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Existence and stability of periodic solutions of an impulsive differential equation and application to CD8 T-cell differentiation

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Abstract

Unequal partitioning of the molecular content at cell division has been shown to be a source of heterogeneity in a cell population. We propose to model this phenomenon with the help of a scalar, nonlinear impulsive differential equation (IDE). In a first part, we consider a general autonomous IDE with fixed times of impulse and a specific form of impulse function. We establish properties of the solutions of that equation, most of them obtained under the hypothesis that impulses occur periodically. In particular, we show how to investigate the existence of periodic solutions and their stability by studying the flow of an autonomous differential equation. A second part is dedicated to the analysis of the convexity of this flow. Finally, we apply those results to an IDE describing the concentration of the protein Tbet in a CD8 T-cell, where impulses are associated to cell division, to study the effect of molecular partitioning at cell division on the effector/memory cell-fate decision in a CD8 T-cell lineage. We show that the degree of asymmetry in the molecular partitioning can affect the process of cell differentiation and the phenotypical heterogeneity of a cell population.

Keywords: Impulsive differential equation - Flow convexity - Cellular differentiation - Unequal partitioning - Immune response

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1 Introduction

Time-dependent processes are often modelled with continuous differential equations. Actually, a lot of biological processes are impacted by brief events occurring on a lower time scale (Kuehn, 2015), for example the evolution of cell phenotype subject to gene expression fluctuation, or population dynamics affected by natural disasters. When such events are brief enough comparing to the process of interest, it can be simpler to consider them as instantaneous. The theory of impulsive differential equations (IDE), initiated by Mil'man and Myshkis (1960), provides suitable mathematical tools for modelling such processes subject to perturbations. IDE have been used to study many different phenomena such as the effects of vaccination (Wang et al., 2015) or of any stress factor on a cell population (Kou et al., 2009), the effects of human activities (hunting, feeding, etc.) on prey-predator dynamics (Liu and Chen, 2003; Liu and Zhong, 2012) or single-species systems (Yan et al., 2004), the consequences of seasonal birth pulses on population dynamics (Tang and Chen, 2002) or dengue fever control via introduction of *Wolbachia*-infected mosquitoes in a non-infected mosquitoes population (Zhang et al., 2016). An impulsive equation is defined by a differential equation, which characterises the evolution of a system between two impulses, an impulse criterion, which decides when impulses occur, and a set of impulses functions which define the effect of impulses on the system. For a general overview of IDE theory, we refer the reader to Bainov et al. (1989); Bainov and Simeonov (1993).

In this paper we focus on the particular case of an autonomous differential equation subject to impulses at fixed times, governed by linear impulse functions. That is, a system in the form of

$$\begin{cases} \frac{dX(t)}{dt} = g(X(t)), & t \in \mathbb{R}^+ \setminus \{\tau_k, k \in \mathbb{N}^*\}, \\ X(\tau_k^+) = (1 + \alpha_k)X(\tau_k^-), & k \in \mathbb{N}^*, \\ X(0) = X_0, \end{cases} \quad (1)$$

We aim at describing the phenomenon of unequal repartition of proteins between daughter cells at cell division (Bocharov et al., 2013), and its consequences on the emergence of different possible fates for a cytotoxic T lymphocyte, known as CD8 T-cell.

Following infection of the organism by a pathogen, CD8 T-cells are activated by antigen presenting cells (APC), proliferate and develop cytotoxic functions, known as effector functions, to fight the infection. In the meantime, 5 to 10% of those lymphocytes develop a memory profile characterised by higher survival properties and abilities to react faster to a subsequent infection (Wherry and Ahmed, 2004). Once the infection is cleared, effector cells die progressively during the so-called contraction phase while memory cells survive in the organism on a long time scale. Even though the mechanisms controlling the fate of each cell are still not well known, it has been shown that high and increasing levels of protein Tbet – a transcription factor expressed by CD8 T-cells and involved in developmental processes – in a CD8 T-cell promote the development of effector profile and repress differentiation toward memory phenotype (Joshi et al., 2007; Kaech and Cui, 2012; Lazarevic et al., 2013).

Moreover, [Chang et al. \(2011, 2007\)](#) have shown that the activation of a cytotoxic T lymphocyte by an APC induces the polarisation of the lymphocyte so that Tbet, and other key factors involved in cell fate, mainly gather to one side of the plane of division of the cell, giving birth to two daughter cells that inherit different amounts of those determinants. They suggested that the asymmetric first division following activation of the cell results in differently fated daughter cells toward effector or memory lineages. Since the polarization of the dividing cell requires APC binding, only the first division after activation can be asymmetric, in the sense that the two daughter cells exhibit clearly distinct fates. However, it has been shown ([Block et al., 1990](#); [Sennerstam, 1988](#)) that, for subsequent divisions, uneven stochastic partitioning of the cellular content at division is still observed. Once repeated over several divisions, this phenomenon of unequal repartition of the proteins can lead to a strong heterogeneity in a cell population coming from the same initial mother cell, resulting in different phenotypes at the end of the differentiation process.

In 2013, [Bocharov et al. \(2013\)](#) highlighted that mathematical tools should be developed in order to take into account the unequal repartition of proteins at cytotoxic T lymphocyte division, in particular for the carboxyfluorescein succinimidyl ester (CFSE) dye, which is used to analyse cell proliferation under the hypothesis that it is symmetrically halved between the daughter cells upon cell division.

Considering a CFSE-labelled lymphocyte population, [Luzyanina et al. \(2013\)](#) built a system of delay hyperbolic partial differential equations structured by a continuous variable representing intracellular CFSE amount and allowing uneven distribution of CFSE between daughter cells. Each equation models the size of the population of lymphocytes that have undergone a given number of divisions. They showed that data are better explained by their model when unequal repartition of CFSE between daughter cells at division is taken into account.

[Mantzaris \(2006, 2007\)](#) introduced a variable number Monte Carlo algorithm in which stochastic division effects such as cell cycle time and repartition of proteins to daughter cells are considered. This algorithm can simulate discrete cell population dynamics, starting from a single cell, along with a deterministic description of the evolution of the quantity of an arbitrary protein in each cell, through an ordinary differential equation. Mantzaris also presented a deterministic partial differential equation of the population density, structured by the quantity of intracellular proteins. He compared the results from both the stochastic and deterministic models and showed that they are very close for big enough population sizes, while stochastic effects are more significant in small cell populations.

[Prokopiou et al. \(2014\)](#) and [Gao et al. \(2016\)](#) developed a multiscale agent-based model describing a discrete population of CD8 T-cells in a lymph node, in the context of the immune response. A system of differential equations, embedded in each cell, describes the concentrations of six intracellular proteins, including Tbet, which control cell differentiation, death and cytotoxicity. When a cell divides, its molecular content is stochastically partitioned between the two daughter cells, resulting in a heterogeneous population. This model is able to qualitatively and quantitatively reproduce the immune response in a lymph node from the activation of an initial population of CD8 T-cells to the development of their effector functions and the beginning of the clonal expansion phase.

It must be noted that the continuous structured population density approach used

by [Luzyanina et al. \(2013\)](#) and [Mantzaris \(2006, 2007\)](#) cannot be used to study single cell fate decision, while no formal analysis can be performed on the stochastic computational algorithms presented by [Mantzaris \(2006, 2007\)](#), [Prokopiou et al. \(2014\)](#) and [Gao et al. \(2016\)](#).

We propose a different approach, that does not focus on a population of cells but rather on the concentration of protein Tbet in a single CD8 T-cell subject to multiple divisions. From the equation on protein Tbet used in [Prokopiou et al. \(2014\)](#) and [Gao et al. \(2016\)](#), we propose an impulsive equation where the differential equation dynamics describes the regulation of Tbet concentration in the cell while the impulses account for the effect of protein partitioning at division in that cell. To our knowledge, this is the first work dealing with IDE from this point of view, and the first one applied to fate decision making in CD8 T-cells. This approach allows us to use theoretical results about IDEs to investigate effects of protein partitioning on cell fate decision making.

The paper is organized as follows. In Section 2 we introduce some properties on the existence, monotonicity and asymptotic behaviour of the solutions of the autonomous IDE with fixed impulse times (1), with the main results shown for the particular case where impulses occur periodically and $\alpha_k \equiv \alpha$ for all $k \in \mathbb{N}^*$. In Section 3, we discuss the existence and stability of periodic solutions of (1). The results mostly rely on the properties of the flow associated to (1). In Section 4 we study the convexity of that flow. In a first part, we show some preliminary results under the assumption that g is a piecewise linear function and then extend our study to any continuously differentiable function. In Section 5, the dynamics of the concentration of protein Tbet in a single CD8 T-cell undergoing several divisions is modelled with an IDE, where an impulse occurs at each division. We use the results from previous sections to study the number of periodic solutions and their stability. In Section 6 we propose an explanation to how a single mother cell can, through multiple divisions, give birth to an heterogeneous cell population, composed of two pools of lymphocytes with opposite phenotypes.

2 Impulsive differential equations: definitions and basic properties

We consider the impulsive system (1), where impulses occur at fixed times τ_k , $k \in \mathbb{N}^*$. Parameters $(\alpha_k)_{k \geq 1}$ and $(\tau_k)_{k \geq 1}$ are two sequences of real numbers, $g : U \rightarrow \mathbb{R}$ is a lipschitz-continuous function, $X_0 \in U$ and U is such that either $U = \mathbb{R}$, or $g(0) = 0$ and $U \in \{\mathbb{R}^-, \mathbb{R}^+\}$.

The definition of U is such that for all $x \in U$ and for all $\alpha > -1$, we have $(1+\alpha)x \in U$ and, in the case where $U \in \{\mathbb{R}^-, \mathbb{R}^+\}$, the condition $g(0) = 0$ ensures that, for any initial condition $X_0 \in U$, the solution of the autonomous equation $\mathbf{d}X/\mathbf{d}t = g(X)$ remains in U .

Definition 1. [Yan and Zhao \(1998\)](#) For any $X_0 \in U$, a real function X defined on \mathbb{R}^+ is said to be a solution of (1) if the following conditions are satisfied:

- (i) X is absolutely continuous on $[0, \tau_1)$ and on each interval (τ_k, τ_{k+1}) , $k \in \mathbb{N}^*$,
- (ii) for any τ_k , $k \in \mathbb{N}^*$, $X(\tau_k^+)$ and $X(\tau_k^-)$ exist in \mathbb{R} (i.e. X may have discontinuity of the first kind only) and $X(\tau_k^+) = X(\tau_k^-)$ (i.e. X is right continuous),

(iii) X satisfies (1).

In the following, we denote the solution of (1) at time t by $X(t; X_0, \alpha_k)$. If for a given $\alpha \in \mathbb{R}$ and for all $k \in \mathbb{N}^*$, $\alpha_k = \alpha$, we simply write $X(t; X_0, \alpha)$. In particular the solution of System (1) without impulse is $X(t; X_0, 0)$.

We introduce the following hypotheses:

(H₁) $0 < \tau_1 < \tau_2 < \tau_3 < \dots$ are fixed values and $\lim_{k \rightarrow \infty} \tau_k = +\infty$.

(H₂) $(\alpha_k)_{k \geq 1}$ is a sequence of real numbers such that, for all $k \geq 1$, $\alpha_k > -1$.

The next proposition states the existence of a solution of (1) and its uniqueness.

Proposition 1. *Let hypotheses (H₁) and (H₂) hold true. Then there exists a unique global solution $X(\cdot; X_0, \alpha_k)$ of (1) and, for all $t \geq 0$, the application $(X_0, (\alpha_k)_{\{k \in \mathbb{N}^*, \tau_k \leq t\}}) \mapsto X(t; X_0, \alpha_k)$ is continuous with respect to both variables. Moreover, for $t \geq 0$,*

$$X(t; X_0, \alpha_k) = X(t) = X_0 + \int_0^t g(X(s))ds + \sum_{\{k \in \mathbb{N}^*, 0 < \tau_k \leq t\}} \alpha_k X(\tau_k^-). \quad (2)$$

Proof. Existence and uniqueness of the solution, as well as formula (2), are given by Theorems 2.3 and 2.6 from Bainov and Simeonov (1993).

The continuity of $X_0 \mapsto X(t; \alpha_k, X_0)$ is proved in Theorem 1.2. from Dishliev et al. (2012) for a more general class of equations but under the hypothesis that there exists $C_g > 0$ such that for all $x \in U$, $|g(x)| < C_g$. In the case of (1), this hypothesis is not necessary.

Indeed, since the functions $X_0 \mapsto X(\cdot; X_0, 0)$ and $x \mapsto (1 + \alpha)x$, $\alpha > -1$, are continuous, it is easy to see that the solutions of (1) are continuous with respect to the initial condition X_0 and with respect to $(\alpha_k)_{\{k \in \mathbb{N}^*, \tau_k \leq t\}}$. Note that $\text{Card}(\{k \in \mathbb{N}^*, \tau_k \leq t\}) =: N < +\infty$ then any topology can be chosen for the continuity regarding parameter $(\alpha_k)_{\{k \in \mathbb{N}^*, \tau_k \leq t\}} \in (-1, +\infty)^N$. □ □

Lemma 1. *Let hypothesis (H₁) hold true, $g(0) = 0$, $U = \mathbb{R}^+$ and $X_0 \in U$. If $(m_k)_{k \geq 1}$, $(\alpha_k)_{k \geq 1}$ and $(M_k)_{k \geq 1}$ are three sequences such that, for all $k \in \mathbb{N}^*$, $-1 < m_k \leq \alpha_k \leq M_k$, then for all $t \in \mathbb{R}^+$,*

$$X(t; X_0, m_k) \leq X(t; X_0, \alpha_k) \leq X(t; X_0, M_k).$$

If $U = \mathbb{R}^-$ Lemma 1 remains true if we reverse the inequalities. If $U = \mathbb{R}$ it is necessary to discuss the sign of the solutions.

Remark 1. *One specific feature of IDE is that two distinct solutions might merge after an impulse depending on the impulse function. In the case of (1), impulses are of the particular form $X(\tau_k^+) = (1 + \alpha_k)X(\tau_k^-)$ and, since for all $\alpha > -1$ the function $X \mapsto (1 + \alpha)X$ is injective and increasing, two distinct solutions cannot cross or merge after an impulse. Indeed, on intervals $[0, \tau_1)$ and (τ_k, τ_{k+1}) , the Cauchy-Lipschitz theorem ensures that distinct solutions cannot overlap. Consequently, under hypotheses (H₁)-(H₂), for a given sequence $(\alpha_k)_{k \geq 1}$ and two initial conditions $X_m < X_M$, for all $t \geq 0$, $X(t; X_m, \alpha_k) < X(t; X_M, \alpha_k)$.*

Hereafter, we study the behaviour of the solutions of (1) when we consider that impulses occur with fixed period $\omega \in \mathbb{R}_*^+$ and that the sequence $(\alpha_k)_{k \in \mathbb{N}^*}$ is constant. To this end, we introduce hypotheses **(H₃)** and **(H₄)**, as follows:

(H₃) $\forall k \in \mathbb{N}^*, \tau_k = k\omega$ with $\omega > 0$ fixed.

(H₄) $\forall k \in \mathbb{N}^*, \alpha_k = \alpha > -1$ with α fixed.

Note that **(H₃)** implies **(H₁)** and **(H₄)** implies **(H₂)**.

We introduce the next definition.

Definition 2. *A solution X of (1) converges to a solution Y if for all $\epsilon > 0$, there exists $t_\epsilon > 0$ such that $|X(t) - Y(t)| < \epsilon$ for $t > t_\epsilon$.*

The following proposition is a particular case of Theorem 12.5 from [Bainov and Simeonov \(1993\)](#).

Proposition 2. *Let hypotheses **(H₃)** and **(H₄)** hold true. Then every bounded solution of (1) converges to a periodic solution.*

Under hypotheses **(H₃)** and **(H₄)**, one can show Lemma 2 and Proposition 3.

Lemma 2. *Let hypotheses **(H₃)** and **(H₄)** hold true and consider the solution $X = X(\cdot; X_0, \alpha)$ of (1). Then,*

- i) *if $X(\omega) > X(0)$ (resp. $< X(0)$) the sequence $(X(k\omega))_{k \in \mathbb{N}^*}$ is strictly increasing (resp. decreasing).*
- ii) *X is ω -periodic if and only if $X(\omega) = X(0)$ (Lemma 12.1 from [Bainov and Simeonov \(1993\)](#)).*

Proof. We first show **i)**. Let us assume that $X(\omega) := (1 + \alpha)X(\omega^-) > X(0)$. We then assume that there exists $k \in \mathbb{N}^*$ such that $X_{k+1} := X((k+1)\omega) > X_k := X(k\omega)$ and we show that $X((k+2)\omega) > X((k+1)\omega)$.

Thanks to the uniqueness of the solution of (1), and by integrating the solutions $Y_1 = Y_1(\cdot; X_{k+1}, \alpha)$ and $Y_2 = Y_2(\cdot; X_k, \alpha)$ of (1) on $[0, \omega)$, the inequality $X((k+1)\omega) > X(k\omega)$ implies that $X((k+2)\omega^-) > X((k+1)\omega^-)$ and then

$$X((k+2)\omega) := (1 + \alpha)X((k+2)\omega^-) > (1 + \alpha)X((k+1)\omega^-) =: X((k+1)\omega).$$

We showed that for all $k \in \mathbb{N}^*$, $X((k+1)\omega) > X(k\omega)$. Similarly, we can show that if $X(\omega) < X(0)$, then for all $k \in \mathbb{N}^*$, $X((k+1)\omega) < X(k\omega)$.

It remains to prove **ii)**. As done in the proof of **i)** we show that if $X(\omega) = X(0)$, then for all $k \in \mathbb{N}^*$, $X(k\omega) = X(0)$. Because X is the solution of an autonomous equation on each interval $[k\omega, (k+1)\omega)$, it follows that for all $k \in \mathbb{N}^*$, for all $t \in [0, \omega)$, $X(t) = X(k\omega + t)$ and then X is ω -periodic. \square

Proposition 3. *Let hypotheses **(H₃)** and **(H₄)** hold true and X be a non-constant periodic solution of (1). Then X is ω -periodic and ω is the smallest period of X .*

Proof. If $\alpha = 0$ (i.e. no impulse) there is no non-constant periodic solution of (1) because the solution of an autonomous scalar differential equation is monotonous. Then, in the rest of the proof, we suppose $\alpha \neq 0$.

We set $\alpha \in (-1, +\infty) \setminus \{0\}$ and let X be a periodic solution of (1) with period $P > 0$. It is easy to show that either $X \equiv 0$, or for all $t \geq 0$, $X(t) > 0$, or for all $t \geq 0$, $X(t) < 0$. Indeed, the sign of X changes at most one time between two impulses and since $\alpha > -1$, the solution X cannot vanish or change its sign due to an impulse. Because X is supposed to be non-constant, for all $t \in \mathbb{R}^+$, $X(t) \neq 0$. Moreover, $(1 + \alpha)X(\omega^-) = X(\omega) \neq 0$ then $X(\omega^-) \neq 0$.

We first show that the smallest period of X is a multiple of ω . Let us assume that X is P -periodic with $\omega \nmid P$. Because of the P -periodicity of X ,

$$X(\omega) = X(P + \omega) \text{ and } X(\omega^-) = X((P + \omega)^-). \quad (3)$$

Moreover, since $X(\omega^-) \neq 0$ and $\alpha > -1$, then

$$X(\omega) = (1 + \alpha)X(\omega^-) \neq X(\omega^-). \quad (4)$$

Consequently, using (3) and (4), we have

$$X(P + \omega) \neq X((P + \omega)^-). \quad (5)$$

On the other hand, since $\omega \nmid (P + \omega)$, $t = P + \omega$ is not an impulsion time, so we have

$$X(P + \omega) = X((P + \omega)^-).$$

This contradicts (5). Therefore, the smallest period of X is a multiple of ω .

Now, it suffices to show that $X(\omega) := (1 + \alpha)X(\omega^-) = X(0)$. Let us assume that $X(\omega) \neq X(0)$. According to Lemma 2, $(X(k\omega))_{k \in \mathbb{N}^*}$ is a strictly monotonous sequence, so for all $k \in \mathbb{N}^*$, $X(k\omega) \neq X(0)$, which is absurd because X is P -periodic with $\omega \mid P$. Finally, the solution X is ω -periodic. \square

In this section, we proved the existence of a solution for System (1) and established some properties for its behaviour. According to Propositions 2 and 3, either a solution of (1) converges to a ω -periodic solution, or it diverges to $\pm\infty$. Consequently, it suffices to study the periodic solutions of (1) to conclude on the asymptotic behaviour of any bounded solution. That is the focus of the next section.

3 Existence and stability of periodic solutions

Throughout this section, hypotheses (\mathbf{H}_3) and (\mathbf{H}_4) hold true. We present results on the existence and stability of periodic solutions of (1).

We introduce φ_ω , the flow of System (1) at time ω and without impulsion, defined by

$$\varphi_\omega : X_0 \in U \mapsto X(\omega; X_0, 0) \in U. \quad (6)$$

Note that, since the first impulse in System (1) occurs at time ω , for any value of α we have

$$\varphi_\omega(X_0) = X(\omega; X_0, 0) = X(\omega^-; X_0, \alpha) = X(\omega; X_0, \alpha)/(1 + \alpha). \quad (7)$$

We can also notice that, thanks to the existence and uniqueness of the solution of (1), φ_ω is strictly increasing on U and it can easily be shown that φ_ω is a bijection from U to U . In addition, if $g \in \mathcal{C}^p(U)$ with $p \in \mathbb{N}^*$, then $\varphi_\omega \in \mathcal{C}^p(U)$ (Cauchy-Lipschitz theorem) where $\mathcal{C}^p(U) = \{h : U \rightarrow \mathbb{R}, h^{(p)} \text{ exists and is continuous}\}$.

For the sake of simplicity, we also introduce the function Γ_ω defined by

$$\Gamma_\omega : X_0 \in U \setminus \{\varphi_\omega^{-1}(0)\} \mapsto \frac{X_0}{\varphi_\omega(X_0)} - 1 \in \mathbb{R}. \quad (8)$$

Remark 2. *As a ratio of two continuous functions, the function Γ_ω is continuous everywhere φ_ω is non-zero, that is on $U \setminus \{\varphi_\omega^{-1}(0)\}$. In particular, if $g(0) = 0$ and $U = \mathbb{R}^+$ (resp. $U = \mathbb{R}^-$), then $\varphi_\omega^{-1}(0) = 0$ and $\Gamma_\omega : \mathbb{R}_*^+ \rightarrow (-1, +\infty)$ (resp. $\Gamma_\omega : \mathbb{R}_*^- \rightarrow (-1, +\infty)$).*

For a given initial condition X_0 and under hypotheses (\mathbf{H}_3) and (\mathbf{H}_4) , if one knows the value of $\varphi_\omega(X_0)$ then one can conclude on the existence and the value of α for which the solution $X(\cdot; X_0, \alpha)$ of (1) is periodic. This is stated in the next proposition.

Proposition 4. *Let hypotheses (\mathbf{H}_3) and (\mathbf{H}_4) hold true. For any initial condition $X_0 \in U$ such that $\varphi_\omega(X_0) \neq 0$, the solution $X = X(\cdot; X_0, \alpha)$ of (1) is periodic if and only if*

$$\alpha = \Gamma_\omega(X_0).$$

Moreover, if $\varphi_\omega(X_0) = 0$, either $g(0) = 0$, and then for any $\alpha \in \mathbb{R}$ the solution $X(\cdot; X_0, \alpha)$ of (1) satisfies $X \equiv 0$, in particular X is periodic; or $g(0) \neq 0$, and then for any choice of $\alpha > -1$, the solution $X(\cdot; X_0, \alpha)$ of (1) is not periodic.

Proof. From Lemma 2 and Proposition 3, $X = X(\cdot; X_0, \alpha)$ is periodic if and only if $X(\omega) = X_0$, that is, from (7), $(1 + \alpha)\varphi_\omega(X_0) = X_0$. If $\varphi_\omega(X_0) \neq 0$, X is periodic if and only if $(1 + \alpha)\varphi_\omega(X_0) = X_0$, that is, from (8), $\alpha = \Gamma_\omega(X_0)$. In the remainder of the proof we assume $\varphi_\omega(X_0) = 0$.

If $g(0) = 0$, then $X \equiv 0$. Indeed $Y \equiv 0$ is a solution of (1) and, for any $\alpha \in \mathbb{R}$, from (7), $X(\omega; X_0, \alpha) = (1 + \alpha)\varphi_\omega(X_0) = 0 = Y(\omega)$. By the uniqueness of the solution, for any $\alpha \in \mathbb{R}$, $X = Y \equiv 0$, in particular X is periodic.

On the other hand, if $g(0) \neq 0$, then $X(0) \neq 0$. Indeed, if $X(0) = 0$, then X is strictly monotonous on $[0, \omega)$ and $\varphi_\omega(X_0) \neq 0$, hence a contradiction. Moreover, for any $\alpha \in \mathbb{R}$, $X(\omega) = (1 + \alpha)\varphi_\omega(X_0) = 0$. Consequently $X(\omega) \neq X(0)$ and thanks to Proposition 3 X is not periodic. \square

In the remainder of this section, we study the stability of the periodic solutions of (1). For the sake of simplicity, for any $\alpha > -1$ and $\omega > 0$ we introduce the function $\gamma_{\omega, \alpha}$ defined by

$$\gamma_{\omega, \alpha} : X_0 \in U \mapsto \varphi_\omega(X_0) - \frac{X_0}{1 + \alpha} \in U. \quad (9)$$

Lemma 3. *Let hypotheses (\mathbf{H}_3) and (\mathbf{H}_4) hold true and let $X_0 \in U$. Let $K^+ := \gamma_{\omega, \alpha}^{-1}(\{0\}) \cap (X_0, +\infty)$ and $K^- := \gamma_{\omega, \alpha}^{-1}(\{0\}) \cap (-\infty, X_0)$. Then*

- i) if $\gamma_{\omega, \alpha}(X_0) > 0$, either $K^+ \neq \emptyset$ and then $X(\cdot; X_0, \alpha)$ converges to the periodic solution $Y(\cdot; Y^+, \alpha)$ where $Y^+ = \min(K^+)$, or $K^+ = \emptyset$ and $X(\cdot; X_0, \alpha)$ diverges to $+\infty$;*

ii) if $\gamma_{\omega,\alpha}(X_0) < 0$, either $K^- \neq \emptyset$ and then $X(\cdot; X_0, \alpha)$ converges to the periodic solution $Y(\cdot; Y^-, \alpha)$ where $Y^- = \max(K^-)$, or $K^- = \emptyset$ and $X(\cdot; X_0, \alpha)$ diverges to $-\infty$;

iii) if $\gamma_{\omega,\alpha}(X_0) = 0$, then $X(\cdot; X_0, \alpha)$ is ω -periodic.

Proof. Proofs of points i) and ii) are similar, hence we only show point i).

We first assume that $\gamma_{\omega,\alpha}(X_0) > 0$ and show that, when $K^+ \neq \emptyset$, Y^+ is well defined. Let us assume that $K^+ \neq \emptyset$. Since $\gamma_{\omega,\alpha}$ is a continuous function and $\{0\}$ is a closed set, then $\gamma_{\omega,\alpha}^{-1}(\{0\})$ is a closed set. Moreover, since $\gamma_{\omega,\alpha}(X_0) > 0$, then $X_0 \notin \gamma_{\omega,\alpha}^{-1}(\{0\})$ and we can write $K^+ = \gamma_{\omega,\alpha}^{-1}(\{0\}) \cap [X_0, +\infty)$. Finally, K^+ is a non-empty closed set which admits a lower bound (X_0), then it admits a minimum $Y^+ > X_0$.

We now prove i). Hereafter, $X = X(\cdot; X_0, \alpha)$. We notice that inequality $\gamma_{\omega,\alpha}(X_0) > 0$ is equivalent to $X(\omega) > X(0)$. Then, according to Lemma 2 and Proposition 2, the sequence $(X(k\omega))_{k \in \mathbb{N}}$ is (strictly) increasing and either X diverges to $+\infty$, or it converges to a periodic solution. Consequently, if there is no periodic solution with initial condition $K_0 > X_0$ (i.e. $K^+ = \emptyset$), then X diverges to $+\infty$. On the other hand, if $K^+ \neq \emptyset$, then we have for all $t \geq 0$, $X(t) \leq Y(t; Y^+, \alpha)$, so X is bounded and converges to a periodic solution of (1). From the definition of Y^+ and since $X(t) \leq Y(t)$ for all $t \geq 0$, we can conclude that X converges to Y .

Point iii) is equivalent to Lemma 2. Indeed, using (7), X is periodic if and only if $X(\omega) = (1 + \alpha)\varphi_\omega(X_0) = X_0$, that is $\gamma_{\omega,\alpha}(X_0) = 0$. \square

Hereafter, we adapt the usual notions of stability used for non-impulsive differential equations to our problem, as follows.

Definition 3. Let hypotheses (\mathbf{H}_3) and (\mathbf{H}_4) be satisfied. Let $X_* \in U$ be such that $\Gamma_\omega(X_*) = \alpha$ and $I \subset U$ be an interval. Then, the periodic solution $X(\cdot; X_*, \alpha)$ is said to be

i) stable if for any neighbourhood V of X_* , there is a neighbourhood W of X_* such that for all $X_0 \in W$ and for all $k \in \mathbb{N}$, $X(k\omega, X_0, \alpha) \in V$;

ii) asymptotically stable if it is stable and there exists a neighbourhood V of X_* such that for any $X_0 \in V$, $\lim_{k \rightarrow +\infty} X(k\omega; X_0, \alpha) = X_*$;

iii) unstable if it is not stable;

iv) attractive on I if for any $X_0 \in I$, the sequence $(|X(k\omega; X_0, \alpha) - X_*|)_{k \in \mathbb{N}}$ decreases and converges to zero;

v) repulsive on I if for any $X_0 \in I$, the sequence $(|X(k\omega; X_0, \alpha) - X_*|)_{k \in \mathbb{N}}$ increases.

Remark 3. As an immediate consequence of Lemma 3, under hypotheses (\mathbf{H}_3) and (\mathbf{H}_4) the stability of a periodic solution $X(\cdot; X_0, \alpha)$ is given by the sign of $\gamma_{\omega,\alpha}$ on both sides of X_0 . The stability of periodic solutions of (1) can however also be sometimes deduced from the graph of Γ_ω . Indeed, let $X_0 \in U$ be such that $\varphi(X_0) > 0$, then, from (8) and (9), $\gamma_{\omega,\alpha}(X_0) > 0$ (resp. $\gamma_{\omega,\alpha}(X_0) < 0$, resp. $\gamma_{\omega,\alpha}(X_0) = 0$) if and only if $\Gamma_\omega(X_0) < \alpha$ (resp. $\Gamma_\omega(X_0) > \alpha$, resp. $\Gamma_\omega(X_0) = \alpha$) and we can conclude on the asymptotic behaviour of $X(\cdot; X_0, \alpha)$ (Lemma 3) based on the graph of Γ_ω . For

example, let $U = \mathbb{R}^+$ and $X_0 > 0$ be such that $\Gamma_\omega(X_0) = \alpha$. If Γ_ω is strictly increasing (resp. decreasing) on a neighbourhood of X_0 , then $X(\cdot; X_0, \alpha)$ is asymptotically stable (resp. unstable).

From Proposition 2, under (\mathbf{H}_3) and (\mathbf{H}_4) , it suffices to determine the initial values of the periodic solutions of System (1) and their stability to conclude on the asymptotic behaviour of all the solutions. In Sections 2 and 3, we have shown that, under hypotheses (\mathbf{H}_3) and (\mathbf{H}_4) , for any $X_0 \in U$ such that $\varphi_\omega(X_0) \neq 0$ there exists a unique $\alpha \in \mathbb{R}$ such that $X(\cdot; X_0, \alpha)$ is a periodic solution of (1) (Proposition 4). Conversely, for any given $\alpha > -1$, we discussed the existence of $X_0 = X_0(\alpha) \in U$ such that the solution $X = X(\cdot; X_0, \alpha)$ of (1) is periodic.

As a consequence of Lemma 3, the initial conditions of the periodic solutions of (1) correspond to the solutions X_0 of equation $\gamma_{\omega, \alpha}(X_0) = 0$, with $\gamma_{\omega, \alpha}$ defined by (9). Necessary and sufficient conditions to determine the stability of the periodic solutions are given in Lemma 3 and Remark 3.

However, in general, the expression of φ_ω , defined by (6), is not known and the exact values of the initial conditions of the periodic solutions of (1) cannot be determined. In that case *ad hoc* studies have to be performed. In Section 4, we give a sufficient condition for φ_ω to be convex. This result will be used in Sections 5 and 6.

4 Convexity of the flow of an autonomous equation

In this section, we aim at investigating the convexity of the flow φ_ω , defined by (6), which can further be useful to determine the number of periodic solutions of (1) when (\mathbf{H}_3) and (\mathbf{H}_4) hold true (Proposition 4). In Section 4.1 we study a Cauchy problem where the right-hand side of the equation is a piecewise linear function and we extrapolate this result to continuously differentiable functions in Section 4.2.

4.1 Preliminary results

We set $a < b \in \mathbb{R}$, $n \in \mathbb{N}^*$ and $(c_i)_{0 \leq i \leq n}$ a subdivision of $[a, b]$ such that $c_0 = a$, $c_n = b$ and, for all $i \in \{0, \dots, n-1\}$, $c_i < c_{i+1}$. Then, we define the piecewise linear function h_n , on $[a, b]$, by

$$h_n(x) = a_{i+1}x + b_{i+1} \quad \text{for } x \in [c_i, c_{i+1}), \quad i \in \{0, \dots, n-1\},$$

where real numbers $(a_i)_{1 \leq i \leq n}$, $(b_i)_{1 \leq i \leq n}$ are such that h_n is positive and continuous, that is

$$h_n(c_0) > 0 \text{ and } \forall 1 \leq i \leq n-1, \quad a_i c_i + b_i = a_{i+1} c_i + b_{i+1} = h_n(c_i) > 0. \quad (10)$$

Then, we introduce the autonomous Cauchy problem

$$\begin{cases} X'(t) &= h_n(X(t)), \quad t \geq 0, \\ X(0) &= X_0. \end{cases} \quad (11)$$

For the sake of simplicity, we set $h_n(c_n) = 0$ such that for any $X_0 \in [a, b)$, the solution of (11) is increasing and remains in $[a, b)$, and $X = c_n = b$ is the unique steady state of (11).

Note that existence and uniqueness for the solution X of System (11) is guaranteed by Cauchy-Lipschitz theorem, most of the time this solution will be denoted by X_n . In particular, the restriction of X_n on each interval $[c_{i-1}, c_i]$, $i \in \{1, \dots, n-1\}$, is solution of a linear equation.

In the following, we first determine the values of t such that $X_n(t) = c_i$ for $i = 0, \dots, n-1$ (Lemma 4) and then we provide an explicit expression for X_n (Proposition 5).

Since $h_n(x) > 0$ for all $x \in [c_0, c_n)$ and $h_n(c_n) = 0$, it is clear that for all $X_0 \in (c_0, c_n)$, X_n is an increasing function and $\lim_{t \rightarrow +\infty} X_n(t) = c_n$. Straightforward calculations show that, if $X_0 \in [c_i, c_{i+1})$ for $i \in \{0, \dots, n-2\}$, then $X_n(t) = c_{i+1}$ if and only if $t = \lambda_{i+1}(X_0)$, where λ_{i+1} is defined for all $x \in [c_i, c_{i+1})$ by

$$\lambda_{i+1}(x) = \begin{cases} \frac{1}{a_{i+1}} \ln \left(\frac{h_n(c_{i+1})}{h_n(x)} \right), & \text{if } a_{i+1} \neq 0, \\ \frac{c_{i+1} - x}{b_{i+1}}, & \text{if } a_{i+1} = 0. \end{cases}$$

By induction, we deduce the expression of $\lambda_k(X_0)$ for any $X_0 < c_k$.

Lemma 4. *Let $i \in \{0, \dots, n-2\}$, $X_0 \in [c_i, c_{i+1})$, and X_n be the solution of (11). Let $k \in \{i+1, \dots, n-1\}$, then $X_n(t) = c_k$ if and only if $t = \lambda_k(X_0)$, defined by*

$$\lambda_k(X_0) = \lambda_{i+1}(X_0) + \sum_{p=i+1}^{k-1} \lambda_{p+1}(c_p). \quad (12)$$

In other words, $\lambda_k(X_0)$ corresponds to the time for the solution X_n of (11) to go from X_0 to c_k . Since $c_n = \lim_{t \rightarrow +\infty} X_n(t)$, we set $\lambda_n(X_0) = +\infty$. If $X_0 \geq c_p$, for $p \in \{0, \dots, n-1\}$, we set $\lambda_p(X_0) = 0$.

Proposition 5. *Let $i \in \{0, \dots, n-1\}$, $X_0 \in [c_i, c_{i+1})$ and X_n be the solution of (11). Then, if $t \in [0, \lambda_{i+1}(X_0))$,*

$$X_n(t) = \begin{cases} \frac{h_n(X_0)}{a_{i+1}} e^{a_{i+1}t} - \frac{b_{i+1}}{a_{i+1}}, & \text{if } a_{i+1} \neq 0, \\ X_0 + b_{i+1}t, & \text{if } a_{i+1} = 0. \end{cases}$$

If $t \in [\lambda_k(X_0), \lambda_{k+1}(X_0))$ for $k \in \{i+1, \dots, n-1\}$, then

$$X_n(t) = \begin{cases} \left(c_k - \frac{b_{k+1}}{a_{k+1}} \right) e^{a_{k+1}(t - \lambda_k(X_0))} - \frac{b_{k+1}}{a_{k+1}}, & \text{if } a_{k+1} \neq 0, \\ c_k + b_{k+1}(t - \lambda_k(X_0)), & \text{if } a_{k+1} = 0. \end{cases} \quad (13)$$

Since, from Lemma 4, $X_n(\lambda_k(X_0)) = c_k$, then to prove Proposition 5 one only has to solve the linear differential equation $X'(t) = a_{k+1}X(t) + b_{k+1}$.

We now consider $\omega > 0$ and φ_ω^n , the flow of (11) at time ω , defined by

$$\varphi_\omega^n : X_0 \in [a, b] \mapsto X_n(\omega) \in [a, b], \quad (14)$$

where X_n is the solution of (11).

Note that, since h_n is continuous, φ_ω^n is also continuous. If we set $t = \omega$, Proposition 5 provides an explicit expression for $\varphi_\omega^n(X_0)$. In the following, we determine the convexity of φ_ω^n by studying its second derivative. However, if $X_0 = c_k$ or $\varphi_\omega^n(X_0) = c_k$, the expression of φ_ω^n is not the same on both sides of X_0 and, in that case, we cannot directly compute the derivative of φ_ω^n . Consequently, we first study the convexity of φ_ω^n everywhere $X_0 \neq c_k$ and $\varphi_\omega^n(X_0) \neq c_k$, and then we conclude on the convexity of φ_ω^n on the whole interval (a, b) . For the sake of simplicity we define

$$J_n = \{c_k, k = 1, \dots, n-1\} \cup \{(\varphi_\omega^n)^{-1}(c_k), k = 1, \dots, n-1\}. \quad (15)$$

Lemma 5. *Let $X_0 \in (a, b) \setminus J_n$ and $i, k \in \{0, \dots, n-1\}$ such that $X_0 \in (c_i, c_{i+1})$ and $\varphi_\omega^n(X_0) \in (c_k, c_{k+1})$. Then, on a neighbourhood of X_0 , φ_ω^n is \mathcal{C}^∞ and strictly convex (resp. strictly concave, resp. affine) if and only if $a_{k+1} > a_{i+1}$ (resp. $a_{k+1} < a_{i+1}$, resp. $a_{k+1} = a_{i+1}$).*

Proof. To prove Lemma 5, it suffices to study the sign of the second derivative of φ_ω^n . Depending on whether a_{i+1} and a_{k+1} are null or not, φ_ω^n can be defined by four different expressions. Here we only provide a proof for the case $a_{i+1} \neq 0$ and $a_{k+1} \neq 0$. Other cases are analogous.

Let $X_0 \in (c_i, c_{i+1})$ such that $\varphi_\omega^n(X_0) \in (c_k, c_{k+1})$, $k > i$. Then, according to equations (12) and (13)

$$\begin{aligned} \varphi_\omega^n(X_0) &= \left(c_k - \frac{b_{k+1}}{a_{k+1}}\right) e^{a_{k+1}(\omega - \lambda_k(X_0))} - \frac{b_{k+1}}{a_{k+1}}, \\ &= \frac{h_n(c_k)}{a_{k+1}} e^{a_{k+1}\omega} \prod_{p=i+1}^{k-1} e^{-a_{k+1}\lambda_{p+1}(c_p)} e^{-a_{k+1}\lambda_{i+1}(X_0)} \\ &= \frac{h_n(c_k)}{a_{k+1}} h_n(X_0)^{\frac{a_{k+1}}{a_{i+1}}} M_{i,k}^{a_{k+1}} - \frac{b_{k+1}}{a_{k+1}}, \end{aligned} \quad (16)$$

where

$$M_{i,k} = e^\omega \left(\frac{1}{h_n(c_{i+1})}\right)^{\frac{1}{a_{i+1}}} \prod_{p=i+1}^{k-1} \left(\frac{h_n(c_p)}{h_n(c_{p+1})}\right)^{\frac{1}{a_{p+1}}} > 0.$$

Consequently

$$\begin{aligned} \frac{d^2 \varphi_\omega^n}{dX_0^2}(X_0) &= \frac{h_n(c_k) M_{i,k}^{a_{k+1}}}{a_{k+1}} \left(\frac{a_{k+1}}{a_{i+1}}\right) \left(\frac{a_{k+1}}{a_{i+1}} - 1\right) a_{i+1}^2 (a_{i+1} X_0 + b_{i+1})^{\left(\frac{a_{k+1}}{a_{i+1}} - 2\right)} \\ &= h_n(c_k) M_{i,k}^{a_{k+1}} (a_{k+1} - a_{i+1}) (a_{i+1} X_0 + b_{i+1})^{\left(\frac{a_{k+1}}{a_{i+1}} - 2\right)}. \end{aligned}$$

Moreover, on any neighbourhood of X_0 included in $(a, b) \setminus J_n$, φ_ω^n is linear and then its second derivative is constant. Finally, the convexity of φ_ω^n is determined by the sign of its second derivative, given by the sign of $(a_{k+1} - a_{i+1})$, as stated in Lemma 5. \square

Thanks to Lemma 5, we can determine the convexity of φ_ω^n on each interval included in $(a, b) \setminus J_n$. To extend this result to the whole interval (a, b) we need to introduce the following lemma.

Lemma 6. *φ_ω^n is continuously differentiable on (a, b) .*

Proof. We know that φ_ω^n is \mathcal{C}^∞ on $(a, b) \setminus J_n$. Let $X_0 \in J_n$ and $i, k \in \{0, \dots, n-1\}$ such that $X_0 = c_i$ or $\varphi_\omega^n(X_0) = c_k$. We want to show that the derivative of φ_ω^n is continuous in X_0 . For the sake of simplicity, we only show the proof for the case where a_i, a_{i+1}, a_k and a_{k+1} are non null, $X_0 = c_i$ and $\varphi_\omega^n(X_0) = c_k$. Other cases are analogous.

Let $X_1 \in (c_{i-1}, c_i)$ such that $\varphi_\omega^n(X_1) \in (c_{k-1}, c_k)$ and $X_2 \in (c_i, c_{i+1})$ such that $\varphi_\omega^n(X_2) \in (c_k, c_{k+1})$

According to (16),

$$\varphi_\omega^n(X_2) = \frac{h_n(c_k)}{a_{k+1}} h_n(X_2)^{\frac{a_{k+1}}{a_{i+1}}} M_{i,k}^{a_{k+1}} - \frac{b_{k+1}}{a_{k+1}}$$

and straightforward calculations lead to

$$\begin{aligned} \varphi_\omega^n(X_1) &= \frac{h_n(c_{k-1})}{a_k} h_n(X_1)^{\frac{a_k}{a_i}} M_{i-1,k-1}^{a_k} - \frac{b_k}{a_k} \\ &= \frac{h_n(c_k)}{a_k} h_n(X_1)^{\frac{a_k}{a_i}} M_{i,k}^{a_k} (h_n(X_0))^{\frac{a_k}{a_{i+1}} - \frac{a_k}{a_i}} - \frac{b_k}{a_k}. \end{aligned}$$

Consequently

$$(\varphi_\omega^n)'(X_2) = h_n(c_k) h_n(X_2)^{\frac{a_{k+1}}{a_{i+1}} - 1} M_{i,k}^{a_{k+1}} \quad (17)$$

and

$$(\varphi_\omega^n)'(X_1) = h_n(c_k) h_n(X_1)^{\frac{a_k}{a_i} - 1} M_{i,k}^{a_k} (h_n(X_0))^{\frac{a_k}{a_{i+1}} - \frac{a_k}{a_i}}. \quad (18)$$

On the other hand, since φ_ω^n is continuous and $\varphi_\omega^n(X_0) = c_k$, it follows that

$$c_k = \varphi_\omega^n(X_0) = \lim_{X_2 \rightarrow X_0} \varphi_\omega^n(X_2) = \frac{h_n(c_k)}{a_{k+1}} h_n(X_0)^{\frac{a_{k+1}}{a_{i+1}}} M_{i,k}^{a_{k+1}} - \frac{b_{k+1}}{a_{k+1}}.$$

That is

$$a_{k+1}c_k + b_{k+1} = h_n(c_k) \left(h_n(X_0)^{\frac{1}{a_{i+1}}} M_{i,k} \right)^{a_{k+1}}. \quad (19)$$

According to (10), $h_n(c_k) = a_{k+1}c_k + b_{k+1} \neq 0$. It follows that

$$h_n(X_0)^{\frac{1}{a_{i+1}}} M_{i,k} = 1.$$

Applying (19) to (17) and (18), we get

$$\begin{aligned} \lim_{X_2 \rightarrow X_0^+} \varphi_\omega^n'(X_2) &= \frac{h_n(c_k)}{h_n(X_0)} \left(h_n(X_0)^{\frac{1}{a_{i+1}}} M_{i,k} \right)^{a_{k+1}} = \frac{h_n(c_k)}{h_n(X_0)}, \\ \lim_{X_1 \rightarrow X_0^-} \varphi_\omega^n'(X_1) &= \frac{h_n(c_k)}{h_n(X_0)} \left(h_n(X_0)^{\frac{1}{a_{i+1}}} M_{i,k} \right)^{a_k} (h_n(X_0))^{\frac{a_k}{a_i} - \frac{a_k}{a_i}} = \frac{h_n(c_k)}{h_n(X_0)}, \end{aligned}$$

and the proof is achieved. \square

Consequently, the convexity of φ_ω^n on (a, b) can be entirely determined, as stated in Proposition 6 hereafter.

Let us note, for all $n \in \mathbb{N}^*$, h_n^* and *h_n the right and left derivatives of h_n respectively. Then, h_n^* and *h_n are defined everywhere in (a, b) and given by

$$\begin{cases} {}^*h_n(x) = h_n^*(x) = h_n'(x) = a_{i+1}, & \text{if } x \in (c_i, c_{i+1}), \\ {}^*h_n(x) = a_i, \quad h_n^*(x) = a_{i+1}, & \text{if } x = c_i. \end{cases} \quad (20)$$

Proposition 6. *Let $X_0 \in (a, b)$. Then φ_ω^n is strictly convex (resp. strictly concave, resp. affine) on a neighbourhood of X_0 if and only if $h_n^*(X_0) - h_n^*(\varphi_\omega^n(X_0)) < 0$ (resp. > 0 , resp. $= 0$) and ${}^*h_n(X_0) - {}^*h_n(\varphi_\omega^n(X_0)) < 0$ (resp. > 0 , resp. $= 0$).*

Proposition 6 is an immediate consequence of Lemmas 5 and 6. Indeed, Lemma 5 provides necessary and sufficient conditions for φ_ω^n to be strictly convex (resp. concave) on any interval included in $(a, b) \setminus J_n$, with J_n defined by (15), and Lemma 6 ensures that φ_ω^n is strictly convex (resp. concave) on a neighbourhood of $X_0 \in J_n$ if and only if it is strictly convex (resp. concave) on both sides of X_0 .

4.2 Generalisation to smooth functions

In this section, we consider the autonomous Cauchy problem

$$\begin{cases} X'(t) = h(X(t)), & t \geq 0, \\ X(0) = X_0 \in [a, b], \end{cases} \quad (21)$$

where $h \in \mathcal{C}^1([a, b], \mathbb{R})$ is a Lipschitz continuous function, positive on (a, b) and satisfying $h(b) = 0$.

We want to study the convexity of φ_ω , the flow associated to System (21) at time $\omega > 0$, defined by (6). Remark that the hypothesis $h(b) = 0$ ensures that for any $X_0 \in [a, b]$, X and φ_ω remain in $[a, b]$.

The main purpose of this section is to prove the following theorem.

Theorem 1. *Let $X_0 \in (a, b)$. If $h'(\varphi_\omega(X_0)) - h'(X_0) > 0$ (resp. < 0), then φ_ω is convex (resp. concave) on a neighbourhood of X_0 .*

Remark 4. *Theorem 1 can be directly adapted to the case where h is a negative function and $h(a) = 0$ by studying the function g defined on $(-b, -a)$ by $g(x) = -h(-x)$.*

In the following, we consider h_n defined in Section 4.1 to be the order n linear interpolation of h on $[a, b]$ with uniform distribution of the nodes. That is, with the notations of Section 4.1,

$$\begin{cases} \forall i \in \{0, \dots, n\}, & c_i = a + i \frac{b-a}{n}, \\ \forall i \in \{0, \dots, n-1\}, & a_{i+1} = \frac{h(c_{i+1}) - h(c_i)}{c_{i+1} - c_i}, \quad b_{i+1} = h(c_i) - a_{i+1} c_i. \end{cases} \quad (22)$$

In particular, h_n is continuous, for all $i \in \{0, \dots, n\}$, $c_{i+1} - c_i = 1/n$ and $h_n(c_i) = h(c_i)$. When there is ambiguity on the order n , we note $a_i = a_i^{(n)}$, $b_i = b_i^{(n)}$ and $c_i = c_i^{(n)}$. The flow φ_ω^n associated with h_n is defined in (14).

Lemma 7. *Let $X_0 \in [a, b]$, then $\lim_{n \rightarrow +\infty} \varphi_\omega^n(X_0) = \varphi_\omega(X_0)$.*

Proof of Lemma 7. Let $X_0 \in [a, b]$ and X_n, X be the respective solutions of (11) and (21). It is easy to show that for any $\epsilon > 0$, there exists $n_\epsilon \in \mathbb{N}^*$ such that for all $n \geq n_\epsilon$ and for all $x \in [a, b]$, $|h(x) - h_n(x)| < \epsilon$. Then for all $x \in [a, b]$, $h(x) - \epsilon \leq h_n(x) \leq h(x) + \epsilon$.

For any $\epsilon > 0$, let us consider X_ϵ and $X_{-\epsilon}$ the respective solutions of $X'_\epsilon = h(X_\epsilon) + \epsilon$ and $X'_{-\epsilon} = h(X_{-\epsilon}) - \epsilon$ satisfying $X_\epsilon(0) = X_{-\epsilon}(0) = X_0$. Then, for all $t > 0$,

$$X_n(t) \in [X_\epsilon(t), X_{-\epsilon}(t)]. \quad (23)$$

It remains to show $\lim_{\epsilon \rightarrow 0^+} X_\epsilon(\omega) = \lim_{\epsilon \rightarrow 0^+} X_{-\epsilon}(\omega) = X(\omega)$. We set $Z = X - X_\epsilon$. Since h is Lipschitz continuous, there exists $C_h > 0$ such that, for all $t \geq 0$,

$$|Z'(t)| = |h(X(t)) - h(X_\epsilon(t)) - \epsilon| \leq C_h |X(t) - X_\epsilon(t)| + \epsilon = |Z(t)| + \epsilon.$$

Using the Gronwall lemma, one gets

$$\forall t \geq 0, |Z(t)| \leq |Z(0)|e^{C_h t} + \frac{\epsilon}{C_h}(e^{C_h t} - 1) = \frac{\epsilon}{C_h}(e^{C_h t} - 1).$$

Consequently $\lim_{\epsilon \rightarrow 0^+} Z(\omega) = 0$ and so $\lim_{\epsilon \rightarrow 0^+} X_\epsilon(\omega) = X(\omega)$. Similarly, we can show $\lim_{\epsilon \rightarrow 0^+} X_{-\epsilon}(\omega) = X(\omega)$. By using the squeeze theorem and (23), it follows that $\lim_{\epsilon \rightarrow 0^+} X_n(\omega) = X(\omega)$. \square

Using Lemma 7 and Proposition 6, we prove Theorem 1.

Proof of Theorem 1. Here we prove the convexity case. Proof for the concavity one is similar. Let $x \in (a, b)$ and $(x_n)_{n \in \mathbb{N}}$ be a sequence such that $\forall n \in \mathbb{N}$, $x_n \in (a, b)$ and $\lim_{n \rightarrow +\infty} x_n = x$. We first show that

$$\lim_{n \rightarrow +\infty} h_n^*(x_n) = \lim_{n \rightarrow \infty} {}^*h_n(x_n) = h'(x), \quad (24)$$

where h_n^* and *h_n are given by (20).

For all $n \in \mathbb{N}^*$, there exists a unique $i \in \{0, \dots, n-1\}$ such that $x_n \in [c_i^{(n)}, c_{i+1}^{(n)})$, we note $i = i_n$. Since $\lim_{n \rightarrow +\infty} x_n = x$ and $\forall i \in \{0, \dots, n-1\}$,

$$\lim_{n \rightarrow +\infty} |c_{i+1}^{(n)} - c_i^{(n)}| = \lim_{n \rightarrow +\infty} 1/n = 0,$$

it follows that

$$\lim_{n \rightarrow +\infty} c_{i_n-1}^{(n)} = \lim_{n \rightarrow +\infty} c_{i_n+1}^{(n)} = x. \quad (25)$$

Using the mean value theorem and the definition of a_i in (22), it follows that for all $n \in \mathbb{N}^*$, there exists $\alpha_n \in (c_{i_n-1}^{(n)}, c_{i_n}^{(n)})$ and $\beta_n \in (c_{i_n}^{(n)}, c_{i_n+1}^{(n)})$ such that

$$a_{i_n} = h'(\alpha_n) \text{ and } a_{i_n+1} = h'(\beta_n). \quad (26)$$

Since $h \in \mathcal{C}^1([a, b], \mathbb{R})$, using (25) and (26), it follows that

$$\lim_{n \rightarrow +\infty} a_{i_n} = \lim_{n \rightarrow +\infty} a_{i_n+1} = h'(x). \quad (27)$$

On the other hand, according to (20), for all $n \in \mathbb{N}^*$,

$$h_n^*(x_n), {}^*h_n(x_n) \in \{a_{i_n}, a_{i_n+1}\}. \quad (28)$$

With (27) and (28) we conclude that (24) is true. In particular,

$$\begin{cases} \lim_{n \rightarrow +\infty} h_n^*(x) = \lim_{n \rightarrow \infty} {}^*h_n(x) = h'(x), \\ \lim_{n \rightarrow +\infty} h_n^*(\varphi_\omega^n(x)) = \lim_{n \rightarrow \infty} {}^*h_n(\varphi_\omega^n(x)) = h'(\varphi(x)). \end{cases} \quad (29)$$

Let $X_0 \in (a, b)$. If we assume $h'(\varphi_\omega(X_0)) > h'(X_0)$, since h' and φ_ω are continuous there exists a neighbourhood I_{X_0} of X_0 such that, for all $y \in I_{X_0}$, $h'(\varphi_\omega(y)) > h'(y)$.

Moreover, (29) implies that there exists a neighbourhood $J_{X_0} \subset I_{X_0}$ of X_0 and $N_0 \in \mathbb{N}^*$ such that for all $n \geq N_0$ and for all $y \in J_{X_0}$,

$${}^*h_n(y) < {}^*h_n(\varphi_\omega^n(y)) \text{ and } h_n^*(y) < h_n^*(\varphi_\omega^n(y)).$$

According to Proposition 6, for all $n \geq N_0$, φ_ω^n is strictly convex on J_{X_0} .

Finally, as the limit of a sequence of strictly convex functions (Lemma 5), φ_ω is convex on J_{X_0} . \square

In the following section, we apply results of Sections 2, 3 and 4 to a special case that motivated that study.

5 Equation on Tbet with impulses

5.1 Introduction

In the multiscale model presented in Prokopiou et al. (2014) and Gao et al. (2016), protein Tbet drives the development of effector properties and cell death in the CD8 T-cell population. In this model, all effector cells develop the same phenotype and the differentiation into memory cells is not considered.

We are interested in studying how unequal partitioning of molecular content at cell division can explain the emergence of two subpopulations of effector CD8 T-cells, with the first ones – characterised by a low level of Tbet – able to develop memory properties and the second ones – characterised by a high level of Tbet – destined to die during the contraction phase (Kaech and Cui, 2012; Lazarevic et al., 2013). We hypothesize that when a CD8 T-cell divides, the intracellular content of the mother cell is not split in two equal parts but asymmetric division occurs and gives birth to two daughter cells with different molecular profiles (Sennerstam, 1988; Block et al., 1990) (here, different concentrations of Tbet). Each daughter cell is supposed to be two times smaller than the mother cell so that the mass is preserved.

To model this phenomenon, we consider the IDE with fixed impulse times (30), which allows us to describe the effect of division, occurring at discrete times τ_k , $k \in \mathbb{N}^*$, on the concentration X of protein Tbet in a single CD8 T-cell throughout several cell divisions,

$$\left\{ \begin{array}{l} \frac{dX(t)}{dt} = \eta \frac{X(t)^n}{\theta^n + X(t)^n} - \delta X(t), \quad t \in \mathbb{R}^+ \setminus \{\tau_k, k \in \mathbb{N}^*\}, \\ X(\tau_k^+) = (1 + \alpha_k)X(\tau_k^-), \quad k \in \mathbb{N}^*, \\ X(0) = X_0 \in \mathbb{R}^+. \end{array} \right. \quad (30)$$

At any time τ_k , $k \in \mathbb{N}^*$, a cell divides and transmits a fraction $(1 + \alpha_k)$ of its concentration of Tbet to one daughter cell (the other one, which is not modelled, receives a concentration $(1 - \alpha_k)X(\tau_k^-)$, so that the mean concentration in the two daughter cells remains equal to $X(\tau_k^-)$) (Prokopiou et al., 2014; Gao et al., 2016).

The dynamics of the concentration X within a single cell between two consecutive divisions is modelled by

$$\frac{dX}{dt} = f(X) := \eta \frac{X^n}{\theta^n + X^n} - \delta X, \quad (31)$$

where n , η , θ and δ are positive constants. The first term on the right hand side of the equation accounts for a positive feedback loop between protein Tbet and its own gene *Tbx21* (Kanhere et al., 2012; Prokopiou et al., 2014; Gao et al., 2016), and parameter δ accounts for protein decay as well as dilution due to cell growth.

A common approach to study an impulsive system such as (30) is to turn it into a differential equation without impulse and study the latter (Yan and Zhao, 1998). Indeed, let us introduce the non-impulsive system (32), obtained from (30),

$$\begin{cases} \frac{d\mathcal{X}(t)}{dt} = P(t)\eta \frac{\mathcal{X}(t)^n}{P(t)^n\theta^n + \mathcal{X}(t)^n} - \delta\mathcal{X}(t), & t \in \mathbb{R}^+ \setminus \{\tau_k, k \in \mathbb{N}^*\}, \\ \mathcal{X}(0) = X_0, \end{cases} \quad (32)$$

where P is the discontinuous function defined by $P(t) = \prod_{0 < \tau_k \leq t} (1 + \alpha_k)^{-1}$. The following proposition is inspired by Theorem 1 from Yan and Zhao (1998), the proof is analogous and will be omitted.

Proposition 7. *Let (\mathbf{H}_1) and (\mathbf{H}_2) hold true. Then X is solution of (30) if and only if $\mathcal{X} : t \mapsto P(t)X(t)$ is solution of (32).*

Remark 5. *Proposition 7 is particularly useful under the strong assumption that function P is periodic on \mathbb{R}^+ (Yan, 2003; Yan et al., 2005; Li and Huo, 2005; Saker and Alzabut, 2007; Kou et al., 2009). Indeed, in that case, the periodicity of \mathcal{X} implies the periodicity of X and then existence and stability of periodic solutions for System (30) can be studied with the non-impulsive system (32), generating smooth solutions. Some authors succeeded in obtaining results on the existence and stability of periodic solutions with weaker hypotheses (Liu and Takeuchi, 2007; Faria and Oliveira, 2016), however for specific right-hand sides of the equation. As mentioned by Liu and Takeuchi (2007), the hypothesis of periodicity of the function P is too restrictive. Moreover it has no simple physical interpretation and is sometimes abusively used, leading to unrealistic conclusions. It would not be relevant for the biological process we are studying to consider that P is periodic, therefore we do not present results obtained under this hypothesis.*

We want to apply the results of Section 4 to System (30). To this end, we first focus on the convexity of f , defined by (31).

Proposition 8. *Assume $n > 1$ and $\Theta = \theta \sqrt[n]{\frac{n-1}{n+1}}$. Then f is strictly convex on $[0, \Theta)$, strictly concave on $(\Theta, +\infty)$ and has an inflexion point in Θ .*

The proof of Proposition 8 is straightforward.

Since f is Lipschitz continuous on \mathbb{R}^+ and $f(0) = 0$, System (30) is a particular case of System (1) where $g = f$ and $U = \mathbb{R}^+$. Consequently the results presented in Section 2 hold true for System (30).

Since we only focus on nonnegative solutions, we only consider nonnegative steady states of the autonomous equation (31), without impulse. Their existence and stability are given by Proposition 9, whose proof is omitted.

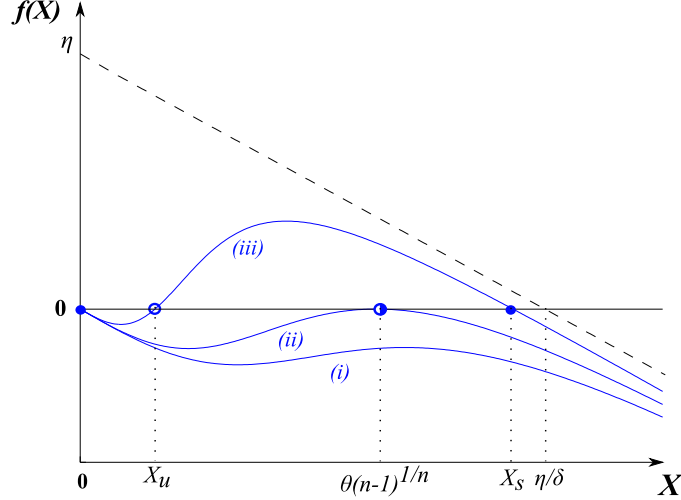


Figure 1: Steady states of (31). Straight blue curves correspond to the different cases given in Proposition 9. Dotted line is the graph of the function $X \mapsto \eta - \delta X$.

Proposition 9. *Let $n > 1$, then*

- (i) *If $\eta(n-1)^{\frac{n-1}{n}} < n\delta\theta$, the trivial solution $X \equiv 0$ is the unique steady state of (31) and it is globally asymptotically stable (on \mathbb{R}_+).*
- (ii) *If $\eta(n-1)^{\frac{n-1}{n}} = n\delta\theta$, equation (31) has exactly two steady states: $X \equiv 0$, which is locally asymptotically stable, and $X \equiv \theta\sqrt[n]{n-1}$ which is unstable (attractive on $[\theta\sqrt[n]{n-1}, +\infty)$ but repulsive on $(0, \theta\sqrt[n]{n-1})$).*
- (iii) *If $\eta(n-1)^{\frac{n-1}{n}} > n\delta\theta$, (31) has exactly three steady states: $0 < X_u < X_s$, such that $X \equiv 0$ is locally asymptotically stable, X_u is unstable and X_s is locally asymptotically stable (with basin of attraction $(X_u, +\infty)$). Moreover $0 < X_u < \theta\sqrt[n]{n-1} < X_s < \eta/\delta$.*

The three statements of Proposition 9 are illustrated on Figure 1. For biological reasons, in the following we will focus on the case when System (31) is bistable, i.e. we will assume that the following hypothesis holds true:

(H₅) $n > 1$ and $\eta(n-1)^{\frac{n-1}{n}} > n\delta\theta$.

Note that under **(H₅)**, $\Theta := \theta\sqrt[n]{(n-1)/(n+1)} < \theta\sqrt[n]{n-1}$. Therefore, according to Proposition 9-(iii), $\Theta \in (0, X_s)$.

5.2 Existence of periodic solutions for (30)

Throughout this section, we suppose that hypotheses **(H₃)**, **(H₄)** and **(H₅)** hold true and we investigate the existence of periodic solutions of (30). We have seen in Proposition 3 that periodic solutions are either constant, or their smallest period is ω .

From now on, φ_ω stands for the flow of System (30) at time ω , as defined in (6). Here $f(0) = 0$ so, according to Remark 2, $\varphi_\omega^{-1}(0) = 0$ and the function Γ_ω , defined

by (8), is continuous on \mathbb{R}_*^+ . Moreover $X(\cdot; X_0, \alpha)$ is periodic if and only if $X_0 = 0$ or $X_0 \in \Gamma_\omega^{-1}(\{\alpha\})$ (Proposition 4). In the rest of this section, we show the following theorem.

Theorem 2. *Let hypotheses (\mathbf{H}_3) , (\mathbf{H}_4) and (\mathbf{H}_5) hold true. Then, there exists $\alpha^* \in (-1, 0)$ and a non-empty interval $I = [I_m, I_M] \subset [X_u, X_s)$ such that*

- *If $\alpha < \alpha^*$, $X \equiv 0$ is the only periodic solution of (30) and for any $X_0 > 0$, $\lim_{t \rightarrow +\infty} X(t; X_0, \alpha) = 0$.*
- *$X(\cdot, X_0, \alpha^*)$ is periodic if and only if $X_0 \in \{0\} \cup I$.*
- *If $\alpha^* < \alpha < \exp(\delta\omega) - 1$, there are exactly 3 periodic solutions of (30): $X \equiv 0$, $X(\cdot; X_l, \alpha)$ and $X(\cdot; X_h, \alpha)$ where $\{X_l, X_h\} = \Gamma_\omega^{-1}(\{\alpha\})$ such that $0 < X_l < X_h$ and these solutions are respectively asymptotically stable and attractive on $[0, X_l)$, unstable and repulsive on $(0, X_l) \cup (X_l, X_h)$ and asymptotically stable and attractive on $(X_l, +\infty)$. Moreover, if $\alpha < 0$, $X_l \in (0, X_u)$ and $X_h \in (X_s, +\infty)$, if $\alpha = 0$, $X_l = X_u$ and $X_h = X_s$, if $\alpha > 0$, $X_l \in (X_u, I_m)$ and $X_h \in (I_M, X_s)$.*
- *If $\alpha \geq \exp(\delta\omega) - 1$, $X \equiv 0$ is the only periodic solution of (30). Moreover, for any $X_0 > 0$, $\lim_{t \rightarrow +\infty} X(t; X_0, \alpha) = +\infty$.*

In addition, if $\Theta \neq X_u$, $I \subset (X_u, X_s)$ and if $\Theta = X_u$, $I = \{X_u\}$.

To prove Theorem 2, we need to apply Proposition 4 and Lemma 3 from Section 3. To this end, we first introduce intermediate results on the behaviour of φ_ω and Γ_ω .

Lemma 8. *Let hypothesis (\mathbf{H}_5) hold true. Then, for any $\omega > 0$,*

$$\lim_{X_0 \rightarrow +\infty} \Gamma_\omega(X_0) = \lim_{X_0 \rightarrow 0^+} \Gamma_\omega(X_0) = \exp(\delta\omega) - 1.$$

Proof. We first rewrite equation (31) in the form $X'(t) = \mathcal{H}(X(t)) - \delta X(t)$ with

$$\mathcal{H} : x \in \mathbb{R}^+ \mapsto \eta \frac{x^n}{\theta^n + x^n} \in [0, \eta).$$

Hence, for all $X_0 \in \mathbb{R}_*^+$,

$$\varphi_\omega(X_0) = X(\omega; X_0, 0) = X_0 e^{-\delta\omega} + e^{-\delta\omega} \int_0^\omega e^{\delta s} \mathcal{H}(X(s)) ds,$$

thus

$$\frac{X(\omega; X_0, 0)}{X_0} = e^{-\delta\omega} + e^{-\delta\omega} \int_0^\omega e^{\delta s} \frac{\mathcal{H}(X(s))}{X_0} ds. \quad (33)$$

Since $\mathcal{H} < \eta$ on \mathbb{R}^+ and $e^{\delta s} \leq e^{\delta\omega}$ for $s \in (0, \omega)$, one obtains

$$0 \leq e^{-\delta\omega} \int_0^\omega e^{\delta s} \frac{\mathcal{H}(X(s))}{X_0} ds \leq \omega \frac{\eta}{X_0}.$$

Then it is clear from (33) that

$$\lim_{X_0 \rightarrow +\infty} \frac{X(\omega; X_0, 0)}{X_0} = \exp(-\delta\omega)$$

and then, from (8), $\lim_{X_0 \rightarrow +\infty} \Gamma_\omega(X_0) = \exp(\delta\omega) - 1$.

We now study the limit of Γ_ω as X_0 goes to zero. Let us recall that if $X_0 < X_u$, then $f(X_0) < 0$ and $X(\cdot; X_0, 0)$ decreases on $[0, \omega]$. Consequently, for $s \in [0, \omega]$, $X(\omega) \leq X(s) \leq X_0$ and we have the inequality

$$\frac{\mathcal{H}(X(s))}{X_0} = \eta \frac{X(s)^n}{(\theta^n + X(s)^n) X_0} \leq \eta \frac{X_0^{n-1}}{\theta^n + X(\omega)^n}.$$

Therefore

$$e^{-\delta\omega} \int_0^\omega e^{\delta s} \frac{\mathcal{H}(X(s))}{X_0} ds \leq \omega \eta \frac{X_0^{n-1}}{\theta^n + X(\omega)^n}.$$

Finally, from (33),

$$\lim_{X_0 \rightarrow 0^+} \frac{X(\omega; X_0, 0)}{X_0} = \exp(-\delta\omega),$$

so $\lim_{X_0 \rightarrow 0^+} \Gamma_\omega(X_0) = \exp(\delta\omega) - 1$. This concludes the proof. \square

Lemma 9. *Let hypothesis (\mathbf{H}_5) hold true, $\omega > 0$ and Θ as defined in Proposition 8. There exists $X_c \in (0, X_s)$ such that φ_ω is convex on $(0, X_c)$ and concave on $(X_c, +\infty)$. Moreover, $X_c = X_u$ if and only if $X_u = \Theta$.*

Proof. Let us assume $\Theta \in (X_u, X_s)$. The proof is similar if $\Theta \in (0, X_u]$. In order to apply Theorem 1, we investigate the sign of $f' - f' \circ \varphi_\omega$ on $(0, +\infty)$.

As steady states of (31), 0 , X_u and X_s are fixed points of φ_ω . Therefore, if $x \in \{0, X_u, X_s\}$, then $f'(x) - f'(\varphi_\omega(x)) = 0$.

If $X_0 \in (0, X_u) \cup (X_s, +\infty)$, then $f(X_0) < 0$ and the solution of (30) decreases on $[0, \omega)$, so $0 < \varphi_\omega(X_0) < X_0$. According to Proposition 8, f is strictly convex on $(0, X_u) \subset (0, \Theta)$ and strictly concave on $(X_s, +\infty) \subset (\Theta, +\infty)$. Consequently, for all $X_0 \in (0, X_u)$, $f'(X_0) - f'(\varphi_\omega(X_0)) > 0$ and for all $X_0 \in (X_s, +\infty)$, $f'(X_0) - f'(\varphi_\omega(X_0)) < 0$.

If $X_0 \in (X_u, X_s)$, then $f(X_0) > 0$, the solution of (30) increases on $[0, \omega)$ and $\varphi_\omega(X_0) > X_0$. Moreover, if $X_0 \in (X_u, \varphi_\omega^{-1}(\Theta))$, then $\varphi_\omega(X_0) \in (X_0, \Theta)$, that is $X_u < X_0 < \varphi_\omega(X_0) < \Theta$. Since f is strictly convex on (X_u, Θ) (Proposition 8), it follows $f'(X_0) - f'(\varphi_\omega(X_0)) < 0$. Similarly, for all X_0 in $[\Theta, X_s)$, $f'(X_0) - f'(\varphi_\omega(X_0)) > 0$. It remains to prove that there exists a unique $X_c \in (\varphi_\omega^{-1}(\Theta), \Theta)$ such that $f'(X_c) - f'(\varphi_\omega(X_c)) = 0$.

Since $f, \varphi_\omega \in \mathcal{C}^\infty(\mathbb{R})$, it follows $f' - f' \circ \varphi_\omega \in \mathcal{C}^\infty(\mathbb{R})$ and then there exists $X_c \in (\varphi_\omega^{-1}(\Theta), \Theta)$ such that $f'(X_c) - f'(\varphi_\omega(X_c)) = 0$. To show the uniqueness of X_c , we suppose there exists $Y_c \in (\varphi_\omega^{-1}(\Theta), \Theta)$, $Y_c > X_c$, such that $f'(Y_c) - f'(\varphi_\omega(Y_c)) = 0$. Since φ_ω is increasing and $\varphi_\omega(X_s) = X_s$, it follows $X_c < Y_c < \Theta < \varphi_\omega(X_c) < \varphi_\omega(Y_c) < \varphi_\omega(\Theta) < X_s$. From Proposition 8, f is convex on $(\varphi_\omega^{-1}(\Theta), \Theta)$ so $f'(X_c) < f'(Y_c)$. On the other hand, from Proposition 8, f is concave on (Θ, X_s) so $f'(\varphi_\omega(X_c)) > f'(\varphi_\omega(Y_c))$. There is a contradiction since $f'(X_c) = f'(\varphi_\omega(X_c))$ and $f'(Y_c) = f'(\varphi_\omega(Y_c))$.

Then $f'(\varphi_\omega(x)) = f'(x)$ if and only if $x \in \{0, X_u, X_c, X_s\}$ and $f'(\varphi_\omega(x)) - f'(x) < 0$ (resp. > 0) if $x \in (0, X_u) \cup (X_c, X_s)$ (resp. if $x \in (X_u, X_c) \cup [X_s, +\infty)$).

According to Theorem 1, and Remark 4 since $f < 0$ on $(0, X_u) \cup (X_s, +\infty)$, we conclude that φ_ω is convex on $(0, X_u)$ and on (X_u, X_c) and concave on (X_c, X_s) and on $(X_s, +\infty)$. Since $\varphi_\omega \in \mathcal{C}^\infty(\mathbb{R}^+)$, then φ_ω is convex on $(0, X_c)$ and concave on $(X_c, +\infty)$.

In the particular case $X_u = \Theta$, f is strictly convex on $(0, X_u)$ and strictly concave on $(X_u, +\infty)$. Thus $f'(\varphi_\omega(x)) - f'(x) < 0$ if $x \in (0, X_u) \cup (X_u, X_s)$ and $f'(\varphi_\omega(x)) - f'(x) < 0$ if $x \in (X_s, +\infty)$. It follows that φ_ω is convex on $(0, X_u)$ and concave on $(X_u, +\infty)$, i.e. $X_c = X_u$. The converse is straightforward. \square

Lemma 10. *Let hypothesis (\mathbf{H}_5) hold true and $\omega > 0$. Then, there exists $\beta > 1$ and $x_\beta > 0$ such that for all $x > 0$, $\varphi_\omega(x) \leq \beta x$ and $\varphi_\omega(x_\beta) = \beta x_\beta$. Moreover, if there exists $x > 0$ such that $\varphi_\omega(x) = \beta x$, then $\varphi'_\omega(x) = \beta$.*

Proof. The function $x \mapsto \varphi_\omega(x)/x$ is continuous on $(0, +\infty)$ and, in particular, on the closed interval $[X_u, X_s]$. Therefore there exists $\beta = \max_{x \in [X_u, X_s]} \varphi_\omega(x)/x$. Moreover, for all $x \in (X_u, X_s)$, $\varphi_\omega(x)/x > 1$ and for all $x \in (0, X_u] \cup [X_s, +\infty)$, $\varphi_\omega(x)/x \leq 1$ (cf. proof of Lemma 9). Consequently $\beta = \max_{x > 0} \varphi_\omega(x)/x$ and $\beta > 1$. Hence there exists $x_\beta \in [X_u, X_s]$ such that $\varphi_\omega(x_\beta) = \beta x_\beta$ and for all $x > 0$, $\varphi_\omega(x) \leq \beta x$.

Let $x > 0$ be such that $\varphi_\omega(x)/x = \beta$. Then $x \in \arg \max_{x > 0} (\varphi_\omega/id)$, where id denotes the identity function on \mathbb{R}^+ . As an extremum of a \mathcal{C}^∞ -function, x satisfies $(\varphi_\omega/id)'(x) = 0$. That is $\varphi'_\omega(x) = \varphi_\omega(x)/x = \beta$. \square

Definition 4. *For any $\omega > 0$, let X_c denotes the inflexion point of φ_ω introduced in Lemma 9, $\beta := \max_{x > 0} (\varphi_\omega(x)/x)$, whose existence is stated in Lemma 10 and $I := \{x > 0, \varphi_\omega(x) = \beta x\}$. It may be noted that X_c , β and I depend on the value of ω .*

Lemma 11. *Let hypothesis (\mathbf{H}_5) hold true and $\omega > 0$. Then $I = [I_m, I_M]$ with $X_c < I_m \leq I_M < X_s$.*

Proof. From Lemma 10 we know that I is non-empty and $I \subset (X_u, X_s)$. Moreover, $I = (\varphi_\omega/id)^{-1}(\{\beta\})$, where id is the identity function and φ_ω/id is continuous on $(0, +\infty)$, so I is a closed set. Let's show that $I \subset (X_c, X_s)$.

By contradiction, let us suppose that there exists $x_0 \in I$ such that $X_u < x_0 \leq X_c$. According to Lemmas 9 and 10, for all $0 < x < x_0$, $\varphi'_\omega(x) \leq \varphi'_\omega(x_0) = \beta$. Then the function $x \mapsto \varphi_\omega(x) - \beta x$ is decreasing on $(0, x_0)$. Consequently, for all $x \in (0, x_0)$, $\varphi_\omega(x) - \beta x \geq \varphi_\omega(x_0) - \beta x_0 = 0$, by definition of x_0 , and in particular $\varphi_\omega(X_u) - \beta X_u \geq 0$.

On the other hand, since X_u is a steady state of (31), then $\varphi_\omega(X_u) = X_u$ and since $\beta > 1$ (Lemma 10), then $\varphi_\omega(X_u) - \beta X_u = X_u(1 - \beta) < 0$. There is a contradiction, so $I \subset (X_c, X_s)$.

It remains to show that I is an interval. If $I = \{x_\beta\}$, given by Lemma 10, the proof is complete. Assume $x_0, y_0 \in I$ with $x_0 < y_0$ and let $z_0 \in (x_0, y_0)$. Since $x_0 > X_c$ and φ_ω is concave on $(X_c, +\infty)$, it follows from Lemma 10 and Definition 4 that

$$\beta = \varphi'_\omega(x_0) \geq \varphi'_\omega(z_0) \geq \varphi'_\omega(y_0) = \beta.$$

Therefore $\varphi'_\omega(z_0) = \beta$ for all $z_0 \in (x_0, y_0)$, so φ_ω is linear. Since $\varphi_\omega(x_0) = \beta x_0$, we deduce $\varphi_\omega(z_0) = \beta z_0$, that is $z_0 \in I$ and I is an interval. \square

Lemma 12. *Let hypothesis (\mathbf{H}_5) hold true, $\omega > 0$, I_m, I_M as defined in Lemma 11 and $x \geq 0$. If $x \in (0, I_m)$, then $\varphi'_\omega(x) > \varphi_\omega(x)/x$ and if $x \in (I_M, +\infty)$, then $\varphi'_\omega(x) < \varphi_\omega(x)/x$. Otherwise, $\varphi'_\omega(x) = \varphi_\omega(x)/x$.*

Proof. We first focus on the interval $(0, X_c) \subset (0, I_m)$. Since φ_ω is convex on $(0, X_c)$ (Lemma 9) and $\varphi_\omega(0) = 0$, from the mean value theorem one has, for all $x \in (0, X_c)$, $\varphi'_\omega(x) \geq \varphi_\omega(x)/x$.

Let us suppose there exists $x_0 \in (0, X_c)$ such that $\varphi'_\omega(x_0) = \varphi_\omega(x_0)/x_0$. Then we claim that for all $x \in (0, x_0)$, $\varphi_\omega(x)/x = \varphi_\omega(x_0)/x_0$. Indeed, from the convexity of φ_ω ,

$$\text{for all } x \in (0, x_0), \varphi_\omega(x)/x \leq \varphi'_\omega(x) \leq \varphi'_\omega(x_0) = \varphi_\omega(x_0)/x_0. \quad (34)$$

Assume there exists $x \in (0, x_0)$ such that $\varphi_\omega(x)/x < \varphi_\omega(x_0)/x_0$. Then, from the mean value theorem, there exists $c \in (x, x_0)$ such that

$$\varphi'_\omega(c) = \frac{\varphi_\omega(x_0) - \varphi_\omega(x)}{x_0 - x} > \frac{\varphi_\omega(x_0) - x\varphi_\omega(x_0)/x_0}{x_0 - x} = \frac{\varphi_\omega(x_0)}{x_0}.$$

There is a contradiction with (34), so for all $x \in (0, x_0)$, $\varphi_\omega(x)/x = \varphi_\omega(x_0)/x_0$.

On the other hand, according to Lemma 8,

$$\lim_{x \rightarrow 0^+} \varphi_\omega(x)/x = \lim_{x \rightarrow 0^+} (\Gamma_\omega(x) + 1)^{-1} = \exp(-\delta\omega).$$

It follows that $\varphi_\omega(x_0)/x_0 = \exp(-\delta\omega)$ and so, for all $x \in (0, x_0)$, $\varphi_\omega(x) = \exp(-\delta\omega)x$. Consequently, φ_ω coincides with the flow of the linear equation $Y'(t) = -\delta Y(t)$. That is impossible since for all $x > 0$, $f(x) > -\delta x$, so the first assumption in the proof is false. We conclude that for all $x_0 \in (0, X_c)$, $\varphi'_\omega(x_0) > \varphi_\omega(x_0)/x_0$.

Now, consider $x_0 \in [X_c, I_m)$ and suppose that $\varphi'_\omega(x_0) \leq \varphi_\omega(x_0)/x_0$. By concavity of φ_ω on $(X_c, +\infty)$ (Lemma 9), for all $x \geq x_0$, $\varphi'_\omega(x) \leq \varphi'_\omega(x_0) \leq \varphi_\omega(x_0)/x_0$. Consequently for any $y \in (x_0, +\infty)$,

$$\varphi_\omega(y) = \varphi_\omega(x_0) + \int_{x_0}^y \varphi'_\omega(x) dx \leq \varphi_\omega(x_0) + \frac{\varphi_\omega(x_0)}{x_0}(y - x_0) = y \frac{\varphi_\omega(x_0)}{x_0}.$$

In particular, $\varphi_\omega(I_m) \leq I_m \frac{\varphi_\omega(x_0)}{x_0}$. However, $\varphi_\omega(I_m) = I_m \beta$ (Lemma 11) and, since $x_0 \notin I = [I_m, I_M]$, $\beta > \varphi_\omega(x_0)/x_0$ (Lemma 10). There is a contradiction. Therefore, for all $x_0 \in [X_c, I_m)$, $\varphi'_\omega(x_0) > \varphi_\omega(x_0)/x_0$.

Similar arguments allow us to conclude that $\varphi'_\omega(x) < \varphi_\omega(x)/x$ for $x \in (I_M, +\infty)$.

Finally, if $x \in [I_m, I_M]$, from Lemmas 10 and 11, $\varphi'_\omega(x) = \beta$ and $\varphi_\omega(x) = \beta x$. That achieves the proof. \square

Now, we can prove Theorem 2.

Proof of Theorem 2. From the definition of Γ_ω in (8), for $x > 0$ one has

$$\Gamma'_\omega(x) = \frac{\varphi_\omega(x) - x\varphi'_\omega(x)}{\varphi_\omega(x)^2}.$$

Then, from Lemma 12, Γ_ω is decreasing on $(0, I_m)$, constant on $[I_m, I_M]$ with $\Gamma_\omega(x) = \alpha^* := (1/\beta) - 1$ (Lemma 10) and increasing on $(I_M, +\infty)$. Moreover from Lemma 8, $\lim_{x \rightarrow +\infty} \Gamma_\omega(x) = \lim_{x \rightarrow 0^+} \Gamma_\omega(x) = \exp(\delta\omega) - 1$. In addition, since X_u and X_s are two steady states of (31), $\Gamma_\omega(X_u) = \Gamma_\omega(X_s) = 0$. So for the different values of α in Theorem 2, we can conclude on $\Gamma_\omega^{-1}(\{\alpha\})$ (see Figure 2 (A)) and then on the number of periodic solutions of (30) (Proposition 4). The stability of the positive periodic

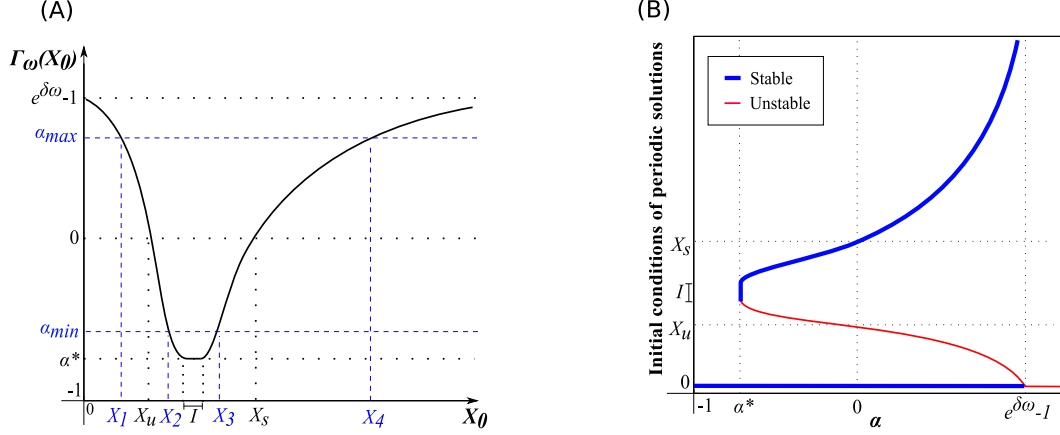


Figure 2: According to the results shown in Section 5 and under hypotheses of Theorem 2 : (A) Qualitative graph of function Γ_ω . Blue dashed lines correspond to critical values mentioned in Proposition 10. (B) Initial conditions of the periodic solutions of (30). For any $\alpha > -1$, the initial conditions X_0 such that $X(\cdot; X_0, \alpha)$ is periodic and stable (resp. unstable) are drawn in bold blue (resp. thin red).

solutions is a consequence of Lemma 3 and can be deduced from the graph of Γ_ω (Remark 3).

Let's consider $\alpha^* \leq \alpha < \exp(\delta\omega) - 1$, then $\Gamma_\omega^{-1}(\{\alpha\}) = \{X_l, X_h\}$ with $X_l \in (0, I_m)$ and $X_h \in (I_M, +\infty)$. From the monotonicity of Γ_ω we conclude that $X(\cdot; X_l, \alpha)$ is unstable and $X(\cdot; X_h, \alpha)$ is asymptotically stable (Remark 3). It remains to study the stability of $X \equiv 0$. Let $X_0 \in (0, X_l)$. Then, for all $t \geq 0$, $0 < X(t; X_0, \alpha) < X(t; X_l, \alpha)$ and, as a periodic function, $X(\cdot; X_l, \alpha)$ is bounded, so $X(\cdot; X_0, \alpha)$ is bounded. According to Proposition 2, $X(\cdot; X_0, \alpha)$ converges to a periodic solution. Since $X(\cdot; X_l, \alpha)$ is unstable, then $X(\cdot; X_l, \alpha)$ converges necessarily to 0, that is $X \equiv 0$ is asymptotically stable.

Let $\alpha \geq \exp(\delta\omega) - 1$, then $\Gamma_\omega^{-1}(\{\alpha\}) = \{\emptyset\}$ and $X \equiv 0$ is the only periodic solution of (30). Moreover, let $X_0 > 0$, then $\Gamma_\omega(X_0) < \alpha$, that is

$$\frac{X_0}{\varphi_\omega(X_0)} - 1 < \alpha \Leftrightarrow X(\omega; X_0, \alpha) := (1 + \alpha)\varphi_\omega(X_0) > X_0.$$

Then, $(X(k\omega; X_0, \alpha))_{k \in \mathbb{N}^*}$ is an increasing sequence (Lemma 2) and either converges to a periodic solution or is unbounded (Proposition 2). $X(\cdot; X_0, \alpha)$ cannot converge to 0 and therefore diverges to $+\infty$.

Similarly, we show that if $\alpha < \alpha^*$, for any $X_0 > 0$, $(X(k\omega; X_0, \alpha))_{k \in \mathbb{N}^*}$ decreases. It follows that $0 < X(\cdot; X_0, \alpha) \leq \max_{t \in [0, \omega)} X(t; X_0, \alpha) < +\infty$. As a bounded solution of (30), $X(\cdot; X_0, \alpha)$ converges to a periodic solution of (30) (Proposition 2), and $X \equiv 0$ is the only one. \square

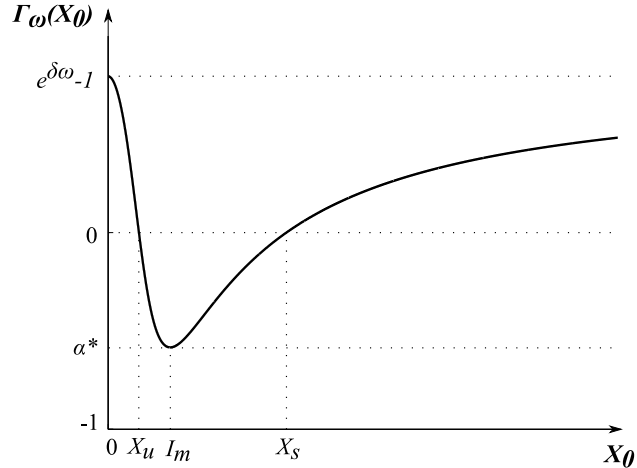


Figure 3: Graph of function Γ_ω with parameters given by (35), X_u , X_s as defined in Proposition 9, I_m from Lemma 11 and α^* from Theorem 2.

5.3 Numerical results

Here, we numerically illustrate the results of Theorem 2 by setting $\omega = 360$ min and using the following set of parameters, satisfying (\mathbf{H}_5) ,

$$\begin{aligned} \eta &= 0.05 \text{ mol.L}^{-1} \text{ min}^{-1}, \quad \theta = 35 \text{ mol.L}^{-1}, \\ \delta &= 4.1 \times 10^{-4} \text{ min}^{-1}, \quad n = 3. \end{aligned} \quad (35)$$

The graph of Γ_ω , defined in (8), with parameters (35) is shown on Figure 3. In particular, we observe that I , in Theorem 2, is reduced to $I = \{I_m\}$ with $I_m \approx 41.5 \text{ mol.L}^{-1}$, $X_u \approx 20.5 \text{ mol.L}^{-1}$, $X_s \approx 119 \text{ mol.L}^{-1}$, $\alpha^* \approx -0.116$ and $\exp(\delta\omega) - 1 \approx 0.159$.

On Figure 4, we illustrate by numerical simulations the 6 qualitatively different cases mentioned in Theorem 2, that is: (A) $\alpha < \alpha^*$ and all positive solutions of (30) converge to the constant solution $X \equiv 0$; (B) $\alpha = \alpha^*$ and for any $X_0 > I_m$ the solution $X(\cdot; X_0, \alpha^*)$ converges to the periodic solution $X(\cdot; I_m, \alpha^*)$ while for any $X_0 < I_m$, the solution $X(\cdot; X_0, \alpha^*)$ converges to the constant solution $X \equiv 0$; (C), (D), (E) are bistable cases: two stable periodic solutions are separated by an unstable one (in particular (D) corresponds to the case without impulse); (F) $\alpha > \exp(\delta\omega) - 1$ and all positive solutions of (30) diverge to $+\infty$.

In this section, we established some qualitative properties of the function Γ_ω , concluded on the existence and stability of periodic solutions of (30) under hypotheses (\mathbf{H}_3) , (\mathbf{H}_4) and (\mathbf{H}_5) and illustrated them with numerical simulations. In the following and last section, we replace (\mathbf{H}_4) by a weaker hypothesis, allowing for stochastic partitioning of the molecular content at cell division, and draw conclusions on the biological problem of protein repartition at cell division.

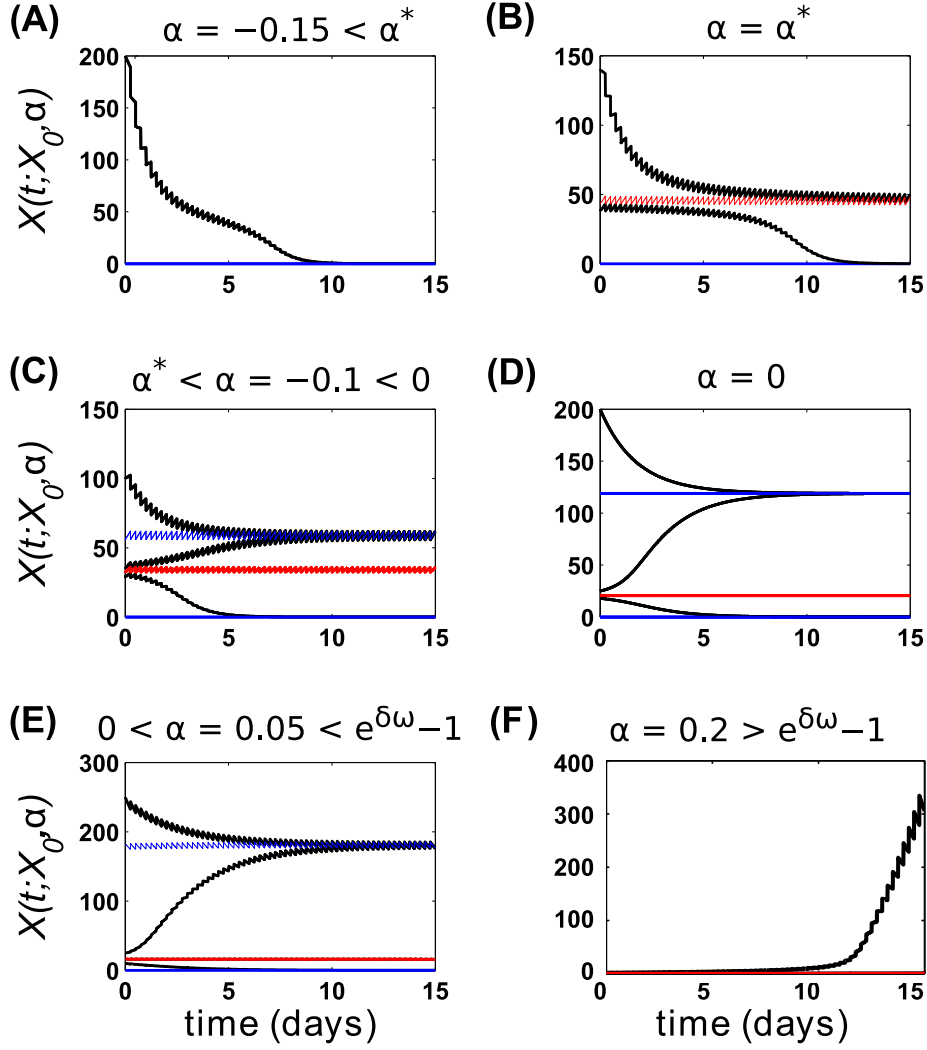


Figure 4: Solutions of System (30) from different initial values and for different values of α corresponding to cases presented in Theorem 2. Stable (resp. unstable) periodic solutions are plotted in blue (resp. red).

6 Application to cell fate decision

In this section, we use results from Section 5 to draw conclusions on the behaviour of (30) when we no longer consider hypothesis (\mathbf{H}_4) to be satisfied. Then, we propose an explanation, based on asymmetric partitioning of Tbet concentration at cell division, for the emergence of two qualitatively different pools of cells, with distinct fates, among a population generated by a single cell.

As done in Prokopiou et al. (2014) and Gao et al. (2016), we suppose that when a cell with concentration X for protein Tbet undergoes its k^{th} division, it gives birth to two daughters cells with concentrations $(1 + \alpha_k)X$ and $(1 - \alpha_k)X$ respectively. Bio-

logically, it would not be so relevant to consider the sequence $(\alpha_k)_{k \in \mathbb{N}^*}$ to be constant (hypothesis **(H₄)**). Consequently, we introduce the weaker hypothesis **(H₆)**,

(H₆) There exists $-1 < \alpha_{min} < \alpha_{max} < +\infty$ such that the sequence $(\alpha_k)_{k \geq 1}$ verifies, for all $k \in \mathbb{N}^*$, $\alpha_k \in [\alpha_{min}, \alpha_{max}] \subset (-1, +\infty)$.

According to Lemma 1, if **(H₆)** holds true, then for any given initial condition X_0 , the solution $X(\cdot; X_0, \alpha_k)$ of (1) is bounded by the two solutions $X(\cdot; X_0, \alpha_{min})$ and $X(\cdot; X_0, \alpha_{max})$ obtained with $m_k = \alpha_{min}$ and $M_k = \alpha_{max}$ respectively. Consequently, although **(H₄)** is very restrictive on parameters $(\alpha_k)_{k \in \mathbb{N}^*}$, studying Systems (1) or (30) with **(H₄)** provides bounds for the less restrictive case **(H₆)**.

The following proposition is then an immediate consequence of Lemma 1 and Theorem 2. Note that X_1, X_2, X_3, X_4 mentioned below are illustrated on Figure 2(A).

Proposition 10. *Let hypotheses **(H₃)**, **(H₅)** and **(H₆)** hold true. Let α^* from Theorem 2 and $\alpha_{min}, \alpha_{max}$ from **(H₆)** satisfy $\alpha^* < \alpha_{min} < 0 < \alpha_{max} < \exp(\delta\omega) - 1$. Then there exists $X_1, X_2, X_3, X_4 \in \mathbb{R}^+$ such that $\Gamma_\omega^{-1}(\{\alpha_{max}\}) = \{X_1, X_4\}$, $\Gamma_\omega^{-1}(\{\alpha_{min}\}) = \{X_2, X_3\}$ and*

$$0 < X_1 < X_u < X_2 < X_3 < X_s < X_4.$$

For the sake of simplicity, the periodic functions $X(\cdot; X_1, \alpha_{max})$, $X(\cdot; X_2, \alpha_{min})$, $X(\cdot; X_3, \alpha_{min})$ and $X(\cdot; X_4, \alpha_{max})$ are denoted hereunder by X_1^p , X_2^p , X_3^p and X_4^p respectively. Then, for any sequence $(\alpha_k)_{k \in \mathbb{N}}$ satisfying **(H₆)**,

- i) if $0 \leq X_0 < X_1$, $X(\cdot; X_0, \alpha_k)$ converges to zero.
- ii) if $X_1 < X_0 \leq X_2$, for all $t \geq 0$, $X(t; X_0, \alpha_k) \in [0, X_4^p(t)] \subset [0, X_4]$.
- iii) if $X_2 < X_0 < X_3$, for all $t \geq 0$, $X(t; X_0, \alpha_k) \in (X_2^p(t), X_4^p(t)) \subset (X_2, X_4)$. Moreover, for any $\epsilon > 0$ there exists $t^* > 0$ such that, for all $t > t^*$, $X(t; X_0, \alpha_k) \in (X_3^p(t) - \epsilon, X_4^p(t)) \subset (X_3 - \epsilon, X_4)$.
- iv) if $X_3 < X_0 \leq X_4$, for all $t \geq 0$, $X(t; X_0, \alpha_k) \in (X_3^p(t), X_4^p(t)) \subset (X_3, X_4)$.
- v) if $X_4 < X_0$, for all $t \geq 0$, $X(t; X_0, \alpha_k) \in (X_3^p(t), X_0) \subset (X_3, X_0)$, moreover for all $\epsilon > 0$ there exists $t^* > 0$ such that for all $t > t^*$, $X(t; X_0, \alpha_k) \in (X_3^p(t), X_4^p(t) + \epsilon) \subset (X_3, X_4 + \epsilon)$.

Remark 6. *For straightforward biological reasons, α_k must be chosen in $(-1, 1)$ so the protein concentration in the daughter cell is positive and at most twice as large as that observed in the mother cell. From now on we consider that for all $k \geq 1$, $\alpha_k \in [q - 1, 1 - q]$ where $q \in (0, 1)$. For example, α_k can be randomly chosen from the uniform law $\mathcal{U}_{[q-1, 1-q]}$ (Prokopiou et al., 2014; Gao et al., 2016).*

Hereinbelow, the concentration of protein Tbet in a cell is associated to its level of differentiation. High concentration of Tbet ($X \approx X_s$) corresponds to an effector phenotype while low concentration ($X \approx 0$) corresponds to a memory phenotype (Joshi et al., 2007; Kaeck and Cui, 2012; Lazarevic et al., 2013). In the following, we say that the fate of a cell is irreversible if Tbet concentration in that cell, modelled by System (30), is definitively higher or definitively lower than X_u . Using the results from Theorem 2 and Proposition 10, we discuss how the value of q , that is, the degree

of asymmetry in the process of protein distribution (see Remark 6), impacts the cell population generated by a single cell and cell fate reversibility. Note that the smaller q , the more asymmetric the distribution.

Let (\mathbf{H}_3) , (\mathbf{H}_5) and (\mathbf{H}_6) hold true with $[\alpha_{min}, \alpha_{max}] = [q - 1, 1 - q]$, $q \in (0, 1)$. According to Theorem 2, the solutions of (30) are bounded if and only if $\alpha_{max} < \exp(\delta\omega) - 1$, that is $q > 2 - \exp(\delta\omega)$. Indeed, if it is not the case, there exists $X_0 > 0$ and a sequence $(\alpha_k)_{k \in \mathbb{N}}$ satisfying (\mathbf{H}_6) such that $X(\cdot; X_0, \alpha_k)$ tends to $+\infty$ (for example, $\alpha_k \equiv \alpha_{max}$). Then, it is reasonable to consider $q > 2 - \exp(\delta\omega)$. Similarly, if $\alpha_{min} < \alpha^*$ (that is $q > 1 + \alpha^*$), for any $X_0 > 0$ there exists a sequence $(\alpha_k)_{k \in \mathbb{N}}$ satisfying (\mathbf{H}_6) such that $X(\cdot; X_0, \alpha_k)$ tends to 0 (for example $\alpha_k \equiv \alpha_{min}$).

On the contrary, if we assume $1 > q > \max(1 + \alpha^*, 2 - \exp(\delta\omega))$, then $[q - 1, 1 - q] \subset (\alpha^*, \exp(\delta\omega) - 1)$ and Proposition 10 holds. In that case, if there exists $K \in \mathbb{N}$ such that $X(K\omega) < X_1$ (resp. $X(K\omega) > X_2$), then for any $(\alpha_k)_{k \geq K}$ satisfying (\mathbf{H}_6) , the concentration $X(\cdot; X_0, \alpha_k)$ of Tbet in the cell's progeny converges to zero (resp. for any $\epsilon > 0$, there exists $K^* > K$ such that for all $k > K^*$, $X(k\omega; X_0, \alpha_k) \in [X_3 - \epsilon, X_4 + \epsilon]$). Consequently the whole cell's progeny will develop a memory (resp. effector) profile, characterised by a low (resp. high) concentration of Tbet. If $X_1 < X(K\omega) < X_2$, cell fate depends on the values of $(\alpha_k)_{k \geq K}$.

In conclusion, if the asymmetry in the repartition of proteins at division is low enough ($1 > q > \max(1 + \alpha^*, 2 - \exp(\delta\omega))$), there exist critical points in the process of differentiation toward memory or effector cell beyond which the differentiation is irreversible for the considered cell and its progeny. Finally, at any time $t = k\omega$, $k \in \mathbb{N}$, the asymptotic state (high or low Tbet level) of a cell's lineage remains undetermined if and only if $X_1 < X(t) < X_2$.

Those results are illustrated on Figure 5.A. Using parameter values from (35), $\omega = 900$ min and $q = 0.8$, such that $1 > q > \max(1 + \alpha^*, 2 - \exp(\delta\omega)) \approx 0.74$. Figure 5.A represents the evolution of the concentration of Tbet in the whole progeny generated by an initial cell with concentration $X_0 = 21 \in (X_1, X_2)$. At each cell division, a coefficient α_k is drawn from the uniform law on $(q - 1, 1 - q)$ and sets the degree of asymmetry of the division. After a few divisions, one observes the emergence of a pool of cells with a low (lower than X_1) concentration for Tbet, associated to irreversible differentiation in memory cells, and a pool of cells with a high (higher than X_2) concentration for Tbet, associated to irreversible differentiation in effector cells. However, at time $t = 12\omega = 180h$, 34 cells are still characterised by a Tbet concentration between X_1 and X_2 : therefore their fates are undetermined (see for instance the cell with the orange trajectory on Figure 5.A).

In practice, the cell cycle length is very short following activation but increases in the following days, when the cells have undergone some divisions (Yoon et al., 2010). It is then meaningful to consider that the cell cycle length increases after each division.

In that case, it is easy to verify that the conclusions of Proposition 10 remain true. In particular, for fixed values of α_{min} and α_{max} , X_1 and X_2 from Proposition 10 respectively increases and decreases when the cycle length ω increases (and converge to X_u if ω tends to infinity). Consequently, while the cycle length increases, the interval of Tbet concentration $[X_1, X_2]$ for which the cell fate remains undetermined shrinks. This suggests that increasing cell cycle length not only slows down the expansion of a cell population but also precipitates cell fate decision.

This is illustrated on Figure 5.B. Parameter values are identical to those from

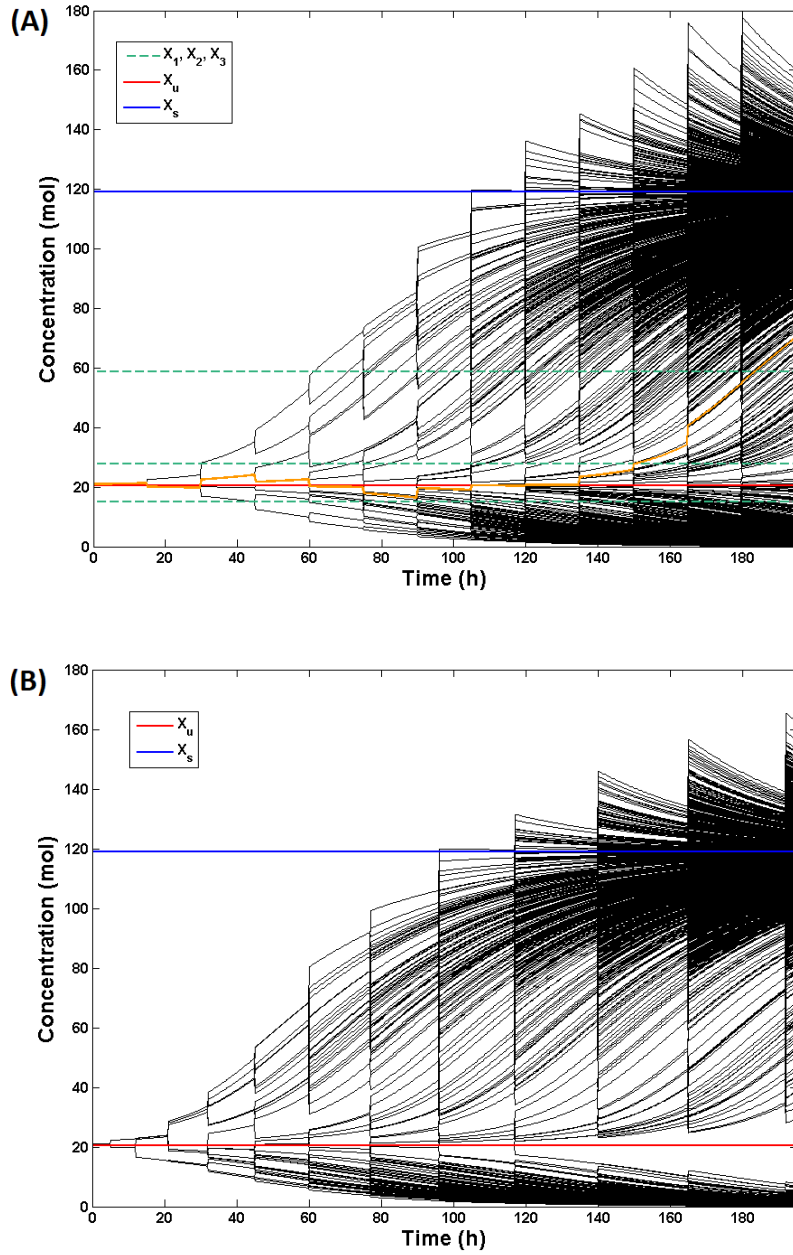


Figure 5: Concentration of Tbet in a cell population generated by an initial cell with $X_0 = 21$ mol. (A) Constant cell cycle length $\omega = 900$ min. (B) Increasing cell cycle length $\omega_k = 300 + 120(k)$ min, $k \in \mathbb{N}$. Dashed green lines in (A), from bottom to top: $X_1 \approx 12$ mol.L⁻¹, $X_2 \approx 32$ mol.L⁻¹, $X_3 \approx 56$ mol.L⁻¹ ($X_4 \approx 361$ mol.L⁻¹, not shown). Orange trajectory in (A) highlights a case where Tbet concentration crosses X_u several times and finally leaves the interval $[X_1, X_2]$ such that, from there, cell fate (effector) is irreversible. Red thick straight line: $X_u \approx 20$ mol.L⁻¹. Blue straight line: $X_s \approx 119$ mol.L⁻¹.

Figure 5.A. except that the cell cycle length starts from 300 min and increases by two hours at each division, then cell cycle lengths are given by $\omega_k = 300 + 120k$ min for $k \in \mathbb{N}$. In particular at time $t = 45h$ cells enter in a cycle of $\omega_5 = 900$ min as in

Figure 5.A. As cell cycle length increases, the interval of Tbet concentration in which cell fate remains undetermined shrinks (not shown) so that more and more cells adopt a definitive fate. At time $t = 160$ h, all the cell fates are irreversible.

Discussion

In this paper, we studied the effect of unequal molecular partitioning at cell division on the emergence of phenotypic heterogeneity in a population of CD8 T-cells. To do so, we introduced the impulsive system (1), characterised by a specific form of impulses but a general form of the reaction term. We proved results on the existence and stability of periodic solutions which represent attractors of the solutions and consequently are biologically relevant. Most of those results rely on the properties of the flow of an autonomous differential equation. Nevertheless, most of the time, an explicit expression of this flow cannot be determined. We then focused on the properties of the flow and gave in Theorem 1 sufficient conditions for the flow to be convex. Those results were applied in Sections 5 and 6 to the case of protein Tbet regulation (described by an autonomous differential equation) and partitioning (described as impulse) in a CD8 T-cell lineage. We investigated how the degree of asymmetry in the molecular partitioning process can affect the differentiation of a CD8 T-cell toward effector or memory phenotype in Theorem 2 and Proposition 10. Associating high concentration of Tbet with effector phenotype and low concentration of Tbet with memory phenotype (Joshi et al., 2007; Kaech and Cui, 2012; Lazarevic et al., 2013), we showed that if the degree of asymmetry is small enough, either the cell concentration of Tbet belongs to a non-trivial interval and the cell can still generate both effector and memory cell, or the cell differentiation is irreversible.

This model is of course too simple to provide biologically realistic quantitative predictions, partly because the process of CD8 T-cell differentiation is too complex to be reduced to a Tbet-mediated differentiation process and partly because stochastic partitioning is not the only source of heterogeneity. In this regard, Huh and Paulsson (2010) emphasised that different sources of heterogeneity (e.g. stochastic partitioning and gene expression noise) have redundant effects and therefore, evaluating the contribution of each source is a challenging task. It could then be instructive to consider an impulsive system in the form of (30) with a stochastic right-hand side function, accounting for gene expression noise. Regarding the simplicity of our model, it is however noticeable that it allows to give insight into a complex biological process by providing theoretical background and original answers to a paramount biological question, and consequently contributes to fill the gap between experimental biology and mathematics.

Regarding the convexity of the flow of an autonomous differential equation, we gave necessary and sufficient conditions to conclude on the strict convexity of the flow when the reaction term of the differential equation is a piecewise linear function (Proposition 6). However, if the reaction term is continuously differentiable, we only concluded on the convexity (not necessarily strict) of the flow in Theorem 1. Based on these results and numerical simulations, one can hypothesise that strict convexity can actually be obtained under Theorem 1's hypotheses. Note that, in that case, Lemma 9 would lead to the strict convexity (resp. concavity) of the flow on $(0, X_c)$ (resp. $(X_c, +\infty)$) and,

as an immediate consequence of Definition 4, we could conclude that I is reduced to a single point, as observed in Figure 3.

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References

- Bainov, D. D., Lakshmikantham, V. and Simeonov, P. (1989), *Theory of Impulsive Differential Equations*, 273 p., World Scientific, Singapore.
- Bainov, D. D. and Simeonov, P. (1993), *Impulsive differential equations: periodic solutions and applications*, 228 p., Longman Scientific & Technical, Harlow.
- Block, D. E., Eitzman, P. D., Wangensteen, J. D. and Srienc, F. (1990), Slit scanning of *saccharomyces cerevisiae* cells: quantification of asymmetric cell division and cell cycle progression in asynchronous culture, *Biotechnol. Prog.* 6(6), 504-512.
- Bocharov, G., Luzyanina, T., Cupovic, J. and Ludewig, B. (2013), Asymmetry of cell division in CFSE-based lymphocyte proliferation analysis, *Front. Immunol.* 4, 264.
- Chang, J. T., Ciocca, M. L., Kinjyo, I., Palanivel, V. R., McClurkin, C. E., DeJong, C. S., Mooney, E. C., Kim, J. S., Steinell, N. C., Oliaro, J., Yin, C. C., Florea, B. I., Overkleeft, H. S., Berg, L. J., Russell, S. M., Koretzky, G. A., Jordan, M. S. and Reiner, S. L. (2011), Asymmetric proteasome segregation as a mechanism for unequal partitioning of the transcription factor T-bet during T lymphocyte division, *Immunity* 34(4), 492-504.
- Chang, J. T., Palanivel, V. R., Kinjyo, I., Schambach, F., Intlekofer, A. M., Banerjee, A., Longworth, S. A., Vinup, K. E., Mrass, P., Oliaro, J., Killeen, N., Orange, J. S., Russell, S. M., Weninger, W. and Reiner, S. L. (2007), Asymmetric T lymphocyte division in the initiation of adaptive immune responses, *Science* 315(5819), 1687-1691.
- Dishliev, A., Dishlieva, K. and Nenov, S. (2012), *Specific asymptotic properties of the solutions of impulsive differential equations. Methods and applications*, 291 p., Academic Publications, Ltd.
- Faria, T. and Oliveira, J. J. (2016), On stability for impulsive delay differential equations and application to a periodic Lasota-Ważewska model, *Discrete Cont. Dyn. Syst. Series B* 21(8), 2451-2472.
- Gao, X., Arpin, C., Marvel, J., Prokopiou, S. A., Gandrillon, O. and Crauste, F. (2016), IL-2 sensitivity and exogenous IL-2 concentration gradient tune the productive contact duration of CD8+ T cell-APC: a multiscale modeling study, *BMC. Syst. Biol.* 10(1), 77.

- Huh, D. and Paulsson, J. (2010), Non-genetic heterogeneity from stochastic partitioning at cell division, *Nat. Genet.* 43(2), 95-100.
- Joshi, N. S., Cui, W., Chandele, A., Lee, H. K., Urso, D. R., Hagman, J., Gapin, L. and Kaech, S. M. (2007), Inflammation directs memory precursor and short-lived effector CD8+ T cell fates via the graded expression of T-bet transcription factor, *Immunity* 27(2), 281-295.
- Kaech, S. M. and Cui, W. (2012), Transcriptional control of effector and memory CD8+ T cell differentiation, *Nat. Rev. Immunol.* 12(11), 749-761.
- Kanhere, A., Hertweck, A., Bhatia, U., Gkmen, M. R., Perucha, E., Jackson, I., Lord, G. M. and Jenner, R. G. (2012), T-bet and GATA3 orchestrate Th1 and Th2 differentiation through lineage-specific targeting of distal regulatory elements, *Nat. Commun.* 3, 1268.
- Kou, C., Adimy, M. and Ducrot, A. (2009), On the dynamics of an impulsive model of hematopoiesis', *Math. Model. Nat. Phenom.* 4(02), 68-91.
- Kuehn, C. (2015), *Multiple time scale dynamics*, 814 p., Springer, New York.
- Lazarevic, V., Glimcher, L. H. and Lord, G. M. (2013), T-bet: a bridge between innate and adaptive immunity, *Nat. Rev. Immunol.* 13(11), 777-789.
- Li, W.-T. and Huo, H.-F. (2005), Global attractivity of positive periodic solutions for an impulsive delay periodic model of respiratory dynamics, *J. Comput. Appl. Math.* 174(2), 227-238.
- Liu, X. and Chen, L. (2003), Complex dynamics of Holling type II Lotka-Volterra predator-prey system with impulsive perturbations on the predator, *Chaos Solitons Fractals* 16(2), 311-320.
- Liu, X. and Takeuchi, Y. (2007), Periodicity and global dynamics of an impulsive delay Lasota-Ważewska model, *J. Math. Anal. Appl.* 327(1), 326-341.
- Liu, Z. and Zhong, S. (2012), An impulsive periodic predator-prey system with Holling type III functional response and diffusion, *Appl. Math. Model.* 36(12), 5976-5990.
- Luzyanina, T., Cupovic, J., Ludewig, B. and Bocharov, G. (2013), Mathematical models for CFSE labelled lymphocyte dynamics: asymmetry and time-lag in division, *J. Math. Biol.* 69(6-7), 1547-1583.
- Mantzaris, N. V. (2006), Stochastic and deterministic simulations of heterogeneous cell population dynamics, *J. Theor. Biol.* 241(3), 690-706.
- Mantzaris, N. V. (2007), From single-cell genetic architecture to cell population dynamics: Quantitatively decomposing the effects of different population heterogeneity sources for a genetic network with positive feedback architecture, *Biophys. J.* 92(12), 4271-4288.
- Mil'man, V. D. and Myshkis, A. D. (1960), On the stability of motion in the presence of impulses, *Siberian Math. J.* 1, 233-237.

- Prokopiou, S. A., Barbarroux, L., Bernard, S., Mafille, J., Leverrier, Y., Arpin, C., Marvel, J., Gandrillon, O. and Crauste, F. (2014), Multiscale modeling of the early CD8 T-cell immune response in lymph nodes: An integrative study, *Computation* 2(4), 159–181.
- Saker, S. and Alzabut, J. (2007), Existence of periodic solutions, global attractivity and oscillation of impulsive delay population model, *Nonlinear Anal. Real. World Appl.* 8(4), 1029-1039.
- Sennerstam, R. (1988), Partition of protein (mass) to sister cell pairs at mitosis: a re-evaluation, *J. Cell Sci* 90(2)(11), 301-6.
- Tang, S. and Chen, L. (2002), Density-dependent birth rate, birth pulses and their population dynamic consequences, *J. Math. Biol.* 44(2), 185-199.
- Wang, Q., J Klinke, D. and Wang, Z. (2015), CD8 + T cell response to adenovirus vaccination and subsequent suppression of tumor growth: modeling, simulation and analysis, *BMC. Syst. Biol.* 9(1), 27.
- Wherry, E. J. and Ahmed, R. (2004), Memory CD8 T-cell differentiation during viral infection, *J. Virol.* 78(11), 5535–5545.
- Yan, J. (2003), Existence and global attractivity of positive periodic solution for an impulsive LasotaWazewska model, *J. Math. Anal. Appl.* 279(1), 111-120.
- Yan, J. and Zhao, A. (1998), ‘Oscillation and stability of linear impulsive delay differential equations, *J. Math. Anal. Appl.* 227(1), 187-194.
- Yan, J., Zhao, A. and Nieto, J. (2004), Existence and global attractivity of positive periodic solution of periodic single-species impulsive Lotka-Volterra systems, *Math. Comput. Model.* 40(5-6), 509-518.
- Yan, J., Zhao, A. and Yan, W. (2005), Existence and global attractivity of periodic solution for an impulsive delay differential equation with Allee effect, *J. Math. Anal. Appl.* 309(2), 489-504.
- Yoon, H., Kim, T. S. and Braciale, T. J. (2010), The cell cycle time of CD8+ T cells responding in vivo is controlled by the type of antigenic stimulus, *PLoS ONE* 5(11), e15423.
- Zhang, X., Tang, S., Cheke, R. A. and Zhu, H. (2016), Modeling the effects of augmentation strategies on the control of dengue fever with an impulsive differential equation, *Bull. Math. Biol.* 78(10), 1968-2010.