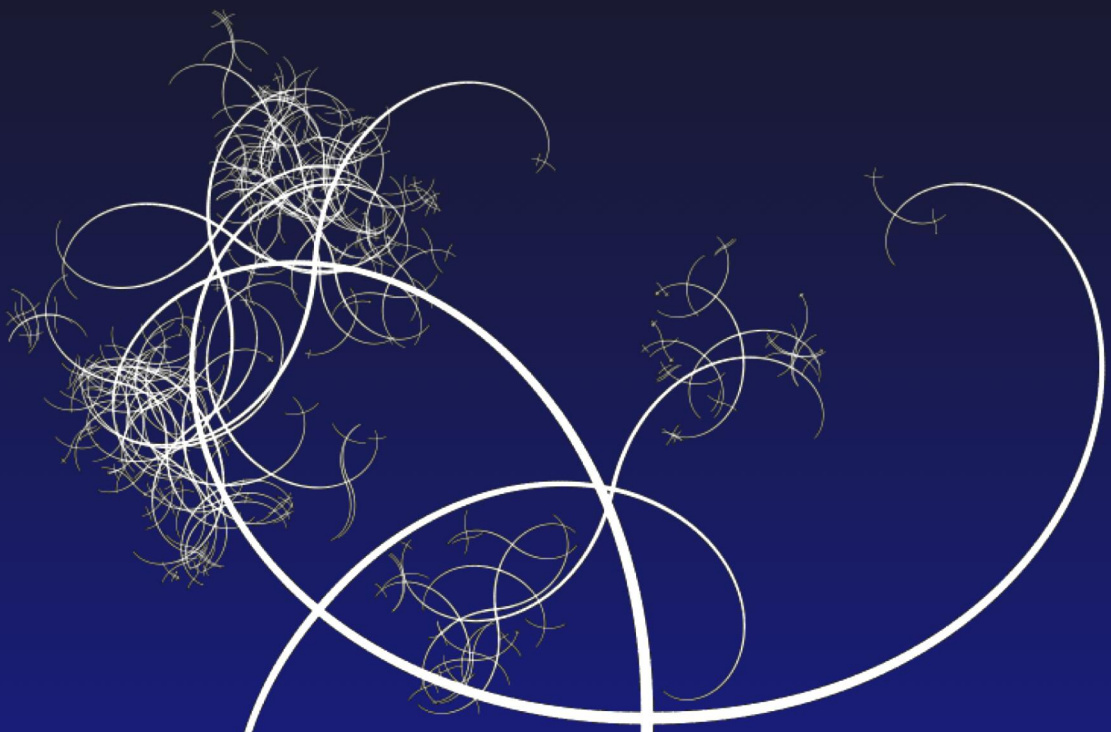
NISSUNA UMANA INVESTIGAZIONE SI PUO DIMANDARE VERA SCIENZIA
S'ESSA NON PASSA PER LE MATEMATICHE DIMOSTRAZIONI
LEONARDO DA VINCI



vol. 8 no. 4 2020

Mathematics and Mechanics *of* Complex Systems

Alberto Maria Bersani, Alessandro Borri,
Francesco Carravetta, Gabriella Mavelli and Pasquale Palumbo

ON A STOCHASTIC APPROACH TO MODEL THE DOUBLE PHOSPHORYLATION/DEPHOSPHORYLATION CYCLE

msp

MATHEMATICS AND MECHANICS OF COMPLEX SYSTEMS
Vol. 8, No. 4, 2020
https://doi.org/10.2140/memocs.2020.8.261

ON A STOCHASTIC APPROACH TO MODEL THE DOUBLE PHOSPHORYLATION/DEPHOSPHORYLATION CYCLE

ALBERTO MARIA BERSANI, ALESSANDRO BORRI,
FRANCESCO CARRAVETTA, GABRIELLA MAVELLI AND PASQUALE PALUMBO

Because of the unavoidable intrinsic noise affecting biochemical processes, a stochastic approach is usually preferred whenever a deterministic model gives too rough information or, worse, may lead to erroneous qualitative behaviors and/or quantitatively wrong results. In this work we focus on the chemical master equation (CME)-based method which provides an accurate stochastic description of complex biochemical reaction networks in terms of the probability distribution of the underlying chemical populations. Indeed, deterministic models can be dealt with as first-order approximations of the average-value dynamics coming from the stochastic CME approach. Here we investigate the double phosphorylation/dephosphorylation cycle, a well-studied enzymatic reaction network where the inherent double time scale requires one to exploit quasisteady state approximation (QSSA) approaches to infer qualitative and quantitative information. Within the deterministic realm, several researchers have deeply investigated the use of the proper QSSA, agreeing to highlight that only one type of QSSA (the total QSSA) is able to faithfully replicate the qualitative behavior of bistability occurrences, as well as the correct assessment of the equilibrium points, accordingly to the not approximated (full) model. Based on recent results providing CME solutions that do not resort to Monte Carlo simulations, the proposed stochastic approach shows some counterintuitive facts arising when trying to straightforwardly transfer bistability deterministic results into the stochastic realm, and suggests how to handle such cases according to both theoretical and numerical results.

1. Introduction

One of the main contributions of mathematicians to the biological field is one of the best-known models of enzyme kinetics put forth during the beginning of the twentieth century by Henri [1901a; 1901b; 1902], and Michaelis and Menten [1913],

Communicated by Victor A. Eremeyev.
MSC2010: primary 34F05, 37L55, 60H10, 60H35; secondary 92C42, 92C45.
Keywords: Michaelis–Menten kinetics, quasisteady state approximation, deterministic and stochastic processes, phosphorylation, chemical master equation, Markov processes.

and later continued by Briggs and Haldane [1925]. 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. These reactions are summarized in the scheme

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C \xrightarrow{k_2} E + P, \tag{1}$$

where k_1, k_{-1}, and k_2 are kinetic parameters associated with the reaction rates (i.e., rate constants).

A very common approximation in the deterministic setting consists of assuming that, after a transient phase, the complex concentration can be considered approximately constant with respect to the substrate dynamics: standard quasisteady state approximation (sQSSA) [Lin and Segel 1988]. The sQSSA utilizes timescale separation to project models of biochemical networks onto lower-dimensional slow manifolds; thus, rapidly fluctuating species are not simulated explicitly (see, among others, [Segel 1988; Segel and Slemrod 1989], and the review paper [Bersani et al. 2015]). In recent decades, many researchers highlighted limits and malfunctioning of the sQSSA, thereby introducing and exploring a new approximation, called total quasisteady state approximation (tQSSA). Under suitable and biologically consistent hypotheses, the tQSSA-based methods were revealed to be very effective in handling the full system of equations, considerably unburdening the computational effort and providing a good approximation at the same time (see, among others, [Laidler 1955; Borghans et al. 1996; Tzafriri 2003; Dell'Acqua and Bersani 2012; Bersani and Dell'Acqua 2012; Bersani et al. 2015]). In the case of reactions that involve only a small number of key regulatory molecules, intrinsic noise is not negligible [Blake et al. 2003; Fedoroff and Fontana 2002], and the enzymatic reaction scheme is more appropriately modeled in a stochastic discrete framework by means of CMEs [van Kampen 2007]. CME-based modeling is a promising approach in systems biology due to its capability of well-fitting experimental data in single-cell experiments, also describing diffusion effects derived from fluctuations and chemical fluxes capable of driving switching from one equilibrium to another. In more detail, the CME provides an accurate stochastic description of complex biochemical networks in terms of the probability distribution of the underlying chemical populations, in contrast to deterministic methods which handle biochemical processes in terms of evolution of the average concentrations for each involved species. Indeed, deterministic models can be dealt with as first-order approximations of the average-value dynamics coming from the stochastic CME approach [van Kampen 2007]. Within the framework of enzymatic reaction networks, many authors investigated the QSSAs via the CME approach, with the aim of providing a good approximation of the full model also in this setting [Cao and Petzold 2005; Gillespie 2001; 2009a;

2009b; Cao et al. 2005; Mastny et al. 2007; Rao and Arkin 2003; Székely and Burrage 2014; Thomas et al. 2011]. In [Barik et al. 2008; MacNamara et al. 2008] the authors studied independently the application of the tQSSA to some well-known biochemical mechanisms providing bistability according to deterministic models.

The work proposed here investigates a CME-based stochastic model of the double phosphorylation/dephosphorylation (PDP) mechanism. This kind of activation/deactivation reaction might be the key to explaining the interactions occurring among the intracellular enzyme networks and several intercellular and macroscopic phenomena, as could be the case highlighted in [George et al. 2019], in the framework of mechanobiology and bone remodeling [Bednarczyk and Lekszycki 2016; Giorgio et al. 2016; 2019; George et al. 2018].

The double PDP cycle is a paradigmatic case of how the application of the sQSSA may provide qualitatively wrong results. With regards to the deterministic approach, several authors (see for example [Ortega et al. 2006; Chickarmane et al. 2007; Kholodenko 2000; Bersani et al. 2011]) studied the appearance of bistable states in the double PDP mechanism, for both the full system and the QSSA settings. In [Dell'Acqua and Bersani 2013; 2011], it is shown that the tQSSA reproduces the behavior of the solutions of the full system for a very wide range of parameters and different initial conditions. On the contrary, the sQSSA can provide misleading results, mainly in the asymptotic concentration values, predicting bistability for large value ranges, whereas the full system (and the tQSSA) shows monostability.

Bistability of several biochemical mechanisms usually in the stochastic framework results in a bimodal stationary probability distribution with randomness allowing for fluctuation around both modes of the distribution, preventing the evolution to stick around just one of the two equilibrium points [Hwang and Velázquez 2013a; 2013b; Bruna et al. 2014; Bazzani et al. 2012; Samoilov et al. 2005]. Within this framework, QSSA still plays an important role to unburden the computational load, though sQSSA may often lead to large errors (both quantitative in matching the wrong modes, and qualitative in failing to catch the bimodal fashion) even when timescale separation holds (see [Kim et al. 2014; 2015] where the stochastic tQSSA is shown to be more accurate than the sQSSA).

Let us underline that references [Kim et al. 2014; 2015] provide very interesting insights into the investigation of stochastic QSS approximations. The common denominator with our work is the way of associating propensities to CME from an ODE initial framework. In more detail, they investigated how different QSS approximations (especially in [Kim et al. 2014], where they consider standard QSSA, total QSSA, and prefactor QSSA) may provide similar results in the deterministic field, but completely different results in the stochastic field. Indeed, the authors showed that, according to a specific setting of the model parameters under investigation, deterministic tQSSA and pQSSA provided the same ODE system

(therefore leading to the same results), while stochastic tQSSA and pQSSA provided a completely different stochastic approximation. In other words, [Kim et al. 2014] provided some *caveat* concerning different QSSAs and provided a criterion to understand in a specific framework whether a stochastic QSSA is reliable or not. Unfortunately these results are not straightforwardly applicable to our case: in [Kim et al. 2015] a unique double time scale enzymatic reaction is considered, while in our manuscript we consider four double time scale enzymatic reactions.

Our study investigates the applicability of both standard and total QSSA to a CME-based stochastic model of the double PDP cycle, showing the preeminent role of the tQSSA. The methodology exploited is the one proposed in [Borri et al. 2013; 2016; Bersani et al. 2014] managing to cope with the computational burden, which usually arises for CMEs, by means of a proper organization of the probabilities in the CME entries. This enables us to characterize the CME dynamics according to a recursive block-tridiagonal structure. In this way, explicit solutions of the CME are achieved for both standard and total QSSA, according to a smart application of the Gauss elimination method [Borri et al. 2016]. This allows us not to resort to Monte Carlo simulation, which may be computationally demanding as well as lead to misleading results unless allowing for enough stochastic realizations (whose number is not known a priori).

Preliminary results have been presented in [Bersani et al. 2014], by introducing the CME-based stochastic model for the double PDP cycle. In the present work we get in deeper details, proposing solutions to the CME according to a wider set that goes beyond the toy-setting of [Bersani et al. 2014], facing real numerical problems arising when dealing with the double time scale. Besides the larger variety of cases here reported, we aim at answering the questions arisen in [Bersani et al. 2014] and to conciliate some counterintuitive behaviors occurring when trying to straightforwardly apply deterministic results to stochastic models. To this end, some new theoretical results on the CME-based stochastic model have been assessed, showing the uniqueness of the stationary probability distribution.

The result of the work is twofold. On the one hand, it shows the preeminent role of the tQSSA, also in the stochastic CME-based model of the double PDP cycle. Its ability to faithfully replicate in the stochastic framework the qualitative behavior of bistability occurrences is shown, as well as the correct assessment of the equilibrium points, in conformity with the nonapproximated model. On the other hand, according to a given setting in the parameter space, and to chosen initial conditions, bistability provided by the deterministic model may be lost in a unimodal distribution when dealing with the stochastic CME. Such a mode coincides with one of the two deterministic equilibria. This counterintuitive result is obtained by means of Monte Carlo simulation for the full system, and is confirmed by the exact CME solution provided by the tQSSA.

The paper is organized as follows. In Section 2 we briefly recall the most important background concerning the sQSSA and the tQSSA for enzymatic reactions. Section 3 proposes the double PDP reactions in detail, dealing with the CME-based stochastic model. Section 4 reports the standard and total QSSA of the double PDP cycle CME-based stochastic model, by first providing them in the deterministic framework. A novel result on the uniqueness of the stationary probability distribution is also provided in this section. In Section 5 we discuss the appearance (or the absence) of stationary bimodality in comparison to bistability arising in deterministic models. Section 6 contains the concluding remarks and perspectives.

2. Introductory notions on sQSSA and tQSSA

The *Michaelis–Menten* (MM) *kinetics* give a very good description of (1), in terms of ordinary differential equations (ODEs). For notational convenience we will use the same symbol to denote both a chemical species and its concentration (i.e., the variables of the ODEs), omitting its dependence on time. We can mathematically describe reaction (1) using the mass action principle — where the growth rates of each reactant are proportional to the instantaneous concentrations of the reactants themselves — and conservation laws. This approach leads to the (full) system

$$\begin{cases} \frac{dS}{dt} = -k_1(E_T - C)S + k_{-1}C, \\ \frac{dC}{dt} = k_1(E_T - C)S - (k_{-1} + k_2)C, \end{cases} \tag{2}$$

with the initial conditions

$$S(0) = S_T, \qquad C(0) = 0, \qquad E(0) = E_T \tag{3}$$

and the conservation laws

$$E + C = E_T, \qquad S + C + P = S_T, \tag{4}$$

where E_T and S_T are the total enzyme and substrate concentrations, respectively.

The MM reaction is characterized by two phases: a short transient phase of rapid increase of the complex C and a second, slower, phase, called the *quasisteady state phase*, where the complex is considered substantially in equilibrium.

The hypothesis of quasisteady state simplifies the reaction, leading to an ODE for the substrate, with initial condition $S(0) = S_T$, while the complex is assumed to be in a quasisteady state, i.e., $\frac{dC}{dt} \approx 0$. The *standard QSSA* (sQSSA) of system (2) is thus achieved:

$$\begin{cases} C \approx E_T \cdot S/(K_M + S), \\ \frac{dS}{dt} \approx -k_2 C \approx -V_{\max} S/(K_M + S), \\ S(0) = S_T, \end{cases} \tag{5}$$

where $K_M = (k_2 + k_{-1})/k_1$ is called the MM constant or affinity constant, and $V_{\max} = k_2 E_T$.

Let us consider again the classical MM kinetics (2). Introducing the total substrate at generic time instant t, $\bar{S}(t) = S(t) + C(t)$, (2) then becomes

$$\begin{cases} \frac{d\bar{S}}{dt} = -k_2 C, \\ \frac{dC}{dt} = k_1[C^2 - (E_T + \bar{S} + K_M)C + E_T \bar{S}], \\ \bar{S}(0) = S_T, \ C(0) = 0, \end{cases} \tag{6}$$

with conservation laws

$$E + C = E_T, \qquad \bar{S} + P = S_T. \tag{7}$$

Assuming that the complex is in a quasisteady state $\left(\frac{dC}{dt} \approx 0\right)$ yields the *total QSSA* (tQSSA) [Borghans et al. 1996], which is valid for a broader range of parameters, with respect to sQSSA, covering both high and low enzyme concentrations:

$$\frac{d\bar{S}}{dt} \approx -k_2 C_-(\bar{S}), \qquad \bar{S}(0) = S_T, \tag{8}$$

where

$$C_-(\bar{S}) = \frac{(E_T + K_M + \bar{S}) - \sqrt{(E_T + K_M + \bar{S})^2 - 4E_T\bar{S}}}{2} \tag{9}$$

is the only biologically allowed solution of $\frac{dC}{dt} = 0$ in the second equation of (6).

3. CME-based stochastic model of the double PDP cycle

The double PDP cycle is one of the most important biochemical mechanisms in intracellular reaction networks. The scheme here investigated is a generalization of the enzymatic reaction network (1) and refers to [Ortega et al. 2006], where both phosphorylation and dephosphorylation are supposed to happen in only one step. Reactions are reported in (10), where M, M_p, and M_{pp} represent the inactive, the mono-phosphorylated, and the double-phosphorylated substrate, respectively, K and P are the kinase (the phosphorylating enzyme) and the phosphatase (the dephosphorylating enzyme), respectively, and C_i are the intermediate complexes:

$$\begin{aligned} M + K &\underset{k_{-11}}{\overset{k_{11}}{\rightleftarrows}} C_1 \xrightarrow{k_{12}} M_p + K, \qquad & M_p + K &\underset{k_{-21}}{\overset{k_{21}}{\rightleftarrows}} C_2 \xrightarrow{k_{22}} M_{pp} + K, \\ M_{pp} + P &\underset{k_{-31}}{\overset{k_{31}}{\rightleftarrows}} C_3 \xrightarrow{k_{32}} M_p + P, \qquad & M_p + P &\underset{k_{-41}}{\overset{k_{41}}{\rightleftarrows}} C_4 \xrightarrow{k_{42}} M + P. \end{aligned} \tag{10}$$

Before building the stochastic model, we write the deterministic full system by exploiting the mass conservation law to reduce the system complexity. Indeed, the

conservation law involves the total substrate M_T,

$$M + M_p + M_{pp} + C_1 + C_2 + C_3 + C_4 = M_T, \tag{11}$$

and the total enzymes K_T and P_T,

$$K + C_1 + C_2 = K_T, \qquad P + C_3 + C_4 = P_T, \tag{12}$$

so that it is possible to reduce the number of deterministic variables (species concentrations) to six independent ones. By taking, for example, $\{M, M_{pp}, C_1, C_2, C_3, C_4\}$ as the set of the independent variables, using the law of mass action, the full system of equations governing the dynamics of the system is therefore

$$\begin{aligned}
\frac{dM}{dt} &= -k_{11}MK + k_{-11}C_1 + k_{42}C_4,\\
\frac{dM_{pp}}{dt} &= -k_{31}M_{pp}P + k_{-31}C_3 + k_{22}C_2,\\
\frac{dC_1}{dt} &= k_{11}MK - (k_{-11} + k_{12})C_1,\\
\frac{dC_2}{dt} &= k_{21}M_pK - (k_{-21} + k_{22})C_2,\\
\frac{dC_3}{dt} &= k_{31}M_{pp}P - (k_{-31} + k_{32})C_3,\\
\frac{dC_4}{dt} &= k_{41}M_pP - (k_{-41} + k_{42})C_4,
\end{aligned} \tag{13}$$

with initial conditions

$$M(0) = M_T, \qquad M_{pp}(0) = 0, \qquad C_i(0) = 0, \tag{14}$$

where $i = 1, \ldots, 4$. Let us observe that, for the sake of brevity, we left in (13) the terms M_p, K, and P, which are related to the six independent variables by (11) and (12):

$$\begin{aligned}
M_p &= M_T - (M_{pp} + C_1 + C_2 + C_3 + C_4 + M),\\
K &= K_T - (C_1 + C_2), \qquad P = P_T - (C_3 + C_4).
\end{aligned}$$

According to a large variety of literature that manages to reformulate the dynamics of a system from an ODE into a CME (chemical master equation) framework (see, e.g., [Bazzani et al. 2012] or [Bersani et al. 2014]), we treat the state variables as discrete copy numbers. In this context, we reinterpret the deterministic reaction rates as probabilities per unit time (or propensities) of a properly defined continuous-time Markov chain (CTMC), i.e., a stochastic process whose

event	reset	propensity
M, K binding	$x_1 \mapsto x_1 - 1$ $x_3 \mapsto x_3 + 1$	$k_{11} x_1 (K_T - x_3 - x_4)$
M, K unbinding	$x_1 \mapsto x_1 + 1$ $x_3 \mapsto x_3 - 1$	$k_{-11} x_3$
M_p release	$x_3 \mapsto x_3 - 1$	$k_{12} x_3$
M_p, K binding	$x_4 \mapsto x_4 + 1$	$k_{21}(M_T - \sum_{i=1}^{6} x_i)(K_T - x_3 - x_4)$
M_p, K unbinding	$x_4 \mapsto x_4 - 1$	$k_{-21} x_4$
M_{pp} release	$x_2 \mapsto x_2 + 1$ $x_4 \mapsto x_4 - 1$	$k_{22} x_4$
M_{pp}, P binding	$x_2 \mapsto x_2 - 1$ $x_5 \mapsto x_5 + 1$	$k_{31} x_2 (P_T - x_5 - x_6)$
M_{pp}, P unbinding	$x_2 \mapsto x_2 + 1$ $x_5 \mapsto x_5 - 1$	$k_{-31} x_5$
M_p release	$x_5 \mapsto x_5 - 1$	$k_{32} x_5$
M_p, P binding	$x_6 \mapsto x_6 + 1$	$k_{41}(M_T - \sum_{i=1}^{6} x_i)(P_T - x_5 - x_6)$
M_p, P unbinding	$x_6 \mapsto x_6 - 1$	$k_{-41} x_6$
M release	$x_1 \mapsto x_1 + 1$ $x_6 \mapsto x_6 - 1$	$k_{42} x_6$

Table 1. Chemical reactions, full system.

trajectories evolve on an n-dimensional lattice, and whose dynamics (in terms of probability of being in a specific state of the CTMC) is described by the CME.

Renaming the independent state variables in the CME-based stochasting setting as $x_1 = M$, $x_2 = M_{pp}$, $x_3 = C_1$, $x_4 = C_2$, $x_5 = C_3$, and $x_6 = C_4$, the reset map associated with the chemical reaction network in (10) is reported in Table 1.

The CME dynamics is $\dot{P} = G\mathcal{P}$, where G is called the infinitesimal generator (or transition matrix) of the CTMC, which is built according to the propensities in Table 1 (see [Borri et al. 2016] for further details), and $\mathcal{P}$ is the vector collecting the time-varying probabilities of all the states of the process. When the dimension of G is large enough to make computationally too demanding the exact solution of the equilibrium equation, $G\mathcal{P} = 0$, one can still employ the Gillespie stochastic simulation algorithm (SSA) [Gillespie 1977; 2001; 2009a; 2009b; Cao et al. 2005], returning one or more statistically correct trajectories of the process, which can be used in a Monte Carlo simulation or in an ergodic setting to obtain a sampled (approximate) equilibrium distribution of the process.

4. QSSA of the double PDP cycle stochastic model

Similarly to the deterministic approach, where the complexes behave as fast variables, while the substrates are the slow variables, the double time scale affects as well the CMEs associated with the reset map detailed in Table 1, slowing down the computational efficiency. Therefore, a need exists to introduce a QSSA also for the stochastic case. Differently from other reactions, as for example the so-called auxiliary (or coupled) reactions [Eilertsen and Schnell 2018], where multiple timescales are present, the four reactions involved in the PDP cycle are characterized by a double timescale. In [Bersani et al. 2014] a way to obtain both standard and total QSSA for the double PDP cycle was shown. In both cases, we start from the ODE version of the QSSA and *stochastify* it by introducing suitably defined one-step processes. Note that, as already described at the end of Section 3, the stochastic approach considered in [Bersani et al. 2014] and here does not consist of perturbing the ODE setting by means of noise terms, which is typical of the stochastic differential equation (SDE)/Langevin approach, but of reinterpreting the deterministic reaction rates as probabilities per unit time (or propensities) of a properly defined CTMC.

With respect to the ODE systems reported below, the continuous state variables actually represent a first-order approximation of the expected value of the copy numbers, which are indeed stochastic variables [van Kampen 2007]. Section 4.1 treats the QSSAs of the ODE system, whereas Section 4.2 concerns the CME-based stochastic version of the QSSAs. Finally, in Section 4.3 a sufficient condition for the uniqueness of the stationary solution for the CME has been provided.

4.1. ***QSSA of the deterministic model of the double PDP cycle.*** With regard to the sQSSA, its ODE version is written by setting the complex dynamics at steady state. In this way complexes are related to substrate and enzyme concentrations by means of algebraic constraints and, after computations (see [Bersani et al. 2011] and references therein for the details), the M and M_{pp} dynamics become

$$\begin{aligned} \frac{dM}{dt} &= -\frac{k_{12}}{K_1} MK + \frac{k_{42}}{K_4} M_p P, \\ \frac{dM_{pp}}{dt} &= \frac{k_{22}}{K_2} M_p K - \frac{k_{32}}{K_3} M_{pp} P, \end{aligned} \tag{15}$$

where $K_i = (k_{-i1} + k_{i2})/k_{i1}$, $i = 1, \ldots, 4$, with

$$K = \frac{K_T}{1 + M/K_1 + M_p/K_2}, \qquad P = \frac{P_T}{1 + M_{pp}/K_3 + M_p/K_4}. \tag{16}$$

Concerning M_p, the sQSSA constrains it to other substrates according to

$$M_p = M_T - M - M_{pp}, \tag{17}$$

thus neglecting the complexes' contribution to the mass conservation law.

Remark. The comparison of the mass conservation law of the full system (11) with (17) leads to the so-called *complex depletion paradox* [Dell'Acqua and Bersani 2013]: the application of the sQSSA implies that, even if the complexes are related to the substrates by their algebraic equations, they are implicitly set equal to zero, because of (17). The consequences are that the sQSSA predicts asymptotic values for the different substrate species which are higher than those predicted by the full system.

For what concerns the tQSSA, as in [Bersani et al. 2011; Dell'Acqua and Bersani 2013; 2011], we set the total substrates at a generic time instant t:

$$\overline{M} = M + C_1, \qquad \overline{M}_p = M_p + C_2 + C_4, \qquad \overline{M}_{pp} = M_{pp} + C_3. \tag{18}$$

In terms of these new variables, the dynamics of the total substrates are given by

$$\begin{aligned} \frac{d\overline{M}}{dt} &= k_{42}C_4 - k_{12}C_1, \\ \frac{d\overline{M}_{pp}}{dt} &= -k_{32}C_3 + k_{22}C_2, \end{aligned} \tag{19}$$

with conservation law

$$\overline{M} + \overline{M}_p + \overline{M}_{pp} = M_T. \tag{20}$$

Moreover, by properly accounting for the QSSA, i.e., complexes' dynamics at steady state, we have the constraints

$$\begin{aligned} (\overline{M} - C_1)(K_T - C_1 - C_2) - K_1C_1 &= 0, \\ (\overline{M}_p - C_2 - C_4)(K_T - C_1 - C_2) - K_2C_2 &= 0, \\ (\overline{M}_{pp} - C_3)(P_T - C_3 - C_4) - K_3C_3 &= 0, \\ (\overline{M}_p - C_2 - C_4)(P_T - C_3 - C_4) - K_4C_4 &= 0. \end{aligned} \tag{21}$$

Remark. It is worth noting that the complex depletion paradox emerging for the sQSSA is not present for the total, since no violation of the mass conservation law occurs. Thus, the tQSSA yields the same asymptotic values for all the reactants (complexes included) as the full system.

4.2. ***QSSA of the CME-based stochastic model of the PDP cycle.*** In agreement with [Bazzani et al. 2012] or [Bersani et al. 2014], we treat the state variables in (15) and (19) as discrete copy numbers that increase by one or decrease by one (according to a one-step process approach [van Kampen 2007]), with propensity provided by the sum of the production or clearance rates of the ODE dynamics for the one-step increase or decrease reaction, respectively. In this way, the reset maps associated with the standard and total QSSA are reported in Tables 2 and 3,

event	reset	propensity
M production	$M \mapsto M+1$	$w_1^{(s)}$
M clearance	$M \mapsto M-1$	$w_2^{(s)}$
M_{pp} production	$M_{pp} \mapsto M_{pp}+1$	$w_3^{(s)}$
M_{pp} clearance	$M_{pp} \mapsto M_{pp}-1$	$w_4^{(s)}$

Table 2. Chemical reactions, sQSSA.

event	reset	propensity
$\overline{M}$ production	$\overline{M} \mapsto \overline{M}+1$	$w_1^{(t)}$
$\overline{M}$ clearance	$\overline{M} \mapsto \overline{M}-1$	$w_2^{(t)}$
$\overline{M}_{pp}$ production	$\overline{M}_{pp} \mapsto \overline{M}_{pp}+1$	$w_3^{(t)}$
$\overline{M}_{pp}$ clearance	$\overline{M}_{pp} \mapsto \overline{M}_{pp}-1$	$w_4^{(t)}$

Table 3. Chemical reactions, tQSSA.

respectively. Regarding the propensities, the ones of the sQSSA, achieved from (15), by exploiting constraints (16) and (17), are

$$w_j^{(s)}(x_1,x_2)=\begin{cases}\dfrac{k_{42}P_T K_3(M_T-x_1-x_2)}{K_3K_4+K_4x_2+(M_T-x_1-x_2)K_3}, & j=1,\\[2ex] \dfrac{k_{12}K_T K_2 x_1}{K_1K_2+K_2x_1+K_1(M_T-x_1-x_2)}, & j=2,\\[2ex] \dfrac{k_{22}K_T K_1(M_T-x_1-x_2)}{K_1K_2+K_2x_1+K_1(M_T-x_1-x_2)}, & j=3,\\[2ex] \dfrac{k_{32}P_T K_4 x_2}{K_3K_4+K_4x_2+K_3(M_T-x_1-x_2)}, & j=4.\end{cases} \tag{22}$$

With regards to the tQSSA, one needs to solve the system of equations (21) with respect to the complexes $C_1,\dots,C_4$ (see, e.g., [Pedersen et al. 2008]), which are functions of the state, and then define

$$w_j^{(t)}(x_1,x_2)=\begin{cases}k_{42}C_4(x_1,x_2), & j=1,\\ k_{12}C_1(x_1,x_2), & j=2,\\ k_{22}C_2(x_1,x_2), & j=3,\\ k_{32}C_3(x_1,x_2), & j=4.\end{cases} \tag{23}$$

4.3. ***Uniqueness of the stationary distribution.*** An important feature to be investigated when dealing with stochastic models coming from CME is whether the stationary distribution is unique, whatever the CME initial conditions. Besides the qualitative behavior properties, the uniqueness of the stationary distribution is

invoked also when dealing with Monte Carlo numerical issues, since it implies the process is ergodic, thus allowing one to resort to a unique very long run of the stochastic sampling algorithm (SSA), by inferring the statistical distribution from the computation of the average recurrence time in each state of the process. With respect to the PDP cycle investigated in this paper, such an issue is of paramount importance: in case of bistability, what one is expected to find from the CME stochastic approach is to have a bimodal stationary distribution, with the modes close to the ODE stable stationary equilibria. However, supposing that bimodality actually occurs, it may happen that any single stochastic realization is able to exhibit only one of the two modes, according to the chosen initial conditions. This is because the model parameters are such that too much time would be required in order to completely explore the space of the admissible states. To know a priori whether the stationary distribution is unique or not allows one to understand if different stochastic realizations provide different stationary distributions because of different initial conditions or just because of the too long time required to obtain a stochastically exhaustive trajectory. To properly address the issue concerning the uniqueness of the stationary distribution, we consider the graph associated with the CTMC of the CME under investigation.

Theorem 1. *The graph associated with the full system is strongly connected.*

Proof. The graph associated with the full system consists of as many nodes as feasible 6-tuples provided by the copy numbers of the independent species M, M_{pp}, C_1, C_2, C_3, C_4, with node A connected to node B if there exists a reaction that updates the species' copy numbers from A to B. Differently from a one-step process, here we have reactions that simultaneously vary couples of state variables, namely M, K binding and unbinding, M_{pp} release, M_{pp}, P binding and unbinding, and M release (see Table 1). The proof consists of showing that, starting from any 6-tuple, there exists a combination of feasible reactions providing any possible one-step update. This fact allows the system to inherit the strong connectivity property associated with one-step processes. The one-step updates we consider, clearly, disregard the ones already provided by the chemical reaction network. Below, for any such one-step update we report the sequence of reactions required to obtain it:

- $M \mapsto M + 1$: provided by the combination of M_p, P binding and M release.
- $M \mapsto M - 1$: provided by the combination of M, K binding and M_p release.
- $M_{pp} \mapsto M_{pp} + 1$: provided by the combination of M_p, K binding and M_{pp} release.
- $M_{pp} \mapsto M_{pp} - 1$: provided by the combination of M_{pp}, P binding and M_p release.

- $C_1 \mapsto C_1 + 1$: provided by the combination of M, K binding, M_p, P binding, and M release.
- $C_3 \mapsto C_3 + 1$: provided by the combination of M_p, K binding, M_{pp}, P binding, and M_{pp} release. □

Theorem 1 proves that the graph associated with the CME here considered is strongly connected, i.e., for any two nodes of the graph there exists a path that connects one node to the other, and vice versa. This is sufficient to prove that there exists a unique *terminal strongly connected component* associated with any of the three graphs (the graph itself, actually), and this proves the uniqueness of the stationary solution for the CME [Bang-Jensen and Gutin 2009].

Remark. To prove the strong connectivity of both the QSSAs is trivial. Indeed, they are built as one-step processes; therefore, the graph associated with them is a complete 2D lattice where any point is reachable from any other by means of one-step independent movement. Therefore, results provided in Section 3 for the full system, dealing with the uniqueness of the stationary probability distribution, hold true also for both standard and total QSSA.

5. ODE bistability versus CME bimodality: a word of caution

This section is devoted to investigating and discussing whether bistability behavior arising from deterministic models of the double PDP cycle transforms into a bimodal distribution of the stationary probability distribution coming from the CME-based stochastic model. To this end, Monte Carlo simulations are carried out when dealing with the original full system, while numerical tools providing the CME analytical solution are exploited for both standard and total QSSAs.

In the following four illustrated cases, the values of the chosen parameters in (10) are $k_{11} = 0.02$, $k_{-11} = 1$, $k_{12} = 0.01$; $k_{21} = 0.032$, $k_{-21} = 1$, $k_{22} = 15$; $k_{31} = 0.045$, $k_{-31} = 1$, $k_{32} = 0.092$; and $k_{41} = 0.01$, $k_{-41} = 1$, $k_{42} = 0.5$.

We set $M_T = 500$ and four different pairs of mass-balance constraints for the kinase and phosphatase:

(a) $K_T = 200$, $P_T = 200$,

(b) $K_T = 600$, $P_T = 400$,

(c) $K_T = 292$, $P_T = 300$,

(d) $K_T = 293$, $P_T = 300$,

with initial condition $M(0) = M_T$ in the deterministic case. Following [Dell'Acqua and Bersani 2011] (see Figures 3 and 4 therein), when we plot the initial value of the kinase MAPKK, i.e., K_T (on the horizontal axis) and the corresponding asymptotic value of M_{pp} (on the vertical axis), we obtain:

(a) The deterministic full system is bistable, with equilibrium points for M_{pp} equal to

$$M_{pp}^1 = 4.30, \qquad M_{pp}^2 = 290.97;$$

the deterministic sQSSA and tQSSA are able to recover the bistable behavior, but with completely different levels of accuracy. In fact, the equilibria are

$$M_{pp}^{s,1} = 2.52, \qquad M_{pp}^{s,2} = 494.35$$

for the sQSSA, and

$$M_{pp}^{t,1} = 4.31, \qquad M_{pp}^{t,2} = 290.86$$

for the tQSSA. This implies that also in this case the tQSSA is much more reliable than the sQSSA, which in fact suffers from the so-called *complex depletion paradox*, as discussed in [Dell'Acqua and Bersani 2011].

(b) The deterministic full system is monostable, with equilibrium point for M_{pp} equal to

$$M_{pp} = 118.89;$$

the deterministic tQSSA is able to reproduce this feature:

$$M_{pp}^t = 118.17.$$

As discussed in [Dell'Acqua and Bersani 2013; 2011], the sQSSA always gives bistability in a larger set of values of K_T than the full system and the tQSSA; in fact, in this case the sQSSA has two stable states

$$M_{pp}^{s,1} = 9.08, \qquad M_{pp}^{s,2} = 496.94,$$

which are very far from the real equilibrium.

(c) The full system is bistable, with equilibrium points for M_{pp} equal to

$$M_{pp}^1 = 5.70, \qquad M_{pp}^2 = 185;$$

the deterministic sQSSA and tQSSA are able to reproduce the bistable behavior, but with completely different levels of accuracy. In fact, the equilibria are

$$M_{pp}^{s,1} = 2.30, \qquad M_{pp}^{s,2} = 494$$

for the sQSSA, and

$$M_{pp}^{t,1} = 5.70, \qquad M_{pp}^{t,2} = 185$$

for the tQSSA. This case also confirms the superiority of the tQSSA with respect to the sQSSA.

(d) This case presents the same features as case (c), but even if it slightly differs in the amount of the mass-balance constraint K_T for the kinase, it is of interest because of the peculiar phenomenon shown by its stochastic counterpart, as described below.

We now compare the behavior of the stochastic representations of full system as well as the sQSSA and tQSSA. With regards to the full system, the state evolves on a 6-dimensional lattice (copy numbers refer to M, M_{pp} and the 4 complexes), formally bounded by the substrate upper bound ($M_T = 500$) and the complexes' upper bounds provided by $\min\{K_T, M_T\}$ for C_1, C_2 and $\min\{P_T, M_T\}$ for C_3, C_4. For instance, concerning case (a), the lattice is inside a 6-dimensional box lattice including $500^2 \times 200^4 \simeq 400$ trillion states. Clearly, not all such states are admissible (e.g., because they can violate the mass conservation laws) but, in any case, there still remain too many states (billions) that prevent any reliable numerical approach to straightforwardly solve the underlining CME. For this reason, the full system is simulated by means of statistical methods, such as the Gillespie stochastic simulation algorithm (SSA) [Gillespie 1977; 2001; 2009a; 2009b; Cao et al. 2005], where Theorem 1 ensures the uniqueness of the stationary probability distribution and the ergodicity of the CTMC associated with the CME.

On the other hand, both the standard and total QSSA evolve on a (lower) 2-dimensional lattice (copy numbers refer to substrates M, M_{pp} or total substrates $\overline{M}$, $\overline{M}_{pp}$, respectively), formally bounded by the 2-dimensional box lattice including $500 \times 500 = 250\,000$ states, that reduce to about 125 000 when accounting for mass conservation laws. These numbers allow one to compute explicitly, and in a computationally very efficient way, the exact theoretical distribution by applying Gaussian elimination, or block-based efficient solvers [Borri et al. 2016], to the CME equilibrium problem. The numerical simulations were performed in the Matlab suite on an Apple MacBook Pro laptop with 2.5 GHz Intel Core i5 CPU and 16 GB RAM. The computation time of the equilibrium distribution is just 3 seconds for the sQSSA and for the tQSSA.

Figure 1 shows the plot of the steady-state marginal distribution of species M_{pp} for case (a). It is apparent that both the standard and total QSSA resemble the full system, although the stochastic modes are not able to catch both equilibrium points coming from the deterministic approach, since apparent unimodal distributions come out. Indeed, the modes of the three distributions substantially reproduce the lower equilibrium point, with the full system and the total QSSA providing a slightly better match than the standard QSSA.

Figure 2 reports the steady-state marginal distribution of species M_{pp} for case (b). Again, we have a very good match between full system and tQSSA, both providing a unimodal distribution, with the mode resembling the unique equilibrium point of

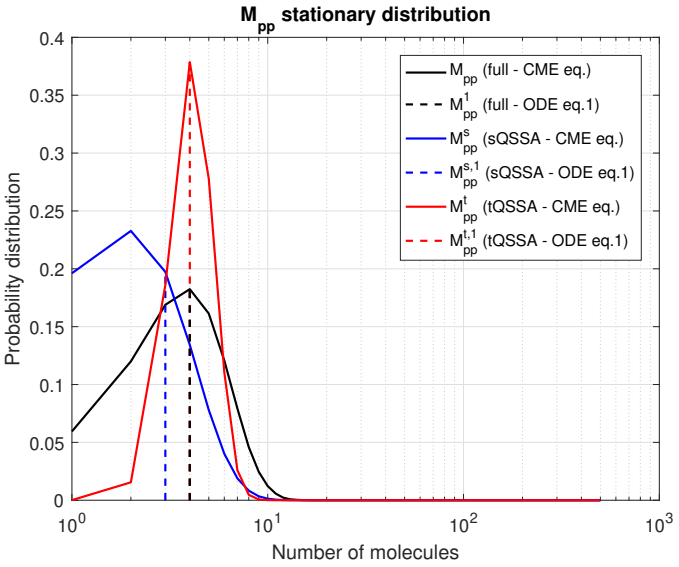


Figure 1. Stochastic setting of the case (a). Steady-state marginal distribution of species M_{pp}. The blue solid line represents the steady-state distribution computed by means of the Gillespie algorithm for the full model of reactions, while the black and red solid lines represent the sQSSA and tQSSA distributions, respectively. The deterministic equilibria are reported in dashed lines. The plot shows that the second modes, which are present in the deterministic counterpart, are not detected. The system appears to be monostable.

the deterministic model; the stochastic sQSSA also exhibits one mode, corresponding to the higher equilibrium of its deterministic counterpart and is hence very far from the modes of full system and tQSSA.

Finally, Figures 3 and 4 show the steady-state marginal distribution of species M_{pp} for cases (c) and (d). Again it is apparent that the Monte Carlo simulation of the full system confirms that only the tQSSA provides a very good approximation. Case (c), for instance, shows that the tQSSA (as well as the full system) provides a bimodal distribution, with the modes corresponding to the equilibrium points of the deterministic model, while the sQSSA provides a unimodal distribution. Moreover, by slightly varying the parameter setting of just 1 copy number, case (d) shows a completely different qualitative behavior, with full and tQSSA providing a unimodal distribution (with the mode resembling the highest of the 2 equilibrium points of the deterministic model), while the sQSSA provides a unimodal distribution completely different from the one coming from the full system.

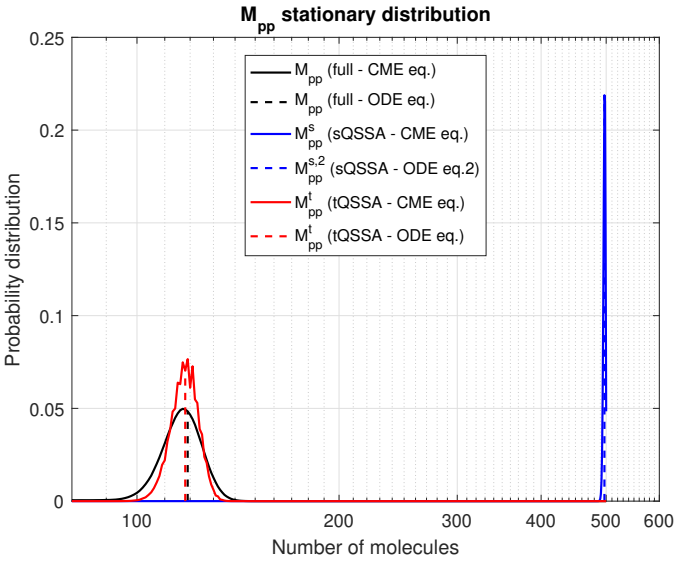


Figure 2. Stochastic setting of the case (b). Steady-state marginal distribution of species M_{pp}. The blue solid line represents the steady-state distribution computed by means of the Gillespie algorithm for the full system of reactions, while the black and red solid lines represent the sQSSA and tQSSA distributions, respectively. The deterministic equilibria are reported in dashed lines. Differently from sQSSA, the tQSSA reproduces with very good approximation the mode of the full system.

One important consideration that comes out from these results is that the stochastic tQSSA seems to be a promising tool for the approximation of the distribution of the full system, when the computation of the exact equilibrium distribution of this system is intractable. Note also that the dimensions of resulting CME matrices are exactly the same in sQSSA and tQSSA, so there is no loss in computational performance in exploiting the latter, which is far more accurate than sQSSA in capturing the position of the modes. Indeed, results from Theorem 1 allow one to trust the stationary distribution as coming from different initial conditions as the unique one, and the correctness of the full system stationary distribution allows one to trust the tQSSA (instead of the sQSSA) as the golden standard to approximate (at least) the steady-state behavior. This result somehow mimics what is already known from the deterministic viewpoint.

Another important consideration concerns the topic of mono/bistability. It is thus important to note that a bistable behavior in the deterministic approach (i.e., in the ODEs) is not (necessarily) associated with a bimodal behavior in the stochastic

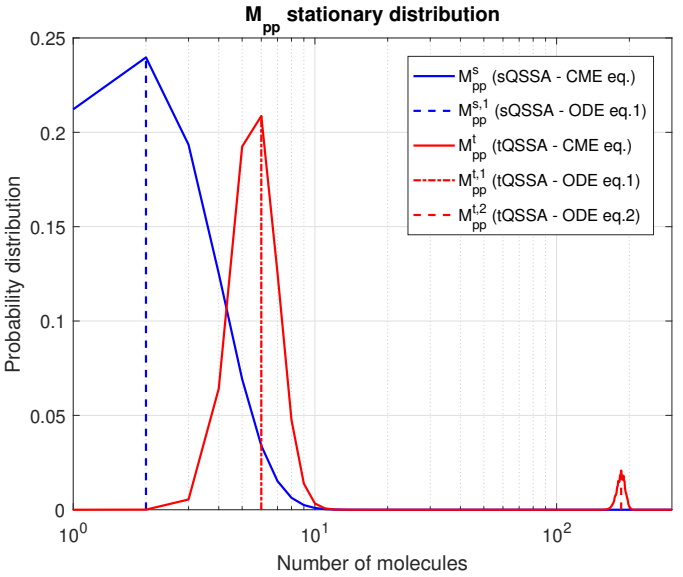


Figure 3. Stochastic setting of the case (c). Steady-state marginal distribution of species M_{pp}. The blue and red solid lines represent the sQSSA and tQSSA distributions, respectively. The deterministic equilibria are reported in dashed lines. The tQSSA reproduces with very good approximation the bistable behavior of the full system, whereas the sQSSA shows a monostable behavior, contrary to what happens in the deterministic setting.

approach (i.e., in the CMEs). Indeed, among the analyzed cases, in cases (a) and (d) numerical simulations show that the stochastic setting presents just one mode, matching one of the two deterministic equilibria. A possible explanation for this phenomenon is that when we consider a deterministic system, we can study its basins of attraction, from which the trajectories flow necessarily towards the same equilibrium point. On the other hand, in the stochastic framework, a trajectory (realization) can always go from any state to any other one during the evolution of the system, in that the graph of reactions is connected and the Markov model is positively recurrent (see [Borri et al. 2016] for further details). So, it is reasonable that, depending on the propensity values, one of the two deterministic equilibrium points can be visited much more often than the other one and that the trajectories (almost) never leave a neighborhood of the *dominating* point, which is stochastically a kind of *black hole*. As a consequence, the other equilibrium point disappears from the plots of the probability distribution and the stochastic system behavior is qualitatively monomodal. Indeed, there is still a way to recover (at least numerically) the bimodal behavior which is not present at a stochastic macroscopic

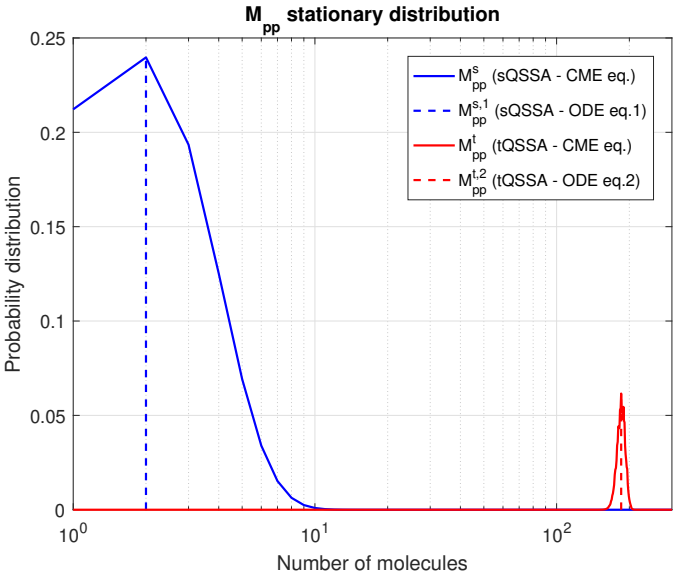


Figure 4. Stochastic setting of the case (d). Steady-state marginal distribution of species M_{pp}. The blue and red solid lines represent the sQSSA and tQSSA distributions, respectively. The deterministic equilibria are reported in dashed lines. Differently from the deterministic setting, both the stochastic QSSAs present only one mode, but while the tQSSA exactly reproduces the second mode of the full system, the sQSSA wrongly reproduces the mode.

level. For the case (a), a plot of the discrete derivative of the steady-state marginal distribution of the species M_{pp}, for sQSSA and tQSSA, is shown in Figure 5, and it shows that both sQSSA and tQSSA exhibit some zero crossings of the derivative corresponding to the second deterministic equilibrium point (around $M_{pp} = 495$ in sQSSA and $M_{pp} = 290$ in tQSSA), which are necessary conditions for the existence of second modes. Anyway, such modes are not detected in Figure 1.

In conclusion, the simulations show the deeper insight of the stochastic approach into the understanding of the qualitative behavior of reaction networks; in particular, stochastic simulations are able to provide information about the actual probability of reaching different equilibrium conditions. This information is lost in the deterministic approach which constitutes just a first-order approximation of the mean value of the CME [van Kampen 2007]. Based on this statement, we can assert that a mode of the stochastic approach always has an equilibrium point as its deterministic counterpart, but that the converse is not always true. Furthermore, in both settings, the superiority of the tQSSA approach compared to the sQSSA is confirmed, in that the former shows a much greater numerical accuracy than

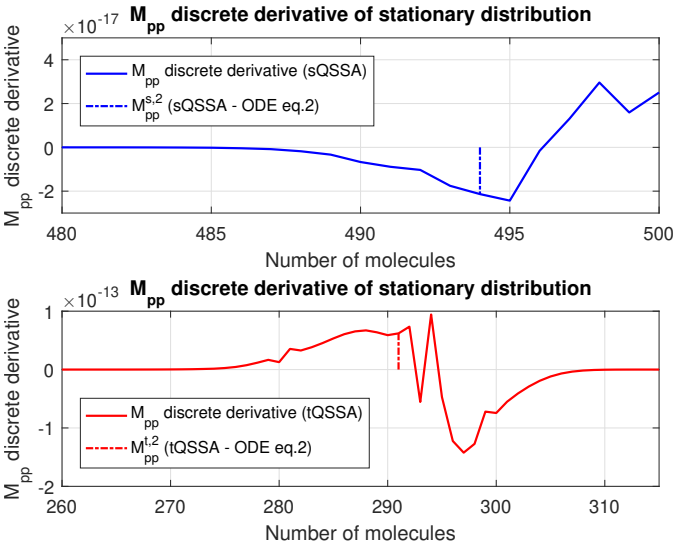


Figure 5. Discrete derivative of the steady-state marginal distribution of species M_{pp} (stochastic case), in case (a), for the sQSSA (top) and tQSSA (bottom) distributions, respectively. The plots show the existence of the second mode (zero crossing of the discrete derivative) in the zone of the second deterministic equilibrium point (reported in dashed line), both in sQSSA and in tQSSA.

the latter in matching the equilibrium points/modes. In the stochastic approach, in addition, the better performance of tQSSA is obtained at the same computational cost as in sQSSA, since the obtained CME dynamical matrices have the same dimension in the two cases.

6. Conclusions

In this work we investigated a stochastic approach for modeling the biochemical reaction cycle of double phosphorylation/dephosphorylation (PDP), which is one of the most important biochemical mechanisms in intracellular reaction networks. The goal of the work is twofold. On the one hand, we aimed to understand whether the QSSA approach could be transferred to the investigation of the qualitative behavior of the double PDP cycle also in the stochastic scheme, usually assessed as the best approach whenever dealing with biochemical processes which are intrinsically noisy and for which the average copy number dynamics is the only (and little informative) result available from a deterministic approach. Our results have somehow extended to the stochastic realm results already established from the deterministic approach: the tQSSA is a superior tool (with respect to sQSSA) to

deal with affordable approximations, since it is able to faithfully replicate the full system results also in those cases where sQSSA fails. On the other hand, proposed results show how deterministic models could produce misleading results if not properly accounted for in the wider setting of the stochastic approach, according to which ODE models can be thought of as a first-order approximation of the average dynamics. Indeed, what emerges is that, in some cases, deterministic bistability does not provide a stationary bimodal distribution.

It is reasonable to state that the apparently paradoxical phenomenon described above may be explained by the following considerations. Continuous-time Markov chains (CTMCs) describe the probabilities for the state of a discrete-event system to stay in a specific point on the state space. According to a given reaction, the dynamics of these probabilities are described by the CME, with reaction rates providing the propensities for the state to jump from one point of the space to another one. We therefore conjecture that the *disappearance* of one mode in the stationary bimodal distribution could occur when the probability of leaving one basin of attraction is so low that it would require a (quite) infinite time to occur. In this case, we can expect a switching phenomenon from one state to the other one after a very long time, in contrast with what occurs in the deterministic case, as observed, in a different context, in [Székely and Burrage 2014]. Under the hypothesis that the ergodicity property of CTMC holds, it is well-known that the stationary probability distribution is unique whatever the initial condition, and that statistical properties can be deduced from a single, sufficiently long realization (stochastic realization) of the stochastic process. The fact that this feature of Markov chains obviously cannot be captured by the deterministic approach could be the reason for the discrepancy between the two different approaches. Waiting for one single (long enough) Gillespie stochastic simulation, instead of running a (large enough) number of them, could be a way to capture the second stable state of a bistable system. The aim of our future work will be to give further explanations of this phenomenon which is still a subject of our studies.

Finally, as already observed in the introduction, in [Kim et al. 2014] it was investigated how different QSS approximations (standard QSSA, total QSSA, and prefactor QSSA) may provide similar results in the deterministic field, while providing completely different results in the stochastic field. In other words, [Kim et al. 2014] provided some *caveat* concerning different QSSAs and provided a criterion to understand in a specific framework whether a stochastic QSSA is reliable or not. Unfortunately these results are not straightforwardly applicable to our case: in [Kim et al. 2015] a unique double time scale enzymatic reaction is considered, while in our manuscript we consider four double time scale enzymatic reactions. However, we plan to extend such results to our more general framework in the continuation of our research.

References

[Bang-Jensen and Gutin 2009] J. Bang-Jensen and G. Gutin, *Digraphs: theory, algorithms and applications*, 2nd ed., Springer, 2009.

[Barik et al. 2008] D. Barik, M. R. Paul, W. T. Baumann, Y. Cao, and J. J. Tyson, "Stochastic simulation of enzyme-catalyzed reactions with disparate timescales", *Biophys. J.* **95**:8 (2008), 3563–3574.

[Bazzani et al. 2012] A. Bazzani, G. C. Castellani, E. Giampieri, D. Remondini, and L. N. Cooper, "Bistability in the chemical master equation for dual phosphorylation cycles", *J. Chem. Phys.* **136**:23 (2012), art. id. 235102.

[Bednarczyk and Lekszycki 2016] E. Bednarczyk and T. Lekszycki, "A novel mathematical model for growth of capillaries and nutrient supply with application to prediction of osteophyte onset", *Z. Angew. Math. Phys.* **67**:4 (2016), art. id. 94.

[Bersani and Dell'Acqua 2012] A. M. Bersani and G. Dell'Acqua, "Is there anything left to say on enzyme kinetic constants and quasi-steady state approximation?", *J. Math. Chem.* **50**:2 (2012), 335–344.

[Bersani et al. 2011] A. M. Bersani, G. Dell'Acqua, and G. Tomassetti, "On stationary states in the double phosphorylation-dephosphorylation cycle", pp. 1208–1211 in *Proceedings of the International Conference on Numerical Analysis and Applied Mathematics 2011* (Halkidiki, Greece, 2011), AIP Conference Proceedings **1389**, American Institute of Physics, College Park, MD, 2011.

[Bersani et al. 2014] A. M. Bersani, A. Borri, F. Carravetta, G. Mavelli, and P. Palumbo, "Quasi-steady-state approximations of the chemical master equation in enzyme kinetics: application to the double phosphorylation/dephosphorylation cycle", pp. 3053–3058 in *53rd IEEE Conference on Decision and Control* (Los Angeles, 2014), IEEE, Piscataway, NJ, 2014.

[Bersani et al. 2015] A. M. Bersani, E. Bersani, G. Dell'Acqua, and M. G. Pedersen, "New trends and perspectives in nonlinear intracellular dynamics: one century from Michaelis–Menten paper", *Contin. Mech. Thermodyn.* **27**:4–5 (2015), 659–684.

[Blake et al. 2003] W. J. Blake, M. Kærn, C. R. Cantor, and J. J. Collins, "Noise in eukaryotic gene expression", *Nature* **422** (2003), 633–637.

[Borghans et al. 1996] J. A. M. Borghans, R. J. de Boer, and L. A. Segel, "Extending the quasi-steady state approximation by changing variables", *B. Math. Biol.* **58**:1 (1996), 43–63.

[Borri et al. 2013] A. Borri, F. Carravetta, G. Mavelli, and P. Palumbo, "Some results on the structural properties and the solution of the chemical master equation", pp. 3771–3776 in *2013 American Control Conference* (Washington, DC, 2013), IEEE, Piscataway, NJ, 2013.

[Borri et al. 2016] A. Borri, F. Carravetta, G. Mavelli, and P. Palumbo, "Block-tridiagonal state-space realization of chemical master equations: a tool to compute explicit solutions", *J. Comput. Appl. Math.* **296** (2016), 410–426.

[Briggs and Haldane 1925] G. E. Briggs and J. B. S. Haldane, "A note on the kinetics of enzyme action", *Biochem. J.* **19**:2 (1925), 338–339.

[Bruna et al. 2014] M. Bruna, S. J. Chapman, and M. J. Smith, "Model reduction for slow–fast stochastic systems with metastable behaviour", *J. Chem. Phys.* **140**:17 (2014), art. id. 174107.

[Cao and Petzold 2005] D. T. Cao, Yang Gillespie and L. R. Petzold, "The slow-scale stochastic simulation algorithm", *J. Chem. Phys.* **122**:1 (2005), art. id. 014116.

[Cao et al. 2005] Y. Cao, D. Gillespie, and L. Petzold, "Multiscale stochastic simulation algorithm with stochastic partial equilibrium assumption for chemically reacting systems", *J. Comput. Phys.* **206**:2 (2005), 395–411.

[Chickarmane et al. 2007] V. Chickarmane, B. N. Kholodenko, and H. M. Sauro, "Oscillatory dynamics arising from competitive inhibition and multisite phosphorylation", *J. Theoret. Biol.* **244**:1 (2007), 68–76.

[Dell'Acqua and Bersani 2011] G. Dell'Acqua and A. Bersani, "Bistability and the complex depletion paradox in the double phosphorylation-dephosphorylation cycle", pp. 55–56 in *Proceedings of the International Conference on Bioinformatics Models, Methods and Algorithms* (Rome, 2011), vol. 1: Bioinformatics, SciTePress, Setúbal, Portugal, 2011.

[Dell'Acqua and Bersani 2012] G. Dell'Acqua and A. M. Bersani, "A perturbation solution of Michaelis–Menten kinetics in a "total" framework", *J. Math. Chem.* **50**:5 (2012), 1136–1148.

[Dell'Acqua and Bersani 2013] G. Dell'Acqua and A. M. Bersani, "Quasi-steady state approximations and multistability in the double phosphorylation-dephosphorylation cycle", pp. 155–172 in *Biomedical Engineering Systems and Technologies*, Communications in Computer and Information Science **273**, Springer, 2013.

[Eilertsen and Schnell 2018] J. Eilertsen and S. Schnell, "A kinetic analysis of coupled (or auxiliary) enzyme reactions", *B. Math. Biol.* **80**:12 (2018), 3154–3183.

[Fedoroff and Fontana 2002] N. Fedoroff and W. Fontana, "Small numbers of big molecules", *Science* **297**:5584 (2002), 1129–1131.

[George et al. 2018] D. George, R. Allena, and Y. Rémond, "Cell nutriments and motility for mechanobiological bone remodeling in the context of orthodontic periodontal ligament deformation", *J. Cell. Immunoth.* **4**:1 (2018), 26–29.

[George et al. 2019] D. George, R. Allena, and Y. Rémond, "Integrating molecular and cellular kinetics into a coupled continuum mechanobiological stimulus for bone reconstruction", *Contin. Mech. Thermodyn.* **31**:3 (2019), 725–740.

[Gillespie 1977] D. T. Gillespie, "Exact stochastic simulation of coupled chemical reactions", *J. Chem. Phys.* **81**:25 (1977), 2340–2361.

[Gillespie 2001] D. T. Gillespie, "Approximate accelerated stochastic simulation of chemically reacting systems", *J. Chem. Phys.* **115**:4 (2001), 1716–1733.

[Gillespie 2009a] D. T. Gillespie, "Deterministic limit of stochastic chemical kinetics", *J. Chem. Phys. B* **113**:6 (2009), 1640–1644.

[Gillespie 2009b] D. T. Gillespie, "A diffusional bimolecular propensity function", *J. Chem. Phys.* **131**:16 (2009), art. id. 164109.

[Giorgio et al. 2016] I. Giorgio, U. Andreaus, D. Scerrato, and F. dell'Isola, "A visco-poroelastic model of functional adaptation in bones reconstructed with bio-resorbable materials", *Biomech. Model. Mechan.* **15**:5 (2016), 1325–1343.

[Giorgio et al. 2019] I. Giorgio, F. dell'Isola, U. Andreaus, F. Alzahrani, T. Hayat, and T. Lekszycki, "On mechanically driven biological stimulus for bone remodeling as a diffusive phenomenon", *Biomech. Model. Mechan.* **18**:6 (2019), 1639–1663.

[Henri 1901a] V. Henri, "Recherches sur la loi de l'action de la sucrase", *C. R. Hebd. Acad. Sci.* **133** (1901), 891–899.

[Henri 1901b] V. Henri, "Über das Gesetz der Wirkung des Invertins", *Z. Phys. Chem.* **39** (1901), 194–196.

[Henri 1902] V. Henri, "Théorie générale de l'action de quelques diastases", *C. R. Hebd. Acad. Sci.* **135** (1902), 916–919.

[Hwang and Velázquez 2013a] H. J. Hwang and J. J. L. Velázquez, "Bistable stochastic biochemical networks: highly specific systems with few chemicals", *J. Math. Chem.* **51**:5 (2013), 1343–1375.

[Hwang and Velázquez 2013b] H. J. Hwang and J. J. L. Velázquez, "Bistable stochastic biochemical networks: large chemical networks and systems with many molecules", *J. Math. Chem.* **51**:8 (2013), 2074–2103.

[van Kampen 2007] N. G. van Kampen, *Stochastic processes in physics and chemistry*, 3rd ed., Lecture Notes in Mathematics **888**, North-Holland, Amsterdam, 2007.

[Kholodenko 2000] B. N. Kholodenko, "Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades", *Eur. J. Biochem.* **267**:6 (2000), 1583–1588.

[Kim et al. 2014] J. K. Kim, K. Josić, and M. R. Bennett, "The validity of quasi-steady-state approximations in discrete stochastic simulations", *Biophys. J.* **107**:3 (2014), 783–793.

[Kim et al. 2015] J. K. Kim, K. Josić, and M. R. Bennett, "The relationship between stochastic and deterministic quasi-steady state approximations", *BMC Syst. Biol.* **9** (2015), art. id. 87.

[Laidler 1955] K. J. Laidler, "Theory of the transient phase in kinetics, with special reference to enzyme systems", *Can. J. Chem.* **33**:10 (1955), 1614–1624.

[Lin and Segel 1988] C. C. Lin and L. A. Segel, *Mathematics applied to deterministic problems in the natural sciences*, 2nd ed., Classics in Applied Mathematics **1**, Society for Industrial and Applied Mathematics, Philadelphia, 1988.

[MacNamara et al. 2008] S. MacNamara, A. M. Bersani, K. Burrage, and R. B. Sidje, "Stochastic chemical kinetics and the total quasi-steady-state assumption: application to the stochastic simulation algorithm and chemical master equation", *J. Chem. Phys.* **129**:9 (2008), art. id. 095105.

[Mastny et al. 2007] E. A. Mastny, E. L. Haseltine, and J. B. Rawlings, "Two classes of quasi-steady-state model reductions for stochastic kinetics", *J. Chem. Phys.* **127**:9 (2007), art. id. 094106.

[Michaelis and Menten 1913] L. Michaelis and M. L. Menten, "Die Kinetik der Invertinwirkung", *Biochem. Z.* **49** (1913), 333–369.

[Ortega et al. 2006] F. Ortega, J. L. Garcés, F. Mas, B. N. Kholodenko, and M. Cascante, "Bistability from double phosphorylation in signal transduction: kinetic and structural requirements", *FEBS J.* **273**:17 (2006), 3915–3926.

[Pedersen et al. 2008] M. G. Pedersen, A. M. Bersani, E. Bersani, and G. Cortese, "The total quasi-steady-state approximation for complex enzyme reactions", *Math. Comput. Simulation* **79**:4 (2008), 1010–1019.

[Rao and Arkin 2003] C. V. Rao and A. P. Arkin, "Stochastic chemical kinetics and the quasi-steady-state assumption: application to the Gillespie algorithm", *J. Chem. Phys.* **118**:11 (2003), 4999–5010.

[Samoilov et al. 2005] M. Samoilov, S. Plyasunov, and A. P. Arkin, "Stochastic amplification and signaling in enzymatic futile cycles through noise-induced bistability with oscillations", *P. Natl. Acad. Sci. USA* **102**:7 (2005), 2310–2315.

[Segel 1988] L. A. Segel, "On the validity of the steady state assumption of enzyme kinetics", *B. Math. Biol.* **50**:6 (1988), 579–593.

[Segel and Slemrod 1989] L. A. Segel and M. Slemrod, "The quasi-steady-state assumption: a case study in perturbation", *SIAM Rev.* **31**:3 (1989), 446–477.

[Székely and Burrage 2014] T. Székely, Jr. and K. Burrage, "Stochastic simulation in systems biology", *Comput. Struct. Biotech. J.* **12**:20–21 (2014), 14–25.

[Thomas et al. 2011] P. Thomas, A. V. Straube, and R. Grima, "Communication: limitations of the stochastic quasi-steady-state approximation in open biochemical reaction networks", *J. Chem. Phys.* **135**:18 (2011), art. id. 181103.

[Tzafriri 2003] A. R. Tzafriri, "Michaelis–Menten kinetics at high enzyme concentrations", *B. Math. Biol.* **65**:6 (2003), 1111–1129.

Received 10 Nov 2019. Revised 24 Jun 2020. Accepted 28 Jul 2020.

ALBERTO MARIA BERSANI: alberto.bersani@uniroma1.it
Dipartimento di Ingegneria Meccanica e Aerospaziale, Università degli Studi di Roma "La Sapienza", Rome, Italy

ALESSANDRO BORRI: alessandro.borri@iasi.cnr.it
Istituto di Analisi dei Sistemi et Informatica "Antonio Ruberti", Consiglio Nazionale delle Ricerche, Rome, Italy

FRANCESCO CARRAVETTA: francesco.carravetta@iasi.cnr.it
Istituto di Analisi dei Sistemi et Informatica "Antonio Ruberti", Consiglio Nazionale delle Ricerche, Rome, Italy

GABRIELLA MAVELLI: gabriella.mavelli@iasi.cnr.it
Istituto di Analisi dei Sistemi et Informatica "Antonio Ruberti", Consiglio Nazionale delle Ricerche, Rome, Italy

PASQUALE PALUMBO: pasquale.palumbo@unimib.it
Dipartimento di Biotecnologie e Bioscienze, Università degli Studi Milano-Bicocca, Milano, Italy

MATHEMATICS AND MECHANICS OF COMPLEX SYSTEMS

msp.org/memocs

MEMOCS (ISSN 2325-3444 electronic, 2326-7186 printed) is a journal of the International Research Center for the Mathematics and Mechanics of Complex Systems at the Università dell'Aquila, Italy.

Cover image: "Tangle" by © John Horigan; produced using the *Context Free* program (contextfreeart.org).

PUBLISHED BY

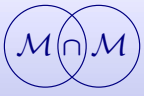
mathematical sciences publishers

nonprofit scientific publishing

http://msp.org/

Mathematics and Mechanics of Complex Systems
vol. 8 no. 4 2020

MEMOCS is a journal of the International Research Center for the Mathematics and Mechanics of Complex Systems at the Università dell'Aquila, Italy.




2326-7186(2020)8:4;1-8