

Analysis and stability of ODE systems for cell differentiation

Jules Guilberteau

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Analysis and stability of ODE systems for cell differentiation

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Internship report
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Master Mathematics of Modelling,
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Introduction

Understanding cell-fate decisions is a major research challenge in biology and medicine. The reversible epithelial–mesenchymal transition (EMT) is an example of process of cell-fate determination, which plays crucial roles in embryonic development, tissue repair and metastasis development [1].

Epithelial cells (E), characterised by a tight cell-cell adhesion and a low mobility can lose these characteristics to become mesenchymal. The mesenchymal phenotype (M) is characterised by a low cell-cell adhesion and a high mobility: the mesenchymal characteristics allow cells to become invasive. There also exists a hybrid phenotype, which presents intermediate characteristics (E/M). These hybrid cells have been observed during the transition from the epithelial phenotype to the mesenchymal phenotype (E to M), but not during the reverse transition (M to E) [2, 3]. In other words, the phenotype adopted by a cell depends not only on the value of external parameters, but also on the previous phenotype adopted: in dynamical systems, this phenomenon is called hysteresis.

The epithelial–mesenchymal transition plays an important role in tumorigenesis: the initial tumor cells lose their epithelial properties to become invasive. Then, they can cross the extracellular matrix toward blood vessels, and find a colonisation niche in a distant organ, where they can form new metastases by becoming epithelial again [1]. The hybrid phenotype seems to play a key role in the spread of metastases. Indeed, cancer cells can migrate as dispersed individual mesenchymal cells, or collectively, as hybrid cells. Collective migrations obviate the need for all cells to be able to detect extrinsic signal for migration, which enables the cells to adapt to different microenvironments [4].

Cell-fate determination between the three phenotypes is induced by numerous internal and external signals (HIF-la, p53, TGF-β, FIGF, FGF, EGF, Notch, Wnt and Hedgehog) [1] which can be seen as an external input I driving a core regulatory unit (Figure 1). This core regulatory unit is composed of four components: two families of transcription factors (TFs), SNAIL and ZEB and two families of microRNAs (miRs), miR-34 and miR-200. [5, 6, 7]. Transcriptions factors are proteins that control the rate of transcription of genetic information, and thus regulate gene expression [8]. As for others proteins, transcription factors synthesis requires messenger RNAs. RNAs expression can be regulated by microRNAs [9]. This regulation is double: microRNAs regulate the process of creation of RNA, called transcription, and the synthesis of transcription factor, called translation.

The core regulation unit can be seen as two interconnected mutually repressive modules: miR-34/SNAIL and miR-200/ZEB. The two transcription factors, SNAIL and ZEB, promote the expression of mesenchymal characteristics, and repress the epithelial expression. [1, 10]. Hence:

- The epithelial phenotype corresponds to a high level of miR-34 and miR-200, and a low level of SNAIL and ZEB.
- The mesenchymal phenotype corresponds to a low level of miR-34 and miR-200 and high level of SNAIL and ZEB.
- the hybrid phenotype corresponds to a medium level of miR-34, miR-200, SNAIL and ZEB.

M. Lu et al have developed an ODE model of the core regulatory unit with six variables [11], which includes ZEB mRNA and SNAIL mRNA. The first objective of this internship is to replicate the numerical results obtained in the article, test the different possible simplifications of the model, and check that they preserve the main biological characteristics of the reversible epithelial–mesenchymal transition. In particular, it is necessary to check that the system has the following two properties:

- Tristability: The model must have exactly three stable equilibrium points, which correspond to the three different phenotypes (E, M, and E/M), for some values of signal I.

- Hysteresis: Since the cells cannot become E/M during the transition from M to E, the system must retain this characteristic.

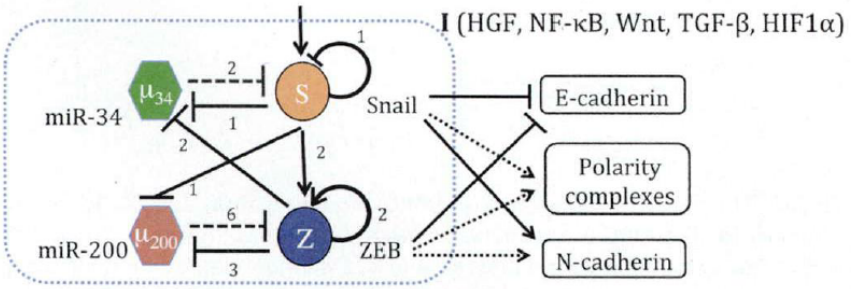


Figure 1: The core regulation unit which induces forward and backward epithelial-mesenchymal transition. Arrows denote activation, and bars inhibition. Solide lines indicate transcriptional regulation, Dashed lines indicate microRNA-mediated translational regulation, and dotted lines indirect regulation. The numbers along the lines specify the number of binding sites corresponding to the regulation process.
E-cadherin is the hallmark of the epithelial phenotype, and N-cadherin and polarity complexes characterise the mesenchymal phenotype. Hence, high levels of transcription factors SNAIL and ZEB promote the mesenchymal phenotype, while high levels of microRNAs miR-34 and miR-200 promote the epithelial phenotype. *source*: [11]

More generally, this model is part of the family of biological switches. Biological switches correspond to multistable systems of ODE, where each stable point corresponds to a possible cellular phenotype. Toggle switches are widely used in order to model cell differentiation, and understanding the way to model them is an important current research topic [12, 13, 14, 15, 16, 17, 18]. Therefore, the second part of this internship was devoted to the study of simple competitive ODEs, in order to model biological toggle switches. We focused on systems of the form:

$$\begin{cases} \dot{x} = g_x H_1(y) - k_x x \\ \dot{y} = g_y H_2(x) - k_y y \end{cases}, \tag{1}$$

where H_1 and H_2 are two decreasing Hill functions, that is to say functions of the shape

$$\begin{aligned} H : \mathbb{R}_+ &\to \mathbb{R}_+ \\ z &\mapsto \frac{1 + \lambda(\frac{z}{z_0})^n}{1 + (\frac{z}{z_0})^n}, \end{aligned}$$

where $n \in \mathbb{N}^*, \lambda \in [0, 1), z_0 \in \mathbb{R}_+^*$.

The particular case where $\lambda_1 = \lambda_2 = 0$ has been studied numerically by Timothy S Gardner, Charles R Cantor, and James J Collins [16]. They have shown that, in this particular case, this system can be monostable or bistable, depending on the parameters. Similarly, Dongya Jia and al. have studied this system in the symmetric case ($g_x = g_y$, $k_x = k_y$, $H_1 = H_2$) [18], and have shown numerically that such systems can also be monostable or bistable, depending on parameters.

Joshua L Cherry and Frederick R Adler [17] have studied analytically the case where $\lambda_1 = \lambda_2 = 0$ and $n_1 = n_2$. They have proven that, in this particular case, the system is monostable if $n_1 = n_2 = 1$, and can be multistable if $n_1 = n_2 > 1$, depending on the other parameters.

Nevertheless, to the best of our knowledge, it has not been shown that this system cannot have more than three equilibria, and precise sets of values which ensures bistability have not been provided.

In the continuity of these articles, the purpose of the second section is to answer the following two questions:

- Can system (1) have more than three equilibrium points? Can it have more than two stable equilibria?
- For which parameters is the system bistable? For which parameters is the system monostable?

We have proven that system (1) cannot have more that three equilibria, and more than two stable equilibra, and we answer the second question for a set of values with a complement of measure zero in the set of parameters. The precise results are given at the beginning of the second section.

1 Numerical study of a model for the reversible Epithelial–Mesenchymal Transition

1.1 Model Description

The purpose of this section is to describe and simulate the model for the EMT developed by M. Lu et al. in [11]. All the parameters used for our simulations, and their biological relevance, are discussed in this article. We indicate them in the Appendix.

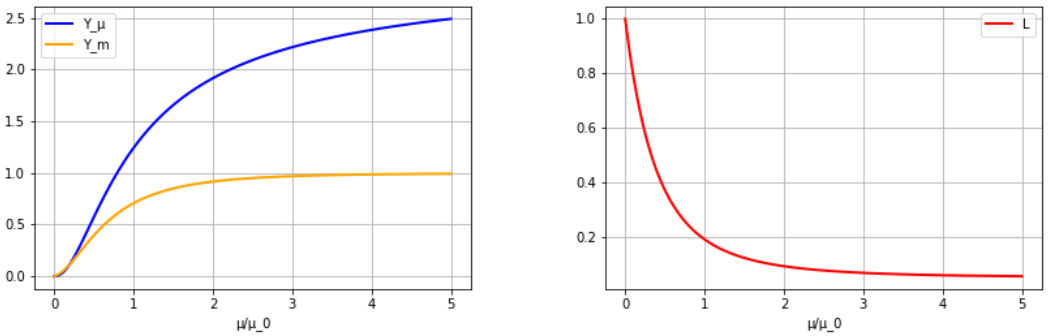
The model is based on a theoretical framework [19] called microRNA-based-chimeric (MBC) circuits. MicroRNA (miR) molecules reversibly bind on microRNA (mRNA) and inhibits the translation of transcription factors (TF) and actively degrades miR and mRNA.

Denoting μ the miR molecules, m the mRNA and B the TF protein, we get the following ODE:

$$\begin{cases} \dot{\mu} = g_\mu - mY_\mu(\mu) - k_\mu \mu \\ \dot{m} = g_m - mY_m(\mu) - k_m m \\ \dot{B} = g_B m L(\mu) - k_B B \end{cases}, \tag{MBC}$$

where:

- g_μ and g_m are the synthesis rates of μ and m respectively. They can be functions of B and external signals.
- k_μ, k_m and k_B are the constant intrinsic degradation rates of μ, m and B respectively.
- g_B is the translation rate of protein B for each mRNA, in the absence of miR.
- $mY_\mu(\mu)$, $mY_m(\mu)$ represents miR active degradation and mRNA active degradation by the miR-mRNA complex respectively.
- $L(\mu)$ represents translation inhibition.



(a) (b)

Figure 2: Values of the functions Y_{mu}, Y_m and L, for six binding sites on the mRNA.

The formulae for Y_μ, Y_m and L depend on the number of binding sites on the mRNA (n), and are given by:

$$Y_\mu(\mu) := \sum_{i=1}^{n} i\gamma_{\mu_i} \binom{n}{i} M_n^i(\mu)$$
$$Y_m(\mu) := \sum_{i=1}^{n} \gamma_{m_i} \binom{n}{i} M_n^i(\mu),$$
$$L(\mu) := \sum_{i=0}^{n} l_i \binom{n}{i} M_n^i(\mu),$$

where $M_n^i := \frac{(\mu/\mu_0)^i}{(1+\mu/\mu_0)^n}$. They are represented, for 6 binding sites, in Figure 2.

The model studied for EMT is composed of two interconnected MBC circuits, miR-34/SNAIL and miR-200/ZEB. In the following two sections, we will study these two MBC circuits independently, and then study the coupled system.

Generalities about Hill functions:

For the two circuits, Hill functions are used in order to model inhibition or activation. A Hill function is a function of the form:

$$H : \mathbb{R}_+ \to \mathbb{R}_+$$
$$z \mapsto \frac{1 + \lambda(\frac{z}{z_0})^n}{1 + (\frac{z}{z_0})^n}$$

with $n \in \mathbb{N}^*$, $\lambda \in \mathbb{R}_+$ $z_0 \in \mathbb{R}_+^*$.

We clearly have: $H \in C^\infty(\mathbb{R}^+)$, $H(0) = 1$ and $\lim\limits_{z \to +\infty} H(z) = \lambda$.

For $z_0 = 1$, we have, for all $z \in \mathbb{R}_+$:

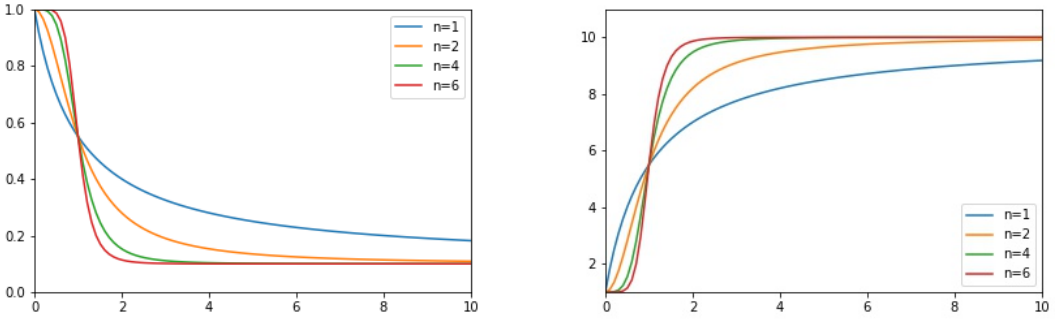
$$H'(z) = \frac{n(\lambda - 1)z^{n-1}}{(1 + z^n)^2}.$$

Hence, H is decreasing if $\lambda < 1$ and increasing if $\lambda > 1$, and constant if $\lambda = 1$ (see Figure 3). Hill functions with $\lambda < 1$ are used to represent inhibition, and Hill functions with $\lambda > 1$ are used to represent activation. The value of λ depends on the amplitude of the regulation, and n denotes the number of binding sites.
In what follows, we will denote by H^+ the increasing Hill functions, and H^- the decreasing ones.

We also have:

$$H''(z) = n(1 - \lambda)z^{n-2} \frac{(n + 1)z^n - (n - 1)}{(1 + z^n)^3},$$

which proves that H has an inflexion point on $\mathbb{R}_+^*$ if and only if $n \geq 2$.



(a) (b)

Figure 3: Examples of decreasing and increasing Hill functions. The parameters for (a) are $\lambda = 0.1$ and $z_0 = 1$. The parameters for (b) are $\lambda = 10$ and $z_0 = 1$, for various values of n.
We observe that all the Hill functions have an inflection point on $\mathbb{R}_+^*$, except when $n = 1$.

1.1.1 miR-34/SNAIL circuit

The miR-34/SNAIL circuit is described by the following MBC model:

$$\begin{cases} \dot{\mu}_{34} & = g_{\mu_{34}} H^-_{S,\mu_{34}}(S) - m_S Y_\mu(\mu_{34}) - k_{\mu_{34}}\mu_{34} \\ \dot{m}_S & = g_{m_S} H^-_{S,m_S}(S) H^+_{I,m_S}(I) - m_S Y_m(\mu_{34}) - k_{m_S} m_S \ , \\ \dot{S} & = g_S m_S L(\mu_{34}) - k_S S \end{cases} \tag{2}$$

where μ_{34}, m_S and S denote miR-34, SNAIL mRNA and SNAIL respectively.

Internal and external signals which promote the epithelial-mesenchymal transition are represented by signal I. $H^-_{S,\mu_{34}}(S)$ corresponds to the inhibition of μ_{34} by S, and $H^-_{S,m_S}(S)$ to the auto-inhibition of S (see Figure 1).

We numerically test this model for various values of I, between 0 and 100.000 (Figure 4). In all cases, the ODE has a unique equilibrium point. Nevertheless, the value of this equilibrium considerably changes depending on the value of I. We will see in the following sections that the value of I impacts the multistability and the values of the coupled system equilibrium points.

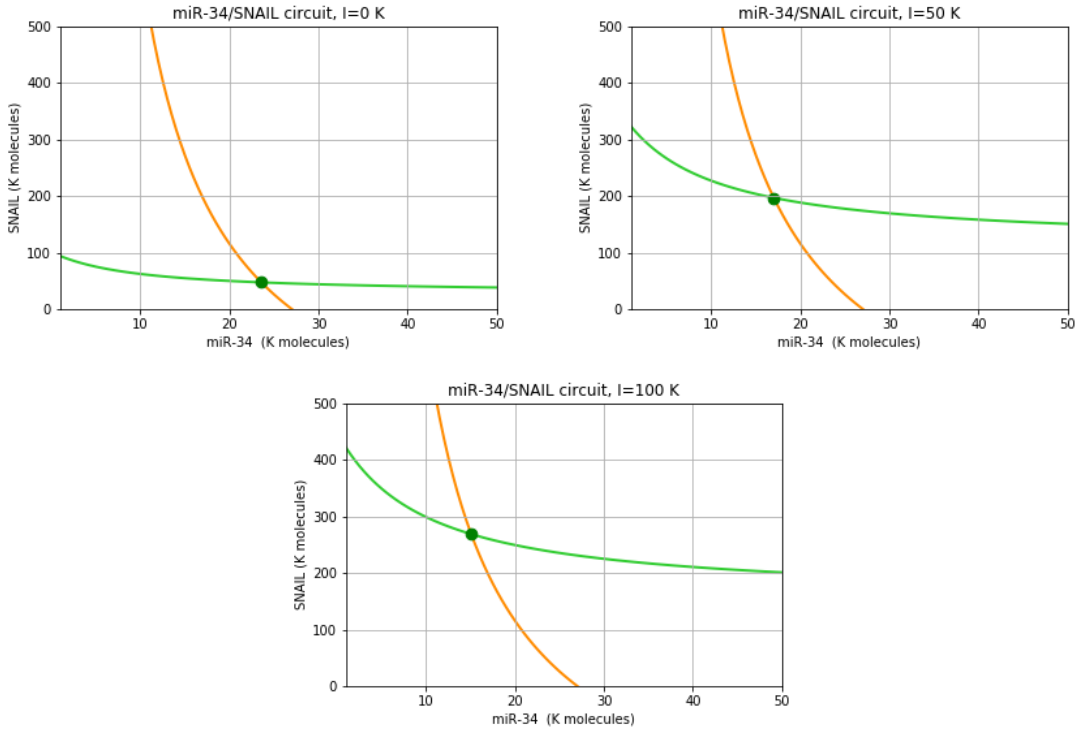


Figure 4: Nullclines for the miR-34/SNAIL circuit. The green curve shows the values of μ_{34} and S for which $\dot{\mu}_{34} = 0$ and $\dot{S} = 0$ in (2), while the orange one represents the values for which $\dot{m}_S = 0$ and $S = 0$, for three different values of I. The stable equilibrium points are denoted by a green dot. We observe that, in each case, the system is monostable.

1.1.2 miR-200/ZEB circuit

This second circuit is also described by a MBC model, driven by S:

$$\begin{cases} \dot{\mu}_{200} &= g_{\mu_{200}} H^-_{Z,\mu_{200}}(Z) H^-_{S,\mu_{200}}(S) - m_Z Y_\mu(\mu_{200}) - k_{\mu_{200}} \mu_{200} \\ \dot{m}_Z &= g_{m_Z} H^+_{Z,m_Z}(Z) H^+_{S,m_Z}(S) - m_Z Y_m(\mu_{200}) - k_{m_Z} m_Z \\ \dot{Z} &= g_Z m_Z L(\mu_{200}) - k_Z Z \end{cases} . \tag{3}$$

$H^-_{Z,\mu_{200}}(Z)$ and $H^-_{S,\mu_{200}}(S)$ represent the miR-200 inhibition by S and Z respectively, and $H^+_{Z,m_Z}(Z)$ and $H^+_{S,m_Z}(S)$ the activation of ZEB by S and Z respectively (see Figure 1).

The main differences between the two circuits is that Z activates m_Z in (3), while S inhibits m_S in (2), and that ZEB mRNAs have six binding sites, instead of two for SNAIL mRNAs.
The auto-activation of Z induces the existence of three equilibrium points for some intermediate values of S, which we associate with the three possible phenotypes (epithelial, hybrid and mesenchymal) (see Figure 5). Moreover, the bifurcation diagram of the circuit driven by S (see Figure 6) shows that the hybrid state can be reached during the transition from the epithelial state to the mesenchymal state, but not during the reverse transition.

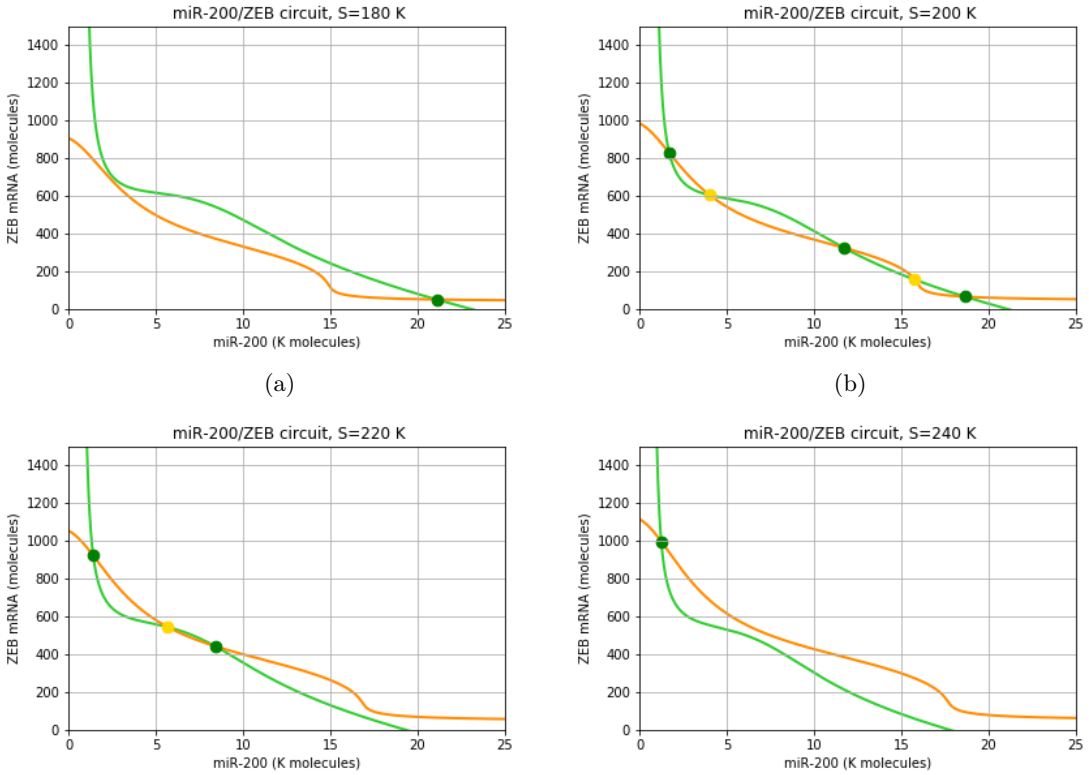
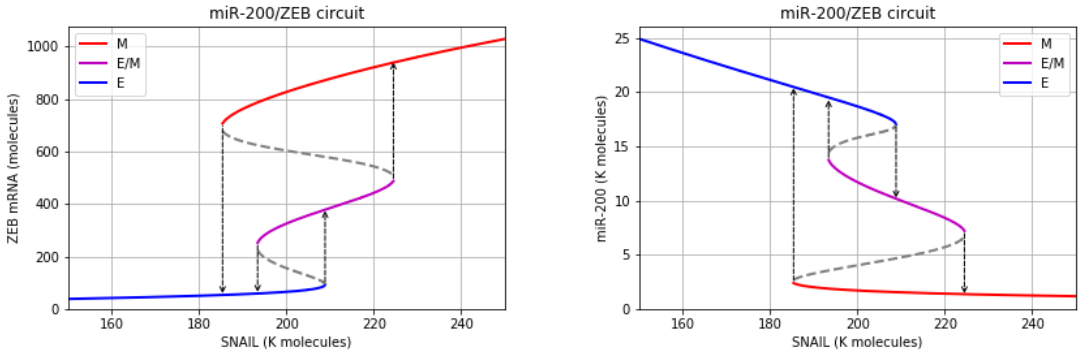


Figure 5: Nullclines for the miR-200/ZEB circuit, driven by the signal S. The green curve shows the values of m_S and Z for which $\dot{\mu}_{200} = 0$ and $\dot{S} = 0$ in (3), and the orange one represents the values for which $\dot{m}_Z = 0$ and $\dot{S} = 0$, for four different values of S. The green dots denote stable equilibrium points, and yellow dots denote unstable equilibrium points.
Depending on the value of the signal S, the system can be monostable ((a) and (d)), bistable (c) or tristable (b). Let us remark that, for a low level of S ($S \equiv 180K$), the unique equilibrium point has a high level of miR-200 and a low level of ZEB, which corresponds to the epithelial phenotype, while with a high level of S ($S \equiv 240K$), the equilibrium point has a high level of miR-200 and a high level of ZEB, which corresponds to the mesenchymal phenotype.



(a) (b)

Figure 6: Bifurcation diagram for the miR-200/ZEB circuit, driven by S. Solid lines represent the stable points, and grey dashed lines the unstable points. We identify the blue lines with the epithelial phenotype, the red line with the mesenchymal phenotype, and the purple one with the hybrid phenotype.
The vertical dashed arrows show transitions between the three states: Starting from the epithelial state (blue) and increasing SNAIL, the circuit reaches the hybrid state (purple), and then the mesenchymal state (red) if SNAIL further increases. On the other hand, starting to the mesenchymal state, and decreasing SNAIL, the circuit directly becomes epithelial, without going through the hybrid state.

1.1.3 Coupled circuit

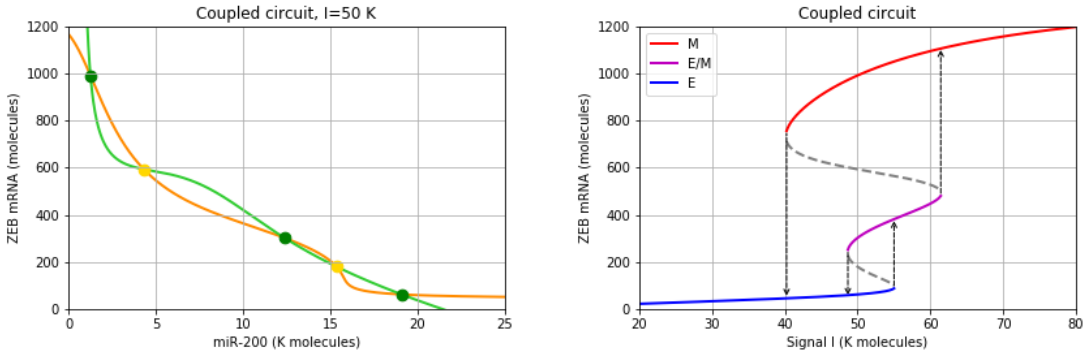
We can now study the complete model, with the two coupled circuits.

$$\begin{cases} \dot{\mu}_{34} &= g_{\mu_{34}} H^-_{S,\mu_{34}}(S) H^-_{Z,\mu_{34}}(Z) - m_S Y_\mu(\mu_{34}) - k_{\mu_{34}}\mu_{34} \\ \dot{m}_S &= g_{m_S} H^-_{S,m_S}(S) H^+_{I,m_S}(I) - m_S Y_m(\mu_{34}) - k_{m_S} m_S \\ \dot{S} &= g_S m_S L(\mu_{34}) - k_S S \\ \dot{\mu}_{200} &= g_{\mu_{200}} H^-_{Z,\mu_{200}}(Z) H^-_{S,\mu_{200}}(S) - m_Z Y_\mu(\mu_{200}) - k_{\mu_{200}}\mu_{200} \\ \dot{m}_Z &= g_{m_Z} H^+_{Z,m_Z}(Z) H^+_{S,m_Z}(S) - m_Z Y_m(\mu_{200}) - k_{m_Z} m_Z \\ \dot{Z} &= g_Z m_Z L(\mu_{200}) - k_Z Z \end{cases} \tag{4}$$

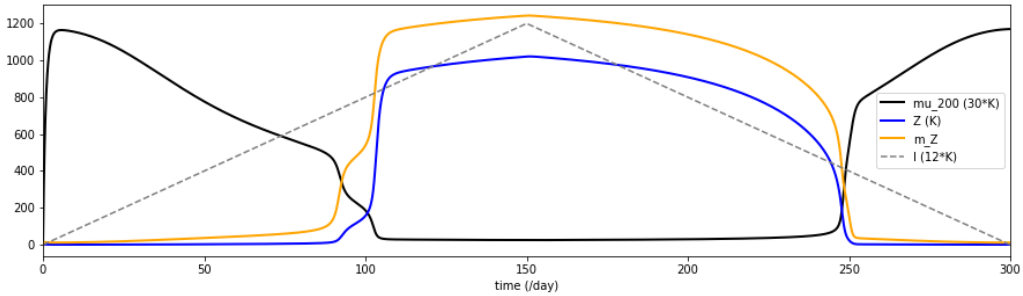
$H^-_{Z,\mu_{34}}(Z)$ represents the inhibition of μ_{34} by ZEB. All the other parameters are described in the previous sections.

We numerically check the following facts (Figure 7):

- The existence of three equilibrium points, for medium values of I ($I \approx 50.000$).
- The existence of hysteresis: in order to check biological observations, a hybrid state must be reached during the transition from the epithelial phenotype to the mesenchymal phenotype, but not during the transition from mesenchymal to epithelial.



(a) (b)



(c) *reproduced simulation [11]*

Figure 7: Numerical simulations for the coupled circuit driven by the signal I.
(a) Nullclines for the coupled circuit, with $I = 50.000$. Green dots denote stable equilibria, and yellow dots unstable equilibria. The graph highlights the tristablility of the system, for $I = 50.000$. (b) Bifurcation diagram of the coupled circuit driven by I. As for the miR-200/ZEB circuit (see Figure 6), the coupled circuit can be monostable, bistable ot tristable, depending on the value of I. The vertical arrows show the transitions between the different states, and show the asymmetry of the circuit. (c) Another illustration of hysteresis: We simulate the effect of an external time-varying signal I. Starting from $I = 0$ at day 0, the signal linearly increases to $I = 100.000$ at day 150, and then linearly decreases to 0 at day 300. We observe that, although I is symmetric, the levels of miR-200, ZEB and ZEB mRNA are asymmetric: indeed, we observe that cells pass through the hybrid state when I increases (around day 100), but not when I decrease (around day 250).

In this section, we showed that the studied ODE preserves the main biological characteristics of the reversible epithelial mesenchymal transition. The purpose of the following section is to simplify this model, while preserving the main properties (three stable states for medium values of the signal I and hysteresis).

1.2 System simplification

1.2.1 Study of the feedback effect from miR-200/ZEB onto miR34/SNAIL

We study (Fig 8) the effect of feedback from miR-200/ZEB to miR34/SNAIL, modeled by $H^-_{Z,\mu_{34}}$ in (4).

The absence of feedback does not affect the system hysteresis, and preserves the tristability for medium values of I.

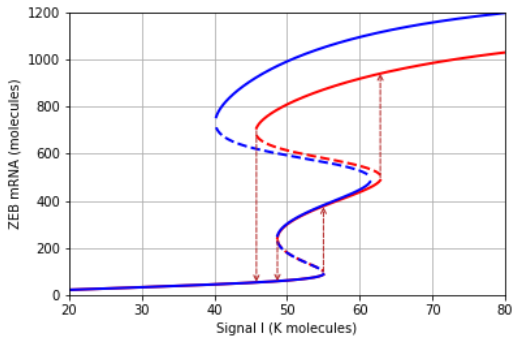


Figure 8: Bifurcation diagrams for the coupled circuit, with respect to the driving signal I. The blue curve corresponds to the circuit with inhibition from ZEB to miR-34, and the red curve corresponds to the case without ZEB feedback. The red dashed arrows represent the transitions between the different states. We note that the ODE without feedback preserves the tristability and the asymmetry of the model.

This shows that we can neglect the feedback from miR-200/ZEB to miR-34/SNAIL, without impacting either the tristability of the sytem or the hysteresis. This allows us to study the miR-34/SNAIL circuit and the miR-200/ZEB circuit independently, by considering S as a driving signal in the second circuit.

1.2.2 From the ODE with three variables to the ODE with two variables

The purpose of this section is to reduce the miR-34/SNAIL and the miR-200/ZEB models which are ODEs with three variables, to ODEs with two variabes.

We consider again the MBC model, and we assume that mRNA quickly reaches equilibrium before substantial changes of the miR and the protein level. This is biologically relevant, because the intrinsic degradation rate of the mRNA is about five to ten times faster than that of the miR and the protein. Thus, we consider that the second equation of the ODE (MBC) is at equilibrium. Hence, by defining:

$$\bar{m}(\mu) := \frac{g_m}{Y_m(\mu) + k_m},$$

we get the *simplified MBC model*:

$$\begin{cases} \dot{\mu} = g_\mu - \bar{m}(\mu) Y_\mu(\mu) - k_\mu \mu \\ \dot{B} = g_B \bar{m}(\mu) L(\mu) - k_B B \end{cases}.$$

By denoting:

$$\begin{aligned} g'_m :=& \frac{g_m}{k_m} \\ P(\mu) :=& \frac{L(\mu)}{1 + \frac{Y_m(\mu)}{k_m}} \\ Q(\mu) :=& \frac{k_m Y_\mu(\mu)}{k_m u (Y_m(\mu) + k_m)}, \end{aligned}$$

we can rewrite this system:

$$\begin{cases} \dot{\mu} = g_\mu - \bar{m}(\mu) Y_\mu(\mu) - k_\mu g'_m Q(\mu) - k_\mu \mu \\ \dot{B} = g_B g'_m P(\mu) - k_B B \end{cases} .$$

Reduced miR-34/SNAIL circuit

Applying this simplification to the miR-34/SNAIL circuit, we get:

$$\begin{cases} \dot{\mu}_{34} &= g_{\mu_{34}} H^-_{S,\mu_{34}}(S) - k_{\mu_{34}} g'_{m_S} H^-_{S,m_S}(S) H^+_{I,m_S}(I) Q(\mu_{34}) - k_{\mu_{34}} \mu_{34} \\ \dot{S} &= g_S g'_{m_S} H^-_{S,m_S}(S) H^+_{I,m_S}(I) P(\mu_{34}) - k_B B \end{cases} \tag{5}$$

- A plot of P (Figure 9) shows that, in this particular case, P can be approximated by the Hill function:

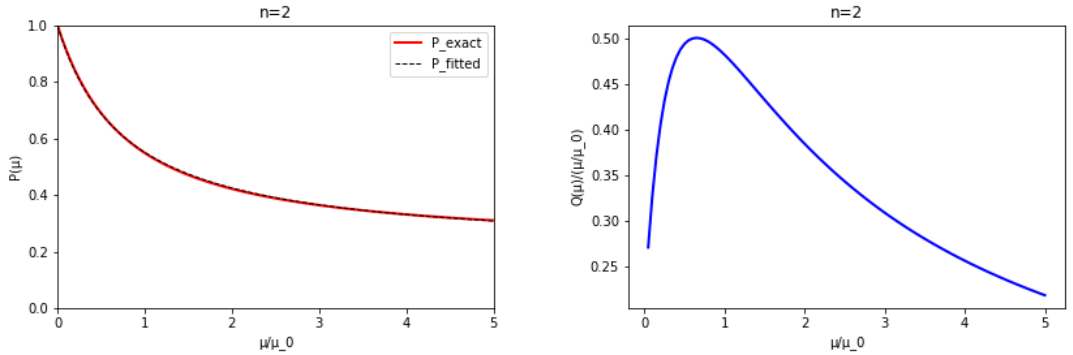
$$\begin{aligned} \mathbb{R}^+ &\to \mathbb{R}^+ \\ z &\mapsto \frac{1 + b\frac{z}{a}}{1 + \frac{z}{a}}, \end{aligned}$$

  with $a := 0.78$, μ^0_{34}, $b = 0.20$.

- A plot of Q (Figure 9) shows that for all $\mu \in \mathbb{R}^+$, $Q(\mu)$ is smaller than or equal to $0.5\mu/\mu^0_{34} = 5e^{-5}\mu$. Since $H^+_{I,m_S} < \lambda_I = 10$, and $g'_{m_S} = \frac{g_{m_S}}{k_{m_S}} = 180$, we have:

$$k_{\mu_{34}} g'_{m_S} H^-_{S,m_S}(S) H^+_{I,m_S}(I) Q(\mu) < 0.09 k_{\mu_{34}} \mu.$$

  Hence, the term $k_{\mu_{34}} g'_{m_S} H^-_{S,m_S}(S) H^+_{I,m_S}(I) Q(\mu_{34})$ can be omitted in system (5).

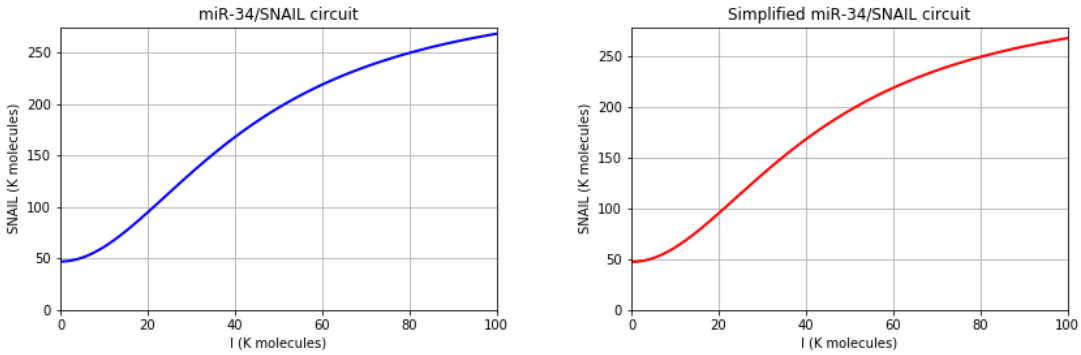


(a) (b)

Figure 9: (a) Plot of the exact function P, (red) and its approximation by a Hill function (black). The parameters for the Hill function are determined by a numerical method of least squares.
(b) Plot of $Q/(\mu/\mu_0)$, which shows that for all $\mu \in \mathbb{R}^+$, $Q(\mu) \leq 0.5\mu/\mu^0_{34} = 5e^{-5}\mu$.
These approximations are possible because miR-34 has two binding sites on SNAIL mRNA.

Lastly, ODE (5) can be rewritten:

$$\begin{cases} \dot{\mu}_{34} & = g_{\mu_{34}} H^-_{S,\mu_{34}}(S) - k_{\mu_{34}}\mu_{34} \\ \dot{S} & = g_S g'_{m_S} H^-_{S,m_S}(S) H^+_{I,m_S}(I) P(\mu_{34}) - k_B B \end{cases} \tag{6}$$



(a) (b)

Figure 10: Bifurcations diagrams for the miR-34/SNAIL circuit driven by the signal I. The blue diagram is for the original circuit (ODE (2)), and the red one for the simplified circuit (ODE (6)). We observe that the simplification almost perfectly preserves equilibrium points.

A comparison between the bifurcation diagram of the initial miR-34/SNAIL circuit and the simplified model (Figure 10) shows that this simplification preserves the uniqueness and the value of the equilibrium point, for all values of I.

Reduced miR-200/ZEB circuit

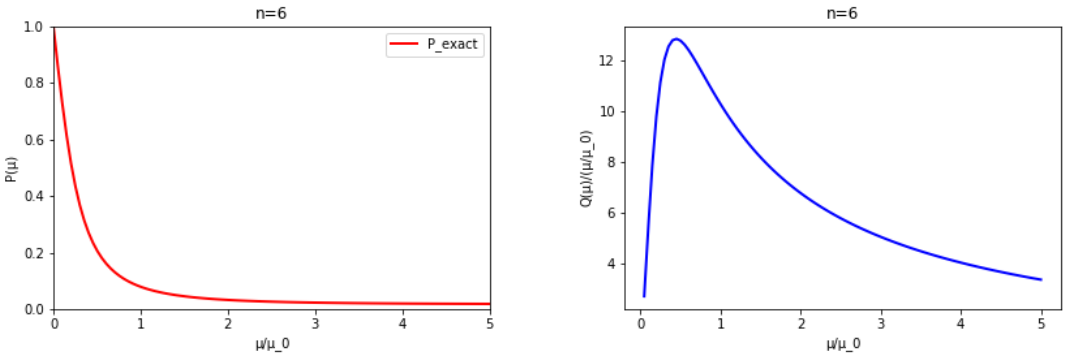
The ODE for the miR-200/ZEB module can be rewritten:

$$\begin{cases} \dot{\mu}_{200} & = g_{\mu_{200}} H^-_{Z,\mu_{200}}(Z) H^-_{S,\mu_{200}}(S) - k_{\mu_{200}} g'_{m_Z} H^+_{Z,m_Z}(Z) H^+_{S,m_Z}(S) Q(\mu_{200}) - k_{\mu_{200}}\mu_{200} \\ \dot{Z} & = g_Z g'_{m_Z} H^+_{Z,m_Z}(Z) H^+_{S,m_Z}(S) P(\mu_{200}) - k_Z Z \end{cases} \tag{7}$$

Since ZEB mRNA has 6 binding sites (instead of 2 for SNAIL mRNA), it will not be possible to do the same simplifications.

- The plot of function P (Figure 11) shows that it does not fit to a Hill function: Indeed, P does not fit to a rank-one Hill function, and does not have an inflexion point, which means that it cannot fit to a Hill function with a higher rank.

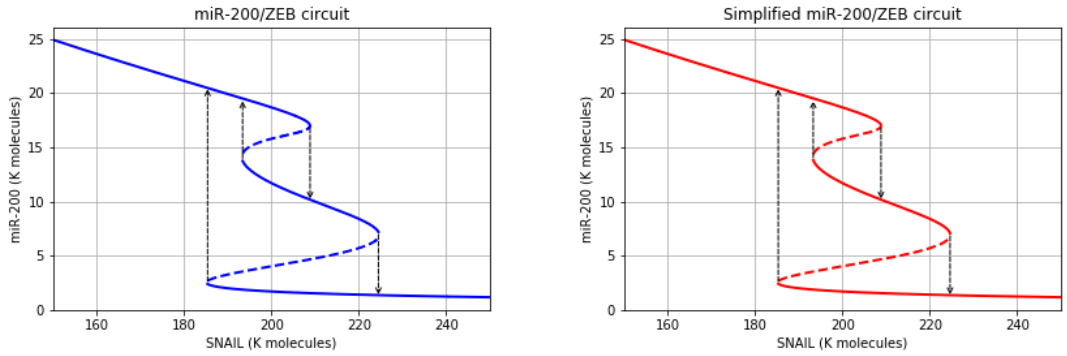
- A plot of Q (Figure 11) shows that, for some values of μ, $Q(\mu)$ can be more than ten times greater than μ/μ^0_{200}. Hence, the system (7) cannot be simplified any further.



(a) (b)

Figure 11: Plot of $P(\mu)$ and $Q(\mu)/(\mu/\mu_0)$, as function of μ/μ_0. As we can see, the values taken by the two functions are significantly different from the miR-34/SNAIL circuit. This is a consequence of the number of miR binding sites on mRNA.

A comparison between the bifurcation diagram of the initial miR-200/ZEB circuit and the simplified model (Figure 10) shows that this simplification preserves the multistability of the system, and the values of the equilibrium points.



(a) (b)

Figure 12: Bifurcations diagrams for the miR-200/ZEB circuit driven by the signal S. The blue diagram is for the original circuit (ODE (3)), and the red one for the simplified circuit (ODE (7)). We observe that the simplification almost perfectly preserves equilibrium points.

A comparison between the bifurcation diagram of the initial miR-200/ZEB circuit and the simplified model (Figure 12) shows that this simplification preserves the multistability and the values of the equilibrium points, for all values of I.

2 Theoretical study of competitive systems for the modelling of biological switches

The purpose of this section is to study competitive systems that model toggle switches. We have focused on competitive systems of the form:

$$\begin{cases} \dot{x} = g_x H_1(y) - k_x x \\ \dot{y} = g_y H_2(x) - k_y y \end{cases}, \tag{8}$$

where $g_x, g_y > 0, k_x, k_y > 0$, and H_1 and H_2 are two decreasing Hill functions with parameters n_1, λ_1, z_{01} and n_2, λ_2, z_{02} respectively.

We recall that Hill functions are functions of the shape

$$\begin{aligned} H : \mathbb{R}_+ &\to \mathbb{R}_+ \\ z &\mapsto \frac{1 + \lambda(\frac{z}{z_0})^n}{1 + (\frac{z}{z_0})^n}, \end{aligned}$$

The purpose of this second section is to answer the following questions:

- Can system (8) have more than three equilibrium point? Can it have more than two stable equilibria?
- For which parameters is the system bistable? For which parameters is the system monostable?

The results of our research are stated in the following two theorems:

Theorem. *Let H be a decreasing Hill function, with parameters, n, λ, z_0, and let us consider the following symmetric ODE:*

$$\begin{cases} \dot{x} = gH(y) - kx \\ \dot{y} = gH(x) - ky \end{cases} \tag{9}$$

with $g, k > 0$.

Then, we have to following result:

If $n = 1$, then system (9) has a unique equilibrium point. Otherwise:

For all $n \geq 2$, there exists $\lambda_0 \in (0, 1)$ such that:

-If $\lambda > \lambda_0$, then (9) has a unique equilibrium point.

-If $\lambda \in [0, \lambda_0]$, then there exists an open interval of $\mathbb{R}_+$ I_λ such that:

- *If $\frac{g}{kz_0} \in I_\lambda$, then (9) has exactly three equilibrium points.*

- *if* $\frac{g}{kz_0} \in \overline{I_\lambda}^C$, *then* (9) *has a unique equilibrium point.*

Moreover, I_λ *is a bounded interval if and only if* $\lambda > 0$.

In each case, the system is monostable if the system has a unique equilibrium, and bistable if it has three equilibria.

The exacts values of λ_0 and I_λ are given in Theorem 2.2.6. The set of values for which the system is tristable is represented in Figure 13.

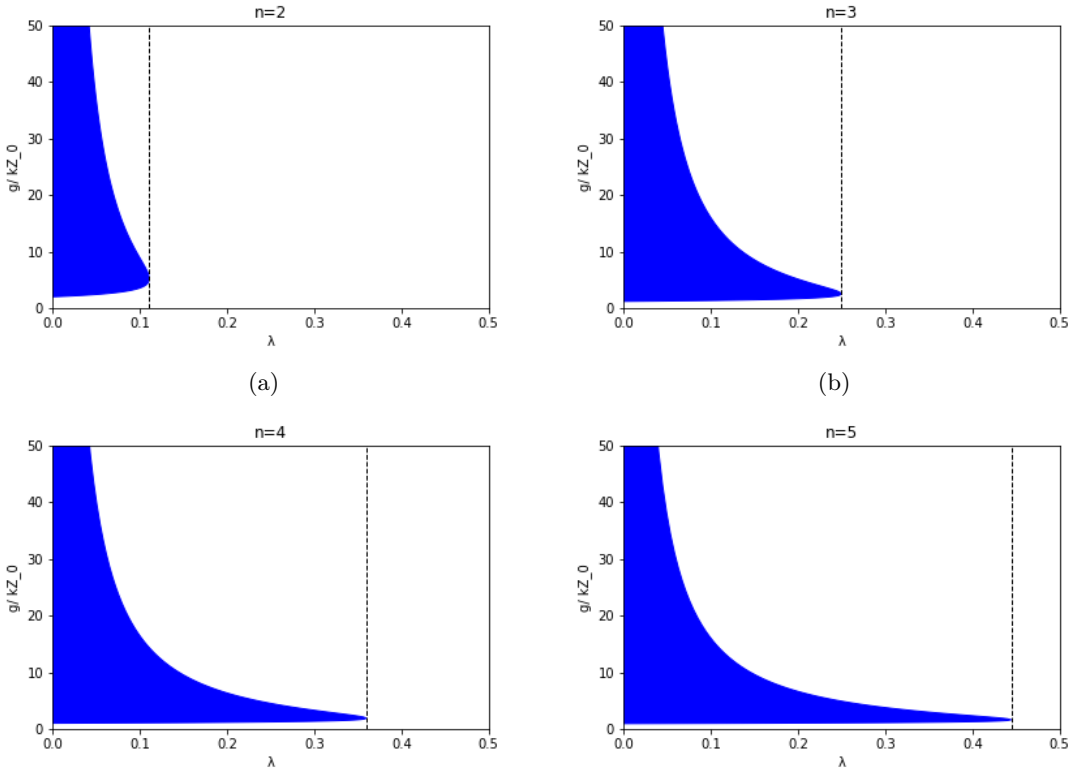
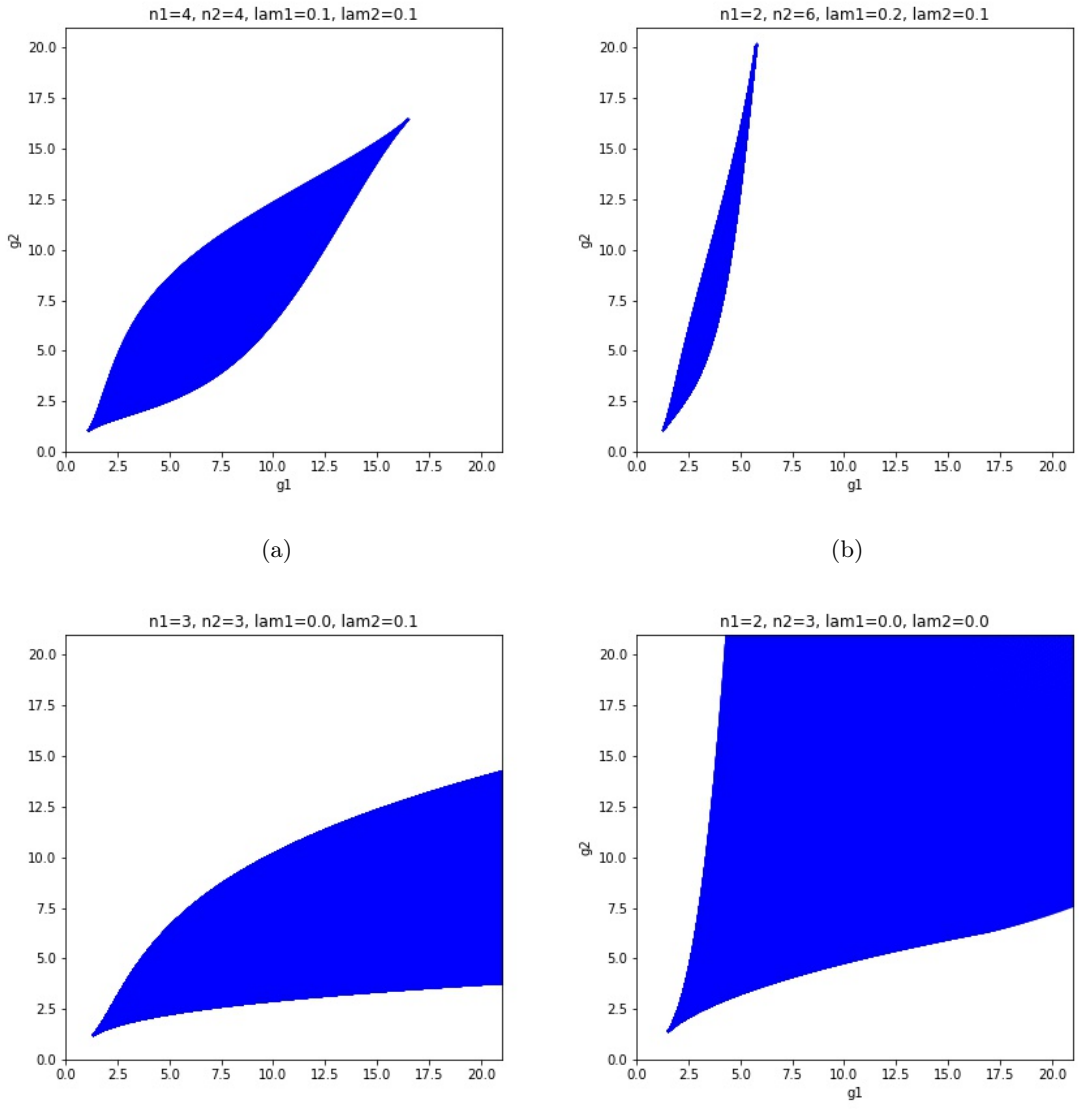


Figure 13: Spaces of parameters for the symmetric system (9), for several values of n. The blue fields represent the values of $(\frac{g}{kz_0}, \lambda)$ for which the system has three equilibria. The vertical dotted lines denote the value of λ_0. For all the parameters in the complement of the closure of the blue set, (9) has a unique equilibrium point. The problem is unsolved only for the values on the boundary of the blue set.

Theorem. *Let us consider:*

$$\begin{cases} \dot{x} = g_x H_1(y) - k_x x \\ \dot{y} = g_y H_2(x) - k_y y \end{cases}, \tag{10}$$

where $g_x, g_y > 0, k_x, k_y > 0$, *and* H_1 *and* H_2 *are two decreasing Hill functions with parameters* n_1, λ_1, z_{01} *and* n_2, λ_2, z_{02} *respectively.*



(a) (b)

(c) (d)

Figure 14: Spaces of parameters for the asymmetric system (10), for several values of n_1, n_2, λ_1 and λ_2. The blue fields represents the values of g_1 and g_2 for which the system has three equilibrium points, while the white field is for the values for which the system has a unique equilibria. The problem remain unsolved only for the values at the boundary. If $\lambda_0 = 0$ (c) or $\lambda_1 = 0$ (d), then the blue set is not bounded.

We denote: $g_1 := \frac{g_x}{k_x z_{02}}$, $g_2 := \frac{g_y}{k_y z_{01}}$ *For all* $n_1, n_2 \in \mathbb{N}^*$, $\lambda_1, \lambda_2 \in [0, 1)$, *there exists* $\rho > 0$ *(which depends on* $n_1, n_2, \lambda_1, \lambda_2$*) such that:*

-If $\rho < 1$, *then* (10) *has a unique equilibrium point, which is stable.*
-If $\rho > 1$, *then there exists* $A \subseteq \mathbb{R}^2_+$, *which depends on* $n_1, n_2, \lambda_2, \lambda_2$ *such that:*

- *If* $(g_1, g_2) \in A$, *then* (10) *has three equilibrium points and is bistable.*

- *If* $(g_1, g_2) \in \overline{A}^C$*, then* (10) *has a unique equilibrium point, which is stable.*

Moreover, A *is bounded if and only if* $\lambda_1 > 0$ *and* $\lambda_2 > 0$.

The exacts values of ρ *and* A *are given in Theorem 2.3.3. The set* A *is represented for different values of* n_1, n_2, λ_1 *and* λ_2 *in Figure 14.*

The objective of what follows is to prove these two theorems.

2.1 Existence, uniqueness and behaviour of solutions

We consider a competitive ODE with two variable of the form:

$$\begin{cases} \dot{x} = g_x F(y) - k_x x \\ \dot{y} = g_y G(x) - k_y y \end{cases} \tag{11}$$

where:

- $g_x, g_y > 0$ correspond to the synthesis rate of x and y respectively, in the absence of regulation.
- $k_x, k_y > 0$ represent the innate degradation rate of x and y respectively.
- $F, G \in C^1(\mathbb{R}^+, (0,1])$ are two decreasing functions, which model the mutual inhibition between x and y.

To ensure that (11) is well-defined, we extend F and G to $\mathbb{R}$ by defining:

$$\forall x \in \mathbb{R}_-, \quad F(x) = F(0), G(x) = G(0).$$

Since, F and G are C^1 on $\mathbb{R}^+$, they are locally Lipschitz continuous on $\mathbb{R}$.

We recall (see [20]) that a C^1 system of differential equations

$$\frac{dx_i}{dt} = F_i(x), \ \forall i \in \{1, ..., n\}$$

is called competitive if $\partial F_i / \partial x_j \leq 0$, for all $i, j \in \{1, ..., n\}$ such that $i \neq j$. Hence, we can easily check that system (11) is competitive.

First of all, let us see what happens in the absence of mutual inhibition, which corresponds to the case where $F = G \equiv 1$. In this case, the two equations are independent, and the explicit solution is given by;

$$\forall t \geq 0 : \begin{cases} x(t) = \frac{g_x}{k_x} + (x(0) - \frac{g_x}{k_x}) e^{-k_x t} \\ y(t) = \frac{g_y}{k_y} + (y(0) - \frac{g_y}{k_y}) e^{-k_y t} \end{cases}$$

For any initial condition, the solution clearly converges to $(\frac{g_x}{k_x}, \frac{g_y}{k_y})$. Hence, for weak regulation, we can expect to find a unique equilibrium point.
In the general case, the following proposition ensures that solutions of (11) exist and that they converge. Nevertheless, the system can of course have multiple equilibria.

The theorem 2.3 of [20] states that, if (x, y) are bounded solutions of a competitive ODE with two variables, then x and y converge to a finite limit. This result applies to our study. More precisely, we have the following proposition:

Proposition 2.1.1. *For any $(x_0, y_0) \in \mathbb{R}_+^2$, there exists a unique solution $(x, y) : \mathbb{R}^+ \to \mathbb{R}$ of the system (11) such that $(x(0), y(0)) = (x_0, y_0)$. Furthermore, x and y are non-negative, bounded and converge to a finite limit.*

Proof. Let $(x_0, y_0) \in \mathbb{R}_+^2$.

Since F and G are locally Lipschitz continuous on $\mathbb{R}$, the right-hand side of the system (11), defined on $\mathbb{R}^2$ by $(x, y) \mapsto (g_x F(y) - k_x x, g_y G(x) - k_y y)$ is also Lipschitz continuous. Hence, the Cauchy-Lipschitz theorem ensures that (11) admits a unique local solution with initial value (x_0, y_0). We call this solution (x, y).

Let $[0, T^*)$ ($T^* \in (0, +\infty]$) be an interval on which this solution is defined.

Since for all $t \in \mathbb{R}$, $F(t) \in (0, 1]$ and $G(t) \in (0, 1]$:

$$\forall t \in [0, T^*),\ \begin{cases} -k_x x < \dot{x} = g_x F(y) - k_x x \leq g_x - k_x x \\ -k_y y < \dot{y} = g_y G(x) - k_y y \leq g_y - k_y y \end{cases} .$$

Hence, by Grönwall's lemma:

$$\forall t \in [0, T^*),\ \begin{cases} 0 \leq x_0 e^{-k_x t} \leq x(t) \leq \frac{g_x}{k_x} + (x_0 - \frac{g_x}{k_x}) e^{-k_x t} \leq \frac{g_x}{k_x} + x_0 \\ 0 \leq y_0 e^{-k_y t} \leq y(t) \leq \frac{g_y}{k_y} + (y_0 - \frac{g_y}{k_y}) e^{-k_y t} \leq \frac{g_y}{k_y} + y_0 \end{cases} ,$$

which proves that x and y are non-negative and bounded (and hence defined on $\mathbb{R}_+$). According to the theorem 2.3 of [20], this is enough to infer that x and y converge to a finite limit.

□

Remark. A direct consequence of this proposition is that the system (11) has at least one equilibrium point. Moreover, if the system has a unique equilibrium point, then all the solutions converge to this equilibrium.

It seems difficult to find general conditions which ensure that the system has a certain number of equilibrium points. For that reason, we will here focus on the case where F and G are two decreasing Hill functions.

In the first part, we will study a canonical system, with symmetric parameters ($g_x = g_y$, $k_x = k_y$, $F = G$), in order to understand the reasoning adapted to this problem. Then, we will extend, as far as possible, these results to the general case of an asymmetric system.

2.2 Study of a simple case: a symmetric system with Hill functions

First, let us study the properties of the symmetric systems, without further hypotheses on F.

2.2.1 General results for symmetric systems

We consider the following symmetric system:

$$\begin{cases} \dot{x} = gF(y) - kx \\ \dot{y} = gF(x) - ky \end{cases} , \tag{12}$$

with $g, k > 0$, and $F : \mathbb{R}^+ \to (0, 1]$ a C^1 decreasing function.

We denote: $f := \frac{g}{k} F$, and we consider the dimensionless system associated with (12):

$$\begin{cases} \dot{x} = f(y) - x \\ \dot{y} = f(x) - y \end{cases} . \tag{13}$$

Let $(x_0, y_0) \in \mathbb{R}_+^2$.
The couple of functions (x, y) is clearly the solution of (12) which satisfies $(x(0), y(0)) = (x_0, y_0)$, if and only if $(\tilde{x}, \tilde{y})$ defined by: $(\tilde{x}(t), \tilde{y}(t)) := (x(\frac{t}{k}), y(\frac{t}{k}))$ for all $t \geq 0$ is a solution of (13), with the same initial value.

Hence, studying one or the other of these two systems is equivalent.

In what follows, we will focus on the problem (13), and (x, y) will always denote the solution of (13) which satisfies $(x(0), y(0)) = (x_0, y_0)$.

The first consequence of the system symmetry is that the trajectories cannot cross the identity line. In other words:

Proposition 2.2.1. *If $x_0 < y_0$ (resp. $y_0 < x_0$, resp. $x_0 = y_0$), then for all $t \geq 0, x(t) < y(t)$ (resp. $y(t) < x(t)$, resp. $x(t) = y(t)$).*

Proof. We assume that : $x_0 < y_0$. We define $z := y - x$. Let us assume that there exists $t > 0$ such that $z(t) \leq 0$. By continuity of z, there exists $t_0 > 0$ such that

$$\forall t \in [0, t_0[,\ z(t) > 0 \text{ and } z(t_0) = 0.$$

Then, on $[0, t_0[$, we have :

$$\begin{aligned} \dot{z} &= \dot{y} - \dot{x} \\ &= f(x) - y - f(y) + x \\ &\geq -z \quad (\text{because } x < y, \text{ hence } f(x) - f(y) > 0). \end{aligned}$$

Thus, by Grönwall's lemma

$$\forall t \in [0, t_0[, z(t) \geq 0, z(t) \geq z(0)e^{-t}.$$

Therefore, by continuity of z

$$z(t_0) \geq z(0)e^{-t_0} > 0.$$

We conclude by contradiction that for all $t \geq z(t) = y(t) - x(t) \geq 0$.
We prove the other two cases with the same method.

□

Another advantage of the symmetric systems is that the equilibrium points can be more easily characterised. In particular, there exists a unique equilibrium point located on the identity line. The following proposition summarises the main characteristics of these equilibria.

Proposition 2.2.2. *The equilibrium points of the system* (13) *have the following properties:*

(i) The system (13) *has a unique equilibrium point of the shape $(\bar{x}, \bar{x})$, with $\bar{x} \in \mathbb{R}^+$. We call this point the* diagonal point *of the system.*

(ii) $(\bar{y}, \bar{z}) \in (\mathbb{R}^+)^2$ is an equilibrium point of (13) *if and only if $\bar{y}$ is a fixed point of $f \circ f$ and $\bar{z} = f(\bar{y})$.*

(iii) If $(\bar{y}, \bar{z})$ is an equilibrium point of (13)*, such that $(\bar{y}, \bar{z}) \neq (\bar{x}, \bar{x})$, then $\bar{y} < \bar{x} < \bar{z}$ or $\bar{z} < \bar{x} < \bar{y}$.*

(iv) If $(\bar{y}, \bar{z})$ is an equilibrium point of (13), then $(\bar{z}, \bar{y})$ is also an equilibrium point of (13).

(v) The ODE (13) has an odd number of equilibrium points.

Proof.

(i) Let $(\bar{x}, \bar{x}) \in \mathbb{R}_+^2$. $(\bar{x}, \bar{x})$ is clearly an equilibrium point of (13) if and only if $\bar{x}$ is a fixed point of f. Since f is decreasing on $\mathbb{R}^+$, $f - \mathrm{id}$ is decreasing, and therefore injective on $\mathbb{R}^+$ Moreover :

$$(f - \mathrm{id})(0) = f(0) > 0 \text{ and } \lim_{x \to \infty} (f - \mathrm{id})(x) = -\infty$$

Hence, by the intermediate value theorem, there exists a unique $\bar{x}$ such that $f(\bar{x}) = \bar{x}$.

(ii) By hypothesis, f is decreasing. Therefore, f is a bijection from $\mathbb{R}^+$ onto $f(\mathbb{R}^+)$. Hence :
$(\bar{y}, \bar{z})$ is an equilibrium point of (13) if and only if

$$\begin{cases} f(\bar{y}) &= \bar{z} \\ f(\bar{z}) &= \bar{y} \end{cases} \iff \begin{cases} f(f(\bar{y})) = f(\bar{z}) = \bar{y} \\ f(\bar{z}) = \bar{y} \end{cases} .$$

(iii) Let $(\bar{y}, \bar{z})$ be an equilibrium of (13) point with $(\bar{y}, \bar{z}) \neq (\bar{x}, \bar{x})$. Then, $\bar{y} \neq \bar{x}$, otherwise, we would have $\bar{z} = \bar{x}$. Since $(\bar{y}, \bar{z})$ is an equilibrium point, $\bar{z} = f(\bar{y})$. Since f is decreasing :
If $\bar{y} < \bar{x}$, then $\bar{z} = f(\bar{y}) > f(\bar{x}) = \bar{x}$.
If $\bar{y} > \bar{x}$, then $\bar{z} = f(\bar{y}) < f(\bar{x}) = \bar{x}$.

(iv) This point is obvious, because of the system symmetry.

(v) This is a direct consequence of the points (i), (iii) and (iv).

□

The previous proposition shows that finding the equilibrium points of (13) is equivalent to finding the fixed points of $f \circ f$. The following one shows that the value of the derivative of f at the diagonal point gives information about the number of equilibrium point of the system, their stability and their basins of attraction.

Proposition 2.2.3. *We recall that $\bar{x}$ denotes the only fixed point of f. Then:*

- *If $f'(\bar{x}) < -1$, then the ODE (13) has at least three equilibrium points. In this case, the equilibrium point $(\bar{x}, \bar{x})$ is unstable, and (x, y) converges to $(\bar{x}, \bar{x})$ if and only if $x_0 = y_0$.*
- *If $f'(\bar{x}) > -1$, then the equilibrium point $(\bar{x}, \bar{x})$ is stable.*

Proof. We denote $\bar{x}$ the unique fixed point of f. We define $h := f \circ f - id$. By hypothesis, $h \in C^1(\mathbb{R}^+)$, and for all $x \geq 0, h'(x) = f'(x)f'(f(x)) - 1$. Hence, if $f'(\bar{x}) < -1$, then $h'(\bar{x}) = (f'(\bar{x}))^2 - 1 > 0$, which means that h' is increasing in $\bar{x}$. In other words, there exists $\varepsilon > 0$ such that : $h(\bar{x} - \varepsilon) < h(\bar{x}) = f(f(\bar{x})) - \bar{x} = 0$. Since $h(0) = f(f(0)) > 0$, according to the intermediate value theorem, there exists $\bar{y} < \bar{x}$ such that : $h(\bar{y}) = 0$, which means that $\bar{y}$ is a fixed point of $f \circ f$.

Hence, according to Proposition 2.2.3, $(\bar{y}, f(\bar{y}))$, $(f(\bar{y}), \bar{y})$, and $(\bar{x}, \bar{x})$ are three equilibrium points of (13).

We will now discuss the stability of $(\bar{x}, \bar{x})$. We recall that the right-hand side of the ODE (13) is

$$\begin{aligned} R : \mathbb{R}^{+2} &\to \mathbb{R}^2 \\ (x, y) &\mapsto (f(y) - x, f(x) - y)) \end{aligned}$$

Hence, for all $(x, y) \in \mathbb{R}^{+2}$, the Jacobian matrix of R at the point (x, y) is

$$J_{R(x,y)} = \begin{pmatrix} -1 & f'(y) \\ f'(x) & -1 \end{pmatrix}$$

In particular

$$J_{R(\bar{x},\bar{x})} = \begin{pmatrix} -1 & f'(\bar{x}) \\ f'(\bar{x}) & -1 \end{pmatrix}.$$

Hence

$$\begin{aligned} \det(J_{R(\bar{x},\bar{x})}) &= 1 - f'(\bar{x})^2 \\ Tr(J_{R(\bar{x},\bar{x})}) &= -2. \end{aligned}$$

Hence:

- If $f'(\bar{x}) < -1$, then $(\bar{x}, \bar{x})$ is linearly unstable.
- If $f'(\bar{x}) > -1$, then $(\bar{x}, \bar{x})$ is stable.

Lastly, let us discuss the basins of attraction in the case where $f'(\bar{x}) < -1$.

According to Proposition 2.1.1, x and y converge.

If $x_0 = y_0$, according to Proposition 2.2.1, $x(t) = y(t)$ for all $t \geq 0$, which means that x and y have the same limit. Since $(\bar{x}, \bar{x})$ is the only equilibrium point on the identity line, x and y converge to $\bar{x}$.

Now, let us assume that $y_0 > x_0$ (the proof for $x_0 > y_0$ is identical). We define: $z := y - x$. According to Proposition 2.2.1, $z > 0$ on $\mathbb{R}_+$, so

$$\dot{z} = f(x) - f(y) + x - y = -\frac{f(x) - f(y)}{x - y} z - z.$$

Since $f'(\bar{x}) < -1$, there exist $\gamma > 0$, $A > 1$ such that:

$$\forall w \in [\bar{x} - \gamma, \bar{x} + \gamma], f'(w) < -A.$$

Let us assume that x and y converge to $\bar{x}$. Then, there exists $t_0 \geq 0$ such that:

$$\forall t \geq t_0 : x(t) \in [\bar{x} - \gamma, \bar{x}) \text{ and } y(t) \in (\bar{x}, \bar{x} + \gamma].$$

Hence, for all $t \geq t_0$:

$$\begin{aligned} \dot{z}(t) &= z(t)(-\frac{f(x(t)) - f'(y(t))}{x(t) - y(t)} - 1) \\ &> z(t) \underbrace{(A - 1)}_{>0} \\ &\geq 0. \end{aligned}$$

Hence, $z(t) > z(t_0) > 0$, which proves that z does not converge to 0. We arrive to a contradiction, thus (x, y) does not converge to $(\bar{x}, \bar{x})$. □

2.2.2 Symmetric systems with Hill functions

We will now study the differential system (12) in the particular case where F is a decreasing Hill function. We consider:

$$\begin{aligned} H : \mathbb{R}_+ &\to \mathbb{R}_+ \\ z &\mapsto \frac{1+\lambda(\frac{z}{z_0})^n}{1+(\frac{z}{z_0})^n}, \end{aligned}$$

with $n \in \mathbb{N}^*$, $\lambda \in [0,1)$, $z_0 > 0$, and we study:

$$\begin{cases} \dot{x} = gH(y) - kx \\ \dot{y} = gH(x) - ky \end{cases} \tag{14}$$

with $g, k > 0$.

The dimensionless system associated with (14) is:

$$\begin{cases} \dot{x} = f_H(y) - x \\ \dot{y} = f_H(x) - y \end{cases}, \tag{15}$$

with

$$\begin{aligned} f_H : \mathbb{R}_+ &\to \mathbb{R}_+ \\ z &\mapsto \underbrace{\frac{g}{kz_0}}_{:=\tilde{g}} H(z_0 z) = \tilde{g}\frac{1+\lambda z^n}{1+z^n}. \end{aligned}$$

Let us define:

$$\tilde{x}(t) := \frac{x(\frac{t}{k})}{z_0}, \tilde{y}(t) := \frac{y(\frac{t}{k})}{z_0}.$$

The couple of functions (x, y) is clearly the solution of (14), with initial value (x_0, y_0) if and only if $(\tilde{x}, \tilde{y})$ is the solution of (15), with initial value $(\frac{x_0}{z_0}, \frac{y_0}{z_0})$.

In order to work with as few parameters as possible, we will work on the second system. In what follows, (x, y) will denote the solution of (15) which satisfies: $(x(0), y(0)) = (x_0, y_0)$. $\bar{x}$ still denotes the only fixed point of f_H.

According to the previous section (Proposition 2.2.2), system (15) has an odd number of equilibrium points; the following proposition states that, in this particular case, the system has in fact either one or three equilibrium points.

Proposition 2.2.4. *System* (15) *(and thus system* (14)*) has either one or three equilibrium points.*

In order to prove this proposition, we will need two lemmas; the first one provides a sufficient for the system to have at most three equilibrium points, while the second proves an inequality which plays a key role in the proof of the proposition.

Lemma 1. *If the ODE* (13) *has five equilibrium points or more, then there exists a non-diagonal equilibrium point (of the form* $(\bar{y}, \bar{z})$*, with* $\bar{y} \neq \bar{z}$*) such that :*

$$f'(\bar{y})f'(\bar{z}) \geq 1.$$

Lemma 2.

$$\forall m \in \mathbb{N}^*, \forall a, b \in \mathbb{R}_+,\ (ab)^m \leq \frac{1}{m+1} \sum_{k=0}^{m} a^{2m-2k} b^{2k}.$$

Moreover, the two sides are equal if and only if $a = b$*.*

Proof of lemma 1. We assume that system (13) has $2n+1$ equilibrium points, with $n \geq 2$. Then, $h := f \circ f - \mathrm{id}$ has $2n+1$ roots.

We denote these roots $y_1 < ... < y_n < x < z_n < ... < z_1$, where x is the only fixed point of f, and for all $i \in \{1, ..n\},\ f(y_i) = z_i$.

Since $h(0) > 0$, $h'(y_1) \leq 0$.

If $h'(y_1) < 0$, then $h(y) < 0$ for all $y \in (y_1, y_2)$, which means that $h'(y_2) \geq 0$.

Hence, $h'(y_1) = 0$ or $h'(y_2) \geq 0$. Since $h'(y_i) = f'(y_i)f'(z_i) - 1$ for all $i \in \{1, ..n\}$, the result follows.

□

Proof of lemma 2. Let $m \in \mathbb{N}^*, a, b \in \mathbb{R}_+$.

We rewrite:

$$\sum_{k=0}^{m} a^{2m-2k} b^{2k} = \sum_{k=0}^{\lfloor \frac{m-1}{2} \rfloor} (a^{2m-2k} b^{2k} + a^k b^{2m-2k}) + \varepsilon(m) a^m b^m,$$

with $\varepsilon(m) = \begin{cases} 0 & \text{if } m \text{ is odd} \\ 1 & \text{if } m \text{ is even} \end{cases}$.

We recall that for all $\alpha, \beta \in \mathbb{R}_+,\ 2\alpha\beta \leq \alpha^2 + \beta^2$.

Hence

$$\forall k \in \{0, ...m\},\ 2(ab)^m = 2(a^{m-k}b^k)(a^k b^{m-k}) \leq a^{2m-2k}b^{2k} + a^{2k}b^{2m-2k}.$$

Thus:

$$\begin{aligned} \sum_{k=0}^{m} a^{2m-2k} b^{2k} &\geq \sum_{k=0}^{\lfloor \frac{m-1}{2} \rfloor} 2(ab)^m + \varepsilon(m)(ab)^m \\ &= [2(\lfloor \frac{m-1}{2} \rfloor + 1) + \varepsilon(m)](ab)^m \\ &= (m+1)(ab)^m. \end{aligned}$$

Since $2(ab)^m < a^{2m} + b^{2m}$ if $a \neq b$, the two sides are equal if and only if $a = b$. □

Proof of Proposition 2.2.4. According to Proposition 2.2.2, system (15) has an odd number of equilibria. Hence, it is enough to show that the system has at most three equilibria.

Let us assume that (15) has several equilibrium points.

Let $(\bar{y}, \bar{z})$ be a non-diagonal equilibrium point of (15) ($\bar{y} \neq \bar{z}$). Then:

$$\begin{cases} \bar{y} &= f_H(\bar{z}) \\ \bar{z} &= f_H(\bar{y}) \end{cases}.$$

Consequently:

$$\begin{aligned} \bar{y} - \bar{z} &= f_H(\bar{z}) - f_H(\bar{y}) \\ &= \tilde{g}\left(\frac{1 + \lambda\bar{z}^n}{1 + \bar{z}^n} - \frac{1 + \lambda\bar{y}^n}{1 + \bar{y}^n}\right) \\ &= \tilde{g}\frac{(1-\lambda)(\bar{y}^n - \bar{z}^n)}{(1 + \bar{y}^n)(1 + \bar{z}^n)}. \end{aligned}$$

$$\begin{aligned} f'_H(\bar{y}) f'_H(\bar{z}) &= n^2\tilde{g}^2\frac{(1-\lambda)^2\bar{y}^{n-1}\bar{z}^{n-1}}{(1 + \bar{y}^n)^2(1 + \bar{z}^n)^2} \\ &= n^2\Big(\underbrace{\tilde{g}\frac{(1-\lambda)(\bar{y}^n - \bar{z}^n)}{(1 + \bar{y}^n)(1 + \bar{z}^n)}}_{=\bar{y}-\bar{z}}\Big)^2\frac{\bar{y}^{n-1}\bar{z}^{n-1}}{(\bar{y}^n - \bar{z}^n)^2} \\ &= n^2\frac{(\bar{y}\bar{z})^{n-1}}{\left(\sum_{k=0}^{n-1}\bar{y}^{n-1-k}\bar{z}^k\right)^2} \end{aligned}$$

(for the last equality, we use the formula $\bar{y}^n - \bar{z}^n = (\bar{y} - \bar{z})(\sum_{k=0}^{n-1}\bar{y}^{n-1-k}\bar{z}^k)$).

Applying lemma 2, with $a = \bar{y}^{1/2}, b = \bar{z}^{1/2}, m = n - 1$, we get:

$$n(\bar{y}\bar{z})^{\frac{n-1}{2}} < \sum_{k=0}^{n-1}\bar{y}^{n-1-k}\bar{z}^k.$$

Thus

$$n^2(\bar{y}\bar{z})^{n-1} < \left(\sum_{k=0}^{n-1}\bar{y}^{n-1-k}\bar{z}^k\right)^2,$$

which proves that

$$f'_H(\bar{y})f'_H(\bar{z}) < 1.$$

We conclude by applying the contrapositive of Lemma 1.

□

This proposition naturally leads to the following corollary:

Corollary 2.2.5.
- *If $f'_H(\bar{x}) > -1$, then $(\bar{x}, \bar{x})$ is the only equilibrium point of (15). Furthermore, for all (x_0, y_0), x and y converge to $\bar{x}$.*
- *If $f'_H(\bar{x}) < -1$, then (15) has exactly three equilibrium points, of the form $(\bar{y}, \bar{z})$, $(\bar{x}, \bar{x})$, $(\bar{z}, \bar{y})$, with $\bar{y} < \bar{x} < \bar{z}$. If $x_0 < y_0$ (resp. $y_0 < x_0$, resp. $x_0 = y_0$), then (x, y) converges to $(\bar{y}, \bar{z})$ (resp. $(\bar{z}, \bar{y})$, resp. $(\bar{x}, \bar{x})$).*

Proof. We assume that $f'_H(\bar{x}) < -1$. According to Proposition 2.2.3, (15) has several equilibrium points, which proves, according to the previous proposition, that the system necessarily has exactly three equilibria.

According to Proposition 2.2.3, (x, y) converges to $(\bar{x}, \bar{x})$ if and only if $x_0 = y_0$. If $x_0 < y_0$ (resp. $y_0 < x_0$), then $x(t) < y(t)$ (resp. $y(t) < x(t)$) for all $t > 0$ (2.2.1), which ensures that x, y converge to $(\bar{y}, \bar{z})$ (resp. $(\bar{z}, \bar{y})$).

Let us denote: $h := f_H \circ f_H - \mathrm{id}$. If h has three equilibrium points (denoting $\bar{y} < \bar{x} < \bar{z}$), the proof of the previous propositions shows that $h'(\bar{y}) < 0$, and so that $h'(\bar{x}) \geq 0$. Hence, $f'_H(\bar{x}) \geq -1$.

□

We can now state the main result of this section, which provides sufficient conditions for the system to have one or three equilibria.

Theorem 2.2.6. *Let us denote $\lambda_0 := (\frac{n-1}{n+1})^2$.*
-If $\lambda > \lambda_0$, or $n = 1$, then (15) has a unique equilibrium point, and this equilibrium point is stable.
-If $n \geq 2$ and $\lambda \in (0, \lambda_0]$, we define:

$$\begin{aligned}
\omega_\lambda^- &:= \frac{n - 1 - (n+1)(\lambda + \sqrt{(1-\lambda)(\lambda_0 - \lambda)})}{2\lambda} \\
\omega_\lambda^+ &:= \frac{n - 1 - (n+1)(\lambda - \sqrt{(1-\lambda)(\lambda_0 - \lambda)})}{2\lambda} \\
g_\lambda^- &:= (\omega_\lambda^-)^{1/n} \frac{1 + \omega_\lambda^-}{1 + \lambda\omega_\lambda^-} \\
g_\lambda^+ &:= (\omega_\lambda^+)^{1/n} \frac{1 + \omega_\lambda^+}{1 + \lambda\omega_\lambda^+}.
\end{aligned}$$

Then, we have the following alternative:

- *If $\frac{g}{kz_0} =: \tilde{g} \in [g_\lambda^-, g_\lambda^+]^C$, then (15) has a unique equilibrium point.*

- *If* $\frac{g}{kz_0} =: \tilde{g} \in (g_\lambda^-, g_\lambda^+)$*, then* (15) *has exactly three equilibrium points.*

-If $n \geq 2$ *and* $\lambda = 0$*, we define:*

$$g_0^- := \big(\frac{1}{n-1}\big)^{1/n} \frac{n}{n-1}.$$

Then:

- *If* $\frac{g}{kz_0} =: \tilde{g} < g_0^-$*, then* (15) *has a unique equilibrium point.*
- *If* $\frac{g}{kz_0} =: \tilde{g} > g_0^-$*, then* (15) *has exactly three equilibrium points.*

In each case, if there exists a unique equilibrium point, for all (x_0, y_0)*,* x *and* y *converge to* $\bar{x}$*. If* (15) *has three equilibrium points,(of the form* $(\bar{y}, \bar{z})$*,* $(\bar{x}, \bar{x})$*,* $(\bar{z}, \bar{y})$*, with* $\bar{y} < \bar{x} < \bar{z}$*), and if* $x_0 < y_0$ *(resp.* $y_0 < x_0$*, resp.* $x_0 = y_0$*), then* (x, y) *converges to* $(\bar{y}, \bar{z})$ *(resp.* $(\bar{z}, \bar{y})$*, resp.* $(\bar{x}, \bar{x})$*).*

Proof. First, let us consider the case where $n = 1$.

We denote $h := f_H \circ f_H - id$. According to Proposition 2.2.2, the number of equilibrium points of the system is equal to the number of positive roots of h. An explicit calculation gives us

$$\forall z \geq 0, \quad h(z) = \frac{\tilde{g} + \lambda\tilde{g}^2 + ((\lambda\tilde{g})^2 - 1)z - (\lambda\tilde{g} + 1)z^2}{1 + \tilde{g} + (\lambda\tilde{g} + 1)z}.$$

Therefore, h has at most two roots. By 2.2.2, we know that the system has an odd number of roots, which proves that (15) has a unique equilibrium point.

We now assume that $n \geq 2$.

By definition:

$$\bar{x} = \tilde{g}\frac{1 + \lambda\bar{x}^n}{1 + \bar{x}^n}.$$

Therefore,

$$\tilde{g} = \bar{x}\frac{1 + \bar{x}^n}{1 + \lambda\bar{x}^n}.$$

Hence:

$$\begin{aligned} f'_H(\bar{x}) &= -n(1-\lambda)\tilde{g}\frac{\bar{x}^{n-1}}{(1+\bar{x}^n)^2} \\ &= -n(1-\lambda)\frac{\bar{x}^n}{(1+\lambda\bar{x}^n)(1+\bar{x}^n)}. \end{aligned}$$

We denote:

$$\begin{aligned}\omega &:= \bar{x}^n\\ P(\omega) &:= (1+\lambda\omega)(1+\omega) - n(1-\lambda)\omega\\ &= \lambda\omega^2 + ((n+1)\lambda + 1 - n)\omega + 1.\end{aligned}$$

We clearly have

$$\begin{aligned}f'_H(\bar{x}) > -1 &\iff P(\omega) > 0\\ f'_H(\bar{x}) < -1 &\iff P(\omega) < 0.\end{aligned}$$

We assume that $\lambda > 0$. Denoting Δ the discriminant of P, we get

$$\begin{aligned}\Delta &= (n+1)^2\lambda^2 + (n-1)^2 - 2(n+1)(n-1)\lambda - 4\lambda\\ &= (n+1)^2\lambda^2 - 2(n^2+1)\lambda + (n-1)^2\\ &= (n+1)^2(\lambda-1)(\lambda - \underbrace{(\frac{n-1}{n+1})^2}_{:=\lambda_0}).\end{aligned}$$

Hence, if $\lambda > \lambda_0$, then $P(\omega) > 0$, and so $f'_H(\bar{x}) > 0$.

If $\lambda \in (0, \lambda_0]$, then:

$$\begin{aligned}P(\omega) > 0 &\iff \omega \in [\omega_\lambda^-, \omega_\lambda^+]^C \iff \bar{x} \in [(\omega_\lambda^-)^{1/n}, (\omega_\lambda^+)^{1/n}]^C\\ P(\omega) < 0 &\iff \omega \in (\omega_\lambda^-, \omega_\lambda^+) \iff \bar{x} \in ((\omega_\lambda^-)^{1/n}, (\omega_\lambda^+)^{1/n}).\end{aligned}$$

Since $\varphi : x \mapsto x\frac{1+\bar{x}^n}{1+\lambda\bar{x}^n} = \frac{\bar{x}}{f_{\lambda,1}(\bar{x})}$

is increasing, we get:

$$\begin{aligned}f'_H(\bar{x}) > -1 &\iff P(\omega) > 0 \iff g = \varphi(x) \in [\varphi((\omega_\lambda^-)^{1/n}), \varphi((\omega_\lambda^+)^{1/n})]^C = [g_\lambda^-, g_\lambda^+]^C\\ f'_H(\bar{x}) < -1 &\iff P(\omega) < 0 \iff g = \varphi(\bar{x}) \in (\varphi((\omega_\lambda^-)^{1/n}), \varphi((\omega_\lambda^+)^{1/n}) = (g_\lambda^-, g_\lambda^+).\end{aligned}$$

If $\lambda = 0$, then:

$$\begin{aligned}P(\omega) > 0 &\iff \omega < \frac{1}{n-1}\\ P(\omega) < 0 &\iff \omega > \frac{1}{n-1}.\end{aligned}$$

Since $\varphi(\frac{1}{n-1}) = (\frac{1}{n-1})^{1/n}\frac{n}{n-1} := g_0^-$, the result follows.

We conclude by applying Corollary 2.2.5. □

Now, we will try to extend the result found in this section to asymmetric systems. Breaking the symmetry adds complexity, first because of the number of parameters, which doubles, and also because we cannot identify a diagonal point as we did in this section, which played a crucial role in the proofs. Nevertheless, we have managed to extend most results.

2.3 Some results in the asymmetric case, with Hill functions

2.3.1 General results for asymmetric systems

We consider again system (11):

$$\begin{cases} \dot{x} = g_x F(y) - k_x x \\ \dot{y} = g_y G(x) - k_y y \end{cases} \tag{11}$$

We recall that $g_x, g_y, k_x, k_y > 0$, and that F and G are two decreasing C^1 functions defined from $\mathbb{R}^+$ onto $(0, 1]$.

We have seen that for all $(x_0, y_0) \in \mathbb{R}^2_+$, there exists a unique solution of (11) with initial solution (x_0, y_0). In this section, (x, y) denotes this solution. We define $f := \frac{g_x}{k_x}F$, $g := \frac{g_y}{k_y}G$.

We recall that, according to Proposition 2.1.1, (11) has at least one equilibrium. The study of $f \circ g$ provides information about these equilibria, as shown in this proposition:

Proposition 2.3.1. *The equilibrium points of* (11) *have the following properties:*

(i) $(\bar{x}, \bar{y}) \in \mathbb{R}^2_+$ *is an equilibrium point of* (11) *if and only if* $\bar{x}$ *is a fixed point of* $f \circ g$ *and* $\bar{y} = g(\bar{x})$.

(ii) If there exists an equilibrium point of (11) $(\bar{x}, \bar{y})$ *such that* $f'(\bar{y})g'(\bar{x}) > 1$, *then* (11) *has at least three equilibria, and* $(\bar{x}, \bar{y})$ *is linearly unstable.*

(iii) If $(\bar{x}, \bar{y})$ *is an equilibrium point of* (11) *such that* $f'(\bar{y})g'(\bar{x}) < 1$, *then* $(\bar{x}, \bar{y})$ *is stable.*

Proof.

(i) By definition, $(\bar{x}, \bar{y})$ is an equilibrium point of (13) if and only if

$$\begin{cases} g_x F(\bar{y}) &= k_x \bar{x} \\ g_y G(\bar{x}) &= k_y \bar{y} \end{cases} \iff \begin{cases} f(\bar{y}) &= \bar{x} \\ g(\bar{x}) &= \bar{y} \end{cases} \iff \begin{cases} f(g(\bar{x})) &= \bar{x} \\ g(\bar{x}) &= \bar{y} \end{cases}.$$

(ii) and (iii) We denote $h := f \circ g - \mathrm{id}$. We assume that there exists $(\bar{x}, \bar{y})$ an equilibrium point of (11) such that $f'(\bar{y})g'(\bar{x}) > 1$. Then, $h'(\bar{x}) = g'(\bar{x})f'(g(\bar{x})) - 1 = f'(\bar{y})g'(\bar{x}) - 1 > 0$. Since $h(0) > 0$ and $h(x) \underset{x\to+\infty}{\longrightarrow} -\infty$, this proves that there exist $\bar{x}_1 \in (0, \bar{x})$, $\bar{x}_2 \in (\bar{x}, +\infty)$ such that: $h(\bar{x}_1) = h(\bar{x}_2) = 0$. According to the first point of this proposition, $(\bar{x}_1, g(\bar{x}_1))$, $(\bar{x}, g(\bar{x}))$, $(\bar{x}_2, g(\bar{x}_2))$ are three equilibrium points of (11).

We will now discuss the stability of $(\bar{x}, \bar{y})$. We recall that the right-hand side of the ODE (13) is

$$\begin{aligned} R : \mathbb{R}^{+2} &\to \mathbb{R}^2 \\ (x, y) &\mapsto (f(y) - x, f(x) - y)) \end{aligned}$$

Hence, the Jacobian matrix of F at the point $(\bar{x}, \bar{y})$ is

$$J_{R(\bar{x},\bar{y})} = \begin{pmatrix} -k_x & g_x F'(\bar{y}) \\ g_y G'(\bar{x}) & -k_y \end{pmatrix}$$

In particular

$$\begin{aligned} \det(J_{R(\bar{x},\bar{y})}) &= k_x k_y - g_x g_y F'(\bar{y}) G'(\bar{x}) \\ \mathrm{Tr}(R_{F(\bar{x},\bar{x})}) &= -k_x - k_y < 0 \end{aligned}$$

Since $f'(\bar{y})g'(\bar{x}) = \frac{g_x g_y}{k_x k_y} F'(\bar{y})G'(\bar{x})$, We clearly have $\det(J_{R(\bar{x},\bar{y})}) > 0$ if and only if $f'(\bar{y})g'(\bar{x}) < 1$, and $\det(J_{R(\bar{x},\bar{y})}) < 0$ if and only if $f'(\bar{y})g'(\bar{x}) > 1$, which concludes the proof.

□

2.3.2 Asymmetric systems with Hill functions

We now study system (11) in the particular case where F and G are two Hill functions. The system can be rewritten

$$\begin{cases} \dot{x} = g_x H_1(y) - k_x x \\ \dot{y} = g_y H_2(x) - k_y y \end{cases}, \tag{16}$$

where

$$\begin{aligned} H_1 : \mathbb{R}_+ &\to \mathbb{R}_+ \\ z &\mapsto \frac{1 + \lambda_1 (\frac{z}{z_{01}})^{n_1}}{1 + (\frac{z}{z_{01}})^{n_1}}, \end{aligned}$$

$$\begin{aligned} H_2 : \mathbb{R}_+ &\to \mathbb{R}_+ \\ z &\mapsto \frac{1 + \lambda_2 (\frac{z}{z_{02}})^{n_2}}{1 + (\frac{z}{z_{02}})^{n_2}}, \end{aligned}$$

with $n_1, n_2 \in \mathbb{N}^*$, $\lambda_1, \lambda_2 \in [0, 1)$, $z_{01}, z_{02} > 0$.

In order to reduce the number of variables, we consider:

$$\begin{cases} \dot{x} = \tilde{g}_x \tilde{H}_1(y) - k_x x \\ \dot{y} = \tilde{g}_y \tilde{H}_2(x) - k_y y \end{cases}, \tag{17}$$

where: $\tilde{g}_x := \frac{g_x}{z_{02}}$, $\tilde{g}_y := \frac{g_y}{z_{01}}$, $\tilde{H}_1(t) := H_1(z_{01}t)$, $\tilde{H}_2(t) := H_2(z_{02}t)$. Then, (x, y) is a solution of (16) if and only if $(\frac{x}{z_{02}}, \frac{y}{z_{01}})$ is a solution of (17).

In what follows, we will denote by (x, y) a solution of (17), $g_1 := \frac{\tilde{g}_x}{k_x}$, $g_2 := \frac{\tilde{g}_x}{k_x}$and $f := \frac{g_x}{k_x} H_1$, $g := \frac{g_y}{k_y} H_2$. Like in the symmetric case, this system has at most three equilibrium points. Nevertheless, in this case it can have two.

Proposition 2.3.2. *System* (17) *has at most three equilibrium points. Moreover, there exists at most one equilibrium point* $(\bar{x}, \bar{y})$ *such that* $f'(\bar{y})g'(\bar{x}) \geq 1$.

In order to prove this proposition, we will use, as in the symmetric case, the inequality of lemma 2. We will also need the following lemma:

Lemma 3. *If the ODE* (11) *has four equilibrium points or more, then there exist at least two equilibrium points (denoted* $(\bar{x}, \bar{y})$ *and* $(\bar{x}', \bar{y}')$*) such that*

$$\begin{cases} f'(\bar{y})g'(\bar{x}) \geq 1 \\ f'(\bar{y}')g'(\bar{x}') \geq 1 \end{cases}.$$

Proof of Lemma 3. We assume that system (11) has n equilibrium points, with $n \geq 4$. Then, according to Proposition 2.3.1, $h := f \circ g - id$ has n roots.

We denote these roots $\bar{x}_1 < ... < \bar{x}_n$.

Since $h(0) > 0$, $h'(\bar{x}_1) \leq 0$.

- We assume that $\bar{x}_1$ is a local minimum point of h. Then, $h'(\bar{x}_1) = 0$. If $h'(\bar{x}_2) < 0$, then for all $x \in (\bar{x}_2, \bar{x}_3)$, $h(x) < 0$, which proves that $h'(\bar{x}_3) \geq 0$.
- We assume that $\bar{x}_1$ is not a local minimum point of h. Then, for all $x \in (\bar{x}_1, \bar{x}_2)$, $h(x) < 0$, and so $h'(\bar{x}_2) \geq 0$. If $\bar{x}_2$ is a local maximum point of h, then $h'(\bar{x}_3) \geq 0$. Otherwise, $h'(\bar{x}_3) = 0$ or $h'(\bar{x}_4) \geq 0$.

In any case, there exist $i_1, i_2 \in \{1, ...4\}$, $i_1 \neq i_2$ such that: $h'(\bar{x}_{i_1}) \geq 0$ and $h'(\bar{x}_{i_2}) \geq 0$.
Since $h'(\bar{y}_i) = f'(\bar{y}_i)g'(\bar{x}_i) - 1$ for all $i \in \{1, ..n\}$, the results follows.

□

Proof of Proposition 2.3.2. We first assume that $n_1 = n_2 = 1$.

In this case, a direct computation yields:

$$\forall z > 0, f(g(z)) - z = \frac{-(\lambda_2 g_2 + 1)z^2 + (g_1 - g_2 - 1 + \lambda_1\lambda_2 g_1 g_2)z + g_1(1 + g_2\lambda_1)}{(\lambda_2 g_2 + 1)z + g_2 + 1},$$

which proves that the system has at most two roots in this case.

We now assume that $n_1 \geq 2$.

Let us assume that (17) has several equilibrium points.

Let $(\bar{x}, \bar{y})$ and $(\bar{x}', \bar{y}')$ be two different equilibrium points of (17). By definition:

$$\begin{cases} \bar{x} &= f(\bar{y}) \\ \bar{y} &= g(\bar{x}) \end{cases},$$

and

$$\begin{cases} \bar{x}' &= f(\bar{y}') \\ \bar{y}' &= g(\bar{x}') \end{cases}.$$

Thus:

$$\begin{aligned} \bar{x} - \bar{x}' &= f(\bar{y}) - f(\bar{y}') \\ &= g_1\left(\frac{1 + \lambda_1 \bar{y}^{n_1}}{1 + \bar{y}^{n_1}} - \frac{1 + \lambda_1 \bar{y}'^{n_1}}{1 + \bar{y}'^{n_1}}\right) \\ &= g_1 \frac{(1 - \lambda_1)(\bar{y}'^{n_1} - \bar{y}^{n_1})}{(1 + \bar{y}^{n_1})(1 + \bar{y}'^{n_1})}. \end{aligned}$$

In the same way

$$\begin{aligned} \bar{y} - \bar{y}' &= f(\bar{x}) - f(\bar{x}') \\ &= g_2 \frac{(1 - \lambda_2)(\bar{x}'^{n_2} - \bar{x}^{n_2})}{(1 + \bar{x}^{n_2})(1 + \bar{x}'^{n_2})}. \end{aligned}$$

On the other hand

$$\begin{aligned} f'(\bar{y})g'(\bar{x}) &= n_1 n_2 g_1 g_2 (1 - \lambda_1)(1 - \lambda_2) \frac{\bar{y}^{n_1 - 1}\bar{x}^{n_2 - 1}}{(1 + \bar{y}^{n_1})^2 (1 + \bar{x}^{n_2})^2} \\ &= n_1 n_2 \frac{(\bar{x} - \bar{x}')(\bar{y} - \bar{y}')\bar{y}^{n_1 - 1}\bar{x}^{n_2 - 1}}{(\bar{x}^{n_2} - \bar{x}'^{n_2})(\bar{y}^{n_1} - \bar{y}'^{n_1})} \frac{(1 + \bar{y}'^{n_1})(1 + \bar{x}'^{n_2})}{(1 + \bar{y}^{n_1})(1 + \bar{x}^{n_2})} \\ &= \frac{n_1 \bar{y}^{n_1 - 1}}{\sum_{k=0}^{n_1 - 1} \bar{y}^{n_1 - 1 - k}\bar{y}'^{k}} \frac{n_2 \bar{x}^{n_2 - 1}}{\sum_{k=0}^{n_2 - 1} \bar{x}^{n_2 - 1 - k}\bar{x}'^{k}} \frac{(1 + \bar{y}'^{n_1})(1 + \bar{x}'^{n_2})}{(1 + \bar{y}^{n_1})(1 + \bar{x}^{n_2})} \end{aligned}$$

(for the last equality, we use the formula $a^n - b^n = (a-b)(\sum_{k=0}^{n-1} a^{n-1-k}b^k)$).
Applying lemma 2, with $a = \bar{x}^{1/2}, b = \bar{x}'^{1/2}, m = n_1 - 1$, and with $a = \bar{y}^{1/2}$ $b = \bar{y}'^{1/2}$, $m = n_2 - 1$ we get

$$n_1(\bar{y}\bar{y}')^{\frac{n_1-1}{2}} < \sum_{k=0}^{n_1-1} \bar{y}^{n_1-1-k}\bar{y}'^{k},$$

and

$$n_2(\bar{x}\bar{x}')^{\frac{n_2-1}{2}} \leq \sum_{k=0}^{n_2-1} \bar{x}^{n_2-1-k}\bar{x}'^{k}.$$

Hence:

$$\begin{aligned} f'(\bar{y})g'(\bar{x}) &< (\frac{\bar{y}}{\bar{y}'})^{\frac{n_1-1}{2}}(\frac{\bar{x}}{\bar{x}'})^{\frac{n_2-1}{2}}\frac{(1+\bar{y}'^{n_1})(1+\bar{x}'^{n_2})}{(1+\bar{y}^{n_1})(1+\bar{x}^{n_2})} \\ &:= \Psi((\bar{x},\bar{y}),(\bar{x}',\bar{y}')). \end{aligned}$$

It clearly holds that;

$$f'(\bar{y}')g'(\bar{x}') < \Psi((\bar{x}',\bar{y}'),(\bar{x},\bar{y})).$$

Since $\Psi((\bar{x}',\bar{y}'),(\bar{x},\bar{y})) = \Psi((\bar{x},\bar{y}),(\bar{x}',\bar{y}'))^{-1}$, $f'(\bar{y})g'(\bar{x}) < 1$, or $f'(\bar{y}')g'(\bar{x}') < 1$.
Hence, there exists at most one equilibrium point of (17) (denoted $(\bar{x},\bar{y})$) such that $f'(\bar{y})g'(\bar{x}) \geq 1$. We conclude by applying the contrapositive to lemma 3.

□

Lastly, this last theorem provides two spaces of parameters for $\tilde{g}_1, \tilde{g}_2$ which ensure that system (17) has one or three equilibrium points.

Theorem 2.3.3. *We define:*

$$\begin{aligned} \phi(\lambda) &:= \frac{1-\sqrt{\lambda}}{1+\sqrt{\lambda}} \\ \rho &:= n_1 n_2 \phi(\lambda_1)\phi(\lambda_2) \end{aligned}$$

If $\rho < 1$, then (17) *has a unique equilibrium point. If $\rho \geq 1$, we define:*
-If $\lambda_1, \lambda_2 > 0$:

$$\begin{aligned} x^{\pm} &:= \left[\frac{1}{\sqrt{\lambda_2}} + \frac{1}{2\lambda_2}(1+\sqrt{\lambda_2})^2\sqrt{\rho-1}\left(\sqrt{\rho-1} \pm \sqrt{\rho-\phi(\lambda_2)^2}\right)\right]^{1/n_2} \\ y^{\pm} &:= \left[\frac{1}{\sqrt{\lambda_1}} + \frac{1}{2\lambda_1}(1+\sqrt{\lambda_1})^2\sqrt{\rho-1}\left(\sqrt{\rho-1} \pm \sqrt{\rho-\phi(\lambda_1)^2}\right)\right]^{1/n_1} \\ \alpha(x) &:= (1+\lambda_1) - (1+\sqrt{\lambda_1})^2(1+\sqrt{\lambda_2})^2\rho\frac{x^{n_2}}{(1+x^{n_2})(1+\lambda_2 x^{n_2})} \\ \beta(y) &:= (1+\lambda_2) - (1+\sqrt{\lambda_1})^2(1+\sqrt{\lambda_2})^2\rho\frac{y^{n_1}}{(1+y^{n_1})(1+\lambda_1 y^{n_1})} \end{aligned}$$

$$
\begin{aligned}
r^{\pm}(x) :&= \left[\frac{-\alpha(x) \pm \sqrt{\alpha(x)^2 - 4\lambda_1}}{2\lambda_1}\right]^{1/n_1} \\
s^{\pm}(y) :&= \left[\frac{-\beta(y) \pm \sqrt{\beta(y)^2 - 4\lambda_2}}{2\lambda_2}\right]^{1/n_2} \\
E :&= \{(x,y) \in (x^-, x^+) \times (y^-, y^+) : r^-(x) < y < r^+(x)\} \\
&= \{(x,y) \in (x^-, x^+) \times (y^-, y^+) : s^-(y) < x < s^+(y)\}.
\end{aligned}
$$

-If $\lambda_1 = 0, \lambda_2 > 0$:

$$
\begin{aligned}
x^{\pm} :&= \left[\frac{1}{\sqrt{\lambda_2}} + \frac{1}{2\lambda_2}(1+\sqrt{\lambda_2})^2\sqrt{\rho-1}\left(\sqrt{\rho-1} \pm \sqrt{\rho - \phi(\lambda_2)^2}\right)\right]^{1/n_2} \\
y^- :&= \left(\frac{1}{\rho-1}\right)^{1/n_1} \\
r^-(x) :&= \left[\frac{(1+x^{n_2})(1+\lambda_2 x^{n_2})}{-\lambda_2 x^{2n_2} + (n_1 n_2(1-\lambda_2) - (1+\lambda_2))x^{n_2} - 1}\right]^{1/n_1} \\
\beta(y) :&= (1+\lambda_2) - (1+\sqrt{\lambda_1})^2(1+\sqrt{\lambda_2})^2\rho\frac{y^{n_1}}{(1+y^{n_1})(1+\lambda_1 y^{n_1})} \\
s^{\pm}(y) :&= \left[\frac{-\beta(y) \pm \sqrt{\beta(y)^2 - 4\lambda_2}}{2\lambda_2}\right]^{1/n_2} \\
E :&= \{(x,y) \in (x^-, x^+) \times (y^-, +\infty) : y > r^-(x)\} \\
&= \{(x,y) \in (x^-, x^+) \times (y^-, +\infty) : s^-(y) < x < s^+(y)\}.
\end{aligned}
$$

-If $\lambda_1 = \lambda_2 = 0$:

$$
\begin{aligned}
x^{\pm} :&= \left(\frac{1}{\rho-1}\right)^{1/n_2} \\
y^- :&= \left(\frac{1}{\rho-1}\right)^{1/n_1} \\
r^-(x) :&= \left(\frac{1+x^{n_2}}{(\rho-1)x^{n_2} - 1}\right)^{1/n_1} \\
s^-(y) :&= \left(\frac{1+y^{n_1}}{(\rho-1)y^{n_1} - 1}\right)^{1/n_2} \\
E :&= \{(x,y) \in (x^-, +\infty) \times (y^-, +\infty) : y > r^-(x)\} \\
&= \{(x,y) \in (x^-, +\infty) \times (y^-, +\infty) : x > s^-(y)\}.
\end{aligned}
$$

In each case, we define:

$$
\begin{aligned}
\Gamma : {\mathbb{R}_+^*}^2 &\to {\mathbb{R}_+^*}^2 \\
(x,y) &\mapsto \left(\frac{x(1+y^{n_1})}{1+\lambda_1 y^{n_1}}, \frac{y(1+x^{n_2})}{1+\lambda_2 x^{n_2}}\right),
\end{aligned}
$$

And we have:

If $(g_1, g_2) \in \Gamma(E)$*, then* (17) *has three equilibrium points, and is bistable.*
If $(g_1, g_2) \in \overline{\Gamma(E)}^C$*, then* (17) *has a unique equilibrium point, and is monostable.*

In order to prove this theorem, we will need this very simple lemma:

Lemma 4. *Let* $P(X) := aX^2 + bX + c$ *a polynomial of degree two.*
We assume that $a > 0$ *and* $c > 0$*. If* P *has two real roots, then they have the same sign. Moreover,* P *has two positive roots if and only if* $b + 2\sqrt{ac} < 0$*.*

Proof of lemma 4. We denote Δ the determinant of this polynomial. If $b \geq 0$, then $P(x) > 0$ for all $x \geq 0$. If $b < 0$, then $P(x) > 0$ for all $x \leq 0$. Hence, P has two distinct positive roots if and only if $\Delta > 0$ and $b \leq 0$. Since $\Delta = b^2 - 4ac = (b - 2\sqrt{ac})(b + 2\sqrt{ac})$, the result immediately follows. □

Proof of theorem 2.3.3. Let $(\bar{x}, \bar{y}) \in \mathbb{R}_+$ an equilibrium points of (15). By definition:

$$\begin{cases} \bar{x} = f(\bar{y}) = g_1 \frac{1+\lambda_1 \bar{y}^{n_1}}{1+\bar{y}^{n_1}} \\ \bar{y} = g(\bar{x}) = g_2 \frac{1+\lambda_2 \bar{x}^{n_2}}{1+\bar{x}^{n_2}} \end{cases}.$$

Hence:

$$\begin{cases} g_1 = \frac{\bar{x}(1+\bar{y}^{n_1})}{1+\lambda_1 \bar{y}^{n_1}} \\ g_2 = \frac{\bar{y}(1+\bar{x}^{n_2})}{1+\lambda_2 \bar{x}^{n_2}} \end{cases}. \qquad (\star)$$

Hence:

$$\begin{aligned} f'(\bar{y})g'(\bar{x}) &= n_1 n_2 (1-\lambda_1)(1-\lambda_2) g_1 g_2 \frac{\bar{x}^{n_2-1}\bar{y}^{n_1-1}}{(1+\bar{x}^{n_2})^2(1+\bar{y}^{n_1})^2} \\ &= n_1 n_2 (1-\lambda_1)(1-\lambda_2) \frac{\bar{x}^{n_2}\bar{y}^{n_1}}{(1+\bar{x}^{n_2})(1+\lambda_2\bar{x}^{n_2})(1+\bar{y}^{n_1})(1+\lambda_1\bar{y}^{n_1})}. \end{aligned}$$

We denote:

$$\begin{aligned} \omega &:= x^{n_2} \\ \delta &:= y^{n_1} \\ C &:= n_1 n_2 (1-\lambda_1)(1-\lambda_2) = (1+\sqrt{\lambda_1})^2(1+\sqrt{\lambda_2})^2\rho \\ D_1(\delta) &:= (1+\delta)(1+\lambda_1\delta) \\ D_2(\omega) &:= (1+\omega)(1+\lambda_2\omega) \\ P(\omega,\delta) &:= \lambda_1 D_2(\omega)\delta^2 + (D_2(\omega)(1+\lambda_1) - C\omega)\delta + D_2(\omega) \\ &= \lambda_2 D_1(\delta)\omega^2 + (D_1(\delta)(1+\lambda_2) - C\delta)\omega + D_1(\delta). \end{aligned}$$

Then:

$$\begin{aligned} f'(\bar{y})g'(\bar{x}) > 1 &\iff P(\omega,\delta) < 0 \\ f'(\bar{y})g'(\bar{x}) < 1 &\iff P(\omega,\delta) > 0. \end{aligned}$$

We now assume that $\lambda_1 > 0$ and $\lambda_2 > 0$: the proof for $\lambda_1 = 0$ or $\lambda_2 = 0$ is similar and simpler, since P becomes a polynomial of degree 1 in function of ω or γ.

According to lemma 4, there exists $\omega, \delta > 0$ such that $P(\omega, \delta) < 0$ if and only if:

$$\begin{aligned} Q_2(\omega) &:= D_2(\omega)(1+\lambda_1) - C\omega + 2\sqrt{\lambda_1}D_2(\omega) < 0 \\ &\iff D_2(\omega)(1+\sqrt{\lambda_1})^2 - C\omega < 0 \\ &\iff \lambda_2\omega^2 + (1+\lambda_2 - \frac{C}{(1+\sqrt{\lambda_1})^2})\omega + 1 < 0 \\ &\iff \lambda_2\omega^2 + (1+\lambda_2 - (1+\sqrt{\lambda_2})^2\rho)\omega + 1 < 0. \end{aligned}$$

According to lemma 4, there exists $\omega > 0$ such that $Q_2(\omega) < 0$ if and only if:

$$(1+\sqrt{\lambda_2})^2 - (1+\sqrt{\lambda_2})^2\rho < 0 \iff \rho > 1.$$

Therefore, $Q_2(\omega) < 0$ if and only if $\rho > 1$ and $\omega \in (\omega^-, \omega^+)$, where ω^-, ω^+ (which depend on n_1, n_2, λ_1 and λ_2), denote the two positive roots of Q_2.

Lastly, $P(\omega, \delta) < 0$ if and only if $\rho > 1$, $\omega \in (\omega^-, \omega^+)$, and $\delta \in (\tilde{r}^-(\omega), \tilde{r}^+(\omega))$, where, for all $\omega \in (\omega^-, \omega^+)$, $\tilde{r}^-(\omega), \tilde{r}^+(\omega)$ denote the two positive roots of $\delta \mapsto P(\omega, \delta)$.

With the same reasoning, by denoting δ_-, δ_+ the two positives roots of $Q_1(\delta) := D_1(\delta)(1+\lambda_2) - C\delta + \sqrt{\lambda_2}D_1(\delta) < 0$, and, for all $\delta \in (\delta^-, \delta^+)$, $\tilde{s}^-(\delta)$ and $\tilde{s}^+(\delta)$, the two positive roots of $\omega \mapsto P(\omega, \delta)$, $P(\omega, \delta) < 0$ if and only if $\rho > 1$, $\delta \in (\delta^-, \delta^+)$, and $\omega \in (\tilde{s}^-(\delta), \tilde{s}^+(\delta))$.

We denote:

$$\begin{aligned} x^- &:= (\omega^-)^{1/n_2}, x^+ := (\omega^+)^{1/n_2} \\ y^- &:= (\delta^-)^{1/n_1}, y^+ := (\delta^+)^{1/n_1} \\ r^-(x) &:= (\tilde{r}^-(\omega))^{1/n_1}, r^+(\omega) := (\tilde{r}^+(\omega))^{1/n_1} \\ s^-(y) &:= (\tilde{s}^-(\delta))^{1/n_2}, r^+(\delta) := (\tilde{r}^+(\delta))^{1/n_2}. \end{aligned}$$

Hence, under the assumption $\rho \geq 1$, $f'(\bar{y})g'(\bar{x}) > 1$ if and only if $(\bar{x}, \bar{y}) \in E$.

Using $(\star)$, this proves that, if $(g_1, g_2) \in \Gamma(E)$, then there exists $(\bar{x}, \bar{y})$ an equilibrium point of (17) such that: $f'(\bar{y})g'(\bar{x}) > 1$, which proves that the system has three equilibrium points. According to Propositions 2.3.2 and 2.3.1, the other two points are stable.

We clearly have: $f'(\bar{y})g'(\bar{x}) \geq 1 \iff (\bar{x}, \bar{y}) \in \overline{E}$. Hence, if $(g_1, g_2) \in \overline{\Gamma(E)}^C \subseteq \Gamma(\overline{E})^C$, then all the fixed points of (17) satisfy: $f'(\bar{y})g'(\bar{x}) < 1$, which proves that the system has a unique equilibrium point. According to Proposition 2.3.1, this equilibrium point is stable. The values of $x^-, x^+, y^-, y^+, r^+, r^-, s^-$ and s^+ are directly computed from the formula of Q_1, Q_2 and P. □

Discussion

The first part of this internship, dedicated to the numerical study of an ODE model for the reversible Epithelial-Mesenchymal Transition (EMT) has allowed us to familiarise with multistable ODE systems, and with the notion of hysteresis. Numerical simulations show that the studied model is consistent with the biological characteristics of the EMT, and that this initial model, an ODE with six variables, can be simplified to two ODE with two variables. The study of these final two ODE drives us to think that the auto-activation of at least one of the variables is necessary in order to have a tristable system with two variables. The next step for the modelling of the EMT would be to study a population of cells rather than a single cell, with a phenotype-structured PDE model.

This numerical study has led us to study simplified competitive systems in order to model toggle switches. Starting with a canonical simplified system, we finally found a more general result, summarised in Theorem 2.3.3.

We managed to prove that systems of the form

$$\begin{cases} \dot{x} = g_x H_1(y) - k_x x \\ \dot{y} = g_y H_2(x) - k_y y \end{cases},$$

where H_1 and H_2 are two decreasing Hill functions, cannot be tristable. This property had been observed numerically [16, 18], but to the best of our knowledge, it had never been proved. We also provide precise sets of parameters which ensure that the system is monostable or bistable. This allows us to determine if a system of this shape is monostable or bistable, without computing the nullclines numerically.

Nevertheless, in the general case, the set of parameters which induces bistability ($\Gamma(E)$ in Theorem 2.3.3) is only defined as the image of a set by a function. The numerical simulations of the spaces of parameters (Figure 14) and the result found in the symmetric case (theorem 2.2.6) hint that $\Gamma(E)$ might be rewritten under the form:

$$\left\{(g_1, g_2) \in I_1 \times I_2 : f^-(g_1) < g_2 < f^+(g_1)\right\}$$

where I_1 and I_2 are two open intervals of $\mathbb{R}^+$, and $f^-, f^+ \in C(I_1, I_2)$. Although we have not managed to rewrite $\Gamma(E)$ under a more explicit form so far, the numerical representations of $\Gamma(E)$ provides precise sets of parameters which ensures the bistability of the system.

We may naturally think that, more generally, we cannot expect to have a tristable circuit under the form:

$$\begin{cases} \dot{x} = g_x F(y) - k_x x \\ \dot{y} = g_y G(x) - k_y y \end{cases},$$

where F and G are two "reasonable" positive and decreasing functions. It would be interesting to find conditions on F and G which ensure that this system cannot be more than tristable.

However, numerical studies [18] have shown that adding auto-activation can induce tristability. This is consistent with the results found in our first part, in which the miR-200/ZEB circuit, where ZEB is auto-

activated, is tristable, while the miR-34/SNAIL circuit, where SNAIL is auto inhibited, is monostable. Hence, it could be relevant to continue our study by considering systems of the form:

$$\begin{cases} \dot{x} = g_x H^+(x) H_1(y) - k_x x \\ \dot{y} = g_y H_2(x) - k_y y \end{cases},$$

where H^+ is an increasing Hill function, which models auto-activation.

Apppendix

i	0	1	2	3	4	5	6
l_i	1.0	0.6	0.3	0.1	0.05	0.05	0.05
γ_{mi}		0.04	0.2	1.0	1.0	1.0	1.0
$\gamma_{\mu i}$		0.005	0.05	0.5	0.5	0.5	0.5

Parameters for the functions L, Y_μ and Y_m.
source: [11]

				Hour^{-1}		Molecules		Molecules/Hour	
$n_{S,\mu_{34}}$	1	$\lambda_{S,\mu_{34}}$	0.1	$k_{\mu_{34}}$	0.05	$S^0_{\mu_{34}}$	300K	g_{m_S}	90.0
n_{S,m_S}	1	λ_{S,m_S}	0.1	k_{m_S}	0.5	$S^0_{m_S}$	200K	$g_{\mu_{34}}$	1.35K
$n_{\mu_{34}}$	2	$\lambda_{Z,\mu_{34}}$	0.2	k_S	0.125	$Z^0_{\mu_{34}}$	600K	$g_{\mu_{200}}$	2.1K
n_I	2	λ_{I,m_S}	10.0	g_S	0.1K	μ^0_{34}	10K	g_{m_Z}	11
$n_{Z,\mu_{200}}$	3	$\lambda_{2,\mu_{200}}$	0.1	$k_{\mu_{200}}$	0.05	$S^0_{\mu_{200}}$	180K		
n_{Z,m_Z}	2	λ_{Z,m_Z}	7.5	k_{m_Z}	0.5	$S^0_{m_Z}$	180K		
$n_{\mu_{200}}$	6	$\lambda_{S,\mu_{200}}$	0.1	k_Z	0.1	μ^0_{200}	10K		
n_{S,m_Z}	2					$I^0_{m_S}$	50K		
$n_{Z,\mu_{34}}$	2					$Z^0_{m_Z}$	25K		
						$Z^0_{\mu_{200}}$	220K		

Parameters for the miR-34/SNAIL circuit, the miR-200/ZEB circuit and the coupled circuit.
source: [11]

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