SMALL-MOLECULE BIOSENSORS FOR HIGH-THROUGHPUT METABOLIC ENGINEERING

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Allosteric transcription factors (aTFs) have proven widely applicable for biotechnology and synthetic biology as ligand-specific biosensors enabling real-time monitoring, selection and regulation of cellular metabolism. However, both the biosensor specificity and the correlation between ligand concentration and biosensor output signal, also known as the transfer function, often needs to be optimized before meeting application needs. In this presentation we outline a versatile and high-throughput method to evolve and functionalize prokaryotic aTF ligand specificity and transfer functions in a eukaryote chassis, namely baker's yeast *Saccharomyces cerevisiae*. From a single round of directed evolution of the aTF ligand-binding domain coupled with various toggled selection regimes, we robustly select aTF variants evolved for change in ligand specificity, increased dynamic output range, shifts in operational range, and a complete inversion of function from activation to repression. Importantly, by targeting only the ligand-binding domain, the evolved biosensors display DNA-binding affinities similar to parental aTFs and are functional when ported back into a non-native prokaryote chassis. The developed platform technology thus leverages aTF evolvability for the development of new biosensors with user-defined small-molecule specificities and transfer functions. Finally, the presentation will highlight examples on biosensor applications for high-throughput metabolic engineering.

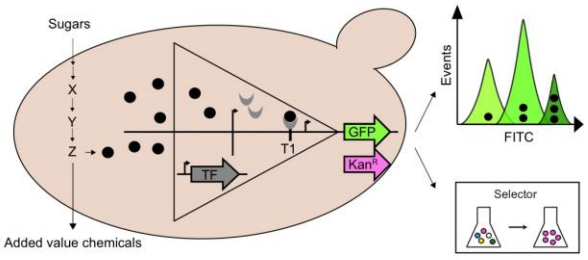

Figure 1 – Schematic outline of a small-molecule biosensor applicable for high-throughput screening and selection of optimal metabolic engineering towards microbial production of added value chemicals