Development of an intracellular glycolytic flux sensor for high throughput applications in E.coli - DTU Orbit (27/04/2019)

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The aim of this PhD project was to construct, test and apply an intracellular, growth-independent and direct measureable glycolytic flux biosensor in E. coli.

Studying the metabolic flux of bacterial cells is of growing interest as it is of fundamental importance to bacterial physiology as well as for in silico modeling and metabolic engineering. The metabolic flux contains information about how efficiently a bacterium can utilize a given carbon source and in which extend it is directed towards the different central metabolic pathways. The knowledge of these fluxes can contribute to the development of efficient production pathways and the identification of possible accumulation points in the engineered pathway. Furthermore it can give information about regulatory networks within the cell.

The developed biosensor is based on the transcription factor Cra and links the metabolic flux to the expression of green fluorescent protein (GFP). The dynamic range of the final biosensor construct covers the whole range of natural, intracellular glycolytic fluxes, induced by different carbon sources and it could also be shown that it is even capable of monitoring a further flux increase.

The sensor was applied to study the flux-altering effects of gene knockouts in E. coli at the single cell level in a vastly parallelized and high-throughput manner. After growth for several generations in rich and minimal media, 2126 gene knockouts, mainly outside of the core metabolism, could be screened. 3 gene knockouts with a high flux and 158 with a low flux phenotype were found, comprising many flagella and phage related genes as well as many so far uncharacterized proteins.

Taken together, the glycolytic flux biosensor offers a tool to screen for metabolic flux changes in an efficient, fast and parallelizable way, opening up for novel screening approaches that enhance our understanding of microbial physiology and can be applied to improve microbial cell factories.

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