Centre for Synthetic Biology

Title
Engineering Artificial Sensory, Signalling and Transport Functions

Overview
The laboratory of Viktor Stein takes a protein-centric approach to synthetic biology as we devise systematic approaches to engineer artificial sensory, signalling and transport functions focussing on the construction of protein switches, optical sensors, protein nanopores and membrane transporters. Strategically, we address fundamental questions exploring the design principles of artificially engineered proteins and develop them towards distinct biotechnological applications. Our work also entails a strong focus on the development and application of enabling technologies. This includes new DNA assembly methods (e.g. iFLinkC[2]) to assemble protein switches and sensors via combinatorial linker libraries and genetic screening systems (e.g. FuN Screen[3]) to study and engineer transport processes across microbial membranes. These high-throughput approaches are complemented by high-resolution analytical methods (e.g. electrophysiological measurements in lipid bilayers[3] and live cell fluorescence microscopy in microfluidics[3]) to better understand the molecular features that underlie the function of artificially engineered proteins.

Research projects are now available for highly motivated Bachelor and Master students with strong interests in protein engineering and synthetic biology. Topics of interests include, but are not limited to:

- Engineering optical protein sensors (e.g. with fluorescent and/or bioluminescent read-outs) against biotechnologically and biomedically relevant analytes.
- Engineering synthetic protease switches and thereof composed signalling circuits to sense and actuate cellular functions following different molecular and physical inputs (e.g. light).
- Re-engineering natural and artificially engineered protein nanopores equipping them with tailored properties and functions while dissecting the underlying design principles.

Experimental Methods
State-of-the-art cloning methods including library assembly; Fluorescence high-throughput genetic screening in microtitre plates and colony on-plate; High-resolution analysis by fluorescence microscopy combined with microfluidics; Recombinant protein expression, purification and characterisation;

Key References

[1] Gräwe A and Stein V. 'Linker Engineering in the Context of Synthetic Protein Switches and Sensors'. *Trends in Biotechnology*, 2021, 39, 731-744.

[2] Gräwe A, Ranglack J, Weyrich A and Stein V 'iFLinkC: An iterative Functional Linker Cloning Strategy for the Combinatorial Assembly and Recombination of Linker Peptides with Functional Domains'. *Nucleic Acids Research*, 2020, 48, e24.

[3] Weber W, Roeder M, Abujubara H, Koeppl H, Tietze A and Stein V 'The Functional Nanopore (FuN) Screen: A Versatile Genetic Assay to Study and Engineer Protein Nanopores in *Escherichia coli*', 2021, bioRxiv, DOI: 10.1101/2021.04.20.440580.