Synthetic biology approaches for protein production optimization in bacterial cell factories - DTU Orbit (28/01/2019)

**Synthetic biology approaches for protein production optimization in bacterial cell factories**

Society’s strong dependence on fossil fuels and petroleum-based products leads not only to a rapid decline of natural oil reserves but contributes massively to global warming and environmental damage. This consequently urges society to look into more sustainable alternatives. Microorganisms present such sustainable alternative if converted into so-called microbial cell factories. Instead of crude oil, cell factories use renewable resources or waste products as source material. The challenge is, however, that microbial production needs to be economically feasible to compete with the classical chemical production. The development of a microbial cell factory typically takes up to 8 years of research and costs over $50 million. The production and selection of heterologous pathway proteins are major bottlenecks encountered in the construction of a cell factory. Thus, new approaches for the optimization of recombinant protein production and screening techniques with high capacity for the identification of the best performing enzymes are continually developed.

This thesis aims to equip researchers with a fundamental knowledge about protein biosynthesis necessary for the understanding of protein production bottlenecks. Moreover, the thesis guides through the possible causes of low protein yields and presents available approaches for optimization of the protein and the host. The main work presented in this thesis provides and applies a new synthetic biology approach for the optimization and selection of recombinant proteins. A major bottleneck during production is translation initiation. By creating sequence libraries of the translation initiation region, protein production can be improved substantially in Gram-negative and Gram-positive bacteria. The design of versatile and tuneable translational coupling devices and their fusion to antibiotic selection markers enables subsequent selection of high-expressing constructs. The approach is a simple and inexpensive alternative to advanced screening techniques. In addition, a second synthetic biology approach provides the means for fast and efficient plasmid backbone swapping and is a versatile tool for the design and construction of optimal protein production constructs.

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