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Stochastic Hybrid Automata with delayed transitions to model biochemical systems with delays

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ABSTRACT

To study the effects of a delayed immune-response on the growth of an immunogenic neoplasm we introduce Stochastic Hybrid Automata with delayed transitions as a representation of hybrid biochemical systems with delays. These transitions abstractly model unknown dynamics for which a constant duration can be estimated, i.e. a delay. These automata are inspired by standard Stochastic Hybrid Automata, and their semantics is given in terms of Piecewise Deterministic Markov Processes. The approach is general and can be applied to systems where (*i*) components at low concentrations are modeled discretely (so to retain their intrinsic stochastic fluctuations), (*ii*) abundant component, e.g., chemical signals, are well approximated by mean-field equations (so to simulate them efficiently) and (*iii*) missing components are abstracted with delays. Via simulations we show in our application that interesting delay-induced phenomena arise, whose quantification is possible in this new quantitative framework.

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1. Introduction

Tumor-immune system interaction involves a number of distinct components, such as effector cells of the innate and adaptive immune systems (e.g., macrophages, natural killers, cytotoxic T lymphocytes) and chemical signals (e.g., cytokines). The immune response to a tumor is triggered by specific neo-antigens, eventually created by a large number of genetic and epigenetic events characterizing tumor cells [43]. Once triggered, the immune system may control and in some case eliminate tumors: this is the so-called *immune surveillance* hypothesis [28]. However, this extremely complex interaction has other possible outcomes, e.g., neoplasm evasion from control, neoplasm constriction in an oscillatory regime or in a microscopic undetectable "dormant" steady-state [23].

Many attempts at modeling this interplay have been pursued by using *Ordinary Differential Equations* (ODEs) or *Delay Differential Equations* (DDEs), e.g., see [37,38,12,45] and references therein. However, to study a neoplasm and its interaction with few immune-system effector cells, an individual-based stochastic representation of the cellular populations seems to be more appropriate. Unfortunately, the exchange of large quantities of chemicals with many kinds of cells (e.g., immune system effector cells, cells of the healthy tissues, endothelial cells), often present in an extremely higher number than cells, makes this interaction "multiscale". Thus, the usual discrete techniques to represent chemical systems, e.g., [30], do not allow an efficient model simulation. In this respect, a hybrid model combining a discrete representation of components at low/intermediate concentrations (e.g., cells) with mean-field equations for abundant entities (e.g., chemicals) seems to be

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the best setting to study this interaction [14,24]. In particular, it allows one to quantitatively estimate the effects of the *intrinsic stochastic fluctuations* of the discrete components which, in the tumor-immune system case, determine the outcome of the interaction. In [14], for instance, by resetting an ODE model of effector cells, tumor cells and cytokines in a hybrid framework, the probability of tumor eradication was evaluated. This biologically plausible outcome was not predictable by the original ODE model formulation [37]. In this sense, a hybrid setting is not only a better computational choice, but can also provide more *informative forecasts*.

A further step can be done to consider, in a hybrid setting, other techniques often used to model chemical systems. We focus here on *delays* used to approximate missing dynamical components, at any level of abstraction. Generally, they are used to abstract complex and often only partially known sequential dynamics in a single step, once an estimate of the duration of the dynamics is available, e.g., [12,45]. In the tumor-immune system case, delays can be used to describe the unavoidable and quite remarkably long lag period in the immune-response, as induced by chemical transportation of signals and the time needed for differentiation/division of effector cells [15]. Even though this abstraction is macroscopical and simplistic, it provides useful insights of this fundamental and complex interaction, especially when a full phenomenological model is missing. Delays are available, in various form, in deterministic models, or in individual-based models as *Continuous-Time Markov Chains* (CTMCs) with deterministic delays [5,4,8]. In terms of formal languages, they are supported in bio-specific process algebras, e.g., see [17] or more generally [13], but their characterization in hybrid systems is, to the best of our knowledge, missing.

Given these premises, in this paper we extend the well-known framework of *Stochastic Hybrid Automata* (SHA [11]) to model *hybrid biochemical systems with delays*. Technically, these automata extend classic SHAs with *non-Markovian delayed transitions* joining both exponential and deterministic distributions, in the control part. In this paper: (*i*) Gillespie models of stochastic chemical reactions [30] are related to SHAs, to settle the background, and (*ii*) Gillespie models with delays [5] are related to SHAs extended with delayed transitions. The semantics of these automata is given in terms of *Piecewise Deterministic Markov Processes* (PDMPs [26]), so to have a well-defined characterization in terms of stochastic processes, as it is for SHAs [6], and an equivalence with delay CTMCs [8] is provided under some conditions.

The paper is structured as follows. In Section 2 background is introduced. In Section 3 our case study is introduced, so to make clear to the reader what we want to model. In Section 4 we define SHAs to model hybrid biochemical systems and show their mapping to PDMPs, in Section 5 we do the analogs for SHAs with delays. In Section 6 we apply such automata to the case study introduced in Section 3. Via numerical analyses (*i*) we study the effect of various delays on tumor mass growth, (*ii*) we quantitatively determine eradication times as probability distributions, (*iii*) perform sensitivity analysis on tumor mass and delay amplitude and (*iv*) we prove, in the oscillatory regime, the existence of a transitory state transition resulting in delay-induced tumor eradication, unpredictable in either the mean-field or the hybrid non-delayed representation of the model.

2. Background

Some notions from stochastic chemical systems are introduced [30]. We consider a system of N distinct species $\mathcal{S} = \{S_1, \ldots, S_N\}$, and denote its state $\mathbf{x}$; component $\mathbf{x}_j$ counts the copies of S_j. The stochastic process $\mathbf{X}_t = \{\mathbf{X}(t) \mid t \in \mathbb{R}\}$ takes values in $\mathbb{N}^N$, if it is generated by a *Continuous Time Markov Chain* (CTMC). When it is hybrid, some of its components take values in $(\mathbb{R}^{\geqslant 0})^N$.

A set of reactions $\mathcal{R} = \{R_1, \ldots, R_M\}$ is described by a *stoichiometry matrix* $M = [\, \nu_1 \quad \ldots \quad \nu_M \,]$ where $\nu_i \in \mathbb{Z}^N$ for $i = 1, \ldots, M$. Each ν_j is a *stoichiometry vector*: when R_i fires in $\mathbf{x}$ it yields the new state $\mathbf{x} + \nu_i$. Each reaction is further described by a *propensity function* $a_i(t)$ so that $a_i(t)\,dt$ is the probability of R_i to fire at time t, within the infinitesimal time $[t, t + dt)$. These are defined according to either well-known or custom kinetics, e.g., mass-action or Michaelis–Menten.[2] When $\mathbf{X}_t$ is time-homogenous we simply write $a_i(\mathbf{x})$ to make $\mathbf{x}$ explicit.

Definition 1. We denote a *chemical system* in this sense as $(\mathcal{R}, \mathcal{S}, M)$.

It is generally convenient to distinguish between the "cause" (the reactants, ν_i^r) and the "effect" (the products, ν_i^p) of a reaction, so we write $\nu_i = -\nu_i^r + \nu_i^p$. In this formulation R_j can fire in $\mathbf{x}$ if enough reactants are present, i.e. if $\nu_i^r \preccurlyeq \mathbf{x}$ where $\preccurlyeq$ is the standard component-wise ordering. So, in a logical sense reactants closely mimicking the biochemical reality are a necessary condition to fire a reaction. The evolution of a chemical system is often evaluated algorithmically realizing a trajectory of $\mathbf{X}_t$ given an initial condition $\mathbf{X}(t_0) = \mathbf{x}_0$, see [30].

Such a trajectory is defined over a state space, which we assume to be *countable*, and that is defined constructively by this operator.

[2] The law of mass-action states that the rate at which a reaction occurs is proportional to the product of the concentrations of its reactants (see [30] for a detailed derivation). Mass-action propensity functions have some desirable properties, e.g., they are *Lipschitz continuous*.

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Assumption 1 *(Reachable state space).* Let $(\mathcal{R}, \mathcal{S}, M)$ be a chemical system with *initial state* $\mathbf{x}_0$, its *reachable state space* $\mathcal{X}_\mathcal{R}$ is recursively computed by

$$\rho_\mathcal{R}^{(0)} = \{\mathbf{x}_0\}, \qquad \rho_\mathcal{R}^{(i+1)} = \bigcup_{j \in \mathcal{R}} \left\{ \mathbf{x} + \nu_j \mid \nu_j^r \preccurlyeq \mathbf{x}, \mathbf{x} \in \rho_\mathcal{R}^{(i)} \right\} \cup \rho_\mathcal{R}^{(i)}. \tag{1}$$

Notice that this is a slight rephrase of the canonical notion of state space which, in general, is independent of the initial condition $\mathbf{x}_0$. When the set of reactions we refer to is clear, we write $\rho^{(i)}$.

Example 1. Consider a unique reaction $A \to B$ with state vector containing species in alphabetical order. We have $\nu = [-1, 0]^{tr} + [0, 1]^{tr} = [-1, 1]^{tr}$. Let $\mathbf{x}_0$ be $(2, 0)$. We have $\mathcal{X}_\mathcal{R} = \rho^{(3)}$ since $\rho^{(0)} = \{\mathbf{x}_0\}$, $\rho^{(1)} = \{\mathbf{x}_0, (1, 1)\}$, and $\rho^{(2)} = \{\mathbf{x}_0, (1, 1), (0, 1)\} = \rho^{(3)}$.

If $\mathcal{X}_\mathcal{R}$ is finite (e.g. ρ has a *least fixed point* as in the above example) model-checking techniques can be applied [1,2]. However, $\mathcal{X}_\mathcal{R}$ is often too large and it might be convenient to reduce it to increase the performance of an analysis. Biological knowledge, for example, can be used to cut-off regions of $\mathcal{X}_\mathcal{R}$ where species grows unrealistically. As alternative, computational techniques can be used: stochastic simulation [20], levels to aggregate intervals of concentrations [17] or the finite state projection method which provides a systematic means of approximating a full system using finite-dimensional subsystems [41].

We remark that building the whole $\mathcal{X}_\mathcal{R}$ is not necessary to perform, for instance, simulations which rely on an on-the-fly generation of a trajectory over $\mathcal{X}_\mathcal{R}$. This is what we actually do for the case study in Section 6.

3. Case study: Delays-induced anti-tumor immune-response

We present here the application which motivated this work: to study the effect of a delayed immune-response on the growth of a neoplasm. The model we study is simple, but contains all the features which inspired this work.

We consider two cell populations, *tumor cells* and immune system *effectors* (T-cells), and the molecular cytokine *Interleukins-2* (IL-2) I. Among many, the role of IL-2, a cytokine expressed by white blood cells (leukocytes), is to induce the proliferation of responsive T-cells and stimulate the production of antibodies. Immune-response plays the role of the tumor-induced recruitment of the effector cells, the biophysical process we want to abstract with a delay [29,44]. Modeling this interaction precisely would require to involve a huge number of molecules we do not consider, the abstraction is then necessary to take into account molecular agents not yet completely characterized; of course, the actual delay will have to be consistent with the timings of the biophysical process it abstracts.

Many different "technical" choices can be done to define such a model. Along the lines of [37,38], let us denote the *population densities* as T_* (tumor cells) and E_* (effector cells), and the interleukins as I_*. A deterministic DDE-based representation of this model is

$$\begin{aligned}
\dot{T}_* &= rT_*(1 - bT_*) - \frac{p_T T_* E_*}{g_T + T_*}, \\
\dot{E}_* &= \frac{p_E I E_*}{g_E + I} - \mu_E E_* + cT_*(t - \theta), \\
\dot{I}_* &= \frac{p_I T_* E_*}{g_I + T_*} - \mu_I I_*.
\end{aligned} \tag{2}$$

In these DDEs, $cT_*(t - \theta)$ is the term modeling the tumor-induced recruitment of effectors. Here, c is a measure of the immunogenicity of the tumor (i.e. "a measure of how different the tumor is from itself" [37]), and θ is the reaction delay. Biologically, c corresponds to the average number of antigens, i.e. secreted antibodies and/or surface receptors on immune system T-cells, expressed by each tumor cell. The lifespan of effectors is μ_E^{-1}, and the average degradation time for IL-2 is μ_I^{-1}. Michaelis–Menten kinetics rule (i) IL-2 production by the tumor immune-system interplay, (ii) effectors recruitment by their interplay with IL-2 and (iii) effectors-induced tumor death. Notice that tumor growth is logistic with plateau $1/b$, that interleukins stimulate the proliferation and recruitment of the effectors and, in turn, are synthesized as the result of the interaction between tumor and immune-system.

The deterministic representation of the tumor-immune system interaction would be suitable only if all the cellular species were so high in number to neglect their intrinsic stochastic fluctuations [7,21], a possibly controversial fact since we study a neoplasm starting with very few cells. Thus, switching these DDEs to an individual-based model is necessary. In principle, one could convert all the species concentrations to *numbers* (by means of a reference volume), and switch to a CTMC with delays [13,4]. With T, E and I being the species converted to numbers, one could derive the set of reactions analogous to the DDEs (as explained, e.g., in [14]). However, as noted in [14] this conversion yields a number of interleukins orders of magnitude bigger than the number of cells, so the CTMC approach would starve in simulating the reactions concerning IL-2.

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The right compromise between speed of simulation of IL-2 dynamics, and ability to capture intrinsic cellular fluctuations seems to be a hybrid approach where cellular populations are discrete, and interleukins are continuous. In Section 6 we will combine these two representations in a hybrid setting, yielding hybrid automata with the delay θ. In brief, these automata will have a discrete control part acting as a counter for the cellular species E and T, and a vector field to describe I_*. The automata will switch probabilistically among the discrete states, and sometimes it might be involved in a delayed transition modeling the immune-response. We will show that by introducing a biologically realistic value for θ some interesting phenomena arise, whose quantification is possible only in a stochastic framework, not in the DDE representation. Also, we show that one of these phenomena is observable only in the presence of a delayed immune-response, so to stress (*i*) the importance of using hybrid systems, when possible, and (*ii*) using delays as an effective abstraction technique.

4. Stochastic Hybrid Automata for chemically reacting systems

Stochastic Hybrid Automata (SHA) add randomness to deterministic Hybrid Automata [19] in various, possibly combined, ways. A common approach switches from ordinary to stochastic equations in the "continuous" part of the automaton [35], while keeping the transitions involving the control part deterministic. Other approaches combine deterministic flows with probabilistic transitions [6,10] or all the above [11].

We recall the definition of SHA as given in [6]. In this case, we restrict to SHAs with no invariant conditions on the real variables or guarded transitions.

Definition 2 *(Stochastic Hybrid Automata with no invariant conditions).* An SHA is a tuple $(Q, V, \delta, \mathcal{J}, \mathcal{V}, [q_0, \mathbf{v}_0, t_0])$ where:

- $Q = \{q_1, \ldots, q_k\}$ is a finite set of *modes*;
- $V = \{v_1, \ldots, v_r\}$ is a finite set of *real variables*, whose values at time t are referred to as $V(t) \in \mathbb{R}^r$;
- $\delta \subseteq Q \times Q$ is a *transition relation* between modes;
- $\mathcal{J} : Q \to (\mathbb{R}^+)^{\mathbb{R}^r \times Q}$ associates with each mode q a *rate function* $\mathcal{J}_q(\mathbf{v}, q')$ defining the probability of jumping from q to q' when $V(t) = \mathbf{v}$;
- $\mathcal{V} : Q \to (\mathbb{R}^r)^{\mathbb{R}^r}$ maps (deterministic) *vector fields* to modes;
- $[q_0, \mathbf{v}_0, t_0]$ is an *initial condition*: $q_0 \in Q$ is the initial mode and $(t_0, \mathbf{v}_0)$ is the initial condition to determine the flow so $t_0 \in \mathbb{R}$, $\mathbf{v}_0 \in \mathbb{R}^r$.

Usually, a mapping between modes and discrete variables defines the "quantitative" interpretation of the control part of the SHA, e.g. in our reference application context each mode counts the amount of a discrete species. In what follows, we make such a mapping explicit only when we translate a chemical system to an SHA, not when we introduce the modeling framework. Finally, to give a sound semantics to an SHA we require each rate function $\mathcal{J}$ to be locally integrable, and each vector field $\mathcal{V}(q)$ to be (locally) Lipschitz continuous.

SHA executions switch probabilistically between modes while left-discontinuous right-continuous (i.e. càdlàg) paths of the random variable $V(t)$ are determined. These executions are conveniently defined in terms of a *Piecewise Deterministic Markov Process* (PDMP [26]). We recall their definition according to [7].

Definition 3 *(PDMP).* A PDMP is a tuple $(\mathbf{Z}, Q, \mathbf{Y}, E, \phi, \lambda, R)$ such that:

- $\mathbf{Z}$ is a set of discrete variables taking values in the countable set $Q \subset \mathbb{R}^k$, the set of *modes* or *discrete states*, thus $q \in Q$ is of the form $(z_1, \ldots, z_k)$. $\mathbf{Y}$ is an n-dimensional vector real variable. The domain of each mode is the open set $E_q \subset \mathbb{R}^n$, and the *hybrid state space* is $E = \bigcup_{q \in Q} \{q\} \times E_q$. A point $\mathbf{x} \in E$ is $\mathbf{x} = (q, \mathbf{y})$, $\mathbf{y} \in E$;
- with each mode $q \in Q$ a *vector field* $F_q : E_q \to R^n$ is associated. The ODE $\dot{\mathbf{y}} = F_q(\mathbf{y})$ is assumed to have a unique solution from each $\mathbf{y}_0 \in E_q$. The *flow* $\phi_q : \mathbb{R}^+ \times E_q \to R^n$ of F_q is assumed continuous in both arguments, and $\phi_q(t, \mathbf{y}_0)$ denotes the point reached at time t starting from $\mathbf{y}_0$;
- $\lambda : E \to \mathbb{R}^+$ is the *jump rate* giving the hazard of each discrete transition, it is assumed to be (locally) integrable;
- $R : E \cup \partial E \times \mathcal{B} \to [0, 1]$ is the *reset kernel* which maps each $y \in E \cup \partial E$ to a probability measure on $(E, \mathcal{B})$, where $\mathcal{B}$ is the Borel σ-algebra of E. $R(\mathbf{x}, A)$ is required to be measurable in the first argument and a probability measure for each $\mathbf{x}$.

Within each mode q a PDMP evolves along the flow ϕ_q, at each time the process can jump spontaneously with hazard given by λ_q, or instantaneously when the boundary ∂E_q of the state space of the current mode q is hit. To capture the evolution of a PDMP we define the random variables T and χ describing the jump time and target state, respectively. Let

$$t_*(\mathbf{x}) = \inf\{t > 0 \mid \phi_q(t, \mathbf{x}) \in \partial E_q\}$$

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be the hitting time of the boundary ∂E_q starting from $\mathbf{x} = (q, \mathbf{y})$, with $\inf \emptyset = \infty$. The survivor function of the next jump T, given current state $\mathbf{x}$, is

$$\mathcal{P}[T \geqslant t] = I_{t<t_*(\mathbf{x})} \exp\left[- \int_0^t \lambda\big(q, \phi_q(s, \mathbf{x})\big)\, ds \right]$$

where I_A is the indicator function of set A. As usual, this distribution can be sampled by solving $\mathcal{P}[T \geqslant t] = u$ for t, with u a uniform sample [3]. Given a sample for T, the target jump point $\chi = (q_1, \mathbf{x}_1)$ of the reset map is sampled from the distribution $R(\phi_q(T_1, \mathbf{x}), \cdot)$. Iterating this way a PDMP simulation is performed. The PDMP is not homogenous when the jump rates λ depend on $\mathbf{x}$, which is changed continuously by the flow $\phi_q(s, \mathbf{x})$. If this is not the case and there are no boundaries (i.e. $t_* = \infty$.), the sequence of variables T_i are homogenous exponentials, the PDMP reduces to a CTMC and the outlined simulation strategy reduces to the well-known Gillespie algorithm [30,31]. Among all possible PDMP executions we restrict to considering the *non-Zeno* ones, namely those which perform with probability one a finite number of jumps in any finite time t, i.e. $\mathbb{E}[\sum_k I_{t>T_k}] < \infty$.

SHAs as in Definition 2 are a model of PDMP with no boundaries [6].

Definition 4 *(SHA semantics).* Let $(Q, V, \delta, \mathcal{J}, \mathcal{V}, [q_0, \mathbf{v}_0, t_0])$ be an SHA, the corresponding PDMP $(\mathbf{Z}, \overline{Q}, \mathbf{Y}, E, \phi, \lambda, R)$ is defined as follows:

- the PDMP has the same variables/modes of the SHA, $\mathbf{Y} = V$ and $\overline{Q} = Q$;
- there are no boundaries, $E_q = \mathbb{R}^{|V|}$ so $\partial E_q = \emptyset$;
- $\lambda(q, \mathbf{y}) = \sum_{(q,q') \in \delta} \mathcal{J}_q(\mathbf{y}, q')$;
- $F_q(\mathbf{y}) = \mathcal{V}_q(\mathbf{y})$ thus $\phi_q(t, \mathbf{y}_0)$ is the solution of the ODE $\dot{\mathbf{y}} = \mathcal{V}_q(\mathbf{y})$ with initial condition $\mathbf{y}_0$;
- the reset kernel for $(q, \mathbf{y})$ is obtained by choosing the reset of one active transition in q with a probability proportional to its rate:

$$R\big((q, \mathbf{y}), A\big) = \sum_{(q,q') \in \delta} \frac{\mathcal{J}_q(\mathbf{y}, q')}{\lambda(q, \mathbf{y})} \delta_{(q', \mathbf{y})}(A)$$

  where $\delta_{(q', \mathbf{y})}(A)$ is the Dirac measure on the point $(q', \mathbf{y})$ assigning probability 1 to $(q', \mathbf{y})$ and 0 to the rest of the space.

Executions of the SHA are executions of the corresponding PDMP starting from the initial mode q_0 and with continuous variables initialized as $\mathbf{y}_0(t_0) = \mathbf{v}_0$.

Proposition 1 *(PDMP soundness). If the* SHA *rate function $\mathcal{J}$ is locally integrable, then the PDMP jump rate λ (a summation over $\mathcal{J}$) is locally integrable. Further, if the* SHA *vector field $\mathcal{V}(q)$ is (locally) Lipschitz continuous, then by the Picard–Lindelöf theorem the PDMP flow ϕ is uniquely determined and continuous, for any initial condition. The measure R is well-defined by the normalization $\lambda(q, \mathbf{y})$ (i.e. is a non-uniform discrete distribution as in [6]).*

Constructing an SHA *from a non-delayed chemical system*. We discuss how to build an SHA from an algebraic Gillespie-like formulation of a chemical system. This corresponds to switching from a CTMC to a PDMP representation. The SHA models a hybrid non-delayed stochastic system where some species are present in a so big number that the differential approximation is appropriate [7,21]. In this case, the SHA is a computationally efficient way to speed up a simulation, otherwise demanding, while retaining the intrinsic stochastic effects for species with few elements.

Definition 5 *(Partitioning).* Let $(\mathcal{R}, \mathcal{S}, M)$ be a chemical system, we partition it to $(\mathcal{R}^{\mathcal{C}}, \mathcal{R}^{\mathcal{D}}, \mathcal{C}, \mathcal{D}, M)$ where $\mathcal{S}$ is split among *discrete* and *continuous* species: $\mathcal{S} = \mathcal{C} \cup \mathcal{D}$, $\mathcal{C} \cap \mathcal{D} = \emptyset$. According to $\mathcal{C}$ and $\mathcal{D}$ reactions are split as well:

(*i*) reactions affecting *only* species in $\mathcal{D}$ are assigned to $\mathcal{R}^{\mathcal{D}}$;
(*ii*) reactions affecting *only* species in $\mathcal{S}$ are assigned to $\mathcal{R}^{\mathcal{C}}$;
(*iii*) if a reaction R_j affects both type of species then all the effects on the species in $\mathcal{C}$ are disregarded (i.e. $m_{j,i}$ in M is set to 0 for $S_i \in \mathcal{C}$).

Species partitioning is done (statically) according to the "numbers" involved. We do not discuss any partitioning criteria but we refer to, e.g., [3,6]. Notice that reactions in (*i*) can be nonlinear in the species in $\mathcal{D}$, and the same holds for the species in $\mathcal{C}$ of (*ii*). In (*iii*), instead, we explicitly remove from the reactants/products all the species which are represented through real variables. If the partitioning is well-founded, such species will be always abundant to neglect the effect of those reactions, thus allowing this simplification. Notice that the propensity functions will still depend on the continuous variables since we are only changing the stoichiometry of the reaction, i.e. $m_{j,i}$ in M. In other words, these could be still nonlinear.

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We write the hybrid stochastic process as $\mathbf{X}(t) = (\mathbf{C}(t), \mathbf{D}(t))$; $\mathbf{x}$ will denote a generic value for $\mathbf{X}(t)$, $\mathbf{c}$ a generic value for $\mathbf{C}(t)$ and $\mathbf{d}$ a value for $\mathbf{D}(t)$.

Definition 6. Given a chemical system $(\mathcal{R}^{\mathcal{C}}, \mathcal{R}^{\mathcal{D}}, \mathcal{C}, \mathcal{D}, M)$ as in Definition 5, the SHA $(Q, V, \delta, \mathcal{J}, \mathcal{V}, [q_0, \mathbf{v}_0, t_0])$ for a given initial configuration $\mathbf{x}_0 = (\mathbf{d}_0, \mathbf{c}_0)$ (at time t_0) is built as follows:

- let $\mathcal{X}_{\mathcal{R}^{\mathcal{D}}}$ be the states reachable from $\mathbf{d}_0$ by applying only discrete reactions as in Definition 1, define $Q = \{q_{\mathbf{d}} \mid \mathbf{d} \in \mathcal{X}_{\mathcal{R}^{\mathcal{D}}}\}$;
- define one variable for each continuous species: $V = \{v_i \mid i \in \mathcal{C}\}$;
- define transitions according to $\mathcal{X}_{\mathcal{R}^{\mathcal{D}}}$: $\delta = \{(q_{\mathbf{d}}, q_{\mathbf{d}+\nu_j}) \mid j \in \mathcal{X}_{\mathcal{R}^{\mathcal{D}}}\}$;
- in mode $q_{\mathbf{d}}$ define $[\mathbf{d}, \mathbf{d}'] = \{j \in \mathcal{R}^{\mathcal{D}} \mid \mathbf{d} + \nu_j = \mathbf{d}'\}$, then the jump rate is

$$\mathcal{J}_{q_{\mathbf{d}}}(\mathbf{v}, q_{\mathbf{d}'}) = \sum_{i \in [\mathbf{d}, \mathbf{d}']} a_{i, q_{\mathbf{d}}}(\mathbf{v}) \tag{3}$$

  where $a_{i,q_{\mathbf{d}}}(\mathbf{v})$ is a mode-specific propensity function[3];
- the i-th component of the $|\mathcal{C}|$-dimensional vector field, $[\mathcal{V}_q(\mathbf{v})]_i$, is

$$\big[\mathcal{V}_q(\mathbf{v})\big]_i = \sum_{j \in \mathcal{R}^{\mathcal{C}}} \nu_{j,i} a_{j,q}(\mathbf{v});$$

- set $q_0 = q_{\mathbf{d}_0}$ and $\mathbf{v}_0 = \mathbf{c}_0$.

In brief, the set of modes covers all the possible values of $\mathbf{D}(t)$: every mode is a "counter", i.e. $q_{\mathbf{d}}$ represents $\mathbf{d}$ so this is the interpretation we give to the modes. The continuous variables determine the values of $\mathbf{C}(t)$. Concerning the jump rates, these are defined by means of the propensity functions of the reactions in $\mathcal{R}^{\mathcal{D}}$, partially evaluated. Notice, also, that all the reactions in $\mathcal{R}^{\mathcal{D}}$ triggering jumps to the same new mode are joint in $[\mathbf{d}, \mathbf{d}']$ to account for the occurrence of any of those reactions ("or" in probability). Finally, each mode contains a set of ODEs, one for each species in $\mathcal{C}$. The i-th component of the vector field is the contribution of all the (continuous) reactions to the species.

The following result states that mass-action propensity functions (see Section 2) define a sound SHA/PDMP, as firstly stated in [6].

Theorem 1 *(SHA soundness). An SHA (Definition 6) for a mass-action chemical system and the resulting PDMP (Definition 4) are sound.*

Proof. As anticipated, mass-action propensity functions are Lipschitz continuous, and so are the mode-specific propensities in Definition 6. So, the SHA is sound and thus, by Proposition 1, the PDMP is sound as well. □

We show an example of application of this definition which contains intuitions about the model introduced in Section 3.

Example 2 *(Intercellular signaling).* We consider a cell of type c in two states: *inactive* (c_{in}) or *active* (c_{act}). Activation is triggered by a *signal molecule* s, at rate k. The signal itself is synthesized at rate α by active cells via *autocrine signaling* and degraded by the environment at rate δ. We do not really mind what the active state means for such cells; this example is purely explanatory. The *Reaction Rate Equations* (RRE) model is

$$\dot{c}_{in} = -ksc_{in}, \qquad \dot{c}_{act} = +ksc_{in}, \qquad \dot{s} = \alpha c_{in} - \delta s, \tag{4}$$

yielding its Gillespie-like [30] stochastic time-homogenous analog (a CTMC)

$$\begin{aligned}
&(\texttt{act}) && c_{in} + s \to c_{act} + s && \text{with rate } a_1(\mathbf{x}) = k\mathbf{x}_{c_{in}}\mathbf{x}_S,\\
&(\texttt{syn}) && \epsilon \to s && \text{with rate } a_2(\mathbf{x}) = \alpha\mathbf{x}_{c_{in}},\\
&(\texttt{deg}) && s \to \epsilon && \text{with rate } a_3(\mathbf{x}) = \delta\mathbf{x}_s,
\end{aligned} \tag{5}$$

where ϵ denotes no reactants/products. Notice that `(syn)` unbounded s according to Definition 1. Independently of this, RREs correctly approximate the CTMC only if c_{in}, c_{act} and s are so large in number to neglect their intrinsic stochastic

[3] This is a *partial evaluation* of $a_i(t)$, yielding it to depend only on the continuous variables in $V(t)$ (thus $a_i(\mathbf{v})$ when $V(t) = \mathbf{v}$) performed via syntactic substitution of the values of the discrete species, i.e. $\mathbf{d}_i$ replaces S_i if $i \in \mathcal{D}$, ν_j is the j-th component of $\mathbf{v}$:

$$a_{i,q_{\mathbf{d}}}(\mathbf{v}) = a_j(t)[\mathbf{d}_i/S_i, \nu_j/S_j]_{i \in \mathcal{D},\, j \in \mathcal{C}}.$$

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fluctuations. If any of these is not sufficiently large, RREs overapproximate. However, as was discussed for IL-2 in Section 3, simulations of the CTMC could find a major computational bottleneck in simulating `(syn)/(deg)` since when the species concentrations are converted to *numbers* (by means of a reference volume), the number of molecules is orders of magnitude bigger than the number of cells. In other words, we are dealing with a *multiscale system*.

A hybrid approach is the solution to achieve a performant simulation, while still observing interesting "facts" concerning cells. In this case we keep discrete the cellular populations and continuous the signal: $\mathcal{C} = \{s\}$ and $\mathcal{D} = \{c_{in}, c_{act}\}$. Activation is hence the only reaction in $\mathcal{R}^{\mathcal{D}} = \{\texttt{act}\}$, and synthesis and degradation are in $\mathcal{R}^{\mathcal{C}} = \{\texttt{syn}, \texttt{deg}\}$. We assume $\mathbf{x}_0 = (\mathbf{x}_{c_{in}}, \mathbf{x}_{c_{act}}, \mathbf{x}_s)$ to contain 3 stem cells, 0 type C cells and signal concentration 10.0, so $\mathbf{d} = (\mathbf{x}_{c_{in}}, \mathbf{x}_{c_{act}})$ and $\mathbf{c} = \mathbf{x}_s$. We have the reachable state (as in Example 1) $\mathcal{X}_{\mathcal{R}^{\mathcal{D}}} = \{(3,0),(2,1),(1,2),(0,3)\}$ so $Q = \{q_{(3,0)}, q_{(2,1)}, q_{(1,2)}, q_{(0,3)}\} = \{q_1, q_2, q_3, q_4\}$ and $V = \{v_s\}$. Also, $\delta = \{(q_1,q_2),(q_2,q_3),(q_3,q_4)\}$ is a path. The jump rates are defined as $\mathcal{J}_{q_i}(\mathbf{x}, q_{i+1}) = a_{1,q_i}(\mathbf{x})$ where the substitution, in this case, yields the mode-specific propensities $a_{1,q_1}(\mathbf{x}) = k3\mathbf{x}_s$, $a_{1,q_2}(\mathbf{x}) = k2\mathbf{x}_s$, $a_{1,q_3}(\mathbf{x}) = k\mathbf{x}_s$ and $a_{1,q_4}(\mathbf{x}) = 0$. Similarly, the unidimensional vector field $\mathcal{V}(q_i)$ is given by $\int_0^t a_{2,q_i}(\mathbf{x}(w)) - a_{3,q_i}(\mathbf{x}(w))\,dw = i\alpha/\delta + e^{-\delta t}$. Finally, the initial condition is $q_0 = q_{(3,0)}$ and $v_s(t_0) = 10.0$.

5. SHAs with deterministic delays

We now introduce SHA *with delayed transitions* (SHAD), the D symbol stands for delayed transitions. In principle, delays can either appear in the discrete transitions, in the vector field, or in both the SHA components. As we anticipated, we consider an SHA where some "control transitions" follow a combination of an exponential (time-homogenous) and a deterministic distribution.

Automata definition. A delayed transition is a probabilistic transition which starts as a non-delayed transition, but completes after some delay. While the transition is started and not completed, i.e. it is scheduled in the terminology of [13], we say that it is ongoing. Ongoing transitions can complete, after some delay, in any mode the automaton might have jumped to (according to its structure). Their completion can induce a switch in the control part of the automaton.

Definition 7 *(Stochastic Hybrid Automata with deterministic delays).* An SHAD is a tuple $(Q, V, \delta, \mathcal{T}, \mathcal{J}, \mathcal{V}, [q_0, \mathbf{v}_0, t_0])$ where:

- $(Q, V, \delta, \mathcal{J}, \mathcal{V}, [q_0, \mathbf{v}_0, t_0])$ is an SHA in the sense of Definition 2;
- $\mathcal{T} = \{\tau_1, \ldots, \tau_n\}$ is a finite set of *delayed transitions* of the form

$$\tau = \left(q_\tau, q'_\tau, \sigma_\tau, f_\tau\right)$$

  where:
  - $q_\tau, q'_\tau \in Q$ are the starting mode and the mode the automata jump to once τ starts, $\sigma_\tau \in \mathbb{R}^+$ is the delay of the transition;
  - $f_\tau : Q \to Q$ is a *completion function* so that the automata instantaneously jumps to $f_\tau(q)$ if an ongoing instance of τ completes in q.

  We require that $\mathcal{J}$ is defined also for all pairs (q_τ, q'_τ), for any τ.

To be well-defined, an SHAD must be able to "react" to the completion of any delayed transition. So, for any τ the completion function should be defined over all the set of modes reachable from q'_τ by means of δ and by the closure with respect to the delayed transitions. In chemical systems with delays f_τ is a total function, as we shall see. Further, the SHAD must satisfy the same mathematical requirements of non-delayed automata, e.g. integrability and continuity, which are then imposed on the embedded SHA.

SHADs can be mapped to PDMPs with boundaries, by extending Definition 4. Intuitively, the continuous part of the state space is augmented with a set of clock "ticking" the time to completion of each delayed transition. Also, modes are extended to store information about the subset of active clocks, i.e. the set of ongoing delayed transitions. This will allow us to use instantaneous/forced PDMP transitions to model the completion of a delayed transition. This way of handling clocks is similar to the clock structures of *Generalized Semi-Markov Processes* [32] used, for instance, in a process algebra with non-Markovian transition [17]. However, using solely PDMPs instead that a joint process ensures to have a well-defined measure on the trajectory space by the theory of PDMPs. Also, in the next section we will prove that some specific PDMP is equivalent, in terms of its constituting probability distributions, to a GMSP [8].

SHADs can yield PDMPs with infinite number of clocks since, in principle, the number of ongoing delayed transition is unbounded. However, for both technical and practical reasons we make the assumption that the maximum number of delayed transitions is some $\kappa \in \mathbb{N}^+$, for each $\tau \in \mathcal{T}$. The following statement suggests that the introduction of κ is safe, in a probabilistic sense.

Proposition 2. *Let $T > 0$ be a* finite time horizon *and $0 < \epsilon \ll 1$ an arbitrary value, there exists $k > 0$ such that the probability of observing k or more delayed transitions within time T is less than ϵ.*

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In principle, at each jump a state-change and hence a rate-change, follow. Rate-changes follow also by the evolution along the SHAD vector field. Let us assume a constant bound $\hat{\lambda} > 0$ for the rate of each delayed transition. The density of two consecutive exponential jumps, i.e. $Z = X + Y$, is given by the *convolution* of the exponentials X and Y, i.e.

$$f_Z(z) = \int_0^z f_X(z-y) f_Y(y)\, dy = \int_0^z \hat{\lambda} e^{-\hat{\lambda}(z-y)} \hat{\lambda} e^{-\hat{\lambda}(y)}\, dy$$

for $0 < z \leqslant T$, which turns out to be Erlang. Generalizing to w exponential jumps yields Z_w to be Erlang with parameters w and $\hat{\lambda}$, so $f_{Z_w}(z) = \hat{\lambda}^w z^{w-1} e^{-\hat{\lambda} z}/(w-1)!$, so the probability of having w jumps within horizon T is known and hence

$$k = \inf_{w \in \mathbb{N}} \left\{ 1 - e^{-\hat{\lambda} T} \sum_{i=0}^{w-1} \frac{(\hat{\lambda} T)^i}{i!} < \epsilon \right\}$$

is the smallest k satisfying Proposition 2, which exists since the summation is monotonic with respect to w. This suggests that shrinking ϵ to 0 makes bounded and unbounded SHADs hard to distinguish. This assumption is also technical: unbounded transitions would require a countable number of PDMP continuous variables, a fact actually not supported by the original definition of PDMPs by Davis [26]. It is important to remark that if a simulation-based approach is adopted, on-the-fly PDMP executions can be constructed without considering such a bound. In any case, we define the SHAD semantics in terms of PDMP with a finite number of continuous variables since the total number of ongoing delayed transitions is $|\mathcal{T}|\kappa$.

Definition 8 *(SHAD semantics).* Let $(Q, V, \delta, \mathcal{T}, \mathcal{J}, \mathcal{V}, [q_0, \mathbf{v}_0, t_0])$ be an SHAD, the corresponding PDMP $(\mathbf{Z}, \overline{Q}, \mathbf{Y}, E, \phi, \lambda, R)$ for at most $\kappa \in \mathbb{N}^+$ ongoing delayed transitions is defined as:

- let $C_* = \bigcup_{\tau \in \mathcal{T}} \{c_{i,\tau} \mid i = 1, \ldots, \kappa\}$ be a set of $\kappa|\mathcal{T}|$ clock variables (κ for each $\tau \in \mathcal{T}$), define $\mathbf{Y} = V \cup C_*$;
- the modes are extended to account for all the possible subsets of ongoing delayed transitions: $\overline{Q} = Q \times \wp(C_\tau)$ so each SHAD mode yields $2^{\kappa|\mathcal{T}|}$ PDMP modes distinguishable for the set of active clocks. We denote by $[q, \mathbf{c}]$ a mode with active set of clocks $\mathbf{c}$, $c_{i,\tau} \in \mathbf{c}$ a generic clock of $\mathbf{c}$[4];
- the hybrid state space is the open set of vectors which have the only components associated with clocks constrained, i.e.

$$E_{[q,\mathbf{c}]} = \left\{ \langle r, c_{1,\tau_1}, \ldots, c_{k,\tau_k} \rangle \;\middle|\; r \in \mathbb{R}^{|V|},\ \forall c_{i,\tau_i} \in \mathbf{c}. c_{i,\tau_i} < \sigma_{\tau_i} \right\};$$

- denote by $\mathbf{c}_\tau = \{c_{i,\tau} \in \mathbf{c}\}$ the clocks assigned to transition $\tau \in \mathcal{T}$ in $\mathbf{c}$ and remark that $\mathcal{J}$ accounts also for the rate to start such transitions. Define for every $[q, \mathbf{c}]$

$$\lambda\big([q, \mathbf{c}], \mathbf{y}\big) = \sum_{(q,q') \in \delta} \mathcal{J}_q\big(\mathbf{y}, q'\big) + \sum_{\tau = (q, q_\tau, \cdot) \in \mathcal{T}} \mathcal{J}_q(\mathbf{y}, q_\tau) I_{|\mathbf{c}_\tau| \leqslant \kappa}$$

  so that exponential and delayed transitions enabled in q compete. Notice that we force the rate of a delayed transition to 0 as soon as $|\mathbf{c}_\tau| = \kappa$;
- the flow discriminates between variables in V and clocks are as follows

$$\big[F_{[q,\mathbf{c}]}(\mathbf{y})\big]_i = \begin{cases} \mathcal{V}_q(\mathbf{y}) & i = 1, \ldots, |V|, \\ 1 & i > |V| \wedge c_{i,\tau} \in \mathbf{c}, \\ 0 & i > |V| \wedge c_{i,\tau} \notin \mathbf{c}. \end{cases}$$

  The first $|V|$ components are assigned the flow as in Definition 4. Time passage for $c_{i,\tau}$ is the usual unitary ODE if $c_{i,\tau}$ is active in $\mathbf{c}$;
- the reset kernel distinguishes between the type of jumps:
  - when $([q, \mathbf{c}], \mathbf{y}) \in E$ the jump is induced (via λ) by the start of a transition (either exponential or delayed). The reset kernel is

$$R\big(([q, \mathbf{c}], \mathbf{y}), A\big) = \sum_{(q,q') \in \delta} \frac{\mathcal{J}_q(\mathbf{y}, q')}{\lambda([q, \mathbf{c}], \mathbf{y})} \delta_{([q', \mathbf{c}], \mathbf{y})}(A) + \sum_{\tau = (q, q_\tau, \cdot) \in \mathcal{T}} \frac{\mathcal{J}_q(\mathbf{y}, q_\tau)}{\lambda([q, \mathbf{c}], \mathbf{y})} \delta_{([q_\tau, \mathbf{c} + \tau], \mathbf{y})}(A) I_{|\mathbf{c}_\tau| \leqslant \kappa}$$

    where $[q_\tau, \mathbf{c} + \tau]$ is the mode where there is a new clock in $\mathbf{c}$ for a transition of type τ, when $|\mathbf{c}_\tau| \leqslant \kappa$;
  - when $([q, \mathbf{c}], \mathbf{y}) \in \partial E$ the jump is induced (hitting a boundary) by the completion of a delayed transition

$$R\big(([q, \mathbf{c}], \mathbf{y}), A\big) = \sum_{\tau = (\cdot, f_\tau) \in \mathcal{T}} \delta_{([f_\tau(q), \mathbf{c} - \tau], \mathbf{y} - (0, \tau))}(A)$$

[4] Notice that we are actually defining more modes than those reachable by the SHAD since, e.g., some $\tau \in \mathcal{T}$ might not enabled in some q. However, these will not be reachable in a probabilistic sense in the PDMP.

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where $([f_\tau(q), \mathbf{c}-\tau], \mathbf{y})$ is the point in the state space where the mode is reset to $f_\tau(q)$, the set of active clocks $\mathbf{c}-\tau$ does not contain the one which triggered the jump and the corresponding component in $\mathbf{y}$ is reset to 0 (denoted $\mathbf{y}-(0,\tau)$);

Executions of this PDMP start from state $([q_0, \emptyset], \mathbf{v}_0)$;

When in $([q,\emptyset],\mathbf{v})$ no delayed transition are ongoing, $t_* = \infty$ since the flow for clocks is 0 and the PDMP behaves as that from Definition 4. When in $[q,\mathbf{c}]$ it holds that $t_* = \min\{\sigma_\tau - c_{i,\tau} \mid c_{i,\tau} \in \mathbf{c}\}$ since time flow is relative for clocks. Thus, the PDMP either hits the boundary of the clock setting the value for t_*, or jumps according to the hazard function. In the former case, it means that the next delayed transition to complete expires before the value sampled according to λ, differently from the latter case where it is still ongoing. In the former case, the reset kernel makes the PDMP switch to a state determined deterministically via the function f_τ of the delayed transition. In this case the clock variable triggering the boundary hit is reset to 0, and in the jumping mode the ODE for that clock is set to 0 by construction. In the latter case, the PDMP jumps according to a transition which can be either exponential or delayed. If it is exponential the current clock evaluations are preserved, as well as the ODEs. If it is delayed the reset kernel drives the automata to a state where a new clock variable is present for the transition just started, provided less then κ transitions of that type are ongoing. This simulation strategy is the rephrased hybrid version of those discussed in [13,5,4] for CTMCs, and is based on the strong Markov property of PDMPs. Its algorithmic formulation can be used to simulate an SHAD without building the PDMP explicitly.

Notice that this formulation is general enough to account for the *purely delayed interpretation* of delays [4]. In brief, under that interpretation the whole application of a reaction is postponed after the delay, so to allow molecules to take part to many reactions in an interleaving fashion. This can be easily obtained by using delayed transitions of the form $\tau = (q, q, \cdot)$.

As for the non-delayed analog, this PDMP is sound when the SHAD is.

Proposition 3 *(PDMP soundness). As for the non-delayed case, let the* SHA *be sound. The jump rate is locally integrable since $I_{|\mathbf{c}_\tau|\leqslant\kappa}$ changes only at discrete mode jumps (i.e. $|\mathbf{c}_\tau|$ increases), or boundary hits (i.e. $|\mathbf{c}_\tau|$ decreases). In both cases, λ is continuous in E among two jumps. The flow ϕ is composite by the* SHAD *flow (assumed to be locally Lipschitz), plus the flow modeling time passage for clocks (clearly Lipschitz). Thus, this new flow still satisfies the hypothesis of the Picard–Lindelöf theorem. For the measurability of R, we can note that $I_{|\mathbf{c}_\tau|\leqslant\kappa}$ is equivalent in R and λ, thus the normalization factor is coherent (i.e. if a delayed transition is off, its contribution does not appear in the normalization factor). So, for points inside E the considerations of Proposition 1 hold. For boundary points, the whole probability mass is assigned to the point determined by f_τ and, by construction, the mode $[f_\tau(q), \mathbf{c}-\tau]$ is inside E, so no jumps outside the state space can be induced.*

SHAD *for chemically reacting systems.* Some notation is introduced: we partition the stochastic reactions $\mathcal{R}^{\mathcal{D}}$ of a chemical system in the sense of Definition 5 as $\mathcal{R}^{\mathcal{D}} = \mathcal{R}^{\mathcal{D}}_{del} \cup \mathcal{R}^{\mathcal{D}}_{ndel}$ with $\mathcal{R}^{\mathcal{D}}_{del} \cap \mathcal{R}^{\mathcal{D}}_{ndel} = \emptyset$ to separate delayed transitions, $\mathcal{R}^{\mathcal{D}}_{del}$, from instantaneous ones. The delay of a reaction will be denoted as $del(j) \in \mathbb{R}^+$ so $\mathcal{R}^{\mathcal{D}}_{del} = \{j \mid del(j) > 0\}$.

Definition 9. Given a chemical system $(\mathcal{R}^{\mathcal{C}}, \mathcal{R}^{\mathcal{D}}_{del}, \mathcal{R}^{\mathcal{D}}_{ndel}, \mathcal{C}, \mathcal{D}, M)$ as in Definition 5, the SHAD $(Q, V, \delta, \mathcal{T}, \mathcal{J}, \mathcal{V}, [q_0, \mathbf{v}_0, t_0])$ for a given initial configuration $\mathbf{x}_0 = (\mathbf{d}_0, \mathbf{c}_0)$ (at time t_0) is built as follows:

- define $\mathcal{R} = \{\nu^r_j, \nu^p_j \mid j \in R^{\mathcal{D}}_{ndel}\}$, let $\mathcal{X}$ be the states reachable from $\mathbf{d}_0$ by applying reactions in $\mathcal{R} \cup R^{\mathcal{D}}_{ndel}$ as in Definition 1, define $Q = \{q_{\mathbf{d}} \mid \mathbf{d} \in \mathcal{X}\}$;
- set V, $\mathcal{V}$, δ (by using the reachable state space $\mathcal{X}$), $\mathcal{J}$ (by using all discrete reactions in $\mathcal{R}^{\mathcal{D}}_{del} \cup \mathcal{R}^{\mathcal{D}}_{ndel}$), q_0 and $\mathbf{v}_0$ as in Definition 6;
- define $\mathcal{T} = \{(q_{\mathbf{d}}, q_{\mathbf{d}+\nu^r_j}, del(j), f_j) \mid j \in \mathcal{R}^{\mathcal{D}}_{del}, \nu^r_j \preccurlyeq \mathbf{d}\}$ where $f_j(q_{\mathbf{d}}) = q_{\mathbf{d}+\nu^p_j}$ for any $\mathbf{d} \in \mathcal{X}$.

Notice that the reachable state space is obtained by considering the reactants and the products of delayed reactions as two separate events. The recursive Definition 1 will produce the state space $\mathcal{X}$ with all modes in which delayed transition can start/complete. When all delayed reactions have reactants, $\nu^r_j \neq \mathbf{0}$ for any $j \in \mathcal{R}^{\mathcal{D}}_{del}$ the recursive definition eventually generates a mode $q_{\mathbf{d}}$ from which no transitions can start because for all j $\nu^r_j \not\preccurlyeq \mathbf{d}$, thus yielding a finite $\mathcal{X}$ (with respect to the delayed reactions). Notice, also, that the this SHAD has a delayed transition for each possible state $q_{\mathbf{d}}$ where a reaction in $\mathcal{R}^{\mathcal{D}}_{del}$ can start. Since a transition can complete in any state we set f_τ as a total function, notice that f_τ is defined over $\mathcal{X}$ by construction. As expected, the jump function accounts also for the start of the delayed transitions.

The following result is analogous to Theorem 1, for SHADs.

Theorem 2 *(*SHAD *soundness). An* SHAD *(Definition 9) for a mass-action chemical system and the resulting PDMP (Definition 8) are sound.*

Proof. As for Theorem 1, the Lipschitz continuous mass-action propensity functions, and the mode-specific analog allow us to apply Proposition 3, so the SHAD and the PDMP are sound. □

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The following proposition states some simple equivalences which hold, by construction, among SHADs and SHAs, and could allow us to apply PDMP/SHA-specific analysis to SHADs.

Proposition 4. *Any* SHAD $\mathcal{A}_D = (Q, V, \delta_d, \mathcal{T}, \mathcal{J}, \mathcal{V}, [q_0, \mathbf{v}_0, t_0])$ *retains a non-delayed analogue* SHA $\mathcal{A}$ *with transition function* $\delta = \delta_d \cup \{(q_\tau, f_\tau(q_\tau)) \mid \tau = (q_\tau, q'_\tau, \sigma, f_\tau) \in \mathcal{T}\}$. *At the semantic level, executions of the PDMP associated with* $\mathcal{A}$ *can be mimicked by the PDMP associated with* $\mathcal{A}_D$, *since the behavior of an* SHAD *is richer. Further, when* $\mathcal{T} = \emptyset$ *the PDMP associated with* $\mathcal{A}_D$ *is the same as the one associated with* $\mathcal{A}$.

This theorem states, at the level of stochastic processes, an equivalence between an SHAD without continuous components and the delay CTMC of [8].

Theorem 3. *Let* $\mathcal{W} = (\emptyset, \mathcal{R}^{\mathcal{D}}_{del}, \mathcal{R}^{\mathcal{D}}_{ndel}, \emptyset, \mathcal{D}, M)$ *be a chemical system* (*as in Definition 5*) *with no continuous components and let* $\mathcal{A}_{\mathcal{W}} = (Q, \emptyset, \delta, \mathcal{T}, \mathcal{J}, \mathcal{V}, [q_0, \mathbf{0}, t_0])$ *be its associated* SHAD. *Fix any maximum number of delayed transitions* $\kappa \in \mathbb{N}^+$, *the PDMP associated with* $\mathcal{A}_{\mathcal{W}}$ *is analogous to the delay CTMC associated with* $\mathcal{W}$ *in the approach of* [8], *where the maximum number of CTMC delayed transitions is limited to* κ.

Proof. In Definition III.3, [8] the semantics of the delay CTMC is given in terms of a GSMP where an unbounded (but finite) number of ongoing delayed transitions are allowed (not shown here).

Let the PDMP be in some state $([q_{\mathbf{d}}, \mathbf{c}], \mathbf{0}) \in E$ shortly denoted $[\mathbf{d}, \mathbf{c}]$: $\mathbf{d}$ is the vector of discrete species, $\mathbf{0}$ is the continuous empty vector and $\mathbf{c}$ are the clocks for the ongoing delayed transitions. This state is analogous to a delay CTMC state $\mathbf{d}$, and to the GSMP state $(\mathbf{d}, A)$ where, in the notation of [8], A is the set of active events in $\mathbf{d}$. In A there are the events associated with the clocks in $\mathbf{c}$, plus other events, as explained in [8]. Here we restrict A to at most κ ongoing delayed transitions, i.e. $|\{e_{\rho,j} \in A\}| \leqslant \kappa$ in the notation of [8].

Since there is no flow for continuous species, the PDMP boundary hit time t_* models the completion of delayed transitions

$$t_*([\mathbf{d}, \mathbf{c}]) = \inf\{t > 0 \mid \phi_{q_{\mathbf{d}}}(t, [\mathbf{d}, \mathbf{c}]) \in \partial E_{q_{\mathbf{d}}}\} = \min_{c \in \mathbf{c}}\{t - c\} = \hat{c}.$$

Notice that we are using the fact that $[F_{[q_{\mathbf{d}}, \mathbf{c}]}(\mathbf{0})]_c = 1$ to model the uniform passage of time for clocks. The survivor function of the PDMP jump is then

$$\mathcal{P}[T \geqslant t] = I_{t<\hat{c}} \exp\left\{-\int_0^t \lambda\big[q_{\mathbf{d}}, \phi_{q_{\mathbf{d}}}(s, [\mathbf{d}, \mathbf{c}])\big]\, ds\right\}$$

and, since $[F_{[q_{\mathbf{d}}, \mathbf{c}]}(\mathbf{0})]_i = 0$ for $i \notin \mathbf{c}$, the jump rate is $\lambda[q_{\mathbf{d}}, \cdot] = \hat{\lambda}$ so

$$\mathcal{P}[T \geqslant t] = I_{t<\hat{c}} \exp\left(-\hat{\lambda}\int_0^t ds\right) = I_{t<\hat{c}} \cdot e^{-\hat{\lambda}t}$$

which is the usual homogenous exponential sojourn time of a CTMC, coupled to the indicator function accounting for the *next delayed transition* to complete (as, e.g., in [5,13]). In the GSMP of [8], T is similarly picked by resolving the competition between *all* the events in A. Since the time-passage is uniform even in the GSMP, i.e. $r(\mathbf{d}, e_{\rho,j}) = 1$ for $e_{\rho,j} \in A$ [8], the next delayed transition completes exactly at $\hat{c}$, and competes with the start of new transitions (with clocks running at speed equal to the rate of the exponential distribution which made them start, i.e. the above distribution). So the two processes have the same distribution of the sojourn time T.

Once sampled T, the two processes either handle the completion of a delayed transition, or start a new transition. In the former case, some f_τ defines the PDMP reset kernel (Definition 8) which, in the context of chemical reactions, is the same stoichiometric summation of the GSMP (Definition III.3, [8] and Definition 9) and both processes move to time $t + \hat{c}$. Differently, when a transition τ starts, either delayed with $\mathbf{c}_\tau < \kappa$ or not, the PDMP reset kernel and the GSMP transition probability are the same non-uniform discrete distribution, since the start rates of each transition are equivalent.

All the above considerations hold even for the initial state $([q_{\mathbf{d}_0}, \emptyset], \mathbf{0}) \in E$, so the two stochastic processes are step-by-step indistinguishable. □

Example 3. Consider a reversible reaction $R_1 : A \to B$ where the reverse $R_2 : B \to A$ is delayed with $del(2) = \lambda$. With initial state $(2, 0)$, two A and no B, when $\lambda = 0$ the set of modes is $\{q_{(2,0)}, q_{(1,1)}, q_{(0,2)}\} = \{q_1, q_2, q_3\}$ and transitions are $\delta = \{(q_i, q_{i+1}) \mid i = 1, 2\} \cup \{(q_i, q_{i-1}) \mid i = 2, 3\}$. The rest of the SHA is built according to Definition 6. When $\lambda > 0$ the R_2 yields the following new reachable states: $(1, 0)$ when R_2 starts in $(1, 1)$, $(0, 1)$ when R_1 starts in $(0, 2)$ and $(0, 0)$ when R_2 starts in $(0, 1)$, so the modes are extended accordingly. Given all the modes the corresponding delayed transition are the following: $\tau_1 = (q_{(1,1)}, q_{(1,0)}, \lambda, f)$, $\tau_2 = (q_{(0,1)}, q_{(0,0)}, \lambda, f)$ and $\tau_3 = (q_{(0,2)}, q_{(0,1)}, \lambda, f)$. In all cases $f(q_{(i,j)}) = q_{(i+1,j)}$. Also, δ contains the transition $(q_{(1,0)}, q_{(0,1)})$ induced by an exponential transition in a state which appears only in the SHAD, not in the SHA. A graphical representation of the SHA and the SHAD is shown in Fig. 1.

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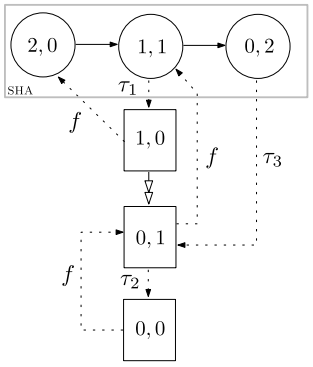

Fig. 1. The SHAD of Example 3. SHA modes are circles, SHAD ones are squares. Full arrows are exponential transitions, delayed transition are drawn explicitly. The completion function is drawn only for the modes where a delayed transition can be ongoing. The double arrow is an interleaving exponential transition modeling the firing of R_1 while an instance of τ_1, i.e. reaction R_2, is ongoing.

6. Case study: Hybrid model and analysis

We now define the hybrid version of the model discussed in Section 3. Results about the non-delayed hybrid version of the model were firstly given in [14,24]; we compare such results with those obtained by an SHAD with a constant delay in the anti-tumor immune-response.

Switching to an SHA/SHAD. As we anticipated in Section 3 the equations for $\dot{E}$ and $\dot{T}$ can be syntactically translated to a set of reactions. It is straightforward to note that converting the logistic growth $rT_*(1-bT_*)$ yields two reactions of the form $T \to 2T$ and $T \to \epsilon$ (ϵ being the null product). This in turn yields, when at least one tumor cell is present, an unbounded number of T in the sense of Definition 1. We remark that this is just a syntactic construction of the state space which does not account for the kinetic property of the logistic function, i.e. a *saturating growth with plateau*. For our case study this, one could use information on regions of realistic parameters in which unbounded cellular growth is predicted to define some cut-offs of the state space [37]. However, since we rely on a simulation-based approach of the underlying SHAD, this does not prevent us to analyze SHAD trajectories to draw our analyses.

When $\theta = 0$ an SHA with instantaneous recruitment can be built by combining a discrete representation of the cellular populations with continuous IL-2. This means interpreting the equations $\dot{E}$ and $\dot{T}$ (as obtained by converting those for $\dot{E}_*$ and $\dot{T}_*$ [14]) in Section 3 as a set of stochastic events. In [37] it is shown that, when $\theta = 0$, the PDMP predicts a desired *tumor eradication via immune surveillance*, whereas the ODE analog does not [37]. This is the main point to stress: in this case the ODE representation over-approximates reality since it does not allow us to predict the most interesting outcome of the interplay in study, i.e. the eradication of the tumor by the immune system. A hybrid setting, instead, allows us to quantitatively determine the *probability of eradication* $\mathcal{P}[T(t) = 0]$ for any t.

When $\theta = 0$ this model is an SHA in the sense of Definition 2. Let us write the system stoichiometry matrix

$$\nu = \begin{pmatrix} 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 1 \end{pmatrix},$$

by applying Definition 6 an SHA with modes in $\mathbb{N} \times \mathbb{N}$ recording the cellular concentrations can be built. The SHA consists of a mode for each possible value of E and T: we write a mode $q = (q_E, q_T)$ to count q_E and q_T effector and tumor cells. Each mode contains the vector field induced by $\dot{I}_*$ (which we write I for simplicity of notation), which in this case becomes linear and has solution for $t > t_q$

$$I(t) = B_q + (I_q - B_q)\exp\big(-\mu_I(t - t_q)\big) \tag{6}$$

with initial condition $I(t_q) = I_q$ when t_q is the mode entrance time and $B_q = [p_I q_T q_E/(g_I V^2 + q_T V)]/\mu_I$. The mode-specific propensity functions are

$$\begin{aligned} a_{1,q}(q) &= r_2 q_T, & a_{2,q}(q) &= r_2 b V^{-1} q_T(q_T - 1), \\ a_{3,q}(q) &= (p_T q_T q_E)/(g_T V + q_T), & a_{4,q}(t) &= \big[p_E q_E I(t)\big]\big/\big[g_E + I(t)\big], \\ a_{5,q}(q) &= \mu_E q_E & a_{6,q}(q) &= c q_T. \end{aligned}$$

Notice that all but $a_{4,q}$ are time-homogenous, i.e. independent of $I(t)$.

So, automata executions as in Definition 4 switch probabilistically between modes, while continuous paths of $I(t)$ are determined, and the jumps between modes are determined by the time-inhomogenous events depending on $I(t)$. Concerning the jump rates, when applying Definition 6 two stochastic events are joint: $a_{2,q}$ and $a_{3,q}$, trigger jumps to the same new mode, i.e. jumps from $q = (q_E, q_T)$ to $(q_E - 1, q_T)$. A graphical representation of a generic state of the SHA is given in Fig. 2.

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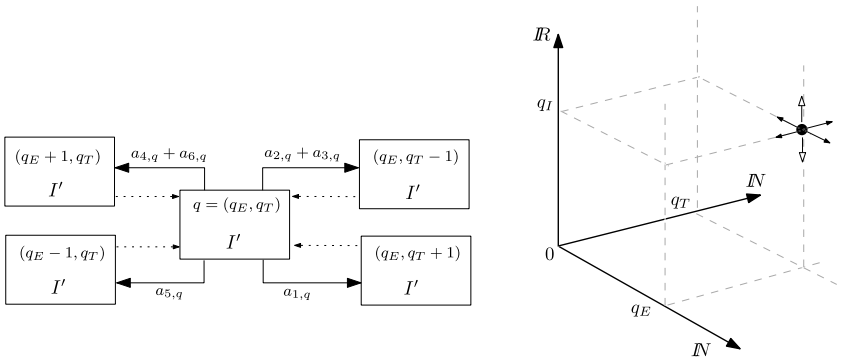

Fig. 2. SHA and the PDMP state-space for $\theta = 0$. For the SHA we draw mode $q = (q_T, q_E)$ with its outgoing transition and part of the incoming ones. We plot the state space $\mathbb{N} \times \mathbb{N} \times \mathbb{R}$ for the PDMP modeled by the SHA. Once the process enters state (q_T, q_E, q_I) the only movement gradient is on the z-axis, i.e. the horizontal component (q_T, q_E) is fixed and q_I changes according to the vertical vector field represented by the empty arrows. Then it jumps on the $\mathbb{N} \times \mathbb{N}$ sub-space, i.e. the horizontal discrete grid denoted by the full arrows.

Executions of this SHA are trajectories of the underlying PDMP with no hitting boundaries and time-dependent jump rates linked to the vector field $I(t)$. The state space for the PDMP is $\mathbb{N} \times \mathbb{N} \times \mathbb{R}^+$ (see Fig. 2). Here, once the process enters a mode there is a vertical movement gradient and a horizontal jump grid (see caption).

The SHAd is built similarly to the SHA. The basic idea is that of applying Definition 9 with the delayed transition induced by a_6, so $\mathcal{R}^{\mathcal{D}} = \{R_6\}$, in every automata mode q_T. These are of the form (q, q, θ, f) where f is the total completion function such that $f(q_E) = q_{E+1}$, as in Definition 9. In what follows, our results are obtained by simulating the PDMP underlying such automaton.

6.1. Results

To investigate the effect of θ on the tumor eradication time and tumor growth we performed simulations of various SHAd configurations. Simulations have been performed by a Java implementation of the PDMP execution for SHAds.

Model parameters. Parameters are taken from [14]. The tumor baseline growth rate is $r = 0.18$ days^{-1}, the organism carrying capacity is $b = 1/10^9$ ml^{-1}. The baseline strength of the killing rate of tumor cells by E, of the IL-2-stimulated growth rate of E and of the production rate for I are, respectively, $p_T = 1$ ml/days, $p_E = 0.1245$ days^{-1} and $p_I = 5$ pg/days. The corresponding 50% reduction factors are $g_T = 10^5$ ml^{-1}, $g_E = 2 \cdot 10^7$ pg/l and $g_I = 10^3$ ml^{-1}, respectively. The degradation rates are $\mu_E = 0.03$ days^{-1} for the inverse of the average lifespan of E and $\mu_I = 10$ days^{-1} for the loss/degradation rate of IL-2. The reference volume is 3.2 ml (blood and bone marrow volumes for mice).

These values pertain to mice [37,36] and are taken from [27,38], where accurate fitting of real data concerning laboratory animals with experimentally induced blood tumors were performed. Volume is estimated by considering the body weight and blood volume of a chimeric mouse [14]. The values of θ and c are given in the captions of figures.

Delay effect on tumor mass growth. We always used the initial condition $(T_0, E_0, I_0) = (1, 0, 0)$ to compare the results of [14]. For $c = 0.02$ (Fig. 2 in [14]), we used $\theta \in \{0, 0.5, 1, 1.5, 2, 2.5, 3\}$; for $\theta > 3$ the tumor mass grows up to the organism carrying capacity $1/b$ [25]. We remark that θ units are *days*, so $\theta > 3$ is a biologically unrealistic [25]. We performed 10^3 simulations for each delay configuration, and we plot in Fig. 3 the averages tumor and effectors growth, i.e. $\langle T(t) \rangle$ and $\langle E(t) \rangle$.

Whatever the delay value the model still predicts tumor eradication, despite the tumor mass grows significantly more for higher θ: at $\theta = 3$ it is 5 times bigger than at $\theta = 0$ ($\approx 10^6$ cells). This, in turn, stimulates the immune-response as shown by the plots of the empirical probability density of the eradication time, i.e. $\mathcal{P}[T(t) = 0]$ with $t \in \mathbb{N}$. Notice that this corresponds to estimating the expected time for the *quasi-absorbing* GMSP state $(0, q_E)$, eventually leading to the absorbing $(0, 0)$ state (natural death of all cells). Plots suggest that, though T grows more rapidly for higher θ, the induced immune response is proportional (the mean peaks are at days 125, 120, 118 and 115). This rather counterintuitive result hints at a functional role of θ in controlling tumor expansion. For Fig. 3 the mean and medians of the distributions are, in order for θ, 127.3 and 126, 120 and 119, 118.4 and 116, 120.1 and 118.5. The confidence intervals are [114, 141], [109, 136], [108, 134] and [109, 137].

To quantitatively determine the sensitivity of $T(t)$ with respect to θ, we used a (numerical) *parametric sensitivity analysis* for CTMCs [22], here contextualized in hybrid systems. We measure variations in the whole $\mathcal{P}[T(t)]$, rather than an aggregated quantity, to capture the effects of small perturbations in a wide range of values for θ. Model sensitivity to θ is defined as a function of the θ itself, that is $s_\theta(t) = \partial \mathcal{P}_\theta[T(t)]/\partial\theta$ where $\mathcal{P}_\theta[T(t)]$ is the probability of the tumor mass, given a value

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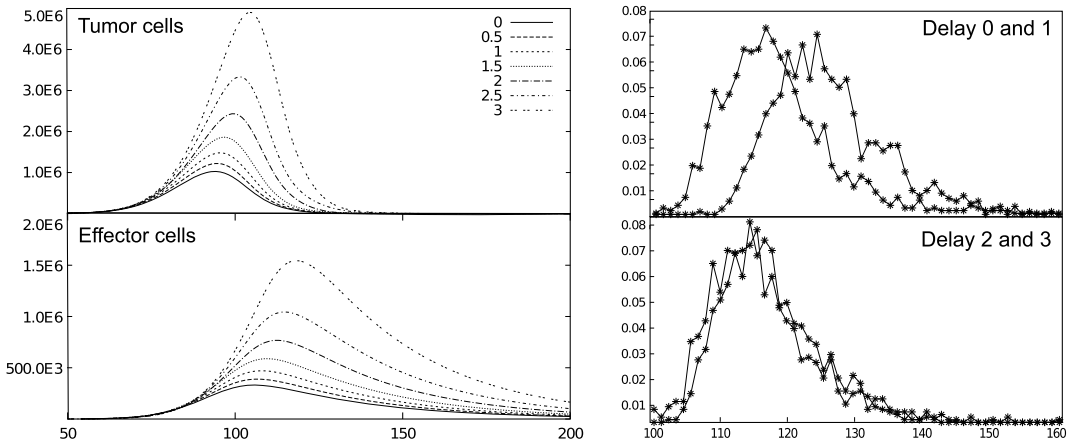

Fig. 3. Tumor and effectors growth and eradication times. On the left we plot the average growth $\langle T(t)\rangle$ and $\langle E(t)\rangle$ as in 10^3 simulations with $c = 0.02$, $\theta \in \{0, 0.5, 1, 1.5, 2, 2.5, 3\}$ (see legend) and $(T_0, E_0, I_0) = (1, 0, 0)$. On the x-axis days are represented, on the y-axis number of cells. On the right we plot the empirical probability density of the eradication time, i.e. $\mathcal{P}[T(t) = 0]$ with $t \in \mathbb{N}$, for $\theta \in \{0, 1, 2, 3\}$. On the y-axis probability density is represented.

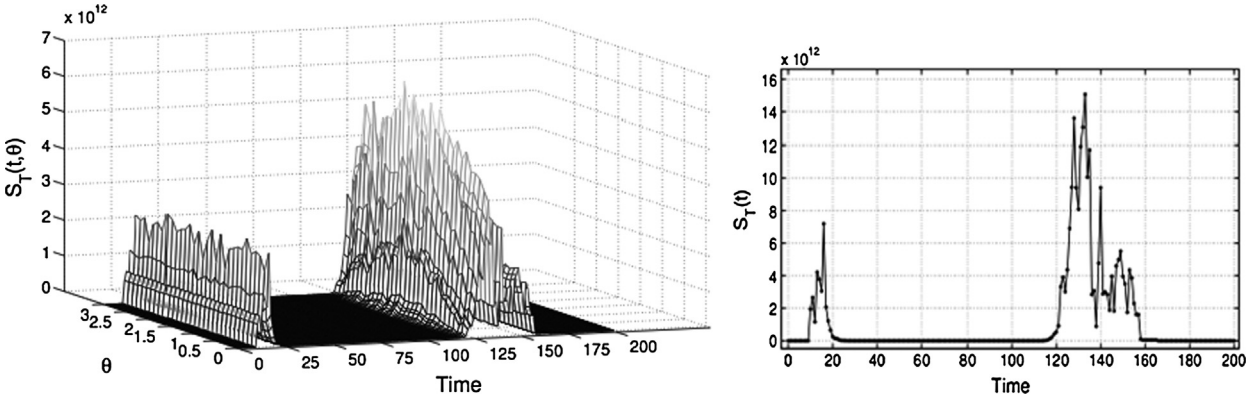

Fig. 4. Sensitivity analysis for the SHAD. On the left we plot a 3-D representation of the sensitivity curves $S_T(t, \theta)$, plotted for each delay in $\Omega\theta$, for each $t \in [0, 200]$, as obtained by 3×10^5 independent simulations. On the right we plot the sensitivity curve $S_T(t)$.

of θ [22]. The sensitivity analysis gives a measure for discrete stochastic systems or, analogously, for the discrete part of hybrid systems, obeying a generic chemical master equation [33], i.e.

$$S_T(t, \theta) = E\big[\big|s_\theta(t)\big|\big] = \int_{\mathbb{N}} \left|\frac{\partial \mathcal{P}_\theta[T(t) = x]}{\partial \theta}\right| \mathcal{P}_\theta\big[T(t) = x\big]\, dx. \tag{7}$$

The dependency of $\mathcal{P}_\theta[T(t)]$ from θ is a curve further generalized as

$$S_T(t) = \int_{\Omega\theta} S_T(t, \theta)\, d\theta \tag{8}$$

where the finite domain $\Omega\theta = \{0.1k \mid 0 \leqslant k \leqslant 30,\ k \in \mathbb{N}\}$ is used.

We performed 10^3 simulations for each delay value, so 3×10^5 independent simulations, and we computed $\mathcal{P}_\theta[T(t)]$ on the range $[0; \max_T]$, where $\max_T$ is the maximum observed value of T for all the values of θ. The sensitivity curves of Eqs. (7) and (8) are shown in Fig. 4. Plots suggest a time-varying sensitivity with maximum influence in the intervals $[10, 25]$ and $[115, 160]$. The overall sensitivity in $[115, 160]$ doubles the maximum $[10, 25]$ (right panel). This suggests that the immune system response influences neoplasm development: (*i*) before its expansion, either preventing or favoring it, or (*ii*) after it has reached its maximum size, inducing reduction/enlargement of the final eradication time.

Delay-induced oscillations. To investigate the role of delays in the oscillatory regime, we performed simulations with $0.03 \leqslant c \leqslant 0.035$, a region for which both the ODE system, Fig. 2D of [37], and the hybrid model, Fig. 7 of [14], predict tumor sustained/dumped oscillations. In Fig. 5 (left) we show simulations with $c = 0.035$, $\theta \in \{0, 1.5\}$ and initial configuration $(T_0, E_0, I_0) = (1, 0, 0)$. The model is simulated for around 10 000 days, i.e. 27 years, a value far beyond the life expectancy of a mouse – on which parameters are fitted – but proving the stability of the equilibrium, if any.

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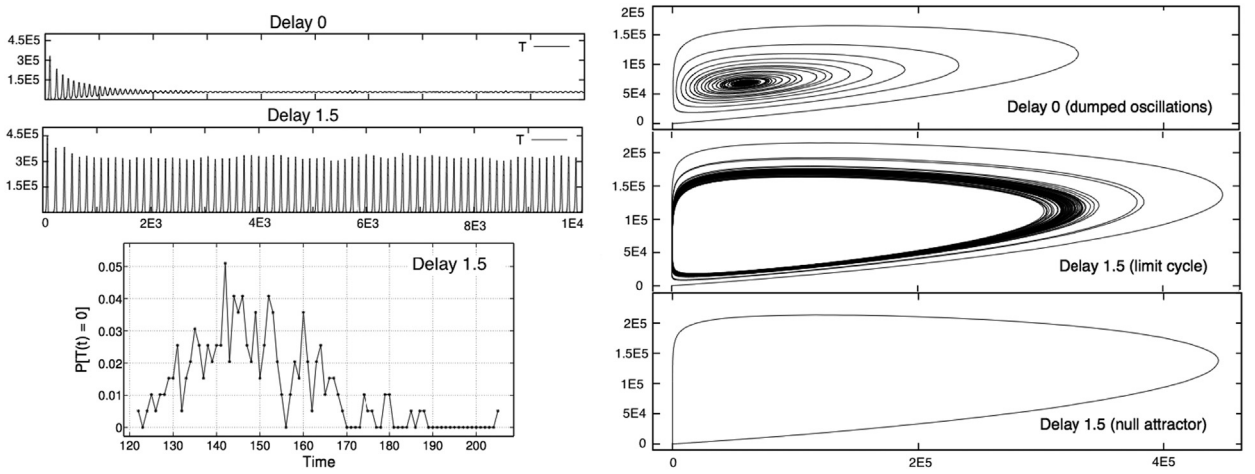

Fig. 5. Stable oscillatory equilibria against transitory state transition. At the top left we plot $T(t)$ and for a single run with $c = 0.035$ and $\theta \in \{0, 1.5\}$. The initial configuration is $(T_0, E_0, I_0) = (1, 0, 0)$. On the x-axis days are represented, on the y-axis number of cells. On the right we plot the phase space of the system restricted to T and E, and we show a stochastic switch to the null attractor for $\theta = 1.5$ whose probability of eradication is estimated by the 196 cases, out of 1000, in which the system jumps to the null attractor for T.

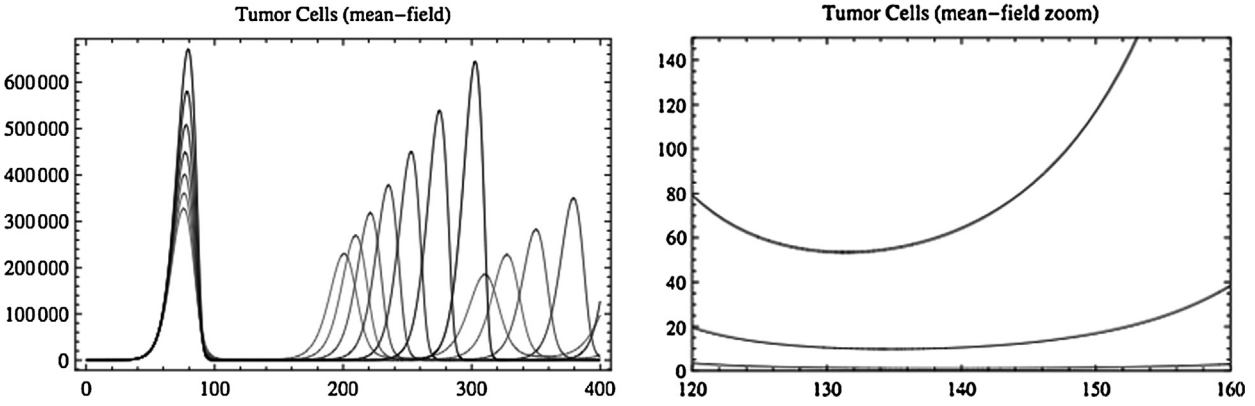

Fig. 6. Mean-field model. Numerical solution of DDEs (2) for $c = 0.035$, $\theta \in \{0, 0.5, 1, 1.5, 2, 2.5, 3\}$ (higher peaks for higher values of θ) and $(T_0, E_0, I_0) = (1, 0, 0)$, $T(t) = 0$ for $t < 0$. Notice the tumor resting period in $t \in [120, 160]$ (right zoom for $\theta \in \{1, 1.5, 2\}$), the length of which depends on θ, is the one in witch the hybrid system probabilistically switches to the null attractor for T. On the x-axis days are represented, on the y-axis number of cells.

Contrary to $\theta = 0$, the tumor mass does not reach a small equilibrium for $\theta = 1.5$. In fact, $T(t) \in (0, 3 \times 10^5]$ with $\theta = 1.5$ while the oscillations are dumped to 10^5 cells for $\theta = 0$. Furthermore, the first oscillation peak is around 4.5×10^5 for $\theta = 1.5$, considerably bigger values than that one reached for $\theta = 0$. These amplified oscillations often arise when models are enriched with delays [42,40,39] and reach very small values as shown in Fig. 5 where a stochastic switch to the null attractor emerges for $\theta = 1.5$. Surprisingly, this result in some simulations showing eradication reached immediately after the first spike of the oscillations for $\theta = 1.5$ (almost 20% of 1000 cases); the same is never observed for $\theta = 0$. This clearly suggests the existence of a *transitory state transition* close to $\theta = 1.5$ with a switch to the null attractor for T so that, for some cases, the tumor gets eradicated. It is interesting to notice that the probability of jumping to the null attractor is non-negligible in the time horizon considered here. Fig. 5 shows the distribution of the eradication time in these cases (the mean is 148.5, the median is 146 and the confidence interval is $[126, 179]$). This conclusion is strengthened by observing that, for $\theta \in \{2, 3\}$, the tumor is always eradicated (in 1000 cases, not shown).

This is even more interesting when compared to the deterministic simulations of the DDE model for $t \in [0, 400]$ and initial condition $T(t) = 0$ at $t < 0$ and $T(0) = 1$, $E(0) = I(0) = 0$, which predict a tumor resting period for $t \in [120, 160]$, the length of which depends on θ (Fig. 6). DDEs predict $T(t) < 1$ for $\theta = 2$ and $T(t) \approx 10$ for $\theta = 1.5$, in accordance with the simulations we performed. In this same period, instead, the hybrid system probabilistically switches to the null attractor for T, thus suggesting the importance of resetting the model in the hybrid setting which, as in [14], is again proved to be more informative.

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7. Conclusions

In this paper we introduce SHAds to model hybrid biochemical systems with delays. This modeling approach uses delays as an abstract model of unknown dynamics [13]. In general, delays can be used to abstract complex and often only partially known sequential dynamics in a single step, once an estimate of the duration of the dynamics is available, or as a model-abstraction technique to reduce model size collapsing multiple steps at once. Despite often simplistic, this turns out to be the best that we can do in many situations, e.g., [12,45].

The hybrid approach we adopt allows us to model those systems for which some components, e.g., cellular populations, have generally low concentrations, whereas other components are present in huge numbers, e.g., chemical signals. The power of the hybrid approach lies in the capability of forecasting behaviors induced by the intrinsic oscillations of the discrete part of the hybrid model. We show through a model of delayed immune-response on the growth of an immunogenic neoplasm (the case study which motivated our work) that behaviors very interesting can be predicted *only* when the standard hybrid approach is augmented with delays, e.g., the transitory state transition in Fig. 5. The interplay of tumor cells and the immune system is an ideal target for this hybrid modeling approach, as first noticed in [14,24].

The kind of automata we build extends standard SHAs [6] with delayed transitions. PDMPs are used to give a well-defined semantics to the automata, as already done for SHAs, and results on their soundness and relation with other approaches such as delay CTMC are proved. We define these automata by starting from the context of Gillespie-like stochastic chemically reacting systems, so we define a pipeline to switch from such a representation to a hybrid one, more efficient to be analyzed and that can be easily augmented with delays.

The clear logical connection with SHAs and their underlying PDMP should allow, in the future, to extend specific analysis techniques to the context of SHAds, e.g., model-checking or convergence in the limit. This last class of results is concerned with the limiting behavior of a sequence of models for increasing population size, the size being the total amount of the discrete species. For this purpose recent literature could be used, e.g., for the non-delayed case [9,7,21] or the delayed case [34,8].

Also, more complex form of delays could be considered, along the line of those used in mean-field models e.g., weak and strong delay kernels [16]. As far as the case study is considered, a further combination of this model with the immunotherapies studied in [24] would be interesting. Also, the model itself could be extended, e.g., the linear antigenic effect $cT_*(t-\theta)$ due to the tumor size could be corrected by assuming a delayed saturating stimulation. Finally, the effects of a perturbed immune-response could be investigated under different settings [18].

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