Designing highly specific protein-based small molecule biosensors

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Genetically-encoded small molecule biosensors are emerging as a powerful tool in synthetic biology for high-throughput phenotyping of metabolic pathway variants. Dynamic change in concentration of certain metabolites can serve as a direct readout of enzymatic activity, function of an operon or effects of regulatory variants on pathway flux. Real time measurement of metabolites allows us to dynamically regulate pathways in response to changes in the metabolic state. Microbial allosteric transcription factors (aTF) are widely used as small molecule biosensors in synthetic biology. The aTFs bind to a wide variety of small molecules such as sugars, phenolics, polycyclic aromatics, alkanes and other industrially useful molecules. Our inability to design a biosensor for a desired molecule is a major hurdle to further expanding the use of biosensors. We currently rely almost exclusively on natural biosensors. Here, we describe a general approach to make designer biosensors for new molecules by redesigning natural aTF specificity. We evaluate tens of thousands of computational design candidates with a high-throughput screen to identify these new allosteric biosensors. We redesigned the lac repressor to respond to four new molecules – gentiobiose, fucose, lactitol and sucralose with activity and specificity comparable to wild-type lac repressor for IPTG. We are currently working on building a suite of biosensors for high-value bioproducts, next-generation biofuels, environmental pollutants and redox cofactors. Biosensors would facilitate strain engineering, bioconversion and environmental bioprospecting.