Supplementary Theory

1 Modeling dynamics of two-compartment system

Consider two cell types A and B. Each cell produces a product along with an associated signaling molecule that diffuses to the opposite cell and inhibits that cell’s production of its product and signaling molecule. Depending on factors such as the strength of inhibition and the proximity of the two cells, over time one cell may be “dominated” by the other, which produces vastly more product. If either cell could theoretically become the dominant cell, then such a system is considered bistable. There are many biological examples of such bistable systems, perhaps most famously the Notch/Delta signaling pathway [10].

Since quantitative factors determine whether a system of cross-repression is monostable or bistable, it can be difficult to experimentally implement a bistable system without theory that numerically describes and predicts the system’s behavior. We present here an adaptation of the theory developed by Ferreira et al. [8] for a general compartmental system of lateral inhibition to the specific case of quorum sensing between colonies of synthetically engineered bacterial cells.

1.1 Setup

We consider a two-compartment system of cross-repression comprising two colonies of bacterial cells of type A and B (Fig 3A in main text). The full system employs orthogonal quorum-sensing (QS) systems. Ultrasensitivity is necessary for bistability, but most QS systems alone are not ultrasensitive, therefore we introduce a repressor such as TetR that binds a promoter with an ultrasensitive response. A channel between the two compartments allows diffusion of AHL between
the colonies. To prevent AHL buildup due to its slow decay rate, we allow the possibility of adding
an extra “drain” channel of the same length as the other channels that connects each compartment
to a reservoir with zero concentration AHL. We incorporate this drainage into the overall decay
rate for AHL.

In exponentially growing populations of bacteria, the volume at which a cell divides increases
with the growth rate such that the increase in volume outpaces the increase in protein, leading
to a net decrease in protein concentration with growth [13, 18]. Such dilution contributes to the
effective decay rate of products within the cell. In our model we assume colonies are diluting
biochemical species concentration similarly to during exponential phase of growth and incorporate
dilution directly into the decay rate of mRNA and protein within cells.

1.2 Ordinary differential equations (ODEs)

The cross-repression system is divided into three modules per cell type, each describing a particular
function of signal reception, repression, and signal production & diffusion (Fig.1). The state space
variables are listed in Table 1. The test parameters are defined and values provided in Table 2.

The repression module $H_A$ describes LasI production by A for a given input concentration of
TetR and is modeled by a repressive Hill function:

\[ H_A : \begin{align*}
\dot{m}_I &= V_I N_I C \left( \frac{1}{1+ \left( \frac{R_A}{R_A^*} \right)^{n_R} + l_I} \right) - \gamma m_I m_A \\
\dot{l}_I &= \epsilon m_I - \gamma I_A
\end{align*} \tag{1} \]

The steady-state concentration of synthase for a constant input $R_A^*$ is then

\[ I_A^* = \frac{\epsilon I_A V_I N_I C}{\gamma I_A} \left( \frac{1}{\left( \frac{R_A^*}{R_A^*} \right)^{n_R} + l_I} \right). \tag{2} \]

The transmission module $t_{x_{A\rightarrow B}}$ encapsulates the catalysis of 3OC12HSL by LasI and the diffusion
of 3OC12HSL to B. It accepts an input $I_A$, the concentration of LasI produced by cell A, and
outputs a vector $X = [X_A, X_B]^T$, where the first entry is the concentration of 3OC12HSL in A
and the second is the concentration of 3OC12HSL in B after diffusion. The equations governing
the behavior of this module are

\[ t_{x_{A\rightarrow B}} : \begin{align*}
\dot{X}_A &= d(X_B - X_A) - \gamma X A + \nu X I_A \\
\dot{X}_B &= d(X_A - X_B) - \gamma X B
\end{align*} \tag{3} \]

where $d(X_B - X_A)$ represents the diffusion balancing of 3OC12HSL between A and B (the term
$d := \frac{D}{l^2}$ includes the diffusion coefficient $D$ and the channel length $l$). The steady-state concentration
of $X_B$ in response to constant input $I_A^*$ is then

\[ X_B^* = \frac{d \nu I_A^*}{\gamma X (\gamma X + 2d)}. \tag{4} \]
The reception module $rx_B$ captures TetR production in B as a function of $X_A$. The activation of repressor mRNA transcription by 3OC12HSL binding to LasR is modeled by a Hill function:

$$
\begin{align*}
\dot{m}_{RB} &= V_{RB} N_{RB} C \left( \frac{X_A}{K_X} \right)^n + l_{RB} - \gamma m_{RB} m_{RB} \\
\dot{R}_B &= \epsilon_{RB} m_{RB} - \gamma_{RB} R_B
\end{align*}
$$

The complementary modules are identical in structure to those above, and are included here only for completeness. The repression module $H_B$ describes LuxI production by B:

$$
\begin{align*}
\dot{m}_{IB} &= V_{IB} N_{IB} C \left( \frac{1}{1 + \left( \frac{X_B}{K_{RB}} \right)^n} + l_{IB} \right) - \gamma m_{IB} m_{IB} \\
\dot{I}_B &= \epsilon_{IB} m_{IB} - \gamma_{IB} I_B
\end{align*}
$$

The transmission module $tx_{B→A}$ covers catalysis of 3OC6HSL by LuxI and the diffusion of 3OC6HSL to A. The output vector $Y = [Y_A, Y_B]^T$ stores the concentrations of 3OC6HSL in A and B respectively:

$$
\begin{align*}
\dot{Y}_A &= d(Y_B - Y_A) - \gamma_Y Y_A \\
\dot{Y}_B &= d(Y_A - Y_B) - \gamma_Y Y_B + \nu_Y I_B
\end{align*}
$$

The reception module $rx_A$ describes repressor production in A as a function of $Y_A$:

$$
\begin{align*}
\dot{m}_{RA} &= V_{RA} N_{RA} C \left( \frac{X_A}{K_X} \right)^n + l_{RA} - \gamma m_{RA} m_{RA} \\
\dot{R}_A &= \epsilon_{RA} m_{RA} - \gamma_{RA} R_A
\end{align*}
$$

The steady-state concentration of repressor for a constant input $Y_A^*$ is given by

$$
R_A^* = \frac{\epsilon_{RA} V_{RA} N_{RA} C \left( \frac{Y_A^*}{K_Y} \right)^n + l_{RA}}{\gamma_{RA} m_{RA}}
$$

The steady-state solutions differ from their complements only in parameter values.

2 Evaluating bistability

Depending on the exact parameter values, the same system of ODEs may describe a monostable or bistable system. For the cross-repression circuit that we have described, one way to determine whether a certain parameter set yields a bistable system is to count the number of steady-state points: If there is one point and it is stable, then the system is monostable; if there are three points total, one unstable and two stable (one each corresponding to the dominance of A or B), then the system is bistable. While steady-state points may be located using a convenient graphical method, it can be more difficult to analytically determine whether they are stable. Fortunately, if the system satisfies a set of technical conditions that classify it as monotone, then the graphical method will also reveal whether the equilibria are stable. Here, we show that our system is monotone and therefore we may use the graphical method to evaluate bistability.
2.1 Monotonicity

Consider the complete system as shown in Fig.1. The entire unit comprises twelve differential equations, two per each of the six modules. In vector form, we represent the entire system with state space

\[ Z := \begin{bmatrix} mI_A \\ I_A \\ X^T \\ mR_B \\ R_B \\ mI_B \\ I_B \\ Y^T \\ m_{RA} \\ R_A \end{bmatrix} \]

as \( \dot{Z} = f(Z) \). Let \( J(\cdot) \) be the Jacobian of \( f(\cdot) \).

For the system to be monotone, evolution of the system with time must preserve ordering in the state space, i.e., for solutions \( \phi(\cdot, \cdot) \) to the differential equation, \( x_1(0) \preceq_K x_2(0) \implies \phi(t, x_1(0)) \preceq_K \phi(t, x_2(0)) \) for all \( t \geq 0 \). The ordering is defined with respect to some positivity cone \( K \) in a Euclidean space. Although physical concentrations cannot be negative, due to the presence of inhibition our system is not monotone with respect to the positive orthant, but rather to a combination of positive and negative orthants. If we redefined the state space as \( \tilde{Z} := [-mI_A, -I_A, -X^T, -mR_B, -R_B, mI_B, I_B, Y^T, m_{RA}, R_A]^T \), then \( \tilde{Z} \) would be monotone with respect to \( \mathbb{R}^{12}_+ \). Hence \( Z \) is monotone with respect to the cone \( K \) spanned by all vectors \( \tilde{Z} \) corresponding to feasible \( Z \) (i.e., \( Z \in \mathbb{R}^{12}_+ \)).

To see that the system is monotone, we apply a graphical method described in [3] for systems with state, input, and output spaces defined by orthants. We proceed by constructing an incidence graph (signed digraph) where each node is a species and each edge describes the relationship between two distinct species: no edge if no direct interaction; + if one promotes the other; and − if one inhibits the other (Fig.2). By \( Z_j \) promotes \( Z_i \) we mean \( J_{ij} := \frac{\partial f_i}{\partial Z_j} = \frac{\partial^2 Z_i}{\partial Z_j \partial Z_i} \geq 0 \) for all \( Z_i, Z_j \in \mathbb{R}_+ \) whereas by \( Z_j \) inhibits \( Z_i \) we mean \( J_{ij} \leq 0 \) for all \( Z_i, Z_j \in \mathbb{R}_+ \).

Because each biochemical species in the system directly affects only one other species in the system in a sequential fashion, the Jacobian for this system is sparse. We consider the entries corresponding to \( H_A, tx_{B \rightarrow A}, \) and \( rx_A; \) because of the symmetry of the system, we know the entries corresponding to \( H_B, tx_{A \rightarrow B}, \) and \( rx_B \) will differ only in exact parameter values. Then the nonzero entries in the Jacobian (except those corresponding to one species’ influence on its own
concentration, which do not appear in the incidence graph) are

$$\mathbf{H}_A : \begin{cases} \frac{\partial \dot{m}_{IA}}{\partial m_{IA}} = V_{IA} N_{IA} C^{-n_{R_A}} \left( \frac{R_A}{K_{R_A}} \right)^{n_{R_A}-1} & \leq 0 \\ \frac{\partial \dot{I}_A}{\partial m_{IA}} = \epsilon_{IA} & \geq 0 \end{cases}$$

$$\begin{align*} \frac{\partial Y_B}{\partial I_0} &= \nu_Y \geq 0 \\ \frac{\partial Y_A}{\partial I_B} &= d \geq 0 \\ \frac{\partial Y_B}{\partial I_A} &= d \geq 0 \end{align*}$$

along with the corresponding entries for the complementary modules.

From a conceptual standpoint, monotonicity means that regardless of context, an element always has the same qualitative effect on itself after its influence is propagated through the network; i.e., the influence of the element on itself is “consistent”. From the graph for this system (shown in Fig.2) we see that there is only one cycle and it is positive in parity; that is, the product of the signs of each edge traversed to complete one cycle is positive, regardless of the direction of travel around the cycle. This implies that the graph is consistent, and hence the system it describes is closed-loop monotone.

### 2.2 Finding steady-state solutions

By Theorem 3 in [3] and [2], the equilibria of a closed-loop monotone system (system with feedback) can be found by examining an open-loop (input-output, or I/O system) monotone system formed by “breaking” the feedback of the original system. For fixed points to exist, the I/O system must have a static input-output characteristic.

In our case, we can define \( T_A \), or \( R_A \rightarrow R_B \), as the cascade of the three modules \( \mathbf{H}_A, \mathbf{t}_A \rightarrow \mathbf{B}, \) and \( \mathbf{r}_A \) having input \( R_A \) and output \( R_B \). With \( \mathbf{T}_B \) defined similarly for the other three modules, then the entire system “broken” at \( R_A \) is the cascade of \( \mathbf{T}_A \) and \( \mathbf{T}_B \). This new I/O system accepts an input \( u \in \mathbb{R}_+ \) and produces an output \( y \in \mathbb{R}_+ \). If the I/O system admits a static input-output characteristic, then the points where \( u = y \) are the steady-state solutions to the closed-loop system.

Since \( \mathbf{H}_A \) and the combined cascade of \( \mathbf{t}_A \rightarrow \mathbf{B} \) and \( \mathbf{r}_A \) each has a unique steady-state solution that is a global and asymptotically stable hyperbolic equilibrium (see Section 5), then the cascade of the three systems, i.e., \( \mathbf{T}_A \), also has a static input-output characteristic. \( \mathbf{T}_B \) is similarly endowed. Therefore the cascade of \( \mathbf{T}_A \) and \( \mathbf{T}_B \) also has a static input-output characteristic, and because the I/O system is also monotone, then its fixed points and the equilibrium points of the closed-loop system correspond.

Define \( \mathbf{T}_A(\cdot) : \mathbb{R} \rightarrow \mathbb{R} \) to be the static I/O characteristic of \( \mathbf{T}_A \), i.e., for constant input \( R_{A_1}^* \), \( \mathbf{T}_A \) produces constant output \( R_{B_1}^* = \mathbf{T}_A(R_{A_1}^*) \), and define \( \mathbf{T}_B(\cdot) : \mathbb{R} \rightarrow \mathbb{R} \) to be the static I/O characteristic of \( \mathbf{T}_B \) for constant input \( R_{B_1}^* \) and output \( R_{A_2}^* \). Then the static I/O characteristic for the cascade of \( \mathbf{T}_A \) and \( \mathbf{T}_B \) is \( \mathbf{T}_B(\mathbf{T}_A(\cdot)) \), which maps constant input \( R_{A_1}^* \) to output \( R_{A_2}^* \). The function \( \mathbf{T}_B(\mathbf{T}_A(\cdot)) \) is nonnegative and sigmoidal, meaning that there must be exactly one or three
intersection points between \( y = u \) and \( y = T_B(T_A(u)) \) (the fixed-point solutions to the I/O system where \( u = y \) or \( R_A^* = R_A^* \)). If there is only one intersection then the I/O characteristic at the intersection must have a slope less than unity \( (T_B'(T_A(y^*))T_A'(y^*)) < 1 \) where \( y^* \) is the intersection point), implying that the corresponding equilibrium is stable. If there are three intersections, then the middle intersection must have a slope greater than one while the higher and lower intersections must have slopes less than one, implying that the middle equilibrium is unstable and the other two equilibria are stable [3]. Hence determining bistability amounts to graphically counting the intersections between \( y = u \) and \( y = T_B(T_A(u)) \). We could also carry out the above analysis with equivalent results for \( T_A(T_B(\cdot)) \) mapping \( R_B^* \) to \( R_B^* \).

3 Multicompartmental systems with symmetry

Up to this point we have considered a system of only two compartments, one each of types A and B. The formation of an interesting pattern, however, requires more than two elements. To that end we will now consider a class of systems with multiple compartments of each type, where each compartment of type A is connected to the same number of compartments of type B, and vice versa. In other words, each compartment of type A or B is essentially indistinguishable from any other. The symmetry present in such a system will allow us to apply the graphical method of analyzing bistability to sets of compartments arranged in particular geometries.

The discussion presented here is not intended as a complete overview of multicompartmental systems of lateral inhibition, but rather as a supplement to our specific experiments. We refer the interested reader to [8] for a more generalized treatment.

3.1 Edge weight matrix

The interior mechanics of all compartments of the same type are the same, so the ODEs governing the behavior of the reception modules \( r_x \) and repression modules \( H \), as defined in Section 11.2, are the same for compartments of the same type. Adding more compartments does, however, change the concentration of diffusible signaling molecules that reach the compartments, and therefore changes the behavior of the transmission modules \( t_x \).

We begin by noting that if we define the \( 2 \times 2 \) matrix

\[
L_2 := d \begin{bmatrix} -1 & 1 \\ 1 & -1 \end{bmatrix}
\]

then we can reformulate (7) as

\[
\dot{Y} = L_2Y - \gamma Y + \begin{bmatrix} 0 \\ \nu Y I_B \end{bmatrix}
\]

with steady-state solution

\[
Y^* = (L_2 + \gamma I)^{-1} \begin{bmatrix} 0 \\ \nu Y I_B \end{bmatrix}.
\]

The matrix \( L_2 \) contains information on the diffusion of AHL between compartments. We now generalize to systems with \( N \) compartments and the corresponding \( N \times N \) matrix \( L_N \) (which we will henceforth designate as simply \( L \)). If all compartments in the system are numbered from 1
to $N$, then the element $[L]_{ij}$ represents the connection strength between compartments $i$ and $j$. Conceptually, the connection strength is the diffusion into compartment $j$ from compartment $i$ (or vice versa) if $i \neq j$, and the total diffusion out of a given compartment to all other compartments if $i = j$. Each element $[L]_{ij}$ is directly proportional to the diffusivity $D$ of AHL and inversely proportional to the square of the distance between $i$ and $j$. In the special case where there are only two compartments, the connection strength between them is identical, hence the elements in $L_2$ are all of magnitude $d$.

Mathematically, we can represent the multicompartmental system as an undirected graph where each vertex is a compartment and each edge is a channel. Let $d_{ij}$ be the edge weight between vertices $i$ and $j$. $L$ is the Laplacian of this graph:

$$\begin{cases} -\sum_{j=1}^{N} d_{ij} & i = j \\ d_{ij} & i \neq j \end{cases}$$

Let $N_A$ be the number of compartments of type A and $N_B$ the number of compartments of type B such that $N_A + N_B = N$. Assume the diffusivity of AHL is constant, all channels have the same length, and compartments of the same type are not connected to each other. Then $d_{ij} = 0$ if $i$, $j$ are of the same type and $d_{ij} = d$ between connected compartments $i$, $j$ of opposite types. Assume each compartment of type A is connected to $q_B$ compartments of type B and each compartment of type B is connected to $q_A$ compartments of type A.

Let the first $N_A$ entries of a row or column of $L \in \mathbb{R}^{N \times N}$ designate compartments of type A and the last $N_B$ entries designate compartments of type B. Then $L$ has the form

$$L = d \begin{bmatrix} -q_B \mathbb{I}_{N_A \times N_A} & F \\ F^T & -q_A \mathbb{I}_{N_B \times N_B} \end{bmatrix}$$

where $F$ is an $N_A \times N_B$ matrix for which $[F]_{ij} = 0$ indicates that the $i$th compartment of type A and the $j$th compartment of type B are not connected by a channel, and $[F]_{ij} = 1$ indicates that they are.

As shown in the following sections, the assumed structure of the system allows us to reduce our $N$-dimensional system to a two-dimensional one, which greatly simplifies the calculations for a steady-state contrasting pattern and enables us to use the graphical method detailed in Section 22.1 to determine when the overall system is bistable.

### 3.2 Model reduction

We use the assumptions and notations from the previous subsection. Define $M \in \mathbb{R}^{N \times 2}$ as

$$M := \begin{bmatrix} \mathbb{I}_{N_A} & 0_{N_A} \\ 0_{N_B} & \mathbb{I}_{N_B} \end{bmatrix}$$

where $\mathbb{I}_n$ designates a length-$n$ vector of all ones and $0_n$ designates a length-$n$ vector of all zeros. Then because all compartments of the same type have the same number of connections to compartments of the opposite type, there exists some $\bar{L} \in \mathbb{R}^{2 \times 2}$ such that

$$LM = M\bar{L}.$$
Let $d_A := dq_B$ be the (nonnegative) total outgoing edge weight for a compartment of type A and $d_B := dq_A$ be the (nonnegative) total outgoing edge weight for a compartment of type B. Because we have assumed no connections between compartments of the same type, $\bar{L}$ has the form

$$\bar{L} = \begin{bmatrix} -d_A & d_A \\ d_B & -d_B \end{bmatrix}.$$  \hfill (14)

We would like to solve for the steady-state value of $X = [X_A, X_B]^T \in \mathbb{R}^N$ in $tx_{A\rightarrow B}$:

$$(-L + \gamma_X I_{N \times N}) X = \begin{bmatrix} \nu_X I_A \kappa_{NA} \\ 0_{N_B} \end{bmatrix}.$$  \hfill (15)

We restrict our search to a subset of solutions for which the variables of interest are identical among compartments of the same type, i.e.,

$$X = M \begin{bmatrix} x_A \\ x_B \end{bmatrix}$$  \hfill (16)

for $x_A, x_B \in \mathbb{R}$. Let $x := [x_A, x_B]^T$. We can then rewrite (15) as

$$(-L + \gamma_X I_{N \times N}) M x = M \begin{bmatrix} \nu_X I_A \\ 0 \end{bmatrix} \Rightarrow M (-\bar{L} + \gamma_X I_{2 \times 2}) x = M \begin{bmatrix} \nu_X I_A \\ 0 \end{bmatrix}$$

which implies that solutions $x^*$ to

$$(-\bar{L} + \gamma_X I) x^* = \begin{bmatrix} \nu_X I_A^* \\ 0 \end{bmatrix} \Rightarrow x^* = (-\bar{L} + \gamma_X I)^{-1} \begin{bmatrix} \nu_X I_A^* \\ 0 \end{bmatrix}$$  \hfill (17)

provide solutions to (15) by way of (16). In other words, $x_A^*$ is the steady-state concentration of $X$ in any compartment of type A and $x_B^*$ is the steady-state concentration of $X$ in any compartment of type B for constant input $I_A^*$. The derivation for $tx_{B\rightarrow A}$ proceeds similarly.

Now recall from (14) that the matrix $\bar{L}$ has form

$$\bar{L} = \begin{bmatrix} -d_A & d_A \\ d_B & -d_B \end{bmatrix}.$$  \hfill (14)

Then the inverse matrix in (17) can be directly evaluated, yielding

$$(-\bar{L} + \gamma_X I)^{-1} = \frac{1}{(d_A + \gamma_X)(d_B + \gamma_X) - d_A d_B} \begin{bmatrix} d_B + \gamma_X & d_A \\ d_B & d_A + \gamma_X \end{bmatrix} = \frac{1}{\gamma_X (\gamma_X + d_A + d_B)} \begin{bmatrix} d_B + \gamma_X & d_A \\ d_B & d_A + \gamma_X \end{bmatrix}.$$  \hfill (17)

Since $d_A, d_B > 0$, the matrix is always invertible provided that $\gamma_X \neq 0$.

In essence, the new multicompartmental system is identical to the two-compartmental system with a revision to the transmission modules:

$$tx_{B\rightarrow A} : \begin{cases} \dot{Y}_A = d_A (Y_B - Y_A) - \gamma_Y Y_A \\ \dot{Y}_B = d_B (Y_A - Y_B) - \gamma_Y Y_B + \nu_Y I_B \end{cases}$$
and similarly for $t\xi_{A\rightarrow B}$. Since $d_A, d_B > 0$ the Jacobian equations from (10) maintain their parity and the analysis developed in Section 22.1 holds. In Section 55 we show that the local stability or instability of a steady state in the reduced system implies local stability or instability of the state in the full system, and therefore we can therefore continue to use the graphical intersection method to determine when the system admits a solution where all cells of the same type are identically (and reversibly) high or low.

3.3 Finite differences within the channel

Thus far, implicit in our definition of $L$ is that we approximate diffusion between compartments using the method of finite differences for a step size of $l$, the channel length. Specifically, we have taken Fick’s diffusion equation in one dimension

$$\frac{dX}{dt} = D \frac{d^2 X}{dr^2}$$

where $X$ is the concentration of some species and $r$ is distance. Under the finite differences approximation, we discretize space along $r$ and approximate the change in concentration at each point as

$$\frac{dX}{dt} \approx D \frac{X(r + \Delta) - X(r)}{\Delta^2} + (X(r - \Delta) - X(r)).$$

In the process of discretizing $r$ we pick two boundary points. To model a single channel connecting a cell of type $A$ to one of type $B$ we simply pick one end, say $r = 0$, to correspond to $A$ and the other end, $r = l$, to correspond to $B$. We assume no diffusion outside the channel. Since $X(0)$ has no neighbors $r < 0$ and $X(l)$ has no neighboring points $r > l$, the approximation at the boundaries is performed using only one difference. At the $r = 0$ boundary we have

$$\frac{dX(0)}{dt} \approx D \frac{X(\Delta) - X(0)}{\Delta^2}$$

and at the $r = l$ boundary

$$\frac{dX(l)}{dt} \approx D \frac{X(l - \Delta) - X(l)}{\Delta^2}.$$ 

If we let $\Delta = l$, $X(0) = X_A$, and $X(l) = X_B$, we recover the familiar

$$\begin{cases} 
\frac{dX_A}{dt} = \frac{D}{l^2} (X_B - X_A) \\
\frac{dX_B}{dt} = \frac{D}{l^2} (X_A - X_B) 
\end{cases}.$$

If $l$ is sufficiently small, this approximation is appropriate. If the channel is too long, however, then setting $\Delta = l$ produces an extremely coarse approximation that may not be an accurate description of the physical process. Suppose now we discretize $r$ such that there are $N_L$ points between $r = 0$ and $r = l$, with a step size of $\Delta = \frac{l}{N_L + 1}$. The equation to describe diffusion is written in matrix form as

$$\begin{bmatrix}
\dot{X}(0) \\
\dot{X}(\Delta) \\
\dot{X}(2\Delta) \\
\vdots \\
\dot{X}(l - \Delta) \\
X(l)
\end{bmatrix} =
\begin{bmatrix}
-1 & 1 & 0 & 0 & \ldots & 0 & 0 & 0 \\
1 & -2 & 1 & 0 & \ldots & 0 & 0 & 0 \\
0 & 1 & -2 & 1 & \ldots & 0 & 0 & 0 \\
\vdots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots \\
0 & 0 & 0 & 0 & \ldots & 1 & -2 & 1 \\
0 & 0 & 0 & 0 & \ldots & 0 & -1 & 1
\end{bmatrix}
\begin{bmatrix}
X(0) \\
X(\Delta) \\
X(2\Delta) \\
\vdots \\
X(l - \Delta) \\
X(l)
\end{bmatrix}.$$
Note that this describes only the diffusion, not the reactions that occur within $X(0)$ and $X(t)$.

We can accommodate multiple cells with multiple channels by adding more terms and associated concentration variables. Imagine that we number the discretized points within a channel from 1 to $N_L$ where 1 is the point nearest to a cell of type $A$ and $N_L$ is the point nearest to a cell of type $B$. Then we assume all channels with the same number form a similar class; i.e., if we have $N_C$ channels we define $M \in \mathbb{R}^{N+N_C N_L \times N_C + 2}$ as

$$M := \begin{bmatrix}
  \mathbb{1}_{N_A} & 0 & N_L & 0 & N_L & \ldots & 0 & N_L \\
  0 & N_L & \mathbb{1}_{N_L} & 0 & N_L & \ldots & 0 & N_L \\
  0 & N_L & 0 & N_L & \mathbb{1}_{N_L} & \ldots & 0 & N_L \\
  \vdots & \vdots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\
  0 & N_L & 0 & N_L & \ldots & 0 & \mathbb{1}_{N_B}
\end{bmatrix}$$

such that

$$\tilde{L} := \frac{D}{\Delta^2} \begin{bmatrix}
  -q_B & q_B & 0 & 0 & \ldots & 0 & 0 & 0 \\
  1 & -2 & 1 & 0 & \ldots & 0 & 0 & 0 \\
  0 & 1 & -2 & 1 & \ldots & 0 & 0 & 0 \\
  \vdots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots \\
  0 & 0 & 0 & 0 & \ldots & 1 & -2 & 1 \\
  0 & 0 & 0 & 0 & \ldots & 0 & -q_A & q_B
\end{bmatrix}$$

with $q_A$ and $q_B$ as defined in Section 33.2. We then rewrite (15) as

$$(-\tilde{L} + \gamma X I) X = \begin{bmatrix}
  \nu X I_A \\
  0_{N_L N_C + 1}
\end{bmatrix}.$$ 

The equation takes a similar form for $Y$.

In the above discussion we have assumed that production takes place only in single compartments, which shrink in size with increasing $N_L$. Let $w$ be the width of a cell colony. To accurately reckon with arbitrary $l$ and $N_C$ we would have to either (a) dilute the concentrations of intercellular components ($l > w$); or (b) designate multiple compartments as production compartments, which would rapidly increase the size of the ODE system ($l < w$). To avoid these situations we set $\Delta = w$ such that $l = kw$ where $k \geq 0$ is an integer. This limitation in the considered range of $l$ is justified by the small range of $l$ that we implement experimentally as well as the minimal effect of $l$ on the theoretically evaluated bistability of the system (for systems with and without multiple diffusive compartments).

### 3.4 Extension to contact-mediated communication

To represent forms of communication other than diffusion, the matrix $L$ representing the Laplacian can be replaced by any matrix with entries appropriately weighted to reflect the strength of the connections between compartments. For example, to model communication for which compartments do not lose concentration of signaling molecule to communicate with neighbors, the diagonal entries can be set to 0. Using this particular form of $L$ instead of the Laplacian in the remainder of our theory for lateral inhibition allows us to model traditional cell-to-cell contact-based mechanisms such as the Notch/Delta system. Note that if $L$ is an asymmetric matrix, dimensionality reduction as described here for particular spatial configurations will in general no longer be mathematically valid.
4 Results: Bistable regimes

4.1 Parameter ranges

We assessed the effect of changing the parameters in Table 3 on the bistability of the system for a given connectivity of compartments—hereafter referred to as a geometry—satisfying the assumptions in Section 33.2. We used a custom MATLAB script to generate “bistability maps” as a function of two parameters at a time to determine in which parameter regimes the system is theoretically bistable. Such maps are useful to elucidate not only whether the system falls in the bistable region, but also its “location” on the bistability map. The location is important for two reasons: (a) the closer a system is to the edge of the bistable region, the smaller a change to the parameters is necessary to push the system into or out of the region; and (b) where the system is in the region determines the system’s exact steady-state values, which in turn determine the observed contrast between the colonies’ expression levels of reporter. These maps are useful in designing bacterial strains to achieve desired behaviors (i.e., bistability, steady-state values) and also for picking geometric configurations that produce those behaviors. For example, a system farther from the edge of the bistable region will be more robust in the sense that perturbations to parameter values (as through experimental error or random variation) are less likely to cause the system to become bistable if it is nominally monostable or monostable if it is nominally bistable.

4.2 Dynamic range matching

In essence, a system of mutual inhibition is equivalent to a pair of switches, each of which turns on for low input and off for high input. The switches cascade to form an open-loop system, which is closed by connecting the output of the second switch to the input of the first. In order for the closed-loop system to be bistable, the input to each switch must span some significant fraction of the dynamic range of the switch; i.e., the minimum output from the first switch should be low enough to turn on the second switch while the maximum output should be high enough to turn it off, and vice versa. The relative dynamic ranges of the switches are determined by the parameters and geometry. The curves in Fig.3 show input/output curves (nullclines) for the first switch with input $X_B$ and output $Y_A$ (modules $r_{XB} \rightarrow H_B \rightarrow t_{XB\rightarrow A}$) and the second switch with input $Y_A$ and output $X_B$ (modules $r_{XA} \rightarrow H_A \rightarrow t_{XA\rightarrow B}$).

5 Additional proofs

5.1 Contrasting steady states emerge if and only if the system can be reduced to the two-compartment case

In Section 33.2 we showed that if compartments of the same type have the same number of neighbors, then the system can be reduced to a two-compartment case for the purposes of identifying contrasting steady states, i.e., those in which all compartments of the same type have the same steady-state concentrations. We now show that if a steady state exists for which (a) all compartments of the same type have the same concentrations and (b) every compartment of type A is connected to at least one compartment of type B and vice versa, then the underlying edge weight matrix $L$ can be reduced to a two-compartment system. We do not need to assume that compartments of the same type must have neighbors only of the opposite type, although a “checkerboard”
of alternating high/low immediately adjacent neighbors will require this arrangement. We do not
even require that all channels be of the same length. We only require that for a fixed compartment
type, the total incoming edge weight from other compartments of the same type as well as the
incoming edge weight from other compartments of the opposite type is the same regardless of the
choice of compartment.

First, we assume that there exists a steady-state solution of the form

\[ X^* = \begin{bmatrix} X_A^* \\ X_B^* \end{bmatrix} = \begin{bmatrix} \mathbb{1}_{N_A} & 0 \\ 0_{N_B} & \mathbb{1}_{N_B} \end{bmatrix} \begin{bmatrix} x_A^* \\ x_B^* \end{bmatrix} =: Mx^* \]

where \( x_A, x_B \in \mathbb{R} \) as in Section 33.2. \( X^* \) a steady-state solution implies that

\[ (-L + \gamma X I_N) X^* = \begin{bmatrix} \nu X I_A \mathbb{1}_{N_A} \\ 0_{N_B} \end{bmatrix} \]

\[ \implies (-L + \gamma X I_N) Mx^* = M \begin{bmatrix} \nu X I_A^* \\ 0 \end{bmatrix} \] (18)

since there cannot be multiple values of \( I_A^* \) that result in the same \( X_A^* \). Now define

\[ L = \begin{bmatrix} L_{11} & L_{12} \\ L_{21} & L_{22} \end{bmatrix} \]

where \( L_{11} \in \mathbb{R}^{N_A \times N_A} \), \( L_{12} = L_{21}^T \in \mathbb{R}^{N_A \times N_B} \), and \( L_{22} \in \mathbb{R}^{N_B \times N_B} \), and observe that

\[ (-L + \gamma X I_N) M = \begin{bmatrix} \sum_{j=1}^{N_A} [L_{11}]_j + \gamma X I_A & \sum_{j=1}^{N_B} [L_{12}]_j \\ \sum_{j=1}^{N_A} [L_{21}]_j & \sum_{j=1}^{N_B} [L_{22}]_j + \gamma X I_B \end{bmatrix} \] (19)

where \([L_{nm}]_j\) denotes the \( j \)th column of \( L_{nm}, n, m = 1, 2 \). Now define

\[ Q_{nm}^i = \begin{cases} -\sum_{j=1}^{N_A} [L_{nm}]_{ij}, & m = 1 \\ -\sum_{j=1}^{N_B} [L_{nm}]_{ij}, & m = 2 \end{cases} \]

Combining (19) with (18) yields

\[ \begin{cases} (Q_{11}^a + \gamma X) x_A^* + (Q_{12}^a) x_B^* = \nu X I_A^*, & a = 1, 2, \ldots, N_A \\ (Q_{21}^b) x_A^* + (Q_{22}^b + \gamma X) x_B^* = 0, & b = N_A + 1, \ldots, N_A + N_B \end{cases} \] (20)

and similarly for \( Y^* \),

\[ \begin{cases} (Q_{11}^b + \gamma Y) y_A^* + (Q_{12}^b) y_B^* = 0, & a = 1, 2, \ldots, N_A \\ (Q_{21}^b) y_A^* + (Q_{22}^b + \gamma Y) y_B^* = \nu Y I_B^*, & b = N_A + 1, \ldots, N_A + N_B \end{cases} \] (21)

Suppose we fix \( a \) and \( b \) and construct a system of four equations from (20) and (21). In these
four equations there are four “unknowns” \((Q_{11}^a, Q_{12}^a, Q_{21}^b, Q_{22}^b)\), suggesting that there is a unique solution to

\[ \begin{bmatrix} x_A^* & x_B^* & 0 & 0 \\ 0 & 0 & x_A^* & x_B^* \\ y_A^* & y_B^* & 0 & 0 \\ 0 & 0 & y_A^* & y_B^* \end{bmatrix} \begin{bmatrix} Q_{11}^a \\ Q_{12}^b \\ Q_{21}^b \\ Q_{22}^b \end{bmatrix} = \begin{bmatrix} \nu X I_A^* - \gamma X x_A^* \\ -\gamma X x_B^* \\ -\gamma Y y_A^* \\ \nu Y I_B^* - \gamma Y y_B^* \end{bmatrix} \] (22)
provided that the LHS matrix is full rank, i.e., $x^*_A x^*_B \neq \frac{y^*_A}{y^*_B}$. This unique solution is the same regardless of the choice of $a$ and $b$, which implies that $Q^a_{11}, Q^a_{12}$ must be the same for all $a$ and $Q^b_{21}, Q^b_{22}$ must be the same for all $b$ (since the solution is unique). This, in turn, implies that we can write

$$LM = M \begin{bmatrix} Q^{11} & Q^{12} \\ Q^{21} & Q^{22} \end{bmatrix} =: M\bar{L}.$$

The only fact left to show, then, is that our system cannot admit a solution for which $x^*_A \neq \frac{y^*_A}{y^*_B}$, which will guarantee a unique solution to (22). Our proof leverages the lateral inhibition structure of the system.

Suppose $x^*_A = \frac{y^*_A}{y^*_B}$ and without loss of generality, define $r := \frac{x^*_A}{y^*_A} = \frac{x^*_B}{y^*_B}$. Substituting $x^*_A = ry^*_A$ and $x^*_B = ry^*_B$ into (20) and (21) for fixed $a,b$ (equivalently (22)), we obtain

$$\begin{align*}
(Q^a_{11} + \gamma X)x^*_A + Q^a_{12}x^*_B &= \nu X I^*_A \\
(Q^a_{11} + \gamma Y)x^*_A + Q^a_{12}x^*_B &= 0 \\
Q^b_{21}x^*_A + (Q^b_{22} + \gamma X)x^*_B &= 0 \\
Q^b_{21}x^*_A + (Q^b_{22} + \gamma Y)x^*_B &= r\nu Y I^*_B
\end{align*}$$

The assumption that every compartment of type A is connected to at least one cell of type B and vice versa guarantees that $Q^a_{12} \neq 0, Q^b_{21} \neq 0$. Rearranging terms, we find

$$\begin{cases}
(\gamma Y - \gamma X)x^*_B = r\nu Y I^*_B \\
(\gamma X - \gamma Y)x^*_A = \nu Y I^*_A
\end{cases}.$$  \hspace{1cm} (23)

Now we know $\gamma X, \gamma Y, \nu X, \nu Y > 0$ and since $x^*_A, x^*_B, I^*_A, I^*_B \geq 0$ (we cannot have negative concentrations) we also know $r \geq 0$. Because of the mutual inhibition relationship we further know that $I^*_A := h_1(rx^*_A)$ is a bounded, nonnegative, nonincreasing function of $x^*_A$ and $I^*_B := h_2(x^*_B)$ is a bounded, nonnegative, nonincreasing function of $x^*_B$. If we are concerned with a nontrivial system we must have $r \neq 0$ because otherwise

$$r = 0 \implies x^*_A = 0 \implies I^*_A = 0 \implies h_1(0) = 0 \implies h_1(z \geq 0) = 0 \forall z,$$

i.e., cells of type A are insensitive to inputs. Similarly, we must have $r$ finite because we could as easily have set up the system in terms of $y^*_A$ and $y^*_B$ with $\frac{1}{r}$ as the ratio of interest, and following the same logic we would have (with slight abuse of notation) $\frac{1}{r} \neq 0$.

Therefore, since $r > 0$, then from (23), nonnegative $x^*_A, I^*_B$ requires $\gamma Y \geq \gamma X$ while nonnegative $x^*_A, I^*_A$ requires $\gamma X \geq \gamma Y$. These two conditions can only be satisfied if $\gamma X = \gamma Y$, which implies that $I^*_A = I^*_B = 0$. But this is impossible from the definition of $h_1, h_2$ in (2) (except in the limit as $R^*_{a1}, R^*_{b1} \to \infty$, which is anyway unattainable because $R^*_{a1}, R^*_{b1}$ are bounded). Hence we cannot have a steady-state contrasting solution for which $x^*_A x^*_B = \frac{y^*_A}{y^*_B}$, implying that the matrix in (22) will have a unique solution. This completes our claim.

5.2 Local (in)stability of full system about reduced system steady states

Let $Z \in \mathbb{R}^{6N}_+$ be the vector of states for the full system where $\dot{Z} = f(Z)$ governed by the equations in Section 11.2. Let $Z^*$ be a steady state where all cells of the same type have identical states, i.e.,
a steady state identified in the reduced system. Assume the states are ordered such that

\[
Z^* := \begin{bmatrix}
Y^* \\
m_{RA}^* I_{NA} \\
R_{A}^* I_{NA} \\
m_{IA}^* I_{NA} \\
I_{A}^* I_{NA} \\
X^* \\
m_{RB}^* I_{NB} \\
R_{B}^* I_{NB} \\
m_{IB}^* I_{NB} \\
I_{B}^* I_{NB}
\end{bmatrix}.
\]

Define

\[
K_{A1} := \left. \frac{\partial \dot{m}_{RA}}{\partial Y_A} \right|_{Y_A^*} = V_{RA} N_{RA} C \frac{n_Y \left( \frac{Y_A^*}{K_Y} \right)^{n_Y - 1}}{K_Y \left( 1 + \left( \frac{Y_A^*}{K_Y} \right)^{n_Y} \right)^2}
\]

\[
K_{A2} := -\left. \frac{\partial \dot{m}_{IA}}{\partial R_A} \right|_{R_A^*} = V_{IA} N_{IA} C \frac{n_{RA} \left( \frac{R_A^*}{K_{RA}} \right)^{n_{RA} - 1}}{K_{RA} \left( 1 + \left( \frac{R_A^*}{K_{RA}} \right)^{n_{RA}} \right)^2}.
\]

\(K_{B1}\) and \(K_{B2}\) are defined analogously with the appropriate subscripts for cell B. Then the Jacobian of the full system evaluated at the steady state \(Z^*\) is given by

\[
J = \begin{bmatrix}
S_A & P_Y \\
P_X & S_B
\end{bmatrix}
\]

where \(J \in \mathbb{R}^{6N \times 6N}\), \(S_A \in \mathbb{R}^{N+4N_A \times N+4N_A}\), \(S_B \in \mathbb{R}^{N+4N_B \times N+4N_B}\), \(P_Y \in \mathbb{R}^{N+4N_A \times N+4N_B}\), and \(P_X \in \mathbb{R}^{N+4N_B \times N+4N_A}\), defined as

\[
S_A := \begin{bmatrix}
L - \gamma_Y I_N & 0 & 0 & 0 & 0 \\
-K_{A1} I_{NA} & 0 & -\gamma_{m_{RA}} I_{NA} & 0 & 0 \\
0 & \epsilon_{RA} I_{NA} & -\gamma_{RA} I_{NA} & 0 & 0 \\
0 & 0 & -K_{A2} I_{NA} & -\gamma_{m_{IA}} I_{NA} & 0 \\
0 & 0 & 0 & \epsilon_{IA} I_{NA} & -\gamma_{IA} I_{NA}
\end{bmatrix}
\]

\[
P_Y := \begin{bmatrix}
0_{N \times N+3N_B} & 0_{N \times N+3N_B} \\
0_{N_A \times N+3N_B} & 0_{N_A \times N+3N_B}
\end{bmatrix}
\]

\[
P_X := \begin{bmatrix}
0_{N \times N+3N_A} & 0_{N \times N+3N_A} \\
0_{N_B \times N+3N_A} & 0_{N_B \times N+3N_A}
\end{bmatrix}
\]

\[
S_B := \begin{bmatrix}
L - \gamma_X I_N & 0 & 0 & 0 & 0 \\
-K_{B1} I_{NB} & 0 & -\gamma_{m_{RB}} I_{NB} & 0 & 0 \\
0 & \epsilon_{RB} I_{NB} & -\gamma_{RB} I_{NB} & 0 & 0 \\
0 & 0 & -K_{B2} I_{NB} & -\gamma_{m_{IB}} I_{NB} & 0 \\
0 & 0 & 0 & \epsilon_{IB} I_{NB} & -\gamma_{IB} I_{NB}
\end{bmatrix}
\]
The matrix measure of a matrix $M$ with respect to the one-norm is defined as

$$\mu(M) := \max_j \left\{ M_{jj} + \sum_{i \neq j} |M_{ij}| \right\}. $$

If there exists an invertible diagonal matrix $D$ such that

$$\mu(DJD^{-1}) < 0, \quad (25)$$

then the mapping described by $J$ is contractive. This implies that the eigenvalues are negative, which for our nonlinear system means that the steady state around which $J$ is linearized is locally stable.

Consider the reduced version of the full system, which has a Jacobian $\bar{J}$ identical in form to (24) for $N_A = 1$, $N_B = 1$, and $L = \bar{L}$ as defined in Section 33.2:

$$\bar{L} := \begin{bmatrix} -d_A & d_A \\ d_B & -d_B \end{bmatrix}$$

where $d_A$ is the total (nonnegative) outgoing edge weight for a cell of type A and $d_B$ is the total (nonnegative) outgoing edge weight for a cell of type B. Let $\bar{D}$ take the form

$$\bar{D} = diag (l_{1A}, l_{1B}, a_1, a_2, a_3, a_4, l_{2A}, l_{2B}, b_1, b_2, b_3, b_4) \quad (26)$$

where $l_{1A}, l_{1B}, a_1, a_2, a_3, a_4, l_{2A}, l_{2B}, b_1, b_2, b_3, b_4 \in \mathbb{R}$ are arbitrary constants. For $\bar{D}$ to satisfy (25), we require

$$-\gamma_Y + \frac{a_1}{l_{1A}} K_{A1} - d_A + \frac{l_{1B}}{l_{1A}} d_A < 0$$

$$-\gamma_Y - d_B + \frac{l_{1A}}{l_{1B}} d_B < 0$$

$$-\gamma_{mR_A} + \frac{a_2}{a_1} \epsilon_R A < 0$$

$$-\gamma_{R_A} + \frac{a_3}{a_2} K_{A2} < 0$$

$$-\gamma_{mI_A} + \frac{a_4}{a_3} \epsilon_I A < 0$$

$$-\gamma_{I_A} + \frac{l_{2A}}{a_4} \nu_X < 0$$

and analogously for columns corresponding to compartments B.

We can combine the inequalities to obtain a single expression if we rearrange them to reflect
the relative sizes of certain constants. Then we obtain

\[
\frac{a_1 K_{A1} + l_{1B} d_A}{\gamma Y + d_A} < l_{1A}
\]

(27)

\[
l_{1A} < \frac{\gamma Y + d_B l_{1B}}{d_B}
\]

(28)

\[
a_1 < \frac{\gamma R_A}{K_{A1}} a_2
\]

\[
a_2 < \frac{\gamma m_{R_A} A_1}{\nu_X}
\]

\[
a_3 < \frac{\gamma R_A}{K_{A2}} a_2
\]

\[
a_4 < \frac{\gamma m_{I_A} A_3}{\nu_I}
\]

\[
l_{2A} < \frac{\gamma (Y a_4)}{\nu_X}
\]

We can rearrange (27) and (28) as

\[
a_1 < \frac{\gamma Y (\gamma Y + d_B + d_A)}{K_{A1} d_B}
\]

whereupon the combination of inequalities yields

\[
l_{2A} < \frac{\gamma I_A \gamma m_{I_A} \gamma R_A \gamma m_{R_A} \gamma Y (\gamma Y + d_B + d_A)}{\nu_X \epsilon I_A K_{A2} \epsilon R_A K_{A1} d_B} l_{1B} =: \frac{1}{C_1} l_{1B}
\]

Similarly, for compartments B,

\[
l_{1B} < \frac{\gamma B \gamma m_{I_B} \gamma R_B \gamma m_{R_B} \gamma X (\gamma X + d_B + d_A)}{\nu_Y \epsilon I_B K_{B2} \epsilon R_B K_{B1} d_A} l_{1A} =: \frac{1}{C_2} l_{2A}
\]

such that

\[
l_{2A} < \frac{1}{C_1 C_2} l_{2A} \implies C_1 C_2 < 1.
\]

Incidentally,

\[
C_1 C_2 = \left( \frac{\epsilon I_A K_{A2}}{\gamma I_A \gamma m_{I_A}} \right) \left( \frac{\epsilon R_B K_{B1}}{\gamma R_B \gamma m_{R_B}} \right) \left( \frac{d_B \nu_X}{\gamma X (\gamma X + d_A + d_B)} \right) \times \left( \frac{\epsilon I_B K_{B2}}{\gamma I_B \gamma m_{I_B}} \right) \left( \frac{\epsilon R_A K_{A1}}{\gamma R_A \gamma m_{R_A}} \right) \left( \frac{d_A \nu_Y}{\gamma Y (\gamma Y + d_A + d_B)} \right)
\]

can be written as

\[
\left( \frac{d I_A^*}{d R_{A_i}^*} \right) \left( \frac{d X_B^*}{d I_A^*} \right) \left( \frac{d R_{A_0}^*}{d X_B} \right) \left( \frac{d Y_A^*}{d I_A} \right) \left( \frac{d R_{A_0}^*}{d Y_A} \right) = \frac{d R_{A_0}^*}{d R_{A_i}^*}, \quad (29)
\]

where \( R_{A_i}^* \) is a constant input and \( R_{A_0}^* \) is the associated output. Hence (29) is the slope of the I/O system evaluated at the steady state \( R_{A_i}^* = R_A^* \), which matches the graphical stability test developed in Section 22.2.
Now consider the full system with Jacobian (24) corresponding to the reduced system for which \( D \) satisfies (25). We construct a \( D \) for the full system that has diagonal entries

\[
[l_1A_{N_A}, l_1B_{I_{N_A}}, a_1I_{N_A}, a_2I_{N_A}, a_3I_{N_A}, a_4I_{N_A}, l_2A_{I_{N_B}}, l_2B_{I_{N_B}}, b_1I_{N_B}, b_2I_{N_B}, b_3I_{N_B}, b_4I_{N_B}]
\]

(30)

where the constants are the same as for \( \tilde{D} \). \( DJD^{-1} \) is then effectively organized into “blocks” that correspond to columns of \( DJD^{-1} \). Let

\[ m_j(M) := M_{jj} + \sum_{i \neq j} |M_{ij}|, \]

i.e., \( \mu(M) = \max_j m_j(M) \). Because \( J \) is linearized about a reduced-system steady state, it is straightforward to see that

\[
m_j(J) = \begin{cases} 
m_1(J), & j = 1, 2, \ldots, N_A \\
m_2(J), & j = N_A + 1, N_A + 2, \ldots, N \\
m_3(J), & j = N + 1, N + 2, \ldots, N + N_A \\
m_4(J), & j = N + N_A + 1, \ldots, N + 2N_A \\
m_5(J), & j = N + 2N_A + 1, \ldots, N + 3N_A \\
m_6(J), & j = N + 3N_A + 1, \ldots, N + 4N_A \\
m_7(J), & j = N + 4N_A + 1, \ldots, N + 5N_A \\
m_8(J), & j = N + 5N_A + 1, \ldots, 2N + 4N_A \\
m_9(J), & j = 2N + 4N_A + 1, \ldots, 3N + 3N_A \\
m_{10}(J), & j = 3N + 3N_A + 1, \ldots, 4N + 2N_A \\
m_{11}(J), & j = 4N + 2N_A + 1, \ldots, 5N + N_A \\
m_{12}(J), & j = 5N + N_A, \ldots, 6N 
\end{cases}
\]

(Note that the equivalence of \( m_j(J) \) to \( m_1(J) \), \( m_2(J) \), \( m_7(J) \), and \( m_8(J) \) for appropriate \( j \) arises out of the form of \( L \), which has diagonal entries \( -d_A \) and \( -d_B \) repeated \( N_A \) and \( N_B \) times respectively, with non-diagonal entries summing to \( d_A \) for the first \( N_A \) columns and \( d_B \) for the last \( N_B \) columns.)

Because \( m_j(J) < 0 \) for \( j = 1, 2, \ldots, 12 \), then \( m_j(J) < 0 \) for \( j = 1, 2, \ldots, 6N \). Therefore for \( J \) linearized about a given reduced-system steady state, the conditions required for \( J \) to be contractive—and therefore for the full system to be locally stable at the steady state—is the same as the graphical condition for the reduced system to be stable or unstable at that steady state, namely, that the slope of the I/O system must be less than 1. Conversely, if (29) is greater than 1, the steady state in the reduced-system subspace is unstable and so the full system (which contains the reduced-system subspace) cannot be stable. Therefore the graphical test developed in Section 33.2 for the stability or instability of steady states in the reduced system is sufficient to determine the local stability or instability of corresponding steady states in the full system.

Note that when \( J \) is a contractive map, the steady state is locally exponentially stable because the real parts of all eigenvalues are negative, therefore analysis as in chapter 9 of [12] ensures that sufficiently small perturbations to system behavior will not destroy the stability of the steady state.
Lastly, this proof only suffices to show local stability of the steady state in the full system. Other solutions representing different spatial patterns may exist and be stable in the full system even though the system reduction as performed herein would not identify them. Equivalently, the fact that the bistable steady state is global in the reduced system but only local in the full system exemplifies how introducing more compartments enlarges the space of possible solutions beyond those that exist in lower-dimensional regimes. Exploring the rich, complex range of behaviors for high-dimensional systems is a challenging and fascinating avenue for future research.

6 Channel length determination

To mimic close-range interaction of lateral inhibition with the diffusion-based system, a PDE model of AHL production, degradation, and diffusion is used to optimize $l$ that allows sufficient diffusion of AHL to the immediate neighbors ($[\text{AHL}] \geq K_d$ at $\Delta x = l$) while not sufficient diffusion between nonadjacent compartments ($[\text{AHL}] < K_d$ at $\Delta x \geq 2l$). Let $\theta(t)$ to be AHL concentration over time at the center of an AHL-producing colony ($x = 0$). During the exponential growth ($t \leq 10h$), the concentration of AHL can be expressed using parameters defined in Table 3 as:

$$
\dot{\theta}(t) = \nu I^* P_0 \exp(\mu t) - (\gamma + \frac{D}{l_{res}^2}) \theta(t)
$$

$$
\rightarrow s\Theta(s) = \nu I^* P_0 \frac{1}{s - \mu} - (\gamma + \frac{D}{l_{res}^2}) \Theta(s)
$$

$$
\Leftrightarrow \Theta(s) = \nu I^* P_0 \frac{1}{(s - \mu)(s + \gamma + \frac{D}{l_{res}^2})} \left[ \exp(\mu t) - \exp(-(\gamma + \frac{D}{l_{res}^2})t) \right]
$$

(31)

where $P_0$ is the initial population size of the colony and $l_{res}$ is the length of channels that connect compartments to reservoir (Section 11.1).

Now, consider diffusion of AHL across a channel. Define $[\text{AHL}](x,t) = \theta(x,t)$ in one-directional, infinite length of channel, with following boundary conditions:

$$
\left\{ \begin{array}{l}
\theta(x,0) = 0, \forall x, \\
\theta(0,t) = \nu I^* P_0 \frac{1}{\mu + \gamma + \frac{D}{l_{res}^2}} \left[ \exp(\mu t) - \exp(-(\gamma + \frac{D}{l_{res}^2})t) \right] , 0 \leq t \leq 10h, \\
\theta(\infty,t) = 0, \forall t.
\end{array} \right.
$$

(32)

Assume the degradation of AHL in the channel is negligible. Then, from Fick’s second law,

$$
\frac{\partial \theta}{\partial t} = D \frac{\partial^2 \theta}{\partial x^2}
$$

$$
\rightarrow s\Theta - 0 = D \frac{\partial^2 \Theta}{\partial x^2}
$$

$$
\Leftrightarrow \Theta(x,s) = A \exp\left(\sqrt{s \frac{\mu}{D}}\right) + B \exp\left(-\sqrt{s \frac{\mu}{D}}\right)
$$
Using boundary conditions Eq. 32,

\[ \Theta(x, s) = \nu I^* P_0 \frac{1}{(s - \mu)(s + \gamma + \frac{D}{\mu_{res}})} \exp(-\sqrt{s/D} \cdot x). \]

\[ \Leftrightarrow \Theta(x, s) = \nu I^* P_0 \frac{1}{\mu + \gamma + \frac{D}{\mu_{res}}} \left( \frac{\exp(-\sqrt{s/D} \cdot x)}{s - \mu} - \frac{\exp(-\sqrt{s/D} \cdot x)}{s + \gamma + \frac{D}{\mu_{res}}} \right). \] (33)

Since there is no simple solution to inversely transform Eq. 33, instead, we will find an upper bound of AHL concentration and use that constant to be the boundary conditions.

Let \( t \leq \tau_f, \)

\[ \theta(0, t) \leq \theta(0, \tau_f) = \nu I^* P_0 \frac{1}{\mu + \gamma + \frac{D}{\mu_{res}}} \left[ \exp(\mu \tau_f) - \exp(-\gamma + \frac{D}{\mu_{res}} \cdot \tau_f) \right] \]

\[ \Leftrightarrow \frac{\nu I^* P_0}{\mu + \gamma + \frac{D}{\mu_{res}}} \left[ \exp(\mu \tau_f) - \exp(-\gamma + \frac{D}{\mu_{res}} \cdot \tau_f) \right] \]

then we get

\[ \Leftrightarrow \theta(0, t) \leq \nu I^* P_0 \frac{1}{\mu + \gamma + \frac{D}{\mu_{res}}} \left[ \exp(\mu \tau_f) - \exp(-\gamma + \frac{D}{\mu_{res}} \cdot \tau_f) \right] \cdot \text{erfc}\left(\frac{x}{2\sqrt{D} t}\right). \] (34)

Using the upper-bound-concentration, we will determine the optimum channel length for short communication time (\( T_{1/2} \) defined as time for a compartment to reach half concentration of the center) between the adjacent colonies while long the communication time between the non-adjacent compartments. \( T_{1/2} \) at location \( x \) can be calculated as

\[ \frac{x}{2\sqrt{D T_{1/2}}} = \text{erfc}^{-1}(0.5) \]

thus,

\[ T_{1/2} = \frac{1}{4D (\text{erfc}^{-1}(0.5))^2}. \] (35)

The objective function and the constraints can be set as follows:

\[ \min_L T_{1/2}(x = l) + (\tau_f - T_{1/2}(x = 2l)), \]

such that

\[ \theta_{C_6}(L, \tau_f) \geq K_{d_{C_6 \rightarrow A}}, \]
\[ \theta_{C_6}(L, \tau_f) < K_{d_{C_6 \rightarrow B}}, \]
\[ \theta_{C_6}(2L, \tau_f) < K_{d_{C_6 \rightarrow A}}, \]
\[ \theta_{C_{12}}(L, \tau_f) \geq K_{d_{C_{12} \rightarrow B}}, \]
\[ \theta_{C_{12}}(L, \tau_f) < K_{d_{C_{12} \rightarrow A}}, \]
\[ \theta_{C_{12}}(2L, \tau_f) < K_{d_{C_{12} \rightarrow B}}. \]
where the functions and parameters represent

\[ [3\text{OC6HSL}](t) = \theta_{C_6}(x,t), \]
\[ [3\text{OC12HSL}](t) = \theta_{C_{12}}(x,t), \]
\[ t = [0, \tau_f] \text{ is the time period when cells are in the exponential growth}, \]
\[ K^{C_6 \rightarrow A}_d \text{ is the dissociation constant of 3OC6HSL on } plux, \]
\[ K^{C_{12} \rightarrow A}_d \text{ is the cross-talk dissociation constant of 3OC12HSL on } plux, \]
\[ K^{C_{12} \rightarrow B}_d \text{ is the dissociation constant of 3OC12HSL on } plas, \]
\[ K^{C_6 \rightarrow B}_d \text{ is the cross-talk dissociation constant of 3OC6HSL on } plas. \]

Using parameter values in Table 3, the optimum channel length is determined to be 4.5 mm \( \leq l \leq \) 9mm (Fig.5A).
**Table 1:** Biochemical species (state space variables) whose behavior is represented by system ODEs. Brackets denote concentration.

| Variable | Description |
|----------|-------------|
| $X_A$    | 3OC12HSL in A (produced by A) |
| $X_B$    | 3OC12HSL in B (diffused from A) |
| $Y_A$    | 3OC6HSL in A (diffused from B) |
| $Y_B$    | 3OC6HSL in B (produced by B) |
| $m_{RA}$ | tetR mRNA in A |
| $m_{RB}$ | tetR mRNA in B |
| $R_A$    | TetR in A |
| $R_B$    | TetR in B |
| $m_{lA}$ | lasI mRNA in A |
| $m_{lB}$ | luxI mRNA in B |
| $l_A$    | LasI in A |
| $l_B$    | LuxI in B |
Table 2: Parameter definitions and values used in the toy system with even parameters, i.e., corresponding parameters between types A and B are equal.

| Parameter | Description | Value |
|-----------|-------------|-------|
| $D_{X/Y}$ | diffusion constant of AHLs | $5 \times 10^{-4}$ |
| $l$       | length of channels | $5 \times 10^{-5}$ |
| $d$       | edge weight constant | $\frac{D}{l^2}$ |
| $\mu$     | dilution due to growth | 0.1 |
| $\gamma_{X/Y}$ | decay rate of AHL + drainage | 0.01 |
| $\nu_{X/Y}$ | production rate of AHLs | 1 |
| $V_{RA/AB}$ | transcription rate of tetR | 1 |
| $N_{RA/AB}$ | copy number of tetR | 1 |
| $C$       | concentration constant | 1 |
| $K_d$     | AHL dissociation constant | 10 |
| $n_{X/Y}$ | Hill coefficient for AHL | 2 |
| $l_{RA/AB}$ | leakiness of plux/plas | 0 |
| $\gamma_{mRA/AB}$ | decay rate of tetR mRNA | 0.105 |
| $\epsilon_{RA/AB}$ | translation rate of TetR | 1 |
| $\gamma_{RA/AB}$ | decay rate of TetR | $\mu$ |
| $K_{RA/AB}$ | dissociation constant of TetR to ptet | 10 |
| $n_{RA/AB}$ | Hill coefficient for TetR to ptet | 4 |
| $l_{IA/AB}$ | leakiness of ptet | 0 |
| $\gamma_{mIA/AB}$ | decay rate of lasI/luxI mRNA | 0.01 |
| $\epsilon_{IA/AB}$ | translation rate of LasI/LuxI | 1 |
| $\gamma_{IA/AB}$ | decay rate of LasI/LuxI | $\mu$ |
Table 3: Parameter definitions and values used in the ODEs for an experimental implementation using the orthogonal QS systems LuxI/LuxR and LasI/LasR.

| Parameter | Description | Value | Reference |
|-----------|-------------|-------|-----------|
| $D$       | diffusion constant of AHLs | $4.9 \times 10^{-4} \text{ mm}^2 \text{ s}^{-1}$ | [6] |
| $l$       | length of channels | 4.5 mm | Section 6 |
| $d$       | edge weight constant | $2.42 \times 10^{-5}$ | $\frac{D}{l^2}$ |
| $\lambda$ | doubling time for *E. coli* | $1.65 \times 10^3 \text{ s}$ | $\log_2 \frac{A}{X}$ |
| $\mu$     | dilution rate due to growth | $4.20 \times 10^{-4} \text{ s}^{-1}$ | $\log_2 \frac{A}{X}$ |
| $\gamma_X$| decay rate of 3OC12HSL | $3.07 \times 10^{-5} \text{ s}^{-1}$ | [11] |
| $\gamma_Y$| decay rate of 3OC6HSL | $4.77 \times 10^{-6} \text{ s}^{-1}$ | [11] |
| $\nu_X$   | production rate of 3OC12HSL | $1 \times 10^{-5} \text{ s}^{-1}$ | Fig.9 |
| $\nu_Y$   | production rate of 3OC6HSL | $8 \times 10^{-5} \text{ s}^{-1}$ | Fig.9 |
| $V_{RA}$  | max transcription rate from *plux* | 0.0308 s$^{-1}$ | [9] |
| $V_{RB}$  | max transcription rate from *plas* | 0.0238 s$^{-1}$ | Fig.7 |
| $N$       | plasmid pSC101* copy number | 4 copies | [14] |
| $C$       | concentration constant | 1 | - |
| $K_X$     | apparent $K_d$ of *plas* | 47.5 nM | Fig.7 |
| $K_Y$     | apparent $K_d$ of *plux* | 8.33 nM | Fig.7 |
| $n_X$     | apparent Hill coefficient of *plas* | 1.83 | Fig.7 |
| $n_Y$     | apparent Hill coefficient of *plux* | 1.91 | Fig.7 |
| $l_{RA}$  | apparent leakiness of *plux* | 0.0208 | Fig.7 |
| $l_{RB}$  | apparent leakiness of *plas* | 0.0261 | Fig.7 |
| $\epsilon_R$ | translation rate of TetR | 0.0320 $\text{ s}^{-1}$ | [7] |
| $\gamma_R$ | decay rate of TetR | $3.07 \times 10^{-4} \text{ s}^{-1}$ | [1] |
| $V_{IA}$  | max transcription rate of *lasI* | 0.0690 s$^{-1}$ | [9] |
| $V_{IB}$  | max transcription rate of *luxI* | 0.0722 s$^{-1}$ | [9] |
| $K_R$     | $K_d$ of TetR for *ptet* | 30 nM | $K_{[TetR-\text{O}]} \cdot \frac{[\text{aTc}]}{K_{[\text{aTc-TetR}]}^\text{ptet}}$ |
| $n_R$     | Hill coefficient of *ptet* | 2 | Fig.8 |
| $l_{IA/B}$ | leakiness of *ptet* | $1.98 \times 10^{10^{-4}}$ | [14] |
| $\gamma_{mRA/B}$ | decay rate of mRNA | 0.002 s$^{-1}$ | [5] |
| $\epsilon_{IA}$ | translation rate of LasI | 0.0718 s$^{-1}$ | [7] |
| $\epsilon_{IB}$ | translation rate of LuxI in B | 0.0751 s$^{-1}$ | [7] |
| $\gamma_{IA/B}$ | decay rate of LasI or LuxI | $4.20 \times 10^{-4} \text{ s}^{-1}$ | $\mu$ |
| $K_{[\text{aTc-TetR}]}$ | $K_d$ of TetR and *aTc* | 0.098 nM | [16] |
| $K_{[\text{TetR-\text{O}]}]$ | $K_d$ of TetR and *tet* operator | 0.27 nM | Estimated from [4] |
Figure 1: Modularized representation of the two-compartment system. Individual modules are represented by boxes, and their steady-state responses can be experimentally characterized. The subscript A or B indicates whether the box represents an event in cell type A or B. The “transmission modules” $tx_{A \rightarrow B}$ and $tx_{B \rightarrow A}$ encapsulate AHL synthesis and diffusion. The “reception modules” $rx_A$ and $rx_B$ encapsulate TetR production activated by cognate AHL. The “repression modules” $H_A$ and $H_B$ encapsulate AHL synthase production repressed by TetR. $Y$ is the vector denoting concentrations of 3OC6HSL, and $X$ denotes 3OC12HSL concentrations. $Rs$ denote intracellular TetR concentrations. $Is$ denote intracellular AHL synthase concentrations.
Figure 2: Network of the biochemical species in the two-compartment system. Sharp arrows indicate activation, or positive parity; flat arrows indicate inhibition/repression, or negative parity. A cycle is any undirected sequence of edges and nodes beginning at one element and ending at the same element (i.e., ignoring direction of arrows). The parity of a cycle is the parity of the product of the signs of all edges traversed to complete the cycle. Since every cycle in the graph is positive in parity, the closed-loop system is monotone. To ensure that the corresponding open-loop system is strongly monotone we require a directed path (i.e., following the arrows) to exist between the input node and every other node, and between every node and the output node. The input and output nodes are determined by where the feedback loop is broken (see Section 22.2). In this system every element is reachable from every other element, so these conditions will be satisfied regardless of where the cycle is broken. Hence the open-loop (input-output) system is also monotone.
Figure 3: Kinetic rates determine whether the cross-repressive switches operate in the appropriate regime to turn each other on and off. Changes in relative output ranges between the two on/off switches for different values of (a) $a_{IA} := \frac{\gamma_{IA} V_{RA} \gamma_{m}}{\epsilon_{IA}}$ and (b) the leakiness of $plux (l_{RA})$. Remaining parameters are as given in Table 2. The intersections between two transfer functions $X_B \rightarrow Y_A$ and $Y_A \rightarrow X_B$ indicate the steady states of the full system. As $a_{IA}$ scales, so does the maximum and minimum output of LasI, which geometrically translates the composite transfer function $Y_A \rightarrow X_B$. Increasing the leakiness increases the minimum output of LasI, which decreases the dynamic range of the composite transfer function $Y_A \rightarrow X_B$. Leakiness reduces ultrasensitivity of $Y_A \rightarrow X_B$, which leads to loss of bistability. The translational movement of $Y_A \rightarrow X_B$ causes mismatched tuning between $X_B \rightarrow Y_A$ and $Y_A \rightarrow X_B$ also leads to loss of bistability.
Figure 4: Schematic qualitatively identifying parameter changes that lead to bifurcation in two-strain circuits in isogenic (parameters identical between strains) and nonisogenic (parameters differ between strains) cases. Yellow background identifies systems that form contrasting patterns. In a balanced system, the cross-representative compartments exhibit identical transfer functions ($T_A(\cdot) = T_B(\cdot)$), while in an unbalanced system, the transfer functions differ. Monostable isogenic populations are homogeneous while nonisogenic populations exhibit contrast in the one-to-one geometry. Geometry may introduce imbalance or offset the biochemical difference, depending on the arrangement. Contrast in balanced systems is only attainable through bistability.
Figure 5: PDE simulation for DLI device design to optimize the inter-compartmental channel length ($l$) and the channel length connecting a compartment and reservoir ($l_{res}$). (A) Graphical representation of constraints on $l$. The inter-compartmental channel length should allow sufficient AHL diffusion in directly adjacent compartments while keeping the AHL concentration in the second closest compartments (distance $2l$) to be below the threshold ($K_{d}$ of AHL-inducible promoters). A constraint can be represented as an area under or above a function, and the $l$ values where all areas overlap indicates appropriate channel length range. The smallest $l$ value satisfying the criteria is optimized for shortest communication time, while largest $l$ value is optimized for longest non-neighbor communication time. $4.5\text{mm} < l < 9 \text{mm}$ satisfies the criteria with the experimentally evaluated parameters given in Table 3. (B) Characterization of $l_{res}$ length and decay time. The channel length connecting a compartment and a reservoir adds extra decaying mechanism for AHL by allowing AHL efflux from every compartment to the bulk solid medium of $AHL \simeq 0$. The efflux diffusion rate from the compartment to medium depends on the length of the channel. The left plot shows the portion of AHL diffused out with respect to time, normalized to the AHL concentration inside the compartment at $t = 0$. The right plot shows the time constant it takes for a portion of the AHL concentration inside the compartment to diffuse outside with respect to the channel length.
Figure 6: Sketch of DLI device preparation. (A) PDMS molds are attached to the bottom of a culture plate and 3.4 mL of 1.5 % agarose-mixed medium is poured into the devices. After solidifying, 0.5 µL of 2.0 OD600 cells are pipetted at the center of each compartment and allowed to grow for 10-12 hours in room temperature to study pattern formation. (B) Various spatial configurations are prepared as PDMS molds. A central compartment with different number of neighbors can be manufactured.
Figure 7: Plate reader assays of output steady-state TetR-sfGFP fluorescence in response to input AHL concentration for the reception modules of Strains A and B in liquid (A) and solid (B) medium. The strains used for reception module characterization lack *ptet* → *luxI* or *lasI*. Steady-state sfGFP fluorescence (t = 10 h) divided by OD600 in liquid medium and steady-state sfGFP fluorescence (t = 10 h) at the center of colonies on solid medium were each normalized to the maximum steady-state values across strains and input AHL conditions. Strain A receiver showed similar threshold AHL concentrations regardless of medium and Strain B receiver showed more AHL-sensitive response in solid than in liquid medium. In both conditions, the approximated $K_d$ of Strain A reception module was higher than Strain B, suggesting higher sensitivity of *plas* in Strain B than *plux* in Strain A. Error bars show standard deviation and solid circles show the average of the measurement (n = 2). The solid curves show best-fit models when measurements were fit to activation Hill functions in the form of $a \frac{[AHL]}{[AHL] + K_d^n}$, where $a$ denotes the maximal production of TetR-sfGFP, $K_d$ denotes the apparent dissociation constant of AHL binding to the promoter and $n$ denotes the apparent Hill coefficient. The following equation parameters were used for the best-fit models: $a = 1$ (Strain A) and 0.771 (Strain B), $K_d = 47.5 \text{ nM}$ (A) and 8.33 \text{ nM}$ (B), $n = 1.91$ (A) and 1.83 (B) for liquid medium, $a = 0.9423$ (A) and 0.6269 (B), $K_d = 34.71 \text{ nM}$ (A) and 0.577 nM (B), n=4 1.088 (A) and 1.556 (B) for solid medium.
Figure 8: Plate reader assays of output $ptet \rightarrow mRFP1$ fluorescence in response to input TetR-sfGFP fluorescence for the repression modules of Strains A ($A$) and B ($B$). The strains used for repression module characterization contain $ptet$ driving $mRFP1$ instead of AHL synthases. Steady-state sfGFP fluorescence and steady-state mRFP1 fluorescence ($t = 10\ h$) were divided by OD600 and then each fluorescence was normalized to the maximum steady-state values across all conditions within the same strain. Different levels of input TetR-sfGFP were induced by AHL to repress mRFP1. As the leaky expression of $tetR-sfGFP$ caused significant repression of mRFP1 even in the absence of any AHL, a range of aTc was added to the medium to sequester basal level of TetR. Re-measurement of mRFP1 fluorescence showed a broad output range when at least 3.1 ng/mL aTc was added to medium. Unexpectedly, aTc also repressed TetR-sfGFP production in Strain A at high concentrations, which constrained viable aTc concentration to be less than 10 ng/mL. Solid circles show the average and error bars show standard deviation of the measurements ($n = 2$). The solid curve represents the best-fit model when the measurements were fit to repressive Hill function in the form of $\frac{1}{1 + x_{max} \left[{TetR}/[K_{Tc}],\frac{[aTc]}{K_{aTc}}\right]^n}$, where $K_{aTc} = 0.098\ nM$, $K_{aTc} = 0.27\ nM$ as determined in Table 3. The resulting fit had a range of Hill coefficient between 1.7 to 2.8 and $x_{max}$ ranged from 1100 nM to 1621 nM across different aTc concentrations.
Figure 9: Plate fluorimeter assay of AHL production and reception by Strains A and B. On a well of 3.4 mL of MOPS EZ Rich solid medium with 5 ng/mL aTc, the complete strains with different ptet promoter strength [15] were seeded at the center, surrounded by receiver strains (Strain A receiver in horizontal direction and Strain B receiver in vertical direction). The top row is seeded with Strain A variants at the center and the bottom row is seeded with Strain B variants at the center. The promoter strength of ptet seems to have little effect on AHL production, as receiver strains showed similar levels of activation regardless of promoter variants within the same strain type.
Figure 10: Single-cell measurements reveals variability in QS promoter activity at various subsaturating AHL concentrations. Each histogram in the stacked plot represents steady-state sfGFP (t = 8 hour) at indicated AHL concentration. The reporter fluorescence showed bimodal distribution in monocultures of Strains A and B at medium levels of AHL. As more external AHL was added, the population fraction with the high fluorescent mode increased. In coculture, most cells belonged to the high fluorescent mode fraction, implying higher AHL concentration in medium compared to the monocultures. This further supports the conclusion that cross-repression in coculture amplified the initial bias in the AHL concentration at steady state. Additionally, Strain B showed highly heterogeneous reporter expression even in a population fraction with the high fluorescence mode.
Figure 11: A plate fluorimeter image of the 1:1 spatial configuration devices with channel length of 9 mm. The image was taken after 12 hours of growth in room temperature. *indicates pre-induced strains with 1 µM AHL and † indicates strains that were biased to be fluorescent by externally added AHL in medium. 9 mm channel length was too long to establish communication between the adjacent compartments, which deviated from the expectation in Section 6. Positive controls were prepared by mixing either 1 µM 3OC6HSL or 3OC12HSL in solid medium on the right-most devices in order to bias the gene expression pattern to be either Strain A fluorescent or Strain B fluorescent. The deviation is possibly caused by approximation of PDE solution using the upper bound AHL concentration.
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