Title: Design and Engineering of Synthetic Control Architectures

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Project Goals: Our objective is to develop a new standard for the engineering of microbial systems based on rational design, engineering, and optimization of hybrid regulatory networks. We envision a future biorefinery that is based on the development of designer organisms that have exquisite and predictable control architectures governing the expression of a range of valuable traits. Computer aided design platforms will guide the assembly of synthetic constructs containing orthogonal heterologous circuits to recode native regulatory networks. Together, these will enable predictable and dynamic control of multiple designer phenotypes such as: i) growth on various feedstocks in consolidated bioprocesses, ii) feedback control to mitigate accumulation of toxic metabolites, iii) production of target molecules (C3-C4 alcohols), and/or iv) robustness to process upsets (e.g. temp., phage). The focus of this proposal is to develop the technical and computational infrastructure to enable this vision. We will develop this platform first in the model organisms E. coli and S. cerevisiae and then in DOE relevant non-model organisms. (Limit to 1,000 characters)

Abstract Text: Please limit such that entire document does not exceed 2 pages.

Genome engineering for improved protein folding and metabolite production in yeast

Climate change requires sustainable solutions that permit renewable synthesis of chemicals and fuels toward decreased consumption of fossil fuels. Advances in metabolic engineering, synthetic biology and systems biology have permitted to increase the turnover of the design-build-test cycle of cell factories with high productivity and titers. Although rational design has gained great momentum, the engineering of complex phenotypes has not been possible until recently due to the lack of mechanistic knowledge of these phenotypes and the limitations of random mutagenesis and rational approaches for engineering such phenotypes.

In recent years our group developed CRISPR Enabled Trackable Genome Engineering (CREATE) and applied it in parallel engineering of regulatory networks in *E. coli* and *S. cerevisiae* regulatory networks to gain access targeted complex phenotypes. With such it was possible to build and screen libraries 100,000 and 80,000 variants in *E. coli* and *S. cerevisiae* respectively. Such libraries were screened for improved tolerances to toxic chemicals from renewable feedstocks and short chain alcohols, molecules of interesting properties for use as biofuels.

We are currently leveraging the CREATE approach in yeast to engineer proteins towards improved folding and functionality focusing on enzymes and transcription factor-based biosensors. We are focusing on enzymes involved in the synthesis of natural products and bulk

chemicals that have low activity because of improper folding. We aim to mitigate misfolding by tuning substrate specificity of molecular chaperones. To that end we have mapped substrate binding sites of two major yeast chaperones and designed libraries towards enhanced folding of target enzymes. We will screen such libraries with a misfolding biosensor based on yeast native response to stress. In parallel, we are working in the development of transcription factor-based biosensors for biofuels. As our target transcription factors are of bacterial origin, transplantation into yeast requires engineering of both the transcription factor protein and the transcription factor-responsive promoter. We will engineer both transcription factor and responsive promoter using the CREATE method with the aim of achieve improved dynamic range, operational range, specificity and sensitivity.

Additionally, we are engineering phosphorylation regulatory networks. Phosphosites are one of the main ways that signaling is regulated within the cell. It plays key roles in almost all cellular pathways including cell division and replication, cell/environment interactions and metabolic function. Naturally, a lot of effort has been done in order to map the phosphoproteomes in various organisms, however studies that experimentally validate this data are rare. Here, we attempt to utilize existing phosphoproteomic data in yeast in order to increase cis-cis-muconic acid and isopentanol production. To this end, we designed and implemented metabolic and transcription factor phosphosite mutation libraries that leverage the CREATE approach in order to validate high-throughput phosphosite editing as host engineering strategy for microbial production.

We expect that leveraging the power of designer genome engineering from CREATE together with biosensor-mediated high-throughput screenings will permit as to access newly target phenotypes as optimized protein folding, enhanced biosensors properties and controls of regulatory networks through phophosite engineering.

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