Synthetic biology in clinically relevant microbes

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July 31, 2012

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Microscopy and Microfluidics

We used a microscopy system similar to our recent studies [1]. Fluorescent images were taken at 4X every 30 seconds using the EMCCD camera (20ms exposure, 97% attentuation) or 2 minutes (2s exposure, 90% attenuation) using a standard CCD camera to prevent photobleaching or phototoxicity.

In each device, *E. coli* cells are loaded from the cell port while keeping the media port at sufficiently higher pressure than the waste port below to prevent contamination (Suppl. Fig 8). Cells were loaded into the cell traps by manually applying pressure pulses to the lines to induce a momentary flow change. The flow was then reversed and allowed for cells to receive fresh media with 0.075% Tween which prevented cells from adhering to the main channels and waste ports.

To measure fluid flow rate before each experiment, we measured the streak length of fluorescent beads (1.0 μm) upon 100 ms exposure to fluorescent light. We averaged at least 1,000 data points for each.

We used several microfluidic devices over the course of the study. For single-cell oscillators (Fig. 1), we used a previously described device consisting of a trapping region and a dynamic switch[2]. Traps have dimensions 40 μm wide x 50 μm long x 0.95 μm high, with the long sides

open to media flow. Since *E. coli* and *S. typhimurium* cells have a 1 μm diameter, the trap maintains growing cells in a monolayer. For colony oscillators (Fig. 2), we used a previously described device consisting of arrays of square trapping regions[1, 3]. Trap dimensions were always 100 μm x 85 μm x 1.65 μm high and spacing between traps was 25 μm. This size allows cells to grow in a colony arrangement rather than a monolayer, while still allowing quantitative measurement of colony fluorescence.

Degradation and Production Rate Quantification

Single cell fluorescence trajectories were obtained from time-lapse movies using custom software previously developed in MATLAB [2]. Each cell fluorescence trajectory represents the median GFP fluorescence signal inside that cell over time. Using built-in MATLAB functions we identified the peaks and troughs for each trajectory. The degradation rate was calculated by taking the amplitude change from peak to the successive trough and dividing by the time change between the peak and the trough. These peak-to-trough sections of the trajectory represent the time when the production of GFP is repressed and the observed dynamics are solely driven by degradation of GFP. Similarly we calculated the net production rate, by calculating the amplitude change from trough to successive peak and dividing by the time change between the trough and the peak. The measurement gives the net production rate, which includes the degradation of the protein.

	Mean Degradation Rate (SE)	**Mean Net Production Rate (SE)**
E. coli	0.024 (0.001)	0.035 (0.002)
S. typhimurium	0.035 (0.002)	0.044 (0.002)

Modeling

To generate the plot in Figure 4D, we used previously described genetic toggle switch model [4]. We included three additional parameters to model the effects of IPTG (C_{IPTG}), ATC (C_{ATC}), and dilution (D) on the synthesis and degradation of proteins:

$$\begin{aligned}\frac{\partial u}{\partial t} &= \frac{C_{IPTG_{(0,1)}}\alpha_u}{1+v^n} - (\gamma_u + D)u \\ \frac{\partial v}{\partial t} &= \frac{C_{ATC_{(0,1)}}\alpha_v}{1+u^n} - (\gamma_v + D)v\end{aligned}$$

In this model, we set n=2 to allow for cooperativity of repression of both promoters. C_{IPTG_0} and C_{ATC_0} were set to 1 for the case of no inducers present. Next, we used metropolis algorithm to find the rest of the parameters to fit the qualitative nature of the curves from Figure 1A. The parameters found to generate the *E. coli* curve were: $C_{IPTG_1} = 1.25$,$C_{ATC_1} = 1.68$,$\alpha_u = 4.28$,$\alpha_v = 5.80$,$\gamma_u = 1.76$,$\gamma_v = 2.37$,$D = 0.11$. The parameters found to generate the *S. typhimurium* curve were: $C_{IPTG_1} = 1.25$, $C_{ATC_1} = 1.68$,$\alpha_u = 11.00$,$\alpha_v = 8.36$,$\gamma_u = 4.86$,$\gamma_v = 3.21$,$D = 0.08$. It is

interesting to note that the optimized parameters show higher production and degradation as well as lower dilution for *S. typhimurium* curve relative to *E. coli* curve, which correlates well with our experimental measurements.

The dynamics of single cell oscillator were modeled using previously described model for activator (a_2) and repressor (r_4) proteins [5]. The production and degradation of these proteins is described by the following set of reactions:

$$
\begin{aligned}
P_{0,0}^{a/r} &\xrightarrow{b_{a/r}} P_{0,0}^{a/r} + m_{a/r} \\
P_{1,0}^{a/r} &\xrightarrow{\alpha b_{a/r}} P_{1,0}^{a/r} + m_{a/r} \\
m_a &\xrightarrow{t_a} m_a + a_{uf} \\
m_r &\xrightarrow{t_r} m_r + r_{uf} \\
a_{uf} &\xrightarrow{k_{fa}} a \\
r_{uf} &\xrightarrow{k_{fr}} r \\
a + a &\underset{k_{-da}}{\overset{k_{da}}{\rightleftharpoons}} a_2 \\
r + r &\underset{k_{-dr}}{\overset{k_{dr}}{\rightleftharpoons}} r_2 \\
r_2 + r_2 &\underset{k_{-t}}{\overset{k_t}{\rightleftharpoons}} r_4 \\
a_{uf} &\xrightarrow{\lambda f(X)} \emptyset \\
r_{uf} &\xrightarrow{f(X)} \emptyset \\
a &\xrightarrow{\lambda f(X)} \emptyset \\
r &\xrightarrow{f(X)} \emptyset \\
a_2 &\xrightarrow{\lambda f(X)} \emptyset \\
r_2 &\xrightarrow{f(X)} \emptyset \\
r_4 &\xrightarrow{f(X)} \emptyset
\end{aligned}
$$

We updated the degradation function $F(X)$ to include dilution as follows:

$$f(X) = \frac{\gamma}{c_e + X} + DX$$

Here, X is the total number of *ssrA* tags in the system (one for each monomeric version, two for dimers, and four for tetramers, including proteins bound to operator sites). We varied the parameter γ from 1x to 2x to evaluate the effect of degradation difference between *E. coli* and *S. typhimurium* on the period of oscillation calculated from single cell model simulations. Dilu-

tion rate was calculated from experimentally measured cell half life as $\frac{ln(2)}{T_{\frac{1}{2}}}$.

To model the dynamics of the quorum-sensing oscillator, we used our previously described model for intracellular concentrations of LuxI (I), AiiA (A), internal AHL (H_i), and external AHL (H_e) [1],

$$\frac{\partial A}{\partial t} = C_A[1-(d/d_0)^4]\ G(\alpha,\tau) - \frac{\gamma_A A}{1+f(A+I)} - DA \quad (1)$$

$$\frac{\partial I}{\partial t} = C_I[1-(d/d_0)^4]\ G(\alpha,\tau) - \frac{\gamma_I I}{1+f(A+I)} - DI \quad (2)$$

$$\frac{\partial H_i}{\partial t} = \frac{bI}{1+kI} - \frac{\gamma_H A H_i}{1+gA} + D(H_e - H_i) - DH_i \quad (3)$$

$$\frac{\partial H_e}{\partial t} = -\frac{d}{1-d}D(H_e - H_i) - \mu H_e + D_1\frac{\partial^2 H_e}{\partial x^2} \quad (4)$$

To model the difference in periods of oscillation between *E. coli* and *S. typhimurium* we varied the degradation parameters γ_A and γ_I. We looked at the changes in the period over different values of the flow rate parameter μ, while varying the degradation prapameters from 1x to 2x of the original model value. To account for the difference in doubling time between the two strains, we introduce exponential decay terms into the model to account for dilution in addition to the enzymatic degradation terms. We add terms $-DI$, $-DH_i$, and $-DH$ to the first three equations respectively, with $D = \frac{ln(2)}{T_{\frac{1}{2}}}$. We then looked at how the change in doubling time affected the period of both strains.

References

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