Development of Biosensors for High-throughput Enzyme Screens

Anthony Meger[1,3]* (ameger@wisc.edu), Kyle Nishikawa,[1] Megan Leander,[1] and **Srivatsan Raman**[1,2,3]

[1]Department of Biochemistry [2]Department of Bacteriology [3]Great Lakes Bioenergy Research Center, University of Wisconsin-Madison

Project Goal: Engineer sugar-responsive biosensors to enable high-throughput screens of cellulase mutants for improved enzymatic activity.

Allosteric TFs (aTF) are useful metabolite sensors which enable directed evolution of enzymes and biosynthetic pathways. Expanding aTFs to respond to new molecules would greatly increase their utility, facilitating the biosynthetic production of fine chemicals, intermediates, and fuels. However, inducer recognition and transcriptional response in aTFs are tightly coupled through allostery, making redesign toward new inducers challenging. Using computational protein design guided by evolutionary analysis, we engineer variants of the *E. coli* lac repressor, LacI, to respond to a variety of fermentable sugars while preserving allosteric function. These sugar biosensors are highly valuable for screening cellulase variants with broader substrate specificities and higher catalytic efficiencies. Superior cellulases have the potential to improve cellulose recovery from biomass and reducing the overall cost of commercial production of biofuels.

References

1. Walker, J. A., Takasuka, T. E., Deng, K., Bianchetti, C. M., Udell, H. S., Prom, B. M., Kim, H., Adams, P. D., Northen, T. R., … Fox, B. G. (2015). Multifunctional cellulase catalysis targeted by fusion to different carbohydrate-binding modules. Biotechnology for biofuels, 8, 220. doi:10.1186/s13068-015-0402-0
2. Taylor, N. D., Garruss, A. S., Moretti, R., Chan, S., Arbing, M. A., Cascio, D., Rogers, J. K., Isaacs, F. J., Kosuri, S., Baker, D., Fields, S., Church, G. M., … Raman, S. (2015). Engineering an allosteric transcription factor to respond to new ligands. Nature methods, 13(2), 177-83.

This material is based upon work supported by the Great Lakes Bioenergy Research Center, U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018409.