Center for Theoretical Biological Physics

RICE

"Customized molecular reporters from regulatory proteins with altered effector specificity"

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12:30 -- 1:30 PM
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Molecular biosensors based on effector-dependent transcriptional regulatory proteins are emerging and powerful biomolecular tools for specific, sensitive, and high-throughput detection/monitoring of intracellular molecules [1]. The naturally occurring proteins couple molecular recognition to changes in gene expression. To direct the evolution of molecular recognition toward compounds of interest, we construct large libraries of regulatory protein variants and screen for ligand responsiveness using a GFP reporter system [2]. These customized molecular biosensors enable high-throughput screening of combinatorial biosynthesis libraries to improve enzymatic or microbial production of metabolites [3, 4]. Our group has shown success engineering AraC, a well-studied native *E. coli* dual-regulatory protein. By generating multiple AraC saturation mutagenesis libraries and using positive and negative FACS-based screening, we have isolated variants with altered specificity toward D-arabinose (D-ara), mevalonate (mev), and triacetic acid lactone (TAL) [2-4]. These novel biosensors and their use in screening for improved biocatalysts will be described.

We then explored the limits of engineering AraC for altered molecular recognition. We sought variants that respond to many compounds including p-coumaric acid, theophylline, propionic acid, vanillin, malonic acid, levulinic acid, and several others. In the majority of cases AraC variants remaining after sorting showed minimal ligand responsiveness, though some promising clones were isolated. Improvements in biosensor properties of variants from saturation mutagenesis library screening are also being sought by further directed evolution (e.g. reduced leaky expression and enhanced sensitivity). Properties of select variants and mutation patterns that correlate with these properties will be described.

(1.) Gredell J.A., Frei C.S. and P.C. Cirino, 2012; (2.) Tang S.Y., Fazelinia H. and P.C. Cirino, 2008; (3.) Tang S.Y., Qian, S., Akinterinwa Ol, Frei C.S., Gredell J.A.l, and P.C. Cirino, 2013; (4.) Tang, S.Y. and P.C. Cirino, *2011.*