

Supporting Information

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Repressilator models without spatial dependence

In the deterministic repressilator model introduced by Elowitz and Leibler,¹ a non-dimensionalized system of ordinary differential equations describes the dynamics of populations of mRNA (m_j) and proteins (p_j), viz.

$$\begin{aligned}\dot{m}_j &= -m_j + \frac{\alpha}{1 + p_{j-1}^n} + \alpha_0, \\ \dot{p}_j &= -\gamma(p_j - m_j),\end{aligned}\tag{1}$$

where the species index j runs from 1 to 3 and is to be interpreted modulo 3. The rate of transcription of mRNA j is regulated by the presence of its repressor protein p_{j-1} . When the repressor is present at high concentrations, the mRNA is transcribed at the ‘leaky’ rate α_0 . In the absence of repressor, the transcription rate is $(\alpha + \alpha_0)$. The corresponding proteins are produced at a rate proportional to the mRNA concentrations. All components decay over time; mRNA species have unit decay constant in non-dimensionalized units and proteins have decay constant γ .

The authors reported that this deterministic repressilator system possesses a steady state that is either stable or unstable depending on the values of the model parameters. In particular, instability of the steady state, which results in the emergence of oscillatory solutions, is favored by large n , large α , small α_0 and small γ .

For simplicity, consider the limit $\alpha_0 = 0$, *i.e.* gene expression can be completely repressed under saturating repressor concentrations. We also make the common simplification of omitting the explicit mRNA level dynamics, assuming that the mRNA populations remain in quasi-equilibrium. This is usually justified since mRNA lifetimes are much shorter than protein lifetimes.² With these considerations, our system of equations reduces to

$$\dot{p}_j = \gamma \left(\frac{\alpha}{1 + p_{j-1}^n} - p_j \right).\tag{2}$$

The dynamics of this simplified, single-step repressilator system were analyzed by Loinger and Biham.³ Consistently with results of Elowitz and Leibler,¹ it was found that the possibility of sustained oscillatory solutions was dependent on the Hill coefficient, n . For $n=1$ and $n=2$, no oscillatory solutions emerge irrespective of the values of other parameters. For $n=3$, sustained oscillations are possible. Thus, the sharpness of the repression modeled by the Hill function is important for determining the dynamics of the system. Notably, it was also found that oscillations were possible with $n=2$ if the mRNA dynamics were explicitly included, as indicated by the earlier results by Elowitz and Leibler. Hence, the behavior of the model system can be sensitive to the assumption of quasi-steady mRNA dynamics in some cases.

In the present work, we design a synthetic system based on the repressilator interaction network but not specifically modeling protein synthesis. We adopt the Hill function form of

repression from the single-step repressilator eqn (2) to model the production rates P_j of three chemical species from discrete sources, as described in the section below.

Repressilator with discrete point sources: stability analysis

Suppose we have three points, \mathbf{X}_j , $j=1,2,3$, in three-dimensional space. Chemical species j is produced at the point \mathbf{X}_j at the rate P_j . The chemical diffuses through the surrounding fluid medium, which is assumed to be quiescent, and degrades with a constant first order rate, γ . The concentration fields, $C_j(\mathbf{x},t)$, are described by

$$\partial_t C_j = D\nabla^2 C_j - \gamma C_j + P_j \delta(\mathbf{x} - \mathbf{X}_j), \quad (3)$$

where D is the diffusion constant and δ denotes the Dirac delta function. The production rates P_j describe the repressilator network: production of species j is reduced by the presence of chemical $(j-1) \bmod 3$ at the point \mathbf{X}_j . Throughout this analysis, the species index is to be interpreted modulo 3. The form of repression is modeled by the Hill function corresponding to the simplified repressilator model, eqn (2):

$$P_j(C_{j-1}(\mathbf{X}_j)) = \frac{P^{\max}}{1 + [C_{j-1}(\mathbf{X}_j)/C^{\text{thresh}}]^n}. \quad (4)$$

Here, P^{\max} is the unrepressed production rate, and C^{thresh} is the threshold concentration around which production drops from high to low. The parameter n is known as the Hill coefficient or cooperativity and determines the sharpness of the transition (see main text, Fig. 1B). The sources are located on the impermeable surface at $z=0$, and the no-flux boundary condition is imposed for the chemicals, $\partial_z C_j|_{z=0} = 0$. Finally, we focus on the symmetric configuration of the sources when they form a regular triangle of size l .

In order to obtain the conditions for the oscillatory behavior of the system, we determine the steady state and study its linear stability. It is convenient to introduce the dimensionless concentrations $c_j = C_j / C^{\text{thresh}}$ so the steady state equations take the form

$$D\nabla^2 c_j - \gamma c_j + \frac{P_0 \delta(\mathbf{x} - \mathbf{X}_j)}{1 + c_{j-1}^n(\mathbf{X}_j)} = 0, \quad (5)$$

where $P_0 = P^{\max} / C^{\text{thresh}}$. The above equation is solved using the Green's function method to obtain

$$c_j(\mathbf{x}) = \frac{2P_0 G_0(|\mathbf{x} - \mathbf{X}_j|)}{1 + \bar{c}_{j-1}^n}, \quad (6)$$

where

$$G_0(R) = \frac{\exp(-\sqrt{\gamma R^2 D^{-1}})}{4\pi DR} \quad (7)$$

is the Green's function, and $\bar{c}_{j-1} = c_{j-1}(\mathbf{X}_j)$. Note that the factor 2 in eqn (6) is due to the non-flux boundary conditions at the surface. Owing to the problem symmetry, all \bar{c}_j are equal, $\bar{c}_j = \bar{c}$, and satisfy the following equation [see eqn (6)]:

$$\bar{c}(1 + \bar{c}^n) = 2P_0 G_0(l). \quad (8)$$

The above equation can be easily solved analytically at $n = 1$ and numerically at arbitrary n .

Now consider small perturbations from equilibrium, $c_j = \bar{c} + \delta c_j$, and linearize the production rates in eqn (3) near the steady state value $\bar{P} = P_0(1 + \bar{c}^n)^{-1}$, writing $P_j = \bar{P} - \bar{S} \delta c_{j-1}$, where

$$\bar{S} = n \bar{c}^{n-1} (1 + \bar{c}^n)^{-1} \bar{P}. \quad (9)$$

Assuming that $\delta c_j(\mathbf{x}, t) = \delta c_j(\mathbf{x}) \exp(\lambda t)$, the linearized eqn (3) is transformed to the eigenvalue problem

$$D\nabla^2 \delta c_j - \gamma \delta c_j - \bar{S} \delta c_{j-1} \delta(\mathbf{x} - \mathbf{X}_j) = \lambda \delta c_j. \quad (10)$$

The steady state is unstable if there exists such eigenvalue λ that $\text{Re}(\lambda) > 0$. Application of the Green's function method to eqn (10) gives the following equation for the value $\xi_j = \delta c_j(\mathbf{X}_{j+1})$:

$$\xi_j = -2\bar{S} G_\lambda(l) \xi_{j-1}. \quad (11)$$

Here, G_λ is the Green's function

$$G_\lambda(R) = \frac{\exp(-\sqrt{(\lambda + \gamma)R^2 D^{-1}})}{4\pi DR}. \quad (12)$$

Applying eqn (11) three times, we obtain the equation for the eigenvalue λ :

$$8\bar{S}^3 G_\lambda^3(l) = -1. \quad (13)$$

It follows from the above equation that the eigenvalue is a complex number as it obeys the equation

$$\bar{S} \frac{\exp(-\sqrt{(\lambda + \gamma)l^2 D^{-1}})}{2\pi Dl} = \sqrt[3]{-1}.$$

Through using the timescale $t^* = 1/\gamma$, lengthscale $l^* = \sqrt{4D/\gamma}$, and production scale $P^* = \frac{1}{2}(4\pi D)^{3/2} \gamma^{-1/2} C^{\text{thresh}}$, we convert the above equation into the following final form:

$$\pi^{1/2} \bar{S} l^{-1} \exp(-2l\sqrt{1 + \lambda}) = \sqrt[3]{-1}. \quad (14)$$

Taking the complex logarithm of both sides of eqn (14) gives

$$2l\sqrt{1 + \lambda} = b - i\pi(1 + 2k)/3, \quad (15)$$

where $b = \log(\pi^{1/2} \bar{S} l^{-1})$ and k is some integer number. It is important to note that the square root of a complex number on the left hand side of the above equation is treated as the principal square root, so solution of eqn (15) requires $b \geq 0$. For a given integer k , the solution λ_k of eqn (15) is

$$\lambda_k = (2l)^{-2} [b^2 - \pi^2(1 + 2k)^2/9 - 2ib\pi(1 + 2k)/3] - 1. \quad (16)$$

The equilibrium state is linearly stable if and only if $\text{Re}(\lambda_k) \leq 0$ for every integer k . By inspection of eqn (16), we see that $\text{Re}(\lambda_k) \leq \text{Re}(\lambda_0)$ for any integer k . Hence, to determine stability of the equilibrium, we need only check the sign of $\text{Re}(\lambda_0)$, which is given by

$$\text{Re}(\lambda_0) = (2l)^{-2} (b^2 - \pi^2/9) - 1 .$$

Recalling that no solution exists if $b < 0$, we can express the condition for linear *instability* of the equilibrium state as $b > \sqrt{4l^2 + \pi^2/9}$, or equivalently,

$$\pi^{1/2} \bar{S} l^{-1} > \exp\left(\sqrt{4l^2 + \pi^2/9}\right) . \quad (17)$$

After introducing the characteristic time- and length-scales, eqn (8) and (9) are rewritten as

$$\bar{c} (1 + \bar{c}^n) = P^{\max} \Theta(l) , \quad (18)$$

$$\bar{S} = \frac{n \bar{c}^{n+1} P^{\max}}{[P^{\max} \Theta(l)]^2} , \quad (19)$$

where $\Theta(l) = \frac{\sqrt{\pi}}{l} \exp(-2l)$. Considering n to be fixed, the stability is therefore determined by the parameters P^{\max} and l . Note that both sides of the inequality eqn (17) vary smoothly with P^{\max} and l . There are no singular points in phase space so we expect regions of (P^{\max}, l) space that yield stable equilibrium states to be separated from regions where the equilibrium is unstable by a boundary curve on which $\text{Re}(\lambda_0) = 0$, *i.e.*,

$$\pi^{1/2} \bar{S} l^{-1} = \exp\left(\sqrt{4l^2 + \pi^2/9}\right) . \quad (20)$$

Let $P^{\text{crit}}(l)$ denote the critical value of the maximum production rate, such that eqn (20) is satisfied for a given separation l . Substituting eqn (19) into eqn (20) and rearranging gives

$$\frac{\bar{c}^{n+1}}{P^{\text{crit}} \Theta(l)} = n^{-1} \exp\left(\sqrt{4l^2 + \pi^2/9} - 2l\right) =: \zeta(l) , \quad (21)$$

We can insert this into the equilibrium condition eqn (18) to obtain

$$1 = \frac{\bar{c} + \bar{c}^{n+1}}{P^{\text{crit}} \Theta} = \zeta + \zeta^{1/(n+1)} (P^{\text{crit}} \Theta)^{-n/(n+1)} .$$

This leads to the expression

$$(P^{\text{crit}})^{n/(n+1)} = \Theta^{-n/(n+1)} \left(\frac{\zeta^{1/(n+1)}}{1 - \zeta} \right) , \quad (22)$$

from which we derive the formula for the critical maximum production rate

$$P^{\text{crit}}(l) = \frac{l}{\sqrt{\pi}} \exp(2l) \frac{1}{(1 - \zeta)} \left(\frac{\zeta}{1 - \zeta} \right)^{1/n} . \quad (23)$$

eqn (23) is the equation of the boundary curve separating stable from unstable behavior near the equilibrium state. These curves are plotted for $n = 2, 3, 4, 5$ in Fig. 2A of the main text. Note,

however, that eqn (22) only has positive real solutions for P^{crit} if $(1 - \zeta) > 0$. This constraint can be expressed as $\zeta(l) = n^{-1} \exp\left(\sqrt{4l^2 + \pi^2/9} - 2l\right) < 1$, or equivalently,

$$n > \exp\left(\sqrt{4l^2 + \pi^2/9} - 2l\right). \quad (24)$$

We now consider different values of n to highlight qualitative sensitivity when n is small.

Case $n = 1$:

The condition expressed by eqn (24) is not met for any $l > 0$. Hence, there is no point in phase space lying on the boundary between stability and instability of the equilibrium state. Since there are no discontinuities in phase space, the equilibrium state is either always stable or always unstable. Arbitrarily choosing $l = P^{\text{max}} = 1$, we find

$$\bar{c}(1 + \bar{c}) = \sqrt{\pi} \exp(-2) \Rightarrow \bar{c} \approx 0.20,$$

$$\bar{S} = \bar{c}^2 \cdot \frac{\exp(4)}{\pi} \approx 0.69,$$

$$\text{Re}(\lambda_0) = \frac{1}{4} \left(\log(\bar{S} \sqrt{\pi})^2 - \pi^2/9 \right) - 1 \approx -1.3 < 0,$$

and therefore conclude that the equilibrium state is always stable when $n = 1$.

Case $n = 2$:

For any $n > 1$, constraint (24) is equivalent to

$$l > \frac{1}{4} \left(\frac{\pi^2}{9 \log n} - \log n \right),$$

which means that for $n = 2$, there is no point in phase space at which $\text{Re}(\lambda_0) = 0$ when

$$l < l^{\text{min}} = \frac{1}{4} \left(\frac{\pi^2}{9 \log 2} - \log 2 \right) \approx 0.22.$$

Examining any point below this minimum separation reveals that the equilibrium state is stable. Hence, for $n = 2$, the source separation must be greater than l^{min} for sustained oscillations. This constraint gives rise to an asymptote $P^{\text{crit}} \rightarrow \infty$ as l approaches l^{min} from above, as seen in Fig. 2A of the main text.

Case $n = 3$:

The critical production rate $P^{\text{crit}}(l)$ exists whenever

$$l > l^{\text{min}} = \frac{1}{4} \left(\frac{\pi^2}{9 \log 3} - \log 3 \right) \approx -0.03,$$

which is satisfied for any separation $l > 0$. Unlike the $n = 2$ case, there is no lower limit to the separation; given any separation between the chemical sources, the equilibrium state can be made unstable, leading to sustained oscillations, by choosing a sufficiently large $P^{\text{max}} > P^{\text{crit}}$. The same is also true for any $n > 3$.

Finite adsorption on boundary

The lower plane boundary $z = 0$ adsorbs some of the chemicals from the fluid with finite capacity. If the total fractional surface coverage is θ , then the rate of adsorption of species j is

$$f_j^{\text{ads}} = K(1-\theta)C_j|_{z=0}, \quad (25)$$

and the boundary condition for the chemical field is

$$D\partial_z C_j|_{z=0} = K(1-\theta)C_j|_{z=0}. \quad (26)$$

We use a finite difference scheme to evolve the chemical fields on a Cartesian grid with spacing Δx . For the purposes of treating the adsorbing boundary condition, consider a one-dimensional subset of the field C_j^k , $k = 1, 2, 3, \dots$ corresponding to grid points with constant x - and y -coordinates and z -coordinates $z^k = (k + \frac{1}{2})\Delta x$. Using this grid definition, we do not have a grid point exactly on the boundary $z = 0$ but we can write a first order approximation

$$C_j^1 = C_j|_{z=0} + \frac{1}{2}\Delta x \cdot \partial_z C_j|_{z=0}. \quad (27)$$

The boundary condition eqn (26) gives an expression for the normal derivative at the boundary, which we substitute into eqn (27) to find the concentration at $z = 0$,

$$C_j|_{z=0} = \frac{2D/\Delta x}{K(1-\theta) + 2D/\Delta x} \cdot C_j^1. \quad (28)$$

From this, we derive an expression for the adsorption term in the discretized model,

$$f_j^{\text{ads}} = K(1-\theta)C_j|_{z=0} = \frac{2DK(1-\theta)/\Delta x}{K(1-\theta) + 2D/\Delta x} \cdot C_j^1. \quad (29)$$

At each time step, this adsorption rate is used to update the concentrations at the grid point adjacent to the boundary according to

$$C_j^1(t + \Delta t) = C_j^1(t) - f_j^{\text{ads}} \Delta t / \Delta x + F_j^1, \quad (30)$$

where F_j^1 is the change in concentration over a time step Δt due to all other processes, such as diffusion from neighboring grid points, chemical production and decay. The surface density of adsorbed chemicals C_j^s is likewise updated according to

$$C_j^s(t + \Delta t) = C_j^s(t) + f_j^{\text{ads}} \Delta t + F_j^s, \quad (31)$$

where F_j^s includes the first order decay of the adsorbed chemicals.

References

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