

## CRISPR-based trackable protein engineering

Eun Joong Oh<sup>1,2\*</sup> ([eun.oh@colorado.edu](mailto:eun.oh@colorado.edu)), Rongming Liu<sup>1,2</sup>, Liya Liang<sup>1,2</sup>, Emily F. Freed<sup>1,2</sup>, Carrie A. Eckert<sup>2,3</sup> and **Ryan T. Gill**<sup>1,2</sup>

<sup>1</sup>University of Colorado Boulder, Boulder; <sup>2</sup>Renewable and Sustainable Energy Institute, Boulder, Colorado; and <sup>3</sup>Bioscience Center, National Renewable Energy Laboratory, Golden, Colorado.

**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 organism *E. coli* and then in DOE relevant non-model organisms.**

Microbes have been harnessed to produce value-added products as cell factories. Many research groups are investigating strategies to use microbial cell factories for sustainable and economical protein production such as enzymes and antibodies. Because the microbes have extensive regulation between metabolic enzymes, protein engineering by error-prone PCR and rational design sometimes leads to disruptive cellular metabolism and regulatory mechanism. In addition, our knowledge is limited to uncover the underlying system controlling metabolic homeostasis<sup>1</sup>. Understanding native regulatory networks will require making libraries of regulatory proteins.

CRISPR Enabled Trackable genome Engineering (CREATE) is a strategy that combines CRISPR/CAS9 editing with multiplexed oligo synthesis, enabling mapping of mutations to traits of interest<sup>2</sup>. In our proof-of-concept study we built editing libraries in *E. coli* following our prior CREATE protocols and then used such editing libraries to build plasmid-based protein libraries in *S. cerevisiae*. Currently, we are attempting to apply CREATE to antibody engineering. CREATE-based single chain antibody mutants provided high-affinity antibodies with trackable high-throughput mapping of desired phenotypes. We could evaluate the contribution of each mutation to the improved affinity.

Our long-term goal is to develop the optimized system for protein engineering including enzymes. CREATE-based enzyme evolution might provide a better understanding of protein fitness landscapes than random mutagenesis in optimizing protein function. We envision that the

CREATE technology will enhance understanding of complex biological networks in protein engineering and further improve biofuel production in microbes.

## **References**

1. Nielsen, J. & Keasling, J. D. Engineering Cellular Metabolism. *Cell* 164, 1185–1197 (2016).
2. Garst, A. D. et al. Genome-wide mapping of mutations at single-nucleotide resolution for protein, metabolic and genome engineering. *Nat. Biotechnol.* 35, 48–55 (2017).

This research is supported by the Office of Biological and Environmental Research in the DOE Office of Science.