Mathematical Analysis of a Multistable Switch Model of Cell Differentiation

JF Rabajante, CO Talaue, BD Aguda

SUPPLEMENTARY MATERIAL

The generalized Cinquin-Demongeot ODE model is as follows

$$\frac{d[X_i]}{dt} = F_i(X) = \frac{\beta_i [X_i]^{c_i}}{\overline{K_i}^{c_i} + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}} + g_i - \rho_i [X_i], \qquad \text{(S1)}$$

$$i = 1, 2, ..., n.$$

The multivariate function H_i is defined by

$$H_i([X_i], [X_2], ..., [X_n]) = \frac{\beta_i [X_i]^{c_i}}{\overline{K_i}^{c_i} + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}}. \qquad \text{(S2)}$$

We investigate the multivariate Hill function by looking at the univariate function defined by

$$H_i^1([X_i]) = \frac{\beta_i [X_i]^{c_i}}{K_i + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}} \qquad \text{(S3)}$$

where each $[X_j]$, $j \neq i$ is taken as a dynamic parameter.

The corresponding polynomial equation to

$$F_i(X) = \frac{\beta_i [X_i]^{c_i}}{K_i + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}} - \rho_i [X_i] + g_i = 0 \qquad \text{(S4)}$$

is

$$
\begin{aligned}
P_i(X) =& \beta_i[X_i]^{c_i} + (g_i - \rho_i[X_i]) \left(K_i + [X_i]^{c_i} + \sum_{j=1, j \neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}} \right) = 0 \\
=& -\rho_i[X_i]^{c_i+1} + (\beta_i + g_i)\,[X_i]^{c_i} - \left(K_i + \sum_{j=1, j \neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}} \right) (\rho_i[X_i]) \\
&+ g_i \sum_{j=1, j \neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}} + g_i K_i = 0 \ \forall i.
\end{aligned} \tag{S5}
$$

Proofs and Notes

SM1. Proof that all state variables are always non-negative.

Proof. Since we are considering only the biologically feasible points, then either $d[X_i]/dt|_{[X_i]=0} = 0$ or $d[X_i]/dt|_{[X_i]=0} > 0$ but $d[X_i]/dt|_{[X_i]=0} \not< 0$ (where $d[X_i]/dt$ is given in the ODE system (S1)). That is, if a component of a state variable is zero then the component will either stay zero or become positive but never negative. Hence, we are sure that the values of the state variables of the generalized Cinquin-Demongeot ODE model (S1) with non-negative initial condition are always non-negative. □

Note that the instantaneous rate of change $d[X_i]/dt|_{[X_i]=0} > 0$ happens only when $g_i > 0$.

SM2. Proof of Theorem 1: Suppose $\rho_i > 0$ for all i. Then the generalized Cinquin-Demongeot ODE model (S1) with $X_0 \in \mathbb{R}^{\oplus n}$ always has a stable component.

Proof. Figures (S1) to (S4) illustrate all possible cases showing the topologies of the intersections of $Y = \rho_i[X_i]$ and $Y = H_i^1([X_i]) + g_i$. We employ

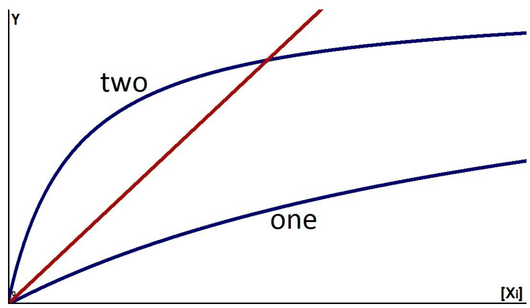

Figure S1: The possible number of intersections of $Y = \rho_i[X_i]$ and $Y = H_i^1([X_i]) + g_i$ where $c_i = 1$ and $g_i = 0$. The value of $K_i + \sum_{j=1,j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$ is fixed.

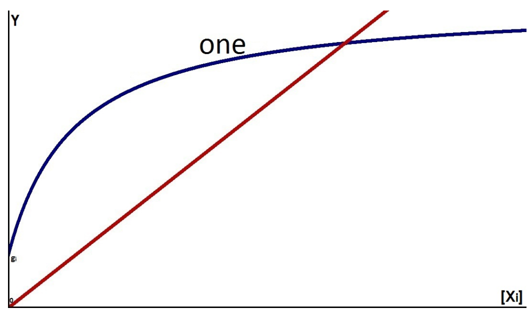

Figure S2: The possible number of intersections of $Y = \rho_i[X_i]$ and $Y = H_i^1([X_i]) + g_i$ where $c_i = 1$ and $g_i > 0$. The value of $K_i + \sum_{j=1,j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$ is fixed.

the geometric analysis shown in Figure (S5) (where we rotate the graph of the curves, making $Y = \rho_i[X_i]$ the horizontal axis) to each topology of the intersections of $Y = \rho_i[X_i]$ and $Y = H_i^1([X_i]) + g_i$. Given specific values of $[X_j]$, $j \neq i$, the univariate Hill curve $Y = H_i^1([X_i])$ and $Y = \rho_i[X_i]$ have the following possible number of intersections (see Figures (S1) to (S4)):

- two intersections (where one is stable);
- one intersection (which is stable); or

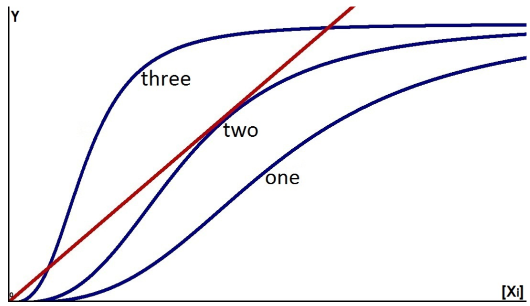

Figure S3: The possible number of intersections of $Y = \rho_i[X_i]$ and $Y = H_i^1([X_i]) + g_i$ where $c_i > 1$ and $g_i = 0$. The value of $K_i + \sum_{j=1,j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$ is fixed.

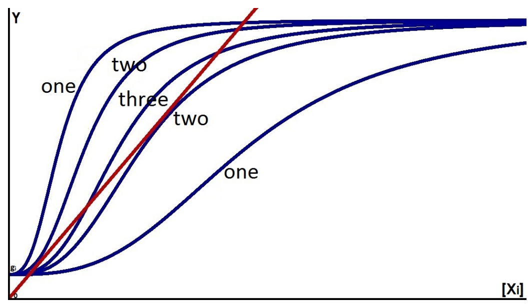

Figure S4: The possible number of intersections of $Y = \rho_i[X_i]$ and $Y = H_i^1([X_i]) + g_i$ where $c_i > 1$ and $g_i > 0$. The value of $K_i + \sum_{j=1,j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$ is fixed.

- three intersections (where two are stable).

We can see that there always exists a stable intersection located in the first quadrant (including the axes) of the Cartesian plane. We can also observe that when there are two or more intersections, the value of one stable intersection is always greater than the value of the unstable intersection — implying that any solution to the ODE is bounded. □

SM3. Proof of the statement: Suppose $\rho_i > 0$ then if both $\beta_i > 0$ and $g_i > 0$

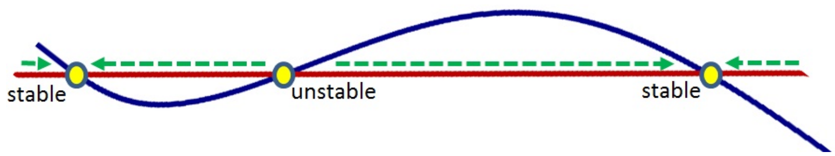

Figure S5: The curves are rotated making the line $Y = \rho_i[X_i]$ as the horizontal axis. Positive gradient means instability, negative gradient means stability. If the gradient is zero, we look at the left and right neighboring gradients.

then g_i/ρ_i cannot be an i-th component of an equilibrium point of the ODE system (S1).

Proof. Suppose $\beta_i > 0$, $g_i > 0$, and g_i/ρ_i is an i-th component of an equilibrium point. Then, from the ODE system (S1),

$$F_i\left([X_1], ..., \frac{g_i}{\rho_i}, ..., [X_n]\right) = \frac{\beta_i\left(\frac{g_i}{\rho_i}\right)^{c_i}}{K_i + \left(\frac{g_i}{\rho_i}\right)^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}} - \rho_i\frac{g_i}{\rho_i} + g_i = 0$$

$$= \frac{\beta_i\left(\frac{g_i}{\rho_i}\right)^{c_i}}{K_i + \left(\frac{g_i}{\rho_i}\right)^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}} = 0$$

implying that $\beta_i\left(g_i/\rho_i\right)^{c_i} = 0$. Thus $\beta_i = 0$ or $g_i = 0$, a contradiction. □

SM4. Proof of Theorem 2: Suppose $\rho_i > 0$. The value $\frac{g_i+\beta_i}{\rho_i}$ is the upper bound of, but will never be equal to, $[X_i]^*$ (where $[X_i]^*$ is the i-th component of an equilibrium point). The equilibrium points of the ODE system (S1) lie in the hyperspace

$$\left[\frac{g_1}{\rho_1}, \frac{g_1+\beta_1}{\rho_1}\right) \times \left[\frac{g_2}{\rho_2}, \frac{g_2+\beta_2}{\rho_2}\right) \times ... \times \left[\frac{g_n}{\rho_n}, \frac{g_n+\beta_n}{\rho_n}\right). \tag{S6}$$

Proof. The minimum value of the multivariate function H_i (S2) is zero which happens when $\beta_i = 0$ or when $[X_i] = 0$. If $H_i([X_1], [X_2], ..., [X_n]) = 0$ then $F_i(X) = g_i - \rho_i[X_i] = 0$, implying $[X_i] = g_i/\rho_i \ \forall i$.

The upper bound of H_i (S2) is β_i. If $H_i([X_1], [X_2], ..., [X_n]) = \beta_i$ then $F_i(X) = \beta_i - \rho_i[X_i] + g_i = 0$, implying $[X_i] = \frac{g_i+\beta_i}{\rho_i} \ \forall i$. However, H_i (S2) is equal to β_i only when $[X_i] = \infty$; hence, $[X_i] = \frac{g_i+\beta_i}{\rho_i}$ is an upper bound but cannot be a component of an equilibrium point. □

Note that the univariate Hill curve $Y = H_i^1([X_i])$ (S3) and $Y = \rho[X_i]$ intersect at infinity when $g_i \to \infty$, $\beta_i \to \infty$ or $\rho_i \to 0$.

SM5. Proof of Proposition 1: Under the assumption that that there is only a finite number of equilibrium points, then the number of equilibrium points of the generalized Cinquin-Demongeot ODE model (S1) (where c_i and c_{ij} are integers) is at most

$$max\{c_1+1, c_{1j}+1 \ \forall j\} \times max\{c_2+1, c_{2j}+1 \ \forall j\} \times ... \times max\{c_n+1, c_{nj}+1 \ \forall j\}.$$

Proof. Suppose there is only a finite number of equilibrium points. The degree of P_i (S5) is $max\{c_i + 1, c_{ij} + 1 \ \forall j\}$. By the Bézout Theorem, the number of complex-valued solutions to the polynomial system is at most $max\{c_1 + 1, c_{1j} + 1 \ \forall j\} \times max\{c_2 + 1, c_{2j} + 1 \ \forall j\} \times \ldots \times max\{c_n + 1, c_{nj} + 1 \ \forall j\}$. It follows that this is the upper bound of the number of real-valued equilibrium points. □

SM6. Proof of Lemma 1: Suppose $c_i = c_{ij} = 1$, $g_i = 0$, $\gamma_{ij} = 1$, $\beta_i = \beta_j = \beta > 0$, $\rho_i = \rho_j = \rho > 0$ and $K_i = K_j = K > 0$, for all i and j. Then the ODE model (S1) has infinitely many non-isolated equilibrium points if $\beta > \rho K$.

Moreover, if $\beta \leq \rho K$ then there is exactly one equilibrium point which is the origin.

Proof. Recall Equation (S5), we have the corresponding polynomial system $P_i(X) = 0$ $(i = 1, 2, ..., n)$:

$$\beta_i[X_i]^{c_i} - \rho_i K_i[X_i] - \rho_i[X_i]^{c_i+1} - \rho_i[X_i] \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$$
$$+g_i K_i + g_i[X_i]^{c_i} + g_i \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}} = 0.$$

Suppose $c_i = c_{ij} = 1$, $g_i = 0$, $\gamma_{ij} = 1$, $\beta_i = \beta_j = \beta > 0$, $\rho_i = \rho_j = \rho > 0$ and $K_i = K_j = K > 0$. Then the polynomial system can be written as $(i = 1, 2, ..., n)$

$$\beta[X_i] - \rho K[X_i] - \rho[X_i]^2 - \rho[X_i] \sum_{j=1, j\neq i}^{n} [X_j] = 0$$
$$\Rightarrow [X_i] \left(\beta - \rho K - \rho[X_i] - \rho \sum_{j=1, j\neq i}^{n} [X_j] \right) = 0$$
$$\Rightarrow [X_i] = 0 \text{ or } \left(\beta - \rho K - \rho[X_i] - \rho \sum_{j=1, j\neq i}^{n} [X_j] \right) = 0. \quad \text{(S7)}$$

Notice that the factor

$$\beta - \rho K - \rho[X_i] - \rho \sum_{j=1, j\neq i}^{n} [X_j]$$
$$= \beta - \rho K - \rho \sum_{j=1}^{n} [X_j]$$

is common to all equations in the polynomial system. Thus, there are infinitely many complex-valued solutions. However, note that we have restricted the state variables to be non-negative, so we do further investigation to determine the conditions for the existence of an infinite number of solutions given strictly non-negative variables. We focus our investigation on real-valued solutions.

Suppose $B = \beta - \rho K$.

Case 1: If $\beta = \rho K$ then $B = 0$. Thus, $B - \rho \sum_{j=1}^{n} [X_j]$ will never be zero except when $[X_j] = 0 \; \forall j = 1, 2, ..., n$ (since $[X_j]$ can take only non-negative values). Hence, the only equilibrium point to the system is the origin.

Case 2: If $\beta < \rho K$ then $B < 0$. Thus, $B - \rho \sum_{j=1}^{n} [X_j]$ will always be negative and will not have any zero for any non-negative value of $[X_j]$. Hence, the only equilibrium point is the origin (that is, $[X_i] = 0 \; \forall i = 1, 2, ..., n$, see Equation (S7)).

Case 3: If $\beta > \rho K$ then $B > 0$. Thus, there exist solutions to the equation $B - \rho \sum_{j=1}^{n} [X_j] = 0$. Notice that the set of non-negative real-valued solutions to $B - \rho \sum_{j=1}^{n} [X_j] = 0$ is a hyperplane (e.g., it is a line for $n = 2$ and it is a plane for $n = 3$). Hence, there are infinitely many non-isolated equilibrium points when $\beta > \rho K$. □

SM7. Stability analysis using the geometry of Hill function, an illustrative example. This is an alternative to the linearization technique using Jacobian matrix.

Consider that all parameters of the ODE system (S1) are equal to 1 except for $c_i = c_{ij} = 3$, $\beta_i = 20$, $\rho_i = 10$, $g_2 = 0$ and $g_3 = 0$, $i, j = 1, 2, 3$. One of the equilibrium points is $([X_1]^* = 0.10103, [X_2]^* = 1.001, [X_3]^* = 0)$. To determine the stability of this equilibrium point we initially look at the intersection of $Y = H_1^1([X_1]) + 1$ and $Y = 10[X_1]$ with $[X_2] = 1.001$ and

$[X_3] = 0$. Then we determine if $[X_1]^* = 0.10103$ is stable or not using Figure (S5). We conclude that $[X_1]^* = 0.10103$ is stable.

Now, we test the stability of $[X_2]^* = 1.001$ by looking at the intersection of $Y = H_2^1([X_2])$ and $Y = 10[X_2]$ with $[X_1] = 0.10103$ and $[X_3] = 0$. Furthermore, we test the stability of $[X_3]^* = 0$ by looking at the intersection of $Y = H_3^1([X_3])$ and $Y = 10[X_3]$ with $[X_1] = 0.10103$ and $[X_2] = 1.001$. The tests reveal that $[X_2]^* = 1.001$ is unstable and $[X_3] = 0$ is stable.

Because of the presence of an unstable component (which is $[X_2]^* = 1.001$), hence, the equilibrium point ($[X_1]^* = 0.10103, [X_2]^* = 1.001, [X_3]^* = 0$) is unstable.

SM8. Proof of Remark 1: Suppose $c_i > 1$. If $[X_i]^* = 0$ (i.e., the i-th component of an equilibrium point of the ODE system (S1) is zero), then $[X_i]^* = 0$ is always a stable component.

Proof. The ODE system (S1) has an equilibrium point with i-th component equal to zero if and only if $g_i = 0$. The only possible topologies of the intersections of $Y = H_i^1([X_i])$ and $Y = \rho_i[X_i]$ are shown in Figure (S6). Notice that zero i-th component is always stable. □

SM9. Proof of Proposition 2: Suppose $c_i = c_{ij} = 1$, $g_i = 0$, $\gamma_{ij} = 1$, $\beta_i = \beta_j = \beta > 0$, $\rho_i = \rho_j = \rho > 0$, $K_i = K_j = K > 0$ and $\beta > \rho K$, for all i and j. Then the origin is an unstable equilibrium point of the ODE system (S1) while the points lying on the hyperplane

$$\sum_{j=1}^{n} [X_j] = \frac{\beta}{\rho} - K \tag{S8}$$

are stable equilibrium points.

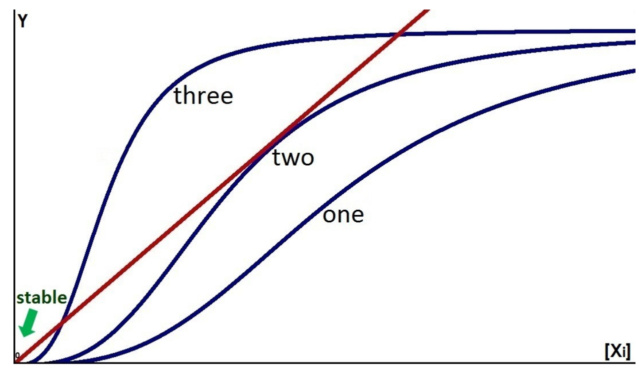

Figure S6: The possible number of intersections of $Y = \rho_i[X_i]$ and $Y = H_i^1([X_i]) + g_i$ where $c > 1$ and $g = 0$. The value of $K_i + \sum_{j=1,j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$ is taken as a parameter. $[X_i]^* = 0$ is always a stable component

Proof. From Corollary 1, the origin and the points lying on the hyperplane (S8) are equilibrium points of the ODE system (S1). Moreover, recall that the graph of the univariate Hill function $Y = H_i^1([X_i])$ (S3) with $c_i = 1$ is hyperbolic.

Suppose $\sum_{j=1,j\neq i}^{n} [X_j] = 0$ in the denominator of H_i^1. At $[X_i] = 0$, the slope of the Hill curve $Y = H_i^1([X_i])$ is

$$\frac{\partial H_i^1}{\partial [X_i]} = \frac{\beta}{K}. \tag{S9}$$

Since $\beta > \rho K$ then $\beta/K > \rho$. This implies that the slope of $Y = H_i^1([X_i])$ at $[X_i] = 0$ is greater than the slope of the decay line $Y = \rho[X_i]$. Therefore, when $\sum_{j=1,j\neq i}^{n} [X_j] = 0$ in the denominator of H_i^1, there are two possible intersections of $Y = H_i^1([X_i])$ and $Y = \rho[X_i]$. The intersection is at the origin (which is unstable) and at $[X_i] = \beta/\rho - K$ (which is stable).

Now, suppose $\sum_{j=1,j\neq i}^{n} [X_j]$ in the denominator of H_i^1 varies. Then the intersection of $Y = H_i^1([X_i])$ and $Y = \rho[X_i]$ is at the origin (which is unstable) and at $[X_i] = \beta/\rho - K - \sum_{j=1,j\neq i}^{n} [X_j]$ (which is stable). Hence, the hyperplane $[X_i] = \beta/\rho - K - \sum_{j=1,j\neq i}^{n} [X_j]$ is a set of stable equilibrium points. See Figure (S7) for illustration.

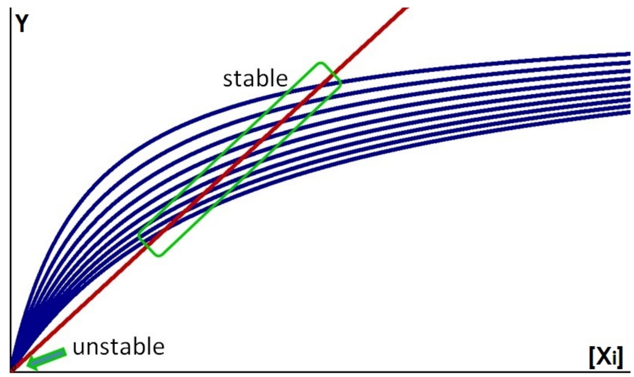

Figure S7: The origin is unstable while the points where $[X_i]^* = \beta/\rho - K - \sum_{j=1,j\neq i}^{n} [X_j]^*$ are stable.

□

SM10. Proof of the statement (see Appendix B): If $c_i > 1$, $g_i = 0$ and

$$\rho_i({K_i}^{1/c_i}) \geq \beta_i \tag{S10}$$

for all i, then the ODE system (S1) has only one equilibrium point which is the origin.

Proof. Let us first consider the case where $[X_j] = 0$, for all $j \neq i$. Recall that the upper bound of H_i^1 is β_i. Moreover, recall that when $[X_i] = K_i^{1/c_i}$ then $H_i^1([X_i]) = \beta_i/2$. Note that $(K_i^{1/c_i}, \beta_i/2)$ is the inflection point of the univariate Hill curve. We substitute $[X_i] = K_i^{1/c_i}$ in the decay function $Y = \rho_i[X_i]$, and if the value of $\rho_i({K_i}^{1/c_i})$ is larger or equal to the value of the upper bound β_i then $Y = H_i^1([X_i])$ and $Y = \rho_i[X_i]$ only intersect at the origin.

Now, as the values of $\gamma_{ij}[X_j]$ for all $j \neq i$ increase then the univariate Hill curve $Y = H_i^1([X_i])$ will just shrink and will definitely not intersect the decay line $Y = [X_i]$ except at the origin. $\square$

SM11. Proof of the statement (see Appendix B): If $c_i = 1$, $g_i = 0$ and $\beta_i/K_i \leq \rho_i$ for all i, then the ODE system (S1) has only one equilibrium point which is the origin.

Proof. Let us first consider the case where $[X_j] = 0$, for all $j \neq i$. Recall that $Y = H_i^1([X_i])$ where $c_i = 1$ is a hyperbolic curve. The partial derivative

$$\frac{\partial H_i^1}{\partial [X_i]} = \frac{\partial}{\partial [X_i]} \left(\frac{\beta_i [X_i]}{K_i + [X_i]} \right) = \frac{K_i \beta_i}{(K_i + [X_i])^2}$$

means that the slope of the hyperbolic curve is monotonically decreasing as $[X_i]$ increases. The partial derivative at $[X_i] = 0$ is

$$\frac{\partial H_i^1}{\partial [X_i]} = \frac{\beta_i}{K_i} \leq \rho_i,$$

which means that the slope of $Y = H_i^1([X_i])$ at $[X_i] = 0$ is less than the slope of the decay line $Y = \rho_i[X_i]$ at $[X_i] = 0$. Hence, the Hill curve $Y = H_i^1([X_i])$ lies below the decay line for all $[X_i] > 0$. $\square$

SM12. Proof of the statement (see Appendix B): In the ODE system (S1), suppose $g_i = 0$ and $c_i = 1$ $\forall i$. Then the origin is a stable equilibrium point when $\rho_i > \beta_i/K_i$ $\forall i$, or an unstable equilibrium point when $\rho_i < \beta_i/K_i$ for at least one i. When $\rho_i = \beta_i/K_i$ for at least one i, then we have a nonhyperbolic equilibrium point, which is an attractor only when $[X_i]$ is restricted to be non-negative and $\rho_j \geq \beta_j/K_j \ \forall j \neq i$.

Proof. The characteristic polynomial associated with the Jacobian of the ODE system (S1) when $X = (0, 0, ..., 0)$ is

$$|\mathbf{J}F(\mathbf{0}) - \lambda \mathbf{I}| = \begin{vmatrix} \frac{\beta_1}{K_1} - \rho_1 - \lambda & 0 & \cdots & 0 \\ 0 & \frac{\beta_2}{K_2} - \rho_2 - \lambda & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & \frac{\beta_n}{K_n} - \rho_n - \lambda \end{vmatrix}$$
$$= \left(\frac{\beta_1}{K_1} - \rho_1 - \lambda\right)\left(\frac{\beta_2}{K_2} - \rho_2 - \lambda\right)...\left(\frac{\beta_n}{K_n} - \rho_n - \lambda\right).$$

The eigenvalues (λ) are $\beta_1/K_1 - \rho_1, \beta_2/K_2 - \rho_2, ..., \beta_n/K_n - \rho_n$. Therefore, the origin is a stable equilibrium point when $\rho_i > \beta_i/K_i \ \forall i$. The origin is an unstable equilibrium point when $\rho_i < \beta_i/K_i$ for at least one i.

If $\rho_j \geq \beta_j/K_j \ \forall j \neq i$ but $\rho_i = \beta_i/K_i$ (for at least one i) then we have a nonhyperbolic equilibrium point. Geometrically, we can see that $[X_i]^* = 0$ is a saddle — stable at the right and unstable at the left of $[X_i]^* = 0$. Hence, if we restrict $[X_i] \geq 0$ for any value of $[X_i]$ then this nonhyperbolic equilibrium point is an attractor. □

SM13. Proof of the statement (see Appendix B): Suppose $\rho_i > 0$, $g_i = 0$ and $c_i > 1 \ \forall i$, then the origin is a stable equilibrium point of the ODE system (S1).

Proof. Since $g_i = 0$ for all i then the origin is an equilibrium point. The characteristic polynomial associated with the Jacobian of the ODE system

(S1) when $X = (0, 0, ..., 0)$ is

$$|\mathbf{J}F(\mathbf{0}) - \lambda\mathbf{I}| = \begin{vmatrix} -\rho_1 - \lambda & 0 & \cdots & 0 \\ 0 & -\rho_2 - \lambda & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & -\rho_n - \lambda \end{vmatrix}$$

$$= (-\rho_1 - \lambda)(-\rho_2 - \lambda)...(-\rho_n - \lambda).$$

The eigenvalues (λ) are $-\rho_1, -\rho_2, ..., -\rho_n$ which are all negative. Therefore, the zero state is a stable equilibrium point. $\square$