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A generic rate law for surface-active enzymes

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ABSTRACT

Many biochemical reactions are confined to interfaces, such as membranes or cell walls. Despite their importance, no canonical rate laws describing the kinetics of surface-active enzymes exist. Combining the approach chosen by Michaelis and Menten 100 years ago with concepts from surface chemical physics, we here present an approach to derive generic rate laws of enzymatic processes at surfaces. We illustrate this by a simple reversible conversion on a surface to stress key differences to the classical case in solution. The available area function, a concept from surface physics which enters the rate law, covers different models of adsorption and presents a unifying perspective on saturation effects and competition between enzymes. A remarkable implication is the direct dependence of the rate of a given enzyme on all other enzymatic species able to bind at the surface. The generic approach highlights general principles of the kinetics of surface-active enzymes and allows to build consistent mathematical models of more complex pathways involving reactions at interfaces.

1. Introduction

Cell membranes are ubiquitous in living systems. Collagen, the most abundant protein in mammals, forms fibers in connective tissues. Carbohydrate polymers like cellulose, chitin and starch are by far the dominant sources of biomass on earth and fulfill important structural and energetic functions. These are common examples of aggregates, macromolecular entities made up by the interaction of similar elements and defined by an interface towards their, typically aqueous, environment. Evidently, spatially heterogeneous systems are the norm rather than an exception in biology.

Yet, surface-active (or interfacial) enzymes have not received the same attention as their classical counterparts, enzymes acting on dissolved compounds. This holds true especially for textbooks and undergraduate curricula. The awareness of surface-active enzymes, of their pervasiveness and their characteristic features seems to be rather low. However, by the very nature of their substrates, lipases, collagenases and amylases cannot be understood exclusively in those terms applying to classical enzymes. If catalytic activity is confined to an interface, constraints not present in aqueous solution become important [1]. This entails characteristic protein domains, mechanisms and kinetic properties of surface-active enzymes.

Probably, the chief reason for the lack of recognition of surface-active enzymes is the lack of consensus. Although Michaelis and Menten's approach [2] cannot be used in every circumstance, it captured the essence of enzymatic catalysis in solution and, by advocating initial-rate measurements, provided a blueprint for experimental design for years to come. A similar breakthrough for surface-active enzymes is missing. There is no canonical kinetic description although specific models [3–8] and conceptual treatments [9–11] have been put forward. It is only consequent that even recent authoritative treatments on enzyme kinetics [12] shy away from discussing interfacial catalysis or do so only with a focus on special systems like membrane surfaces [13, Section 7.12]. Marangoni's textbook [14, Ch. 10] and especially Berg and Jain's substantial contribution [1] are notable exceptions. The latter, unfortunately, had a limited impact on the mainstream, apparently due to the focus on membrane surfaces and lipases. While the fragmentation of disciplines is unavoidable and to a certain degree necessary, we believe that this was at the expense of developing a better conceptual understanding of surface-active enzymes.

As a contribution to this understanding, we propose to use generic rate laws for surface-active enzymes. Generic rate laws are

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useful to analyze characteristic properties for a given class of enzymes by deriving a mathematical form that is invariant towards certain mechanistic details. At the same time a generic rate law suggests the appropriate modification of a mathematical term in the rate law whenever a concrete situation applies. For example, Rohwer and Hofmeyr [15] discuss kinetic and thermodynamic contributions to control by means of a generic rate equation.

In addition to in vitro characterization, the development of generic rate laws is also driven by attempts to model metabolic systems consistently if detailed mechanistic information is missing or judged unnecessary [16–19]. Again, it is commonly acknowledged that Michaelis–Menten-like rate laws alone are insufficient to understand regulatory properties of enzymes and pathways. Still, in recognition of Occam's razor we are thankful having at least a simple foundation onto which we can build, layer by layer, more complexity as needed. Interfaces, in particular, force us to recognize this additional complexity.

Many metabolic models evade a mechanistically correct description of interfacial processes by treating aggregated substrates as external (i.e. source or sink) or applying classical rate laws to describe their turnover [20,21]. Both strategies become insufficient if interfacial reactions are crucial to understand regulatory features. An impressive example emerged recently in plant biology. Leaves of flowering plants store starch during the day to provide sink organs with carbon during the night. This turnover appears to be precisely controlled at different levels [22,23] but intriguingly the insoluble nature of starch and enzymes acting at its interface turn out to be crucial. Mutant plants lacking a native starch interface or functional surface-active enzymes show less capabilities to grow under stress and changing environments, conditions which a sessile organism hardly can avoid.

2. Methods

Interfacial catalysis spans reaction spaces where different concentration measures apply. We propose some notational conventions to reduce the burden of bookkeeping. Interfacial species are typically denoted by an asterisk [1], a convention we will abide by. Furthermore, it is convenient to distinguish between cis- ($*$X) and trans-elements (U$*$) akin to the terminology used in gene regulation. A cis-element denotes a species that is either a genuine component of the substrate surface or originates from it. On the contrary, trans-elements are diffusible species usually residing in the aqueous phase. The notation for interfacial complexes is straightforward: cis–cis complexes ($*$X+$*$Y → $*$XY), trans–trans complexes (U$*$ + V$*$ → UV$*$) and cis–trans complexes (U$*$ + $*$X → U$*$X). Square brackets ($[\cdot]$) and angle brackets ($\langle\cdot\rangle$) denote concentrations per unit volume and per unit surface area, respectively.

A biochemical rate law is a single equation describing the rate of an enzymatically catalyzed reaction. Since these reactions are made up of several chemical steps (association, dissociation, catalysis) representing the mechanism of the enzyme, a rate law implies certain approximations. These approximations allow to reduce the set of ordinary differential equations (ODEs), describing the dynamics of the complete system, to a single ODE for product formation which is usually assumed to be the rate-limiting step. Two well-known assumptions are the rapid-equilibrium (RE) approximation, that was used by Michaelis and Menten [2], and the (standard) quasi-steady state (sQSS) approximation due to Briggs and Haldane [24]. The validity of these and other approximations from a mathematical viewpoint has been studied extensively elsewhere [25].

Here, we will apply Cha's method [26] assuming a partial equilibrium mechanism. We apply the RE approximation for enzyme adsorption, assuming this process to be close to equilibrium at the time scale of catalytic turnover. The sQSS approximation is then applied to the resulting reaction scheme to derive the steady state rate equation. This hybrid approach allows to formulate the rate law for surface-active enzymes in terms of the so-called *adsorption isotherm*. This well-known concept from surface science is used to quantify the partitioning of an adsorbate (here enzyme) between two phases (here aqueous solution and substrate surface) at equilibrium for a given temperature, hence isotherm. Adsorption isotherms, unlike equilibrium constants, define the mass action ratio at equilibrium not by a single number but by some, in general implicit, equation $[E*]_{eq}/[E]_{eq} = f([E*]_{eq})$ (eq denotes equilibrium concentrations). The confinement to equilibria may appear to be a severe restriction. Still, the observed diversity of adsorption isotherms [27–29] allows to cover a wide range of adsorption mechanisms and to study their effects on the rate of catalysis. Also, the RE approximation usually leads to simpler rate laws and is often advocated as a first approach [12,30]. It should be noted that the equilibrium assumption only relates to binding and dissociation processes and does not preclude lateral diffusion of the enzyme. Similarly, the RE approximation in the original Michaelis–Menten approach does not preclude the enzyme, the substrate or the complex to move in solution.

3. Results and discussion

To illustrate our generic approach, we assume a simple reversible uni–uni mechanism at the interface (Fig. 1). Upon adsorption of the enzyme E at the interface of the aggregated substrate (area A), the trans-species E$*$ binds the cis-reactant $*$S in a bimolecular reaction to form the cis–trans complex E$*$S. The catalytic step immediately follows and releases the cis-product $*$P and E$*$. The enzyme can either desorb or engage in another catalytic cycle. The two-step process of adsorption followed by finding the reaction partner at the interface assumes that either the enzyme, or the reactant or both can perform lateral movements.

Before we embark on the derivation of the rate law for surface-active enzymes, we will consider adsorption as an isolated process which is essentially completed and, thus, in equilibrium during the phase of catalytic turnover. There is some experimental evidence [31,32] justifying the assumption of such a temporal hierarchy and several studies of interfacial enzyme kinetics used adsorption isotherms [5,33,34], albeit without applying Cha's method.

Following this, we will derive the rate law and study some of its qualitative properties, most notably the effects of substrate amount, surface properties and enzyme amount.

3.1. Adsorption equilibrium

Following Langmuir's seminal work [35], we may think of adsorption as a bimolecular reaction between the enzyme E and an empty "elementary space" $*$ at the surface. We define this space such that its surface area equals the parking area of the adsorbate. In this case a simple stoichiometric relation applies, E+$*$ = E$*$, and we can write the on-rate as

$$v_{on} = k'_{on}[*][E], \tag{1}$$

where k'_{on} is a second-order rate constant.

Langmuir's model assumes that the adsorption sites are independent and that adsorbed molecules do not interact, essentially behaving like a two-dimensional ideal gas. We seek a phenomenological description that allows a departure from these restrictive assumptions. To this end, we introduce the so-called *available area function*, Φ, into enzyme kinetics. This concept from surface physical chemistry [28,36] allows to describe many different adsorption scenarios. The value of Φ lies between 0 and 1, and quantifies the

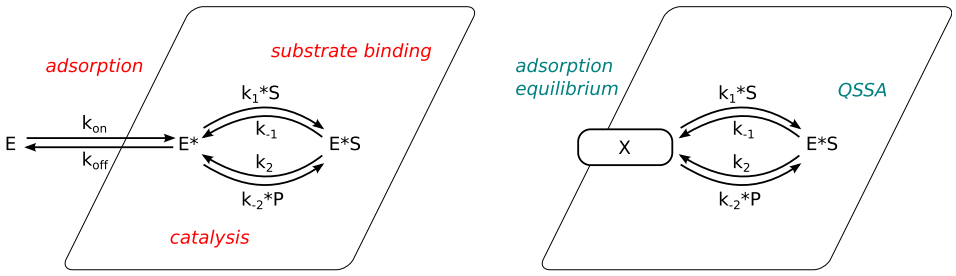

Fig. 1. Left: Kinetic scheme of a simple enzymatic reaction converting a single surface substrate $*S$ into a product $*P$. Right: Schematic representation of the approximations applied to derive the rate law. X is the rapid equilibrium segment (rapid binding and dissociation of the enzyme to the surface), QSSA = Quasi-Steady State Assumption.

unoccupied fraction of the surface area. To motivate this concept with Langmuir's model, Φ can be expressed in terms of the elementary spaces introduced above. To see this, consider the *maximum* surface concentration of elementary spaces n_0, a constant that is characteristic for a particular adsorbate since it describes the number density in a complete monolayer (dimension NL^{-2}). The unoccupied fraction of surface area is then actually equal to the fraction of empty elementary spaces

$$\Phi = \frac{\langle * \rangle}{n_0}, \tag{2}$$

where $\langle * \rangle = [*]/[A]$ and $[A]$ is the substrate surface area per unit volume (dimension L^{-1}). Note, that relation (2) is not a definition of the available area function but merely holds in terms of Langmuir's model. The fraction of the surface covered by the bound enzyme $E*$ is

$$\theta = \frac{\langle E* \rangle}{n_0}, \quad \theta \in [0,1], \tag{3}$$

known as the fractional surface coverage (*coverage* for short). If we consider adsorption as an isolated process, $E*$ is the only species contributing to surface coverage and Langmuir's model entails the conservation relation

$$1 = \theta + \Phi. \tag{4}$$

However, the relation between available area function and coverage can be much more complicated, in particular nonlinear, if we abandon some of the basic assumptions in Langmuir's model. The virtue then of using the available area function is that it can be used as a proxy for a whole class of different mechanisms by writing it generally as

$$\Phi = 1 - f(\theta) \tag{5}$$

for monolayer adsorption. For reasons of symmetry one may call $f(\theta)$ the *excluded area function* [see also [37]]. We only demand that this function fulfills $f(\theta = 0) = 0$ and $f(\theta) \leqslant 1$ for any admissible $\theta \in [0,1]$. In the derivation of the enzymatic rate laws below, these equations have to be generalized to reflect that also other species, in particular interfacial enzyme-substrate complexes $E*S$, contribute to the coverage.

In the following we will use the on-rate in the general form

$$v_{on} = k_{on}\Phi[A][E], \tag{6}$$

where $[*] = \langle * \rangle [A]$ has been replaced using Eq. (2) and $k_{on} = k'_{on} n_0$ (dimension LT^{-1}) is the new rate coefficient of adsorption, related to the activation energy of adsorption as defined by Ramsden [36]. Finally, to derive the equilibrium condition, we have to specify a desorption rate. Assuming a simple decay of adsorbed species we set

$$v_{off} = k_{off}[E*]. \tag{7}$$

Now let $k_A = k_{on}/k_{off}$, then the equilibrium condition $v_{on} = v_{off}$ leads to the following equation, which defines the adsorption isotherm *implicitly*,

$$\frac{[E*]_{eq}}{[E]_{eq}} = k_A[A]\Phi_{eq}, \tag{8}$$

or, in terms of coverage,

$$\frac{\theta_{eq}}{[E]_{eq}} = K\Phi_{eq}, \tag{9}$$

where $K = k_A/n_0$ (dimension $N^{-1}L^3$) is the affinity or Langmuir adsorption constant. This general equilibrium relation, whether in the form of Eq. (8) or Eq. (9), is central to our discussion of the properties of surface-active enzymes. As indicated above, specifying a particular isotherm amounts to choosing an appropriate function $\Phi(\theta)$.

3.2. Modeling different adsorption isotherms

3.2.1. Single-enzyme case

Before we continue with the derivation of the interfacial rate law, it is interesting to compare the effects of different adsorption models. We will here focus on the comparison of the intuitive Langmuir model with the more realistic random sequential adsorption (RSA) model [28,38], for which both the available area function and the corresponding isotherms are shown in Fig. 2, respectively. For instance, the classical Langmuir adsorption isotherm can be derived in explicit form by setting $\Phi = 1 - \theta$ in Eq. (9), the solid line in Fig. 2, leading to

$$\theta_{eq} = \frac{K[E]_{eq}}{1 + K[E]_{eq}} = \frac{e_{eq}}{1 + e_{eq}}, \tag{10}$$

where $e_{eq} = K[E]_{eq}$ is the scaled (dimensionless) equilibrium concentration of enzyme in solution. As shown in the inset of Fig. 2, the Langmuir ("LNG") isotherm is a hyperbolic relation akin to the classical Michaelis–Menten rate law, albeit with different meanings of the variables. The characteristic constant K^{-1} is now the equilibrium concentration of free enzyme in solution when half of the surface is covered ($\theta = 1/2$).

In RSA, the surface is treated as a continuum and there are no well-defined elementary spaces. As a consequence, novel effects like "jamming" (i.e. $\Phi \approx 0$ for $\theta < 1$) appear. That is, although there is enough space in total, the distribution of adsorbates is such that no incoming particle finds a gap that is big enough on the surface. In one-dimensional RSA this is suggestively called the car-parking problem. Even for this rather intuitive model, in two dimensions no closed expression for the function $\Phi(\theta)$ exists. However, the saturation limit $\theta_\infty \approx 0.547$ at which $\Phi(\theta_\infty) = 0$ can be calculated [28] and a good and simple polynomial approximation for Φ has been derived [39]. This approximation, along with the corresponding isotherm, is shown in Fig. 2 (dashed curve). The phenomenon of

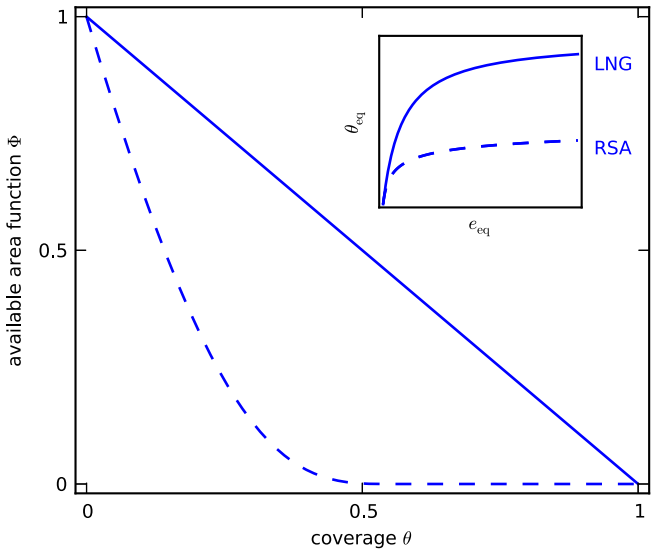

Fig. 2. Available area function and adsorption isotherm. The main figure shows the available area functions for Langmuir's model ("LNG") and random sequential adsorption ("RSA"). The inset shows the corresponding adsorption isotherms that apply at equilibrium in terms of coverage $\theta = [E*]/(n_0[A])$ and enzyme in solution $e = K[E]$.

jamming, which is reflected by a saturation limit of much lower than $\theta = 1$, leads to a stronger reduction of the available area when compared to the Langmuir model. To illustrate the consequences, we depict in Fig. 3 the fraction of adsorbed enzyme as a function of total enzyme amount and surface area. It can be seen that for the RSA model (dashed lines), the fraction of adsorbed enzyme is consistently lower when compared to Langmuir's model (solid lines), while the difference for a given amount of enzyme depends on the surface area. Not surprisingly, a smaller substrate surface area sequesters less enzyme in both models. However, it is worth noting the differences in the shape. For high surface area ($a \geqslant 5$) the adsorbed fraction in RSA is more sensitive to total enzyme amount than in LNG. For smaller surface areas ($a \leqslant 1$) both adsorption models behave more and more similar, especially for higher enzyme concentrations ($e_0 \geqslant 1$), and become insensitive to enzyme amount for very small surface areas ($a = 0.1$).

3.2.2. Multi-enzyme systems – competitive and multi-site adsorption

Heterogeneous reactions in biological systems commonly involve many enzymes that are active at one and the same interface. Starch is a classical example, where dikinases and phosphatases modify the interface and enable different amylases to efficiently degrade it. Thus, we have to consider enzymes competing for the same adsorption sites, so-called competitive adsorption. On the other hand, direct competition may be less severe if the enzymes adsorb at different sites on the substrate, so-called multi-site adsorption. Again, starch provides an excellent example. The interactions between amylopectin side-chains generally lead to a rigid, crystal-like interface but transition to a more dynamic, liquid-like interface can be triggered by surface-active enzymes by disrupting hydrogen bonds [40]. It is conceivable that enzymes have significantly different affinities for these physically different targets or "patches" [36] at the interface. Both solvation forces [36], in aqueous systems usually established by hydrogen bonding, and electrostatic interactions [41,42] can differ considerably between enzyme and substrate surface.

We want to demonstrate here that the available area function is a suitable concept for understanding these effects. However, for the sake of clarity we focus exclusively on the Langmuirian case. For RSA, available area functions have been derived for binary mixtures [28,39] and other cases [43]. In many of the more complicated cases Monte Carlo simulations are the only way to get reliable approximations of Φ.

The available area function for a system with competitive adsorption reads

$$\Phi = 1 - \sum_{i \in \mathsf{E}} \theta_i, \tag{11}$$

where E denotes an index set to enumerate the different species, for example E = {1,2,3}. This leads, by applying Eq. (9) for each species, to the following family of adsorption isotherms,

$$\theta_{\mathrm{eq},j} = \frac{K_j [E]_{\mathrm{eq},j}}{1 + \sum_i K_i [E]_{\mathrm{eq},i}}, \quad j \in \mathsf{E}. \tag{12}$$

This well-known result shows that increasing the concentration of any of the enzymes $i \neq j$, causing a higher $\theta_{\mathrm{eq},i}$ and $[E]_{\mathrm{eq},i}$, leads to a decrease in $\theta_{\mathrm{eq},j}$ for all $j \neq i$, that is to a displacement at the interface. The effect on the available area function depends on the relative magnitudes of the affinity parameters K_i that govern how some change $\Delta\, e_{0,i}$ translates into a change $\Delta\theta_{\mathrm{eq}} = \sum_{i \in \mathsf{E}} \Delta\theta_{\mathrm{eq},i}$. A moderate increase in a high-affinity enzyme can have the same effect as a higher increase in a low-affinity enzyme. The coupling between competitive enzymes, represented by the available area function, Eq. (11), will be further discussed below in relation with the rate law for surface-active enzymes.

The case of multi-site adsorption for a single enzyme reflects a scenario in which one enzyme binds with different affinities to different regions (patches) on the surface. Strictly speaking, this is not a multi-enzyme system, but there is more than one trans-species present at the surface. Now the rate of adsorption at a site k does not simply depend on the total concentration of empty elementary spaces $[*]$ but on the elementary spaces of type k, denoted $[*_k]$, respectively. If we derive, in analogy to Section 3.1, the implicit adsorption isotherm, we arrive at

$$\frac{[E*]_{\mathrm{eq},k}}{[E]_{\mathrm{eq}}} = k_{\mathrm{A},k} \alpha_k [A] \Phi_{\mathrm{eq},k}, \quad k \in \mathsf{S}, \tag{13}$$

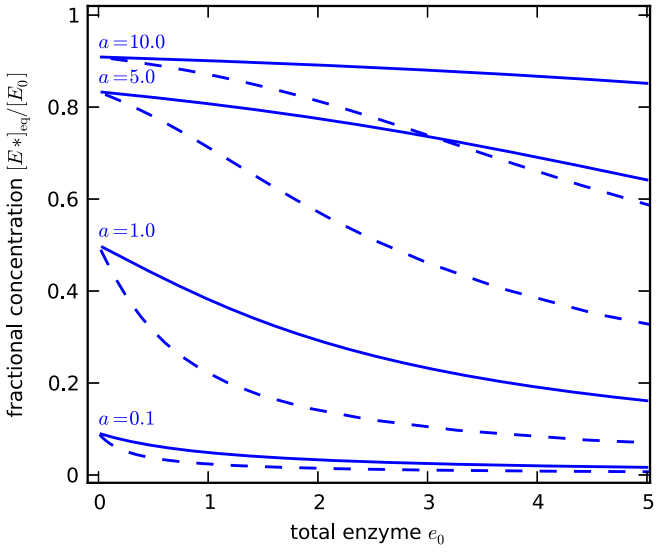

Fig. 3. Fraction of adsorbed enzyme as a function of total enzyme amount and surface area. Enzyme concentration and surface area are expressed in dimensionless units, $e_0 = [E_0]k_A/n_0$ and $a = [A]k_A$, respectively. Dashed lines represent results for RSA, solid lines for Langmuir's model.

where S is the index set enumerating the different surface types and $\alpha_k = A_k/A$ is the surface fraction made up of type k sites. We further assume that the surface types do not overlap. This implies $\sum_{k\in S}\alpha_k = 1$ and we have, for each type, an independent available area function

$$\Phi_k = 1 - \theta_k, \quad k \in S. \tag{14}$$

If n_0 is assumed site-independent we can again divide Eq. (13) by $n_0[A]$ to obtain the implicit isotherm

$$\frac{\theta_{eq,k}}{[E]_{eq}} = K_k \alpha_k \Phi_{eq,k}, \quad k \in S. \tag{15}$$

Replacing Φ_k leads to the multi-site Langmuir adsorption isotherm

$$\theta_{eq} = \sum_{k\in S}\theta_{eq,k} = \sum_{k\in S}\alpha_k \frac{K_k[E]_{eq}}{1 + K_k[E]_{eq}}. \tag{16}$$

Note, that this reduces to the single-site isotherm if $\alpha_k = 1$ for any k. Moreover, α_k is equal to the maximum coverage possible at site k.

3.2.3. Practical aspects

It is clear from Eq. (9) and Fig. 2 that the shape of the adsorption isotherm is determined by the available area function alone, the parameter K only entering as a scaling factor. However, in practical terms it is difficult to infer the adsorption behavior of an enzyme using this equation because the coverage θ cannot be measured directly. If the adsorption process can be observed in isolation, for example for an enzyme with inactivated or truncated catalytic domain but active binding/adsorption domain, the total enzyme concentration is $[E_0] = [E]_{eq} + [E*]_{eq}$. Measuring the free enzyme E at equilibrium after applying different amounts, E_0, gives the equilibrium amount of bound enzyme E*. Then, the ratio on the left-hand side in Eq. (8) can be plotted against $[E*]_{eq}$ to reveal the shape of the available area function. As shown in the main panel in Fig. 2, a linear dependence is a strong indicator of Langmuir-type adsorption, while a convex curve could suggest, for example, the random sequential adsorption (RSA) model. Moreover, exploiting that $\Phi_{eq}(\theta = 0) = 1$, this plot also allows to estimate k_A by extrapolation if $[A]$ is known, which should be the case in a properly designed experiment.

One should keep in mind, however, that one and the same isotherm can result from different mechanisms. This limits our ability to infer mechanisms from isotherms but at the same time allows an empirical approach by proposing several basic shapes that represent typically observed cases, an approach taken by Giles [27,29]. Purely empirical approaches, however, are in danger of introducing inconsistencies by proposing isotherms that are thermodynamically impossible [29]. We believe, therefore, that using the available area function, which has a firm interpretation in terms of statistical mechanics, helps in safeguarding against such inconsistencies.

As regards studying enzyme adsorption in isolation one may point to the example of carbohydrate-binding modules (CBMs) [44], noncatalytic domains of carbohydrate-active enzymes, that have been cloned and purified to study their adsorption properties on cellulose and starch [45–48].

3.3. Rate law for surface-active enzymes

According to our assumption of adsorption equilibrium and the reaction pathway depicted in Fig. 1, the adsorbate species E and $E*$ form the rapid-equilibrium segment X whose concentration is given by

$$[X] = [E]_{eq} + [E*]_{eq}. \tag{17}$$

Following Cha [26], we proceed by defining a reference species and writing down the corresponding fractional concentration factors for the mechanism. Taking the free enzyme E as reference, these factors read

$$f_E = \frac{[E]_{eq}}{[X]} = \frac{1}{1 + \frac{[E*]_{eq}}{[E]_{eq}}}, \tag{18}$$

and

$$f_{E*} = \frac{[E*]_{eq}}{[X]} = \frac{\frac{[E*]_{eq}}{[E]_{eq}}}{1 + \frac{[E*]_{eq}}{[E]_{eq}}}. \tag{19}$$

After applying the RE approximation, the sQSS assumption is applied to the enzyme intermediate(s) in the resulting partial equilibrium reaction scheme,

$$X + {}^*S \underset{k_{-1}}{\overset{k_1 f_{E*}}{\rightleftharpoons}} E{}^*S \tag{20}$$

$$E{}^*S \underset{k_{-2} f_{E*}}{\overset{k_2}{\rightleftharpoons}} X + {}^*P. \tag{21}$$

As in Cha's approach, apparent (or composite) rate constants appear in this scheme whenever the elementary reaction depends on the concentration of X. Since these reactions take place at the interface, E is not used, thus f_E does not appear in the condensed scheme. Apart from the composite rate constants and the interfacial species, reminding us to use surface concentrations, we see that this scheme is formally identical to the general reversible Michaelis–Menten mechanism. Thus, instead of calculating the solution (see Appendix A) or applying the King–Altman [49] method to it, we can immediately write down the net steady-state rate

$$v = \mathrm{d}[*P]/\mathrm{d}t \tag{22}$$

by analogy. Here, this is done in terms of specificity constants (k_S, k_P) and Michaelis constants (K_{mS}, K_{mP}), leading to

$$v = \frac{f_{E*}[E_0](k_S\langle *S\rangle - k_P\langle *P\rangle)}{1 + f_{E*}\left(\frac{\langle *S\rangle}{K_{mS}} + \frac{\langle *P\rangle}{K_{mP}}\right)}, \tag{23}$$

where $[E_0]$ is the total concentration of enzyme per unit volume and the specificity and Michaelis constants are defined in the usual way [12] (see Eqs. (A.12) and (A.13)).

It is apt to point out already here two limiting cases depending on the value of f_{E*}. If no enzyme is adsorbed at the surface ($f_{E*} = 0$) we, trivially, have $v = 0$. If all of the enzyme in the rapid-equilibrium segment is present in the form E* (i.e. $f_{E*} = 1$), the rate law reads

$$v = \frac{[E_0](k_S\langle *S\rangle - k_P\langle *P\rangle)}{1 + \frac{\langle *S\rangle}{K_{mS}} + \frac{\langle *P\rangle}{K_{mP}}}, \tag{24}$$

which resembles the classical case with the difference of having surface concentrations for the reactants.

The more interesting behavior, of course, lies between these physical extremes. To investigate this regime further and formulate a genuine interfacial rate law, we can exploit the implicit adsorption isotherm derived above. Note, that with Eq. (8) the fractional concentration factor, Eq. (19), can be written as

$$f_{E*} = \frac{k_A[A]\Phi_{eq}}{1 + k_A[A]\Phi_{eq}}, \tag{25}$$

leading to

$$v = \frac{k_A[A]\Phi_{eq}[E_0](k_S\langle *S\rangle - k_P\langle *P\rangle)}{1 + k_A[A]\Phi_{eq}\left(1 + \frac{\langle *S\rangle}{K_{mS}} + \frac{\langle *P\rangle}{K_{mP}}\right)}. \tag{26}$$

This interfacial rate law can be interpreted by considering that the free area, $[A^{free}] = [A]\Phi_{eq}$, serves as the first "substrate" of the enzyme, to which it has to bind before exerting any catalytic activity

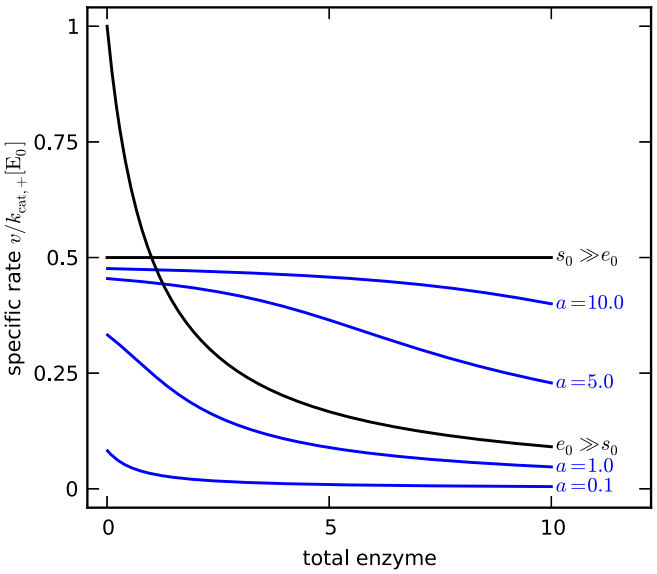

Fig. 4. Effect of enzyme amount on rate laws. The scaled specific rate is shown in dependence of total enzyme, given as $e_0 = [E_0]K$ for the surface-active enzyme (blue, with Langmuir isotherm) and $e_0 = [E_0]K_m$ for two cases (substrate and enzyme excess, respectively) where enzymes act in solution (black). The initial substrate concentration is set to $s_0 = 1$, that is $\langle *S_0 \rangle = K_{mS}$ (blue) and $[S_0] = K_m$ (black), respectively. The surface area parameter $a = k_A[A]$ is varied.

on the surface, much in analogy to a bisubstrate ordered mechanism of an enzyme working in solution [50].

A key difference to enzymes in solution is the appearance of the available area function Φ in this generic surface-active rate law, which implies a nonlinear dependence of both the denominator and numerator on the total enzyme concentration. Whereas in the classical case (with substrate excess) the rate scales proportionally with the enzyme concentration for any fixed substrate concentration, this is no longer the case for surface-active enzymes. Only for the limiting case of irreversible adsorption ($k_A \to \infty \Rightarrow f_{E*} = 1$), sometimes referred to as the scooting mode of surface-active enzymes [1], the rate law in Eq. (26) becomes equal to Eq. (24), thus proportional to enzyme concentration and insensitive to free surface area.

The decreasing effect of increasing enzyme amount on the rate of surface-active enzymes, mediated through Φ, is best illustrated by comparing the dependence of the *specific* activity on total enzyme amount as shown in Fig. 4. Here, we define the specific activity in dimensionless form as $v/[E_0]k_{cat,+}$ with $k_{cat,+} = k_2$ and use dimensionless versions of the parameters (see figure caption). Characteristically, the specific activity in the Michaelis–Menten case, which assumes substrate excess, is insensitive to $[E_0]$ ($s_0 \gg e_0$, black). For comparison, the black hyperbola shows the specific activity of the classical mechanism in solution if the enzyme is in excess ($e_0 \gg s_0$), as advocated by McLaren and Packer [9] for colloidal substrates in suspension. However, for comparable parameter combinations this hyperbolic dependence on enzyme concentration does not resemble those found with a true adsorption model (blue curves), where we find different shapes depending on the surface areas a (for simplicity, we show the one-way initial rate with Langmuirian adsorption, see Appendix B). For increasing surface area the shape of the function appears to switch from convex to concave, approaching the insensitivity of the classical Michaelis–Menten case. In Appendix B the suggested equality is proven for $a \to \infty$.

In general, the available area function depends not only on the concentration of the enzyme under consideration, because all other enzyme species able to bind to the surface further reduce the available area for this enzyme. Thus, Φ becomes a function of all surface-binding species,

$$\Phi = \Phi(\theta_i, i \in E). \tag{27}$$

For the special case of Langmuir's model, this function has the simple form of Eq. (11). It now becomes apparent that the available area function Φ plays an important role in defining the interaction and competition between several agents on one surface by implementing a monolayer model with hard-sphere exclusion at the surface. This phenomenon is fundamentally different from the case in solution. There, enzymes may also compete for various substrates, however, the overall effect is a result of the reaction network in which the enzymes interact, but the form of the rate equation is the same, regardless whether or not competing enzymes are present.

3.4. Effect of substrate properties

We now proceed to investigate direct practical consequences of the derived rate law Eq. (26). Often, when kinetic properties of surface-active enzymes are investigated, the experiments are carried out in analogy to initial rate assays for enzymes in solution [51,52]: The activity is monitored for different amounts of substrate, e.g. the activity of β-amylase is measured for varying amounts of starch, measured by mass. If other information is unavailable, this may lead to difficulties in interpreting the results, because it is actually the surface of the applied substance which serves as substrate. Surface and mass are related by

$$[A] = a_s[M], \tag{28}$$

where $[M]$ is the mass concentration (mass per volume, unit ML^{-3}), and a_s is the specific surface area (unit L^2M^{-1}), which depends on the geometry and size distribution (polydispersity) of the substrate. Distributing a given amount of mass to smaller but more particles leads to a larger a_s. A general equation for a_s of polydisperse spheroids is applied to starch granules by Tatsumi et al. [53] and Levine et al. [54] present an approach to map a combination of more complicated shapes to a distribution of spheres that preserve total area and volume.

Since the mass is easy to control experimentally, it is useful to express the rate law in terms of $[M]$,

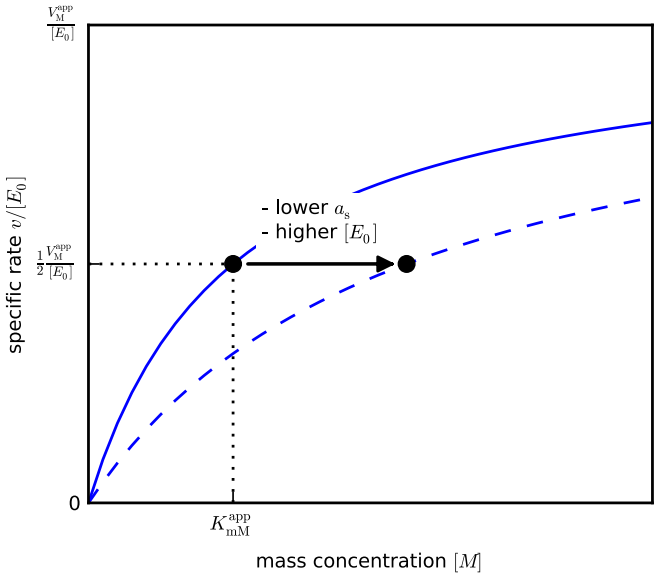

Fig. 5. Effect of specific surface area on apparent Michaelis constant in standard assays. Blue curves show the specific rate in terms of $[M]$, see Eq. (29). For completeness, the effect of $[E_0]$ is also illustrated.

$$v = \frac{k_A a_s \Phi_{eq}[M][E_0](k_S\langle *S\rangle - k_P\langle *P\rangle)}{1 + k_A a_s \Phi_{eq}[M]\left(1 + \frac{\langle *S\rangle}{K_{mS}} + \frac{\langle *P\rangle}{K_{mP}}\right)} = \frac{V_M^{app}\frac{[M]}{K_{mM}^{app}}}{1 + \frac{[M]}{K_{mM}^{app}}}, \quad (29)$$

with the *apparent* Michaelis constant

$$K_{mM}^{app} = \frac{1}{k_A a_s \Phi_{eq}\left(1 + \frac{\langle *S\rangle}{K_{mS}} + \frac{\langle *P\rangle}{K_{mP}}\right)} \quad (30)$$

and apparent limiting rate

$$V_M^{app} = [E_0] \cdot \frac{k_S\langle *S\rangle - k_P\langle *P\rangle}{1 + \frac{\langle *S\rangle}{K_{mS}} + \frac{\langle *P\rangle}{K_{mP}}}. \quad (31)$$

Fig. 5 illustrates the dependence of the apparent Michaelis constant K_{mM}^{app} on the key parameters specific surface area a_s and total enzyme concentration $[E_0]$. The fact that the specific surface area, a_s, enters the expression for the apparent Michaelis constant, Eq. (30), in the denominator shows that for smaller particles (higher a_s) a lower K_{mM}^{app}-value is observed than for larger particles and vice versa. This understanding is critical to avoid misinterpretations of enzymatic assays and to ensure reproducibility by having the parameter a_s under proper control [53,55]. In general, one may say that assays with surface-active enzymes have more degrees of freedom such that concentration alone does not suffice to describe the reaction rate.

What makes the situation even more difficult is that the apparent Michaelis constant does also depend on the total amount of enzyme. Because the available area function $\Phi([E_0])$ is monotonously decreasing for increasing enzyme concentration, the K_{mM}^{app}-value appears larger if more enzyme is applied in the assay, as illustrated in Fig. 5.

4. Concluding remarks

Notably, interfacial reactions have been studied for a long time in chemistry, because it has been recognized already in the 19th century that the surface of certain substances considerably enhances the speed of chemical reactions [56] and application of surface coverage to derive rate laws has a long tradition [57]. However, the situation for these technologically important processes is fundamentally different to biochemical processes on surfaces. In chemistry it is the surface itself which acts as a catalyst, to which the reacting species must adsorb. In contrast, in the biochemical processes we are interested in here, the catalysts are the adsorbates and the substrate surface is the adsorbent. So here, the enzymes must first adsorb before they mediate a chemical transformation of the surface.

A surface-active enzyme can only attack immediately accessible parts of the substrate confined to the interface. Although these reactants may be part of a larger molecule, like chain ends of amylopectin in the case of starch, it targets these sites as individual chemical species. Both, the characteristics of the interface and the interfacial reactants complicate the proper choice of observables to unambiguously characterize reaction rates [1]. Artificial model substrates with well-defined interfacial properties are arguably a very convenient choice to characterize surface-active enzymes. For instance, the use of chips with self-assembled monolayers [8,31,32,58,59] and lipid monolayers [60] allow control over the surface number density of the substrate $\langle *S\rangle$. Coupled with techniques like surface-plasmon resonance spectroscopy they allow for real-time monitoring of adsorption and catalysis. In cases where we want to describe activity on the native substrate we are often confined to indirect measurements of $\langle *S\rangle$, like electron microscopy to measure the size of a unit cell in crystalline substrates [see e.g. [61], for starch].

In a typical mean-field approach to enzyme kinetics in dilute solution it is usually sufficient to consider the concentration of substrates and products as the sole variables. In contrast, suspensions of aggregated substrates possess additional degrees of freedom: the large particles may have different sizes (polydispersity) and shape (polymorphism) and pores may be present. All these aspects affect the surface area of the substrate, a key factor for adsorption and activity of surface-active enzymes. Failure to take into account these complications, for example in enzyme assays, can lead to non-reproducible results, simply because one or several sources of variation are not under proper control or unknown.

So far, no treatment has been developed to theoretically describe the rate of surface-active enzymes in terms of *general* adsorption isotherms. Here, we overcome this limitation by presenting a generic rate law for surface-active enzymes and thus provide a theoretical framework in which this important class of processes can be studied and incorporated into mathematical models of more complex pathways. Our formalism generalizes previous approaches, which usually focused only on specific examples, such as phospholipases [5,50]. The generalization makes use of the available area function, a concept originally developed for surface physical chemistry. Its use provides flexibility with respect to different adsorption models, because the choice of adsorption model is reflected by its functional form, i.e. by how the available area depends on the covered fraction of the surface. Although only for the simplest case of Langmuir's model analytic expressions for the isotherm exist, the ability to easily adapt rate laws of surface-active enzymes to more realistic scenarios like random sequential adsorption (RSA) seems particularly beneficial.

The approach we have employed here exemplarily to derive the rate law for a reversible uni-uni reaction on a surface can easily be generalized to other surface-active enzymatic mechanisms. Once the relevant rate laws for the enzymes of interest have been established, they can be used for the formulation of mathematical models of arbitrarily complex pathways, much as current ODE-based models of metabolic networks are generated from Michaelis–Menten and related rate laws. However, the key difference to the classical case in solution is the presence of the available area function in the rate laws, which in turn depends on all enzymes adsorbing to a particular surface and thus represents an important coupling term which does not exist in solution. When applying our approach for the dynamic description of a pathway involving several competing enzymes, it is therefore critical that the available area function correctly reflects the competition of enzymes adsorbing at the surface. Finally, with our concept presented here, it is now possible to develop a sound description of processes involving reactions in both phases simultaneously. This will be of particular importance for a correct simulation of fundamental processes such as starch or cell-wall synthesis and degradation.

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Appendix A. Calculating the steady-state solution

The derivation of the rate law is completely analogous to the classical case. However, for the surface rate v^*, there is a slight modification to Cha's partial equilibrium schemes. The reaction

rates at the interface depend on surface concentrations, hence $[X]$ has to be weighted by

$$\bar{f}_{E*} = \frac{f_{E*}}{[A]} \tag{A.1}$$

instead of f_{E*}. This amounts to using $\langle E* \rangle = \bar{f}_{E*}[X]$ instead of $[E*] = f_{E*}[X]$ in the algebraic equations to derive the rate law.

The surface rate law is defined by the net rate through the rate-limiting product formation at steady state,

$$v^* = \frac{\mathrm{d}\langle *P \rangle}{\mathrm{d}t} = k_2 \langle E*S \rangle_{ss} - k_{-2}\bar{f}_{E*}[X]_{ss}\langle *P \rangle, \tag{A.2}$$

an equation with two unknowns. We eliminate $[X]_{ss}$ by using the general conservation relation

$$[E_0] = [X] + [E*S] \Rightarrow [X] = [E_0] - [A]\langle E*S \rangle, \tag{A.3}$$

leading to

$$v^* = k_2 \langle E*S \rangle_{ss} - k_{-2}\bar{f}_{E*}([E_0] - [A]\langle E*S \rangle_{ss})\langle *P \rangle, \tag{A.4}$$

$$= \langle E*S \rangle_{ss}(k_2 + k_{-2}\bar{f}_{E*}[A]\langle *P \rangle) - k_{-2}\bar{f}_{E*}[E_0]\langle *P \rangle \tag{A.5}$$

$$= \langle E*S \rangle_{ss}(k_2 + k_{-2}f_{E*}\langle *P \rangle) - k_{-2}\bar{f}_{E*}[E_0]\langle *P \rangle. \tag{A.6}$$

To eliminate the second unknown, $\langle E*S \rangle_{ss}$, the sQSSA is applied to the cis–trans complex E*S, that is

$$0 \approx k_1\bar{f}_{E*}[X]\langle *S \rangle - (k_{-1} + k_2)\langle E*S \rangle_{ss} + k_{-2}\bar{f}_{E*}[X]\langle *P \rangle. \tag{A.7}$$

With Eq. (A.3) this leads to

$$\langle E*S \rangle_{ss} = \frac{\bar{f}_{E*}[E_0](k_1\langle *S \rangle + k_{-2}\langle *P \rangle)}{(k_{-1} + k_2) + k_1 f_{E*}\langle *S \rangle + k_{-2}f_{E*}\langle *P \rangle}. \tag{A.8}$$

Note, that we have the usual dimensionless fractional concentration factors in the denominator. Replacing this into the rate law definition in the form (A.6) gives, after some terms cancel out,

$$v^* = \frac{\bar{f}_{E*}[E_0](k_2k_1\langle *S \rangle - k_{-1}k_{-2}\langle *P \rangle)}{(k_{-1} + k_2) + f_{E*}(k_1\langle *S \rangle + k_{-2}\langle *P \rangle)}. \tag{A.9}$$

Usually, however, we are interested in the turnover rate in the whole reactor volume,

$$v = \frac{\mathrm{d}[*P]}{\mathrm{d}t} \tag{A.10}$$

$$= [A]\frac{\mathrm{d}\langle *P \rangle}{\mathrm{d}t} + \langle *P \rangle \frac{\mathrm{d}[A]}{\mathrm{d}t}. \tag{A.11}$$

Thus, if the surface area does not change, or the second term is significantly smaller than the first one, it is valid to simply multiply v^* by $[A]$ to obtain v. Given the following definitions for the empirical parameters,

$$k_S = \frac{k_1k_2}{k_{-1} + k_2}, \quad K_{mS} = \frac{k_{-1} + k_2}{k_1} \tag{A.12}$$

for the forward reaction and

$$k_P = \frac{k_{-1}k_{-2}}{k_{-1} + k_2}, \quad K_{mP} = \frac{k_{-1} + k_2}{k_{-2}} \tag{A.13}$$

for the backward reaction, this leads to the rate law in Eq. (23).

Appendix B. Calculating the specific activity as a function of total enzyme concentration

In Fig. 4 we use the initial rate and assume $\langle *P \rangle \approx 0$ for Eq. (26). Using the dimensionless quantities $s_0 = \langle *S_0 \rangle / K_{mS}$ and $a = k_A[A]$, and $k_{cat,+}$ for the forward rate constant we can write the scaled specific forward rate as

$$v' = \frac{v}{k_{cat,+}[E_0]} = \frac{a\Phi_{eq}s_0}{1 + a\Phi_{eq} + a\Phi_{eq}s_0}. \tag{B.1}$$

To evaluate how the right-hand side depends on the enzyme concentration, Φ has to be known explicitly. To this aim we have to solve the following system of nonlinear algebraic equations:

$$0 = \Phi e - \theta_E, \qquad \text{(adsorption equilibrium, see } Eq.(8))$$
$$0 = 1 - f(\theta_E, \theta_{ES}) - \Phi, \qquad \text{(monolayer conservation)}$$
$$0 = e_0 - e - a(\theta_E + \theta_{ES}), \qquad \text{(enzyme conservation)}$$
$$0 = \theta_E s_0 - \theta_{ES}. \qquad \text{(QSSA)}$$

In line with the dimensionless rate law (B.1) we use additional dimensionless quantities, defined by $\{e,e_0\} = \{[E]_{eq}K,[E_0]K\}$. We omit the subscripts here for legibility, however, note that the system is solved for equilibrium (Φ, e, θ_E) and steady state (θ_{ES}) variables. The excluded area function f has to be chosen according to the adsorption model. With Langmuir's model we have the simple linear relation

$$f(\theta_E, \theta_{ES}) = \theta_E + \theta_{ES}$$

and the system can be solved by any computer algebra system for $\Phi(a,s_0,e_0)$ to obtain the solution for Eq. (B.1) by substitution. Here, Sage [62] was used for the symbolic calculations underlying Fig. 4. The result is a complicated expression, which for $s_0 = 1$ reduces to

$$\Phi_{eq} = \frac{2\,a + 2\,e_0 - \sqrt{4\,a^2 - 8\,ae_0 + 4\,e_0^2 + 4\,a + 4\,e_0 + 1} + 1}{2\left(\left(\sqrt{4\,a^2 - 8\,ae_0 + 4\,e_0^2 + 4\,a + 4\,e_0 + 1} - 1\right)a - 2\,a^2 + 2\,ae_0\right)}. \tag{B.2}$$

However, in the general case one can show that $\lim_{a\to\infty}\Phi_{eq} = 1$, hence

$$\lim_{a\to\infty} v' = \lim_{a\to\infty}\frac{\Phi_{eq}s_0}{\frac{1}{a} + \Phi_{eq} + \Phi_{eq}s_0} = \frac{s_0}{1 + s_0}, \tag{B.3}$$

which is the dimensionless form of the Michaelis–Menten rate law divided by the limiting rate $V = k_{cat}[E_0]$.

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