Figure S1: Plasmids used in this study.
Figure S2: A Hill function accurately fits the relationship between corrected absorbance and CFU/ml in LBM medium. A log-linear fit of the form \( \log_{10}(A_{600} - c_3) = c_1 \log_{10}(r) + c_2 \) is shown for comparison. The small standard deviations of the experimental data at high absorbances make this log-linear fit less predictive than the Hill function.
Figure S3: Periodic dilution results for network architecture c, pluxRp-101 and pPSSUB-102. This figure shows the corrected absorbance and fluorescence dynamics over the duration of the periodic dilution experiment. $A_{600}^0$ is the initial absorbance of the overnight culture used to start the periodic dilution experiment. The system exhibited the same fluorescence trend regardless of the initial condition: an initial burst followed by a gradual decay. Each plot uses the same shading to highlight differences in the measured fluorescence values.
Figure S4: Periodic dilution results for network architecture b, pluxG-102C and pPSSUB-102. This figure shows the corrected absorbance and fluorescence dynamics over the duration of the periodic dilution experiment. $A_{600}^o$ is the initial absorbance of the overnight culture used to start the periodic dilution experiment. $+10\ \mu M\ 3OC6HSL$ indicates that the overnight culture was induced with $10\ \mu M\ 3OC6HSL$. Each plot uses the same shading to highlight differences in the measured fluorescence values.
Figure S5: Individual periodic dilution experiments for testing of architecture b (pluxG-102C and pPSSUB-102) for bi-stability. Here, initial cultures were either grown to a low absorbance ($A_{600} < 0.2$), grown to a high absorbance ($A_{600} > 0.7$), or dosed with 10 µM 3OC6HSL. In each experiment, these different initial conditions led to very similar steady-state profiles. The variation in the steady-state data from day to day, plots (a-c), led to the variability in the average results, plot (d).
Figure S6: Cell viability for the periodic dilution experiments as determined by serial dilution and plating. Each plot corresponds to a periodic dilution experiment in the main text as noted.
<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Constructed Plasmid</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-G-NotI</td>
<td>aagcgcggccgcctcgtataagccatttccgctcg</td>
<td>pluxG-102,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pluxG-102C</td>
</tr>
<tr>
<td>3-XhoI-luxR</td>
<td>gaactcctcgaggatccccgtacttaatttttaaagtatgg</td>
<td>pluxG-102</td>
</tr>
<tr>
<td>5-G2-lux</td>
<td>ctgcgcaaaaaaatattaaagaggagaaaggtacccatg</td>
<td>pluxG-102</td>
</tr>
<tr>
<td>3-G2-lux</td>
<td>ctctcttttaaatggttctgtcgaagtttttgctg</td>
<td>pluxG-102</td>
</tr>
<tr>
<td>3-KpnI-GC</td>
<td>ctatgaggggacctggtcggttcttttaaagtattggtctgtcgaagttttg</td>
<td>pluxG-102C</td>
</tr>
<tr>
<td>5-AatII-luxI</td>
<td>ctgagggaggtccttaaattaagactgcttttttataactgtg</td>
<td>pluxG-103</td>
</tr>
<tr>
<td>5-Rseq</td>
<td>ttcatagccgtaaacaatggctcgc</td>
<td>pluxG-103</td>
</tr>
<tr>
<td>5-SalI-plux</td>
<td>gaactgtgcagccctctctcgcgatcaacgttct</td>
<td>pluxRp-101</td>
</tr>
<tr>
<td>3-pluxC</td>
<td>cttttattttctgctttttatcttttctctcttttatcagc</td>
<td>pluxRp-101</td>
</tr>
<tr>
<td>5-luxRC</td>
<td>gagaatatagcatgaaaacataatgccgaacgacac</td>
<td>pluxRp-101</td>
</tr>
<tr>
<td>5-seq</td>
<td>catagccggaatatgtccctcccac</td>
<td>pluxRp-101</td>
</tr>
<tr>
<td>5-Rp-SalI</td>
<td>agcaatcaccctatggaactgtcg</td>
<td>pluxRp-103E</td>
</tr>
<tr>
<td>3-HindIII-luxRa</td>
<td>gctttctacaagctttaatttatattattattctgtatg</td>
<td>pluxRp-103</td>
</tr>
<tr>
<td>5-Rp3</td>
<td>gataaaagagggagaaggtacatgaaaacataaatg</td>
<td>pluxRp-103</td>
</tr>
<tr>
<td>3-Rp3</td>
<td>cattttgtttttctagttcctctctctcttttatc</td>
<td>pluxRp-103</td>
</tr>
<tr>
<td>3-KpnI-RpE</td>
<td>ctatgaggtacaccggtttttctacccgatcaggtatttcgac</td>
<td>pluxRp-103E</td>
</tr>
</tbody>
</table>

Table S1: Primers used in this study.
Table S2: Kinetic mechanism used to model the behaviors of network architecture b. G, C, and Q refer to green fluorescent protein, bacterial cells, and the DNA encoding constitutive expression, respectively.

S1 Derivation of the Quorum-Sensing Mathematical Model

In this section, we derive the mass balances used to construct the quorum-sensing mathematical model. We consider only architecture b, in which luxR is constitutively expressed and luxI and gfplva are under the control of the p(luxI) promoter. The other two configurations can be similarly derived.

Given the kinetic mechanism of Table S2, we can write mass balances for each of the species of interest

\[
\frac{dc_A}{dt} = (-2r_1 + n_a(r_3 + r_4))V_c - r_6V 
\]

\[
\frac{dc_R}{dt} = (-2r_1 + r_8 - r_5)V_c 
\]

\[
\frac{dc_R}{dt} = (r_1 - r_2)V_c 
\]

\[
\frac{dc_{P0}}{dt} = -r_2 
\]

\[
\frac{dc_{P1}}{dt} = r_2 
\]

\[
\frac{dc_G}{dt} = (n_G(r_3 + r_4) - r_7)V_c 
\]

\[
\frac{dc_c}{dt} = r_9V 
\]

in which

- \(V = \) reactor volume,
### Table S3: Parameters used for simulation of the quorum-sensing models

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Initial Value</th>
<th>Reference</th>
<th>Revised Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_1$</td>
<td>10. nM⁻¹</td>
<td></td>
<td>50. nM⁻¹</td>
</tr>
<tr>
<td>$K_2$</td>
<td>0.01 nM⁻¹</td>
<td>[8]</td>
<td></td>
</tr>
<tr>
<td>$k_3$</td>
<td>20. hr⁻¹</td>
<td>[4]</td>
<td></td>
</tr>
<tr>
<td>$k_4$</td>
<td>$4.05 \times 10^{-6}$ hr⁻¹</td>
<td>[4]</td>
<td>0.1 hr⁻¹</td>
</tr>
<tr>
<td>$k_5$</td>
<td>1.386 hr⁻¹</td>
<td>[3]</td>
<td></td>
</tr>
<tr>
<td>$k_6$</td>
<td>0.0289 hr⁻¹</td>
<td>[5]</td>
<td></td>
</tr>
<tr>
<td>$k_7$</td>
<td>1.0397 hr⁻¹</td>
<td>[1]</td>
<td></td>
</tr>
<tr>
<td>$k_8$</td>
<td>0.4819 hr⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n_A$</td>
<td>1. × 10³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n_G$</td>
<td>1.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$c_{P_T}$</td>
<td>41.5 nM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$c_{Q_T}$</td>
<td>41.5 nM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V$</td>
<td>5. ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{one}$</td>
<td>10⁻¹⁵ l</td>
<td>[2]</td>
<td></td>
</tr>
<tr>
<td>$k_G$</td>
<td>1. nM⁻¹</td>
<td></td>
<td>50. nM⁻¹</td>
</tr>
<tr>
<td>$f_{bgd}$</td>
<td>0</td>
<td></td>
<td>−150</td>
</tr>
</tbody>
</table>

**Initial values for parameters were obtained from the literature when possible. The revised values reflect adjustments made to parameters to fit the experimental results for the shuffled architectures.**

- $V_{one} =$ volume of one cell,
- $V_c =$ total intracellular volume = $c_c V_{one}$, and
- $c_{c,max} =$ max cell density.

Here, we assume that cell replication maintains a constant plasmid concentration. We assume that fluorescence ($f$) is proportional to the GFPLVA concentration ($c_G$) plus a scaling factor $f_{bgd}$, i.e.,

$$f = k_G c_G + f_{bgd} \quad (2)$$

A list of parameters used in this paper are presented in Table S3. Parameters such as $k_9$ that do not affect the steady state are not reported in this table.

Assuming that reactions one and two ($r_1$ and $r_2$) are at equilibrium, we obtain the following reduced model:

$$\frac{d(c_A V)}{dt} + 2 \left( \frac{d(c_{R} V_c)}{dt} + V_c \frac{dc_{P_1}}{dt} \right) = (n_a(r_3 + r_4)) V_c - r_6 V \quad (3a)$$

$$\frac{d(c_{R} V_c)}{dt} + 2 \left( \frac{d(c_{R} V_c)}{dt} + V_c \frac{dc_{P_1}}{dt} \right) = (r_8 - r_5) V_c \quad (3b)$$

$$K_1 = \frac{c_{R}}{c_{A R}} \quad (3c)$$

$$K_2 = \frac{c_{P_1}}{c_{P_5} c_{R}} \quad (3d)$$

$$c_{P_T} = c_{P_0} + c_{P_1} \quad (3e)$$

$$\frac{d(c_G V_c)}{dt} = (n_G(r_3 + r_4) - r_7) V_c \quad (3f)$$

$$\frac{d(c_v V)}{dt} = r_9 V \quad (3g)$$
in which \( c_P \) is the total plasmid concentration per cell.

By setting the derivatives of equation (3), one can solve for the steady states of the system to yield:

\[
0 = -k_6\alpha c_A^2 + (k_3\alpha\beta - k_6) c_A + k_4\beta
\]
\[\alpha = K_1K_2 \] (4a)
\[\beta = n_aV_c c_{c,\text{max}} c_P \] (4b)
\[c_G = \frac{n_gc_P (k_4 + K_1K_2k_3c_Ac_R)}{k_7 (1 + K_1K_2c_Ac_R)} \] (4d)

Equation (4) indicates that the steady-state concentration of the 3OC6HSL signalling molecule is a quadratic function.

In a similar fashion, one can solve for the steady states of network architecture a

\[
c_A = \frac{n_aV_c k_8}{k_6} c_{c,\text{max}} \] (5a)
\[c_R = \frac{k_8}{k_5} \] (5b)
\[c_P = c_{P_0} + c_{P_1} \] (5c)
\[c_G = \frac{n_gc_P (k_4 + K_1K_2k_3c_Ac_R)}{k_7 (1 + K_1K_2c_Ac_R)} \] (5d)

and network architecture c

\[
0 = -k_6\alpha c_A^3 + k_3\alpha\beta c_A^2 - k_6c_A + k_4\beta
\]
\[\alpha = K_1K_2 \] (6a)
\[\beta = n_aV_c c_{c,\text{max}} c_P \] (6b)
\[c_G = \frac{n_gc_P (k_4 + K_1K_2k_3c_Ac_R)}{k_7 (1 + K_1K_2c_Ac_R)} \] (6d)

Equations (5) and (6) indicate that the steady-state concentration of the 3OC6HSL signalling molecule for network architectures a and c are linear and cubic functions, respectively.

S2 Effect of Positive Feedback on the p(luxR) Promoter

Previous works indicate that luxR is capable of positively stimulating transcription from the p(luxR) promoter to a small degree [6, 7], thereby resulting in positive feedback on the p(luxR) promoter. In contrast, we have assumed that the p(luxR) promoter is constitutive. In this section, we numerically explore the effects of this positive feedback.

The experimental results of Sitnikov et al. [7] suggest that the positive feedback from the p(luxR) promoter (1.9-fold stimulation) is roughly twenty times less than that from the p(luxI) promoter (37-fold stimulation). Consequently, we explore increasing the positive feedback from the p(luxR) promoter on network architectures a and b. To do so, we define the amplification factor of promoter j (\( \alpha_j \)) as the fold increase in the expression levels due to positive feedback. We consider manipulating the amplification factor for the p(luxR) promoter, \( \alpha_{p(luxR)} = \frac{k_3'}{k_4'} \), by altering \( k_3' \) while leaving \( k_4' \) at a constant value. Parameter values are the same as the revised values in Table S3, noting that \( k_3' = k_8 \) and \( k_4' \) is variable.
Figure S7: Effect of increasing the amplification factor $\alpha_{p(luxR)}$ for the $p(luxR)$ promoter for network architectures a and b. Plot (a): increasing $\alpha_{p(luxR)}$ for network architecture a. Plot (b): increasing $\alpha_{p(luxR)}$ for network architecture b. Plot (c): comparing all three network architectures for $\alpha_{p(luxR)} = 3$. Plot (d): comparing all three network architectures for $\alpha_{p(luxR)} = 10$. 
<table>
<thead>
<tr>
<th>Transcription Factor Binding</th>
<th>Degradation</th>
<th>Cell growth (logistic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2R + 2A \xrightleftharpoons[K_1]{K_0} R^*$</td>
<td>$R, A, G \xrightarrow{k_5, k_6, k_7} \text{degraded}$</td>
<td>$C \xrightarrow{k_0} 2C$</td>
</tr>
<tr>
<td>$R^* + P_0 \xrightleftharpoons[K_2]{K_3} P_1$</td>
<td>$r_9 = k_9 c \left(1 - \frac{c}{c_{\text{max}}} \right)$</td>
<td></td>
</tr>
</tbody>
</table>

Architecture a

- $P_0 \xrightarrow{k_4} P_0 + n_g G$
- $P_1 \xrightarrow{k_3} P_1 + n_g G$
- $P_0 \xrightarrow{k_3'} P_0 + R + n_a A$
- $P_1 \xrightarrow{k_3'} P_1 + R + n_a A$

Architecture b

- $P_0 \xrightarrow{k_4} P_0 + n_g A + n_g G$
- $P_1 \xrightarrow{k_3} P_1 + n_g A + n_g G$
- $P_0 \xrightarrow{k_3'} P_0 + R$
- $P_1 \xrightarrow{k_3'} P_1 + R$

Table S4: Kinetic mechanisms used to model the behaviors of architectures a and b assuming that the $p(luxR)$ promoter exhibits positive feedback. Positive feedback from the $p(luxI)$ promoter is denoted by the $k_3$ and $k_4$ rate constants, while positive feedback from the $p(luxR)$ promoter is denoted by the $k_3'$ and $k_4'$ rate constants.

Because the $p(luxI)$ promoter has an amplification factor in the model of $\alpha_{p(luxI)} = k_3/k_4 = 200$, we restrict our attention to amplification factors for the $p(luxR)$ promoter of $0 \leq \alpha_{p(luxR)} \leq 10$ to account for the experimental observations of Sitnikov et al.[7]. In Figure S7 (a) and (b), we see that the amplification from the $p(luxR)$ promoter increases the sharpness of the steady-state quorum-sensing response for both network architectures a and b. Additionally, these figures demonstrate that these values of $\alpha_{p(luxR)}$ maintain the graded and threshold responses of network architectures a and b, respectively. Figure S7 (c-d) compares the steady-state quorum-sensing responses of all three network architectures for the same value of $\alpha_{p(luxR)}$. These two figures demonstrate that increasing the number of lux regulatory elements under control of the $p(luxI)$ promoter increases the sharpness of the response and reduces the location of the induction threshold for the given set of parameters. Such phenomena result because the positive feedback from the $p(luxI)$ promoter is significantly greater than that from the $p(luxR)$ promoter.

**S3 Effect of Prolonged Quorum-Sensing Induction on Architecture c**

Prolonged maximal induction of the quorum-sensing circuit negatively impacts cellular function for the architecture c configuration of pluxRp-101 and pPSSUB-102. As seen in Figure S3, the dynamic fluorescence readings exhibit a weak maximum in intensity, then begin to slowly decrease in value. Determination of cell viability by serial dilution and plating confirms the negative impact on cellular function: Figure S6 (a) demonstrates that the number of viable cells for pluxRp-101 and pPSSUB-102 are consistently lower than those for architectures a and b. Additionally, the plated cells for pluxRp-101 and pPSSUB-102 were consistently smaller than those for architectures a and b (data not shown). Interestingly, pluxRp-103E and pPSSUB-102 only displayed similar effects when exposed to a 3OC6HSL concentration of 1.0 $\mu$M (or greater than 100 nM) as shown in Figures S8 and S9. Since over-expression of LuxR, LuxI, and GFPLVA from $p(luxI)$ causes such limitations on growth, there is a strong selective pressure to down-regulate the quorum-sensing system. We speculate that the response to this pressure leads to the observed decreases in fluorescence.
Figure S8: Steady states of pluxRp-103E and pPSSUB-102 as a function of 3OC6HSL induction. The result for the experiment induced at 1.µM 3OC6HSL does not lead to a steady state.
Figure S9: Periodic dilution results for pluxRp-103E and pPSSUB-102 as a function of 3OC6HSL induction. This figure shows the absorbance and fluorescence dynamics over the duration of the periodic dilution experiment. Induction with a 3OC6HSL concentration of 100 nM or lower leads to a stable steady state. Induction with a 3OC6HSL concentration of 1 µM does not achieve a steady state in the duration of the experiment. Each plot uses the same shading to highlight differences in the measured fluorescence values.
References


