Supplementary Information
Rapid and tunable post-translational coupling of genetic circuits

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Additional Experimental Results

Degradation tag experiments

In addition to exploring the effect of variable-length linker (TS repeats) on the phase-shift in module degradation (Supplementary Fig. 1c-f), we tested a well characterized AAV degradation tag (5). In Andersen *et al*, GFP-AAV was shown to have 50% higher half-life than GFP-LAA. In this study, downstream module (CFP-AAV) showed a delay in degradation relative to the driver module (GFP-LAA) that was similar to that of the 2 TS-linker sequence (Supplementary Fig. 1b bottom). Further characterization is required to determine the differences in the mechanism of action between variable-length TS linker sequence before the SspB binding region and the AAV degradation tag. While CFP to GFP bleed-over is more significant than GFP to CFP bleed-over, the CFP to GFP bleed-over is not relevant to our experiment in Figure 1a, where the induced protein (GFP) drives the protein level of the coupled protein (CFP). Thus, we performed an experiment to test the potential for bleeding from sfGFP into CFP fluorescence channel by activation sfGFP with 10nM AHL in a strain that lacked CFP fluorophore. We saw no change in CFP fluorescence while sfFGP increased as expected (Supplementary Fig. 1b top).

NFB helps H_2O_2 synchronize oscillations between colonies

We defined the inter-pulse (wait) time as the time between the 10% downlsope point of one peak and 10% upsope point of the following peak (Supplementary Fig. 1a).The mean QS inter-pulse time decreased with addition of IPTG (0.5mM) to the coupled system, while the time of each pulse stayed constant. In addition, we find that QS trajectories from the coupled oscillator system showed significantly lower variability without IPTG as compared to 0.5mM IPTG (Supplementary Fig. 3a-b). These results suggests that stronger NFB (0mM IPTG) associated with higher NFB protein production (1) leads to shorter and more robust inter-pulse behavior in the coupled system. In large biopixel devices, less robust colony-level oscillations prevent H_2O_2 from effectively coupling neighboring pixels, resulting in unsynchronized QS oscillations (No NFB in Supplementary Fig. 3c). NFB reduces inter-pulse duration noise, which allows H_2O_2 to synchronize QS oscillations in neighboring colonies in biopixel devices (0.1mM IPTG in Supplementary Fig. 3c). Increasing NFB strength, further

H_2O_2 increases protein degradation rate

Our analysis of H_2O_2 synchronized quorum clock trajectories showed decrease in the period and increase in the amplitude of oscillations (Fig. 4b Top). H_2O_2 synchronization leads to clear reduction of the degradation time in these trajectories (Supplementary Fig. 4a). One of the significant contributors to the decrease in the period is the increase in the activity of ClpXP targeted proteins, which we quantified as the rate of CFP fluorescence decrease from the peak time to the 10% downslope time. Supplementary Figure 4b shows a significant increase in the ClpXP degradation rate (3X) due to H_2O_2 coupling.

Model Formulation

QS oscillator

To describe dynamic behavior of uncoupled QS oscillator, we expanded on the delay-differential equation model presented in (2). In addition to the equations for LuxI (I), AiiA (A), internal AHL (H_i), external AHL (H_e), we included AHL substrate (S), consisting of acyl-ACPs and S-adenosylmethionine (SAM) (6), to account for the slowing down of H_i production while the number of LuxI molecules is still on the rise. Transcription, translation, and maturation rate of proteins are combined into a single time-delay parameter τ_H. Transcriptional activation by the LuxR and AHL complex (2 of each LuxR and AHL molecules) give delayed production term $P(\tau_H)$, which depends on the past concentration of internal AHL, $H_i(t-\tau_H)$. We assumed a constant level of LuxR since it is not tagged for fast degradation and has a large amount of genetic copies on the plasmid (it is on colE1 twice and p15A once). We used hill coefficient of 4 in accordance with (7) to account for high AHL cooperativity possibly due to AHL-LuxR polymerazation. Diffusion of AHL through cell membrane is described by terms proportional to D, while dilution of external AHL is described by the term proportional to μ. Cell density parameter d was incorporated into the system to account for the difference in the total cell volume and media volume. Enzymatic degradation terms proportional to γ_I and γ_A describe enzymatic degradation of LuxI and AiiA respectively through Michaelis-Mentent kinetics. Different values of k_I and k_A represent different preferential binding dynamics of LuxI and AiiA to ClpXP.

$$\frac{\partial A}{\partial t} = C_A P(\tau_H) - \frac{\gamma_A (A/k_A)}{1 + A/k_A + I/k_I} \tag{1}$$

$$\frac{\partial I}{\partial t} = C_I P(\tau_H) - \frac{\gamma_I (I/k_I)}{1 + A/k_A + I/k_I} \tag{2}$$

$$\frac{\partial H_i}{\partial t} = \frac{bI(S/k_S)}{1 + S/k_S} - \frac{\gamma_H A(H_i/k_H)}{1 + H_i/k_H} + D(H_e - H_i) \tag{3}$$

$$\frac{\partial H_e}{\partial t} = -\frac{d}{1-d} D(H_e - H_i) - \mu H_e \tag{4}$$

$$\frac{\partial S}{\partial t} = S_0 - S - \frac{bI(S/k_S)}{1 + (S/k_S)} \tag{5}$$

$$P(\tau_H) = \alpha_0 + \frac{\alpha_H (H(t-\tau_H)/h0)^4}{1 + (H(t-\tau_H)/h0)^4}$$

Experimentally relevant scaled parameters used with this model are described in Extended Data Table 1.

NFB oscillator

To describe dynamic behavior of NFB oscillator, we used a single delay-differential equation for LacI (L) based on (8). Transcription, translation, and maturation of proteins are lumped together into time-delay parameter τ_L. Transcriptional inactivation of LacI gives the delayed production term $Q(\tau_L)$, which depends on the past concentration of LacI, $L(t-\tau_L)$. Enzymatic degradation

of LacI is described by the term proportional to γ_L through Michaelis-Mentent kinetics. Parameter C in production expression Q represents the effect of IPTG on the strength of LacI repression.

$$\frac{\partial L}{\partial t} = Q(\tau_L) - \frac{\gamma_L(L/k_L)}{1 + L/k_L} \tag{6}$$

$$Q(\tau_L) = \frac{\alpha_L}{1+(L(t-\tau_L)/C)^2}$$

The dynamics of the above model accounted for most of the experimental results. To resolve the amplitude increase in the NFB oscillator when coupled to the QS oscillator during the QS pulse we had to include reporter dynamics with equations for YFP precursor (Y_p) and mature YFP (Y_m). These additional equations are not required to explain the QS dynamics in the coupled system. Experimentally relevant scaled parameters used with this model are shown in Extended Data Table 1.

$$\frac{\partial L}{\partial t} = Q(\tau_L) - \frac{\gamma_L(L/k_L)}{1 + L/k_L + Y_p/k_L + Y_p/k_L} \tag{7}$$

$$\frac{\partial Y_p}{\partial t} = Q(\tau_L) - \frac{\gamma_L(Y_p/k_L)}{1 + L/k_L + Y_p/k_L + Y_m/k_L} - Y_p \tag{8}$$

$$\frac{\partial Y_m}{\partial t} = Y_p - \frac{\gamma_L(Y_m/k_L)}{1 + L/k_L + Y_p/k_L + Y_m/k_L} \tag{9}$$

$$Q(\tau_L) = \frac{\alpha_L}{1+(L(t-\tau_L)/C)^2}$$

Coupled NFB and QS oscillators

Coupling of the two oscillators was accomplished by increasing the effective "queueing" effect through CplXP degradation (9). In the uncoupled case, the degradation of the two oscillator components would be independent, $\frac{ClpXP}{1+QS} + \frac{ClpXP}{1+NFB}$, while in the coupled scenario, $\frac{ClpXP}{1+QS+NFB}$, the degraded components end up in the same degradation term. To couple NFB and QS oscillators through ClpXP degradation, we added LuxI and AiiA from QS system to the degradation expressions in NFB system and LacI (L) from NFB system to the degradation expression in QS system.

$$\frac{\partial A}{\partial t} = C_A P(\tau_H) - \frac{\gamma_A (A/k_A)}{1 + A/k_A + I/k_I + L} \quad (10)$$

$$\frac{\partial I}{\partial t} = C_I P(\tau_H) - \frac{\gamma_I (I/k_I)}{1 + A/k_A + I/k_I + L} \quad (11)$$

$$\frac{\partial H_i}{\partial t} = \frac{bI(S/k_S)}{1 + S/k_S} - \frac{\gamma_H A(H_i/k_H)}{1 + H_i/k_H} + D(H_e - H_i) \quad (12)$$

$$\frac{\partial H_e}{\partial t} = -\frac{d}{1-d} D(H_e - H_i) - \mu H_e \quad (13)$$

$$\frac{\partial S}{\partial t} = S_0 - S - \frac{bI(S/k_S)}{1 + (S/k_S)} \quad (14)$$

$$\frac{\partial L}{\partial t} = Q(\tau_L) - \frac{\gamma_L (L/k_L)}{1 + L/k_L + A + I} \quad (15)$$

$$P(\tau_H) = \alpha_0 + \frac{\alpha_H (H(t-\tau_H)/h0)^4}{1 + (H(t-\tau_H)/h0)^4}$$
$$Q(\tau_L) = \frac{\alpha_L}{1 + (L(t-\tau_L)/C)^2}$$

Experimentally relevant scaled parameters used with this model are described in Extended Data Table 1. We varied the flow μ, IPTG concentration C, and arabinose concentration α_L to recapture many of the experimental findings.

Leader cell wait time shortening

To understand the multicellular dynamics of QS pulse activation we constructed a model with two identical cells that share external AHL (H_e). We first considered a QS only system consisting of two cells with slightly different constitutive production of AiiA and LuxI. In this system, the slower cell couples to the faster one, suggesting that cells whose QS pulse fires first cause QS pulse activation in the nearby cells through AHL cell-to-cell communication (Supplementary Fig. 2a). Next we added NFB to cell 1 in a two-cell system, resulting in period shortening of that cell. As the result, when the two cells were linked through external AHL, the slower cell 2 (without NFB), coupled to the faster cell 1 (Supplementary Fig. 2b). Consequently, even though NFB might be out of phase in different cells, the onset of QS pulse in the faster cells can initiate the propagation of the QS pulse through the rest of the cells in the nearby region. This effect further reduces cell-cell QS variability, which we see from period variability reduction in a 20-cell model (Supplementary Fig. 2d). We added noise to constitutive production of AiiA and LuxI proteins ($\alpha_0 = 0.6 \pm 0.1$) of each of the 20 cells and showed period variability reduction in synched vs unsynched cells (Supplementary Fig. 2e).

QS and H_2O_2 coupled through queueing

To describe dynamic behavior of QS oscillator in response to H_2O_2 produced during LuxI fluorescent reporter expression, we added a differential equation describing production and degradation of H_2O_2 (V_i and V_e) to the QS oscillator delay-differential equation model. We assumed that the production of H_2O_2 is dependent on the concentration of LuxI, which is under the same

promoter as the CFP fluorescent protein. Degradation of H_2O_2 by catalase is proportional to its concentration. H_2O_2 affects the QS oscillator in two characteristic ways. First, ArcA, which is under normal conditions partially represses Lux promoter, is inactivated under oxidizing conditions triggered by H_2O_2 , relieving Lux repression and increasing LuxI and AiiA production. We model this phenomenon by adding a multiplier to the production term that is dependent on H_2O_2 concentration. Second, H_2O_2 has been shown to reduce ClpXP load, leading to increased rate of AiiA and LuxI degradation. Again, we model this behavior by adding a multiplier in front of the degradation term, dependent on H_2O_2 concentration. Finally, H_2O_2 can freely diffuse across cell membrane, which we describe a diffusion term characterized by diffusion parameter D_V. Extracellular H_2O_2 (V_e) can further leave the system with the rate propotional to its concentration.

$$\frac{\partial A}{\partial t} = C_A P(\alpha_H, \tau) - (1 + V_i)\frac{\gamma_A (A/k_A)}{1 + A/k_A + I/k_I} \quad (16)$$

$$\frac{\partial I}{\partial t} = C_I P(\alpha_H, \tau) - (1 + V_i)\frac{\gamma_I (I/k_I)}{1 + A/k_A + I/k_I} \quad (17)$$

$$\frac{\partial H_i}{\partial t} = \frac{bI(S/k_S)}{1 + S/k_S} - \frac{\gamma_H A(H_i/k_H)}{1 + H_i/k_H} + D(H_e - H_i) \quad (18)$$

$$\frac{\partial H_e}{\partial t} = -\frac{d}{1-d} D(H_e - H_i) - \mu H_e \quad (19)$$

$$\frac{\partial S}{\partial t} = S_0 - S - \frac{bI(S/k_S)}{1 + (S/k_S)} \quad (20)$$

$$\frac{\partial V}{\partial t} = \frac{\delta(I/C_I)}{1 + I/C_I} - V_i + D_V(V_e - V_i) \quad (21)$$

$$\frac{\partial V_e}{\partial t} = \frac{d}{1-d} D_V(V_e - V_i) - \mu_V * V_e \quad (22)$$

$$P(\tau_H) = (1 + f_p V)(\alpha_0 + \tfrac{\alpha_H (H(t-\tau_H)/h0)^4}{1+(H(t-\tau_H)/h0)^4})$$

H_2O_2 increases QS period robustness

As we have mentioned before, reduction in inter-pulse duration leads to reduction in period variability arising from noise. Incorporating H_2O_2 effects on QS oscillator into our model (see above) results in several major changes in QS trajectory. First, as expected the amplitude of QS and the downslope time of QS decrease with addition of H_2O_2 (Supplementary Fig. 4c). The result of these two effects also results in shortening of inter-pulse duration, which leads to more robust QS oscillations (Supplementary Fig. 4d). We simulated the model to obtain at least 50 period measurement for period CV calculation. The noise was introduced into the model through addition of a noisy production term ($\alpha_v = \pm 0.1$) to the delayed production term $P(\tau_H) = \alpha_v + (1 + f_p V)(\alpha_0 + \frac{\alpha_H (H(t-\tau_H)/h0)^4}{1+(H(t-\tau_H)/h0)^4})$.

Interestingly, our model shows that individual effects of H_2O_2 activation of lux promoter and increase in ClpXP activity result in the increase the CV of the QS period (Supplementary Fig. 4d). With respect to increased ClpXP activity, higher CV is mainly due to the resulting longer inter-pulse duration (Supplementary Fig. 4c green). Increased lux promoter activity, however, leads to more variable degradation due to higher pulse amplitude variability. The two countering

H_2O_2 effects seem to cancel each other's variability generating more robust QS oscillations.

Fitting model parameters to experimental results

To fit the NFB period data from experiments we used the following parameter scaling functions for the LacI production term ($Q(\tau_L) = \frac{\alpha_L}{1+(L(t-\tau_L)/C)^2}$) to fit IPTG and arabinose (ARA) concentrations:

$$alpha_L \propto A_A + D_A \frac{(\frac{ARA}{C_A})^{H_A}}{(1+\frac{ARA}{C_A})^{H_A}}$$

$$A_A = 0.2758, D_A = 1.6291, C_A = 0.5638, H_A = 0.9029$$

$$C \propto A_C + D_C \frac{(\frac{IPTG}{C_C})^{H_C}}{(1+\frac{IPTG}{C_C})^{H_C}}$$

$$A_C = 0.0968, D_C = 60.8510, C_C = 8.2451, H_C = 0.4334$$

Similarly we fit the model flow term μ to the experimental flow values using the following function

$$\mu = A_\mu \mu^2 + B_\mu \mu + C$$

$$A_\mu = 1.2e-7, B_\mu = 0.0022, C_\mu = -0.11$$

Model parameter values

C_A = 1 (AiiA copy number); C_I = 4 (LuxI copy number); γ_A = 8 (ClpXP degradation of AiiA); γ_I = 8 (ClpXP degradation of LuxI); K_A = 1 (AiiA binding affinity to ClpXP); K_I = 0.2 (LuxI binding affinity to ClpXP); α_0 = 0.6 (Lux promoter basal production); α_H = 3 (Lux promoter AHL induced production); $h0$ = 0.1 (AHL promoter binding affinity); τ_H = 1 (delay in LuxI and AiiA production); b = 1 (AHL synthesis rate by LuxI); k_S = 25 (AHL substrate binding affinity to LuxI) ; S_0 = 50 (basal AHL substrate production); γ_H = 1 (AHL degradation rate by AiiA); k_H = 0.1 (AHL binding affinity to AiiA); D = 0.8 (AHL diffusion across the membrane); d = 0.1 (cell density); μ = 0.5 (flow rate); α_L = 1 (LacI/YFP production rate); C = 0.0025 (LacI promoter binding affinity); τ_L = 0.7 (delay in LacI/YFP production); k_L = 0.001 (LacI/YFP binding affinity to ClpXP); γ_L = 0.05 (ClpXP degradation of LacI/YFP); δ = 1 (H_2O_2 production due to QS fluorophores); C_I = 2 (Michaelis constant); f_p = 1.3 (strength of H_2O_2 activation of LuxI promoter); D_V = 8 (H_2O_2 diffusion across membrane); μ_V = 0 (extracellular H_2O_2 dilution)

References

[1] Stricker, J. *et al.* A fast, robust and tunable synthetic gene oscillator. *Nature* **456**, 516–519 (2008).

[2] Danino, T., Mondragón-Palomino, O., Tsimring, L. & Hasty, J. A synchronized quorum of genetic clocks. *Nature* **463**, 326–330 (2010).

[3] Prindle, A. *et al.* A sensing array of radically coupled genetic/biopixels/'. *Nature* **481**, 39–44 (2011).

[4] Mondragón-Palomino, O., Danino, T., Selimkhanov, J., Tsimring, L. & Hasty, J. Entrainment of a population of synthetic genetic oscillators. *Science Signaling* **333**, 1315 (2011).

[5] Andersen, J. B. *et al.* New unstable variants of green fluorescent protein for studies of transient gene expression in bacteria. *Applied and environmental microbiology* **64**, 2240–2246 (1998).

[6] Parsek, M. R. & Greenberg, E. P. Acyl-homoserine lactone quorum sensing in gram-negative bacteria: a signaling mechanism involved in associations with higher organisms. *Proceedings of the National Academy of Sciences* **97**, 8789–8793 (2000).

[7] Müller, J., Kuttler, C., Hense, B. A., Rothballer, M. & Hartmann, A. Cell–cell communication by quorum sensing and dimension-reduction. *Journal of mathematical biology* **53**, 672–702 (2006).

[8] Mather, W., Bennett, M. R., Hasty, J. & Tsimring, L. S. Delay-induced degrade-and-fire oscillations in small genetic circuits. *Physical review letters* **102**, 068105 (2009).

[9] Cookson, N. A. *et al.* Queueing up for enzymatic processing: correlated signaling through coupled degradation. *Molecular systems biology* **7** (2011).