been investigated extensively with computational approaches there is a lack of experimental knowledge about their role and function in their native organism. It was shown that several *de novo* proteins of *D. melanogaster* are involved in spermatogenesis and DNA-binding. Mechanistic explanations of these functions are still eluding. One possible mechanism could be liquid-liquid phase separation in which one or more polymer form a coacervate.

**Objectives:** Candidate *de novo* proteins that are predicted to undergo liquid-liquid phase separation (PSpredictor, fuzdrop) will be ordered, amplified and cloned into expression vectors. Optimal expression conditions will be identified using SDS-PAGE and Western Blot. Proteins will be expressed and purified using BL21 cells and His-tag affinity purification. Small amounts of protein will be incubated under different conditions (pH, salt concentration, temperature etc.) and analysed using light microscopy for droplet formation. Following positive results further sophisticated tests will be conducted. International collaborations are possible.



**Figure 1:** AlphaFold2 predicted structure of *de novo* protein Goddard. Blue terminus is predicted to undergo liquid-liquid phase separation

## 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

## 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