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ORIGINAL PAPER

Modeling the action of drugs on cellular enzymes by means of optimal control techniques

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Abstract In this paper we apply Optimal Control techniques, based on the solution of Hamilton–Jacobi–Bellman equations, to simulate the action of a drug on some sub-networks of an intracellular signal transduction network. The cost functional to be minimized is chosen in order to take into account, in the models, different factors, like the toxicity of some reactants, the drug action and the costs of the drug itself. Numerical results are also shown, to test the model.

Keywords Hamilton–Jacobi equations · Viscosity solutions · Michaelis–Menten kinetics · Intracellular signal transduction · Drug discovery

1 Introduction

In the last few decades the rapid evolution of experimental tools to study the cell biology at molecular level has deeply modified our view of the cellular machinery and of hierarchical processes within it. For example, the well established paradigm "one gene–one protein" and the unidirectional flux of genetic information have been challenged by

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recent astonishing findings. We now know that information processing within the cell is a very complex task, involving an intricate network of interacting "compartments", each with a well defined role and molecular interactions. We are observing the evolution and proliferation of several "-ome" compartments: proteome, metabolome, interactome and so on, which act as modules of a more complex biochemical network regulating all the aspects of cell life. So far, the knowledge about the primary (i.e. genomic) information is not enough to understand the cell behavior and, more importantly, the cell malfunctioning. In fact, the epigenetic regulation of genes and the processing of RNA transcripts can modulate protein expression in many ways: the knowledge of the primary structure of the gene provides only a limited—though necessary—information on many of the intracellular processes. Moreover, due to the alternative splicing process of the RNA, the same gene can express different isoforms of a protein (up to tens of isoforms) depending on the cell type, i.e., the intracellular environment.

Recently, several techniques have been developed to interfere with intracellular processes at different levels. For example, the RNA interference (RNAi) technique [1,2] is a promising tool to regulate the protein expression without a direct action on the DNA regulation. The injection of complementary RNA oligonucleotides (anti-sense oligonucleotide, ASO) can regulate the protein expression by targeting the double-stranded complex to degradation and modulating the role of the protein within the global intracellular interaction network.

According to [3], "anti-sense drugs are being researched to treat cancers (including lung cancer, colorectal carcinoma, pancreatic carcinoma, malignant glioma and malignant melanoma), diabetes, ALS, Duchenne muscular dystrophy and other diseases such as asthma and arthritis with an inflammatory component. Most potential therapies have not yet produced significant clinical results, though one anti-sense drug, *fomivirsen* (marked as *Vitravene*) has been approved by the US Food and Drug Administration (FDA) as a treatment for *cytomegalovirus retinitis*."

Thus a theoretical study is needed to optimize the protocols and provide some information to understand, for example, the reason of failure of RNAi-based therapies.

Moreover, with the successes of the Systems Biology program [4], a relatively new area of cell biology research is the implementation of mathematical models for the study of the emerging network properties of the cell.

The mathematical models of biochemical processes have been proposed at the beginning of the last century [5–9] and have been used for the quantitative study of simple metabolic processes. Since 1996, when Huang and Ferrell [10] proposed a model for the so-called MAPK cascade of intracellular signal transduction, many interdisciplinary research groups have proposed kinetic models for some identified (discrete) protein–protein interaction subnetworks (modules). They are described in terms of systems of first order nonlinear ordinary differential equations (ODEs) describing the rate of change of the concentrations of molecular species. Many of the first models were based on the mass conservation principle according to which a single molecule can interact with other species and change the activity, but the total mass must be conserved. These models have been extended to involve other processes affecting protein concentrations, like gene expression, protein degradation and RNA metabolism. The long-term aim of these studies is the integration of the "modules" in a "virtual cell", in order to reproduce the global behavior of a specific cell type and to use it for

basic, pharmacological and medical research. The ODE system describing the behavior of a (sub)network depends on a set of parameters, i.e., the initial concentration of dynamical molecular species and the kinetic constants which quantitatively describe the velocity of every single reaction. The kinetic constants have a precise pharmacological meaning: a drug targeted to a specific molecule can affect the kinetics of its reaction(s), modifying the behavior of global protein–protein interaction networks. The theoretical study of protein networks and the related numerical results identify the "sensitive" nodes (i.e., interactions) on which the global behavior mainly depends and the dose and chemical structure of the potential drug. In this sense, a reliable mathematical modeling can be a valid tool for pharmacological pre-clinical research. The effect of a drug can be simulated by varying one or more parameters simultaneously, and comparing the response of the system (network) to a reference ("health") dynamical state of the cell (described by the time course of the concentration of the network components). This can be seen as a "static" intervention on the network, i.e., the effect of the drug is to modify the kinetic of one or more reactions fixing the value of the kinetic constant(s) to a new value. Moreover, the mathematical models can simulate the perturbation produced by an external stimulus on the intracellular network once it has interacted with plasma membrane and has been internalized by a specific process (for example, endocytosis).

Very recent medical and pharmaceutical research is actively focusing on the study of highly specific drugs, which are able to enter a cell and selectively react with targeted enzymes.

Very often the drug molecules are introduced by means of nanostructures, which release them inside the cell (see for example [11–18]). The experiments involve nanotechnology devices, in particular molecular transporters; due to the high impermeability of cell membranes to foreign substances and the need for intracellular delivery of molecules via cell-penetrating transporters for drug, gene, or protein therapeutics [15].

The recently developed technique of carbon nanotube (CNT) internalization is an example of such a promising process of targeting drugs within the cell. Among nanomaterials of industrial relevance, CNTs are produced in increasing amounts for several industrial applications, because these structures are endowed with extremely advantageous chemical and mechanical features.

In particular, the use of functionalized CNTs as carriers of biologically active molecules holds great promise. In fact, CNTs have been shown to effectively breach the cell-membrane barriers and to deliver and enable functionality of extracellular agents, such as peptides, proteins, nucleic acids and drug molecules into cells. These innovative carriers present a lower toxicity, a fact that boosts their potential for biomedical applications [16,17].

Moreover, nanotubes-based optical biosensors may be used to detect specific targets inside the human body, e.g. tumor cells, wrapping the tubes by a protein which can link only the targeted cells.

We can model the effects of a drug, once it has been internalized by the cell, "modulating" the value of one or more kinetic constants, simulating the time course of the injected drug (targeting a reaction or a RNA), and forcing the system to replicate the health reference state. With a theoretical approach, a double information can

be inferred: identification of the drug targets and the drug administration protocol (for example, short pulse or a specific time course).

In our opinion, the most appropriate mathematical approach is based on Optimal Control techniques.

Optimal control problems can be formulated in terms of an objective function to be minimized, subject to possible control input constraints and the system dynamics is usually described by ordinary or partial differential equation systems.

In general frameworks, optimal control can be applied to high-dimensional dynamical systems with state and control constraints in the form of general equality and inequality constraints. Their use has been extended to large-scale problems in real-world applications (see [19] for a nice review). For example, in [20] the authors apply Optimal Control techniques to the study of networks of passive electric circuits interconnecting transducers, with the aim of determining the optimal electric parameters for the dissipation of the mechanical energy. The equations governing the network are quite similar to those ones describing our models.

Moreover, several authors have treated manipulation of self-organization dynamics and pattern formation in space and time by means of Optimal Control tools, as in the case of open flow reactors or complex cellular dynamics.

In this paper we apply an approach based on Hamilton–Jacobi–Bellman (HJB) equation [21,22], with the aim of simulating the optimal action of a drug on specific enzymatic intracellular targets.

We show that it is possible to control the behavior of the concentrations of predetermined enzymes adequately modifying the rate constants governing the reactions.

The paper is organized as follows.

In Sect. 2, we briefly recall the mathematical aspects of enzyme–enzyme interactions, based on the so-called Michaelis–Menten kinetics.

In Sect. 3, we give a short resume of the theory of viscosity solutions of the HJB equations, applied to Optimal Control problems.

In Sects. 4 and 5, we apply the theory to the study of the optimal product degradation in a Michaelis–Menten reaction and to the study of the control of an activated enzyme in the phosphorylation—dephosphorylation cycle, or Goldbeter–Koshland switch [23–25], respectively.

We also discuss some numerical results, obtained in [26], based on our theoretical models. The simulations confirm what is expected from our point of view. The controls introduced in the models show a behavior which is consistent with experimental studies and intuition.

Once validated the techniques, our scope is to extend in the future our researches and these techniques to more complex interaction networks, as proposed in Sect. 6.

2 Mathematical modeling of intracellular signal transduction pathways: Michaelis–Menten Kinetics

One of the principal components of the mathematical approach to Systems Biology is the model of biochemical reactions set forth by Henri in 1901 [5–7] and Michaelis and Menten in 1913 [8], and further developed by Briggs and Haldane in 1925 [9]. This

formulation considers a reaction where a substrate S binds an enzyme E reversibly to form a complex C. The complex can then decay irreversibly to a product P and the enzyme, which is then free to bind another molecule of the substrate.

This process is summarized in the scheme

$$E + S \underset{d}{\overset{a}{\rightleftarrows}} C \xrightarrow{k} E + P, \tag{1}$$

where a, d and k are kinetic parameters (supposed constant) associated with the reaction rates.

The fundamental step is modeling all of the intermediate reactions, including binding, dissociation and release of the product using mass action and conservation laws. This leads to an ODE for each involved complex and substrate. We refer to this as the full system.

From now on we will indicate with the same symbols the names of the enzymes and their concentrations. For (1) the equations are

$$\frac{dS}{dt} = -a(E_T - C)S + d\ C, \tag{2}$$

$$\frac{dC}{dt} = a(E_T - C)S - (d + k)C. \tag{3}$$

with the initial conditions

$$S(0) = S_T, \quad C(0) = 0, \tag{4}$$

and the conservation laws

$$E + C = E_T, \quad S + C + P = S_T. \tag{5}$$

The initial conditions give the concentrations of S and C at the beginning of the reaction, and their time development is described by the ODEs, while E and P are linked to S and C through the conservation laws. Here E_T is the total enzyme concentration assumed to be free at time $t = 0$. Also the total substrate concentration, S_T, is free at $t = 0$. This is the so-called Michaelis–Menten (MM) kinetics [8,9,24]. Let us observe that the system (2), (3) admits only one asymptotic solution for $t \to \infty$, obtained by setting the derivatives equal to zero. This solution is given by $C = S = 0$, so that from the conservation laws $P = S_T$ and $E = E_T$. This means that all the substrate eventually becomes product due to the irreversibility, while the enzyme eventually is free and the complex concentration tends to zero.

The assumption that the complex concentration is approximately constant after a short transient phase leads to the usual Michaelis–Menten (MM) approximation, or *standard quasi steady-state approximation* (sQSSA). It leads to an ODE for each substrate while the complexes are assumed to be in a quasi-steady state (i.e., $\frac{dC}{dt} \approx 0$):

$$\frac{dS}{dt} \approx -kC \approx -\frac{V_{\max} S}{K_M + S}, \quad S(0) = S_T, \tag{6}$$

where

$$V_{\max} = k\, E_T, \quad K_M = \frac{d+k}{a}. \tag{7}$$

The advantage of a quasi steady-state approximation is that it reduces the dimensionality of the system, passing from two equations (*full system*) to one (*MM approximation or sQSSA*) and thus speeds up numerical simulations greatly, especially for large networks as found *in vivo*. Moreover, the kinetic constants in (1) are usually not known, whereas finding the kinetic parameters for the MM approximation is a standard *in vitro* procedure in biochemistry. See e.g. [24] for a general introduction to this approach. We stress here that this is an approximation to the full system, and that it is not always valid (for example, it is valid when the enzyme concentration is much lower than either the substrate concentration or the Michaelis constant K_M, i.e., $E_T \ll S_T + K_M$ [27,28]). This condition is usually fulfilled for in vitro experiments, but often breaks down in vivo [29,30].

In the last years, following a pioneering paper by Laidler [31], several authors [25,32–39] have proposed, studied and applied a new approximation, called total quasi-steady state approximation (tQSSA), which is valid in a more general setting (i.e., for larger parameter ranges).

We refer to [40] for a nice general review of the kinetics and approximations of (1).

As mentioned above, to simulate physiologically realistic *in vivo* scenarios, in particular complex reaction networks, one faces the problem that any QSSA approximation is no longer valid; that is why we decide, in this paper, to study the kinetic reactions by means of the full ODE systems, without any QSSA approximation.

3 Mathematical background: Hamilton–Jacobi–Bellman equation and viscosity solutions

Optimal control can be applied to high-dimensional dynamical systems with state and control constraints in the form of general equality and inequality constraints. Their use has been extended to large-scale problems in real-world applications (see [19] for a nice review).

Other authors have studied the application of Optimal Control techniques to analyze how dynamic perturbations of a given system can produce a desired behavior in a dynamical system. For example, in [41] the authors aim at controlling two of the most important examples of self-organized processes exhibiting oscillations: the glycolytic cycle and the circadian clock [42]. In the former case they apply multiple shooting techniques for the solution of inverse problems with time-dependent input controls for Ordinary Differential Equations /Differential Algebraic Equations to detect appropriate temporally varying input signals leading to specific experimentally measured output. In the latter case they use a multiple-shooting mixed integer approach to a bang-bang control scenario aimed at annihilation of biochemical oscillators; in this case a target-oriented external manipulation induces the desired behavior.

As underlined by the authors, "the latter approach might be valuable for the development of drugs and treatment strategies aimed at so called dynamic diseases that are caused by malfunctions in the dynamics of cellular signaling and metabolism."

In this section we introduce an approach based on HJB equation [21,22] in order to simulate the optimal action of a drug on specific enzymatic intracellular targets.

Since we are focusing on biological phenomena occurring inside a cell, whose life time is finite, we will consider only control problems with finite horizon, where we define with T the cell life time, or the duration time of the reactions under consideration.

Let us consider the following HJB first order nonlinear partial differential equation, in the unknown v:

$$F(x, v(x), Dv(x)) = 0, \tag{8}$$

where $F : \Omega \times I\!R \times I\!R^N \to I\!R$ is a continuous function, $\Omega \subset I\!R^N$ is an open bounded subset of $I\!R^N$ and $Dv(x)$ represents the gradient of v at the point x. Here v is a bounded continuous function on Ω.

It is well known that to assume everywhere differentiability is a too restrictive assumption on v.

Since v in general can be non differentiable, but at least continuous, we are looking for weak solutions of Eq. (8). At this purpose, assuming that v is continuous in Ω, let us consider respectively the *superdifferential* of v at the point x

$$D^+v(x) := \left\{ p \in I\!R^N \;\middle|\; \limsup_{y \to x} \frac{v(y) - v(x) - p \cdot (y - x)}{|y - x|} \leq 0 \right\} \tag{9}$$

and the *subdifferential* of v at the point x

$$D^-v(x) := \left\{ q \in I\!R^N \;\middle|\; \liminf_{y \to x} \frac{v(y) - v(x) - q \cdot (y - x)}{|y - x|} \geq 0 \right\}. \tag{10}$$

Definition 1 A continuous function u is called a *viscosity solution* of (8) if the following conditions are satisfied:

$$\begin{aligned} &\textbf{(a)} \quad F(x, u(x), p) \leq 0 \quad \forall x \in I\!R^N,\ \forall p \in D^+u(x) \\ &\textbf{(b)} \quad F(x, u(x), q) \geq 0 \quad \forall x \in I\!R^N,\ \forall q \in D^-u(x). \end{aligned} \tag{11}$$

Any u satisfying (11) **(a)** is called a *viscosity subsolution* of (8); any u satisfying (11) **(b)** is called a *viscosity supersolution* of (8).

An alternative way of defining viscosity solutions of the HJB equation is based on test functions.

Lemma 1 [22] *Let $u \in C(\Omega)$. Then,*

(a) $p \in D^+u(x)$ if and only if there exists $\phi \in C^1(\Omega)$ such that $D\phi(x) = p$ and $u - \phi$ has a local maximum at x;

(b) $p \in D^- u(x)$ *if and only if there exists* $\phi \in C^1(\Omega)$ *such that* $D\phi(x) = p$ *and* $u - \phi$ *has a local minimum at* x.

Definition 2 A function $u \in C(\Omega)$ is a *viscosity subsolution* of the HJB equation in Ω if, for any $\phi \in C^1(\Omega)$,

$$F(x_0, u(x_0), D\phi(x_0)) \leq 0 \tag{12}$$

at any local maximum point $x_0 \in \Omega$ of $u - \phi$.

A function $u \in C(\Omega)$ is a *viscosity supersolution* of the HJB equation if, for any $\phi \in C^1(\Omega)$,

$$F(x_1, u(x_1), D\phi(x_1)) \geq 0 \tag{13}$$

at any local minimum point $x_1 \in \Omega$ of $u - \phi$.

A function $u \in C(\Omega)$ is a *viscosity solution* of the HJB equation in Ω if it is simultaneously a viscosity subsolution and supersolution.

The definition of viscosity solution is consistent with the classical notion of solution.

Proposition 1 [22] *If* $u \in C^1(\Omega)$ *is a classical solution of the HJB equation, that is,* u *is differentiable at any* $x \in \Omega$ *and*

$$F(x, u(x), Du(x)) = 0 \qquad \forall x \in \Omega, \tag{14}$$

then u *is a viscosity solution of the HJB equation.*

If $u \in C^1(\Omega)$ *is a viscosity solution of the HJB equation, then* u *is a classical solution of the HJB equation.*

In general, we cannot expect regularity of the solution u. *Nevertheless the next proposition says that any viscosity solution of HJB is also a generalized solution:*

Proposition 2 [22] *If* u *is locally Lipschitz continuous and it is a viscosity solution of the HJB equation, then*

$$F(x, u(x), Du(x)) = 0 \qquad \textit{almost everywhere in } \Omega. \tag{15}$$

Under suitable assumptions, uniqueness of viscosity solutions is obtained by Pontryagin Maximum Principle or comparison results.

For the problems here considered we must state the Dynamic Programming Principle (DPP) and derive from it the appropriate HJB equation for the value function.

Let us consider a control system where the state is described by the solution y_x of the system of differential equations (or *dynamics* of the system)

$$\begin{cases} \dot{y_x}(t) = f(y_x(t), v(t)) & \forall t > 0 \\ y_x(0) = x \in I\!R^N \end{cases} \tag{16}$$

where f is a continuous mapping from $\mathbb{R}^N \times V$ to $\mathbb{R}^N$, V is a compact metric space, called the *space of controls*, and v, the *control*, is a measurable function defined on $]0, T[$ with values in V:

$$v \in \mathcal{C} = \{\alpha \ :]0, T[\to V \ ; \ \alpha \text{ measurable}\}.$$

Let us suppose that there exist $C_f, M_f \in \mathbb{R}$ s.t.

$$\begin{cases} |f(x,v) - f(y,v)| \leq C_f \ |x-y|, |f(x,v)| \leq M_f \quad \forall x, y \in \mathbb{R}^N, v \in V, \\ f \text{ is a continuous mapping from } \mathbb{R}^N \times V \text{to} \mathbb{R}^N \end{cases} \tag{17}$$

where the constant C_f does not depend on $v \in V$, but on f.

From now on we will omit the dependence of y_x on v.

Let us introduce the *cost functional*

$$J(x,t,v(\cdot)) := \int_0^t \ell(y_x(s), v(s)) \cdot e^{-\lambda s} ds \ + e^{-\lambda t} g(y_x(t)) \tag{18}$$

where ℓ is the so called *instantaneous cost or running cost*, satisfying suitable regularity conditions (see [22], p. 99).

Here $e^{-\lambda t}$ represents the so called discount factor and $\lambda \geq 0$ is the discount rate. Finally, g is the *terminal cost*, s.t. $g \in BUC(R^N)$, where $BUC(\Omega)$ stands for the set of bounded uniformly continuous functions on Ω, i.e., there exist $\omega_g, G_g \in \mathbb{R}$ s.t.

$$\left\{ |g(x) - g(y)| \leq \omega_g |y - x| \quad , \quad |g(x)| \leq G, \quad \forall x, y \in \mathbb{R}^N \right. \tag{19}$$

The form of f, g and λ depends on the control problem we want to study.

The aim is to minimize the functional J; thus we act on the control, with the main goal of finding the *optimal control* which gives the minimum of J.

Introducing the *value function* u, defined by

$$u(x,t) = \inf_{v \in \mathcal{C}(]0,T[,V)} J(x,t,v(\cdot)) \tag{20}$$

we are ready to state the following

Theorem 1 Dynamic Programming Principle [21,22] *Let* (17) *and* (19) *hold and* $\lambda \geq 0$. *Then for all* $x \in \mathbb{R}^N$, *for all* $t \in [0, T]$ *and* $0 < \tau \leq t$, u *satisfies*

$$u(x,t) = \inf_{v \in \mathcal{C}} \int_0^\tau \ell(y_x(s), v(s)) \cdot e^{-\lambda s} ds + u(y_x(\tau), t - \tau) e^{-\lambda \tau}. \tag{21}$$

Theorem 2 [22] *Let* (17)–(19) *hold,* $\lambda \geq 0$ *and* $g \in BUC(\mathbb{R}^N)$. *Then the value function u associated to the control problem with finite horizon is the unique viscosity solution in* $BUC(\mathbb{R}^N \times [0, T])$ *of the evolution equation*

$$u_t + \lambda u + H(x, u, Du(x)) = 0 \text{ on } \mathbb{R}^N \times (0, T) \tag{22}$$

together with the initial condition

$$u(x, 0) = g(x) \quad \text{for all} \quad x \in \mathbb{R}^N , \tag{23}$$

where

$$H(x, u, p) = \sup_{v \in V} \{-f(x, v) \cdot p - \ell(x, v))\} . \tag{24}$$

Under rather general conditions on the data, including the boundedness of ℓ, the value function is continuous and bounded in $\mathbb{R}^N$.

4 Optimal Control and the HJB equation: effects of a drug on cellular enzyme degradation

When we consider reaction (1) we can introduce a term which describes the biochemically relevant phenomenon of degradation of some enzymes. The degradation can be induced or accelerated by some drugs, that can directly act on a specific enzyme, which can be considered toxic for the cell. We want to perform the zero limit of P, by controlling its degradation rate and also taking into account practical limitations, like the toxicity and/or the costs of the drug.

Due to the kinetic nature of the mathematical model, the degradation process is described by a first order reaction. The control by a drug is modeled by a varying kinetic rate $\alpha(t)$, whose time-dependence reflects the optimal administration protocol. In the present work, we want to simulate the drug "optimal" time-course in order to completely eliminate the product of a Michaelis–Menten reaction, with a scheduled time course.

In the framework of the Dynamic Programming approach we can exhibit a mathematical formulation of the above described problem.

Introducing in (2), (3) a suitable control $\alpha(t)$ we obtain the supplementary equation

$$\frac{dP}{dt} = k\ C(t) - \alpha(t) P(t) \tag{25}$$

where the term $\alpha(t) P(t)$ represents the degradation of P.

Let us remark that the presence of the first order degradation term does affect the second conservation law in (5), because modifies the total number of substrate molecules.

Thus the independent equations are now three, in S, C and P.

We impose that the dynamical system evolves inside a subset Ω of $\mathbb{R}^3$

$$\Omega = \{(S, C, P) \in \mathbb{R}^3 \mid S \geq 0\ ,\ C \geq 0\ ,\ P \geq 0\} \tag{26}$$

Interpreting the function $\alpha(t)$ as a control, we want to determine the admissible controls α such that

$$(S(t), C(t), P(t)) \in \Omega\ , \tag{27}$$

$$\lim_{t \to +\infty} P(t) = 0 \tag{28}$$

and $\alpha \in [0, K]$ for some $K > 0$.

The upper bound K can be interpreted as the need of modeling the presence of financial costs and/or toxicity of the drug. In other words, if the drug is expensive and/or toxic, it would be preferred to introduce it inside the cell only at low concentrations.

Obviously, if we could use the drug at high concentrations and consequently consider high values of K, we could expect that an immediate and high drug administration could lead quite instantaneously the product P to very low concentrations.

Actually, we must calibrate α in order to control in the optimal way the presence of P and of the drug inside the cell.

Let us define the set of admissible controls $\mathcal{C}$ as the set of measurable functions $\alpha : [0, T] \to [0, K]$, for some $K > 0$, such that

$$(S(t), C(t), P(t)) \in \Omega \text{ for each } t \in (0, T). \tag{29}$$

where T represents the duration time of the reaction.

We rewrite system (2), (3), (25) in the vector form

$$\frac{dy}{dt} = g(y(t), k_\alpha(t)) \tag{30}$$

where

$$y(t) = (S(t), C(t), P(t))^T \in \mathbb{R}^3 \tag{31}$$

$$y(0) = (S_T, 0, 0)^T =: x \in \mathbb{R}^3 \tag{32}$$

and

$$\begin{aligned} g(y(t), \alpha(t)) = &(-a(E_T - C(t))S(t) + d\ C(t)\ , \\ &a(E_T - C(t))S(t) - (d + k)C(t)\ ,\ k\ C(t) - \alpha(t)P(t))^T . \end{aligned} \tag{33}$$

In our first models we have decided, for the sake of simplicity, to put in (18) $\lambda = 0$ and $g \equiv 0$.

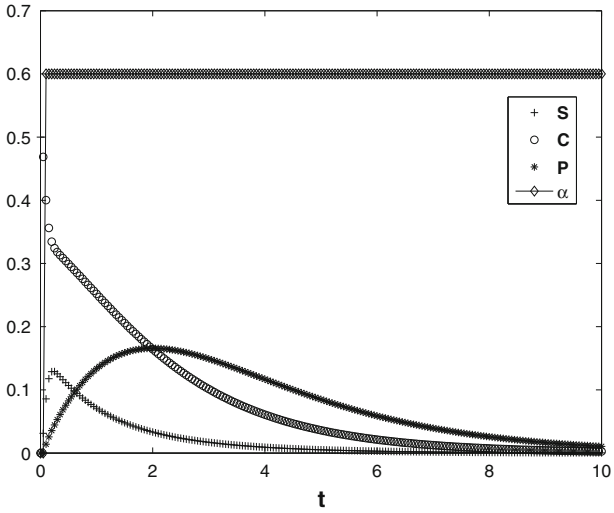

Fig. 1 Degradation of the product P by means of optimal control of the kinetic rate α. If the cost functional does not take into account the costs related to α, the control immediately reaches the upper bound, rapidly degrading P. The characteristic model parameter values are $K = 0.6$, $E_T = 0.5$, $S_T = 0.5$, $T = 10$, $a = 37.5$, $d = 2.4$, $k = 0.6$, $\ell(y_x, \alpha) = P^2$

Thus, following the previous section, for any "admissible control" we define a cost functional, depending on the choice of the initial condition $y(0) = x$, as

$$J_x(\alpha(\cdot)) := J(x, t, \alpha(\cdot)) = \int_0^t \ell(y(s), \alpha(s))ds. \tag{34}$$

To force the system to degrade P, we consider ℓ depending on P and on α, because we want to take into account the cost (or the toxicity) of the drug, too.

As shown in Figs. 1, 2, the choice of the function ℓ is fundamental. In fact, a suitable choice of the cost ℓ allows us to give optimal strategies to send P to zero. Figure 1 shows that if the cost does not depend on α (e.g., $\ell(P, \alpha) = P^2$), than the optimal strategy is to choose α as large as possible; in this case P rapidly degrades. In our test we set as upper bound $K = 0.6$. Figures 2 and 3 show that, if $\ell(P, \alpha) = P^2 + \alpha^2$, it is more convenient to degrade P only partially.

The optimal choice depends on T, as well. In fact, as shown in Fig. 2 and 3, when T is sufficiently large, the system chooses to inoculate high drug levels just for a short lapse of time, in order to rapidly degrade P. Though initially α reaches very high levels, influencing the value of J, its total contribution can be smoothed in a long time interval $[0, T]$.

On the other hand, when T is small, high α levels heavily influence J; thus the best choice for the system is to degrade only partially P, using relatively low α levels.

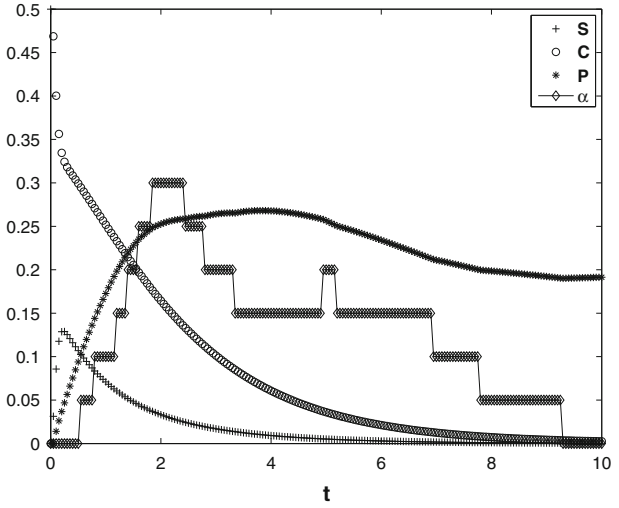

Fig. 2 Degradation of the product P by means of optimal control of the kinetic rate α. The characteristic model parameter values are $K = 0.6, E_T = 0.5, S_T = 0.5, T = 10, a = 37.5, d = 2.4, k = 0.6, \ell(y_x, \alpha) = P^2 + \alpha^2$. For small values of T it is less costly for the system not to degrade all the product P

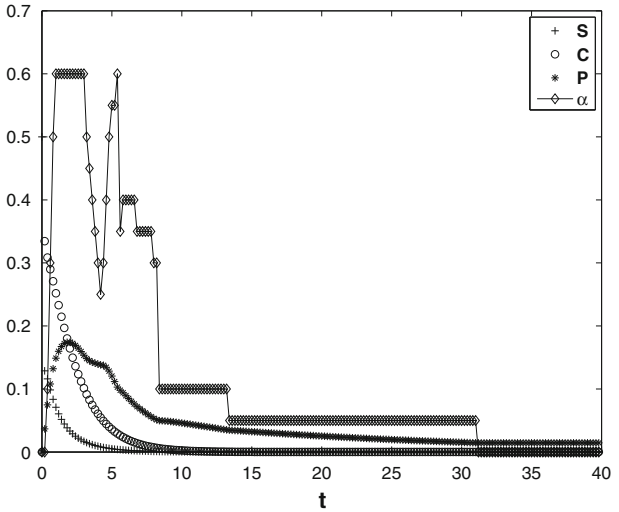

Fig. 3 Degradation of the product P by means of optimal control of the kinetic rate α. The characteristic model parameter values are $K = 0.6, E_T = 0.5, S_T = 0.5, T = 100, a = 37.5, d = 2.4, k = 0.6, \ell(y_x, \alpha) = P^2 + \alpha^2$. For large values of T it is less costly to allow the control to reach initially high values in order to rapidly degrade P. In the figure it is shown the enzyme temporal behavior only up to $T_0 = 40$

5 Optimal Control and the HJB equation: control of the substrate concentration

The second example studies the phosphorylation-dephosphorylation cycle, or Goldbeter–Koshland switch [23–25]

$$\begin{aligned} S + E_1 &\underset{d_1}{\overset{a_1}{\rightleftarrows}} C_1 \xrightarrow{k_1} E_1 + S^*, \\ S^* + E_2 &\underset{d_2}{\overset{a_2}{\rightleftarrows}} C_2 \xrightarrow{k_2} E_2 + S, \end{aligned} \tag{35}$$

where the substrate S is respectively phosphorylated (i.e. activated) and dephosphorylated (i.e. inactivated) by means of a kinase E_1 and a phosphatase E_2. S^* represents the phosphorylated substrate.

The reaction is mathematically described by the system

$$\begin{cases} \frac{dS}{dt} = -a_1(E_{1T} - C_1)S + d_1C_1 + k_2C_2 \\ \frac{dS^*}{dt} = -a_2(E_{2T} - C_2)S^* + d_2C_2 \cdot C_1 \\ \frac{dC_1}{dt} = a_1(E_{1T} - C_1)S - d_1C_1 \\ \frac{dC_2}{dt} = a_2(E_{2T} - C_2)S^* - (d_2 + k_2)C_2 \end{cases} \tag{36}$$

the initial conditions

$$S(0) = S_T \ , \ S^*(0) = 0 \ , \ C_i(0) = 0 \tag{37}$$

and the conservation laws

$$S_T = S + C_1 + C_2 + S^*, \ E_{iT} = E_i + C_i, \ i = 1, 2. \tag{38}$$

This is one of the most commonly found regulatory mechanisms in many intracellular reaction networks, like, for example, the Raf-MEK-ERK kinase cascade (or, shortly, MAPK cascade) [43–48].

The MAPK cascade is a fundamental component of both normal and pathological cell regulatory networks, because transduces signals from activated membrane receptors into the cell to regulate, for example, proliferation, apoptosis and differentiation [49–59].

The MAPK module functions as a switch both in the cytoplasm and at the membrane. Recruitment and activation of the MAPK module components to the plasma membrane provides the cell with a circuit configuration that is both switch-like and sensitive, which allows cells to generate a digital output from low-level analog inputs. These two properties may be crucial for cells to reliably fire in response to stimulation with physiological levels of growth factors in vivo [52].

ERK is the component of the MAPK cascade able to translocate to the nucleus and translate the extracellular signal into a specific gene expression pattern.

ERK activation ultimately results in stimulation and modulation of gene transcription and its steady state concentration levels, amplitude, duration and subcellular compartmentalization are critical determinants of the biological response in different cell types [60–68].

In fact, cells can use transient and sustained activation of ERKs to determine different responses.

There are multiple signal transduction pathways leading to ERK activation.

Since the external signal activates the intracellular network as a whole, each pathway could specifically contribute to the level and duration of ERK activation and could be selectively used by different receptors to regulate the amplitude and duration of signaling.

For example ERK (MAPK) activation is sustained for several hours following NGF stimulation, but it is short lived after EGF stimulation [69].

"Strong" ERK activation can promote cell cycle arrest in fibroblasts, differentiation of PC12 cells and survival of carcinoma cells. Conversely, "weak" ERK activation can result in proliferation of fibroblasts and PC12 cells and apoptosis in carcinoma cells. The magnitude of ERK activation is also responsible for various outcomes in the selection and specification of certain T-cell populations [70,71]. Some proteins (e.g., IMP) function as a signal threshold regulator for the ERK pathway by imposing a stimulus-responsive inhibitory mechanism that must itself be inhibited for signal transduction to occur [61,72,73].

These observations suggest that these kinds of proteins function as threshold modulators, controlling sensitivity of the cascade to stimulus by directly limiting the assembly of functional Raf-MEK complexes [61].

Moreover, in PC12 cells differentiation or proliferative responses to receptor tyrosine kinases can be based on the duration of ERK activation.

On the other hand, transient activation can have very different consequences with respect to sustained activation (proliferation).

In other cells the converse may be true: for example, in fibroblasts, sustained activation of ERKs is associated with proliferation, not differentiation [74].

The balance of phosphorylation and dephosphorylation establishes the threshold of activation in MAPK circuits [52].

Consequently it is very important to control in some sense the concentration levels of the different enzymes involved in the reaction.

Let us remark that, as shown in [23,25], in this reaction the reactant concentrations (including the complexes) asymptotically tend to values different from zero, still respecting the conservation law (38). In fact, because of the presence of a cycle, if we neglect degradation or inhibition phenomena, the complexes are permanently created and permanently produce phosphorylated and dephosphorylated substrate.

The action of a drug could influence the steady state values of the reactants.

In this model we want to study the effect of a drug on the asymptotic concentrations, in order to maintain the concentration level of the phosphorylated substrate above and/or below a priori determined thresholds. The effects to be avoided are in that

case due to anomalous concentrations of the phosphorylated substrate S^* not obeying certain predetermined necessary bounds.

Following the mass action principle for every enzyme species and substituting to the kinetic rate a_2 a control α, the reaction is governed by the system

$$\begin{cases} \frac{dS}{dt} = -a_1(E_{1T} - C_1)S + d_1C_1 + k_2C_2 \\ \frac{dS^*}{dt} = -\alpha(t)(E_{2T} - C_2)S^* + d_2C_2 + k_1 \cdot C_1 \\ \frac{dC_1}{dt} = a_1(E_{1T} - C_1)S - (d_1 + k_1) \cdot C_1 \\ \frac{dC_2}{dt} = \alpha(t)(E_{2T} - C_2)S^* - (d_2 + k_2)C_2 \end{cases} \tag{39}$$

the initial conditions

$$S(0) = S_T\ ,\ S^*(0) = 0\ ,\ C_i(0) = 0 \tag{40}$$

and the conservation laws

$$S_T = S + C_1 + C_2 + S^*,\ E_{iT} = E_i + C_i,\ i = 1, 2. \tag{41}$$

Let us remark that the presence of the time dependent kinetic parameter α does not affect the conservation laws, because its variations do not modify the total number of substrate molecules, but only the relative velocities of the reactions and thus the number of free, bound and activated molecules.

To keep S^* in a δ-neighborhood of an a priori determined constant value m, we solve a PDE like (22), (24), where the running cost can be chosen in such a way that the system is penalized whenever the S^* concentration assumes values not belonging to the range $[m - \delta, m + \delta]$.

We can choose ℓ in different ways, for example either

$$\ell(x, c) = c \cdot ([S^* - (m + \delta)]_+)^2 + ([-S^* + (m - \delta)]_+)^2. \tag{42}$$

or

$$l(x) = c \cdot [(S^* - (m + \delta))((S^* - (m - \delta))]_+, \tag{43}$$

where $[f(x)]_+$ represents the positive part of f and c is a predetermined penalty term.

In this model we decide to use for ℓ the form given in (43). In this way whenever S^* assumes values either below the bound $(m - \delta)$ or above $(m + \delta)$, the term in the square brackets is positive and the system is penalized; when S^* belongs to the range $(m-\delta\ ,\ m+\delta)$, the term in the square brackets is negative and there is no penalization at all. In our model the control α can assume values in the range [0.0, 1.0]

Let us remark that this choice gives quite different results in terms of optimal strategies in Fig. 4 ($m = 0.3, \delta = 0.1$), 5 ($m = 0.6, \delta = 0.1$) and 6 ($m = 0.1, \delta = 0.1$), where we have chosen the following parameter values and initial conditions:

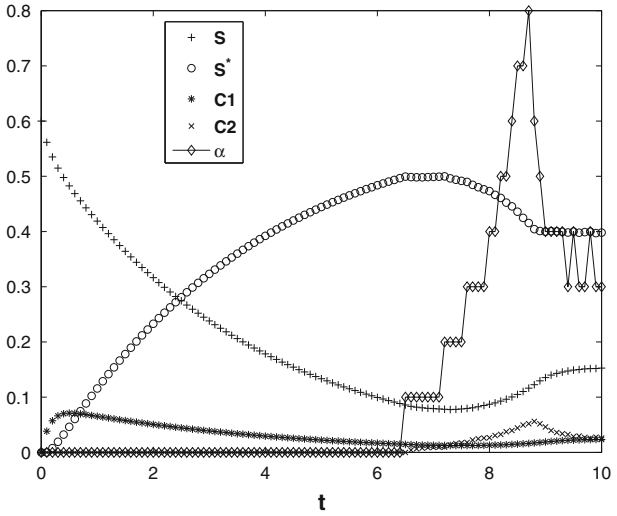

Fig. 4 Control of the substrate concentration. $m = 0.3, \delta = 0.1, a_1 = 0.8\ ,\ d_1 = d_2 = 2.0\ ,\ k_1 = k_2 = 2.0\ ,\ E_{1T} = E_{2T} = 0.8\ ,\ S_T = 0.6$. After a lasting phase, in which the control is inactive, it begins to act (assuming values different from 0), whenever the system needs to lower S^* concentration, bringing back it within the interval [0.3, 0.5]

$$a_1 = 0.8,\ d_1 = d_2 = 2.0,\ k_1 = k_2 = 2.0,\ E_{1T} = E_{2T} = 0.8,\ S_T = 0.6, c = 10.$$

In absence of control ($a_2 = 0.8$), S^* asymptotically tends to a value close to 0.26.

Due to the choice of ℓ in (43), in the three cases the control begins to assume values different from zero only when the concentration of S^* begins to exceed the upper bound $(m + \delta)$.

Taking into account the fact that setting $a_2 = 0$ inhibits the dephosphorylation reaction, leading to a monotone increase of S^*, in Fig. 4 we see that the control acts at different steps with different amplitudes; in Fig. 5 the control does not need to act on S^* because the inhibition of the dephosphorylation mechanism naturally brings S^* concentration within the interval [0.5, 0.7]. In Fig. 6 quite immediately the control reaches its maximum allowable value, accelerating the dephosphorylation reaction and inducing low levels of S^* concentration.

6 Problems and perspectives

The rapid growth of nanotechnologies and of their applications in Medicine and Pharma and the development of drugs acting on specific intracellular targets imply the need of more and more refined mathematical models, able to reproduce in the best allowable way the cell behavior.

Optimal Control techniques appear one of the most appropriate and promising tools to reach the scope of simulating and tuning targeted drug effects on specific parts of the cells, even molecules and enzymes, in order to obtain an accurate response to the need of controlling the drug action, in terms of optimal dosage.

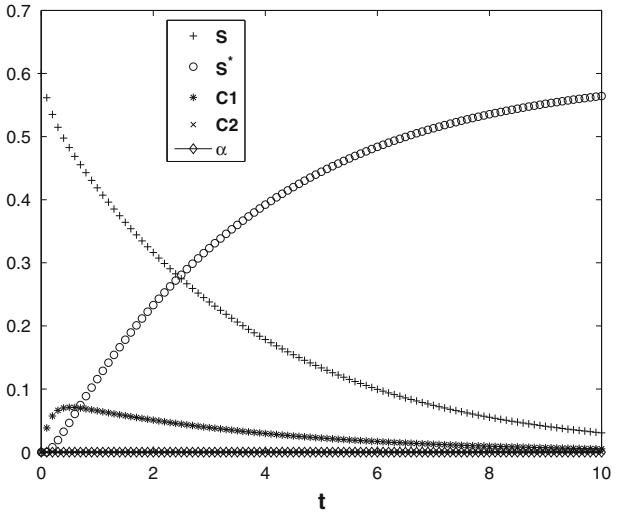

Fig. 5 Control of the substrate concentration. $m = 0.6, \delta = 0.1. a_1 = 0.8$, $d_1 = d_2 = 2.0$, $k_1 = k_2 = 2.0$, $E_{1T} = E_{2T} = 0.8$, $S_T = 0.6$. The control is always inactive because setting its value equal to zero completely inhibits dephosphorylation and the system phosphorylates all the substrate S, thus S^* tends to $S_T = 0.6$

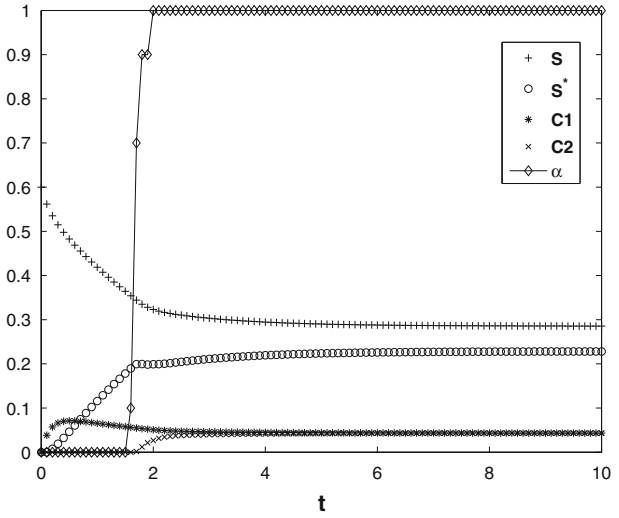

Fig. 6 Control of the substrate concentration. $m = 0.1, \delta = 0.1. a_1 = 0.8$, $d_1 = d_2 = 2.0$, $k_1 = k_2 = 2.0$, $E_{1T} = E_{2T} = 0.8$, $S_T = 0.6$. Since S^* concentration must be maintained at a low level, i.e., $S^* \in [0.0, 0.2]$, quite immediately the control reaches its maximum value, in order to support the dephosphorylation mechanism

In this paper we have applied the theory of Optimal Control and of the viscosity solutions of the HJB equations to the analysis of two study cases concerning enzyme reactions (Michaelis–Menten reaction with product degradation and

Goldbeter–Koshland switch), where one of the kinetic parameters characterizing the reaction acts as a control, in order to force some reactants to tend to predetermined asymptotic values.

The numerical results, here reported and obtained in [26] by means of Parallel Computing techniques, show a very good agreement between the theoretical models and the experience, mainly for what concerns, in the first example, the balance between product toxicity and drug costs and/or toxicity.

Taking into account the complexity of the intracellular reaction networks, these study cases can be considered as first examples of the application of the Optimal Control techniques and can be extended to more complex networks, where several controls can be inserted, in order to impose scheduled constraints on the behaviors of several enzymes involved in the same network, acting on their reaction rates.

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