Quorum-Sensing Synchronization of Synthetic Toggle Switches: A Design based on Monotone Dynamical Systems Theory

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Supporting Information

SI-1 Toggle B2

Kobayashi et al [1] consider a number of genetic toggle switches, interfaced with a QS signaling pathway. Specifically, their *E. coli* strain “B2” (Fig. SI-1.1) detects as well as produces (through the synthetase encoded by the expressed gene luxI, which converts common precursor metabolites) acyl-homoserine lactone (AHL) signaling molecules. AHL is a QS signaling pathway from *Vibrio fischeri*. Functionally, toggle B2 enables an *E. coli* population to measure population density through AHL, because AHL signaling can be reversibly transported to the medium via diffusion, contributing to the AHL density in the culture [1]. To achieve an in-depth understanding of dynamic properties of coupled QS and toggle constructs, Kuznetsov et al. [2] developed and studied a mechanistic mathematical model of a population (or, equivalently, an ensemble) comprising $N$ toggles, see Fig. 1 (bottom panel), corresponding to Toggle B2. Their study revealed important multiple functions, namely bistability as well as stable oscillations, that an ensemble of Toggles B2 was capable of exhibiting. Analytical conditions for bistability were found, and a time separation was introduced to obtain a stable limit cycle for a population of interacting cells.

In bistable circuits (toggles), transitions such as those caused by fluctuations due to low copy numbers of species per cell, or due to local environmental “noise” can force individual cells to change expression state at random [1]. This noise effect can spontaneously lead to the emergence of heterogeneous (mixed) populations consisting of cells in different expression states, which appear as bimodal population distributions when the corresponding protein levels are measured [1]. To investigate the effect of a spontaneous toggle switching in single and coupled cellular systems, leading to bimodal population distributions, Wang et al. [3] developed models for a single cell and a multi-cellular toggle system comprising $N$ cells, respectively. In their models, the dynamics of the repressor proteins LacI and $\lambda CI$ is described by the two ODE equations developed in [4]. The AI-interfacing employed in the population model [3] corresponds to a signaling pathway which is slightly different from the signaling pathway in Toggle B2.
Figure SI-1.1. Toggle B2: Density-Dependent Gene Activation. Notations and abbreviations. Plasmids: pTSM$b^2$, pCIR$b$ and pAHL$a$; genes: gfp, cI857, lacI, luxR, and luxI; promoters: $P_{trc}$, $P_{L^*}$, and $P_{lux}$; AHL, acyl-homoserine lactone. Figure adapted from Fig. 6(A) in [1]. (Fig. SI-1.1) as suggested in [1] and described earlier. The main difference is in the description of the expression of the gene that encodes LuxI, see [3] for more details.

SI-2 Model Derivation

Here, we describe the main assumptions and steps used to derive mass-balance equations for the S- and A-models formulated in the main text.

SI-2.1 Mass-Balance Equations

The derivation of the S- and A-models includes the development of two modules:

I. A transcription-translation module describing biosynthesis of repressor proteins.

II. A metabolic module describing biosynthesis of autoinducers.

A general and systematic discussion of both modules can be found in [5, 6]. The derivation of the first module for the A-model, relevant to our work, is given in [2]. Because one of our modeling objectives is to ultimately describe how the analysis of the mathematical models can be mechanistically interpreted in terms of tuning synthetic toggle “dials” by implementable experimental interventions as reviewed in [7], including modifications of ribosome-binding sites (RBS), carboxy-terminal tags, etc., [4, 8, 9], we will derive mass-balance equations at the level of molecular detail sufficient to suggest plausible modeling predictions.
A transcription-translation module can be described by a basic two-stage model \([2,5,6]\).

\[
\frac{dn_x}{dt} = n_A k_x - r_x n_x, \quad \text{(SI-2.1a)}
\]

\[
\frac{dn_X}{dt} = k_X n_x - r_X n_X. \quad \text{(SI-2.1b)}
\]

Here, \(n_x\) is the number of mRNA transcripts per cell for gene \(x\), and \(n_X\) is the number of protein molecules per cell; \(n_A\) is the number of active promoters from which the mRNA of gene \(x\) is transcribed at an average rate \(k_x\); \(k_X\) is the averaged translation rate; \(r_x\) and \(r_X\) are the effective first-order rate constants associated with degradation of the mRNA and proteins, respectively.

Since mRNA molecules are usually degraded rapidly compared to other cellular processes, a quasi-steady state for the equation (SI-2.1a) can often be assumed \([2]\), yielding

\[
\frac{dn_x}{dt} = \frac{n_A k_x}{r_x}. \quad \text{(SI-2.2)}
\]

Using (SI-2.2) in the right-hand side of the equation (SI-2.1b), we obtain

\[
\frac{dn_X}{dt} = k_X \frac{k_x n_A}{r_x} - r_X n_X. \quad \text{(SI-2.3)}
\]

The ratio \(b_x = k_X/r_x\) in equation (SI-2.3) is called a burst parameter of the protein X \([2]\). Using \(b_x\) in (SI-2.3) yields

\[
\frac{dn_X}{dt} = b_x k_x n_A - r_X n_X. \quad \text{(SI-2.4)}
\]

Assuming log-phase growth of \(E.\ coli\), the volume \(V(t)\) of the growing bacterium can be approximated by the expression \(V(t) = V_0 \exp(\lambda t)\), and equation (SI-2.4) can be rewritten in a concentration form,

\[
\frac{d[X]}{dt} = b_x k_x [P_A] - (r_X + \mu) [X]. \quad \text{(SI-2.5)}
\]

Here, \([X](t) = n_X(t)/V(t)\) and \([P_A](t) = n_A(t)/V(t)\).

The concentration of active promoters, \([P_A]\), can be computed, using an appropriate Hill function \([6]\). For example, we use

\[
[P_{YA}] = \frac{[P_Y]}{1 + ([Y]/K_Y)^n_Y} \quad \text{(SI-2.6)}
\]

for the repressor protein Y binding to the promoter P\(_Y\) with the dissociation constant \(K_Y^{n_Y}\). In (SI-2.6), \([P_Y]\) is the total concentration of all promoters P\(_Y\), while \([P_{YA}]\) is the concentration of active promoters not bound with the repressor protein Y. Recall that the cooperativity described by the Hill exponent \(n_Y\) can arise from \([4–6]\):

(i). Multimerization of repressor proteins;

(ii). Cooperative binding of repressor multimers to multiple operator sites in the promoter.

Analogously, we use a Hill-function

\[
[P_{GA}] = [P_G] \frac{([G]/K_G)^n_G}{1 + ([G]/K_G)^n_G} \quad \text{(SI-2.7)}
\]
for the autoinducer G binding to the promoter $P_G$ with the dissociation constant $K_{G}^{G}$ and the Hill exponent $n_G$. The concentration of all active promoters $P_A$ can now be obtained from (SI-2.6) and (SI-2.7) as

$$P_A = P_{YA} + P_{GA}. \quad (SI-2.8)$$

Using expression (SI-2.8), the equation (SI-2.5) can finally be updated as

$$\frac{d[X]}{dt} = \frac{b_x k_x[P_Y]}{1 + ([Y]/K_Y)^n_Y} + \frac{b_x k_x[P_G]}{1 + ([G]/K_G)^n_G} - (r_X + \mu) [X]. \quad (SI-2.9)$$

Here, all parameters are described below in Table SI-3.2.

Similar mass balanced equations can be derived for the repressor protein Y, and synthases U and W. For example, the mass balance equation for the synthase U is

$$\frac{d[U]}{dt} = \frac{b_u k_u[P_Y]}{1 + ([Y]/K_Y)^n_Y} - (r_U + \mu) [U]. \quad (SI-2.10)$$

Analogously, we can write down a mass balance equation for the autoinducer concentration $G$, that is, [C14-HSL], governed by the synthase U (CinI),

$$\frac{d[G]}{dt} = k_G [U] + D_G (G_e - G) - (r_G + \mu) [G]. \quad (SI-2.11)$$

Here, $k_G$ is the maximum production rate of C14-HSL by CinI (Table SI-3.4), $D_G$ is the export rate of C14-HSL (Table SI-3.4), and $[G_e]$ is the extracellular concentration of C14-HSL.

Assuming that the concentration of the enzyme U reaches its quasi-steady state rapidly [10], one can obtain from (SI-2.10) that

$$[U] = \frac{b_u k_u [P_Y]}{r_U + \mu} \times \frac{[P_Y]}{1 + ([Y]/K_Y)^n_Y}. \quad (SI-2.12)$$

Using (SI-2.12) in the equation (SI-2.11) yields

$$\frac{d[G]}{dt} = \frac{b_u k_u k_G}{r_U + \mu} \times \frac{[P_Y]}{1 + ([Y]/K_Y)^n_Y} - (r_G + \mu) [G]. \quad (SI-2.13)$$

Here, the definitions and the values of all parameters are given in Table SI-3.4. A similar mass balanced equation can be derived for the second autoinducer R (C4-HSL), and we omit the details.

### SI-2.2 Nondimensionalization

To nondimensionalize mass balance equations, as for example, the mass balance equations (SI-2.9) and (SI-2.13), we use the following dimensionless state variables, which are similar to those introduced in [2],

$$t' = (r_d + \mu) t, \quad x = \frac{[X]}{K_X}, \quad y = \frac{[Y]}{K_Y}, \quad g = \frac{[G]}{K_G}, \quad g_e = \frac{[G_e]}{K_G}, \quad r = \frac{[R]}{K_R}, \quad r_e = \frac{[R_e]}{K_R}. \quad (SI-2.14)$$
Here, we assume that all protein degradation rates can be set experimentally so that the following equalities can be obtained approximately [8],

\[ r_X = r_X = r_U = r_W = r_d = \frac{\ln 2}{\tau_{1/2}}, \quad \tau_{1/2} = 4 \text{ min.} \]  

(SI-2.15)

The procedure of setting all protein degradation rates or, equivalently, all protein half-lives approximately equal to a prescribed value close to mRNA half-lives [8] is required to balance the toggle [4]. We discuss the balancing procedure and relevant experimental interventions in Sect. SI-2.2.

Using the dimensionless variables (SI-2.14), all original modeling mass balances can be nondimensionalized, yielding the S- and A-models formulated in the main text, where the prime is dropped from dimensionless time \( \tau' \). In this case, dimensional and dimensionless parameters are related to one another as:

1. For the dimensionless rates, we obtain:

\[ a_1 = \frac{b_x k_x [P_Y]}{K_X (r_d + \mu)}, \quad a_2 = \frac{b_y k_y [P_X]}{K_Y (r_d + \mu)}, \quad a_3 = \frac{b_x k_x [P_G]}{K_X (r_d + \mu)}, \quad a_4 = \frac{b_y k_y [P_R]}{K_Y (r_d + \mu)}, \quad a_5 = \frac{b_u k_u k_G [P_Y]}{K_G (r_d + \mu)^2}, \quad a_6 = \frac{b_w k_w k_R [P_X]}{K_R (r_d + \mu)^2}. \]  

(SI-2.16a)

2. For dimensionless diffusion and degradation parameters, we obtain:

\[ d_g = \frac{D_G}{r_d + \mu}, \quad d_r = \frac{D_R}{r_d + \mu}, \quad \delta_g = \frac{r_G + \mu}{r_d + \mu}, \quad \delta_r = \frac{r_R + \mu}{r_d + \mu}, \quad \delta_e = \frac{\mu e}{r_d + \mu}. \]  

(SI-2.17a)

Molecular and biophysical parameter values used in the expressions (5) - (6) will be estimated in Sect. SI-2.2 while dimensionless parameters will be estimated in Sect. SI-2.2. In this section, we only mention that due to [8], we can set

\[ K_X = K_Y = 40 \text{ monomers per cell.} \]  

(SI-2.18)

We could not find any estimation of values for the two parameters \( K_G \) for C14-HSL and \( K_R \) for C4-HSL in the literature despite the fact that more and more precise measurements of kinetic parameters become available [11]. We estimate the order of magnitude of \( K_G \) and \( K_R \) as follows.

In the detailed experimental results on the C4-HSL-mediated quorum sensing regulatory system of the opportunistic Gram-negative bacterium \( Aeromonas \) hydrophila, the concentration of C4-HSL was found to be of order of magnitude equal to 10 \( \mu \)M [12]. In \( E. coli \) biology, it is convenient to use nM units [13], because relative to the effective \( E. coli \) volume [14], the value of 1 nM corresponds to one molecule per cell. This fact is widely used in the literature [8]. Therefore, the above estimate of 10 \( \mu \)M corresponds to \( 10^4 \) C4-HSL signaling molecules per cell.

Another ad-hoc rule of \( E. coli \) biology used in a number of studies with the Cornell \( E. coli \) computer model [15-18], resulting in a number of relevant predictions such as ribosomal-protein limitations, lac-control, plasmid stability, and etc. [14][19][24], is that, the coarse-grained estimation for the dissociation equilibrium constant to be used in the Hill function can be calculated as 25% of the intracellular modifier (reference) concentration. In our case, this yields a coarse-grade estimate of \( 0.25 \times 10^4 \) C4-HSL signaling molecules per cell,

\[ K_G = K_R = 2.5 \times 10^3 \text{ molecules per cell.} \]  

(SI-2.19)

The values for other parameters will be estimated below.
SI-2.3 Toggle Balancing (Symmetrization)

As it was observed experimentally \[4,8\], synthetic circuits can operate and deliver the engineered traits only if special molecular constrains are fulfilled,

(i) repressor protein half-lives are close to mRNA halflifes \[8\];

(ii) repressor protein half-lives are approximately equal \[4,8\].

Constrain \(\text{(ii)}\) is required for “balancing” the given circuit \[4\]. Moreover, both works suggest experimental interventions to fulfill the above constraints \([4,8]\). Such and similar interventions are termed a “tuning dials” in the review \[7\].

It is mathematically convenient for us to generalize the above balancing procedure by the procedure of “symmetrization” of two antagonistic, mutually repressing toggle subsystems by selecting synthetic (tuned) parameter values that would make two antagonist subsystems symmetric to one another. In other words, we assume that an ideal S toggle has mirror symmetry corresponding to permutations between the two antagonistic subsystems. Symmetry usually helps with analytical analysis of nonlinear mathematical models.

Specifically, we “symmetrize” (balance) biosynthesis kinetic rates, using constraints

\[
a_1 = a_2, \quad a_3 = a_4 \quad \text{and} \quad a_5 = a_6. \tag{SI-2.20}
\]

Appropriate molecular interventions, which can be used to set the relationships \(\text{SI-2.20}\) approximately under certain experimental conditions, are reviewed in \([7]\). Similarly, we symmetrize “diffusion” parameters,

\[
d_g = d_r = d, \tag{SI-2.21}
\]

and the autoinducer “degradation” or “utilization” (“load”) parameters,

\[
\delta_g = \delta_r = \delta_e = \delta. \tag{SI-2.22}
\]
SI-3 Estimation of Parameter Values

SI-3.1 Toggle Parameters

In our estimations, we use general biochemical calculations \[25,26\]. First, we collect generic prokaryotic and specific \textit{E. coli} parameter values in Table SI-3.1.

Table SI-3.1. Generic prokaryotic and \textit{E. coli} specific values of model parameters

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_m\text{RNA}$</td>
<td>Rate of transcription by RNA polymerase in prokaryotes</td>
<td>80</td>
<td>bp/sec</td>
<td>[27]</td>
</tr>
<tr>
<td>$\tau_m\text{RNA}$</td>
<td>Typical half-life time for 80% of genes in \textit{E. coli}</td>
<td>3 - 8</td>
<td>min</td>
<td>[28]</td>
</tr>
<tr>
<td>$r_{aa}$</td>
<td>Rate of translation by the ribosome in prokaryotes</td>
<td>20</td>
<td>aa/sec</td>
<td>[27]^a</td>
</tr>
<tr>
<td>$k_P$</td>
<td>Rate of translation by the ribosome in prokaryotes</td>
<td>1.71</td>
<td>sec$^{-1}$</td>
<td>estimated in [SI-3.3]</td>
</tr>
<tr>
<td>$T$</td>
<td>\textit{E. coli} replication period under specific nutrition conditions</td>
<td>25</td>
<td>min</td>
<td>[14]</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Intracellular specific dilution rate due to \textit{E. coli} cell growth</td>
<td>$\ln 2/T$</td>
<td>min$^{-1}$</td>
<td>[14]</td>
</tr>
<tr>
<td>$\mu_e$</td>
<td>Extracellular dilution rate due to flow</td>
<td>0.1</td>
<td>min$^{-1}$</td>
<td>[29]</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Total volume fraction of cells in chamber</td>
<td>0.8</td>
<td></td>
<td>[30]</td>
</tr>
<tr>
<td>$N$</td>
<td>Number of \textit{E. coli} cells in an overnight population culture</td>
<td>$10^9$ (OD$_{600} = 1$)</td>
<td>cells/ml</td>
<td>[14]</td>
</tr>
</tbody>
</table>

^a This estimate is smaller than the estimate 33 aa/sec used in [11].

A general rate of translation of protein P in prokaryotes ($k_P$). Suppose that an mRNA transcript of protein P contains $n_{aa}$ amino acids. Then, for one ribosome to transcribe P from its mRNA transcript, assuming a translation rate of 20 amino acids per second (Table SI-3.1), it will take time

$$t_P = \frac{n_{aa}}{20}. \quad (SI-3.1)$$

The above estimates yields the rate per ribosome which is

$$\frac{1 \text{ molecule}}{t_P} = \frac{20}{n_{aa}} \text{ sec}^{-1}. \quad (SI-3.2)$$

Given that the coding region of protein P is $n_{aa} \times 3$ nucleotides long, and that a ribosome can attach every 35 nucleotides, we can estimate that $n_{aa} \times 3/35$ ribosomes can be attached per mRNA molecule. We, thus, obtain

$$k_P = \frac{20}{n_{aa}} \times \frac{n_{aa} \times 3}{35} = 1.71 \text{ protein molecules sec}^{-1}. \quad (SI-3.3)$$

To illustrate our parameter estimation procedure, we derive parameter values for the Lac-repressor subsystem only. Parameter values for all other subsystems can be derived similarly.

$k_x$ : Fully induced strength of promoters $P_Y$ ($P_{tet}$) and $P_G$. One $lacI$ mRNA transcript is 1204 bases long (Table SI-3.2). To transcribe one molecule of $lacI$ mRNA from one gene with a rate of 80 bases per second (Table SI-3.2) takes

$$\frac{1204 \text{ bases}}{80 \text{ bases/sec}} = 15.05 \text{ sec.} \quad (SI-3.4)$$
Table SI-3.2. Parameter values of the LacI-repressor subsystem.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Transcription:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$L_{\text{lacI}}$ Size of $\text{lacI}$ gene</td>
<td>1204</td>
<td>bp</td>
<td>[31]</td>
</tr>
<tr>
<td></td>
<td>$\Gamma_x$ Repressed strength of promoter $P_{\text{tet}}$</td>
<td>$5.0 \times 10^{-4}$</td>
<td>molecules/cell/sec</td>
<td>[8]</td>
</tr>
<tr>
<td></td>
<td>$k_x$ Fully induced transcription rate$^a$</td>
<td>$6.65 \times 10^{-2}$</td>
<td>species/cell/sec</td>
<td>estimated in (SI-3.5)</td>
</tr>
<tr>
<td></td>
<td>$K_Y$ The number of TetR to repress$^b$ $P_{\text{tet}}$</td>
<td>40</td>
<td>monomers/cell</td>
<td>estimated in (SI-2.18)</td>
</tr>
<tr>
<td></td>
<td>$K_G$ The number of C14-HSL to activate C14-HSL</td>
<td>$2.5 \times 10^3$</td>
<td>molecules/cell</td>
<td>estimated in (SI-2.19)</td>
</tr>
<tr>
<td></td>
<td>$\tau_{1/2,\text{lacI}}$ Half-life of $\text{lacI}$ mRNA</td>
<td>3.8</td>
<td>min</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td>$r_x$ Rate of $\text{lacI}$ mRNA degradation</td>
<td>$3.04 \times 10^{-3}$</td>
<td>sec$^{-1}$</td>
<td>estimated in (SI-3.7)</td>
</tr>
<tr>
<td></td>
<td>$n_Y$ The number of subunits in TetR</td>
<td>2</td>
<td></td>
<td>[33]</td>
</tr>
<tr>
<td></td>
<td>$n_G$ Hill coefficient of C14-HSL</td>
<td>3</td>
<td></td>
<td>a reference value$^d$</td>
</tr>
<tr>
<td></td>
<td><strong>Translation:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$L_{\text{LacI}}$ Size of one subunit in tetrameric LacI</td>
<td>360</td>
<td>aa/subunit</td>
<td>[34]</td>
</tr>
<tr>
<td></td>
<td>$k_X$ Rate of LacI translation</td>
<td>1.71</td>
<td>molecules/cell/sec</td>
<td>estimated in (SI-3.3)</td>
</tr>
<tr>
<td></td>
<td>$\tau_{1/2,\text{LacI}}$ Half-life of LacI protein</td>
<td>4</td>
<td>min</td>
<td>[8]</td>
</tr>
<tr>
<td></td>
<td>$r_X$ Rate of LacI degradation</td>
<td>$1.16 \times 10^{-3}$</td>
<td>sec$^{-1}$</td>
<td>estimated in (SI-3.7)</td>
</tr>
</tbody>
</table>

*$^a$ Fully induced strength of promoters $P_{\text{tet}}$ and $P_{\text{cin}}$.

*$^b$ For the sake of brevity, “to repress” means “to half-maximally repress.”

*$^c$ Fully induced strength of promoters $P_{\text{tet}}$ and $P_{\text{cin}}$.

*$^d$ For the sake of brevity, “to activate” means “to half-maximally activate.”

Then, per gene the estimate (SI-3.4) yields

$$k_x = \frac{1}{15.05 \text{ sec}} = 6.65 \times 10^{-2} \text{ lacI mRNA (molecules/cell/sec)}.$$  (SI-3.5)

The estimate (SI-3.4) is one order of magnitude less than the estimate 0.5 lacI mRNA (molecules/cell/sec) provided in [8].

**$r_x$ : Rate of lacI mRNA degradation.** The calculation of degradation rates for proteins is based on the known protein half-lives,

$$r_x = \frac{\ln 2}{t_{1/2}}.$$  (SI-3.6)

We obtain (Table SI-3.2),

$$r_x = \frac{\ln 2}{3.8 \times 60 \text{ sec}} = 3.04 \times 10^{-3} \text{ sec}^{-1}.$$  (SI-3.7)

**$r_X$ : Rate of LacI (X) degradation.** We obtain (Table SI-3.2),

$$r_d = r_X = \frac{\ln 2}{4 \times 60 \text{ sec}} = 2.89 \times 10^{-3} \text{ sec}^{-1}.$$  (SI-3.8)

We use the estimate (SI-3.8) for all proteins in the model.
### Table SI-3.3. Parameter values of the TetA-repressor subsystem.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transcription:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$L_{tetR}$</td>
<td>Size of tetR gene</td>
<td>905</td>
<td>bp</td>
<td></td>
</tr>
<tr>
<td>$\Gamma_y$</td>
<td>Repressed strength of promoter $P_{lac}$</td>
<td>$5 \times 10^{-4}$</td>
<td>molecules/cell/sec</td>
<td></td>
</tr>
<tr>
<td>$k_y$</td>
<td>Fully induced transcription rate$^a$</td>
<td>$8.87 \times 10^{-2}$</td>
<td>molecules/cell/sec</td>
<td></td>
</tr>
<tr>
<td>$K_X$</td>
<td>The number of LacI to repress$^b$ $P_{lac}$</td>
<td>40</td>
<td>monomers/cell</td>
<td></td>
</tr>
<tr>
<td>$K_R$</td>
<td>The number of C4-HSL to activate$^c$ $P_{rhl}$</td>
<td>$5 \times 10^4$</td>
<td>monomers/cell</td>
<td></td>
</tr>
<tr>
<td>$\tau_{1/2,tetR}$</td>
<td>Half-life of tetR mRNA</td>
<td>0.5</td>
<td>min</td>
<td></td>
</tr>
<tr>
<td>$r_y$</td>
<td>Rate of tetR mRNA degradation</td>
<td>$2.31 \times 10^{-2}$</td>
<td>sec$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$n_X$</td>
<td>The number of subunits in LacI</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n_R$</td>
<td>Hill coefficient of C4-HSL</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Translation:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$L_{TetR}$</td>
<td>Size of one subunit in tetrameric TetR</td>
<td>207</td>
<td>aa/subunit</td>
<td></td>
</tr>
<tr>
<td>$k_Y$</td>
<td>Rate of TetR translation</td>
<td>1.71</td>
<td>molecules/cell/sec</td>
<td></td>
</tr>
<tr>
<td>$\tau_{1/2,TetR}$</td>
<td>Half-life of TetR protein</td>
<td>4</td>
<td>min</td>
<td></td>
</tr>
<tr>
<td>$r_Y$</td>
<td>Rate of TetR degradation</td>
<td>$1.16 \times 10^{-3}$</td>
<td>sec$^{-1}$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Fully induced strength of promoters $P_{lac}$ and $P_{tet}$.

$^b$For the sake of brevity, “to repress” means “to half-maximally repress.”

$^c$For the sake of brevity, “to activate” means “to half-maximally activate.”

$^d$Equilibrium dissociation constant for LacI is $7.7 \times 10^{-8}$ M $^{40}$.

### Table SI-3.4. Parameter values of the 3-OH-C14-HSL/CinI-signaling subsystem.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transcription:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$L_{cinI}$</td>
<td>Size of cinI gene$^a$</td>
<td>663</td>
<td>bp</td>
<td>estimated</td>
</tr>
<tr>
<td>$\Gamma_u$</td>
<td>Repressed strength of promoter $P_{lac}$</td>
<td>$5 \times 10^{-4}$</td>
<td>molecules/cell/sec</td>
<td></td>
</tr>
<tr>
<td>$k_u$</td>
<td>Fully induced strength of promoter $P_{tet}$</td>
<td>$13.27 \times 10^{-2}$</td>
<td>molecules/cell/sec</td>
<td></td>
</tr>
<tr>
<td>$\tau_{1/2,cinI}$</td>
<td>Half-life of cinI mRNA</td>
<td>6.6</td>
<td>min</td>
<td>arbitrary$^b$</td>
</tr>
<tr>
<td>$r_u$</td>
<td>Rate of cinI mRNA degradation</td>
<td>$1.75 \times 10^{-3}$</td>
<td>sec$^{-1}$</td>
<td></td>
</tr>
<tr>
<td><strong>Translation:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$L_{CinI}$</td>
<td>Size of CinI aa-sequence</td>
<td>221</td>
<td>aa</td>
<td><a href="http://string-db.org">http://string-db.org</a></td>
</tr>
<tr>
<td>$k_U$</td>
<td>Rate of CinI translation</td>
<td>1.71</td>
<td>molecules/cell/sec</td>
<td></td>
</tr>
<tr>
<td>$\tau_{1/2,CinI}$</td>
<td>Half-life$^a$ of CinI protein</td>
<td>4</td>
<td>min</td>
<td></td>
</tr>
<tr>
<td>$r_U$</td>
<td>Rate of protein CinI degradation</td>
<td>$1.16 \times 10^{-3}$</td>
<td>sec$^{-1}$</td>
<td></td>
</tr>
<tr>
<td><strong>Signaling:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_G$</td>
<td>Maximal production rate of CinI</td>
<td>2</td>
<td>min$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$r_G$</td>
<td>Degradation rate of C14-HSL</td>
<td>0.002</td>
<td>hr$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$D_G$</td>
<td>Export rate of C14-HSL</td>
<td>2.1</td>
<td>min$^{-1}$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$The coding region of the gene has been estimated from its protein sequence size provided in the same table as $221 \times 3 = 663$.

$^b$This estimate corresponds to a general (or typical) pattern for mRNA half-lives in *E. coli* $^{44}$. Note that $^8$ use a generic half-life parameter value of 2 min.

$^c$Data for 3-OH-C12-HSL is used.
Table SI-3.5. Parameter values of the C4-HSL/RhlI-signaling subsystem.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transcription:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$L_{rhlI}$</td>
<td>Length of $rhlI$ gene</td>
<td>603</td>
<td>bp</td>
<td>[45]</td>
</tr>
<tr>
<td>$\Gamma_w$</td>
<td>Repressed strength of promoter $P_{lac}$</td>
<td>$5 \times 10^{-4}$</td>
<td>molecules/cell/sec</td>
<td>estimated in Table SI-3.2</td>
</tr>
<tr>
<td>$k_w$</td>
<td>Fully induced strength of promoter $P_{lac}$</td>
<td>$13.27 \times 10^{-2}$</td>
<td>molecules/cell/sec</td>
<td>estimated</td>
</tr>
<tr>
<td>$\tau_{1/2,rhlI}$</td>
<td>Half-life of $rhlI$ mRNA</td>
<td>6.6</td>
<td>min</td>
<td>[46]</td>
</tr>
<tr>
<td>$r_w$</td>
<td>Rate of $lacI$ mRNA degradation</td>
<td>$1.75 \times 10^{-3}$</td>
<td>sec$^{-1}$</td>
<td>estimated</td>
</tr>
<tr>
<td><strong>Translation:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$L_{LacI}$</td>
<td>Length of RhlI protein aa-sequence</td>
<td>196</td>
<td>aa</td>
<td>[47]</td>
</tr>
<tr>
<td>$k_W$</td>
<td>Rate of RhlI translation</td>
<td>1.71</td>
<td>molecules/cell/sec</td>
<td>estimated in Table SI-3.2</td>
</tr>
<tr>
<td>$\tau_{1/2,RhlI}$</td>
<td>Half-life of RhlI protein</td>
<td>4</td>
<td>min</td>
<td>estimated in Table SI-3.2</td>
</tr>
<tr>
<td>$r_X$</td>
<td>Rate of $LacI$ mRNA degradation</td>
<td>$1.16 \times 10^{-3}$</td>
<td>sec$^{-1}$</td>
<td>estimated</td>
</tr>
<tr>
<td><strong>Signaling:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_R$</td>
<td>Maximal production rate of C4-HSL by RhlI</td>
<td>16</td>
<td>min$^{-1}$</td>
<td>[41]</td>
</tr>
<tr>
<td>$r_R$</td>
<td>Degradation rate of C4-HSL</td>
<td>0.02</td>
<td>hr$^{-1}$</td>
<td>[42]</td>
</tr>
<tr>
<td>$D_R$</td>
<td>Export rate of C4-HSL</td>
<td>3.0</td>
<td>min$^{-1}$</td>
<td>[43]</td>
</tr>
</tbody>
</table>

*The half-life data for lasI mRNA is used because the degradation of rhlI is positively regulated by LasI [46] and, so, could have a longer half-life. This estimate is in line with a general (or typical) pattern for mRNA half-lives in *E. coli* [44]. Note that [8] use a genetic half-life parameter value of 2 min (Table SI-3.4).*

**SI-3.2 Dimensionless parameter values**

Using data from Table SI-3.1 and the estimate SI-3.8, we obtain

$$ \mu = \frac{\ln 2}{25 \times 60} = 0.46 \times 10^{-3} \text{sec}^{-1}, \quad r_d + \mu = 3.35 \times 10^{-3} \text{sec}^{-1}. \quad (SI-3.9) $$

To estimate rates $a_i, i = 1 \ldots 6$, defined in (5), we assume that the equalities $b_x = b_y = b_u = b_w = 10$ can be approximately set by using RBS-related interventions [7]. Also, to avoid competition for ribosomes, only a few plasmids bearing promoters $P_X, P_Y, P_G$, and $P_R$ can be used. By selecting $[P_X] = [P_Y] = [P_G] = [P_R] = 1$ copies per cell, we obtain

$$ a_1 = a_3 = \frac{10 \times (6.65 \times 10^{-2}) \times 1}{40 \times (3.35 \times 10^{-3})} = 4.96 \approx 5, \quad (SI-3.10a) $$

$$ a_2 = a_4 = \frac{10 \times (8.87 \times 10^{-2}) \times 2}{40 \times (3.35 \times 10^{-3})} = 6.61 \approx 7, \quad (SI-3.10b) $$

$$ a_5 = a_6 = \frac{10 \times (13.27 \times 10^{-2}) \times (2/60) \times 2}{(2.5 \times 10^3) \times (3.35 \times 10^{-3})^2} = 3.15 \approx 3. \quad (SI-3.10c) $$

Next, from (6), we obtain

$$ d_g = \frac{2.1/60}{3.35 \times 10^{-3}} = 10.44, \quad (SI-3.11a) $$

$$ d_r = \frac{3/60}{3.35 \times 10^{-3}} = 14.40, \quad (SI-3.11b) $$

$$ \delta_g = \delta_r \approx \frac{\mu}{r_d + \mu} = \frac{0.46 \times 10^{-3}}{3.35 \times 10^{-3}} = 0.14, \quad (SI-3.11c) $$

$$ \delta_e = \frac{0.1/60}{3.35 \times 10^{-3}} = 0.50. \quad (SI-3.11d) $$
We find the estimated values of the parameters to be of the same order of magnitude as the corresponding parameter values estimated and used in \cite{2, 4, 8, 35, 37}. Not enough is yet known about molecular interactions inside host cells to obtain highly precise descriptions \cite{7}; it is common to computationally evaluate the effect of different values for rate parameters and even for Hill exponents \cite{2, 4, 8}. Following \cite{2, 4, 8}, where genetic circuits built from similar elements have been studied, we have explored sets of parameter values which are close to the estimates given in (SI-3.10) and (SI-3.11), which ensure bistability in both S- and A-models, see the main text.
SI-4 Alternative Definitions of Monotone Systems and Order Preservation

We defined monotonicity using graph partitions because this is the easiest way to present the concept. However, the usual definition found in textbooks is not phrased in that form. We explain here how our definition is equivalent to the usual one as well as to another property. For further remarks on these equivalences, see [48]. A signed graph (such as the species influence graph obtained by looking at signs of Jacobian entries) \( G \) is said to be balanced (see Harary [49]) if every undirected closed loop in the graph \( G \) has a net positive sign, that is to say, an even number, possibly zero, of negative arrows. Equivalently, any two (undirected) paths between two nodes must have the same net sign. By undirected loops or paths, we mean that one is allowed to transverse an edge either forward or backward. A spin assignment \( \Sigma \) for the graph \( G \) is an assignment, to each node \( v_i \), of a number \( \sigma_i \) equal to “+1” or “−1” (a “spin,” to borrow from statistical mechanics terminology). If there is an edge from node \( v_j \) to node \( v_i \), with label \( J_{ij} \in \{±1\} \), we say that this edge is consistent with the spin assignment \( \Sigma \) provided that:

\[
J_{ij}\sigma_i\sigma_j = 1
\]

which is the same as saying that \( J_{ij} = \sigma_i\sigma_j \), or that \( \sigma_i = J_{ij}\sigma_j \). An equivalent formalism is that in which edges are labeled by “0” or “1,” instead of 1 and −1 respectively, and edge labels \( J_{ij} \) belong to the set \( \{0, 1\} \), in which case consistency is the property that \( J_{ij} \oplus \sigma_i \oplus \sigma_j = 0 \) (sum modulo two). One says that \( \Sigma \) is a consistent spin assignment for the graph \( G \) (or simply that \( G \) is consistent) if every edge of \( G \) is consistent with \( \Sigma \). In other words, for any pair of vertices \( v_i \) and \( v_j \), if there is a positive edge from node \( v_j \) to node \( v_i \), then \( v_j \) and \( v_i \) must have the same spin, and if there is a negative edge connecting \( v_j \) to \( v_i \), then \( v_j \) and \( v_i \) must have opposite spins. (If there is no edge from \( v_j \) to \( v_i \), this requirement imposes no restriction on their spins.) It is easy to see that if there is a consistent spin assignment for \( G \), then the graph is balanced. Conversely, if \( G \) is balanced then there is a consistent spin assignment for \( G \): to see this, simply label one node arbitrarily, and follow paths to label other nodes consistently. (If the graph is not connected, repeat the procedure in each connected component.)

For any spin assignment \( \Sigma \), let \( A_1 \) be the subset of nodes labeled +1, and let \( A_{-1} \) be the subset of nodes labeled −1. The set of all nodes is partitioned into \( A_1 \) and \( A_{-1} \). Conversely, any partition of the set of nodes into two subsets can be thought of as a spin assignment. With this interpretation, a consistent spin assignment is the same as a partition of the node set into two subsets \( A_1 \) and \( A_{-1} \) in such a manner that all edges between elements of \( A_1 \) are positive, all edges between elements of \( A_{-1} \) are positive, and all edges between a node in \( A_1 \) and a node in \( A_{-1} \) are negative. In summary, our definition of monotonicity, given in terms of partitions of state variables, amounts to the same as the requirement that there exist at least one consistent spin assignment for its associated graph \( G \), or equivalently, that its graph \( G \) is balanced.

Supposing that a system is monotone, with a consistent spin assignment \( \Sigma = \{\sigma_i, i = 1, \ldots, n\} \), we introduce following the relation among vectors \( x \in \mathbb{R}_{\geq 0}^n \):

\[
x \preceq y
\]

means that

\[
\sigma_i x_i \leq \sigma_i y_i \quad i = 1, \ldots, n.
\]
This is a componentwise inequality that requires $x_i \leq y_i$ if node $i$ has been assigned a positive spin, and $x_i \geq y_i$ if instead node $i$ has been assigned a negative spin. Let $y(t)$ and $z(t)$ be any two solutions of the system $dx/dt = f(x)$, and suppose that $\sigma_i y_i(0) \leq \sigma_i z_i(0)$ for each $i = 1, \ldots, n$. Then, Kamke’s Theorem states that $\sigma_i y_i(t) \leq \sigma_i z_i(t)$ for all $t \geq 0$ and coordinate $i = 1, \ldots, n$. This is the usual definition of monotonicity: if states start at time zero in a certain order, then they must remain forever in the same order. Conversely, a flow that preserves an order of this type must be monotone in the sense that we have defined the concept. See the textbook [50] for a proof, and [51] for extensions with systems with external inputs.

The order preservation property has a variety of important implications for our model. For parameters viewed as constant states, it allows us to conclude the monotonicity of stable branches in bifurcation diagrams, as illustrated by the results described in Monotone Parametric Dependencies in the S design. A different implication concerns the domain of attraction of equilibria. Suppose that we consider an initial state $x(0)$ that is coordinate-wise less, in the monotone order, than a given equilibrium $E$ (in a possibly multistable system). Comparing to $E$ the solution $x(t)$ starting from this initial state $x(0)$, we know that $x(t)$ must remain less than $E$ for all times. Thus, an equilibrium to which $x(t)$ converges must be upper bounded by $E$. In particular, if the equilibrium $E$ is minimal (with respect to the coordinate-wise order), it follows that this trajectory converges to $E$. Similar conclusions apply to maximal equilibria $E$ and initial states $x(0)$ that are coordinate-wise larger than $E$. One obtains in this manner a rich amount of information about the basin of attraction of equilibria in monotone systems.
SI-5 Symmetry

In this section, we formalize the symmetry of

- a single S toggle embedded into an environment \((N = 1)\),
- a population of \(N\)-identical S toggles interacting via a common environment \((N \geq 2)\).

SI-5.1 Symmetry of the S Model

As is routine in physics and engineering, symmetry-based simplifications often lead to important insights into complex phenomena \([52]\), and we also use symmetry to discuss bifurcations in S toggle populations. We observe that under a special condition imposed on the parameter values,

\[
a_1 = a_2, \quad a_3 = a_4, \quad a_5 = a_6, \quad d_g = d_r, \quad \delta_g = \delta_r,
\]  
(SI-5.1)

a single S toggle embedded into an external environment is described by the S model (1) with \(N = 1\) which has a \(\mathbb{Z}_2\)-symmetry group generated by involution \(I\) \([52–54]\),

\[
I : (x,y,g,r,g_e,r_e) \rightarrow (y,x,r,g,r_e,g_e).
\]  
(SI-5.2)

Consider the fixed-point subspace \(\text{Fix}(\mathbb{Z}_2) \in \mathbb{R}^6\) of the group \(\mathbb{Z}_2\), see \([52]\),

\[
\text{Fix}(\mathbb{Z}_2) = \{z = (x,y,g,r,g_e,r_e) \in \mathbb{R}^6 | Iz \equiv z\}.
\]  
(SI-5.3)

We ignore the trivial equilibria that belong to \(\text{Fix}(\mathbb{Z}_2)\), that is, equilibria of the S model (1) for which the following equalities hold, \(x = y, g = r, \) and \(g_e = r_e\), corresponding to identically the same levels of LacI and TetR, and C14-HSL and C4-HSL, respectively. Let us denote the equilibrium of the S model (1) by \(z_0\), and let us assume that \(z_0 \notin \text{Fix}(\mathbb{Z}_2)\). Now, because the S model is invariant with respect to the involution \(\text{SI-5.2}\), and because \(z_0 \notin \text{Fix}(\mathbb{Z}_2)\), we obtain that both \(z_0\) and \(Iz_0\), \(Iz_0 \neq z_0\), are different equilibria of the S model (1), see \([52,53]\).

The equilibria \(z_0\) and \(Iz_0\) are called relative equilibria \([55]\). All bifurcations for the relative equilibria occur simultaneously at the same values of free parameters. We generalize G- and R-homogeneous populations states as relative equilibria, which means that as soon as the S toggle has a G-state, it will also have the corresponding R-state, implying bistability.

The general case of \(N \geq 2\) is slightly more complicated as a population of identical S toggles has a symmetry group obtained after combinations of permutations among all cells in the given population and the toggle involution \([\text{SI-5.2}]\), which we denote \(G = \mathbb{Z}_2 \times S_N\) for brevity. Here, \(S_N\) is a symmetric group of order \(N \geq 1\), and \(\mathbb{Z}_2\) is the toggle involution \([\text{SI-5.2}]\) applied to all toggles simultaneously. For example, for \(N = 2\), we will have one permutation,

\[
P : (x_1, y_1, g_1, r_1, x_2, y_2, g_2, r_2, g_e, r_e) \rightarrow (x_2, y_2, g_2, r_2, x_1, y_1, g_1, r_1, g_e, r_e),
\]  
(SI-5.4)

and the involution,

\[
I : (x_1, y_1, g_1, r_1, x_2, y_2, g_2, r_2, g_e, r_e) \rightarrow (y_1, x_1, r_1, g_1, y_2, x_2, r_2, g_2, r_e, g_e).
\]  
(SI-5.5)
SI-5.2 Symmetry Breaking

The symmetry-breaking (pitchfork) bifurcation discussed around Fig. 7 in the main text has co-dimension one for all typical systems with $\mathbb{Z}_2$-symmetry \[52,53\]. To understand the symmetry-breaking occurring at the BP-point shown in Fig. 7, we need first to define the symmetry of the original symmetric (1:1)-mixed state. We observe that the original symmetric (1:1)-mixed state is invariant with respect to transformation $g$,

$$g = \mathcal{P} \circ \mathcal{I} = \mathcal{I} \circ \mathcal{P}, \quad g \circ g = \text{id} \quad (\text{SI-5.6})$$

where id is the identity. Here, $\mathcal{P}$ and $\mathcal{I}$ are as defined in (SI-5.4) and (SI-5.5), respectively. The transformation (SI-5.6) forms subgroup $\Sigma_{(1,1)}$ of the group $\mathbb{Z}_2 \times S_2$, see SI-5.1 Symmetry of the S Model, which consists of two elements, that is, $\Sigma_{(1,1)} = \{\text{id}, g\}$. The subgroup $\Sigma_{(1,1)}$ is called the isotropy subgroup \[52\] of the original (1:1)-mixed state. We further observe that the two (1:1)-states bifurcating from the original $\Sigma_{(1,1)}$-symmetric (1:1)-state at the BP-point (Fig. 7) are not invariant with respect to the isotropy subgroup $\Sigma_{(1,1)}$. Indeed, they are mapped one to one another by the transformation (SI-5.6). This observation motivates using the “symmetry-breaking” terminology \[52\] with respect to the loss of the isotropy subgroup symmetry by the (1:1)-mixed state at the BP-point. Due to the isotropy subgroup $\Sigma_{(1,1)}$ of the original (1:1)-mixed state, involution $g$ defined in (SI-5.6) maps panel (A) to panel (D), and panel (B) to panel (C) within Fig. 7 of the main text. That is, $g : \text{LP}_1 \rightarrow \text{LP}_2$, $g : \text{LP}_2 \rightarrow \text{LP}_1$, and $g : \text{BP} \rightarrow \text{BP}$, see the coordinates of the three critical points, BP, LP$_1$, and LP$_2$, at the end of the caption of Fig. 7.

SI-5.3 A Remark on Bifurcations in Symmetric vs. Non-Symmetric Models

Mathematical models are idealizations of complex phenomena, based on certain assumptions, and there is a long established tradition to use symmetries in mathematical physics to clarify and explain complex phenomena. We use symmetry as another mathematical simplification alternative to simplifications arising from biological assumptions.

For our modeling studies with the S model, it may be difficult and even impossible to construct identical promoters which would correspond to identical values of parameters, that is, for example, $a_3 \neq a_4$. Therefore, it is required to discuss an appropriate interpretation of bifurcation diagrams computed for the S model.

First of all, we note that all LP-points will typically persist under small non-symmetric perturbations. However, all BP-points corresponding to pitchfork bifurcations will typically disappear under non-symmetric perturbations. They will typically be replaced by LP-bifurcation points. In such cases, in the small vicinity of the original BP point after a non-symmetric perturbation, we will typically have three branches of solutions, one branch of solutions which do not change their stability, and other two branches of solutions, stable and unstable, which will emanate from or collide with one another at the LP bifurcation point. Outside of the small vicinity of the perturbed BP-point, the bifurcation diagrams for both symmetric and non-symmetric models will be typically qualitatively the same. Such situations are mathematically very well studied and are described in the corresponding literature \[52,53\].
SI-6 Exponential Stability of Cellular Populations

A systematic analysis of dynamical mathematical models begins with finding equilibrium solutions followed by the analysis of their exponential stability [56]. The next step is often to carry out (local) bifurcation analysis of the equilibrium solutions, allowing for the exploration of “stability boundaries” in the parameter space [53]. Both stability and bifurcation analyses rely on the computation of the eigenvalues from the corresponding model linearizations [53].

A nontrivial specificity of the computation of eigenvalues for the stability and bifurcation analyses of the A- and S-population models is that both models with \( N > 1 \) are invariant with respect to the action or the given linear representation of the symmetric group \( S_N \) of permutations among \( N \)-cells [52]. It is known that irreducible representations of groups enforce multiple eigenvalues of matrices that commute with their linear representations, a well-known fact following from Schur’s Lemma in the representation theory of Lie groups [52].

To take into account the necessity to deal with multiple eigenvalues in the situations when the value of \( N \) is \textit{a priori} unknown, we have developed a general approach to the analysis of exponential stability [56] in arbitrary populations of identical cells, independently of \( N \), as described below.

A conceptually similar reduction approach (without any discussion of the multiplicity problem) on the exponential orbital stability of periodic solutions in systems of identical and slightly different oscillators coupled via a medium was developed by E. E. Shnol [57]. In his work, an averaging technique over the entire cellular population was used in both cases of homogeneous and mixed populations. Later, G. Katriel [58] has rediscovered the reduction result for homogeneous populations only, using Floquet Theory [59]. We note that the Schur’s formula [60] can also be used to compute multipliers of periodic solutions in systems of coupled oscillators, using the linearizations of the corresponding Poincaré maps, in the very similar way as it is done for the case of equilibrium solutions in this work.

SI-6.1 A General Population Model of Identical Cells

In this SI, we use Schur’s formula [60] to compute explicitly the characteristic polynomials for the corresponding model linearizations. The most important implication of Schur’s formula is that it can be easily seen that the values of the eigenvalues are independent of \( N \geq 2 \).

To describe the general exponential stability analysis, we first introduce an appropriate notation as follows. Let \( S \) and \( z \) be “generalized” global (extracellular) and local (intracellular) state variables, respectively, \( \dim S = m \geq 1 \) and \( \dim z = k \geq 2 \). Using the generalized variables, both the S-model (1) and the A-model (2) can then be rewritten in the following general form, which we call a G-model,

\[
\frac{dS}{dt} = H_0(S) + \frac{\rho}{N} \sum_{i=1}^{N} H(S, z_i), \quad 0 \leq \rho \leq 1, \tag{SI-6.1a}
\]

\[
\frac{dz_i}{dt} = h(S, z_i), \quad i = 1, \ldots, N. \tag{SI-6.1b}
\]

The G-model [SI-6.1] includes \( m + Nk \) equations.
SI-6.2 A Homogeneous Population

In the case of a homogeneous population of identical cells, we have \( z_i(t) \equiv z(t) \). As a result, the G-model (SI-6.1) reduces to a system of \((m+k)\)-differential equations,

\[
\begin{align*}
\frac{dS}{dt} &= H_0(S) + \rho H(S, z), \quad 0 \leq \rho \leq 1, \\
\frac{dz}{dt} &= h(S, z).
\end{align*}
\]

(SI-6.2a)

(SI-6.2b)

Observe that the model (SI-6.2) describes a single cell placed in a “free”, non-constant medium.

**Definition 1.** Let \((S_0, z_0)\) be an equilibrium solution of the model (SI-6.2). Then, \((S_0, z_0)\) corresponds to a homogeneous population equilibrium solution,

\[
(S_0, z_0, \ldots, z_0) = (S_0, N \times z_0),
\]

(SI-6.3)

of the full G-model (SI-6.1) for any \( N \geq 2 \). Notation \( N \times z_0 \) means that \( z_0 \) is repeated \( N \)-times in \((S_0, z_0, \ldots, z_0)\).

Although the model (SI-6.2) is sufficient to study the existence of homogeneous population equilibrium solutions (SI-6.3), it is not enough to establish the exponential stability of the corresponding solutions (SI-6.3). Let \((S_0, N \times z_0)\) be a homogeneous population equilibrium solution of the G-model (SI-6.1) with any fixed \( N \geq 2 \). To analyze the exponential stability of \((S_0, N \times z_0)\) in the “full” G-model (SI-6.1), we need to compute the eigenvalues of the corresponding Jacobian matrix \( J_N \),

\[
J_N = \begin{pmatrix}
A & \frac{\rho}{N} B & \frac{\rho}{N} B & \cdots & \frac{\rho}{N} B \\
C & D & O & \cdots & O \\
& C & O & D & \cdots & O \\
& & \vdots & \vdots & \ddots & \vdots \\
& & & C & O & \cdots & D
\end{pmatrix}.
\]

(SI-6.4)

In (SI-6.4), each of three matrices, \( B \), \( C \), and \( D \), is repeated \( N \)-times; \( A \) and \( D \) are square matrices of sizes \( m \) and \( k \), respectively; \( B \) and \( C \) are rectangular matrices of sizes \( m \times k \) and \( k \times m \), respectively,

\[
A = \frac{\partial H_0}{\partial S} + \rho \frac{\partial H}{\partial S}, \quad B = \frac{\partial H}{\partial z}, \quad C = \frac{\partial h}{\partial S}, \quad D = \frac{\partial h}{\partial z}.
\]

(SI-6.5)

All partial derivatives in the expressions (SI-6.5) are evaluated at \((S_0, z_0)\) which depends on all G-model parameters with the one important exception that they are independent of \( N \) because \((S_0, z_0)\) is obtained using (SI-6.2). Notation \( O \) corresponds to zero submatrices of appropriate sizes.

We call a square matrix stable if all its eigenvalues have strictly negative real parts. The following theorem holds for \( J_N \).

**Theorem 1.** (I). Statements (a), (b), and (c) are equivalent.

(a). The matrix \( J_N \) is stable for all \( N \geq 2 \).
(b). The matrix $J_1$ and its submatrix $D$ are both stable.

(c). The matrix $J_2$ is stable.

(II). The matrix $J_N$ has typically $k$ different eigenvalues, each of multiplicity $N - 1$ in the following sense. Let $\{\lambda_1, \ldots, \lambda_{m+k}\}$ be the set of eigenvalues of matrix $J_1$, and let $\{\mu_1, \ldots, \mu_k\}$ be the set of eigenvalues of its submatrix $D$. Then,

$$\{\lambda_1, \ldots, \lambda_{m+k}, (N - 1)(\mu_1, \ldots, \mu_k)\} \quad \text{(SI-6.6)}$$

is the set of all eigenvalues of matrix $J_N$ for any $N \geq 2$, where $\{\mu_1, \ldots, \mu_k\}$ is repeated $(N - 1)$-times.

Proof. Let $\lambda$ be a complex number, $\lambda \in \mathbb{C}$. Consider a new matrix $M_\lambda = J_N - \lambda I_{m+Nk}$, where $I_{m+Nk}$ is the identity matrix of size $m + nk$. To find eigenvalues of $J_N$, we need to write down the corresponding characteristic equation $P(\lambda) = 0$, $P(\lambda) = \det M_\lambda$. Let us represent matrix $M_\lambda$ in the form

$$M_\lambda = \begin{pmatrix} A_\lambda & B \\ C & D_\lambda \end{pmatrix}. \quad \text{(SI-6.7)}$$

Here, matrices $A_\lambda = A - \lambda I_m$, $B = \frac{1}{N} (\mathbf{B}, \ldots, \mathbf{B})$, $C = (\mathbf{C}, \ldots, \mathbf{C})^T$, and $D_\lambda = \text{diag}(D_\lambda, \ldots, D_\lambda)$ with $D_\lambda = D - \lambda I_k$. Next, assume for a moment that $D^{-1}_\lambda$ exists. Then, Schur’s formula can be used to compute $\det M_\lambda$ [60],

$$\det M_\lambda = \det D_\lambda \cdot \det (A_\lambda - BD_\lambda^{-1}C). \quad \text{(SI-6.8)}$$

Next, we compute

$$BD_\lambda^{-1}C = B \left( D_\lambda^{-1}C \right) = \frac{1}{N} (\mathbf{B}, \ldots, \mathbf{B}) \begin{pmatrix} D_\lambda^{-1}C \\ D_\lambda^{-1}C \\ \vdots \\ D_\lambda^{-1}C \end{pmatrix} = BD_\lambda^{-1}C. \quad \text{(SI-6.9)}$$

For the determinant of the block diagonal $D_\lambda$, we obtain $\det D_\lambda = (\det D_\lambda)^N$. Substituting [SI-6.9] into [SI-6.8] yields

$$\det M_\lambda = (\det D)^N \cdot \det (A_\lambda - BD_\lambda^{-1}C). \quad \text{(SI-6.10)}$$

Using the Schur’s formula for the product $\det D \cdot \det (A_\lambda - BD_\lambda^{-1}C)$ in the “backward” direction, we can rewrite [SI-6.10] in the following equivalent form

$$\det M_\lambda = (\det D_\lambda)^{N-1} \cdot \det \left( A_\lambda \begin{pmatrix} B \\ C \end{pmatrix} D_\lambda \end{pmatrix}. \quad \text{(SI-6.11)}$$

The expression [SI-6.11] can now be rewritten simply as

$$P(\lambda) = (\det D_\lambda)^{N-1} \cdot \det (J_1 - \lambda I_{m+k}). \quad \text{(SI-6.12)}$$

Recall that the expression [SI-6.12] has been proven under a restrictive condition $\det D_\lambda \neq 0$, see above, which means that $\lambda$ is not an eigenvalue of the matrix $D$. This restriction can be
removed, for example, as follows. Let $\lambda_0$ be an eigenvalue of the matrix $D$. Then, we obtain for the polynomial $P(\lambda)$ by continuity

$$P(\lambda_0) = \lim_{\lambda \to \lambda_0} P(\lambda) = \lim_{\lambda \to \lambda_0} (\det D - \lambda I_k)^{N-1} \cdot \det (J_1 - \lambda I_{m+k}) = 0.$$  

(SI-6.13)

It follows from (SI-6.13) that (SI-6.12) holds for all $\lambda \in \mathbb{C}$.

Finally, we observe from (SI-6.12) that to compute all eigenvalues of the Jacobian matrix $J_N$ for any $N \geq 2$, it is sufficient to compute the eigenvalues of either two smaller matrices, $D$ and $J_1$, or one matrix $J_2$. The latter may be practically slightly easier than computing the eigenvalues for $D$ and $J_1$ separately. The proof of the theorem follows.  

Consider a differential equation

$$\frac{dz}{dt} = h(S_0, z),$$  

(SI-6.14)

where $S_0$ is a fixed parameter corresponding to the equilibrium $(S_0, N \times z_0)$ of the full G-model (SI-6.1). In contrast to equation (SI-6.2), equation (SI-6.14) describes a single cell placed into a constant environment, which can be interpreted as an environment shaped by the large population of cells and which does not “sense” any changes in a single cell. Additionally, consider a cascade model

$$\frac{dS}{dt} = H_0(S) + \rho H(S, z_1), \quad 0 \leq \rho \leq 1,$$

(SI-6.15a)

$$\frac{dz_j}{dt} = h(S, z_j), \quad j = 1, 2.$$  

(SI-6.15b)

Observe that the variable $z_2$ is absent from the first equation (SI-6.15a) and, hence, (SI-6.15) cannot be obtained from (SI-6.1) by simply setting $N = 2$.

Then, using the definition of exponential stability [56], the first statement of Theorem 1 can be reformulated as the following corollary which admits an intuitive interpretation of the fact why the case of $N = 2$ is sufficient to study the exponential stability of homogeneous population solutions.

**Corollary 1.** Let $(S_0, N \times z_0)$ be an equilibrium solution of the G-model (SI-6.1). Then, statements (a) - (d) are equivalent.

(a). $(S_0, N \times z_0)$ is exponentially stable in the G-model (SI-6.1) for any $N \geq 2$.

(b). $(S_0, z_0)$ is exponentially stable in the reduced model (SI-6.2), and $z_0$ is exponentially stable in the single-cell model (SI-6.14).

(c). $(S_0, z_0, z_0)$ is exponentially stable in the G-model (SI-6.1) at $N = 2$.

(d). $(S_0, z_0, z_0)$ is exponentially stable in the cascade model (SI-6.15).

A comparison of Statements (a) and (b) of Corollary 1 leads to a conclusion that the given population consisting of identical cells is stable with respect to any small perturbation if and only if (i) the population is stable with respect to any small uniform perturbation of the entire population described by system (SI-6.1) and, simultaneously, (ii) a majority of unperturbed cells forces a single slightly perturbed cell to re-join back the unperturbed majority.
Indeed, system (SI-6.14) used in Statement (b) means that the entire population does not sense small perturbations in a single cell because $S_0$ is fixed in (SI-6.14).

Note that both conditions in Statement (b) can be reformulated, using the cascade model (SI-6.15) from statement (d). Finally, because the stability property is independent of the number $N$ of identical cells in the population, the simple case of $N = 2$ can be used as given by statement (c).

### SI-6.3 A Mixed Population Split into Two Subpopulations

Suppose now that the given population consisting of $N$, $N \geq 4$, identical cells is split into two different subpopulations of sizes $N_1 \geq 2$ and $N_2 \geq 2$, respectively, where $N = N_1 + N_2$. We always assume that each subpopulation consists of at least two cells. Then, the two different homogeneous subpopulations can be described by two state variables $z_1$ and $z_2$, respectively, where $z_1 \neq z_2$, that is, $z_{ip}(t) \equiv z_1(t)$ for some subset of indexes $i_p$, $p = 1, \ldots, N_1$, and $z_{iq}(t) \equiv z_2(t)$, for another subset of indexes $i_q$, $q = 1, \ldots, N_2$. It follows that the equation (SI-6.1a) from the G-model (SI-6.1) simplifies as follows

$$
\frac{dS}{dt} = H_0(S) + \frac{\rho}{N} \sum_{i=1}^{N} H(S, z_i) = \rho \left( \beta_1 H(S, z_1) + \beta_2 H(S, z_2) \right). \tag{SI-6.16}
$$

In (SI-6.16), $\beta_j$ is the fraction of the $j$-th subpopulation, $\beta_j = N_j/N$, $j = 1, 2$, $\beta_1 + \beta_2 = 1$. In this case, the entire G-model (SI-6.1) reduces to the following three equations

$$
\dot{S} = H_0(S) + \rho \left( \beta_1 H(S, z_1) + \beta_2 H(S, z_2) \right), \quad \beta_j \in \mathbb{Q}, \quad \beta_1 + \beta_2 = 1, \tag{SI-6.17a}
$$

$$
\dot{z}_j = h(S, z_j), \quad j = 1, 2. \tag{SI-6.17b}
$$

**Definition 2.** Let $(S_0, z_{10}, z_{20})$, $z_{10} \neq z_{20}$, be a non-uniform equilibrium solution of the reduced system (SI-6.17). Then, $(S_0, z_{10}, z_{20})$, $z_{10} \neq z_{20}$ corresponds to a mixed population equilibrium solution,

$$(S_0, z_{10}, \ldots, z_{10}, z_{20}, \ldots, z_{20}) = (S_0, N_1z_{10}, N_2z_{20}), \tag{SI-6.18}$$

of the full G-model (SI-6.1). The solution (SI-6.18) describes a mixed population of $N$ identical cells, split into two (non-identical) subpopulations of sizes $N_1 > 0$ and $N_2 > 0$, respectively, $N_1 + N_2 = N$. Notation $N_jz_{j0}$ means that $z_{j0}$ is repeated $N_j$-times in $(S_0, z_{10}, \ldots, z_{10}, z_{20}, \ldots, z_{20})$, $j = 1, 2$.

Due to the condition $\beta_1 + \beta_2 = 1$ used in (SI-6.17a), there formally exists a continuum of different fractions $\beta_1 : \beta_2$, $\beta_1 \in \mathbb{R}$ and $\beta_2 \in \mathbb{R}$. Of course, in the biological sense, only rational values $\beta_1 \in \mathbb{Q}$ and $\beta_2 \in \mathbb{Q}$ are allowable, leading to infinitely many fractional ($\beta_1 : \beta_2$)-configurations in the subdivision of the original population into two different subpopulations. Simple examples of such situations can be easily presented (Fig. SI-6.1).

For the sake simplicity of the exponential stability analysis, we will always assume that both $\beta_1$ and $\beta_2$ are real numbers, that is, $\beta_j \in \mathbb{R}$, $j = 1, 2$.

Let $(S_0, N_1z_{10}, N_2z_{20})$ be a mixed population equilibrium solution of the G-model (SI-6.1) with any fixed $N \geq 4$, see (SI-6.3). To analyze the exponential stability of $(S_0, N_1z_{10}, N_2z_{20})$, we
Figure SI-6.1. Examples of \((p : q)\)-populations. The left panel corresponds to the case of a (10:0)-homogeneous population; the middle panel corresponds to the case of a (5:5)- or, equivalently, (1:1)-mixed population, and the right panel corresponds to a (9:1)-mixed population.

To compute the eigenvalues of the corresponding Jacobian matrix \( J_N \) obtained from the G-model (SI-6.1),

\[
J_N = \begin{pmatrix}
A & \frac{\rho}{N}B_1 & \cdots & \frac{\rho}{N}B_1 & \frac{\rho}{N}B_2 & \cdots & \frac{\rho}{N}B_2 \\
C_1 & D_1 & \cdots & D_1 & O & \cdots & O \\
\vdots & \vdots & \ddots & \vdots & \vdots & \ddots & \vdots \\
C_1 & O & \cdots & D_1 & O & \cdots & O \\
C_2 & O & \cdots & O & D_2 & \cdots & O \\
\vdots & \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\
C_2 & O & \cdots & O & O & D_2 &
\end{pmatrix}
\]  \hspace{1cm} (SI-6.19)

In the matrix (SI-6.19), submatrices \(B_i, C_i, \) and \(D_i\), are repeated \(N_j\)-times; \(A\) and \(D_i\) are square matrices of sizes \(m\), and \(k\), respectively; \(B_i\) and \(C_i\) are rectangular matrices of sizes \(m \times k\) and \(k \times m\), respectively, and

\[
A = \frac{\partial H_0}{\partial S} + \sum_{j=1}^{2} \rho_j \frac{\partial H(S_0, z_{j0})}{\partial S}, \hspace{1cm} (SI-6.20a)
\]

\[
B_j = \frac{\partial H(S_0, z_{j0})}{\partial z}, \hspace{1cm} C_j = \frac{\partial h(S_0, z_{j0})}{\partial S}, \hspace{1cm} D_j = \frac{\partial h(S_0, z_{j0})}{\partial z}, \hspace{1cm} j = 1, 2. \hspace{1cm} (SI-6.20b)
\]

Consider the Jacobian matrix \(Q_2\) of size \(m + 2k\) for the system (SI-6.17), computed at \((S_0, z_{10}, z_{20})\),

\[
Q_2 = \begin{pmatrix}
A & \rho_1B_1 & \rho_2B_2 \\
C_1 & D_1 & O \\
C_2 & O & D_2
\end{pmatrix}
\]  \hspace{1cm} (SI-6.21)

Theorem 2. (I). Statements (a) and (b) are equivalent.

(a). The matrix \(J_N\) is stable for all \(N \geq 4\), and with any \(N_1 \geq 2\) and \(N_2 \geq 2\) such that \(N_1 + N_2 = N\).

(b). Matrix \(Q_2\), and its two submatrices, \(D_1\) and \(D_2\), are stable.

(II). Matrix \(J_N\) has typically \(2k\) different multiple eigenvalues in the following sense. Let \(\{\lambda_1, \ldots, \lambda_{m+2k}\}\) be the set of eigenvalues of \(Q_2\), let \(\{\mu_1, \ldots, \mu_k\}\) be the set of eigenvalues of \(D_1\), and let \(\{\sigma_1, \ldots, \sigma_k\}\) be the set of eigenvalues of \(D_2\). Then,

\[
\{\lambda_1, \ldots, \lambda_{m+k}, (N_1 - 1)(\mu_1, \ldots, \mu_k), (N_2 - 1)(\sigma_1, \ldots, \sigma_k)\}, \hspace{1cm} (SI-6.22)
\]
is the set of all eigenvalues of the matrix $I_N$ for any $N \geq 4$. In (SI-6.22), the set $\{\mu_1, \ldots, \mu_k\}$ is repeated $(N_1 - 1)$-times, and the set $\{\sigma_1, \ldots, \sigma_k\}$ is repeated $(N_2 - 1)$-times. To have a nonzero value of multiplicity $N_j - 1$ in (SI-6.22), condition $N_j \geq 2$ and, hence, $N \geq 4$, are natural requirements, $j = 1, 2$. In other words, the latter two conditions guarantee that both matrices $D_1$ and $D_2$ exist. Otherwise, Theorem 2 does not make any sense.

**Proof.** The proof Theorem 2 can be carried out, using a simple modification of the proof of Theorem 1. For this reason, we only provide a brief sketch of the proof for Theorem 2. Similarly to the proof of Theorem 1, we need to write down a characteristic equation $P(\lambda) = 0$ Here $P(\lambda) = \det M_\lambda$, and matrix $M_\lambda$ can be defined as in (SI-6.7), using appropriate submatrices,

\[
A_\lambda = A - \lambda I_m, \quad (SI-6.23a)
\]
\[
B = \frac{1}{N} (B_1, \ldots, B_1, B_2, \ldots, B_2), \quad (SI-6.23b)
\]
\[
C = (C_1, \ldots, C_1, C_2, \ldots, C_2)^T, \quad (SI-6.23c)
\]
\[
D_\lambda = \text{diag} (D_{1\lambda}, \ldots, D_{1\lambda}, D_{2\lambda}, \ldots, D_{2\lambda}), \quad D_\lambda = D_i - \lambda I_k. \quad (SI-6.23d)
\]

In the above submatrix definitions, the matrices with index $j$ are repeated $N_j$-times, $i, j = 1, 2$. In this case, $\det M_\lambda$ can also be computed using Schur’s formula, see (SI-6.8). However, (SI-6.9) should be replaced by

\[
\mathbb{B}D_\lambda^{-1}C = \rho_1 B_1 D_{1\lambda}^{-1} C_1 + \rho_2 B_2 D_{2\lambda}^{-1} C_2. \quad (SI-6.24)
\]

For the block diagonal matrix $D_\lambda$, we obtain $\det D_\lambda = (\det D_{1\lambda})^{N_1} \cdot (\det D_{2\lambda})^{N_2}$. Now, similarly to (SI-6.10), we will have

\[
\det M_\lambda = (\det D_1)^{N_1} \cdot (\det D_2)^{N_2} \cdot \det \left( A_\lambda - \rho_1 B_1 D_{1\lambda}^{-1} C_1 - \rho_2 B_2 D_{2\lambda}^{-1} C_2 \right). \quad (SI-6.25)
\]

Using the Schur’s formula in the “backward” direction, we will then have

\[
P(\lambda) = (\det D_1 - \lambda I_k)^{N_1-1} \cdot (\det D_2 - \lambda I_k)^{N_2-1} \cdot \det Q_2. \quad (SI-6.26)
\]

The rest can be proved as in the proof for Theorem 1. The proof of Theorem 2 follows. \hfill \Box

Consider the following cascade model

\[
\frac{dS}{dt} = H_0(S) + \rho \left( \beta_1 H(S, z_1) + \beta_2 H(S, z_3) \right), \quad \beta_1 + \beta_2 = 1, \quad (SI-6.27a)
\]
\[
\frac{dz_j}{dt} = h(S, z_j), \quad j = 1, \ldots, 4. \quad (SI-6.27b)
\]

Variables $z_2$ and $z_4$ are absent from the first equation (SI-6.27a) and, hence, the cascade system (SI-6.27) cannot be obtained from the G-model (SI-6.1) by simply setting $N = 4$. Now, Theorem 2 can be reformulated in terms its Corollary 2 as follows.

**Corollary 2.** Let $(S_0, N_1 \times z_{10}, N_2 \times z_{20})$ be a mixed population equilibrium solution of the G-model (SI-6.1). Then, Statements (a) - (c) are equivalent.

(a). $(S_0, N_1 \times z_{10}, N_2 \times z_{20})$ is exponentially stable in the G-model (SI-6.1) for any $N \geq 4$, and with any $N_1 \geq 2$ and $N_2 \geq 2$ such that $N_1 + N_2 = N$.

(b). $(S_0, z_{10}, z_{20})$ is exponentially stable in the reduced model (SI-6.17), and each $z_{j0}$ is exponentially stable in the single-cell model (SI-6.14), $j = 1, 2$.

(c). $(S_0, z_{10}, z_{10}, z_{20}, z_{20})$ is exponentially stable in the cascade model (SI-6.27).
SI-6.4 A Mixed Population Split into Several Subpopulations

The case of a mixed population split into two subpopulations with densities $\rho_1 = \beta_1 \rho$ and $\rho_2 = \beta_2 \rho$ can be generalized to the case of a mixed population split into $L$-different subpopulations with densities $\rho_1, \ldots, \rho_L$, where $\rho_1 + \ldots + \rho_L = \rho$, $L \geq 3$ as follows.

Let subpopulation $j$ consist of $N_j$ cells, and let subpopulation $j$ correspond to variable $z_j$, that is, we have $z_j(t) \equiv z_j(t)$, where $j' \in \{i_1, i_2, \ldots, i_N\} \subset \{1, 2, \ldots N\}$, $j = 1, \ldots, L$. In this case, the G-model (SI-6.1) reduces to the following equations

\[
\dot{S} = H_0(S) + \sum_{j=1}^{L} \rho_j H(S, z_j), \quad \sum_{j=1}^{L} \rho_j = \rho,
\]

(SI-6.28a)

\[
\dot{z}_j = h(S, z_j), \quad \rho_j = \beta_j \rho, \quad \beta_j = \frac{N_j}{N}, \quad j = 1, \ldots, L.
\]

(SI-6.28b)

**Definition 3.** Let $(S_0, z_{10}, \ldots, z_{L0})$ be a non-uniform equilibrium solution of the system (SI-6.28), where $z_{j0} \neq z'_{j0}$ for all $j \neq j'$. Then, $(S_0, z_{10}, \ldots, z_{L0})$, corresponds to a mixed population equilibrium solution,

\[
(S_0, N_1 z_{10}, \ldots, N_L z_{L0}),
\]

(SI-6.29)

of the full G-model (SI-6.1). The solution (SI-6.29) describes a mixed population of $N$ identical cells, which is split into $L$ subpopulations of the corresponding sizes $N_j \geq 2$, $N_1 + \ldots + N_L = N$. Notation $N_j z_{j0}$ means that $z_{j0}$ is repeated $N_j$-times in the vector-form solution of the the full G-model (SI-6.1), $N_j \geq 2$, $j = 1, \ldots, L$.

Consider the Jacobian matrix $Q_L$ for the reduced system (SI-6.28), computed at $(S_0, z_{10}, \ldots, z_{L0})$,

\[
Q_L = \begin{pmatrix} A & \rho_1 B_1 & \cdots & \rho_L B_L \\
C_1 & D_1 & \cdots & O \\
\vdots & \vdots & \ddots & \vdots \\
C_L & O & \cdots & D_L \end{pmatrix}.
\]

(SI-6.30)

In (SI-6.30), all submatrices are defined as in (SI-6.20), where $j = 1, 2$ should be replaced by $j = 1, \ldots, L$. Below, we formulate Theorem 3 and Corollary 3 without any proof because they are similar to Theorem 2 and Corollary 1 respectively.

**Theorem 3.** (I). Statements (a) and (b) are equivalent.

(a). The Jacobian $J_N$ computed for the G-model at the given equilibrium (SI-6.29) is stable for all $N \geq 2L$, and with any $N_j \geq 2$, $j = 1, \ldots, L$, such that $N_1 + \ldots N_L = N$.

(b). Matrix $Q_L$ and its submatrices $D_j$, $j = 1, \ldots, L$, are stable.

(II). Matrix $J_N$ has typically $kL$ different multiple eigenvalues in the following sense. Let \{\(\lambda_1, \ldots, \lambda_{m+kL}\)\} be the set of eigenvalues of $Q_L$, and let \{\(\mu_1^{(j)}, \ldots, \mu_k^{(j)}\)\} be the set of eigenvalues of $D_j$, $j = 1, \ldots, L$. Then,

\[
\left\{ \lambda_1, \ldots, \lambda_{m+k}, (N_1 - 1) \left( \mu_1^{(1)}, \ldots, \mu_k^{(1)} \right), \ldots, (N_L - 1) \left( \mu_1^{(N_L)}, \ldots, \mu_k^{(N_L)} \right) \right\}
\]

(SI-6.31)

is the set of all eigenvalues of the matrix $J_N$ for any $N \geq 2L$. In (SI-6.31), each set \{\(\mu_1^{(j)}, \ldots, \mu_k^{(j)}\)\} is repeated $(N_j - 1)$-times with all $N_j \geq 2$, $j = 1, \ldots, K$.  

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Consider the following cascade model

$$\frac{dS}{dt} = H_0(S) + \sum_{j=1}^{L} \rho_j H(S, z_{2j-1}), \quad \sum_{j=1}^{L} \rho_j = \rho,$$

(SI-6.32a)

$$\frac{dz_j}{dt} = h(S, z_j), \quad j = 1, \ldots, 2L.$$  

(SI-6.32b)

State variables $z_{2j}$ with even indeces are absent from the first equation (SI-6.32a) of the cascade model (SI-6.32).

**Corollary 3.** Let $(S_0, N_1 z_{10}, \ldots, N_L z_{L0})$ be a mixed equilibrium solution of the G-model (SI-6.1), where $N_{j0} \neq N_{j0}'$ for all $j \neq j'$. Then, Statements (a) - (c) are equivalent.

(a). $(S_0, N_1 z_{10}, \ldots, N_L z_{L0})$ is exponentially stable in the G-model (SI-6.1) for any $N \geq 2L$, and with any $N_j \geq 2$, $j = 1, \ldots, L$, such that $N_1 + \ldots N_L = N$.

(b). $(S_0, z_{10}, \ldots, z_{L0})$ is exponentially stable in the reduced model (SI-6.28), and each $z_{j0}$ is exponentially stable in the single-cell model (SI-6.14), $j = 1, \ldots, L$.

(c). $(S_0, z_{10}, z_{10}, z_{20}, z_{20}, \ldots, z_{L0}, z_{L0})$ is exponentially stable in the cascade model (SI-6.32).
SI-7 Additional Figures

Figure SI-7.1

In accordance with predictions from Fig. 3, we observe that an increase in the values of $\delta_g$ lead to suppressed levels in $x_1$ (LacI) as well as to elevated levels in $y_1$ (TetR). This is illustrated in Fig. SI-7.1. The almost constant dependencies in Fig. SI-7.1 (C) and (D) can be explained by suppressed levels of TetR and C4-HSL in the G-population.

Figure SI-7.1. Examples of monotone parametric dependencies for the repressor-protein levels in the G-homogeneous state. Red solid curves correspond to a weak coupling among all toggles ($d = 0.1$), while black solid curves correspond to a strong coupling among all toggles ($d = 10$).

Analogously (Fig. SI-7.1), an increase in the values of $\delta_g$ should also lead to suppressed levels in $g_1$ (C14-HSL) as well as to elevated levels in $r_1$ (C4-HSL), while an increase in the values of $\delta_r$ should lead to elevated levels in $g_1$ (C14-HSL) and, simultaneously, to decreased levels in $r_1$ (C4-HSL). This is illustrated in Fig. SI-7.2. Constant dependencies in Fig. SI-7.2 (C) can be explained by suppressed levels of TetR and C4-HSL in the G-population.
Figure SI-7.2

Figure SI-7.2. Examples of monotone parametric dependencies for the signaling species levels in the G-homogeneous state. Red solid curves correspond to a weak coupling among all toggles \((d = 0.1)\), while black solid curves correspond to a strong coupling among all toggles \((d = 10)\).
Figure SI-7.3

Figure SI-7.3. Examples of monotone parametric dependencies for the signaling species levels in the (1:1)-mixed state. All explanations are as in Fig. 5.
Figure SI-7.4. Examples of monotone parametric dependencies for the repressor-protein levels in the (9:1)-mixed state (a 90% large G-subpopulation.) Green and red solid curves correspond to stable solution branches, while all blue curves correspond to unstable solution branches. Red filled circles correspond to an LP-bifurcation point. In panels (A) and (B), projections of stable and unstable solution branches coincide and, so, only the stable solution branches are shown.

The monotone parametric dependencies for a (9:1)-mixed state corresponding to a spontaneous synchronization error are illustrated in Fig. SI-7.4 for a large G-subpopulation comprising 90% of all cells, and in Fig. SI-7.5 for a small R-subpopulation comprising 10% of all cells in the given (9:1)-mixed state.

We observe that LP-bifurcation points are present in both panels (A) and (B), and are absent from both panels (C) and (D) in Fig. SI-7.4 and Fig. SI-7.5. To explain this observation we have to recall the difference between parameters $\delta_g$ and $\delta_r$. As discussed earlier, a decrease in the values of $\delta_g$ can be interpreted in terms of the improved communication between the toggles within the large subpopulation, while a decrease in the values of $\delta_r$ can be interpreted in terms of the improved communication between the toggles within the small subpopulation.
Figure SI-7.5. Examples of monotone parametric dependencies for the repressor-protein levels in the (9:1)-mixed state (a 10% small R-subpopulation.) Green and red solid curves correspond to stable solution branches, while all blue curves correspond to unstable solution branches. In panel (D), projections of stable and unstable solution branches coincide. Red filled circles in panels (A) and (B) correspond to an LP-bifurcation point.
Figure SI-7.6. Homogeneous populations of S and A toggles. Dependencies of G-homogeneous populations on the values of the parameter $d$ are shown. Top panels (A) and (B) correspond to a G-homogeneous population of S toggles. Panel (A) presents (dimensionless) levels of the activated LacI, while levels of the repressed TetR are of order of magnitude about $10^{-3}$ and are not shown. Panel (B) presents levels of C14-HSL. The green curve corresponds to the intracellular levels, while the black plot corresponds to extracellular levels of C14-HSL, respectively. Panels (C) and (E) present levels of the activated LacI and C4-HSL obtained for the A toggle settled at the G-state (LacI > TetR). Panels (D) and (F) present levels of the activated TetR and C4-HSL obtained for the A toggle settled at the R-state (TetR > LacI).
We observe from Fig. [SI-7.6] that the intracellular and extracellular levels of the QS signaling molecule C14-HSL become asymptotically indistinguishable from one another as \( d \to \infty \). The asymptotic behavior of the S toggle for large values of \( d \) can be analytically understood after introducing a small parameter \( \varepsilon = d^{-1} \) into the S-model (1) which becomes a singularly-perturbed problem [61]. Setting formally \( \varepsilon = 0 \) in the singularly-perturbed problem as required by the theory of singular perturbations [61], the differential equations (SI-8.1c) and (SI-8.1d) can be reduced to elementary algebraic equations \( g = g_e \) and \( r = r_e \), respectively.
A (1:1)-mixed population of A-toggles

Green and red color-coded curves in panels (A) and (B) correspond to the intracellular concentrations of LacI and TetR, respectively, while a black color-coded curve in panel (C) corresponds to the extracellular concentration of C4-HSL. In panel (C), the green color-coded curve corresponds to the concentration of C4-HSL within the G-subpopulation, that is, LacI > TetR as in panel (A), while the red color-coded curve corresponds to the R-subpopulation, that is, TetR > LacI as in panel (B).

Figure SI-7.7. A (1:1)-mixed population of A-toggles.
Figure SI-7.8. A (9:1)- and (1:9)-mixed population of A toggles. Here, all notations and color-coding schemes are as in Fig. 9. Panels (A) and (B) correspond to the (9:1)-mixed population, within which the transcription signature LacI ≫ TetR dominates in proportion 9:1 (i.e., with 90% of green cells and 10% of red cells), while panels (C) and (D) correspond to the (1:9)-mixed population, within which the opposite transcription signature TetR ≫ LacI as well dominates in proportion 9:1 (i.e., with 90% of red cells and 10% of green cells.)
Figure SI-7.9. Lack of any self-correction capability for spontaneous errors by A toggles. The left panels correspond to the reference (10:0)-state (G-state), while the right panels correspond to the reference (0:10)-state (R-state). The initial conditions in the left panels correspond to nine “green” cells and one “red” cell. The initial conditions in the right panels correspond to one “green” cell and nine “red” cells.
SI-8 Modification of the S and A Models to Describe Sequestration of AAA+ protease ClpXP

To probe competition and sequestration effects for AAA+ proteases ClpXP in the context of our monotone theory-based modeling studies described in the main text, we have modified S and A models by adding the corresponding Michaelis-Menten type degradation [37]. Because all scaling procedures used for this case are similar to the scaling procedures described in detail earlier, we omit laborious technical details.

SI-8.1 Modification of the S Model

A dimensionless modified $S_m$ model is

$$\frac{dx_i}{dt} = \gamma_x + \frac{a_1}{1 + y_i^{nx}} + \frac{a_3 g_i^{nG}}{1 + g_i^{nG}} - \delta x_i - \frac{k_{ssrA} x_i}{1 + Z_i}, \quad (SI-8.1a)$$

$$\frac{dy_i}{dt} = \gamma_y + \frac{a_2}{1 + x_i^{nx}} + \frac{a_4 r_i^{nR}}{1 + r_i^{nR}} - \delta y_i - \frac{k_{ssrA} y_i}{1 + Z_i}, \quad (SI-8.1b)$$

$$\frac{dg_i}{dt} = \gamma_g + \frac{a_5}{1 + y_i^{ny}} + d\left(g_e - g_i\right) - \delta_g g_i, \quad (SI-8.1c)$$

$$\frac{dr_i}{dt} = \gamma_r + \frac{a_6}{1 + x_i^{rx}} + d\left(r_e - r_i\right) - \delta_r r_i, \quad i = 1, \ldots, N, \quad (SI-8.1d)$$

$$\frac{dg_e}{dt} = \frac{\rho}{N} \sum_{i=1}^{N} d\left(g_i - g_e\right) - \delta_e g_e, \quad 0 \leq \rho \leq 1, \quad (SI-8.1e)$$

$$\frac{dr_e}{dt} = \frac{\rho}{N} \sum_{i=1}^{N} d\left(r_i - r_e\right) - \delta_e r_e. \quad (SI-8.1f)$$

Here, all state variables are as defined for the original (non-modified) S model (1), see the main text. New parameters in (SI-8.1b) and (SI-8.1b) include: $k_{ssrA}$, a maximal degradation rate for ssrA tagged proteins, $\delta$ is an intracellular dilution rate due to cell growth. A new term $Z_i$ used in (SI-8.1b) and (SI-8.1b) is

$$Z_i = x_i/K_{MX} + y_i/K_{MY}. \quad (SI-8.2)$$

(SI-8.2) assumes that the degradation kinetics of all ssrA-tagged proteins via ClpXP is the same and, hence, can be described with the same Michaelis-Menten equation [37]. Parameters, $K_{MY}$ and $K_{MY}$, used in (SI-8.2) are scaled Michaelis constants. All other parameters in (SI-8.1a) - (SI-8.1f) are as defined for the original (non-modified) S model (1).
SI-8.2 Modification of the A Model

A dimensionless modified A_m model is

\[
\frac{dx_i}{dt} = \gamma_x + \frac{a_1}{1 + y_i^{\text{nY}}} + \frac{a_4 r_i^{\text{nR}}}{1 + r_i^{\text{nR}}} - \delta x_i - \frac{k_{\text{ssrA}}}{1 + Z_i} x_i, \quad \text{(SI-8.3a)}
\]

\[
\frac{dy_i}{dt} = \gamma_y + \frac{a_2}{1 + x_i^{\text{nX}}} - \delta y_i - \frac{k_{\text{ssrA}}}{1 + Z_i} y_i, \quad \text{(SI-8.3b)}
\]

\[
\frac{dr_i}{dt} = \gamma_r + \frac{a_6}{1 + x_i^{\text{nX}}} + d(r_e - r_i) - \delta_r r_i, \quad i = 1, \ldots, N, \quad \text{(SI-8.3c)}
\]

\[
\frac{dr_e}{dt} = \frac{\rho}{N} \sum_{i=1}^{N} d(r_i - r_e) - \delta_e r_e. \quad \text{(SI-8.3d)}
\]

Here, all state variables and parameters are as defined for the S_m model [SI-8.1].

SI-8.3 Reference parameter values

For the sake of simplicity and as an important extreme situation, we assume that the reference number of AAA+ protease ClpXP molecules per cell is of the same order of magnitude as the reference number of ssrA-tagged protein molecules, i.e., LacI and TetR (SI-2.2 Nondimensionalization). In other words, we assume that the number of AAA+ protease ClpXP molecules per cell is about 40 monomers per cell, that is, ssrA-tagged LacI and TetR should compete for AAA+ protease ClpXP. A set of all dimensionless parameter values used in the modified models can be found in Table SI-8.1, and is computed based on the data obtained from [37].

Table SI-8.1. Dimensionless parameter values used in the computational modeling.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description of dimensionless parameters</th>
<th>Value</th>
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<tr>
<td>(k_{\text{ssrA}})</td>
<td>a maximal degradation rate for ssrA tagged proteins</td>
<td>10.0</td>
</tr>
<tr>
<td>(K_{MX})</td>
<td>a parameter reciprocal to the non-monotonicity degree for LacI</td>
<td>33.0</td>
</tr>
<tr>
<td>(K_{MY})</td>
<td>a parameter reciprocal to the non-monotonicity degree for TetR</td>
<td>33.0</td>
</tr>
<tr>
<td>(\delta)</td>
<td>an intracellular dilution rate due to cell growth</td>
<td>0.7</td>
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</table>
References


