A modular strategy for feedback control using designed proteins

Andrew H. Ng[1,2,3], Robert A. Langan[4,5], Galen Dods[2], Scott E. Boyken[4,5,6], Jennifer A. Samson[1], Lucas M. Waldburger[1], David Baker[4,5,6], John E. Dueber[1], Hana El-Samad[2]

[1]Department of Bioengineering, University of California, Berkeley, Berkeley, CA, [2]Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA, [3]The UC Berkeley - UCSF Graduate Program in Bioengineering, [4]Department of Biochemistry, University of Washington, Seattle, WA, [5]Institute for Protein Design, University of Washington, Seattle, WA, [6]Howard Hughes Medical Institute, University of Washington, Seattle, WA

1) Motivation: Enabling biomolecular feedback

Feedback control has enabled complex applications in traditional engineering disciplines, but its applications in biology have lagged behind largely due to a lack of tools. If designed properly, a feedback controller can mitigate the effect of disturbances to a process, such as an engineered cell. Here, we tackle the challenge of building a modular feedback controller inside a cell using genetic parts. A feedback controller can be broken down into three essential modules:

1) Sensing: Measuring the output of a process

2) Controller: Calculating corrective input based on error between setpoint and output

3) Actuation: Implementing signal from controller to process

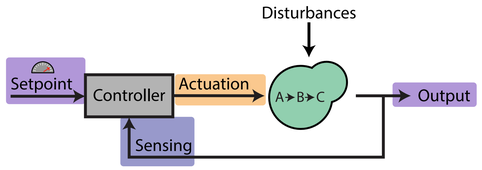


Figure 1: Block diagram illustrating connection of a biological process (engineered cell) with a feedback controller

A modular feedback controller would have broad implications in synthetic biology applications demanding precise outputs, such as metabolic engineering and cellular therapy.

2) Designed Latching Orthogonal Cage Key pRoteins (LOCKR) for inducible protein degradation

Here we present **L**atching **O**rthogonal **C**age **K**ey p**R**oteins (LOCKR), pairs of designed proteins[1] that have the ability to cage a signal peptide of interest until exposed to the cognate Key (Figure 2A). We have embedded the cODC murine degron[2] in the Latch (Figure 2B), enabling degradation of a protein of interest via fusion to the Cage in the presence of the Key (Caged-degron). We tested the functionality of the Caged-degron in yeast by fusing it to a synthetic transcription factor[3] (SynTF), and titrating the concentration of the SynTF-Caged-degron and Key-CFP independently using estradiol (E2) and progesterone (Pg)[4] (respectively) (Figure 3A). We indirectly measure the amount of SynTF in the cell via YFP produced off of the pSynTF promoter (Figure 3B), and at the same time measure the amount of Key (Figure 3C).

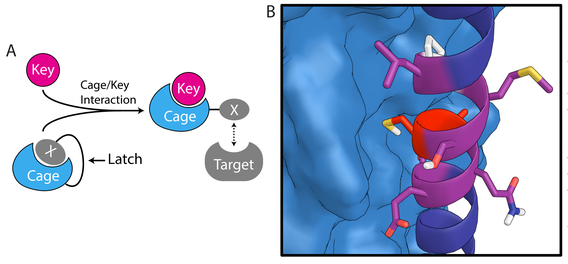
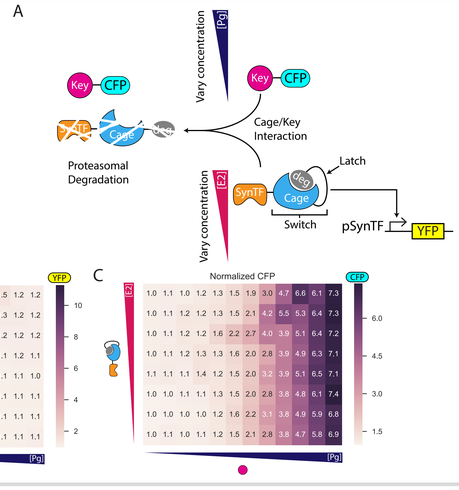


Figure 2 (Left): ***(A)*** LOCKR is a six helix bundle where the sixth helix has been destabilized to weakly interact with the other five helices (Cage). The sixth helix (Latch) can be embedded with a signal peptide of interest such that when the Key outcompetes the Latch for binding with the Cage, the signal peptide is revealed. ***(B)*** Structure of Cage (light blue) interacting with the Latch (dark blue) containing the cODC degron (purple). The two key residues of the degron that interact with the proteasome are shown in red and are hidden in the when no Key is present.

Figure 3 (Right, below): ***(A)*** Experimental design of dual induction of SynTF-RFP-Caged-degron and Key-CFP via E2 and Pg (activating GEM and Z3PM transcription factors respectively). The output of SynTF is measured via pSynTF which produces a YFP. Varying the concentration of each protein in the same cell allows us to evaluate the dynamic range of the Caged-degron via observation of the concentration of YFP or RFP. ***(B, C)*** Heatmap of YFP (B) and CFP (C) with varying concentrations of E2 and Pg. Fluorescence values were normalized by OD600, then normalized to the uninduced strain ([0,0] dose of [E2,Pg]) to show the fold change over the background.



3) LOCKR as a strategy for feedback control of an inducible synthetic transcription factor

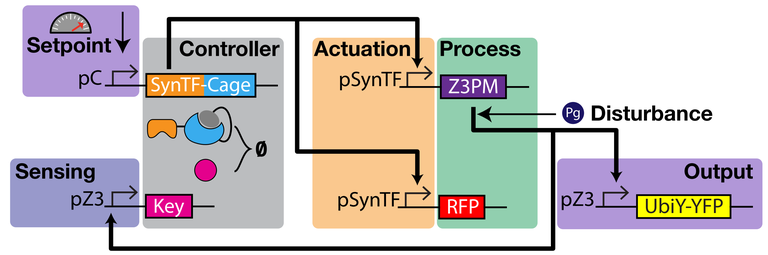


Figure 4 (Above): Design of a feedback control circuit utilizing the LOCKR parts as a controller. The strength of the constitutive promoter(pC) driving SynTF fused to Caged-degron (shown as Cage) determines the setpoint of the output by setting the rate of production of the actuating molecule (SynTF). The SynTF actuates a signal to the process via the pSynTF promoter driving Z3PM and RFP (here RFP acts as a proxy measurement for the amount of Z3PM). When activated by Pg, Z3PM drives transcription from the pZ3 promoter, which acts both as the output and sensor in the system. The output is monitored via a degron (UbiY) tagged YFP produced by the output pZ3 promoter, while the Key produced by the sensing pZ3 promoter performs negative feedback on the SynTF by triggering its degradation via interaction with the Caged-degron.

Because the Caged-degron degrades any protein fused to it in the presence of the Key, LOCKR can perform negative feedback as long as the actuation molecule of interest tolerates a C-terminal fusion, and the output of interest can be sensed via transcription. As a proof-of-principle we designed a genetic circuit to perform feedback control of the inducible synthetic transcription factor Z3PM using SynTF as the actuation molecule (Figure 4). Below, we discuss the specific realization of each module of our controller:

Controller: Caged-degron fused to the actuation molecule of interest (here SynTF) and Key produced by sensing promoter (pZ3). The two inputs are the setpoint (pC driving SynTF) and sensing promoter.

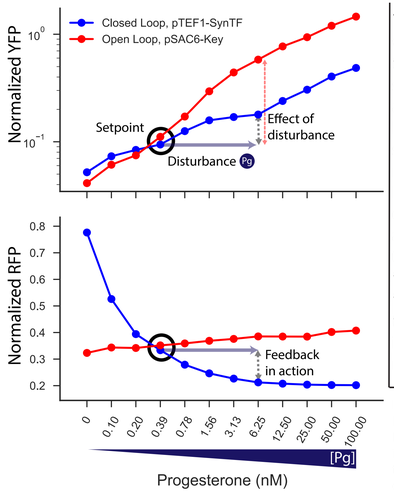
Actuation: Molecule fused to Caged-degron that can activate the process either via a biological process such as transcription or phosphorylation (SynTF produces Z3PM via pSynTF)

Process: Molecule or molecules that are controllable via the actuation input and whose output is measurable via transcription (Z3PM, with RFP as a measurement of the amount of Z3PM)

Output: Molecule of interest that requires feedback control (YFP) and is directly produced by the process. The level of output is determined by the setpoint (pC driving SynTF)

Sensing: Promoter that is sensitive to the output (pZ3) driving production of the Key. This closes the feedback loop on the actuation molecule.

4) Feedback control attenuates disturbances by degrading the actuation molecule



To measure the effect of our feedback controller, we compare our closed loop (CL) feedback controller (shown in Figure 4) with an open loop (OL) system, where the sensing module is replaced with a constitutive promoter driving expression of the Key. We compare the CL and OL circuits at a given setpoint, which consists of a given constitutive promoter (pTEF1-SynTF for both CL and OL, and pSAC6-Key for OL), and a dose of Pg (0.39nM) where both outputs are equivalent (Figure 5, see circle). Given a disturbance shifting the concentration of Pg from 0.39nM to 6.25nM, the OL YFP output increases 5.2 fold, whereas the CL YFP output only increases 1.9 fold for the same change in Pg. This remarkable disturbance rejection is facilitated by a 36% decrease in the amount of Z3PM (observed in the RFP channel) in CL.

Figure 5 (Left): Steady-state fluorescence measurements for closed loop and open loop strains over a dose response of progesterone. Fluorescence values are measured on a flow cytometer and normalized by cell size. For a disturbance of Pg from the indicated setpoint the difference between the CL and OL strains is illustrated in the output (YFP) as well as the amount of Z3PM (RFP).

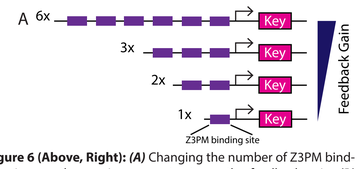
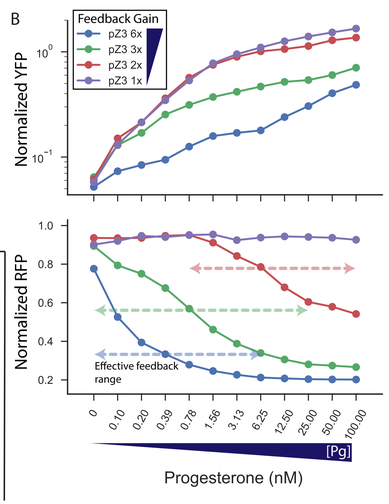


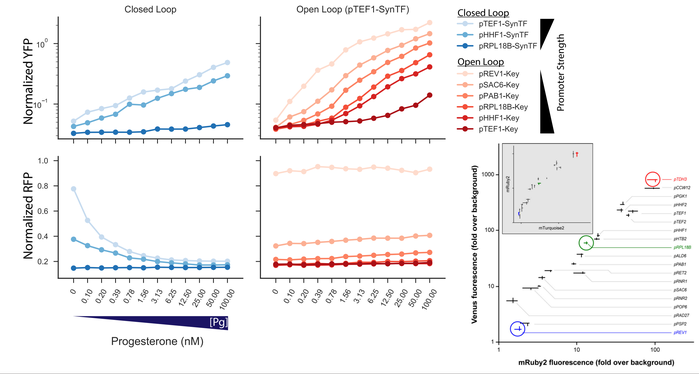
Figure 6 (Above, Right): ***(A)*** Changing the number of Z3PM binding sites on the sensing promoter tunes the feedback gain. ***(B)*** Steady-state fluorescence measurements for strains with different feedback gains. Effective range of feedback is shown and determined by the range of Pg over which the Z3PM concentration can still be influenced



In a practical application of our feedback controller, the user would need to specify the region over feedback is required. To achieve this, one can simply reduce the feedback gain by modulating the number of Z3PM binding sites on the sensing pZ3 promoter (Figure 6A). Dialing down the feedback gain from 6x (the feedback gain that was used in the experiment in Figure 5) to 3x increases the steady-state output due to the reduced disturbance rejection, but extends the effective feedback range all the way to 25nM (Figure 6B). As the feedback gain is decreased to 1x, the steady-state output begins to approximate OL behavior, as seen from the Pg invariant amount of Z3PM measured as RFP.

5) Process output setpoint can be predictably tuned via simple manipulations

Figure 7 (Below): Steady-state fluorescence measurements for closed loop and open loop strains over a dose response of progesterone. Fluorescence values are measured on a flow cytometer and normalized by cell size. In CL different constitutive promoters (see inset for promoter strengths[5]) for SynTF were used to change the setpoint, whereas in OL the same constitutive promoter was used for the SynTF (pTEF1) while different strength constitutive promoters were used to drive expression of the Key.



In order to gain greater control over the setpoint of our circuit, we swapped the actuation module from SynTF to GEM, which is inducible via E2 and activates transcription from the pGal1 promoter (Figure 8A). In this circuit configuration, the constitutive promoter driving GEM is fixed, and the setpoint is determined by the amount of E2. We compare a CL circuit with an OL circuit in which the Key is driven off a fixed constitutive promoter, and vary the amount of E2 fed to each circuit and generate a dose response to Pg (Figure 8B). Using GEM as an actuation node, we can achieve precision control over the setpoint (setting the amplitude of the dose response curve in YFP), while maintaining the same feedback behavior over the process (same effective feedback range as observed when using SynTF as the actuation node).

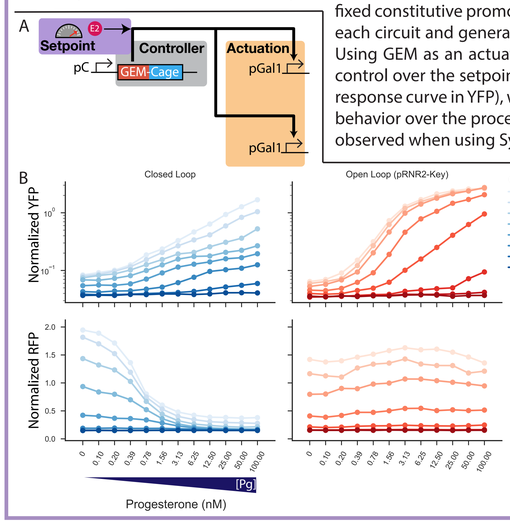


Figure 8 (Left): ***(A)*** Swapping GEM for SynTF as the actuation module for the original circuit design in Figure 4. All other modules remain the same, except for the ones shown in this Figure. ***(B)*** Steady-state fluorescence measurements for closed loop and open loop strains with GEM as the actuation module over a dose response of progesterone. Fluorescence values are measured on a flow cytometer and normalized by cell size. Setpoints were determined by the amount of E2 fed to the circuit.

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References: 1) Boyken et al., Science 2016. 2) Takeuchi et al., *J Biochem.*, 2008 3) Khalil et al., *Cell* 2012. 4) Mace et al., *ACS Synth Biol* 2016. 5) Lee et al., *ACS Synth Biol* 2015.