Designing, Modeling, and Constructing Coherence Detection Synthetic Gene Circuits Based on Protein Oligomerization

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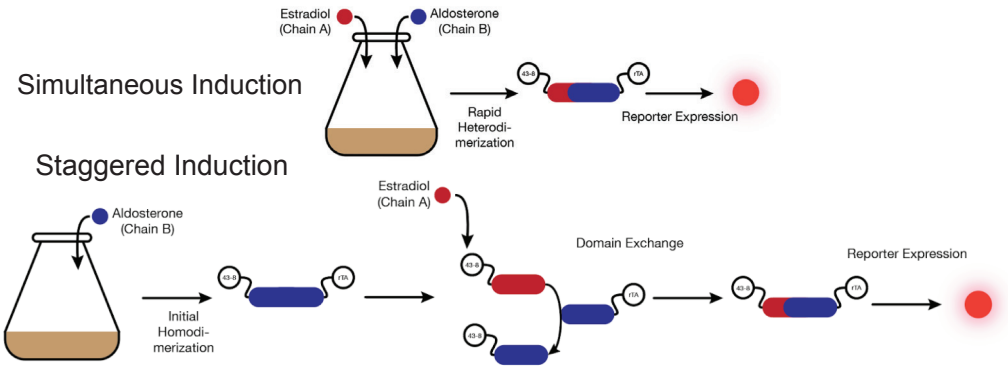
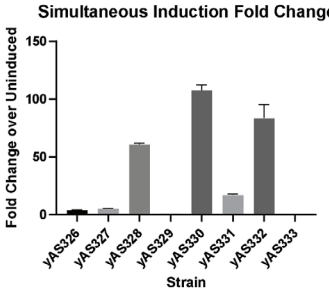
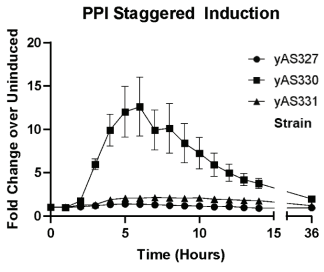
Synthetic biology is an emerging multidisciplinary field that involves not only the modification of existing DNA, but also the synthesis of DNA de novo. We designed a genetic circuit in Saccharomyces cerevisiae that utilizes molecular assembly kinetics to perform “input coherence detection”. These circuits can detect when two distinct input signals are present simultaneously, allowing for greater specificity and fine-tuned output control to make a synthetic circuit more akin to natural biological circuits. Our system leverages oligomerizing protein-protein pairs that can form both homodimers and heterodimers. Each oligomerizing domain is fused to half of a synthetic transcription factor and controlled by a distinct chemical input. In the simultaneous presence of both hormones, co-expression of both protein domains drives formation of heterodimers to activate circuit output. Staggered induction will alternatively drive production of homodimeric complexes that delay circuit activation. Through a series of experiments, we measured fluorescent output in response to varied temporal input sequences via flow cytometry. We then developed a computational model of the circuit based on protein-protein assembly kinetics, from which we derived characteristic circuit parameters, such as induction and decay time constants. Further work includes identifying topological improvements to the circuit architecture to adjust for unwanted couplings that may influence its behavior. By modeling our synthetic circuit under physiologically relevant conditions, we have expanded our toolbox of protein-protein pairs for future applications in vivo. Our work could contribute to downstream therapeutics, such as CAR T-cell therapy or biosensors to recognize pathogens in the body.







$$\frac{d[AA]}{dt} = \frac{k_{act_1}[Est]^{n_1}}{[Est]^{n_1} + (EC_{50_1})^{n_1}} - k_{dil_1}[AA]$$

$$\frac{d[BB]}{dt} = \frac{k_{act_2}[Ald]^{n_2}}{[Ald]^{n_2} + (EC_{50_2})^{n_2}} - k_{dil_2}[BB]$$

$$\frac{d[mKate2]}{dt} = \frac{k_{act_3}[AB]^{n_3}}{[AB]^{n_3} + (EC_{50_3})^{n_3}} - k_{dil}[mKate2]$$