A General Strategy for Sensing Small Molecules in Eukaryotes

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Project Goals: Microbes engineered to express synthesis pathways for useful molecules usually require extensive pathway and metabolic optimization to become efficient producers. Selections from large libraries of engineered or random variants can be a highly effective optimization method, but this requires the microbe to be able to sense target molecules and transduce information about their presence into growth or death signals[1]. However, such biosensors do not exist for many microbially producible useful molecules because they have been absent from the microbes' evolutionary histories. To address this issue, our lab previously developed methods for engineering the specificity of natural allosteric transcription factors like LacI to efficiently and specifically respond to novel ligands[2]. However, because the structural requirements for preserving allostery constrains the flexibility of ligand retargeting, we sought more modular methods for generating biosensors that operated by other mechanisms.

We describe a method to create biosensors starting with a computationally designed ligand-binding domain (LBD). The LBD is fused to a reporter and is destabilized by mutation such that the fusion accumulates only in cells containing the target ligand. We illustrate the power of this method by developing biosensors for digoxin and progesterone. Addition of ligand to cells expressing a biosensor activates transcription in yeast, mammalian cells and plants, with a dynamic range of up to ~100-fold. We use the biosensors to improve the biotransformation of pregnenolone to progesterone and to regulate CRISPR activity. In concert with computational LBD design approaches, this method should enable the generation of biosensors for a broad range of molecules.

References

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