Genome Wide Identification of Bacterial Membrane Capacity Determinants

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Project Goals: The production of fuels and bioproducts **in microbial hosts require significant development and optimization of not only the biosynthetic pathway but also the host chassis. However, the initial choice of microbial host has a considerable yet often overlooked impact the success of the project. The goals of this project are focused on evaluating the determinants governing membrane capacity, as membrane-bound proteins are key to many aspects of the host chassis optimization. We assembled a high-throughout method to rapidly screen a saturated transposon library in *Escherichia coli* covering all non-essential genes for single gene mutants that increase the overall cellular membrane capacity.**

The production of fuels and bioproducts in a microbial host requires significant development and optimization of any given heterologous gene pathway. However, the initial choice of microbial host for gene expression has a considerable yet often overlooked impact the success of the project, as the microbial host must withstand a range of environmental and physiological perturbations to yield high levels of the desired product. In this project we have specifically aimed to understand the determinants governing membrane capacity, as membrane-bound proteins play a central role in many functions relevant to optimal carbon utilization and bio-production. Examples include cellular transporters that regulate the import of the carbon source, sensors that respond to the composition of the cytoplasm and most importantly, the export of toxic intermediates or end-products from the intracellular-milieu.

While eminently useful, the overexpression of such membrane proteins often causes growth inhibition placing an inherent upper limit on expression. Membrane-protein overexpression can result in nonfunctional or mislocalized protein complexes, morphological deformities, trigger cellular stress response and a metabolic shunt to acetate. We hypothesized that it would be possible to increase the total cellular capacity for membrane protein expression by inactivating single genes that are dispensable for growth under laboratory conditions.

In this study, we assembled a high-throughout method to rapidly screen a saturated transposon library in *Escherichia coli* covering all non-essential genes for single gene mutants which increased the overall cellular membrane capacity. We assessed membrane protein expression

using the fluorescence of a membrane protein fused to GFP as a proxy. We report the discovery of single gene knockouts that significantly improve the expression of candidate membrane proteins in these mutant strains. These candidate mutant alleles were then validated using independently derived isogenic deletion mutants. We provide evidence that the overexpression of these membrane-proteins, achieved using expression in gene deletion backgrounds, leads to the desired improvement in the related phenotypic fitness. Our observations provide a microbial-host chassis that may be immediately applicable to any membrane protein expression system without further optimization of transcription or translation.

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