Mathematical Analysis of a Multistable Switch Model of Cell Differentiation

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Abstract

Non-binary simultaneous decision network (SDN) of gene regulation represents a cell differentiation process that involves more than two possible cell lineages. This SDN can predict the existence of multistable master switches. To investigate the qualitative behavior of the dynamics of the SDN, we employ geometric techniques in the analysis of the network's associated system of ordinary differential equations (ODE). We determine the location and the maximum number of equilibrium points given a set of parameter values. Varying the values of some parameters, such as efficiency of transcription, intensity of exogenous stimulus and protein degradation rate, can decrease the size of the basin of attraction of an undesired steady state as well as increase the size of the basin of attraction of a desired steady state. A sufficient change in some parameter values can silence or reactivate gene transcription that re-

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sults in cell-fate switching. We further show that increasing the amount of exogenous stimulus can shutdown multistability of the system such that only one stable equilibrium point remains. Using the simultaneous decision model, we can predict the temporal state of a population of differentiating cells and investigate possible conditions for reprogramming.

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1. Highlights

1. We prove properties of the generalized Cinquin-Demongeot model of gene regulation.
2. The nature of the steady states can be examined using the geometry of the Hill function.
3. Varying parameter conditions can induce dedifferentiation or transdifferentiation.
4. We model a mesenchymal cell differentiation system using simultaneous decision network.

2. Introduction

In this study, we are interested in the analysis of gene interaction networks that orchestrate the differentiation of stem cells to various cell lineages that

make up an organism [1, 2, 3, 4, 5]. Differentiated cells can be induced to switch phenotype (transdifferentiation) [6, 7, 8, 9] or to switch back to a pluripotent state (dedifferentiation) through cellular programming [10, 11, 12, 13, 14]. We are motivated by the prospects of utilizing stem cells in regenerative medicine, in revolutionizing drug discovery, and in the control of cancer stem cells that had been hypothesized to maintain the growth of tumors [15, 16, 17, 18, 19].

According to Waddington's model [20], cell differentiation is similar to a ball rolling down a landscape of hills and valleys. The ridges of the hills can be regarded as unstable equilibrium points while the parts of the valleys where the ball can stay without rolling further (i.e., at relative minima of the landscape) can be regarded as stable equilibrium points or attractors. An attractor is suggested to represent a specific cell type. A mathematical model representing the dynamics of stem cells is therefore expected to exhibit multistability [21, 22, 23, 24]. Cinquin and Demongeot [25] formulated a gene regulatory network (GRN) that can account for the differentiation of one cell to more than two possible lineages (multistability). Depicted in Figure (1) are two models of cell differentiation. The simultaneous decision network (lower half of Figure (1)) is one of the possible representations of Waddington's model where there are possibly many cell lineages involved. This representation is an alternative model to the binary or boolean hierarchic decision network shown in the upper half of Figure (1) [25, 26, 27, 28].

Cinquin and Demongeot [25] translated the simultaneous decision network (SDN) with auto-activation and mutual inhibition into an ordinary differential equation (ODE) model. Auto-activation is a common property

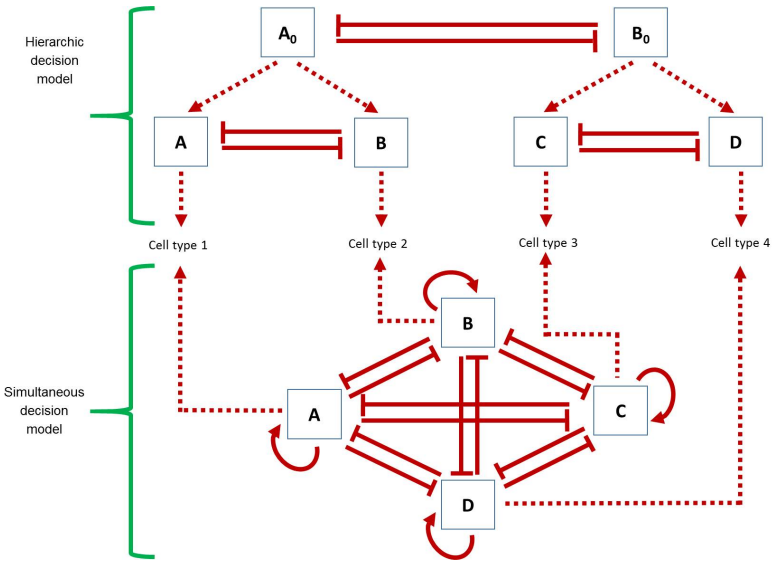

Figure 1: Hierarchic decision model and simultaneous decision model [25, 26]. Bars represent repression or inhibition, while arrows represent activation. The nodes in the simultaneous decision model represent proteins involved in gene expression.

of cell fate-determining factors known as "master" switches [25]. All elements in the original Cinquin-Demongeot ODE model are symmetric, that is, each node has the same relationship with all other nodes. In this paper, we generalize the Cinquin-Demongeot ODE model by considering symmetric and asymmetric protein interactions, and by having more adjustable parameters to predict a wider range of steady states. The state variables of the ODE model represent the concentration of the transcription factors (TFs) involved in gene expression.

Stability and bifurcation analysis of the generalized Cinquin-Demongeot ODE model is performed to help understand the dynamics of cell differentiation. We determine the biologically feasible (non-negative real-valued)

coexisting stable equilibrium points of the generalized Cinquin-Demongeot ODE model for a given set of parameters. We then identify if varying the values of some parameters, such as those associated with the exogenous stimuli, can steer the system toward a desired state.

Furthermore, we present a case where the generalized Cinquin-Demongeot ODE model can be used. We represent a phenomenological gene regulatory network of a mesenchymal cell differentiation system [29] using the simultaneous decision model. This GRN is composed of four nodes consisting of pluripotency and differentiation modules. The differentiation module represents a circuit of transcription factors that activate osteogenesis, chondrogenesis, and adipogenesis. We show that increasing the efficiency of transcription and effect of exogenous stimuli as well as decreasing the protein degradation rate can induce cells to undergo dedifferentiation or transdifferentiation.

3. ODE model representing GRN dynamics

A state $X = ([X_1], [X_2], ..., [X_n])$ represents a temporal stage in the cell differentiation or programming process. We define $[X_i]$ as a *component* (coordinate) of a state which represents the concentration of the transcription factor X_i. A stable state (stable equilibrium point) $X^* = ([X_1]^*, [X_2]^*, ..., [X_n]^*)$ represents a certain cell phenotype, e.g., pluripotent, tripotent, bipotent, unipotent, or fully (terminally) differentiated cell.

In [25], Cinquin and Demongeot suggested to extend their model to include combinatorial interactions and non-symmetrical networks (i.e., each node does not have the same relationship with other nodes and all equations in the system of ODEs do not have equal parameter values). We in-

clude more adjustable parameters to the original Cinquin-Demongeot model to represent a wider range of situations. In this generalized model, some cell differentiation factors can be stronger than others. We generalize the Cinquin-Demongeot ODE model as follows:

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

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

where n is the number of nodes. To have biological significance, we restrict the parameters to be non-negative real numbers. The parameter β_i is the speed or efficiency of transcription (or strength of the unrepressed TF expression relative to the first-order degradation), ρ_i is the assumed first-order degradation (exponential decay) constant of $[X_i]$, and γ_{ij} is a coefficient associated with the inhibition of X_i by X_j. If $\gamma_{ij} = 0$ then X_j does not inhibit the growth of $[X_i]$. We consider

$$g_i = \alpha_i s_i + e_i \qquad (2)$$

to represent basal or constitutive expression (e_i) of the corresponding TF [30, 31] plus the effect of the exogenous stimulus with concentration s_i and rate α_i. In this paper, we assume fixed concentration of exogenous stimulus s_i.

We define the multivariate function H_i by

$$H_i([X_i], [X_2], ..., [X_n]) = \frac{\beta_i [X_i]^{c_i}}{\overline{K_i}^{c_i} + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij} [X_j]^{c_{ij}}} \qquad (3)$$

which comes from the classical Hill equation [32, 33]. The terms $\sum_{j=1,j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$ in the denominator reflect the inhibitory influence of other TFs on the growth of $[X_i]$. For simplicity, let $K_i = \overline{K_i}^{c_i} > 0$, which is the threshold constant. If all $[X_j] = 0$ for all $j \neq i$ then the function value of H_i is equal to $\beta_i/2$ when $K_i = [X_i]^{c_i}$. The parameter $c_i \geq 1$ represents the Hill constant and affects the steepness of the Hill curve associated with $[X_i]$, and denotes auto-activation. The parameter c_{ij}, $j \neq i$ denotes inhibition. These exponents represent the sigmoidal kinetics possibly induced by multiple cellular processes [34]. In addition, the lower bound of H_i (3) is zero and its upper bound is β_i. Thus, the parameter β_i can also be interpreted as the maximal expression rate of the corresponding TF.

We only consider the biologically feasible points — those that are real-valued and non-negative. The initial value $X_0 = ([X_1]_0, [X_2]_0, ..., [X_n]_0)$ should always be biologically feasible. It follows that the flow of the ODE model (1) (where $X_0 \in \mathbb{R}^{\oplus n}$ can be any initial condition) is always in $\mathbb{R}^{\oplus n}$ (that is, all state variables are always non-negative).

For simplification, only the transcription regulation process is considered in modeling cell differentiation. By using an ODE model, we assume that the time-dependent macroscopic dynamics of the GRN are continuous in both time and state space. We assume continuous dynamics because the process of lineage determination involves a temporal extension, that is, cells pass through intermediate stages [35]. ODEs are primarily used to represent the average dynamics of phenomenological (coarse-grained) regulatory networks [35].

3.1. Geometry of the multivariate Hill function

The Hill function defined by Equation (3) is a multivariate sigmoidal function when $c_i > 1$ and a multivariate hyperbolic function when $c_i = 1$. We can investigate the multivariate Hill function by looking at the univariate function defined by

$$H_i^1([X_i]) = \frac{\beta_i[X_i]^{c_i}}{K_i + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}} \tag{4}$$

where each $[X_j]$, $j \neq i$ is taken as a dynamic parameter. This means that we project the high-dimensional space onto a two-dimensional plane. If $c_i = 1$, the graph of the univariate Hill function in the first quadrant of the Cartesian plane is hyperbolic (for any value of $[X_j]$, $j \neq i$). If $c_i > 1$, the graph of the univariate Hill function in the first quadrant is sigmoidal (for any value of $[X_j]$, $j \neq i$).

It is always true that

$$\frac{\beta_i[X_i]^{c_i}}{K_i + [X_i]^{c_i}} \geq \frac{\beta_i[X_i]^{c_i}}{K_i + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}} \tag{5}$$

for any value of $[X_j]$ $\forall j$. In other words, when the value of

$$K_i + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}} \tag{6}$$

in the denominator of $H_i^1([X_i])$ increases, the graph of the Hill curve, $Y = H_i^1([X_i])$, shrinks (see Fig. (2)). Note that each $[X_j]$, $j \neq i$ is taken as a dynamic parameter but varying these parameters does not change the geometry of the univariate Hill function.

Moreover, when the value of c_i increases, the graph of $Y = H_i^1([X_i])$ gets steeper. If we add a term g_i to $H_i^1([X_i])$ then the graph of $Y = H_i^1([X_i])$ in the Cartesian plane is translated upwards by g_i units. The geometric properties of the Hill function is essential in understanding the nature of the steady states.

4. Equilibrium points

To find the equilibrium points, we need to solve the multivariate equation $F_i(X) = 0\ \forall i$ (where F_i is given in the ODE system (1)). This implies that we need to determine the real solutions to

$$\frac{\beta_i[X_i]^{c_i}}{K_i + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}} + g_i = \rho_i[X_i]\ \forall i. \tag{7}$$

That is, we identify the intersections of the $n+1$-dimensional curve induced by $H_i([X_1], [X_2], ..., [X_n]) + g_i$ (left hand side of Equation (7)) and the $n+1$-dimensional hyperplane induced by $\rho_i[X_i]$ (right hand side of Equation (7)).

For easier analysis, we examine the intersections of the univariate functions defined by $Y = H_i^1([X_i]) + g_i$ and $Y = \rho_i[X_i]$ while varying the value of $K_i + \sum_{j=1, j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$ in the denominator of the univariate Hill function $H_i^1([X_i])$ (see Figure (2) for illustration). In the univariate case, we can look at $Y = \rho_i[X_i]$ as a line in the Cartesian plane passing through the origin with slope equal to ρ_i. Given non-negative state variables and parameters in the ODE system (1), if $g_i > 0$ then $\rho_i > 0$ is a necessary and sufficient condition for the existence of an equilibrium point. Now, the following definition will help us in identifying the stable equilibrium points (for the proof of the theorems/propositions, see the Supplementary Material).

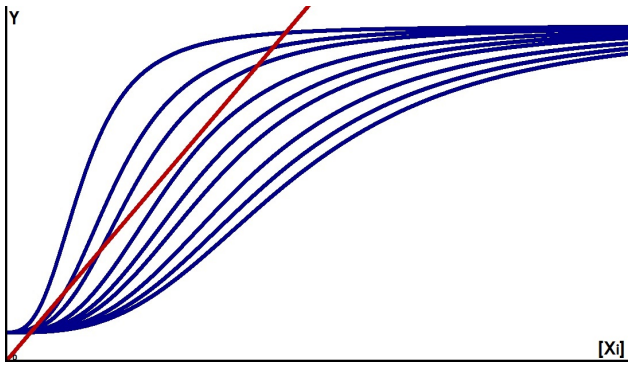

Figure 2: The intersections of $Y = \rho_i[X_i]$ and $Y = H_i^1([X_i]) + g_i$ with varying values of $K_i + \sum_{j=1,j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$, an example.

Definition 1. *Stable component* and *stable equilibrium point.* Under the assumption that the value of $\sum_{j=1,j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$ in the denominator of the univariate Hill function (4) is fixed, if $[X_i]$ converges to $[X_i]^*$ for all initial conditions $[X_i]_0$ near $[X_i]^*$, then we say that $[X_i]^*$ is stable; otherwise, $[X_i]^*$ is unstable. The equilibrium point $X^* = ([X_1]^*, [X_2]^*, ..., [X_n]^*)$ of the ODE system (1) is stable only if all its components are stable. .

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

The stable component can be a coordinate of an equilibrium point or an attractor that induces sustained oscillations. These sustained oscillations are due to the varying value of $\sum_{j=1,j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$ in the denominator of the Hill function that causes change in the topology of the intersections of $Y = H_i^1([X_i]) + g_i$ and $Y = \rho_i[X_i]$. One example of such oscillating system is a

repressilator of the form

$$\frac{d[X_1]}{dt} = \frac{[X_1]^2}{1+[X_1]^2+[X_2]^2} - .1[X_1] + .1 \tag{8}$$

$$\frac{d[X_2]}{dt} = \frac{[X_2]^2}{1+[X_2]^2+[X_3]^2} - .1[X_2] + .1 \tag{9}$$

$$\frac{d[X_3]}{dt} = \frac{[X_3]^2}{1+[X_3]^2+[X_1]^2} - .1[X_3] + .1$$

$$[X_1]_0 = .1,\ [X_2]_0 = .2,\ [X_3]_0 = .3$$

4.1. Location of equilibrium points

Suppose $\rho_i > 0$ then if both $\beta_i > 0$ and $g_i > 0$ then g_i/ρ_i cannot be an i-th component of an equilibrium point of the ODE system (1). Moreover, if $g_i, \rho_i > 0$ then $[X_i] = g_i/\rho_i$ can only be an i-th component of an equilibrium point of the ODE system (1) if $\beta_i = 0$.

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 (1) lie in the hyperspace*

$$\left[\frac{g_1}{\rho_1}, \frac{g_1+\beta_1}{\rho_1}\right) \times \left[\frac{g_2}{\rho_2}, \frac{g_2+\beta_2}{\rho_2}\right) \times ... \times \left[\frac{g_n}{\rho_n}, \frac{g_n+\beta_n}{\rho_n}\right). \tag{10}$$

Note that if we have multiple stable equilibrium points lying on the hyperspace (10) then possible strategies for increasing the basin of attraction of a stable equilibrium representing a certain cell type are by increasing the value of g_i and β_i, and by decreasing the value of ρ_i. However, the value and the number of stable equilibrium points may change when doing this strategy.

The case where the trajectory converges to the origin $(0, 0, ..., 0)$ is trivial. The zero state neither represents a pluripotent cell nor a cell differentiating into the cell lineages considered in the scope of the given GRN. Note that the generalized Cinquin-Demongeot ODE model (1) has an equilibrium point with i-th component equal to zero (i.e., $[X_i]^* = 0$) if and only if $g_i = 0$. The zero state $(0, 0, ..., 0)$ of the ODE system (1) can only be an equilibrium point if and only if $g_i = 0$ for all i. Zero steady state happens when exponential decay is faster than the activation of the TFs. The zero steady state is not important in cell differentiation, but to have a comprehensive analysis of the generalized Cinquin-Demongeot model, a discussion about zero steady state is included in Appendix B.

4.2. Cardinality of equilibrium points

In this section, we use the Bézout Theorem [36] to determine the possible maximum number of equilibrium points. Bézout Theorem does not give the exact number of equilibrium points but only the upper bound. However, we need to note that when at least two polynomials in a polynomial system have a non-constant common factor then the polynomial system has infinitely many complex-valued solutions.

Suppose c_i and c_{ij} are integers for all i and j. The corresponding polynomial equation to

$$F_i(X) = \frac{\beta_i [X_i]^{c_i}}{K_i + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij} [X_j]^{c_{ij}}} - \rho_i [X_i] + g_i = 0 \qquad (11)$$

is

$$P_i(X) = \beta_i [X_i]^{c_i} + (g_i - \rho_i [X_i]) \left(K_i + [X_i]^{c_i} + \sum_{j=1, j\neq i}^{n} \gamma_{ij} [X_j]^{c_{ij}} \right) = 0. \quad (12)$$

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 (1) (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 of Proposition (1) is by Bézout Theorem applied to Equation (12). From Proposition (1), the maximum number of equilibrium points is dependent on the value of c_i and c_{ij} as well as on n. According to Cinquin and Demongeot, manipulating the strength of cooperativity (c_i and c_{ij}) is of minimal biological relevance [25]. Nevertheless, the possible dependence of the number of equilibrium points on n (dimension of our state space) has a biological explanation. The value of n represents the number of proteins that are involved in the regulation of gene expression that affects the state (potency) of the cell. As n increases then it is possible that the number of possible combinations of expressed genes also increases.

It is necessary to check if all equations in the polynomial system (12) have no common factor of degree greater than zero, because if they do then there will be infinitely many complex-valued solutions. We determine the set of parameter values (where the strengths of cooperativity are integer-valued) that would give rise to a system of equations having a non-constant common factor. We have found one case (which is a Michaelis-Menten symmetric system) where such common factor exists.

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 (1) 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.

Corollary 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. If $\beta > \rho K$ then the equilibrium points of the ODE system (1) are the origin and the non-isolated points lying on the hyperplane with equation*

$$\sum_{j=1}^{n} [X_j] = \frac{\beta}{\rho} - K, \ [X_j] \geq 0 \ \forall j. \tag{13}$$

Note that if $[X_i] = 0$ and $g_i = 0$, then the n-dimensional system reduces to an $n-1$-dimensional system. For example, the equilibrium points of the form $([X_1]^*[X_2]^*, [X_3]^*, 0)$ of a system with $n = 4$ and $g_4 = 0$ are exactly the equilibrium points of the corresponding system with $n = 3$.

In the next subsection, we determine the stability of the equilibrium points of the generalized Cinquin-Demongeot ODE model (1) for a given set of parameters.

4.3. Stability of equilibrium points

One strategy for determining the stability of an equilibrium point is by considering Definition (1) (see SM7 in the Supplementary Material for illustration). To determine if the equilibrium point $([X_1]^*, [X_2]^*, ..., [X_n]^*)$ is stable, we need to check if $[X_i]$ converges to $[X_i]^*$ for all initial conditions $[X_i]_0$ near $[X_i]^*$, $i = 1, 2, ..., n$ (i.e., all the components of the equilibrium point are stable).

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

Remark (1) is important because this implies that when the i-th TF (where $g_i = 0$) is switched-off then it can never be switched-on again, unless we introduce an exogenous stimulus or we introduce some stochastic noise. Dedifferentiation, such as activating silenced TFs that induce pluripotency, has been shown to be possible through deterministic [37, 38] and stochastic [39, 40, 41, 42, 43, 44] cellular reprogramming.

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 (1) while the points lying on the hyperplane (13)*

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

are stable equilibrium points.

In GRNs, the existence of infinitely many non-isolated equilibrium points can be biologically volatile. A small perturbation in the initial value of the system can lead the trajectory of the system to converge to a different attractor. The basin of attraction of each stable non-isolated equilibrium point may not be as large compared to the basin of attraction of a stable isolated equilibrium point. This special phenomenon represents competition where the co-expression, extinction and domination of the TFs depend on the value of each TF, and the dependence among TFs is a continuum. The existence

of an attracting hyperplane is also discovered by Cinquin and Demongeot in [25].

The size of the basin of attraction of an equilibrium point depends on the number of existing equilibrium points and on the size of the hyperspace (10). Note that the hyperspace (10) is fixed for a given set of parameter values, and the basin of attraction of each existing equilibrium point is distributed in this hyperspace. If there are multiple stable equilibrium points then there are multiple basins of attraction that share the region of the hyperspace.

5. Bifurcation of parameters

Varying the values of some parameters can decrease the size of the basin of attraction of an undesired equilibrium point as well as increase the size of the basin of attraction of a desired equilibrium point. We can mathematically manipulate the parameter values to ensure that the initial condition is in the basin of attraction of our desired attractor.

We can force the i-th component of an equilibrium point to dominate other components by increasing β_i or g_i, or by decreasing ρ_i. Decreasing the value of K_i or sometimes the value of c_i minimizes the size of the basin of attraction of the lower-valued stable intersection of $Y = H_i^1([X_i]) + g_i$ and $Y = \rho_i[X_i]$, thus, the chance of converging to an equilibrium point with $[X_i]^* > [X_j]^*$ $j \neq i$ may increase. However, the effect of K_i and c_i in increasing the value of $[X_i]^*$ is not as indispensable compared to β_i, g_i and ρ_i, since K_i and c_i do not affect the upper bound of the hyperspace (10). Note that increasing the value of c_i or of c_{ij} may result in an increased number of equilibrium points (by Proposition (1)).

It is possible to obtain an equilibrium point that has an i-th component sufficiently dominating other components by introducing an exogenous stimulus. Here, we assume that changes in exogenous stimuli are represented in the variations of the parameter g_i. The parameter g_i is assumed as a constant production term that enhances the concentration of X_i, which affects the maximum value of the multivariate Hill function (altering the effect of the efficiency of transcription β_i).

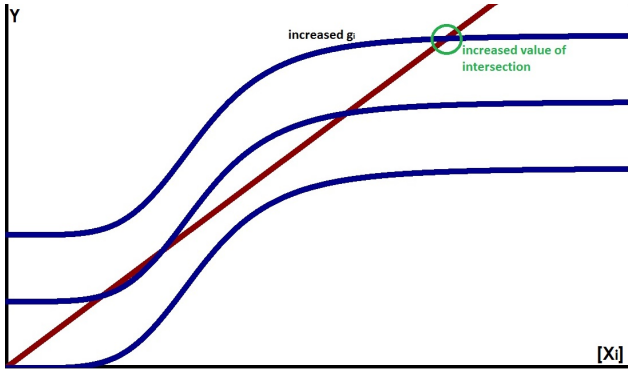

Figure 3: Increasing the value of g_i can result in an increased value of $[X_i]$ where $Y = H_i^1([X_i]) + g_i$ and $Y = \rho_i([X_i])$ intersects.

If we increase the value of g_i then the value of $[X_i]$ where $Y = H_i^1([X_i]) + g_i$ and $Y = \rho_i([X_i])$ intersect also increases. We can force such increased value of $[X_i]$ to be the only intersection even if the value of $\sum_{j=1,j\neq i}^{n} \gamma_{ij}[X_j]^{c_{ij}}$ increases (see Figure (3) for illustration). The possible value of the equilibrium component $[X_i]^*$ can be increased if we amplify the effect of g_i up to a sufficient level. Since $[X_i]$ inhibits $[X_j]$, then as the value of $[X_i]^*$ increases, the value of $[X_j]$, $j \neq i$ where $Y = H_j([X_j]) + g_j$ and $Y = \rho_j([X_j])$ intersect decreases. We can force such decreased value of $[X_j]$ to be the only intersection.

Note that if $g_j = 0$, then it is possible to make $[X_j] = 0$ the only intersection of $Y = H_j([X_j])$ and $Y = \rho_j([X_j])$. Therefore, by sufficiently changing the value of g_i we can have a sole stable equilibrium point where the i-th component dominates the others ($[X_j]^*$, $j \neq i$). For any initial condition, the trajectory of the ODE model (1) will converge to this sole equilibrium point. Hence, we predict that it is possible to manipulate the potency and fate of a stem cell by varying the value of g_i.

Example 1. Consider that all parameters in the generalized Cinquin-Demongeot ODE model (1) are equal to 1 except for $c_i = c_{ij} = 2$, $\gamma_{ij} = 1/8$, $\rho_i = 1/21$ and $g_i = 0$, for $i, j = 1, 2$. The nonlinear ODE system is of the form:

$$\begin{aligned} \frac{d[X_1]}{dt} &= \frac{[X_1]^2}{1 + [X_1]^2 + \frac{1}{8}[X_2]^2} - \frac{1}{21}[X_1] \\ \frac{d[X_2]}{dt} &= \frac{[X_2]^2}{1 + [X_2]^2 + \frac{1}{8}[X_1]^2} - \frac{1}{21}[X_2]. \end{aligned} \tag{14}$$

This system has 9 equilibrium points which is equal to the Bézout upper bound of the number of possible equilibrium points (see Appendix A for the numerical results). There are only 4 stable equilibrium points out of the 9. The four attractors represent a bipotent cell, two fully differentiated cells and a zero state.

Now, suppose we introduce $g_1 = 0.5$. Then there will be exactly one attractor, and this attractor represents a fully differentiated cell. The fully differentiated cell expresses the gene associated with $[X_1]$.

6. The MacArthur et al. GRN

In this section, we present a GRN (originally illustrated by MacArthur et al. as Figures 1 and 2 in [29]) where the generalized Cinquin-Demongeot

ODE model can be employed. This gene network shows the coupled interaction among stem-cell-specific transcription factors and lineage-specifying transcription factors induced by exogenous stimuli. The interaction depicted in the GRN involves the differentiation of multipotent stem cells to three mesenchymal stromal stem cells, namely, cells that form bones (osteoblasts), cartilages (chondrocytes), and fats (adipocytes).

The MacArthur et al. GRN [29] is composed of a pluripotency module (a circuit consisting of OCT4, SOX2, NANOG and their heterodimer and heterotrimer) and a differentiation module (a circuit consisting of RUNX2, SOX9 and PPAR-γ) [29, 45]. The transcription factors RUNX2, SOX9 and PPAR-γ activate the formation of bone cells, cartilage cells and fat cells, respectively. In mouse embryonic stem cells, RUNX2 is stimulated by retinoic acid (RA) and BMP4; SOX9 by RA and TGF-β; and PPAR-γ by RA and Insulin.

The TF proteins OCT4, SOX2, NANOG, OCT4-SOX2, OCT4-SOX2-NANOG, SOX9, RUNX2 and PPAR-γ are the nodes in the original MacArthur et al. GRN [29]. The path NANOG $\rightarrow$ OCT4-SOX2-NANOG $\rightarrow$ OCT4 $\rightarrow$ OCT4-SOX2 $\rightarrow$ SOX2 $\rightarrow$ OCT4-SOX2-NANOG $\rightarrow$ NANOG is one of the positive feedback loops of the gene network. A positive feedback loop that contains OCT4, SOX2, NANOG and their multimers can be regarded as an auto-activation loop of the pluripotency module.

Furthermore, both the OCT4-SOX2-NANOG and OCT4-SOX2 multimers inhibit SOX9, RUNX2 and PPAR-γ. However, SOX9, RUNX2 and PPAR-γ inhibit OCT4, SOX2 and NANOG. This implies that the pluripotency module and the differentiation module mutually inhibit each other.

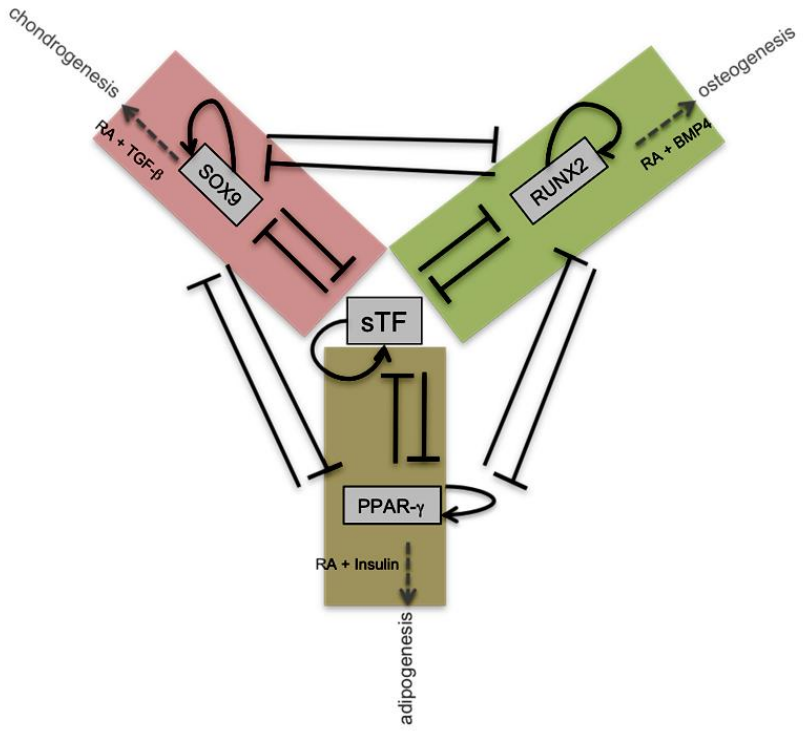

Figure 4: The simplified MacArthur et al. GRN representing the mesenchymal cell differentiation system. Bars represent repression or inhibition, while arrows represent activation

Since the pluripotent module exhibits auto-activation and mutual inhibition with all the TFs in the differentiation circuit, then we can simplify the pluripotency module as one node while preserving the essential qualitative dynamics. We denote the pluripotency module as the sTF (stemness transcription factor). From eight nodes, we only have four nodes as represented by the coarse-grained biological network in Figure (4). Since each node undergoes auto-activation and inhibition by the other nodes (as shown by the arrows and bars) then the simplified GRN is in the SDN form that can be

translated into a Cinquin-Demongeot ODE model.

In our simplified network, we have four nodes and thus, $n = 4$. Let $[X_1] = [RUNX2]$, $[X_2] = [SOX9]$, $[X_3] = [PPAR{-}\gamma]$ and $[X_4] = [sTF]$. The parameter s_i represents the effect of the growth factors stimulating the differentiation towards the i-th cell lineage, specifically, $s_1 = [RA + BMP4]$, $s_2 = [RA + TGF{-}\beta]$ and $s_3 = [RA + Insulin]$. The parameter s_4 represents the amount of exogenous stimulus for activating the sTF. One limitation of a coarse-grained phenomenological model is that it excludes time-delays that may arise from the deleted molecular details. However, a phenomenological model is sufficient to address the general principles of cell differentiation and cellular programming, such as the temporal behavior of the dynamics of the GRN [46, 35].

MacArthur et al. [29] conducted numerical simulations to investigate the behavior of the system and tried to analytically analyze the system but only for a specific case — when the pluripotency module is switched-off. The ODE model that they analyzed when the pluripotency module is switched-off follows the original Cinquin-Demongeot [25] formalism with $c = 2$. MacArthur et al. [29] analytically proved that the three cell types (tripotent, bipotent and terminal states) are simultaneously stable for some parameter values. Based on their deterministic computational analysis, the pluripotency module cannot be reactivated once silenced, that is, it becomes resistant to reprogramming. They argued that the pluripotency module can be reactivated by introducing stochastic noise to the system [29]. In this paper, we show that there can be cases where dedifferentiation is possible even without the aid of stochasticity. Adding a sufficient amount of exogenous stimulus s_4 (if

possible) can silence the expression of genes and can reactivate pluripotency. Various experimental studies have investigated the effect of external stimuli on cell differentiation and reprogramming [47, 48, 49, 50, 51]. Moreover, increasing β_4 (efficiency of sTF) and decreasing ρ_4 (degradation rate of sTF) can steer the system towards pluripotency as long as the initial condition is not turned-off. Regulation of the degradation rate of proteins can play a big role in cell differentiation [52, 53, 54].

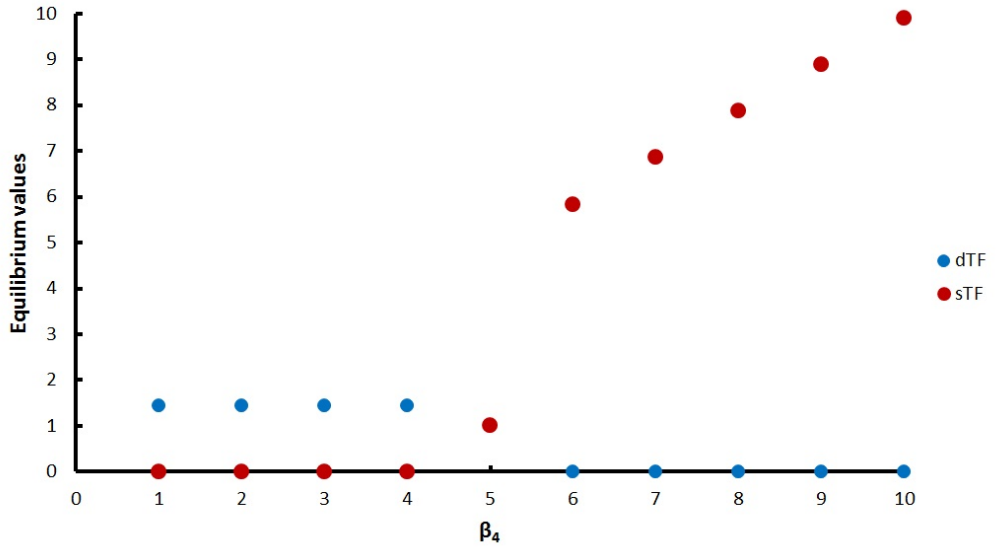

Figure 5: Varying the value of β_4 can switch equilibrium states. Initial values are set to $[X_1]_0 = 1$, $[X_2]_0 = 1$, $[X_3]_0 = 1$, $[X_4]_0 = 1$. dTF refers to X_1, X_2 and X_3.

Example 2. Suppose the hypothetical nonlinear ODE system associated with the simplified MacArthur et al. GRN has the following form (assume

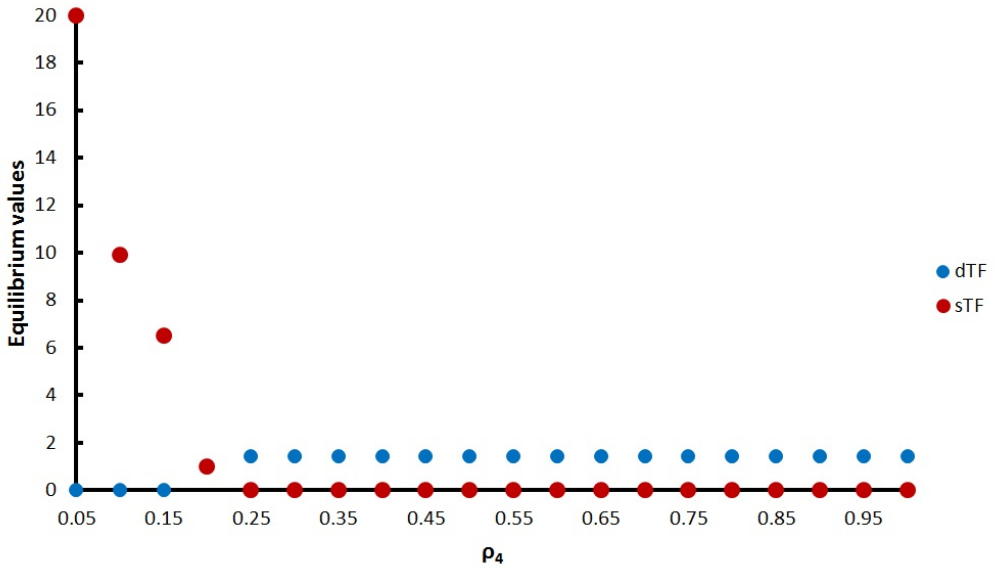

Figure 6: Varying the value of ρ_4 can switch equilibrium states. Initial values are set to $[X_1]_0 = 1$, $[X_2]_0 = 1$, $[X_3]_0 = 1$, $[X_4]_0 = 1$. dTF refers to X_1, X_2 and X_3.

symmetric differentiation module):

$$\begin{aligned}
\frac{d[X_1]}{dt} &= \frac{[X_1]^2}{1+[X_1]^2+[X_2]^2+[X_3]^2+[X_4]^2} - 0.20[X_1] \qquad (15)\\
\frac{d[X_2]}{dt} &= \frac{[X_2]^2}{1+[X_1]^2+[X_2]^2+[X_3]^2+[X_4]^2} - 0.20[X_2]\\
\frac{d[X_3]}{dt} &= \frac{[X_3]^2}{1+[X_1]^2+[X_2]^2+[X_3]^2+[X_4]^2} - 0.20[X_3]\\
\frac{d[X_4]}{dt} &= \beta_4 \frac{[X_4]^2}{1+[X_1]^2+[X_2]^2+[X_3]^2+[X_4]^2} - \rho_4[X_4] + g_4.
\end{aligned}$$

Figure (5), (6) and (7) show possible 'mathematical' strategies towards reprogramming to pluripotency. The figures illustrate how the regulation of g_4 (through exogenous stimulus s_4), β_4 (efficiency of sTF) and ρ_4 (decay rate of sTF) can change the fate of cells. Similarly, varying g_i (through s_i), β_i and

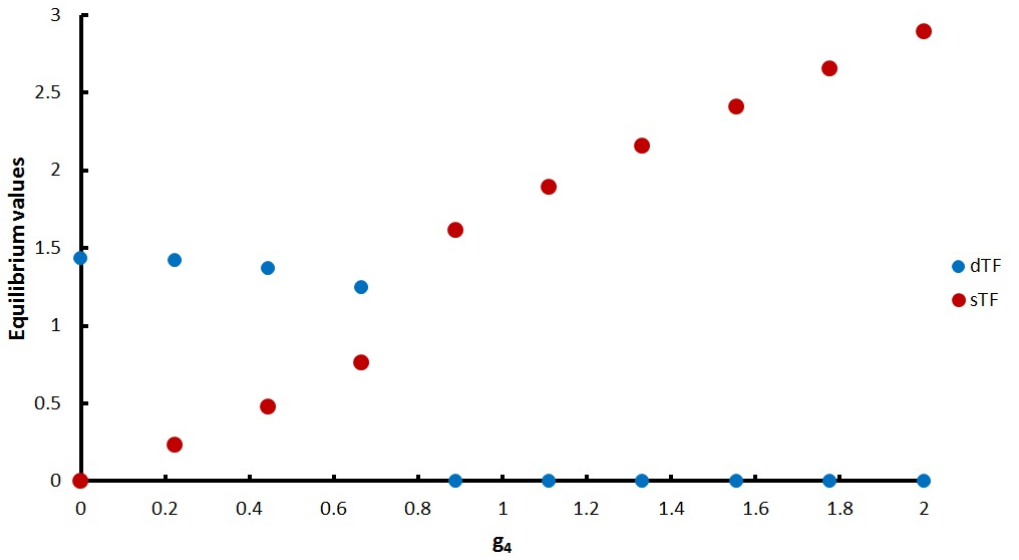

Figure 7: Varying the value of g_4 can switch equilibrium states even though the initial condition of sTF is turned-off. Initial values are set to $[X_1]_0 = 1$, $[X_2]_0 = 1$, $[X_3]_0 = 1$, $[X_4]_0 = 0$. dTF refers to X_1, X_2 and X_3.

ρ_i, $i = 1, 2, 3$ can regulate cell differentiation or transdifferentiation towards osteogenesis, chondrogenesis and adipogenesis, respectively.

By using the generalized Cinquin-Demongeot model, we can predict the temporal steady state of a population of cells given certain parameter values and initial condition. The predictions of the model (including results of parameter perturbation) can help understand the mesenchymal cell differentiation system and provide insights on the possible conditions for cellular programming (dedifferentiation and transdifferentiation).

6.1. Biological interpretation of equilibrium points

A TF is switched-off or inactive if its concentration is approximately zero, and switched-on otherwise. Moreover, we say that $[X_i] \neq 0$ sufficiently dominates $[X_j]$ if $[X_j]/[X_i] < \epsilon \leq 1$, where ϵ is an acceptable tolerance constant.

If no component representing a node from the differentiation module sufficiently dominates $[sTF]$ (e.g., $[sTF] > [OCT4]$, $[sTF] > [SOX2]$ and $[sTF] > [PPAR - \gamma]$) and sTF is switched-on, then the state represents pluripotency. If all the components of a state are approximately equal and switched-on (i.e., genes are equally expressed), then this situation represents a priming state.

The situation with at least one component from the differentiation module sufficiently dominating $[sTF]$ represents differentiated state (partial or full). If exactly three components from the differentiation module are approximately equal and sufficiently dominate $[sTF]$, then the state represents tripotency. If exactly two components from the differentiation module are approximately equal and sufficiently dominate all other components, then the state represents bipotency. If exactly one component from the differentiation module sufficiently dominates all other components, then the state represents unipotency.

If sTF is switched-off, then the cell had lost its ability to self-renew. If exactly one TF from the differentiation module remains switched-on and all other TFs including sTF are switched-off, then the state represents fully differentiated cells.

It should be clear that sTF and differentiation genes cannot be turned-

on at the same time in a single cell. In this mathematical model, a steady state describes a population of cells. For example, a condition where sTF is switched-on and a differentiation gene is also switched-on implies that there is a probability that a daughter cell could be in the pluripotent steady state or in a differentiated state. Hence, we expect to find some cells that are pluripotent and some that are differentiated in a population of cells.

7. Conclusions

We are able to show the qualitative dynamics of the non-binary simultaneous decision network by investigating the mathematical properties of the generalized Cinquin-Demongeot ODE model. The simultaneous decision network can represent multistability that may give rise to co-expression or to domination by some transcription factors. Manipulating the values of some parameters, such as efficiency of transcription, decay rate of proteins and effect of exogenous stimuli, can influence the expression of genes and the potency of stem cells. The introduction of an exogenous stimulus is a possible deterministic strategy for controlling cell fate towards a chosen lineage or for reprogramming cells back to pluripotency. Deterministic cellular programming can result in a system with a sole attractor, which can regulate the effect of moderate stochastic noise in gene expression.

Suppose the solution to our system tends to an equilibrium point with silenced transcription factor. If we want to reactivate this transcription factor then one strategy is to add an exogenous stimulus. The idea of introducing a sufficient amount of stimulus is to make the solution of our system escape a certain equilibrium point. However, it is sometimes impractical or infea-

sible to continuously add such a constant amount of inducement to control cell fate. Consequently, we may rather consider an exogenous stimulus that varies through time. Introducing a varying amount of stimulus can affect cell fate if there are multiple stable equilibrium points and if the convergence of trajectories is dependent on the initial condition.

Random noise can be introduced to the ODE model. Stochasticity can induce cells to switch lineages or to switch back to a pluripotent state. However, this technique is not always efficient, especially in the absence of multistability. If deterministic cellular programming is not perpetually possible, combining deterministic and stochastic techniques could be done, such as by supplementing the effect of exogenous stimulus with stochastic fluctuations.

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Appendix A. Numerical results

For Example 1:

Note that a trajectory converging to the zero state is a trivial case because the zero state does not represent a cell differentiating into bone, cartilage or fat. The trivial case may either represent a cell differentiating towards other cell lineages (e.g., towards becoming a neural cell) which are not in the domain of our GRN.

The approximate values of the equilibrium points of the ODE system (14) are

$([X_1]^* = 18.62, [X_2]^* = 18.62)$ — stable (bipotent),

$([X_1]^* = 20.89, [X_2]^* = 3.11)$ — unstable,

$([X_1]^* = 3.11, [X_2]^* = 20.89)$ — unstable,

$([X_1]^* = 0.05, [X_2]^* = 0.05)$ — unstable,

$([X_1]^* = 0, [X_2]^* = 0.05)$ — unstable,

$([X_1]^* = 0.05, [X_2]^* = 0)$ — unstable,

$([X_1]^* = 0, [X_2]^* = 20.95)$ — stable (terminal state),

$([X_1]^* = 20.95, [X_2]^* = 0)$ — stable (terminal state), and

$([X_1]^* = 0, [X_2]^* = 0)$ — stable (trivial case).

When $g_1 = 0.5$ is introduced, the sole equilibrium is $([X_1]^* = 31.48, [X_2]^* = 0)$.

Appendix B. Zero steady state

If all parameters are equal to 1 except for $c_i = c_{ij} = 2$ and $g_i = 0$ for all i, j, then the only equilibrium point is the origin. Actually, this kind of system is the original Cinquin-Demongeot ODE model [25] without "leak"

where speed of transcription is equal to 1 and $c = 2$. In general, suppose $c_i \geq 1$ and $g_i = 0$ for all i, then the origin is the only equilibrium point of the ODE model (1) if and only if the univariate curve $Y = H_i^1([X_i])$ lies below the decay line $Y = \rho_i[X_i]$ (i.e., $H_i^1([X_i]) < \rho_i[X_i]$, $\forall[X_i] > 0$) for all i. This phenomenon indicates that exponential decay is faster than the activation of the TFs. We expect that the associated gene expression will be silenced.

If $c_i > 1$, $g_i = 0$ and

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

for all i, then the ODE system (1) has only one equilibrium point which is the origin. Moreover, if $c_i = 1$, $g_i = 0$ and $\beta_i/K_i \leq \rho_i$ for all i, then the ODE system (1) has only one equilibrium point which is the origin.

The following statements present cases where the solution to the ODE system (1) converges to the zero state (depending on the initial condition):

- In the ODE system (1), 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 $\rho_j \geq \beta_j/K_j$ $\forall j \neq i$.

- 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 (1).

In some cases where a stable zero equilibrium component is unwanted, a

modified Cinquin-Demongeot model can be used:

$$\frac{d[X_i]}{dt} = \frac{\beta_i \exp(c_i([X_i] - \delta_i))}{K_i + \exp(c_i([X_i] - \delta_i)) + \sum_{j=1, j\neq i}^{n} \gamma_{ij} \exp(c_{ij}[X_j])} + g_i - \rho_i[X_i], \quad (B.2)$$

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

The parameter δ_i shifts the sigmoidal kinetics of the i-th TF to higher values of $[X_i]$.