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Near Activation and Differential Activation in Enzymatic Reactions

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ABSTRACT

A framework that introduces the concept of near-activation complexation and differential free energy of activation is presented to extend the capabilities of the classical transition-state theory in enzymatic reactions. In our approach, reaching a near-equilibrium energy level is assumed to be necessary for complexation near the activation point, whereas an additional differential energy level is required for the near-equilibrium complex to activate and release reaction products. Integration of these energy levels within the transition-state theory explains the thermodynamic nature of the Michaelis–Menten (affinity) constant and its relationship with the rate constant under the quasi-steady-state assumption. The concepts of near-activation complexation and differential free energy of activation were tested on 57 independent experiments of NH_4^+ and NO_3^- uptake by various microalgae and bacteria at temperatures ranging between 1 and 45°C. Results showed that near-activation complexation was always favored, whereas the differential energy of activation led to an apparent energy barrier consistent with earlier observations. Temperature affected all energy levels within this framework but did not alter substantially their thermodynamic features. The approach (1) mutually links the thermodynamics and kinetics of Michaelis-Menten and rate constants with a mathematical expression; (2) describes the likelihood of formation of sub-, super-, and activated complexes; and (3) shows direction and thermodynamic likelihood of each reaction branch within the transition state.

INTRODUCTION

The Michaelis–Menten(-Monod) framework (MM) is one of the most common reaction kinetics as it can describe reactions of chemical and biochemical nature in various contexts. The MM kinetic order has a lower bound between zero and one, whereas its upper bound depends on the number of MM terms and proportionality products. In the simplest case (the lower bound), MM kinetics under the quasi-steady-state approximation include only two parameters, that is, the reaction rate constant k and the Michaelis–Menten (affinity) constant K_s . There are two powerful approaches that describe a MM reaction; (1) the kinetic approach, which is used to assess the mass fluxes between reactants, activated complex, and products over the course of a reaction; and (2) the thermodynamic approach, which is used to describe the likelihood of that reaction to occur as well as the energetic state of reactants, (activated) complex, and products. The two approaches are mostly complementary and provide unique insights of a reaction from different perspectives. The quantities that both approaches share are k and K_s , which are necessary in the kinetic equations to determine the reaction velocity, and in the reaction thermodynamics to assess its energetics. However, the two approaches have some drawbacks in that the kinetic equations lack a thermodynamic link between k and K_s , whereas the reaction thermodynamics does not properly address the mass fluxes and conservation at the activation point. These two aspects are explored in greater detail in the following two argumentations and are essential for the development of this communication.

Consider an enzymatic reaction that consumes a substrate S and produces a product P through an enzyme E . Using the 1913 Michaelis–Menten reaction framework in [1](#), S is assumed to attach to E to form an activated complex $C^\ddagger$ in equilibrium with S and E , which releases P and the free, unchanged enzyme E as

where k^+ and k^- are the rate constants of the forward and backward equilibrium reactions between reactants (S and E) and complex ($C^\ddagger$), and k is the reaction rate constant^{*}.

Argumentation 1: On the kinetic equations and the link between rate constant and Michaelis–Menten constant. By describing the fluxes of matter in Eq. [1](#) using first-order kinetics [1](#) and assuming that $C^\ddagger$ is in quasi-steady-state ($d[C^\ddagger]/dt \approx 0$) at a small concentration ($[C^\ddagger] \approx 0$), one can write the Michaelis–Menten constant K_s as [3](#)

$$K_s = \frac{k^- + k}{k^+} \quad (2)$$

Although this relationship establishes a mathematical function either for $k = k(K_s)$ or for $K_s = K_s(k)$ that links the rate constants of all matter fluxes of Eq. [1](#) to the Michaelis–Menten

constant, it does not allow the quantification of these functions in common situations. In fact, when k and K_s are estimated from experiments where $[S]$ or $[P]$ are directly measured, there is an infinite number of (k^+, k^-) couples that satisfy Eq. 2.

An advance to circumvent this shortcoming was proposed in the GEBIK and GEBIF equations 4, 5, where the Briggs–Haldane assumption of quasi-steady-state was relaxed and the rate constants k^+ , k^- , and k were estimated from the isotopic expression accumulated in the activated complex, while K_s was calculated a posteriori using Eq. 2†. Although the method successfully described the kinetics at the activated complex, as well as variable and inverse isotopic effects observed in S and P , a generalized use still has to be tested. Note that missing an interpretative tool of Eq. 2 based on a theoretical (possibly thermodynamic) foundation does not preclude one to analyze a reaction kinetics as long as experimental data are available to estimate k and K_s . Yet, a comprehensive understanding of aspects intrinsic to Eq. 2 will remain hidden when one is interested in thermodynamic effects. For example, a reaction occurring at different temperatures will show different k (as formulated in the Arrhenius' law) and one may question if these differences would only involve k or should affect also K_s , as presumable from Eq. 2. There is empirical evidence that K_s in enzymatic reactions is sensitive to temperature 6-9 and analyses showed that an Arrhenius'-like law may also apply to K_s 10; yet, a robust framework or explanation of those observations and analyses is currently not available. We show in this work a thermodynamic interpretation of, and provide a mathematical relationship between k and K_s of Eq. 2.

Argumentation 2: On the equilibrium and mass fluxes within the transition-state theory. Eyring's theory of absolute reaction rates is currently one of the most powerful approaches to describe k by means of first principles within the transition-state theory 11, 12. One should ideally be able to calculate k at any temperature T using the absolute frequency factor $K_b T/h$, with $K_b = 1.38 \times 10^{-23}$ J/K and $h = 6.626 \times 10^{-34}$ J·s the Boltzmann and Planck constants, respectively, and the Gibbs free energy of activation $\Delta G^{0,\ddagger}$ at standard conditions as 11

$$k = \frac{K_b T}{h} e^{-\Delta G^{0,\ddagger}/RT} \quad (3)$$

The crucial step in this second argumentation is that the activated complex $C^\ddagger$ in the transition-state theory is assumed to be in equilibrium with the reactants S and E . This condition allows one to conveniently write the kinetic equations describing the chemical system by using the quasi-steady-state assumption for $C^\ddagger$. However, if on the one hand quasi-steady-state and equilibrium $(S + E) \rightleftharpoons C^\ddagger$ are expressions of the same process within activation, and fit in well with each

other in kinetic terms, on the other hand, they do not exactly match in thermodynamic terms. In fact: exclude from Eq. 1 the pathway that releases P and E , and only consider the equilibrium assuming S , E , and $C^\ddagger$ are mixed 13, 14. In this case, the equilibrium constant of activation

$$K^\ddagger = \frac{k^+}{k^-} \quad (4)$$

implies that the forward and backward mass fluxes are

$$k^+[S][E] = k^-[C^\ddagger] \quad (5)$$

In thermodynamics terms, $K^\ddagger$ can be written using the fundamental Boltzmann relationship as 12

$$K^\ddagger = e^{-\Delta G^{0,\ddagger}/RT} \quad (6)$$

where $\Delta G^{0,\ddagger}$ is normally described as the energy barrier of the Arrhenius' law (when entropy and temperature are implicitly accounted for). Note also that the exponential term in Eq. 6 can be

interpreted as a probability factor $\text{Pr}[(S + E) \rightarrow C^\ddagger] \propto e^{-\Delta G^{0,\ddagger}/RT}$ for the reactants to form $C^\ddagger$ at an energy level $\Delta G^{0,\ddagger}$ above the one where they are inert (i.e., according to the Boltzmann distribution function 14). When release of products is accounted for as in the transition-state theory, and the activated complex $C^\ddagger$ passes the energy barrier $\Delta G^{0,\ddagger}$ with a frequency $K_b T/h$ 12, then equilibrium between $(S + E)$ and $C^\ddagger$ is not satisfied anymore given that the mass flux in the forward equilibrium reaction would exceed that in the backward reaction by $k[C^\ddagger]$, that is, Eq. 5 becomes

$$k^+[S][E] > k^-[C^\ddagger] \quad (7)$$

The level of approximation introduced by assuming equilibrium in this circumstance is measured by this excess mass flux; assuming a transmission coefficient $\tau = 1$ (discussed in greater detail in the Discussion section), if $k[C^\ddagger] \gg k[C^\ddagger]$, then the equilibrium condition may not lead to a great error. This condition may be met in slow reactions, but fast reactions may depart from this situation and lead to greater errors. The equilibrium Gibbs free energy of activation, therefore, only partially captures how mass fluxes are partitioned at the activation point as brought to light in 15, but an alternative approach that revises these contrasting aspects of the transition-state theory has not yet been proposed in the literature.

Given these two preparatory argumentations, the aim of this communication is to elaborate a theoretical advance to harmonize the kinetic and thermodynamic approaches in the description of equilibrium and activation in enzymatic reactions of the Michaelis–Menten type. The approach extends previous developments of the absolute reaction rate theory and the kinetic equations consolidated in this context by introducing novel elements that allow (1) a description of the thermodynamic link between reaction rate and Michaelis–Menten constants and (2) an explanation of the mass flux partitioning near and at the activation point using a thermodynamic approach. The approach is theoretical in nature, but evidence of its validity is given using 57 data

sets of enzymatic reactions of NH_4^+ and NO_3^- uptake at temperatures ranging from 1 to 45°C by various microalgal and bacterial strains.

THEORETICAL FRAMEWORK

Sections below introduce the working hypotheses and derivations of the approach presented in this work.

Equilibrium Complexation

Consider only equilibrium complexation in Eq. 1; this is thermodynamically determined by $K^\ddagger = e^{-(G^{\ddagger,+} - G^{\ddagger,-})/RT} = e^{-\Delta G^{\ddagger,\ddagger}/RT}$; $K^\ddagger$ represents the fraction of occurrences that reactants ($S + E$) reach complexation $(S + E) \rightleftharpoons C$ at Gibbs free energy level $G^{0,+}$ from energy level $G^{0,-}$, which equals the energy of formation $G^0(S + E) = G^0(S) + G^0(E)$ of the reactants (Fig. 1). It also implies that equilibrium complexation is governed by the relative position of $G^{0,+}$ and $G^{0,-}$ (Fig. 1a). In a general sense, $G^{0,+}$ and $G^{0,-}$ can be located relative to each other in any order depending on whether equilibrium is toward the reactants ($G^{0,+} > G^{0,-}$ and $K^\ddagger < 1$) or the complex ($G^{0,+} < G^{0,-}$ and $K^\ddagger > 1$), or whether these are in equilibrium ($G^{0,+} = G^{0,-}$, and $K^\ddagger = 1$); these cases are explored in greater detail in the section Subactivated, Activated, and Superactivated complex.

Figure 1

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Conceptual representation of (a) the Gibbs free energy of equilibrium, (b) the Gibbs free energy in the transition-state-theory, and (c) the Gibbs free energy of near-activation complexation and differential activation in this work, with the blue curve representing the apparent energy barrier.

Approach to equilibrium is intended here as the situation in which the reacting complex $(S + E) \rightleftharpoons C$ moves along the reaction coordinate toward $C^\ddagger$, but does not yet cross activation at point $C^\ddagger$ in the reaction coordinate (Fig. 2, see the equilibrium reaction). The distance δ between C and $C^\ddagger$ can be imagined to decrease during transition toward equilibrium. During equilibrium complexation in the absence of product release, the mass flux moving forward

to C equals that backward to $(S + E)$. $\Delta G^{0\ddagger}$, referred to as the Gibbs free energy of activation in the transition-state-theory (Fig. 1b), is considered to describe only the energy related to equilibrium complexation $(S + E) \rightleftharpoons C$ in this framework after argumentation 2, and does not imply activation and release of products $C^\ddagger \rightarrow (P + E)$.

Figure 2

[Open in figure viewer](#)[PowerPoint](#)

Conceptual representation along the reaction coordinate of (1) equilibrium complexation in the equilibrium reaction of MM kinetics of Eq. 1 in the absence of product release and (2) near-activation complexation for the process $(S + E) \rightleftharpoons C$ and differential activation for the process $C^\ddagger \rightarrow (P + E)$ in the MM kinetics of Eq. 1 with near-activation distance $\delta_a \rightarrow 0$.

The mass flux when reaction products are not released was conceptually represented in Fig. 1a as a circular flow from the reactants up to the equilibrium complex, and then down vertically to the same energy level of the reactants along the reaction coordinate. This representation should only be taken as an indication that mass moves backward, but we do not have an actual reason to exclude that the backward mass flux could follow a different pathway within the energy limits depicted by the arrows. Likewise, the curved forward reaction pathway climbing up to $\Delta G^{0,+}$ from $G^{0,-}$ is only an indication, which may not reflect the actual shape of the energy surface (or curve in this representation).

Near Activation and Differential Activation (in the Gibbs Sense)

Once an equilibrium complexation that balances the mass fluxes in the forward and backward directions is established without release of products (Fig. 2, equilibrium complexation), it is hypothesized in this framework that an event may occur by which the equilibrium complex C escapes equilibrium toward the activation point $C^\ddagger$. This event corresponds to the decreasing of the distance δ between C and $C^\ddagger$ to $\delta = \delta_a$, where δ_a is a limit distance that separates

the two processes $(S + E) \rightleftharpoons C$ (equilibrium) and $C^\ddagger \rightarrow (P + E)$ (activation and release of products) along the reaction coordinate, and it is called here near-activation distance (Fig. 2). Assuming continuity of the two processes $(S + E) \rightleftharpoons C$ and $C^\ddagger \rightarrow (P + E)$, the distance δ_a can be conceptualized to decrease and ideally become $\delta_a \rightarrow 0$. In the limit $\delta_a \rightarrow 0$, the writing $C \cong C^\ddagger$ is used to represent the near-activation complex for process $(S + E) \rightleftharpoons C \cong C^\ddagger$ at the Gibbs free energy level of the equilibrium complex, and the activated complex of process $C \cong C^\ddagger \rightleftharpoons (P + E)$ characterized by a differential Gibbs free energy level G_λ^0 above $G^{0,-}$.

The likelihood that the equilibrium complex C at energy level $G^{0,+}$ approaches the activation point $C^\ddagger$ at energy G_λ^0 can be expressed by means of a Boltzmann function as much as the likelihood for the reactants to move from $G^{0,-}$ to $G^{0,+}$ is expressed by $e^{-\Delta G^{0,\ddagger}/RT}$ in Eq. 6. Hence, the analogue likelihood for the reactants to reach G_λ^0 from $G^{0,-}$ can be expressed as $e^{-(G_\lambda^0 - G^{0,-})/RT}$ (Fig. 1c); only after $C^\ddagger$ has reached G_λ^0 , reaction products may be released at rate k^0 at standard conditions written as

$$k^0 = \frac{K_B T}{h} e^{-(G_\lambda^0 - G^{0,-})/RT} \quad (8)$$

Introduction of the differential activation energy factor

$$\lambda = \frac{G_\lambda^0 - G^{0,-}}{G^{+,0} - G^{0,-}} \quad (9)$$

allows one to write $(G_\lambda^0 - G^{0,-}) = \lambda \Delta G^{0,\ddagger}$ and simplify Eq. 8 to

$$k^0 = \frac{K_B T}{h} (K^\ddagger)^\lambda \quad (10)$$

Term $(G_\lambda^0 - G^{0,+})$ represents an additional contribution to the Gibbs free energy for equilibrium complexation $\Delta G^{0,\ddagger}$ that is necessary for products to be formed during the course of a reaction. The differential Gibbs free energy of activation $(G_\lambda^0 - G^{0,+})$ can be associated with the work required to divert part of the mass flux of the backward equilibrium reaction toward the reaction direction. Diversion would therefore occur at the expense of some energy associated with maintaining the reactants and near-activated complex near equilibrium. This diversion would also imply that equilibrium would persistently be disproportioned by the mass flux $k[C^\ddagger]$ toward $(P + E)$, and the mass balance at the activation point would be such that $k^*[S][E] = (k + k)[C^\ddagger]$. Note that this writing is the same as the kinetic equation expressing the quasi-steady-state assumption for $C^\ddagger$ in the classical formulation in 3 once the mass conservation law for the total undegraded enzyme $E_0 = E + C^\ddagger$ is used.

Following the concept depicted in Fig. 2 for the particular point corresponding to $C \cong C^\ddagger$, we have introduced the concepts of “near-activation complexation” for the process $(S + E) \rightleftharpoons C \cong C^\ddagger$ of substrate consumption and “differential activation” for the process $C \cong C^\ddagger \rightarrow (P + E)$ of product release in the limit $\delta_a \rightarrow 0$. Panel in Fig. 1c represents the Gibbs free energy level associated with inert, near-activation complex, and differential activation complex relative to the kinetic reaction along the reaction coordinate as depicted in Fig. 2. The blue curve in Fig. 1c expresses the apparent Gibbs free energy level to be crossed over relative to that of the reactant. As an additional note, the complex $C \cong C^\ddagger$ is treated as a molecule that undergoes all thermodynamic and kinetic laws of regular molecules in the transition state theory and is treated in this way for near-activation and differential activation in this framework. This concept is further discussed in the Discussion section.

Equation 10 provides an expression for the reaction rate constant k that is only slightly different from that of the theory of absolute reaction rates by Eyring, but allows for further elaborations as shown in the next sections.

Examining the sequence of events for kinetic reactions depicted in Fig. 2, near-activation complexation $(S + E) \rightleftharpoons C \cong C^\ddagger$ and differential activation $C \cong C^\ddagger \rightarrow (P + E)$ occur at some energy levels of C and $C^\ddagger$ that may differ between each other, and between that of the inert reactants and products. Intermediates that have different Gibbs free energy have been hypothesized in a number of occasions and have been linked to a lowering of the activation energy caused by the binding energy between E and S 16. Multiple intermediates may also be hypothesized depending on the enzymatic mechanisms, each possibly resulting in different Gibbs free energy levels 17. It is however important to recognize that even enzymatic kinetics reactions that foresee only one enzyme binding to a substrate in the absence of other enzymes or coenzymes ideally lead to a comprehensive network of equilibrium intermediates that do not necessarily appear in a linear sequence over the reaction coordinate. The best example of this network of intermediates was comprehensively investigated in 18, which included a nonenzymatic and an enzymatic pathway. Excluding the nonenzymatic pathway, the framework presented here introduces a different approach in the description of the transition state of enzymatic reactions as compared to the above. The main difference is in the accounting of a near-activation complexation $(S + E) \rightleftharpoons C \cong C^\ddagger$ and a differential activation $(C \cong C^\ddagger \rightarrow (P + E))$ that share $C \cong C^\ddagger$. $(S + E) \rightleftharpoons C \cong C^\ddagger$ and $C \cong C^\ddagger \rightarrow (P + E)$ describe therefore continuous, near-equilibrium processes ideally concurrent in the reaction coordinate (i.e., $\delta_a \rightarrow 0$). This conceptualization has made it possible to define a differential activation energy $\lambda\Delta G^{0\ddagger}$ without the explicit introduction of additional forward and backward rate constants

between the near-activation complexation and differential activation points. As shown later, this approach has also made it possible to derive an explicit expression for the thermodynamic relationship between the reaction rate constant k and Michaelis–Menten constant K_s .

Activation and Release Probability Factors

Two factors can be identified that contribute to the probability $\text{Pr}[(S + E) \rightarrow (P + E)]$ that reaction in Eq. 1 occurs. This probability is determined by the simultaneous probability $\text{Pr}[(S + E) \rightarrow C \cong C^\ddagger]$ that $(S + E)$ moves toward $C \cong C^\ddagger$ and the probability $\text{Pr}[C \cong C^\ddagger \rightarrow (P + E)]$ that $C \cong C^\ddagger$ releases $(P + E)$. These probabilities are reflected in Eq. 10 by the scaling $\text{Pr}[(S + E) \rightarrow C \cong C^\ddagger] \propto e^{-\Delta G^{\ddagger,0}/RT}$ and $\text{Pr}[C \cong C^\ddagger \rightarrow (P + E)] \propto e^{-(\lambda-1)\Delta G^{\ddagger,0}/RT}$, with their product scaling as

$$\text{Pr}[(S + E) \rightarrow (P + E)] \propto e^{-\frac{\lambda \Delta G^{\ddagger,0}}{RT}} = (K^\ddagger)^\lambda \quad (11)$$

The actual probabilities introduced above can be written after normalizing them by the partition function $\text{Pr}[(S + E) \rightarrow C \cong C^\ddagger] + \text{Pr}[C \cong C^\ddagger \rightarrow (P + E)]$ of the Boltzmann distribution 14. In this way, it is possible to determine the probability f by which $C \cong C^\ddagger$ will turn back toward the reactants and the probability f that $C \cong C^\ddagger$ will continue toward the products. Under the hypothesis of near-activation complexation ($\delta_a \rightarrow 0$), $\text{Pr}[C \cong C^\ddagger \rightarrow (S + E)] \simeq \text{Pr}[(S + E) \rightarrow C \cong C^\ddagger]$, and f and f can be written as

$$f^- = \frac{e^{-\frac{\lambda \Delta G^{\ddagger,0}}{RT}}}{e^{-\frac{\Delta G^{\ddagger,0}}{RT}} + e^{-\frac{\lambda \Delta G^{\ddagger,0}}{RT}}} = \frac{(K^\ddagger)^{\lambda-1}}{1 + (K^\ddagger)^{\lambda-1}} \quad (12a)$$

$$f = \frac{e^{-\frac{\Delta G^{\ddagger,0}}{RT}}}{e^{-\frac{\Delta G^{\ddagger,0}}{RT}} + e^{-\frac{\lambda \Delta G^{\ddagger,0}}{RT}}} = \frac{1}{1 + (K^\ddagger)^{\lambda-1}} \quad (12b)$$

with $f + f = 1$. Whether $f > f$ (or otherwise) is determined by the relative position of $G^{0,-}$, $G^{0,+}$, and G_λ^0 . For example, relative to the conceptual drawing in Fig. 1c, $f > f$ because $\lambda \Delta G^{0,\ddagger} > 0$ is greater than $\Delta G^{0,\ddagger} > 0$.

The probabilities f and f can also be interpreted as the mass flux fractions from the activation point toward $(S + E)$ and $(P + E)$, respectively; for example, take unit flux $f^- = 1$ for the forward reaction $(S + E) \xrightarrow{k^+} C \cong C^\ddagger$, then f is the backward flux $(S + E) \xleftarrow{k^-} C \cong C^\ddagger$ and f is the reaction flux $C^\ddagger \xrightarrow{k} (P + E)$.

Subactivated, Activated, and Superactivated Complex

As mentioned earlier, there is no necessary reason for the near-activation and differential activation energy $\Delta G^{0,\ddagger}$ and $\lambda \Delta G^{0,\ddagger}$ to be located at any particular level relative to each other and,

if we exclude constraints relative to $G^{0,-}$ (discussed later), six ideal cases can be predicted on the basis of simple considerations around $K^\ddagger$ and λ . Each one of these six cases may be more or less likely, and a brief analysis is introduced below. In any of these six cases, we will refer to the activated complex as “superactivated” when $G_\lambda^0 > \max\{G^{0,-}, G^{0,+}\}$, “subactivated” when $G_\lambda^0 < \min\{G^{0,-}, G^{0,+}\}$, and “activated” in all the other cases.

The three cases when $K^\ddagger < 1$ correspond to $\lambda \geq 1$, $0 \leq \lambda < 1$, and $\lambda < 0$, respectively (Figs. 3a–3c). In these cases, the equilibrium $(S + E) \rightleftharpoons C \rightleftharpoons C^\ddagger$ favors $(S + E)$ whereas release of reaction products requires either an energy input when $\lambda \geq 1$ or an energy release when $0 \leq \lambda < 1$ and $\lambda < 0$. The three cases also correspond to complex “superactivation” (i.e., $G_\lambda^0 > G^{0,+}$), “activation” (i.e., $G^{0,+} < G_\lambda^0 < G^{0,-}$), and “subactivation” (i.e., $G_\lambda^0 < G^{0,-}$). The Gibbs free energy of activation $\lambda\Delta G^{0,\ddagger}$ represents here the apparent energy barrier that $C^\ddagger$ crosses, which is depicted as a blue curve in Fig. 3. Among these cases, superactivation and activation are the most likely situations; subactivation is less likely given that there would not be an apparent energy barrier for $C^\ddagger$ to release reaction products.

Figure 3

[Open in figure viewerPowerPoint](#)

Conceptual Gibbs free energy levels along the reaction coordinate for various combinations of near-activation equilibrium $K^\ddagger$ and differential Gibbs free energy of activation $\lambda\Delta G^\ddagger$. Green and red arrows represent positive and negative quantities, respectively; blue curves represent apparent energy lines. Configurations in (a), (b), and (d) are possible, whereas (c), (e), and (f) are only theoretical and unlikely.

The three cases when $K^\ddagger > 1$ are mirrored from above and correspond to $\lambda < 0$, $0 \leq \lambda < 1$, and $\lambda \geq 1$, respectively (Figs. 3d–3f). In these cases, the equilibrium $(S + E) \rightleftharpoons C \cong C^\ddagger$ favors $C^\ddagger$, with superactivation (i.e., $G_\lambda^0 > G^{0,-}$), activation (i.e., $G^{0,+} < G_\lambda^0 < G^{0,-}$), and subactivation (i.e., $G_\lambda^0 < G^{0,+}$) leading to thermodynamic implications similar to those highlighted before.

Among all possible thermodynamic configurations, those depicted in Figs. 3a, 3b, and 3d are the most likely in Michaelis–Menten reactions, while 3c, 3e, and 3f are unlikely given that no energy barrier is formed.

Thermodynamic Link between K_s and k

By substituting Eq. 4 into Eq. 2, one can write the Michaelis–Menten constant at standard conditions as

$$K_s^0 = \frac{1}{K^\ddagger} + \frac{k^0}{k^{0,+}}$$

Additionally, by analogy with equilibrium reactions, and following the reasoning in 6, the standard rate constant for the forward near-equilibrium complexation reaction $k^{0,+}$ can be written as (blue curve, Fig. 1c)

$$k^{0,+} = \frac{K_B T}{h} e^{-(G^{0,+} - G^{0,-})/RT} \quad (13)$$

With $G^{0,+} - G^{0,-} = \Delta G^{0,\ddagger}$, and with the reaction rate constant k^0 as in Eq. 8, K_s^0 becomes

$$K_s^0 = \frac{1}{K^\ddagger} + (K^\ddagger)^\lambda k^0 \quad (14)$$

By isolating $K^\ddagger$ in Eq. 10 and substituting it into Eq. 14, one can write K_s^0 as an explicit function of k^0 as

$$K_s^0 = \left(\frac{h k^0}{K_B T} \right)^{-\frac{1}{\lambda}} + \left(\frac{h k^0}{K_B T} \right)^{\frac{\lambda-1}{\lambda}} \quad (15)$$

Equations 10 and 14 are key outcomes of this approach after introduction of near-activation and differential activation in Michaelis–Menten enzyme kinetics. On the one hand, they show that

both the reaction rate constant and the Michaelis–Menten constant are functions of the near-activation complexation constant $K^\ddagger$ and the differential activation energy factor λ , thus they allow to mathematically link the two constants as in Eq. 15 and fill in the gap highlighted in argumentation 1 of the Introduction. On the other hand, they correct the definition of equilibrium in the transition-state theory by introducing the condition of “near-activation complexation” between reactants and complex at a distance $\delta_a \rightarrow 0$ from activation, which implies an additional energy level at the activation point for the reaction to release the products as highlighted in argumentation 2. More importantly, the mathematical problem has not substantially changed after introduction of the differential Gibbs free energy of activation; that is, the two kinetic quantities k and K_s of a reaction have been substituted by the thermodynamic quantities $K^\ddagger$ (or equivalently $\Delta G^{0,\ddagger}$) and λ . In practice, the kinetic problem expressed in the (k, K_s) coordinates is unambiguously coupled to the thermodynamic problem in the $(K^\ddagger, \lambda)$ coordinates as $(k, K_s) \leftrightarrow (K^\ddagger, \lambda)$.

It is possible to express the contribution of temperature in an explicit form using the definition of Gibbs free energy $\Delta G = \Delta H - T\Delta S$, with H and S the enthalpy and entropy, respectively, as

$$k = \frac{K_b T}{h} (K^\ddagger)^\lambda = \frac{K_b T}{h} e^{-\frac{\lambda(\Delta H^\ddagger - T\Delta S^\ddagger)}{RT}} \quad (16a)$$

$$K_s = \frac{1}{K^\ddagger} + (K^\ddagger)^{\lambda-1} = \frac{1 + e^{-\frac{\lambda(\Delta H^\ddagger - T\Delta S^\ddagger)}{RT}}}{e^{-\frac{\Delta H^\ddagger - T\Delta S^\ddagger}{RT}}} \quad (16b)$$

Equations 16a and 16b confirm the nonlinearity of k in T investigated since Arrhenius' work, but also provides an answer to the question addressed in argumentation 1 of Introduction; that is, what is the temperature dependence of the MM affinity constant? In both k and K_s , nonlinearity with T is accentuated by the differential activation energy factor λ . Note also that while k is an exponential function, K_s is not strictly exponential given that the sum of exponential functions is not an exponential function. Equation 16b addresses therefore the approach initially empirically proposed in 10, where Arrhenius'-like equations were used to express the effect of T on K_s , to a more rigorous accounting of this effect. As compared to Eqs. 10 and 14, and to the quasi-steady-state MM kinetics, the full form of Eqs. 16a and 16b include one additional degree of freedom; hence, λ , $\Delta H^\ddagger$, and $\Delta S^\ddagger$ can only be determined if experiments at different temperatures are available, and the kinetics and thermodynamic problems are not unambiguous (i.e., $(k, K_s) \leftarrow (\lambda, \Delta H^\ddagger, \Delta S^\ddagger)$).

Experimental Validation

To test application of the concept of differential Gibbs free energy of activation, 57 independent data sets of NH_4^+ and NO_3^- uptake by various microalgal and bacterial strains from [8](#) were used (Table [II](#), columns 4 and 5). Chemostat cultures of isolated strains were conducted at various temperatures, and growth to steady state was monitored at various times by measuring optical density changes. The reaction rate constant k was obtained from a first-order linear regression analysis of the semilogarithmic plot of optical density versus time, whereas K_s was determined from residual substrate concentrations of samplings at various dilution (more details are available in [8](#)). Parameters obtained with this technique were considered equivalent to those used in the Michaelis–Menten kinetics [2](#), [19](#) and in the GEBIK equations [4](#), [5](#) solved under the quasi-steady-state hypothesis. Data relative to the reaction rate constant k expressed with the units of s^{-1} were assumed to include any stoichiometric coefficients, whereas the Michaelis–Menten constant K_s was expressed in molal concentration.

These data sets are particularly valuable because experiments were repeated at temperatures between 1 and 45°C for most incubations, thus allowing us to retrieve a statistically comprehensive validation of the work presented here.

RESULTS

The following sections report analyses and interpretations of the thermodynamic characteristics of the kinetic parameters of NH_4^+ and NO_3^- uptake by various microalgae and bacteria. Detailed analyses of the microbiological and ecological implications were beyond the scope of this work and can be found in [8](#).

Determination of λ , $\Delta H^\ddagger$, and $\Delta S^\ddagger$

Arrhenius plots of experimental k and K_s values from [8](#) (Table [II](#) in Appendix) highlight an intermediate strength in their dependence on temperature, with a greater variance in K_s than in k (Figs. [4a](#) and [4b](#)).

Figure 4

[Open in figure viewerPowerPoint](#)

Arrhenius plot of experimental and modeled values of (a) reaction rate constants k and (b) Michaelis–Menten constants K_s of NH_4^+ and NO_3^- uptake. Experimental data are listed in Appendix, Table 7.

These values of k and K_s were grouped by microbial types (algae and bacteria) and substrate used (NH_4^+ and NO_3^-); each of the four groups included 9–22 experiments conducted with different strains at various temperatures; hence, the full temperature dependencies of k and K_s were investigated as a function of λ , $\Delta H^\ddagger$, and $\Delta S^\ddagger$ using Eqs. 16a and 16b, which were fitted with a

nonlinear fitting algorithm to experimental values. Estimated λ , $\Delta H^\ddagger$, and $\Delta S^\ddagger$ values (Table I) show that regression through experimental points was variably good among the four groups and that, overall, Eqs. 16a and 16b could capture k and K_s dependence on temperature qualitatively well (see curves in Fig. 4). Note that λ and $\Delta H^\ddagger$ were both negative, whereas $\Delta S^\ddagger$ was positive in all four groups of experiments. This pattern will be investigated in greater detail in the next section.

Table I. Thermodynamic Parameters (Columns 4–6) Determined for Kinetic Parameters in Table II Using Eqs. 16a and 16b

1	2	3	4	5	6
Organism	S	T (°C)	λ	$\Delta H^{\ddagger}$ (KJ/mol)	$\Delta S^{\ddagger}$ (J/K mol)
Algae	NH_4^+	3.0–25.0	–2.218	–8.50	99.29
Algae	NO_3^-	1.0–25.0	–2.608	–9.77	75.32
Bacteria	NH_4^+	4.0–45.0	–2.251	–5.44	106.26
Bacteria	NO_3^-	4.0–45.0	–2.622	–4.53	91.21

Near-Activation Complexation and Differential Activation

Estimated λ , $\Delta H^\ddagger$, and $\Delta S^\ddagger$ values were used to calculate $K^\ddagger$ and $\Delta G^\ddagger$ relative to near-activation complexation, and $\lambda \Delta G^\ddagger$ for differential activation using temperatures T between 0 and 50°C in Eqs. 16a and 16b.

The first result of application of this approach is that $K^\ddagger$ was found to be always greater than 1; that is, $\Delta G^\ddagger < 0$ in all data sets implied that the equilibrium between reactants and complex was always toward $C \approx C^\ddagger$ (Figs. 5a and 5b), a result that was anticipated in the conceptual Figs. 3d–3f. The second result is that λ was always negative, meaning that the differential Gibbs free

energy of activation $\lambda\Delta G^\ddagger$ was positive. The apparent energy pathway depicted in Fig. 3d can be conceptually used to interpret the thermodynamics of these reactions, where $C \equiv C^\ddagger$ is to be a superactivated complex to release P and E . The pattern observed in $K^\ddagger$ and $\lambda\Delta G^\ddagger$ was persistent over all reactions and microbial populations, suggesting that there was no actual thermokinetic separation between NH_4^+ and NO_3^- uptake by different microorganisms. In particular, a superactivated complex was observed in all instances, with $G_\lambda > G_i(S + E)$.

Figure 5

[Open in figure viewer](#)[PowerPoint](#)

Thermodynamic parameters as a function of the temperature T : (a) near-activation equilibrium constant $K^\ddagger$; (b) Gibbs free energy of near-activation complexation $\Delta G^\ddagger$; and (c) differential Gibbs free energy of activation $\lambda\Delta G^\ddagger$. Thermodynamic parameters are relative to NH_4^+ and NO_3^- uptake by the four microbial groups of Table 5.

Temperature showed various effects on these thermodynamic parameters. $K^\ddagger$ was generally decreasing over T ranging between 0 and 50°C in all samples. Excluding psychrophobic and thermophobic metabolic slowdown of mesophilic microorganisms at low and high temperatures, these results suggest that NH_4^+ and NO_3^- uptake implied that there was a strong natural tendency for the enzyme to bind to the substrate, but also suggest that this tendency was reduced with increasing temperatures possibly due to higher vibrational energy reducing its binding effectiveness (especially evident with uptake of NH_4^+). This trend was detected in the corresponding Gibbs free energy $\Delta G^\ddagger$, which decreased by about 5 KJ/mol in all cases (Fig. 5b). The apparent energy barrier given by the product $\lambda\Delta G^\ddagger$ rose nearly linearly above $G_i(S + E)$ (cfr. Fig. 3d) as the temperature increased (Fig. 5d). On the one hand, this rise in $\lambda\Delta G^\ddagger$ made the reactants less likely to reach the activation point from $G_i(S + E)$ but, on the other side, more reactants were formed as a result of equilibrium complexation moving in favor of $(S + E)$ at higher temperatures. This trade-off overall resulted in a higher frequency for near-activation complexes $(S + E) \rightleftharpoons C \simeq C^\ddagger$ to climb up the energy barrier (see also next section). Whether an increasing temperature overall facilitated the reaction is clearly shown by experimental and

modeled k values increasing with T (Fig. 4a), results which are reflected in the scaling $k \propto T(K^\ddagger)^\lambda$ of Eq. 10.

Reaction Probability

With the values of λ and $\Delta G^\ddagger$ determined above, one can calculate the probability f for the reaction to occur once near-activation complexation is achieved. Application of Eqs. 12a and 12b shows that f was particularly small and between 10^{-22} and 10^{-20} for both NH_4^+ and NO_3^- (Fig. 6). These low values of f are explained by the fact that near-activation complexation was always found to favor the near-equilibrium complex, suggesting that $C \cong C^\ddagger$ was highly likely to be formed and, simultaneously, to release back the reactants as prescribed by $f = 1 - f \approx 1$ of Eqs. 12a and 12b.

Figure 6

[Open in figure viewer](#) [PowerPoint](#)

Probability f that an activated complex release the product P and E during NH_4^+ and NO_3^- uptake as a function of the temperature.

Overall, an average trend was observed for f to increase with temperature, a result that aligns with the increasing reaction rate with increasing temperature already shown in Fig. 4.

DISCUSSION

Introduction of near-activation and differential activation Gibbs free energy $\Delta G^{0,\ddagger}$ and $\lambda\Delta G^{0,\ddagger}$ in the transition-state theory has allowed us to represent the energy requirement for an enzymatic kinetic reaction to release reaction products. A number of achievements have been reached, including: temperature and mutual dependence of reaction rate constant and Michaelis–Menten constant, the identification of sub- and superactivated complexes, and the representation of mass flux partitioning at the activation point. Other aspects have been highlighted as a consequence and are discussed below.

While $\Delta G^{0,\ddagger}$ represents the energy barrier that $C^\ddagger$ has to pass to release the reaction products in the transition-state theory, it represents only the energy associated with near-activation complexation in this approach, which is a state of near-equilibrium between inert reactants and activation complex. The actual energy barrier is described by the product $\lambda\Delta G^{0,\ddagger}$ between the differential activation energy factor λ and $\Delta G^{0,\ddagger}$. This implies that the apparent energy line followed by a reacting complex along the reaction coordinate reaches $\lambda\Delta G^{0,\ddagger}$, as depicted by the blue line in Fig. 1c. The concept of energy barrier is not removed in this approach, but its definition is enriched by accounting for near-activation complexation energy and for differential activation energy required by $C \cong C^\ddagger$ to escape equilibrium $(S + E) \rightleftharpoons C \cong C^\ddagger$ toward the reaction direction. Evidence that an energy barrier $\lambda\Delta G^{0,\ddagger} > 0$ is formed during enzymatic reactions was found in all 57 data sets tested here for NH_4^+ and NO_3^- uptake.

An interesting finding was the negative value of $\Delta G^{0,\ddagger}$ in all tested experiments. Values $\Delta G^{0,\ddagger} < 0$ mean that equilibrium is toward the complex (i.e., $K^\ddagger > 1$) and may be explained by a highly specialized capability of enzymes to bind to and react with specific substrates. This result may also be explained by the initial substrate concentration being greater than K_s in all experiments with the exception of data sets 38–41 of NO_3^- uptake by microalgae (Table II). Other effects of complementarity caused by additional binding groups as defined in 16 were not taken into account in our analyses but could not be excluded a priori. Additionally, an equilibrium favoring the complex could imply $[C^\ddagger] > [S][E]$, meaning that the condition $[C^\ddagger] \approx 0$ for quasi-steady-state assumed in the Michaelis–Menten kinetics may not be satisfied if the concentrations of S and E are high; a high $C^\ddagger$ concentration could also imply that reactants are immobilized therein, and $C^\ddagger$ should be measurable during the course of a reaction if free and stable enough. It is difficult to anticipate if these conditions are met in reactions mediated by enzymes that occur either intra- or extramembrane. Earlier work in 20, 21 show that some intracellular switches can concurrently act to maintain a concentration of unmodified and modified protein by two converter enzymes, an equilibrium reflected in the coupled protein–enzyme complexes. We do not currently have an hypothesis or explanation of whether $C^\ddagger$ could actually accumulate for a

certain period during a reaction but, to the best of our knowledge, complexes with a measurable concentration have not been reported in the literature. In relation to tests carried out here, we note that the product $[S][E]$ is expected to be small given that $[E]$ may be orders of magnitude smaller than $[S]$, hence $[E]$ is most likely the limiting compound for the formation of $C \cong C^\ddagger$. Therefore, depending on $K^\ddagger$, it is possible that even if $C \cong C^\ddagger$ is formed and remains stable before releasing the reaction product, its concentration may be too small to be detected with current techniques.

Table II. Kinetic Parameters (Columns 4 and 5) Determined in [8](#) for Various Microalgae and Bacteria (A and B, Respectively, Column 6)

1	2	3	4	5	6	7
Set	S	T (°C)	k (1/s)	K_s	Organism	Strain
1	NH_4^+	5	3.30×10^{-2}	4.50×10^{-8}	A	<i>Chaetoceros</i> sp.
2	NH_4^+	3	2.90×10^{-2}	7.56×10^{-8}	A	<i>Chaetoceros</i> sp.
3	NH_4^+	22	2.84×10^{-2}	2.97×10^{-7}	A	<i>Chaetoceros curvisetum</i>
4	NH_4^+	17	2.52×10^{-2}	2.52×10^{-7}	A	<i>Chaetoceros curvisetum</i>
5	NH_4^+	12	1.90×10^{-2}	9.54×10^{-8}	A	<i>Chaetoceros curvisetum</i>
6	NH_4^+	25	6.80×10^{-2}	4.50×10^{-7}	A	<i>Dunaliella tertiolecta</i>
7	NH_4^+	20	8.70×10^{-2}	5.40×10^{-7}	A	<i>Dunaliella tertiolecta</i>

1	2	3	4	5	6	7
Set	S	T (°C)	k (1/s)	K _s	Organism	Strain
8	NH ₄ ⁺	15	5.90 × 10 ⁻²	3.24 × 10 ⁻⁷	A	<i>Dunaliella tertiolecta</i>
9	NH ₄ ⁺	10	5.80 × 10 ⁻²	5.40 × 10 ⁻⁷	A	<i>Dunaliella tertiolecta</i>
10	NH ₄ ⁺	15	6.14 × 10 ⁻²	4.03 × 10 ⁻⁷	B	<i>Vibrio logei</i>
11	NH ₄ ⁺	10	5.30 × 10 ⁻²	3.74 × 10 ⁻⁷	B	<i>Vibrio logei</i>
12	NH ₄ ⁺	8	4.80 × 10 ⁻²	2.72 × 10 ⁻⁷	B	<i>Vibrio logei</i>
13	NH ₄ ⁺	6	4.90 × 10 ⁻²	1.33 × 10 ⁻⁷	B	<i>Vibrio logei</i>
14	NH ₄ ⁺	4	3.91 × 10 ⁻²	2.41 × 10 ⁻⁷	B	<i>Vibrio logei</i>
15	NH ₄ ⁺	15	7.40 × 10 ⁻²	9.00 × 10 ⁻⁸	B	<i>Hydrogenophaga pseudoflava</i>
16	NH ₄ ⁺	10	6.00 × 10 ⁻²	1.89 × 10 ⁻⁷	B	<i>Hydrogenophaga pseudoflava</i>
17	NH ₄ ⁺	5	6.00 × 10 ⁻²	6.66 × 10 ⁻⁸	B	<i>Hydrogenophaga pseudoflava</i>

1	2	3	4	5	6	7
Set	S	T (°C)	k (1/s)	K_s	Organism	Strain
18	NH_4^+	15	1.20×10^{-1}	2.38×10^{-7}	B	<i>Brevibac. sp.</i>
19	NH_4^+	10	8.00×10^{-2}	2.79×10^{-7}	B	<i>Brevibac. sp.</i>
20	NH_4^+	5	5.50×10^{-2}	2.77×10^{-7}	B	<i>Brevibac. sp.</i>
21	NH_4^+	20	1.28×10^{-1}	2.97×10^{-7}	B	<i>Klebsiella oxytoca</i>
22	NH_4^+	15	1.05×10^{-1}	2.45×10^{-7}	B	<i>Klebsiella oxytoca</i>
23	NH_4^+	10	9.20×10^{-2}	2.30×10^{-7}	B	<i>Klebsiella oxytoca</i>
24	NH_4^+	35	1.72×10^{-1}	1.34×10^{-6}	B	<i>E. coli</i>
25	NH_4^+	25	6.40×10^{-2}	2.84×10^{-7}	B	<i>E. coli</i>
26	NH_4^+	15	3.70×10^{-2}	1.55×10^{-7}	B	<i>E. coli</i>
27	NH_4^+	45	1.44×10^{-1}	6.91×10^{-7}	B	<i>B. stearothermophilus</i>

1	2	3	4	5	6	7
Set	S	T (°C)	k (1/s)	K_s	Organism	Strain
28	NH_4^+	40	9.60×10^{-2}	5.53×10^{-7}	B	<i>B. stearothermophilus</i>
29	NH_4^+	35	7.80×10^{-2}	2.03×10^{-7}	B	<i>B. stearothermophilus</i>
30	NH_4^+	30	6.70×10^{-2}	3.06×10^{-7}	B	<i>B. stearothermophilus</i>
31	NH_4^+	25	5.80×10^{-2}	2.09×10^{-7}	B	<i>B. stearothermophilus</i>
32	NO_3^-	5	3.30×10^{-2}	1.55×10^{-7}	A	<i>Chaetoceros</i> sp.
33	NO_3^-	3	2.90×10^{-2}	5.08×10^{-7}	A	<i>Chaetoceros</i> sp.
34	NO_3^-	1	2.20×10^{-2}	2.91×10^{-7}	A	<i>Chaetoceros</i> sp.
35	NO_3^-	22	2.80×10^{-2}	1.05×10^{-7}	A	<i>Chaetoceros curvisetum</i>
36	NO_3^-	17	2.70×10^{-2}	2.05×10^{-7}	A	<i>Chaetoceros curvisetum</i>
37	NO_3^-	12	2.00×10^{-2}	3.53×10^{-7}	A	<i>Chaetoceros curvisetum</i>
38	NO_3^-	25	7.80×10^{-2}	1.18×10^{-6}	A	<i>Dunaliella tertiolecta</i>

1	2	3	4	5	6	7
Set	S	T (°C)	k (1/s)	K _s	Organism	Strain
39	NO ₃ ⁻	20	1.00 × 10 ⁻¹	3.93 × 10 ⁻⁶	A	<i>Dunaliella tertiolecta</i>
40	NO ₃ ⁻	15	9.50 × 10 ⁻²	4.77 × 10 ⁻⁶	A	<i>Dunaliella tertiolecta</i>
41	NO ₃ ⁻	10	4.50 × 10 ⁻²	4.40 × 10 ⁻⁶	A	<i>Dunaliella tertiolecta</i>
42	NO ₃ ⁻	15	6.77 × 10 ⁻²	4.28 × 10 ⁻⁷	B	<i>Vibrio logei</i>
43	NO ₃ ⁻	10	5.30 × 10 ⁻²	1.02 × 10 ⁻⁶	B	<i>Vibrio logei</i>
44	NO ₃ ⁻	8	3.75 × 10 ⁻²	5.70 × 10 ⁻⁷	B	<i>Vibrio logei</i>
45	NO ₃ ⁻	6	3.73 × 10 ⁻²	7.32 × 10 ⁻⁷	B	<i>Vibrio logei</i>
46	NO ₃ ⁻	4	3.81 × 10 ⁻²	9.36 × 10 ⁻⁷	B	<i>Vibrio logei</i>
47	NO ₃ ⁻	20	1.43 × 10 ⁻¹	1.07 × 10 ⁻⁶	B	<i>Klebsiella oxytoca</i>
48	NO ₃ ⁻	15	1.16 × 10 ⁻¹	1.69 × 10 ⁻⁶	B	<i>Klebsiella oxytoca</i>

1	2	3	4	5	6	7
Set	S	T (°C)	k (1/s)	K_s	Organism	Strain
49	NO_3^-	10	8.70×10^{-2}	2.27×10^{-6}	B	<i>Klebsiella oxytoca</i>
50	NO_3^-	35	2.12×10^{-1}	7.35×10^{-6}	B	<i>E. coli</i>
51	NO_3^-	25	1.50×10^{-1}	5.46×10^{-6}	B	<i>E. coli</i>
52	NO_3^-	20	1.70×10^{-1}	7.59×10^{-6}	B	<i>E. coli</i>
53	NO_3^-	15	8.00×10^{-2}	5.56×10^{-6}	B	<i>E. coli</i>
54	NO_3^-	45	1.44×10^{-1}	2.38×10^{-6}	B	<i>B. stearothermophilus</i>
55	NO_3^-	40	9.80×10^{-2}	1.65×10^{-6}	B	<i>B. stearothermophilus</i>
56	NO_3^-	35	7.10×10^{-2}	1.54×10^{-6}	B	<i>B. stearothermophilus</i>
57	NO_3^-	30	5.80×10^{-2}	1.85×10^{-6}	B	<i>B. stearothermophilus</i>

In addition to the above, $\Delta G^{0\ddagger} < 0$ may be associated with a low-energy intermediate equilibrium complex [16-18](#), [22](#), a concept which is consolidated in the binding of enzyme and reactants before reaching activation. However, we underline that a key difference proposed in this work is that the near-activation complex and the activated complex are conceptualized as having a small (perhaps infinitesimal) distance δ in the reaction coordinate (see the “near-equilibrium complexation” in [Fig. 2](#)) and were treated in this framework as they were one entity (hence the writing $C \cong C^\ddagger$).

The transition-state-theory does not say much about the Michaelis–Menten constant. However, if the quasi-steady-state assumption is used to assert that the activated complex is in equilibrium with reactants while it releases products, one can note that [Eqs. 2](#) and [4](#) have a similar structure. In fact, if $K^\ddagger$ could be written as $K^\ddagger = k^+/(k^- + k)$ when reaction products are released, then the Michaelis–Menten constant could be expressed as $K_s = 1/K^\ddagger$. If Eyring's formulation of the reaction rate constant k is maintained in its original formulation, then the two equations governing the reaction parameters could be written as $k = K_b T/h \cdot K^\ddagger$ and $K_s = 1/K^\ddagger$, so that only one parameter instead of two would describe the reaction kinetics in full. Note that this case corresponds exactly to the case of [Eqs. 16a](#) and [16b](#) for $\Delta G^\ddagger$ with $\lambda = 1$, and with $k^- = k$ as for the original derivation of the transition state by Eyring reported in [6](#). We have not expanded on this special case of $K^\ddagger = k^+/(k^- + k)$ as this aspect may require further conceptualization and testing.

As an additional note, the approach proposed here applies to the quasi-steady-state approximation of the Michaelis–Menten kinetics; the total quasi-steady-state more recently proposed in [23](#) conveniently approximates the kinetics over a wider range of kinetic parameters and initial conditions [24](#). The working hypotheses proposed here may therefore be tested on the total quasi-steady-state approximation framework for further validation.

A particular attention has to be paid to the transmission coefficient in the absolute reaction rate theory: Eyring introduced the transmission coefficient τ ranging between 0 and 1 to account for the fraction of $C^\ddagger$ that turns back toward the reactants after passing over $\Delta G^\ddagger$, so that the mass flux toward the products is actually expressed by $\tau K_b T/h \cdot [C^\ddagger]$. Note that $\tau = 1$ does not mean that all reactants reaching activation state will release the products, but that all activated complexes that have passed the activation state will release the products. The literature is quite loose in this respect, with the majority assuming that $\tau = 1$ is an acceptable value [11](#), [25](#) suggested that $\tau = 1$ can be used in most reactions, whereas $\tau \ll 1$ may represent the transmission coefficient in adiabatic, unimolecular reactions [26](#). In the approach presented here, we have considered $\tau = 1$, so that all activated complexes that have passed the energy barrier

$\lambda\Delta G^{0\ddagger}$ will release the products, and does not have to be confused with the mass flux fraction f of complexes that will return to reactants from the energy level $\Delta G^{0\ddagger}$.

While the approach presented here is derived for enzyme-mediated reactions, it is possible to infer that nonenzymatic reactions may also show a similar thermodynamic characteristic in that concerns with the differential energy of activation as long as an activated complex has to be formed before reaction products are released. Rather, it is more complicated to relate in a direct or indirect way how other processes that interfere with a reaction affect the differential energy of activation. It is presumed that competition by enzymes, competitive inhibition for substrate consumption, and noncompetitive reaction inhibition in a multisubstrate multienzyme system [27](#) may affect to some extent equilibrium between reactants and near-activation complex, and the differential energy of activation by raising the apparent energy barrier $\lambda\Delta G^{0\ddagger}$. It has become evident in [28](#) that energy conservation at cellular level may have an expression in terms of Gibbs free energy and that a reduction in energy difference between that available in the environment and that required by cells may affect the reaction rate substantially. This aspect may be investigated to a better extent in relation to the thermal limits of enzyme actions and may be linked to an interpretation based on the differential Gibbs free energy factor λ introduced in this work.

CONCLUSIONS

The concepts of near-activation and differential activation (Gibbs free) energy were developed in their theoretical components and applied to a comprehensive set of values of reaction rate and Michaelis–Menten constants of NH_4^+ and NO_3^- uptake by various microbial populations. The key element of the approach is the introduction of a thermodynamic parameter, the differential activation energy factor λ , that allows a representation of the energy requirement for an enzymatic kinetic reaction to release the reaction products. Introduction of λ in the transition-state theory, and application of the framework showed that a near-equilibrium complexation always favored the formation of a complex, and that a differential activation energy barrier formed at the activation point, which decreased the likelihood for the near-equilibrium complex to release the reaction products. This finding corresponded to a negative Gibbs free energy of near-activation complexation $\Delta G^{0\ddagger}$ and in a negative differential activation energy factor λ , that is, a positive differential activation energy barrier $\lambda\Delta G^{0\ddagger}$ had to be crossed to release the products. An explicit thermodynamic expression was found to mutually link the reaction rate constant k and the Michaelis–Menten constant K_s with the temperature, and showed that K_s depends on temperature in a similar way as k , but also showed that an Arrhenius-like

equation can only approximate it. In virtue of this, Michaelis–Menten enzymatic kinetic equations can be solved either in the (k, K_s) or in the $(\lambda, K^\ddagger)$ spaces unambiguously, or by estimating λ , $\Delta H^\ddagger$, $\Delta S^\ddagger$ from experiments at two different temperatures, and calculating k and K_s as proposed in this work.

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Appendix A

- *Note that [2](#) did not propose any hypotheses on the way reactants form an activated complex; this hypothesis was developed a decade later in [3](#).
- †As a reflection posterior to those works, we highlight that isotopic methods are essential tools to give a rich insight on chemical kinetics.