Development of modular biomolecular integral control for synthetic biology applications

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1) Antithetic feedback control as a mechanism for achieving biomolecular integral control

PID (Proportional-Integral-Derivative) controllers have been the workhorse of modern control theory since they were originally conceived. Some utility can be gained from simpler controllers, such as P, PI, or PD controllers, but these controllers face their limitations. In particular, controllers lacking an integral term are not guaranteed to reach zero steady-state error based on the internal model principle[1]. For the controller applications we are considering in synthetic biology, zero steady-state error is an important design consideration, so we are beginning our development of a biological PID controller with integral control. Integral control has long been studied in the context of biological systems, where integral plays an essential role in systems that perform robust perfect adaptation[2,3]. To build integral control into a synthetic gene network, we take inspiration from Briat et al.[4], who proposed antithetic feedback control as a method to achieve integral control. A diagram illustrating the mechanism of the controller is shown on the right (Figure 1). In brief, a reference molecule Z_1 actuates a signal by producing the first molecule in a plant to be controlled. The output of the plant produces Z_2, a control species that performs a bimolecular annihilation with Z_1. The system is modeled to the right of the figure. We plan to construct the network by using a synthetic transcription factor fused to half of a leucine zipper as Z_1 to implement the actuation reaction, and to have the output of the plant produce a half of a leucine zipper fused to a targeting domain as Z_2. We propose to use the controller to control the noise rheostat, a dual inducible transcription factor cascade.

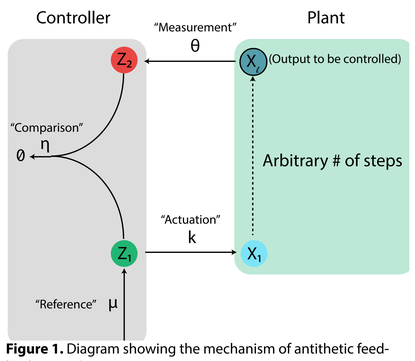


Figure 1. Diagram showing the mechanism of antithetic feedback control

Assume that the plant has 0 steps, where $X_l = X_1$. Then the system can be modeled using the following ODEs, if the rate of annihilation is assumed to be much greater than the rate of dilution/degradation.

$$\frac{dZ_1}{dt} = \mu - \eta * Z_1 * Z_2$$
$$\frac{dZ_2}{dt} = \theta * X_l - \eta * Z_1 * Z_2$$
$$\frac{dX_l}{dt} = k * Z_1 - \gamma * X_l$$

The "error" can be represented as the difference between the fluxes of the the two control species, Z_1 and Z_2

$$\frac{dZ_1}{dt} - \frac{dZ_2}{dt} = \mu - \theta * X_l$$

The steady-state value of the output is then as follows: $X_l = \frac{\mu}{\theta}$

2) Controller Engineering

Controller in open loop can be viewed as a "leaky" comparator

$$\frac{dZ_1}{dt} = \mu_1 - \eta * Z_1 * Z_2 - \gamma * Z_1$$
$$\frac{dZ_2}{dt} = \mu_2 - \eta * Z_1 * Z_2 - \gamma * Z_2$$
$$\frac{dX_l}{dt} = k * Z_1 - \gamma * X_l$$

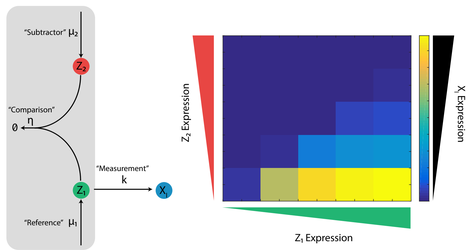
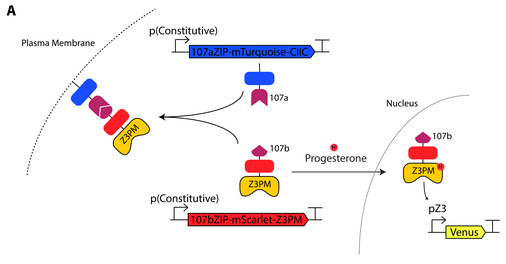


Figure 2. Characterizing the bimolecular annihilation in open loop by titrating the amounts of Z_1 and Z_2. Simulation results are shown on the right.

At steady-state, measured output: $X_l = \frac{k}{\gamma} \frac{-(\eta(\mu_1 - \mu_2) - \gamma^2) \pm \sqrt{(\eta(\mu_1 - \mu_2) - \gamma^2)^2 + 4\gamma^2\eta\mu_1}}{2\gamma\eta}$

A leucine zipper interaction and targeting domain perform a functional annihilation of a transcription factor



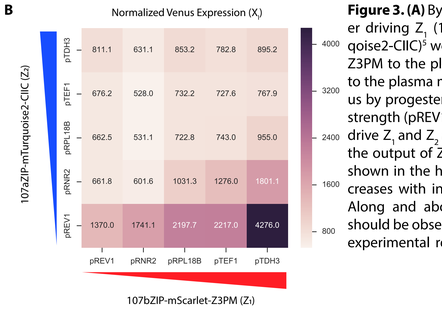


Figure 3. (A) By varying the strength of the constitutive promoter driving Z_1 (107bZIP-mScarlet-Z3PM) and Z_2 (107aZIP-mTurquoise2-CIIC)[5] we can explore how efficient the sequestration of Z3PM to the plasma membrane then it is induced to enter the nucleus by progesterone. **(B)** 5 constitutive promoters of increasing strength (pREV1, pRNR2, pRPL18B, pTEF1, pTDH3) were used to drive Z_1 and Z_2 at different ratios in the same cell. We measure the output of Z_1 as the fluorescent protein Venus (X_l), which is shown in the heatmap. We observe that Venus production increases with increasing Z_1, and decreases with increasing Z_2. Along and above the right diagonal, minimal fluorescence should be observed as predicted by the model. Our preliminary experimental results look similar to the results of the model.

3) Plant Engineering

Characterizing the transfer function of inducible transcription factors

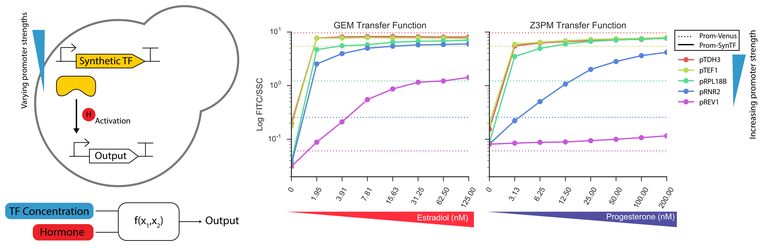


Figure 4. Expression from the pGal1 and pZ3 promoters (activated by GEM and Z3PM, respectively) can be tuned by both changing the concentration of total transcription factor in the cell (by selecting different constitutive promoters), or by changing the concentration of activating hormone.

Studying the controllability of the noise rheostat

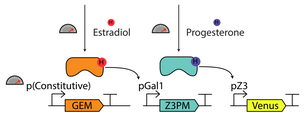
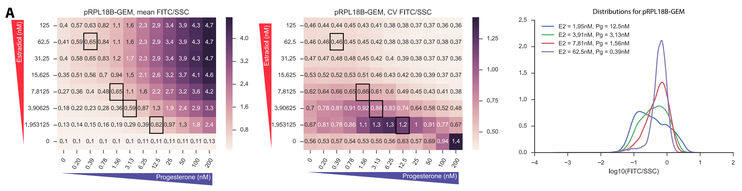


Figure 5. (Left) The noise rheostat[6] is a cascade of two inducible transcription factors. Expression from the pZ3 promoter can be tuned by: 1) changing the constitutive promoter driving GEM, 2) estradiol concentration, and 3) progesterone concentration.



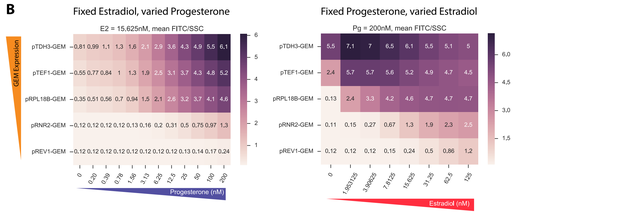


Figure 6. (A) The same mean expression from pZ3 can be achieved at different concentrations of estradiol and progesterone (black boxes, left panel), but the noise (CV) in expression greatly varies at each of the different concentrations (middle panel). The shapes of the distributions with similar means but different CV are shown in the right panel. **(B)** For the noise rheostat to be controllable via feedback control, changes in GEM concentration must be able to influence the output of pZ3. In the left and right panels, we show that changes in the mean output of pZ3 at a given promoter strength driving GEM as a result of changing either progesterone (left) or estradiol (right) at a fixed dose of the other hormone can be rejected by changing the concentration of GEM (selecting a different constitutive promoter). This suggests the system is controllable by changing the concentration of GEM.

4) Optogenetic system engineering

LOVTRAP[7] can be used to dynamically release and sequester proteins from different locations in the cell

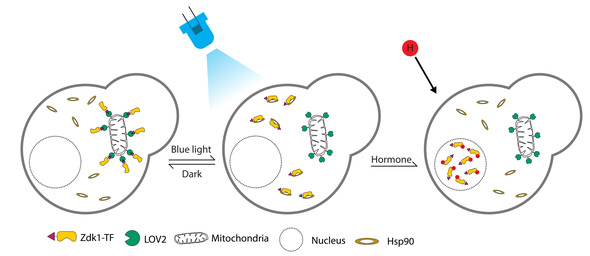


Figure 7. Zdk1 is an engineered peptide that binds the asLOV2 domain in the dark. By targeting a LOV domain to the mitochondria, we can sequester any cargo of interest fused to Zdk1 to the mitochondria until induction with blue light. By fusing an inducible transcription factor (Z3PM)to Zdk1, expression from the pZ3 promoter will be dependent not only on hormone concentration to activate the transcription factor, but also light to release transcription factor from the mitochondria.

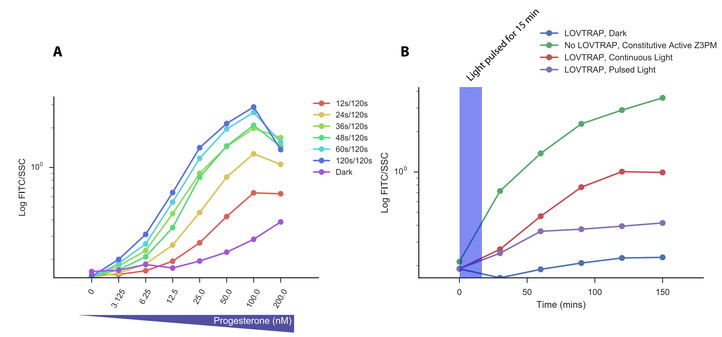


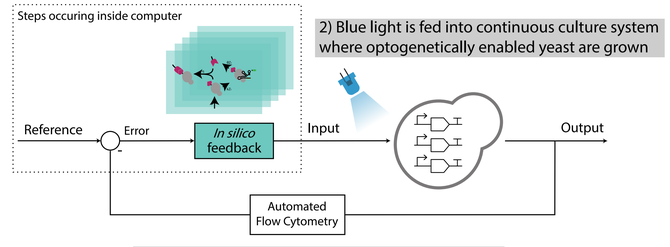
Figure 8. (A) Expression from pZ3 driving Venus is tunable by both hormone concentration and by increasing amounts of blue light. By increasing the length of the duty cycle, available Z3PM concentration is increased, similar to increasing the concentration of Z3PM by changing the constitutive promoter (Figure 4). **(B)** Light induction of the Zdk1-Z3PM is reversible. A pulse of blue light (purple) produces a burst of transcription (fluorescent reporter production stops after ~60 min), whereas continuous light (red) produces increasing amounts of the fluorescent reporter with time. Z3PM without LOVTRAP (green) and Z3PM with LOVTRAP in the dark (blue) are shown as the upper and lower bounds of expression from pZ3

5) Future Directions

We are planning to further develop our current capabilities for automated flow cytometry to act as a prototyping for genetic control circuits. This involves developing a larger suite of optogenetic tools at our disposal, as well as upgrading our continuous culture system to work in higher throughput to test many circuit designs at once.

Workflow for Optogenetic Prototyping

1) Computer compares measurement and reference, then computes an input based on a model of the controller



3) Culture dynamics are dynamically measured using automated flow cytometry hardware

References: 1) Francis and Wonham, *Automatica* 1976. 2) Barkai and Leibler *Nature*, 1997. 3) Muzzey et al., *Cell* 2009. 4) Briat et al., *Cell Systems* 2016. 5) Chen et al., *ACS Synth. Biol.* 2015 6) Mace et al., *ACS Synth Biol* 2016. 7) Wang et al., *Nat. Methods* 2016.