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Citation: J. Chem. Phys. **136**, 054111 (2012); doi: 10.1063/1.3681942
View online: http://dx.doi.org/10.1063/1.3681942
View Table of Contents: http://jcp.aip.org/resource/1/JCPSA6/v136/i5
Published by the American Institute of Physics.

Stochastic mapping of the Michaelis-Menten mechanism

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(Received 19 October 2011; accepted 17 January 2012; published online 6 February 2012)

The Michaelis-Menten mechanism is an extremely important tool for understanding enzyme-catalyzed transformation of substrates into final products. In this work, a computationally viable, full stochastic description of the Michaelis-Menten kinetic scheme is introduced based on a stochastic equivalent of the steady-state assumption. The full solution derived is free of restrictions on amounts of substance or parameter values and is used to create stochastic maps of the Michaelis-Menten mechanism, which show the regions in the parameter space of the scheme where the use of the stochastic kinetic approach is inevitable. The stochastic aspects of recently published examples of single-enzyme kinetic studies are analyzed using these maps. © *2012 American Institute of Physics*. [doi:10.1063/1.3681942]

I. INTRODUCTION

Highly selective and extremely efficient enzyme catalysis is in the heart of biochemical processes and provides the molecular foundation of life itself. The small size and the high variation in the types of proteins in a single cell, which is usually considered a chemical reactor spatially distinct from other cells (although not unconnected to them), necessarily lead to the conclusion that at least some of the essential enzymes in it should be present in amounts that do not exceed a few individual molecules. Therefore, describing chemical reactions at very low amounts of substance (zeptomol or yoctomol), which is seldom of significant interest for usual chemical processes, is highly important in biochemistry. This point is further strengthened by the fact that measuring enzyme activity provides the foundation of numerous specialized diagnostic methods, which are highly desirable to be implemented using the smallest possible amounts of human sample.

The Michaelis-Menten mechanism has been an extremely productive tool to interpret experimental findings in enzyme kinetics ever since its postulation about a century ago.[1–5] The simplest form of the Michaelis-Menten mechanism involves the reversible reaction of an enzyme (E) and a substrate (S) to give an enzyme-substrate adduct (ES), and the subsequent formation of the product (P) with simultaneous regeneration of the original enzyme,

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \overset{k_2}{\rightarrow} \mathrm{E} + \mathrm{P}. \tag{1}$$

In typical experiments, the substrate is used in large excess over the enzyme, and it is also quite common that substrate binding to the enzyme is orders of magnitude faster than product formation. Consequently, only two parameters can usually be determined in the scheme given in Eq. (1): k_2 and a combination parameter $K_\mathrm{M} = (k_2 + k_{-1})/k_1$, which is called Michaelis constant. The Michaelis-Menten equation, which gives the rate of product formation, is normally given in the following form:

$$\frac{d[\mathrm{P}]}{dt} = \frac{k_2[\mathrm{E}]_0[\mathrm{S}]}{K_\mathrm{M} + [\mathrm{S}]}. \tag{2}$$

Although enzyme action very often follows more complex schemes, the Michaelis-Menten equation is often still useful for interpreting such cases.[3–5]

With the advance of detection technology, it is now possible to study the activity of a single enzyme.[6–13] For example, the activity of a single cholesterol oxidase molecule could be observed through fluorescent microscopy by detecting the emission from the enzyme's fluorescent active site, flavin adenine dinucleotide.[6] In addition to following single-molecule events, these measurements also gave evidence of slow fluctuations in protein conformation.[6] Another work investigated individual β-galactosidase enzyme molecules and concluded that the Michaelis-Menten equation, interpreted in a microscopic fashion, still holds even for a fluctuating single enzyme.[11] A somewhat earlier article reported the development of a method based on confocal fluorescence microscopy in order to study the catalytic activity of lipase enzyme molecules on a molecular level.[10] Finally, the method of alternating laser excitation fluorescence resonance energy transfer was also employed to measure reaction rates in systems using the RNA-cleaving 8–17 deoxyribozyme enzyme at the single-molecule level in real time.[13]

These experimental examples clearly demonstrate that usual deterministic kinetics, which is the background of Eq. (2), cannot be used for cases where the number of involved particles is very low and consequently cannot be useful for single-enzyme kinetics. This phenomenon has been predicted theoretically and kinetic descriptions of systems containing a single enzyme based on various stochastic approaches were published multiple times.[14–27]

Staff developed a stochastic model for the reversible Michaelis-Menten mechanism with one substrate, one intermediate, and one product focusing on the state of equilibrium and showed that fluctuations are small with large particle numbers.[14] In a mathematically rather advanced but

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seldom cited paper, Arányi and Tóth[15] gave a complete stochastic description of the time evolution of individual state probabilities with a single-enzyme molecule and an unrestricted number of substrate molecules using generating functions.[15] They also pointed out that using the stochastic mean in comparisons with experimental data could be a favorable practice. In a study of the action of a single horseradish peroxidase enzyme molecule by fluorescence spectroscopy, Edman *et al.*[16] postulated a two-state dynamic model that was based on deterministic equations but aimed at describing random fluctuations. In another stochastic analysis of the single-molecule Michaelis-Menten system including both enzyme dynamics and substrate turnover, it was shown that the system can exhibit oscillatory features in the non-equilibrium steady state in certain regions of the parameter space.[19] Rao and Arkin[20] applied the Gillespie algorithm[28] for describing enzyme kinetics at very low molecule numbers. They also used the quasi-steady-state assumption in their model, which will be discussed in more detail in Sec. II of the present work. Basu and Mohanty[23] widened the scope of stochastic single-molecule Michaelis-Menten kinetics by including two-dimensional diffusion effects. A Monte Carlo simulation study of single enzyme and multi-enzyme systems took the combined effect of stochastic noise and spatial diffusion into account.[26] The Michaelis-Menten approximation has also been compared with the slow-scale stochastic simulation algorithm and it was shown that the validity conditions of discrete stochastic models at low particle numbers are similar to the deterministic case.[27] Several relevant reviews have also been published focusing on various aspects such as single-molecule enzymology in general,[17] experimental results in detection of single-molecule processes,[18] stochastic models of *in vivo* reactions,[21] kinetic fluctuations in biochemically relevant chemical processes,[24] and irreversible processes coupled with diffusion.[25]

Experimental evidence shows that the Michaelis-Menten mechanism is useful for understanding single-enzyme kinetics.[5,7,10,11,27] A particularly popular method is based on using the waiting time τ for product formation to occur, the expectation of which is related to the parameters by an equation fully analogous to Eq. (2),

$$\frac{1}{\langle\tau\rangle} = \frac{k_2[\mathrm{S}]}{K_\mathrm{M} + [\mathrm{S}]}. \tag{3}$$

Equation (3) is referred to as the single-molecule Michaelis-Menten equation.[12,22]

Recent advance in the stochastic modeling of absolute asymmetric reactions shows that it is often possible to obtain meaningful analytical solutions for systems exhibiting moderate levels of complexity, and give reasonably complete general mathematical descriptions of those chemical schemes.[29–31] This paper attempts to carry out a similar analysis for the Michaelis-Menten mechanism.

There are three main objectives of this work. The first is to give an appropriate mathematical framework for a full stochastic treatment of the Michaelis-Menten mechanism for any number of species, which is more complete and more general than previously published approaches. The second is to explore those conditions in the parameter space of initial concentrations and rate constant values under which the use of the stochastic approach is inevitable, an analysis that might be referred to as stochastic mapping. The third is to relate these theoretical results to experimental data published earlier by various groups and highlight the implications for experiment design. Generally, mathematical results will only be stated in the text, the proofs are deposited separately in the supplementary material.[32]

II. RESULTS AND DISCUSSION

A. Full stochastic description of the Michaelis-Menten mechanism

The continuous time discrete state stochastic (CDS) approach[33] will be applied to the Michaelis-Menten mechanism. This has already been done for the case of a single-enzyme molecule and the solution has been obtained by using the method of generating functions.[15] The present work will treat the more general case when the initial number of enzyme molecules is e_0, and the initial number of substrate molecules is s_0 in the kinetic scheme displayed as Eq. (1).

In contrast to the deterministic approach, which views matter as a continuum, CDS deals with individual molecule numbers.[30,33] A state of the system is identified by giving the number of molecules present. For the present scheme, one can give the actual number of free enzyme molecules (e) and the number of uncomplexed substrate molecules (s) to identify a given state. By mass conservation, the number of enzyme-substrate adducts (es) will be $es = e_0 - e$, and the number of product molecules formed will be $p = s_0 - s - e_0 + e$. The overall number of different possible states (m) is given as follows:

$$m = \left(s_0 - \frac{e_0}{2} + 1\right) \times (e_0 + 1). \tag{4}$$

This formula is given for the usual case of $s_0 \geq e_0$. For $s_0 < e_0$, a fully analogous formula obtained by exchanging s_0 and e_0 could be used. This is true for all forthcoming discussions. The established formalism of the CDS approach gives a differential equation for $P_{e,s}(t)$ functions, which give the probability that the system is exactly in state (e,s) at a given time moment t. The relevant master equation for the scheme given in Eq. (1), already stated with various simplifications in the earlier literature,[15,20] takes the following form:

$$\begin{aligned}\frac{dP_{e,s}(t)}{dt} = &-\left[\frac{k_1}{N_\mathrm{A}V}es + (k_{-1}+k_2)(e_0-e)\right]P_{e,s}(t)\\ &+\frac{k_1}{N_\mathrm{A}V}(e+1)(s+1)P_{e+1,s+1}(t)\\ &+k_{-1}(e_0-e+1)P_{e-1,s-1}(t)\\ &+k_2(e_0-e+1)P_{e-1,s}(t).\end{aligned} \tag{5}$$

It should be noted that the CDS approach usually uses rate constants that are different from the deterministic ones.[30,33] In this work, however, all rate constants are used with the deterministic values for internal consistency (e.g., k_1 has the

units of $M^{-1}s^{-1}$), which explains the origins of the appearance of volume (V) and the Avogadro constant (N_A) in the master equation. Equation (5) is a system of linear differential equations, which can be solved exactly. For direct computations at relatively low particle numbers ($m \leq 10^4$), the use of an enumerating function[30] is necessary, which gives a unique integer number between 1 and m to every possible state. In this case, a suitable enumerating function is

$$f(e,s) = \begin{cases} (s_0 - s - e_0 + e + 1)(e_0 + 1) - e & \text{if } e \leq s \\ (s_0 - s - e_0 + e + 1)(e_0 + 1) - \dfrac{(e - s - 1)(e - s)}{2} - e & \text{if } e > s \end{cases}. \tag{6}$$

Using this enumerating function and a direct method (such as matrix exponential functions), Eq. (5) can be solved for relatively small values of m. The expectations and standard deviations for the number of ES and P molecules have additional significance and also give potential ways to relate the theoretical calculations to experimental results.[15] These values can be calculated directly based on their definitions:

$$\langle \mathrm{ES} \rangle (t) = \sum_{\text{all } m \text{ states}} (e_0 - e) P_{e,s}(t), \tag{7}$$

$$\sigma_{\mathrm{ES}}(t) = \sqrt{\sum_{\text{all } m \text{ states}} [(e_0 - e)^2 P_{e,s}(t)] - [\langle \mathrm{ES} \rangle (t)]^2}, \tag{8}$$

$$\langle \mathrm{P} \rangle (t) = \sum_{\text{all } m \text{ states}} (s_0 - s - e_0 + e) P_{e,s}(t), \tag{9}$$

$$\sigma_P(t) = \sqrt{\sum_{\text{all } m \text{ states}} [(s_0 - s - e_0 + e)^2 P_{e,s}(t)] - [\langle \mathrm{P} \rangle (t)]^2}. \tag{10}$$

B. Stochastic equivalent of pre-equilibrium and steady-state approximations

In deterministic kinetics, the pre-equilibrium and steady-state approximations are often used, mostly for intermediates. Such is the case in the classic approach to Michaelis-Menten kinetics as well, where the usual form displayed in Eq. (2) is customarily derived from the scheme in Eq. (1) by using a steady-state assumption for the enzyme-substrate adduct ES. Mathematically, these approximations replace one of the differential equations with a non-differential equation and give the concentration of the intermediate as an explicit function of other concentrations so that the time dependence remains only implicit.[34] When these approximations are useful, the primary reason for the success is that the time resolution and the concentration sensitivity of the experiments are insufficient to make a significant difference between the full and the approximation model. A stochastic equivalent of this approach was developed in this work to enable calculations for larger particle numbers. A somewhat similar simplified calculation method using CDS stochastic kinetics was published in the Michaelis-Menten mechanism by Rao and Arkin.[20] However, in this earlier work, the deterministic Michaelis-Menten equation (Eq. (2) here) was used to derive steady-state concentrations of the enzyme-substrate adduct ES.[20] This is an approximation as the process involves a second-order reaction (the formation of ES) and the stochastic expectations of variables are by no means the same as deterministic concentrations.[33] Another work focusing on quasi-steady-state model reductions in stochastic kinetics also used the Michaelis-Menten kinetics as one of the examples.[34] Here, singular perturbation analysis was used to derive a reduced set of equations, which is similar to the one used by Rao and Arkin[20] in that it does not rely on the stochastic particle distribution for the enzyme substrate complex ES or the enzyme E. In contrast, the present work uses a fully stochastic approach in this sense.

The deterministic pre-equilibrium and steady-state approximations decrease the number of concentrations whose time dependence needs to be calculated (e.g., Eq. (2) in the mentioned work about quasi-steady-state model reductions[34]), whereas the stochastic equivalent reduces the number of states whose probability needs to be calculated as a function of time. This is done by assuming that the function $P_{e,s}(t)$ can be obtained as a product of a time dependent R function and an S value, which is characteristic of the state but does not depend on time,

$$P_{e,s}(t) = R_{s_0 - s + e - e_0}(t) S_{e_0 - e, s_0 - s + e - e_0}. \tag{11}$$

In essence, this assumption states that the probability of the formation of a given number of ES adducts can be obtained simply from the initial number of enzyme molecules and untransformed product molecules without explicit inclusion of time, which is essentially the same assumption made during the usual deterministic derivation of the Michaelis-Menten equation. With the new notation introduced by Eq. (11), Eq. (5) is transformed into the following, more compact form:

$$\frac{dR_p(t)}{dt} = k_2 \langle \mathrm{ES} \rangle_{p-1} R_{p-1}(t) - k_2 \langle \mathrm{ES} \rangle_p R_p(t). \tag{12}$$

The new quantity $\langle \mathrm{ES} \rangle_p$ is the steady-state expectation for the number of ES molecules when there are p molecules of product formed. By definition, it can be calculated as

$$\langle ES \rangle_p = \sum_{i=0}^{\alpha} i S_{i,p}, \quad \text{where } \alpha = \min(e_0, s_0 - p). \tag{13}$$

Using statistical thermodynamics and partition functions, S values can be calculated by the following formula:

$$S_{i,p} = \frac{\binom{e_0}{i} \frac{(s_0-p)!}{(s_0-p-i)!}(V N_A K_M)^{-i}}{\sum_{i=0}^{\alpha} \binom{e_0}{i} \frac{(s_0-p)!}{(s_0-p-i)!}(V N_A K_M)^{-i}}. \quad (14)$$

Furthermore, the expectation and the standard deviation are analytically given as follows:

$$\langle ES \rangle_p = \frac{\sum_{i=1}^{\alpha} i \binom{e_0}{i} \frac{(s_0-p)!}{(s_0-p-i)!}(V N_A K_M)^{-i}}{\sum_{i=0}^{\alpha} \binom{e_0}{i} \frac{(s_0-p)!}{(s_0-p-i)!}(V N_A K_M)^{-i}} = \alpha \frac{{}_1F_1(-\alpha+1, |e_0-s_0+p|+1, -K_M N_A V)}{{}_1F_1(-\alpha, |e_0-s_0+p|+1, -K_M N_A V)}, \quad (15)$$

$$\sigma_{ES,p} = \sqrt{\frac{\langle ES \rangle_p K_M}{V N_A} - (e_0 - \langle ES \rangle_p)(s_0 - p - \langle ES \rangle_p)}. \quad (16)$$

Equation (15) uses ${}_1F_1$, the confluent hypergeometric function. It should be noted that Eqs. (15) and (16) basically give the stochastic description of an equilibrium state of the reaction E + S ⇌ ES with $1/K_M$ as its equilibrium constant. This solution (with some confusing typographical errors) has already been published in the earlier stochastic literature[35] not related to the Michaelis-Menten mechanism. It is also notable that a similar line of thought was used in a recent, Monte Carlo simulation study of the Soai reaction based on stochastic kinetics.[36]

Solving Eq. (12) is much less computationally memory intensive than solving the Eq. (5) and is possible for much larger initial molecule numbers. The expectation and standard deviation for the number of product molecules is given as

$$\langle ES \rangle (t) = \sum_{i=0}^{s_0} \langle ES \rangle_i R_i(t), \quad (17)$$

$$\sigma_{ES}(t) = \sqrt{\sum_{i=0}^{s_0} \left[(\sigma_{ES,i}^2 + [\langle ES \rangle_i]^2) R_i(t) \right] - [\langle ES \rangle (t)]^2}, \quad (18)$$

$$\langle P \rangle (t) = \sum_{i=0}^{s_0} i R_i(t), \quad (19)$$

$$\sigma_P(t) = \sqrt{\sum_{i=0}^{s_0} i^2 R_i(t) - \left(\sum_{i=0}^{s_0} i R_i(t) \right)^2}. \quad (20)$$

The stochastic steady-state approximation provides an excellent approximation of the desired results. This is shown in Fig. 1, where a comparison between the expectations and standard deviation calculated by the full and steady-state equations is given for a relatively small system, where both

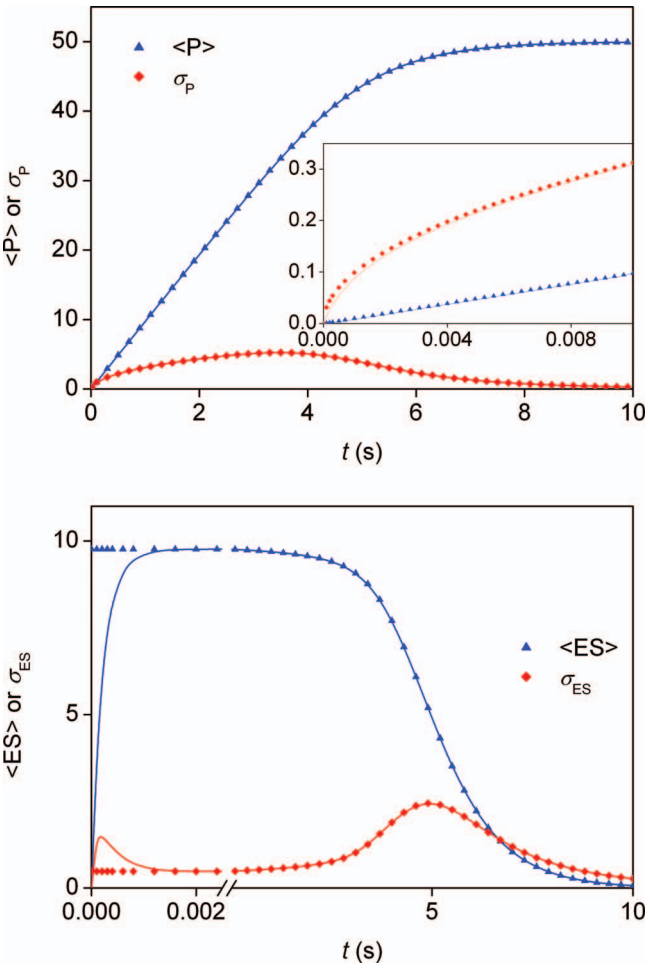

FIG. 1. Comparison of expectations (⟨⟩) and standard deviations (σ) obtained for the enzyme-substrate adduct (ES) and the product (P) obtained with the full solution and the stochastic steady-state approximation in the Michaelis-Menten mechanism. Solid lines: full solution; markers: steady-state approximation. $k_1/N_A V = 100\ s^{-1}$, $k_{-1} = 100\ s^{-1}$, $k_2 = 1\ s^{-1}$, $e_0 = 10$, and $s_0 = 50$.

calculations were viable. Figure S1 (supplementary material) (Ref. 32) gives a similar comparison for a different parameter set. The excellent agreement proves that the steady-state method is useful. At this point, it should be noted that the steady-state assumption is always associated with some loss of information. In this special case, this loss refers to the prediction of the number of ES molecules in some initial period of the reaction (Fig. 1 for ES). This dead time can be estimated parametrically by the following formula:

$$t_d = \frac{1}{k_\Psi} \ln \frac{20 k_\Psi N_A V + k_1 \langle ES \rangle_0}{k_\Psi N_A V + k_1 \langle ES \rangle_0}$$
$$k_\Psi = k_1 \frac{e_0 + s_0 - 2\langle ES \rangle_0}{N_A V} + k_{-1}. \quad (21)$$

Figure S2 in the supplementary material[32] gives a comparison between values calculated by this formula and those obtained from the full solutions of the system and shows an excellent agreement. Events within this initial time are very often inconsequential as they are below the limit provided by the time resolution of experimental data. This aspect of the

stochastic steady-state approximation bears some similarities to fast variable elimination in stochastic kinetics.[37]

C. Stochastic maps of the Michaelis-Menten mechanism

Stochastic mapping means exploring the parameter space of a given kinetic scheme to decide in which region the use of the stochastic kinetic approach is inevitable. Outside this region, the computationally much less intensive deterministic approach can be used for a comparison with measured data. Stochastic maps critically depend on the experimental property followed during the course of reaction. The stochastic region of a given scheme is the set of parameter values for which the stochastic approach shows that the relative standard error of the followed property is larger than a pre-set critical value, which will be 1% in this work following the convention used in an earlier work concerned with the effect of parity violation on the formation of biological chirality.[38] The cornerstone of this method is Kurtz's theorem,[39] which states that the deterministic description of any system is obtained as the limit of the stochastic description at infinite volume. From this, it also follows that when the standard deviation of the expectation based on the stochastic approach is small, then this expectation is very close to the deterministic property. In the present analysis, two important properties are selected, the first is the number of product molecules formed, and the second is the enzyme activity. These are exactly the ones for which Eqs. (7)–(10) and (17)–(20) were stated.

The number of product molecules formed is usually an important property in kinetic investigations. The expectation of this quantity can be directly calculated from the approximation introduced here. The stochastic map of the Michaelis-Menten mechanism based on product formation is shown in Fig. 2 and includes different values of e_0 (i.e., not only single-molecule cases). The map has composite parameters on both axes. The x axis shows k_2t, in other words, the time in units of $1/k_2$. The values of time t and rate constant k_2 do not influence the stochastic map individually, only through their product. The y axis shows the initial substrate concentration in K_M units. Two additional parameters influence the map. These are e_0, the initial number of enzyme molecules, and the overall volume of the system (V). The effects of these parameters are also shown in Fig. 2 by drawing several border lines. It was clear from the calculations that there is a high-volume and a low-volume limit on the map, which are also shown in the graph. This may be surprising at first sight. However, the phenomenon of volume dependence is well known from earlier stochastic literature.[30,33,39] In the map of Fig. 2, it is necessary to show these limits because the description of the system depends on the number of substrate molecules present and not only the concentration. However, if the molecule number were chosen as an independent variable, the volume dependence would still remain and the map would lose one very practical factor: the dimensionless nature of the y axis. It should be kept in mind that the extreme case of $s_0 = 1$ also sets a lowest meaningful volume in the representation used in Fig. 2, which is $V_{min} = 1/([S]_0 N_A)$.

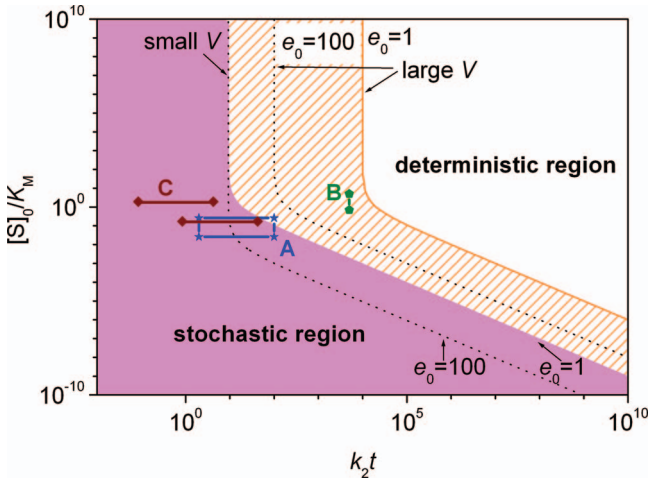

FIG. 2. Stochastic map of the Michaelis-Menten mechanism for the number of product molecules formed. In addition to the quantities shown on the x and y axes, the map depends on the initial number of enzyme molecules (e_0) and the overall volume (V) as well. Solid lines for boundaries are drawn for $e_0 = 1$, dotted lines represent $e_0 = 100$. Points A, B, and C are experimental points from the literature ($e_0 = 1$ for all of them). A: Ref. 11; B: Ref. 10; C: Ref. 7.

Published experimental data from three earlier single-enzyme kinetic studies are also shown on the map in Fig. 2. These data were measured at the high-volume limit (i.e., relatively large numbers of substrate molecules present). The three sets of points clearly fall into the stochastic region, which shows that only a stochastic evaluation of these experiments is meaningful. In fact, a stochastic evaluation was used in these earlier studies, but it was limited in scope. Unfortunately, a deeper analysis of these data does not seem feasible because, unsurprisingly, the published results are focused on those for which a meaningful evaluation was also published. It seems that the entire experimental design was devised keeping the limited evaluation method in mind.

Enzyme activity is often of high importance in medical applications. The expectation of this parameter can be simply calculated as the product of the expectation of enzyme-substrate adduct molecules ($\langle ES\rangle(t = 0) = \langle ES\rangle_0$) and rate constant k_2,

$$v = k_2 \langle ES\rangle_0 . \tag{22}$$

Figure 3 shows the stochastic map of the Michaelis-Menten mechanism based on enzyme activity. This is somewhat simpler than the map drawn based on the number of product molecules. The variables in this stochastic map are $N_A V/K_M$ and s_0 ($= [S]_0 N_A V$). The boundary between the deterministic and stochastic regions is defined by a line, which is dependent on the value of e_0 and mostly relates to quantities on the two axes in an inversely proportional manner. Another possible representation ($K_M - [S]_0$) of the same map is shown in the lower graph of Fig. 3, although the concept of the smallest meaningful volume should also be considered here. This alternative representation is only drawn for the single-enzyme case with some experimental data included. Again, the experimental points are firmly in the stochastic region. For higher number of enzyme molecules, the graph would be very similar with a somewhat shifted boundary.

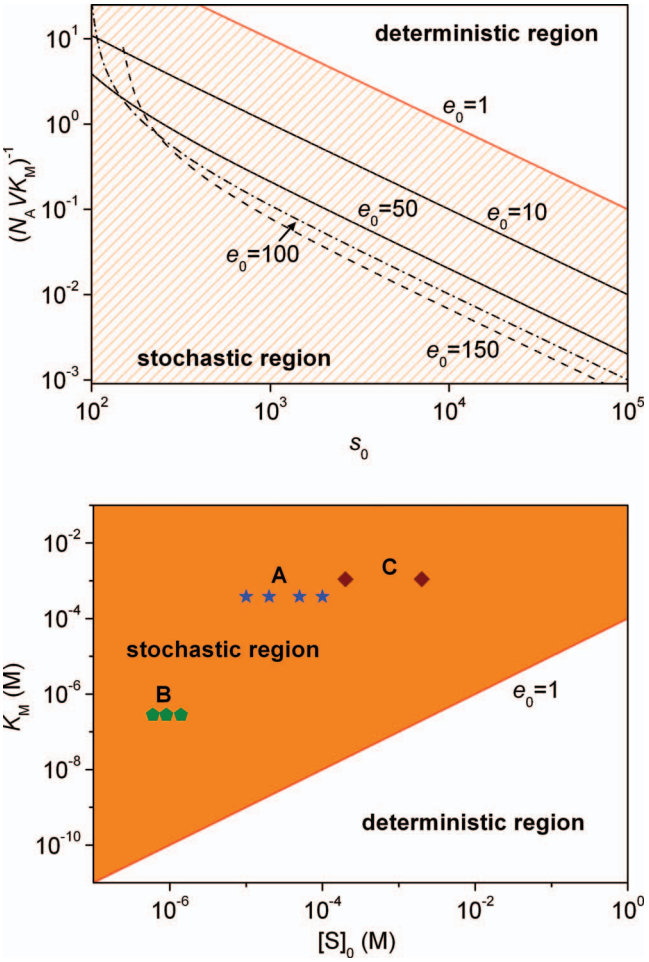

FIG. 3. Stochastic maps of the Michaelis-Menten mechanism for enzyme activity. In addition to the quantities shown on the x and y axes, the map depends on the initial number of enzyme molecules (e_0) and several boundary lines are shown for different values of e_0. Points A, B, and C are experimental points from the literature ($e_0 = 1$ for all of them). A: Ref. 11; B: Ref. 10; C: Ref. 7.

A paramount fact must be noticed from the two stochastic maps presented. Even for single-enzyme kinetics, there are certain conditions when some parameters can be successfully obtained from the deterministic model. Conversely, even when the number of initial particles are quite large, certain properties can only be correctly calculated with the stochastic approach. It is also highly important to notice that the maps depend on the overall volume. This may be counterintuitive at first sight, but it is a well-established characteristic of stochastic kinetics.[29,39] It should also be noted that the mathematical approach here does not assume any limitations on the number of enzyme or substrate molecules initially present. All stochastic maps given in Figs. 2 and 3 are drawn for several different e_0 values so that the effects of increasing initial numbers of enzyme molecules can be seen easily. Generally speaking, the boundary between the deterministic and stochastic region shifts toward higher values of K_M as e_0 increases. This means that more enzyme molecules are needed in a system to be in the deterministic region when the enzyme binds the substrate less strongly. However, the actual relationships describing the boundaries are usually complex and it is much better to consult the map with several different e_0 values than to rely on a simple evaluation of tendencies.

Most of the earlier experimental data were evaluated solely based on the τ waiting times.[11,22] The expectation for this quantity is shown in Eq. (3) for $e_0 = 1$. The analogy of this formula with deterministic Eq. (2) is rather tempting and one might think that it could be completed by simply multiplying with e_0 for a general case ($e_0 > 1$). However, this is mathematically incorrect. It can be shown that for more than one enzyme molecule, the correct expression is given as follows:

$$\langle \tau \rangle = \frac{1}{k_2 \langle ES \rangle_0}. \tag{23}$$

As expected, this equation is converted into Eq. (3) for $e_0 = 1$ as the equations $\langle \mathrm{ES} \rangle_0 = s_0/(N_\mathrm{A} V K_\mathrm{M})/[1 + s_0/(N_\mathrm{A} V K_\mathrm{M})]$ and $[\mathrm{S}]_0 = s_0/(N_\mathrm{A} V)$ hold. For $e_0 > 1$, this formula cannot be simplified in an analogous manner.

The stochastic description given in this paper is full, i.e., any experimentally measured property can be derived based on it. Therefore, the experimental design of forthcoming work on kinetics with small numbers of molecules need and must not be limited to a narrow selection of parameters.

III. CONCLUSION

It has been conclusively shown that the stochastic analog of the steady-state approximation developed in this work is suitable for giving a full stochastic description of the Michaelis-Menten mechanism. Stochastic maps can be created based on this description in order to differentiate the cases when using the stochastic description is inevitable from the ones the mathematically less demanding deterministic solution can be justifiably used. In this differentiation, the volume of the reactor plays an important role, which should be kept in mind when modeling chemical processes in extremely small volumes such as individual cells. The necessity to use the stochastic approach to chemical kinetics is not limited to studies on single enzymes, it could arise in multi-enzyme systems as well depending on other system parameters.

ACKNOWLEDGMENTS

This work was supported by the TÁMOP 4.2.1/B-09/1/KONV-2010-0007 project, which is co-financed by the European Union and the European Social Fund.

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