CSM2024: Homework 3 (85 points)

1. (25 points) **Double positive feedback**

   In this problem we will analyze the double positive feedback circuit defined by the equations

$$\frac{dx}{dt} = f(y) - \alpha x, \qquad \frac{dy}{dt} = g(x) - \alpha y.$$

   (a) (10 points) Phase plane analysis. Assuming reasonable functional forms for $f(y)$ and $g(x)$ use phase plane analysis to demonstrate that the double positive feedback loop can be bistable. Include nullclines for x, y , as well as fixed points in your plots. Include all values of model parameters.

   The nullclines for x, y are the set of points where $\frac{dx}{dt}, \frac{dy}{dt} = 0$:

$$\frac{dx}{dt} = 0 = f(y) - \alpha x \implies x = \frac{f(y)}{\alpha}$$

$$\frac{dy}{dt} = 0 = g(x) - \alpha y \implies y = \frac{g(x)}{\alpha}$$

   So, the nullclines for each variable are:

$$x : x = \frac{f(y)}{\alpha}$$

$$y : y = \frac{g(x)}{\alpha}$$

   We will assume that f, g are in the form of Hill functions:

$$f(y) = \frac{\beta_y y^n}{1 + y^n}, \quad g(x) = \frac{\beta_x x^n}{1 + x^n}$$

   Thus, the nullclines are:

$$x : x = \frac{\beta_y y^n}{\alpha(1 + y^n)}$$

$$y : y = \frac{\beta_x x^n}{\alpha(1 + x^n)}$$

   And, we have our system defined by the following equations:

$$\frac{dx}{dt} = \frac{\beta_y y^n}{1 + y^n} - \alpha x$$

$$\frac{dy}{dt} = \frac{\beta_x x^n}{1 + x^n} - \alpha y$$

   Plotting the nullclines, we obtain the following:

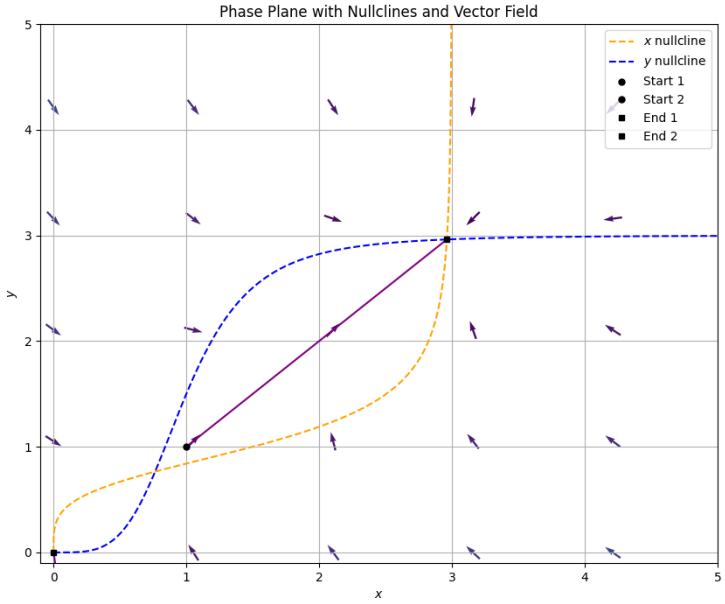


Figure 1: Phase plane plot with nullclines for x and y.

In figure 1, we see that there are two stable fixed points: one at $(0, 0)$, and another at $(2.96, 2.96)$. The model parameters that were used are summarized as follows:

β_x	β_y	α	n
3	3	1	4

The plot demonstrates that the double positive feedback loop can be bistable.

(b) (5 points) Effect of cooperativity. Show that in the absence of cooperative interactions the system is always monostable.
In the absence of cooperativity, i.e., $n = 1$, we can plot the nullclines for the system using the updated value for n (shown in figure 2).

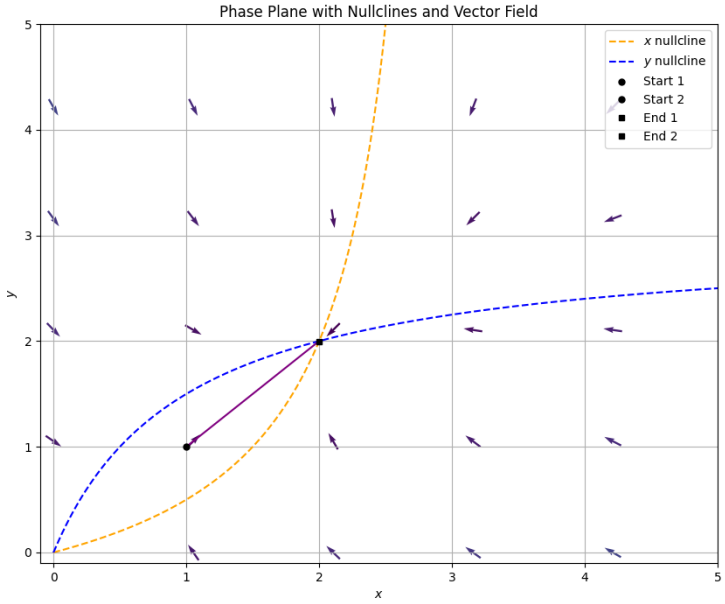


Figure 2: Phase plane plot with $n = 1$

Figure 2 shows the presence of one stable fixed point, illustrating that in the absence of cooperative interactions (when $n = 1$), the system is monostable.

(c) (10 points) Positive autoregulation. Now consider a model in which both x and y additionally undergo noncooperative positive autoregulation. Write differential equations for this system assuming AND logic for the effect of auto- and crossregulation. Find parameters of your model for which the system is bistable and make a phase plane plot showing nullclines, fixed points and example trajectories.

With AND logic, we need both terms (autoregulation and cross-regulation) to act together to stimulate x and y production. We can represent the production rates of x and y as products of two Hill functions, one for autoregulation and one for cross-regulation:

$$\frac{dx}{dt} = \frac{\beta_{xy} y^n}{1 + y^n} \cdot \frac{\beta_x x^n}{1 + x^n} - \alpha x$$

$$\frac{dy}{dt} = \frac{\beta_{yx} x^n}{1 + x^n} \cdot \frac{\beta_y y^n}{1 + y^n} - \alpha y$$

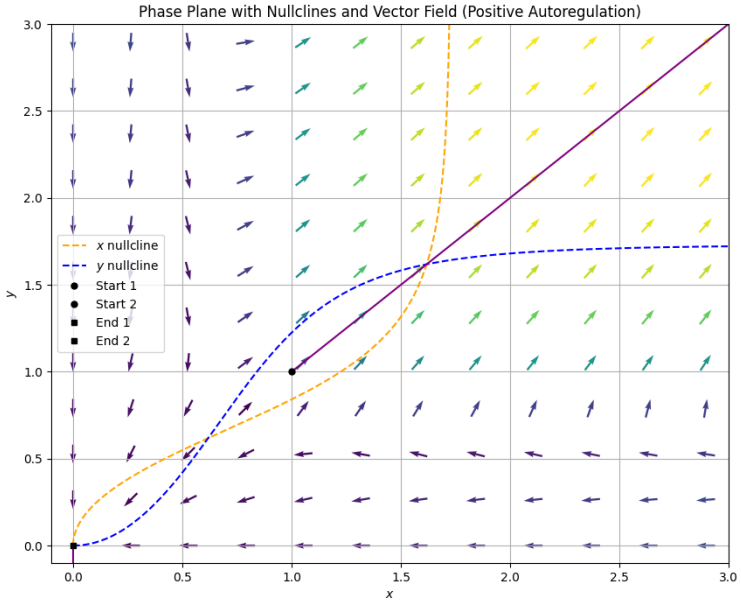


Figure 3: Phase plane plot for noncooperative positive autoregulation

Using this system, we obtain the phase plane diagram shown in figure 3 above. The model parameters are as follows:

β_{xy}	β_{yx}	β_x	β_y	α	n
3	3	4	4	1	4

2. (25 points) **Analysis of a 2D ODE system** Consider the following 2D equations for a model describing an enzyme substrate and its product:

$$\frac{dS}{dt} = v_0 - k_1 P^2 S \qquad \frac{dP}{dt} = k_1 P^2 S - k_2 P.$$

(a) (5 points) Fixed points. Find an expression for the fixed point(s) of the systems as a function of the parameters.

The fixed point(s) occur when $\frac{dS}{dt} = 0$ and $\frac{dP}{dt} = 0$:

$$\frac{dS}{dt} = v_0 - k_1 P^2 S = 0 \implies v_0 = k_1 P^2 S \implies S = \frac{v_0}{k_1 P^2}$$

$$\frac{dP}{dt} = k_1 P^2 S - k_2 P = 0 \implies k_1 P^2 S = k_2 P \implies S = \frac{k_2}{k_1 P}, (P \neq 0)$$

Now, we have two expressions for S, we can set them equal to each other and solve:

$$S = \frac{v_0}{k_1 P^2} = \frac{k_2}{k_1 P} \implies v_0 = k_2 P \implies P = \frac{v_0}{k_2}$$

So:

$$S = \frac{k_2}{k_1 P} = \frac{k_2}{k_1 (\frac{v_0}{k_2})^2} = \frac{v_0 k_2^2}{k_1 v_0^2} = \frac{k_2^2}{k_1 v_0}$$

Thus, we have that the fixed points occur at $S^* = \frac{k_2^2}{k_1 v_0}$ and $P^* = \frac{v_0}{k_2}$

(b) (5 points) Jacobian. Determine the Jacobian for this system at the fixed point(s). Based on this result what types of behavior(s) do you expect the system to exhibit and why?

The Jacobian matrix is the matrix containing the partial derivatives for the system. We have our 2D equations, both functions of S and P:

$$\frac{dS}{dt} = f(S, P) = v_0 - k_P^2 S$$

$$\frac{dP}{dt} = g(S, P) = k_1 P^2 S - k_2 P$$

With our equations redefined as functions, we can define the Jacobian as:

$$J = \begin{bmatrix} \frac{\partial f}{\partial S} & \frac{\partial f}{\partial P} \\ \frac{\partial g}{\partial S} & \frac{\partial g}{\partial P} \end{bmatrix}$$

After computing the partial derivatives, we get:

$$J = \begin{bmatrix} -k_1 P^2 & -2k_1 PS \\ k_1 P^2 & 2k_1 PS - k_2 \end{bmatrix}$$

Now, evaluating at the fixed points ($S^* = \frac{k_2^2}{k_1 v_0}$ and $P^* = \frac{v_0}{k_2}$), we get:

$$J = \begin{bmatrix} -k_1 \left(\frac{v_0}{k_2}\right)^2 & -2k_2 \\ k_1 \left(\frac{v_0}{k_2}\right)^2 & k_2 \end{bmatrix} = \begin{bmatrix} -\frac{k_1 v_0^2}{k_2^2} & -2k_2 \\ \frac{k_1 v_0^2}{k_2^2} & k_2 \end{bmatrix}$$

Based on this result, we can examine the determinant and trace of J to discover insights about the eigenvalues λ for this matrix.

$$Tr(J) = k_2 - \frac{k_1 v_0^2}{k_2^2}$$

$$Det(J) = -\frac{k_1 v_0^2}{k_2} + 2\frac{k_1 v_0^2}{k_2} = \frac{k_1 v_0^2}{k_2}$$

Assuming that k_1, v_0, and k_2 are positive constants, then $Det(J) > 0$. This means that we do not expect a saddle point in this system (occurs when $Det(J) = 0$).
Now let us examine the value of Δ:

$$\Delta = Tr(J)^2 - 4Det(J) = (k_2 - \frac{k_1 v_0^2}{k_2^2})^2 - 4(\frac{k_1 v_0^2}{k_2})$$

Since $Tr(J)^2 > 0$ (any quantity squared is positive), and we previously discussed that $Det(J) > 0$, then we know that $\Delta = Tr(J)^2 - 4Det(J) < 0$. This means that the behavior of this system will mimic some sort of a spiral (spiral sink, spiral source, or center spiral).

(c) (10 points) Stability analysis. Taking $k_1 = v_0 = 1$, analyze the stability of the fixed point(s) as a function of k_2. What different possible stability classes do you find? Plot an illustrative trajectory from each class and be sure to label your plots with the corresponding value of k_2.

Using $k_1 = v_0 = 1$, we can update the trace and determinant of J:

$$Tr(J) = k_2 - \frac{1}{k_2^2}$$

$$Det(J) = \frac{1}{k_2}$$

Now, we can use these quantities to calculate Δ:

$$\Delta = Tr(J)^2 - 4Det(J) = (k_2 - \frac{1}{k_2^2})^2 - \frac{4}{k_2} = k_2^2 - \frac{6}{k_2} + \frac{1}{k_2^4}$$

We can create a plot to analyze the stability of the system as a function of k_2:

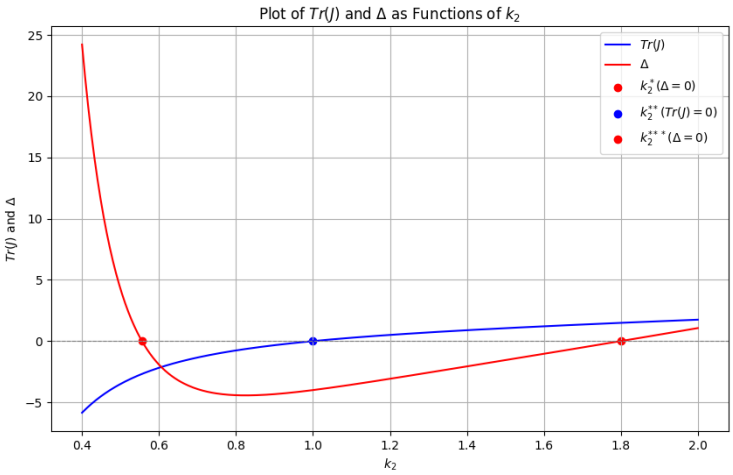


Figure 4: Plot showing the trace and delta of J as a function of k_2.

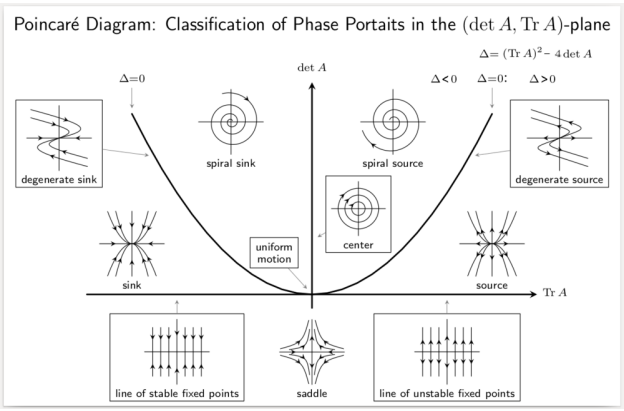


Figure 5: Poincare diagram

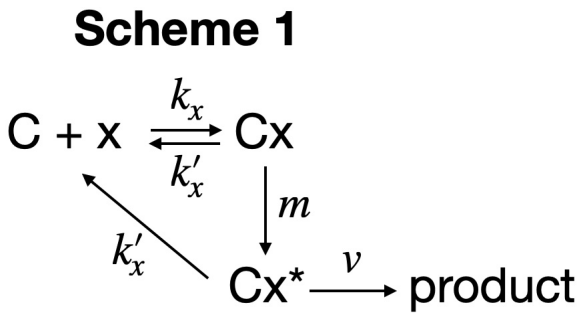
We find that the critical points (points at which Δ or $Tr(J)$ are equal to zero occur at: $k_2^* = 0.556$, $k_2^{**} = 1.0$, and $k_2^{***} = 1.8$. Let us now look at each section of the graph and determine the stability class for each, referencing the Poincare diagram:

k_2 **value**	Δ	$Tr(J)$	**Stability class**
$(0,k_2^*)$	+	-	sink
k_2^*	0	-	degenerate sink
$(k_2^*,\ k_2^{**})$	-	-	spiral sink
k_2^{**}	-	0	center
(k_2^{**}, k_2^{***})	-	+	spiral source
k_2^{***}	0	+	degenerate source
(k_2^{***}, ∞)	+	+	source

3. (25 points) **Kinetic proofreading model**

In this problem you will analyze two reaction schemes for their potential to exhibit kinetic proofreading using a steady state analysis. In each scheme the species X represents either the correct ligand c or the wrong ligand w to be paired with codon C.

(a) (10 points) Determine the steady state error rate F for Scheme 1:



Don't make any assumption (as we did in class) about the relative magnitudes of k'_x and m but you may assume that $k'_x \gg v$.

At the steady state, $\frac{dCx^*}{dt} = 0$.

$$\frac{dCx^*}{dt} = 0 = Cx \cdot m - Cx^* \cdot k'_x - Cx^* \cdot v$$

and

$$\frac{dCx}{dt} = 0 = C \cdot x \cdot k_x - Cx \cdot m - Cx \cdot k'_x$$

$$Cx^* \cdot k'_x + Cx^* \cdot v = Cx \cdot m \implies Cx^* = \frac{Cx \cdot m}{k'_x + v}$$

$$Cx \cdot m + Cx \cdot k'_x = C \cdot x \cdot k_x \implies Cx = \frac{C \cdot x \cdot k_x}{m + k'_x}$$

For the correct ligand c:

$$R_{correct} = v \cdot Cc^* = \frac{v \cdot Cc \cdot m}{k'_c + v} = \frac{v \cdot C \cdot c \cdot k_c \cdot m}{(k'_c + v)(m + k'_c)}$$

For the wrong ligand w:

$$R_{wrong} = \frac{v \cdot C \cdot w \cdot k_w \cdot m}{(k'_w + v)(m + k'_w)}$$

Error rate:

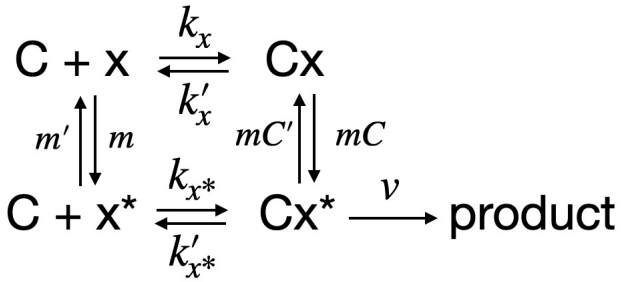
$$F = \frac{R_{wrong}}{R_{correct}} = \frac{v \cdot C \cdot w \cdot k_w \cdot m/(k'_w + v)(m + k'_w)}{v \cdot C \cdot c \cdot k_c \cdot m/(k'_c + v)(m + k'_c)} = \frac{w}{c} \cdot \frac{k_w(k'_c + v)(m + k'_c)}{k_c(k'_w + v)(m + k'_w)}$$

Since $k'_x \gg v$:

$$F = \frac{w}{c} \cdot \frac{k_w k'_c(m + k'_c)}{k_c k'_w(m + k'_w)}$$

(b) (10 points) Determine the steady state error rate F for Scheme 2:

Scheme 2



Again, don't make any assumption about the relative magnitudes of k'_x and mC but you may assume that $k'_x \gg v$. (Hint: consider the constraints on the rate constants imposed by detailed balance).

$$Cx = C \cdot x \cdot \frac{k_x}{k'_x}$$

$$Cx^* = Cx \cdot \frac{mC}{mC'}$$

For the correct ligand c:

$$R_{correct} = v \cdot Cc^* = v \cdot Cc \cdot \frac{mC}{mC'} = \frac{v \cdot C \cdot c \cdot k_c \cdot mC}{k'_c \cdot mC'}$$

For the wrong ligand w:

$$R_{wrong} = v \cdot Cw^* = v \cdot Cw \cdot \frac{mC}{mC'} = \frac{v \cdot C \cdot w \cdot k_w \cdot mC}{k'_w \cdot mC'}$$

Error rate:

$$F = \frac{R_{wrong}}{R_{correct}} = \frac{v \cdot C \cdot w \cdot k_w \cdot mC/k'_w \cdot mC'}{v \cdot C \cdot c \cdot k_c \cdot mC/k'_c \cdot mC'} = \frac{w}{c} \cdot \frac{k_w \cdot k'_c}{k_c \cdot k'_w}$$

(c) (5 points) Explain how your results demonstrate that kinetic proofreading requires an input of energy.

In our derived expressions, we see that the error rate F depends on the rate of association and dissociation for both the correct and incorrect ligands. The presence of this intermediate step (of "checking" the ligand) and the subsequent irreversible reaction of product creation allows for increased discrimination between correct and incorrect ligands. Specifically, it acts as a "checkpoint" that enables incorrect ligands to dissociate at a higher rate, reducing the error rate F beyond what is achievable in an equilibrium process. This extra suppression of the error rate is only possible because energy is being continuously consumed to drive the reaction through the irreversible transition. So, kinetic proofreading requires energy input to sustain the irreversibility and the checkpoints that enable selective correction, allowing the system to achieve a lower error rate than what equilibrium alone would permit.

4. (10 points) **Condition for Turing patterns**

In 1952, Turing proposed that two diffusible, interacting molecules can generate repeating patterns, such as spots or stripes in 2D space, serving as a foundational theory for biological pattern formation. The concentrations of two molecular species u, v in such systems are described by the equations:

$$\frac{\partial u}{\partial t} = D_1 \frac{\partial^2 u}{\partial^2 x} + f(u, v)$$

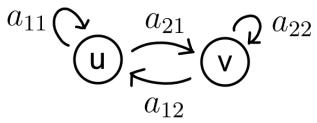
$$\frac{\partial v}{\partial t} = D_2 \frac{\partial^2 v}{\partial^2 x} + g(u, v)$$

Turing's key insight was to (1) consider a system where the homogeneous state (u_0, v_0) is stable in the *absence* of diffusion and (2) then find the conditions for which small perturbations grow over time in the *presence* of diffusion. While diffusion is often associated with a spatially smoothing effect, Turing showed that diffusion can destabilize the homogeneous state into patterns (hence, "Turing instability").

In 1972, Gierer and Meinhardt extended this analsysis to find the exact conditions for diffusion-induced instability. This revealed that the two diffusible molecules must be a pair of "Short-range activator and Long-range inhibitor", where, range refers to the length scale of molecular diffusion. Saving calculations, the final conditions are:

$$a_{11} + a_{22} < 0$$
$$D_1 a_{22} + D_2 a_{11} > 0$$

where $a_{11} = \frac{\partial f}{\partial u}\big|_{u=u_0}$ and $a_{22} = \frac{\partial g}{\partial v}\big|_{v=v_0}$ are coefficients following the linearization of the system around the fixed point (u_0, v_0). The network diagram with linearized reaction rates is sketched here:



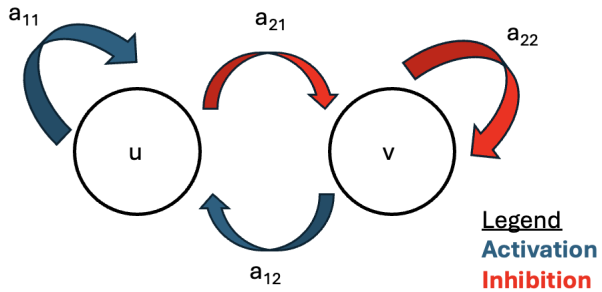
(a) (5 points) Explain how the two inequalities can be physically interpreted as the "Short-range activator and Long-range inhibitor" condition. Hint: You can assume $a_{11} > 0$ for simplicity. Think about the relative signs of a_{11}, a_{22} and the magnitudes of D_1, D_2.

The first inequality, $a_{11} + a_{22} < 0$, states that the sum of the self-regulation terms a_{11} and a_{22} should be negative. Since we assume $a_{11} > 0$, then a_{22} must be sufficiently negative for the inequality to hold true. The positive value for a_{11} means that u acts as an activator, enhancing its own production/ accumulation. The negative value for a_{22} means that v acts as an inhibitor on itself. Thus, the inequality $a_{11} + a_{22} < 0$ enforces a balance where the combined self-regulation of u and v does not lead to runaway growth, stabilizing the system locally in the absence of diffusion.

The second inequality, $D_1 a_{22} + D_2 a_{11} > 0$, involves both the self-regulation terms and the diffusion coefficients D_1 and D_2, which describe the spatial spread of each molecule. Since $a_{11} > 0$ and $a_{22} < 0$, then the diffusion rate of the inhibitor v (D_2) must be greater than that of the activator u (D_1). Since the activator u has a lower diffusion rate D_1, this means that it will have a shorter diffusion range. Conversely, the inhibitor, v, has a larger diffusion rate D_2, which means that it has a longer diffusion range, allowing it to spread out more and inhibit regions outside of the activator's influence. Thus, we see that these condition align with the "Short-range activator, Long-range inhibitor" description as desired.

(b) (5 points) Using an additional inequality $a_{11}a_{22} > a_{12}a_{21}$, identify the signs of a_{12}, a_{21} and re-write the network diagram above by replacing each arrow with those that indicate positive/negative regulation. There are two possible network diagrams.

The given inequality, $a_{11}a_{22} > a_{12}a_{21}$, expresses that the product of the self-regulation terms a_{11} and a_{22} is greater than the product of the cross regulation terms a_{12}, which describes the influence of v on u, and a_{21}, which describes the influence of u on v. Since we have already defined that $a_{11} > 0$ and $a_{22} < 0$, then we know that the product $a_{11}a_{22} < 0$, i.e., is negative. For the inequality to be true, then the product $a_{12}a_{21}$ must also be negative, and smaller than the product of the self-regulation terms. So, we know that the cross regulation terms must have opposing signs, and that either a_{12} or a_{21} is negative. We will choose the case where $a_{21} < 0$, i.e., u acts as an inhibitor on v. So, from this, then we know that $a_{12} > 0$, i.e., that v acts as an activator on u. This results in the following network diagram:



Collaboration Statement

Problem Number	Collaborators	Resources
1	N/A	Lecture notes (bistability)
2	N/A	Lecture notes (oscillators)
3	N/A	Lecture notes (kinetic proofreading)
4	N/A	N/A