A Much better replacement of the Michaelis-Menten equation and its application

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Abstract

Michaelis-Menten equation is a basic equation of enzyme kinetics and gives an acceptable approximation of real chemical reaction processes. Analyzing the derivation of this equation yields the fact that its good performance of approximating real reaction processes is due to Michaelis-Menten curve \cite{15}. This curve is derived from Quasi-Steady-State Assumption(QSSA), which has been proved always true and called Quasi-Steady-State Law by Banghe Li et al \cite{19}.

Here, we found a quartic equation $A(S, E) = 0$ \cite{22}, which gives more accurate approximation of the reaction process in two aspects: during the quasi-steady state of a reaction, Michaelis-Menten curve approximates the reaction well, while our quartic equation $A(S, E) = 0$ gives better approximation; near the end of the reaction, our equation approaches the end of the reaction with a tangent line same to that of the reaction, while Michaelis-Menten curve does not. In addition, our quartic equation $A(S, E) = 0$ differs to Michaelis-Menten curve less than the order of $1/S^3$ as $S$ approaches $+\infty$.

By considering the above merits of $A(S, E) = 0$, we suggest it as a replacement of Michaelis-Menten curve. Intuitively, this new equation is more complex and harder to understand. But, just because its complexity, it provides more information about the rate constants than Michaelis-Menten curve does.

Finally, we get a better replacement of the Michaelis-Menten equation by combing $A(S, E) = 0$ and the equation $dP/dt = k_2 C(t)$.

\textit{keywords} : rate constants of enzyme kinetics; quasi-steady-state assumption; quasi-steady-state law.

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1 Introduction

Enzymes are biological catalysts in almost all life processes. Enzyme kinetics as an important branch of enzymology studies the rate of reaction and the change of rate under different conditions. It is essential to describe the reaction mechanism[1].

In 1902, Adrian Brown studied the rate of hydrolysis of sucrose by yeast enzyme $\beta$-fructofuranosidase, which was considered as the first case study of enzyme kinetics[2]. Victor Henri proposed two reaction mechanisms which contains only one substrate and one product forming a substrate-enzyme complex[3, 4]. One of them became the basic model of enzyme kinetics:

$$E + S \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} C \overset{k_2}{\rightarrow} P + E,$$

where $E$, $S$, $C$, $P$ represent enzyme, substrate, enzyme-substrate complex and product, respectively. And $k_1$, $k_{-1}$, $k_2$ represent the rate constants of corresponding reaction steps.

Since Briggs and Haldane proposed the quasi-steady-state-assumption (QSSA) in 1925[5], this simplest model has been thoroughly studied under QSSA[1, 6, 7]. By QSSA, Briggs and Haldane obtained the classic Michaelis-Menten equation:

$$v_0 = \frac{V_{\text{max}}S_0}{K_M + S_0},$$

where $v_0$ is the initial velocity of the reaction, $K_M$ is the Michaelis constant defined as $K_M = (k_{-1} + k_2)/k_1$ and $V_{\text{max}}$ is the so-called maximal velocity in many literatures, which is actually the supremum of the velocity but is never reached. Michaelis-Menten equation soon became the basic equation of enzyme kinetics[1]. All the experimental results so far show that Michaelis-Menten equation provides a good description of enzyme kinetics processes for large ensemble of
enzyme molecules when the concentration of substrate exceeds that of enzyme greatly. At the single-molecule level, the enzyme molecule moves according to thermal fluctuation and reacts stochastically with substrate molecules\[8, 9\]. By statistical analysis of the stochastic behaves, Michaelis-Menten equation also holds\[10, 11\].

After Briggs and Haldane’s work, Lineweaver and Burk\[12\] found that the reciprocal form of Michaelis-Menten equation gave a linear relation between $1/v_0$ and $1/S_0$, i.e.

$$\frac{1}{v_0} = \left(\frac{K_M}{V_{max}}\right)\frac{1}{S_0} + \frac{1}{V_{max}}. \quad (2)$$

This linear relation can be used to estimate the kinetics parameters with least square method. Although this estimation sometimes may lead to relative poor accuracy\[13, 14, 15\], many textbooks recognized its value on simplicity and visualization\[1, 16, 17\]. Michaelis-Menten equation do waste too much information on progress curve. In fact, Michaelis-Menten equation is derived from the quadratic equation $dE/dt = 0$ which can describe the whole process of the chemical reaction except the initial transient period provided $S_0 \gg E_0$\[18\].

The validity of the Michaelis-Menten equation is strongly dependent on the validity of QSSA. Many biologists tested QSSA through biological experiments or computational experiments. But no one can confirm its validity during the next 80 years until recently Banghe Li et al. gave the rigorous description of this assumption and proved it mathematically\[19\]. Thus, from now on, this assumption is called the Quasi-Steady-State Law(QSSL). Moreover, this assures the validity of the Michaelis-Menten equation. This may be the first application of qualitative theory of dynamical systems into this basic enzyme kinetics model.

To quote the QSSL, we first introduce the basic model of enzyme kinetics. The enzyme kinetics is a branch of chemical kinetics\[1\]. Thus, according
to the law of mass action the time evolution of concentrations of reactants is determined by the following differential equations:

\[
\begin{align*}
\frac{dS}{dt} &= -k_1 S(t)E(t) + k_{-1}C(t) \\
\frac{dE}{dt} &= -k_1 S(t)E(t) + (k_{-1} + k_2)C(t) \\
\frac{dC}{dt} &= k_1 S(t)E(t) - (k_{-1} + k_2)C(t) \\
\frac{dP}{dt} &= k_2 C(t)
\end{align*}
\]

with the initial condition

\[(S(0), E(0), C(0), P(0)) = (S_0, E_0, 0, 0).\]

where \(E(t)\), \(S(t)\), \(C(t)\) and \(P(t)\) denote the concentrations of enzyme, substrate, enzyme-substrate complex and product at time \(t\) during the process, respectively. Under the two conservation laws

\[
\begin{align*}
E(t) + C(t) &= E_0 \\
S(t) + C(t) + P(t) &= S_0,
\end{align*}
\]

these differential equations are equivalent to system of differential equations consisted of \((S(t), E(t))\), \((S(t), P(t))\) or \((P(t), E(t))\), i. e.

\[
\begin{align*}
\frac{dS}{dt} &= -k_1 S(t)E(t) + k_{-1}(E_0 - E(t)) \\
\frac{dE}{dt} &= -k_1 S(t)E(t) + (k_{-1} + k_2)(E_0 - E(t))
\end{align*}
\]

\[
\begin{align*}
\frac{dS}{dt} &= -k_1 (S(t) + P(t) + E_0 - S_0)S + k_{-1}(S_0 - S(t) - P(t)) \\
\frac{dP}{dt} &= k_2(S_0 - S(t) - P(t))
\end{align*}
\]
or

\[
\begin{align*}
\frac{dP}{dt}(t) &= k_2(E_0 - E(t)) \\
\frac{dE}{dt}(t) &= -k_1 E(t)(S_0 - P(t) - E_0 + E(t)) + (k_{-1} + k_2)(E_0 - E(t))
\end{align*}
\]  

(12)

Equation (11) is often used to analyze the basic model, but the other two forms are in fact equivalent to it, and sometimes are more convenient. These systems are nonlinear, and cannot be integrated explicitly. However, they can be further simplified with the QSSL.\cite{19}

**Quasi-Steady-State Law 1**: Given any small positive number \(\varepsilon > 0\), there is a proper positive number \(U\) such that \(C(t)\) will go upwards from 0 at \(t = 0\) to \(E_0 - \varepsilon\) in a period less than \(\varepsilon\), then it will stay in the interval between \(E_0\) and \(E_0 - \varepsilon\) until \(S(t)/S_0 < \varepsilon\), if \(S_0 > U\).

**Quasi-Steady-State Law 2**: Given any small positive number \(\varepsilon > 0\), there is a proper positive number \(U\) such that \(|\frac{dC}{dt}(t)|\) will be less than \(\varepsilon\) after a fast initial period less than \(\varepsilon\) and keep this state until \(S(t)/S_0 < \varepsilon\), if \(S_0 > U\).

Michaelis-Menten equation is derived from the quadratic equation \(\frac{dE}{dt} = 0\), which is assured to be an acceptable approximate solution of the process after the initial transient period until \(S\) is nearly exhausted provided \(S_0 \gg E_0\) by QSSLs. This article provides another equation which approximates the whole process of the chemical reaction better than \(\frac{dE}{dt} = 0\) does. This replacement is first introduced in our former paper \cite{21}. In \cite{21}, we provided an improved method to measure all rate constants in the simplest enzyme kinetics model using this replacement with the aid of Michaelis-Menten equation. This method improved the approach in \cite{22} greatly. Here, we do deep analysis of this equation and found that all the three rate constants in the simplest enzyme kinetics model can be measured without Michaelis-Menten equation. The results are better than those gotten from using the Michaelis-Menten equation only, which shows
that this equation can replace the Michaelis-Menten equation.

The mathematical background can be found in many fundamental books on mathematical biology [23, 24] or ordinary differential equations [25, 26].

This article is organized as follows. Section 2 introduces the deviations of Michaelis-Menten curve and Michaelis-Menten equation which is not novel and can be read in many commentaries [27]. Section 3 gives our corresponding replacements of the curve and equation, and the merits for the replacements are given in section 4. Section 5 gives an application of the replacement of the Michaelis-Menten curve, and the conclusion comes in Section 6. Some subtle mathematics are left in Appendix.

# 2 Michaelis-Menten curve versus Michaelis-Menten equation

## 2.1 Derivation of Michaelis-Menten curve

Let \( t_1 \) be the time when the reaction attains its steady-state. According to QSSL2, after the initial transient, that is \( t > t_1 \), the reaction come to the steady-state:

\[
\frac{dC}{dt}(t) \approx 0,
\]

which is equivalent to

\[
\frac{dE}{dt}(t) \approx 0.
\]

Therefore, during the quasi-steady state of a reaction, the relationship about the concentrations \( S(t) \) and \( E(t) \) can be approximated by the following equation

\[
0 = -k_1 S(t)E(t) + (k_{-1} + k_2)(E_0 - E(t)),
\]
which yields

\[ E_0 - E(t) = E_0 S(t)/(S(t) + K_M). \]  \hspace{1cm} (16)

We name the curve of enzyme and substrate determined by the equation (15) or (16) as Michaelis-Menten curve.

### 2.2 Derivation of Michaelis-Menten equation

According to equation (6) and the Michaelis-Menten curve (16), we have

\[ \frac{dP}{dt}(t) = k_2 E_0 S(t)/(K_M + S(t)), \]  \hspace{1cm} (17)

or equivalently

\[ v(t) = k_2 E_0 S(t)/(K_M + S(t)). \]  \hspace{1cm} (18)

Let \( v_0 \) denote the initial velocity of the reaction, which is indeed the velocity when the reaction attains its steady-state, i.e. \( dP/dt(t_1) \). Equation (17) becomes

\[ v_0 = V_{\text{max}} S(t_1)/(K_M + S(t_1)), \]  \hspace{1cm} (19)

where \( V_{\text{max}} = k_2 E_0 \). It may be assumed that

\[ S(t) \approx S_0, \]  \hspace{1cm} (20)

when \( 0 < t \leq t_1 \) \[20\] \[28\] (A rigorous proof is given in Appendix). Therefore, the Michaelis-Menten equation is obtained

\[ v_0 = V_{\text{max}} S_0/(K_M + S_0). \]  \hspace{1cm} (21)
Notice that, if $v_0$ is considered as a function of $S_0$, $v_0$ is increasing and

$$\lim_{S_0 \to +\infty} v_0 = V_{\text{max}}.$$ 

This is why biologists define $k_2E_0$ as $V_{\text{max}}$. They consider it as the maximal initial velocity. However, as we have shown, it can not be attained.

2.3 The determinant of Michaelis-Menten curve

By distinguishing Michaelis-Menten curve from Michaelis-Menten equation, we see clearly that the good performance of Michaelis-Menten equation approximating the real reactions is due to Michaelis-Menten curve.

Hence, if we find another curve which is a better approximation, then we can improve the classical Michaelis-Menten equation. Fortunately, we find one.

The following section gives our better replacements of Michaelis-Menten curve and Michaelis-Menten equation, respectively.

3 Replacements of Michaelis-Menten curve and Michaelis-Menten equation

For brevity here, we just give the formulas of the replacements of Michaelis-Menten curve and Michaelis-Menten equation, respectively. Their merits and motivations are given later.

3.1 Replacements of Michaelis-Menten curve

The replacement of Michaelis-Menten curve is
\[ E(E_0 - E)[k_1 SE - k_1(E_0 - E)] + SE_0[k_1 SE - (k_{-1} + k_2)(E_0 - E)] = 0. \quad (22) \]

We simply denote the left hand side of the above quartic equation as \( A(S, E) \).

### 3.2 Replacements of Michaelis-Menten equation

Just like equation (16) represents an explicit solution \( E(S) \) of equation (15), there is an explicit solution of equation (22) or \( A(S, E) = 0 \), too. Due to the complexity of the form, we denote \( E(S) = x_2(S) \) here and give its detail in Appendix 7.3.

Hence, we get a replacement for Michaelis-Menten equation.

\[ v_0 = k_2(E_0 - x_2(S_0)), \quad (23) \]

where \( x_2 = -(36abc - 108a^2d - 8b^3 + 12\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a^{1/3}/12a + (3ac - b^2)/(3a(36abc - 108a^2d - 8b^3 + 12\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a^{1/3}) - b/(3a) + (1/2)\sqrt{3}i((36abc - 108a^2d - 8b^3 + 12\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a^{1/3}/6a - 2(3ac - b^2))/(3a(36abc - 108a^2d - 8b^3)\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a^{1/3})), a = -k_1S - k_{-1}, b = (k_1S + 2k_1E_0, c = -k_{-1}E_0^2 + (k_1S + k_{-1} + k_2)E_0S \text{ and } d = -(k_{-1} + k_2)E_0^2S. \]

The detail form of equation (23) is somewhat complicated. However, for the purpose of applications, using the curve (22) instead of equation (23) is sufficient.

The following section will show that curve \( A(S, E) = 0 \) approximates real reactions better than Michaelis-Menten curve does, and hence the replacement of Michaelis-Menten equation is better than Michaelis-Menten equation.
4 Motivation and Derivation of the Replacement

The replacement of Michaelis-Menten curve was given first in [21]. In Section 4 of [21], we have given the motivation and derivation of the equation. For the convenience of the readers, we recap those here. For more information, please read [21].

This paper adopts the same notations. To be precisely, they are listed below again.

The first quadrant of the phase plane $S - E$ is divided into five regions as

\[
\begin{align*}
L_1 &= \{(S, E) : Q(S, E) = 0, S \geq 0\}, \\
L_2 &= \{(S, E) : P(S, E) = 0, S \geq 0\}, \\
R_1 &= \{(S, E) : E > \hat{E}, (S, \hat{E}) \in L_1\}, \\
R_2 &= \{(S, E) : \hat{E} > E > \hat{E}, (S, \hat{E}) \in L_1, (S, \hat{E}) \in L_2\}, \\
R_3 &= \{(S, E) : E < \hat{E}, (S, \hat{E}) \in L_2\}.
\end{align*}
\]

where

\[
\begin{align*}
P(S, E) &= -k_1SE + k_{-1}(E_0 - E), \quad (24) \\
Q(S, E) &= -k_1SE + (k_{-1} + k_2)(E_0 - E). \quad (25)
\end{align*}
\]

The whole process of the reaction $(S(t), E(t))$ can be drawn on the $S - E$ plane. Since $S(t)$ decreases when $t$ increases, we can consider $E$ to be a function of $S$.

\[
dE/dS = (-k_1SE(S) + (k_{-1} + k_2)(E_0 - E(S)))/(-k_1SE(S) + k_{-1}(E_0 - E(S))) \quad (26)
\]
The solutions with its initial condition on the curve $L_2$ will vertically enter the region $R_2$. Then, the concentration of substrate decreases and that of enzyme increases. In fact, these solutions will stay in $R_2$ forever and finally approaches the singular point. For sufficiently large initial concentration of substrate, these solutions go almost horizontally in $R_2$, but at last they will approach the singular point with a certain slope. Therefore, there is an inflection point on each of these solutions.

We have

$$
\frac{d^2 E}{dS^2} = \frac{k_1 k_2 A(S, E)}{(k_1 SE(S) - k_{-1}(E_0 - E(S)))^3}.
$$

Thus, the collection of inflection points satisfies $d^2 E/dS^2 = 0$, that is $A(S, E) = 0$. As this system satisfies the existence and uniqueness condition of differential systems, any two different solutions will not intersect. Thus, the curve $A(S, E) = 0$ is just beneath the real process on the $S - E$ phase plane.

This is how we find the replacement $A(S, E) = 0$.

5 Reasons for the replacement being much better

We will give reasons that (22) is a much better replacement of Michaelis-Menten curve in this section.

In [21], we have observed that there is a part of $A(S, E) = 0$ lying in the region $R_2$ which approximates the real process well. It is denoted as $L_3$. In fact $L_3$ is the replacement of Michaelis-Menten curve. Next, we will show that $L_3$ is a better approximation of real reaction than $L_1$ which is the Michaelis-Menten curve $Q(S, E) = 0$ [25]. We only need to show that $L_3$ approximates
real reaction better than $L_1$ does.

5.1 Comparison in the major process of a reaction

When the reaction begins, $S$ and $E$ would decrease until they pass through the curve $L_1$. In this period of the reaction, neither Michaelis-Menten equation nor its replacement $L_3$ can approximate the solution well.

Here, the major process of a reaction means that $S$ and $E$ are in the region of $R_2$ excluding the end of the reaction. The following two subsections will show that $A(S, E) = 0$ gives a more approximation of the real reaction processes than $L_1$ (Michaelis-Menten curve) by numerical instances under different conditions.

5.1.1 A case when QSSL condition violates

we choose $k_1 = 1$, $k_2 = 1$, $k_{-1} = 1$, $E_0 = 10$ but $S_0 = 20$. In this example the QSSL can not be used for $S_0$ is not sufficient large compared with $E_0$. So $L_1$ may not be a good approximation of the solution. Before the reaction process approaches the region $R_2$, both approximation of the solution are too bad. However, after the solution enters the region $R_2$, $L_3$ gives a good approximation of the solution but $L_1$ doesn’t, c. f. Fig 2

5.1.2 Cases when QSSL condition holds

We denote $(S^*(t), E^*(t))$ to be the solution with initial condition that $(S^*(0), E^*(0)) = (S_0, E_0)$, $\hat{E}(S^*)$ to be the explicit form of approximate solution $L_1$ and $\hat{E}(S^*)$ to be the explicit form of approximate solution $L_3$. $E^*(t)$ is greater than $\hat{E}(S^*(t))$ for all $t > 0$, which is proved in Appendix 6.1. $\hat{E}(S^*(t))$ is smaller than $E^*(t)$ when $t > \hat{t}$, which is proved by Lemma 3 in [19]. Here, $\hat{t}$ is the time the real process touches the curve $L_3$. That is to say, after the reaction enters the region $R_2$, the real process lies between these two approximations. We choose $k_1 = 1$,
\(k_2 = 1, \ k_{-1} = 1, \ E_0 = 10\) and \(S_0 = 1000\) as the second example. In this example the QSSL can be used, so \(L_1\) is a good approximation of the solution.

During the reaction process, when \(S^*(t) < 989.8\) or \(t > 0.03\), the difference between \(\hat{E}(S^*(t))\) and \(E^*(t)\) is less than the difference between \(\hat{E}(S^*(t))\) and \(E^*(t)\). That is to say \(L_3\) is a better approximation of the solution after the initial transient period, i.e. less than 0.03.

We have also done another 250 numerical experiments. \(k_1, \ k_2\) and \(k_{-1}\) are chosen from \(\{1, \ 3, \ 5, \ 7, \ 9\}\), and \(E_0 = 0.5, \ S_0 = 20\) or 40. In each case, we divide \(S(\hat{t})\) into 7 equal pieces with 6 point, which we denote from small to large as \(S_1, \ \cdots, \ S_6\). We calculate the distances of \(E^*\) and \(\hat{E}\) and the distances of \(E^*\) and \(\tilde{E}\). Table 1 shows the rate of these two numbers at the six points. These show that the curve \(L_3\) approximate the solution better, and the smaller \(S\) is the better \(L_3\) does.

5.2 Comparison near the ends of the reactions

Our new equation \(A(S, E) = 0\), that is \(L_3\), approaches the end of reactions with a tangent line same to that of the reaction processes, while Michaelis-Menten curve does not. The following is the proof.

\(L_3\) can be regarded as a graph of a function taking \(S\) as independent variable and \(E\) as dependent variable. The explicit form is given in Appendix 7.3.

Rewrite \(A(S, E) = 0\) as \((-k_1 S - k_{-1})(E - E_0)^3 + (-2 k_1 SE_0 - k_{-1} E_0)(E - E_0)^2 + (SE_0 k_{-1} - k_1 E_0^2 S + k_2 E_0 S + k_1 S^2 E_0) (E - E_0) + k_1 S^2 E_0^2 = 0\). Divide each side of the equation by \(S^2\) and let \(S \to 0\). Then,

\[
k_{-1} (dE/dS(0))^2 + (k_1 E_0 - (k_2 + k_{-1})) dE/dS(0) - k_1 E_0 = 0. \tag{28}
\]
Solving it, we get

\[
dE/dS(0) = -\left(k_1 E_0 - (k_{-1} + k_2) + \sqrt{(k_1 E_0 + k_{-1} + k_2)^2 - 4k_1 k_2 E_0} \right)/2k_{-1}
\]  

(29)

where the other root is dropped for the slope must be negative. This slope is just the slope of the solution \((S^*(t), E^*(t))\) entering the point \((0, E_0)\). Thus, this part of \(A(S, E) = 0\) give well approximation of \((S^*(t), E^*(t))\) even when \(S(t)\) is very small. So, we confirm this part of \(A(S, E)\) is a better approximation of a real reaction.

### 5.3 Comparison of the behaviors for large \(S\)

In [21], we saw that \(L_3\) almost coincide with \(L_1\) when \(S\) is sufficiently large. In fact, this can be proved. \(A(S, E) = 0\) equals to \(E(E_0 - E)[k_1 SE - k_{-1}(E_0 - E)] + SE_0[k_1 SE - (k_{-1} + k_2)(E_0 - E)]\). Let \((\hat{S}, \hat{E})\) be the point on \(L_1\) and \((\tilde{S}, \tilde{E})\) on \(L_2\). Then, \(A(\hat{S}, \hat{E}) = k_2 \hat{E}(E_0 - \hat{E})^2 > 0\) and \(A(\hat{S}, \tilde{E}) = -k_2 \hat{S}E_0(E_0 - \tilde{E}) < 0\). Thus, there must be one point \(E^*\) between \(\tilde{E}\) and \(\hat{E}\) such that \(A(\hat{S}, E^*) = 0\). This proved that for each \(S > 0\) there is a point of curve \(A(S, E) = 0\) lies in \(R_2\). In fact, there is only one. The proof is given in Appendix 7.3.

Moreover, we can prove that, as \(\hat{S} \to +\infty\), \((E^* - \hat{E})/(E^* - \tilde{E}) \to 0\). Since

\[
0 = E^*(E_0 - E^*)[k_1 \hat{S}E^* - k_{-1}(E_0 - E^*)] + \hat{S}E_0[k_1 \hat{S}E^* - (k_{-1} + k_2)(E_0 - E^*)],
\]

it can be proved that

\[
(k_1 \hat{S}E^* - (k_{-1} + k_2)(E_0 - E^*)/(k_1 \hat{S}E^* - k_{-1}(E_0 - E^*)) = -E^*(E_0 - E^*)/(\hat{S}E_0).
\]

(30)

For

\[
k_1 \hat{S}\tilde{E} - (k_{-1} + k_2)(E_0 - \tilde{E}) = 0,
\]

(31)
\[ k_1 \hat{S} \hat{E} - k_{-1} (E_0 - \hat{E}) = 0 \quad (32) \]

and

\[ k_1 \hat{S} E^* - (k_{-1} + k_2)(E_0 - E^*) - (k_1 \hat{S} \hat{E} - (k_{-1} + k_2)(E_0 - \hat{E})) = (k_1 \hat{S} + k_{-1} + k_2)(E^* - \hat{E}), \]

\[ k_1 \hat{S} E^* - k_{-1} (E_0 - E^*) - (k_1 \hat{S} \hat{E} - k_{-1} (E_0 - \hat{E})) = (k_1 \hat{S} + k_{-1})(E^* - \hat{E}), \]

(30) can be written as

\[ (k_1 \hat{S} + k_{-1} + k_2)(E^* - \hat{E})/(k_1 \hat{S} + k_{-1})(E^* - \hat{E}) = -E^*(E_0 - E^*)/(\hat{S}E_0). \quad (33) \]

Letting \( \hat{S} \to +\infty \) on both side of (33),

\[ (E^* - \hat{E})/(E^* - \hat{E}) \to 0 \]

for \( E^* \to 0 \) when \( \hat{S} \to +\infty \).

Thus, there is a part of \( A(S, E) = 0 \) in region \( R_2 \) asymptotically approaching to \( L_1 \) when \( S \) approaches \( +\infty \).

According to (33),

\[ E^* - \hat{E} = -E^*(E_0 - E^*)(k_1 \hat{S} + k_{-1})(E^* - \hat{E})/(\hat{S}E_0(k_1 \hat{S} + k_{-1} + k_2)). \quad (34) \]

Note that \( E^* \leq \hat{E}, E_0 - E^* \leq E_0 \) and \( E^* - \hat{E} \leq \hat{E} - \hat{E} \). Because of (31),

\[ \lim_{S \to +\infty} \hat{E} \hat{S} = K_M. \]

The difference of (31) and (32) is

\[ k_1 \hat{S}(\hat{E} - \hat{E}) - (k_{-1} + k_2)(E_0 - \hat{E}) + k_{-1}(E_0 - \hat{E}) = 0. \]

\( \hat{S} \to +\infty \) implies \( \hat{S}(\hat{E} - \hat{E}) \to k_2 E_0/k_1 \). These together with (31) yields \( E^* - \hat{E} = O(1/\hat{S}^3) \).
6 Application

The above section has shown that the curve $A(S, E) = 0$ approximates the trajectory $(S(t), E(t))$ of the reaction better than the curve $Q(S, E) = 0$ does. In this section, we will show that $A(S, E) = 0$ not only gives more information about the relationships among the three rate constants but also gives more accurate evaluations of these constants.

6.1 $A(S, E) = 0$ gives more information about the rate constants

For convenience, we repeat $A(S, E)$ and $Q(S, E)$ here again as

\[
A(S, E) = E(E_0 - E)[k_1 SE - k_{-1}(E_0 - E)] + SE_0[k_1 SE - (k_{-1} + k_2)(E_0 - E)],
\]

\[
Q(S, E) = -k_1 SE + (k_{-1} + k_2)(E_0 - E).
\]

Rearranging the items of the right side of $A(S, E)$ yields that

\[
A(S, E) = k_1(-SE^3 + E_0 SE^2 + E_0 S^2 E)
+ k_2(E_0 SE - E_0^2 S)
+ k_{-1}(-E^3 + 2E_0 E^2 + E_0 SE - E_0^2 E - E_0^3 S).
\]

By comparing equations $A(S, E)$ and $Q(S, E)$, we find that given some values of $(S, E)$, we can calculate all the three values of $k_1$, $k_2$ and $k_{-1}$ up to a common multiplier by equation $A(S, E) = 0$, but we only get two values of $k_1$ and $(k_{-1} + k_2)$ up to a common multiplier by equation $Q(S, E) = 0$. In other words, $Q(S, E)$ only contains the information about the Michaelis constant $K_M$ as a whole, but $A(S, E)$ contains the information of $a = k_2/k_1$ and $b = k_{-1}/k_1$, which also yield Michaelis constant by $K_M = a + b$. 

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Moreover, by including an additional equation

\[ \frac{dP}{dt} = k_2(E_0 - E). \]  

This is just (6), from which \( k_2 \) can be measured, \( Q(S, E) = 0 \) only provides the information about \( K_M \) and \( k_2 \), while \( A(S, E) = 0 \) provides that of all the three rate constants \( k_1, k_2 \) and \( k_{-1} \).

Since equations (6) and \( Q(S, E) = 0 \) consist of the origin of Michaelis-Menten equation, the compounding of equations (6) and \( A(S, E) = 0 \) gives more information about the rate constants than Michaelis-Menten equation does.

6.2 \( A(S, E) = 0 \) gives more accurate evaluations of the rate constant

As an example, we design a numerical experiment to show that compared with \( Q(S, E) = 0 \), \( A(S, E) = 0 \) not only gives more information about the relationships among the three rate constants but also gives more accurate evaluations of these constants. In the example, we set the rate constants as \( k_1 = 0.3 \), \( k_2 = 0.2 \) and \( k_{-1} = 0.1 \), and the initial concentrations of enzyme and substrate as \( E_0 = 0.5 \) and \( S_0 = 20 \). Some points of \((S, E)\) are measured on the trajectory of the reaction, and then the results are calculated by \( Q(S, E) = 0 \) and \( A(S, E) = 0 \), respectively. All the results are listed in Table (2).

In table (2), we only list the concentrations of substrate, and do not list the corresponding concentrations of enzyme for brevity. \( a : h : b \) means that the concentrations of substrate are measured from \( S = a \) to \( S = b \) with a step length \( h \). After measured these values of \( S \) and their corresponding values of \( E \), \( K_M \) can be calculated by \( Q(S, E) = 0 \) and \( A(S, E) = 0 \), respectively.

For different sets of points \((S, E)\) as chosen in table (2), \( K_M^Q \) is always closer to its exact value 1 than \( K_M^A \). It is even the case, when there are only two points
in the set, such as \((S = 3, E = 0.1234)\) and \((S = 19, E = 0.0250)\).

Another phenomena observed from this table is that for both equations \(Q(S, E) = 0\) and \(A(S, E) = 0\), \(K_M\) is more accurate when the data set is measured closer to the core region of the steady state of the reaction. Such a phenomena also gives another support that both \(Q(S, E) = 0\) and \(A(S, E) = 0\) approximate the real reaction well at the quasi-steady state, moreover \(A(S, E) = 0\) is better than \(Q(S, E) = 0\).

If \(k_2\) is measured by equation (6), then \(k_1\) and \(k_{-1}\) are all known due to equation \(A(S, E)\). In this example, we assume that \(k_2 = 0.200\), and hence, the estimated values of \(k_1\) and \(k_{-1}\) are listed in the table, too.

Now, we have completely shown that compared with \(Q(S, E) = 0\), \(A(S, E) = 0\) not only gives more information about the relationships among the three rate constants but also gives more accurate evaluations of these constants. Thus, we claim that \(A(S, E) = 0\) is a better replacement of Michaelis-Menten curve, and combined with (6) gives a better replacement of Michaelis-Menten equation.

7 Conclusion

In this article, we propose another curve that can replace the Michaelis-Menten curve and another equation that can replace the Michaelis-Menten equation. We used this new curve to estimate all the rate constants of the basic enzyme kinetics model. Results show that this replacement does very well. The Michaelis-Menten curve only gives information about \(K_M\). The Michaelis-Menten equation, which is derived by combining Michaelis-Menten curve and (6), only gives information about \(K_M\) and \(k_2\). By contrasting to Michaelis-Menten curve, the replacement curve gives more information. And then, the replacement equation gives information about \(k_1\), \(k_{-1}\) and \(k_2\). Numerical experiments show that these replacements not only give more information about the relationships among the
three rate constants but also give more accurate evaluations of these constants. We did not give the mathematical meaning and reasoning that the replacement curve gives better approximate than Michaelis-Menten curve during the major process. Instead, we only give some numerical examples. We hope to do so in future work.

8 Appendix

8.1 $S(t)$ in the initial transient period of a reaction

To obtain the Michaelis-Menten equation, (20) is assumed in former literatures. Here, we prove it under the conditions in QSSLs. That is to say if $S_0$ is much more larger than $E_0$, $S(t)$ is nearly equal to $S_0$ when $0 < t \leq t_1$. To be more precise and rigorous, we state it as a lemma below.

**Lemma:** Given $E_0$ and any small positive number $\varepsilon > 0$, there is a proper positive number $U_1$ such that $|dC/dt(t)|$ will be less than $\varepsilon$ after a fast initial period $t_1$ less than $\varepsilon$, and keep this state until $S(t)/S_0 < \varepsilon$, if $S_0 > U$. Moreover, $|S(t)/S_0| \geq 1 - \varepsilon$, for $0 < t \leq t_1$.

**Proof:** The first part of the theorem is just the QSSL2. According to Lemma 3 in [19], $dS/dt(t) < 0$ for $t > 0$. For equation (3),

$$|dS/dt(t)| = | - k_1 S(t) E(t) + k_{-1} C(t) |$$

$$= k_1 S(t) E(t) - k_{-1} C(t)$$

$$\leq k_1 S(t) E(t)$$

$$\leq k_1 S_0 E_0. \quad (36)$$

Because of QSSL2, we could find $U_1$ and $U_2$ satisfies the following two statements, respectively. Given $E_0$ and any small positive number $\varepsilon/(k_1 E_0) > 0$, there is a proper positive number $U_1$ such that $|dC/dt(t)|$ will be less than
\[ \varepsilon / k_1 E_0 > 0 \] after a fast initial period less than \( \varepsilon / k_1 E_0 > 0 \), and keep this state until \( S(t)/S_0 < \varepsilon / k_1 E_0 > 0 \), if \( S_0 > U_1 \). Given any small positive number \( \varepsilon > 0 \), there is a proper positive number \( U_2 \) such that \( |dC/dt(t)| \) will be less than \( \varepsilon \) after a fast initial period less than \( \varepsilon \) and keep this state until \( S(t)/S_0 < \varepsilon \), if \( S_0 > U_2 \). Choose \( U \) such that \( U > U_1 \) and \( U > U_2 \). Then, if \( S_0 > U \), the first statement of the theorem is proved. Moreover, for (36)

\[ S(t)/S_0 \geq (S_0 - \varepsilon k_1 S_0 E_0/(k_1 E_0))/S_0 = 1 - \varepsilon. \] (37)

when \( 0 < t \leq t_1 \). This completes the proof. □

Now

\[ v_0 = V_{\text{max}} S(t_1)/(K_M + S(t_1)) = V_{\text{max}} S_0/(K_M S_0/(S(t_1) + S_0)) \approx V_{\text{max}} S_0/(K_M + S_0), \]

because \( 1 \leq S_0/S(t_1) \leq 1/(1 - \varepsilon) \), and \( \varepsilon \) can be arbitrarily small.

### 8.2 The convexity of \( (S^*(t), \ E^*(t)) \)

The solution \( (S^*(t), \ E^*(t)) \) do not have any inflection point at all, i.e. \( (S^*(t), \ E^*(t)) \) do not go across \( A(S, \ E) = 0 \) and \( (S^*(t), \ E^*(t)) \) lies above \( L_3 \). Assume \( (S(t), \ E(t)) \) is a solution of system (10), and at time \( t_2 \) it intersects with \( A(S, \ E) = 0 \) at \( (S(t_2), \ E(t_2)) \). Consider \( A(S(t), \ E(t)) = E(t)(E_0 - E(t))[k_1 S(t) E(t) - k_1 (E_0 - E(t))] + S(t) E_0 [k_1 S(t) E(t) - (k_1 + k_2)(E_0 - E(t))]. \)

Differentiate it with respect to \( t \), and note that \( dS/dt = P(S, \ E), \ dE/dt = Q(S, \ E) \):

\[ dA/dt = -Q(E_0 - E)P + EQP - E(E_0 - E)dP/dt - PQ E_0 dQ/dt. \] (38)
For simplicity, we write $A$ as $A(S(t), E(t))$, $P$ as $P(S(t), E(t))$, $Q$ as $Q(S(t), E(t))$, $E$ as $E(t)$ and $S$ as $S(t)$. Simple calculation shows that

$$\frac{dP}{dt} = -k_1PE - k_1SQ - k_{-1}Q$$  \hspace{1cm} (39)$$

and

$$\frac{dQ}{dt} = -k_1PE - k_1SQ - k_{-1}Q - k_2Q.$$  \hspace{1cm} (40)$$

As $A(S(t_2), E(t_2)) = 0$,

$$SQE_0 = -E(E_0 - E)P$$  \hspace{1cm} (41)$$

at point $t = t_2$. By putting (39), (40) and (41) in (38),

$$\frac{dA}{dt}(t_2) = -2P(S(t_2), E(t_2))Q(S(t_2), E(t_2))(E_0 - E(t_2)).$$  \hspace{1cm} (42)$$

For (??), $dA/dt(t_2) > 0$. Therefore, if the solution $(S(t), E(t))$ of (10) has one point $t_0$ in the region $R_2$ satisfying $A(S(t_0), E(t_0)) > 0$, then $A(S(t), E(t)) \geq 0$ for $t > t_0$. We have proved that $A(\hat{S}, \hat{E}) = k_2\hat{E}(E_0 - \hat{E})^2 > 0$, where $(\hat{S}, \hat{E})$ is on $L_1$. Assume at time $t_4$, $(S^*(t), E^*(t))$ reached the curve $L_1$. Thus, $A(S^*(t_4), E^*(t_4)) > 0$. For continuity, there is a $\varepsilon$, such that for any $t_4 + \varepsilon > t_5 > t_4$, $(S(t_5), E(t_5))$ is in the region $R_2$ and $A(S^*(t_5), E^*(t_5)) > 0$. Then, $A(S^*(t), E^*(t)) > 0$ for $t \geq t_4$. Moreover, $A(S^*(t), E^*(t)) > 0$ for $0 < t < t_4$, i.e. $(S(t), E(t)) \in R_1$, can be verified by straight calculation. According to (27), $(S^*(t), E^*(t))$ is convex.

8.3 The explicit form

In this subsection, we talk about the explicit form of the curve $L_3$. $A(S, E) = 0$ is a three degree equation of $E$. Thus, for each $S > 0$, there are at most
three real solutions of \( E \). We have proved that there is at least one solution of \( E \) in the region \( R_2 \) for any \( S > 0 \). As in Section 3.2, we have proved that \( A(\hat{S}, \hat{E}) = k_2\hat{E}(E_0 - \hat{E})^2 > 0 \) and \( A(\hat{S}, \hat{E}) = -k_2SE_0(E_0 - E) < 0 \). Note that,

\[
\lim_{E \to \infty} A(\hat{S}, E)/E^3 = -k_1\hat{S} - k - 1
\]

(43)

for any \( \hat{S} > 0 \). Thus, when \( E \) is positively sufficiently large, \( A(\hat{S}, E) < 0 \). And when \( E \) is negatively sufficiently large, \( A(\hat{S}, E) > 0 \). For the continuity of \( A(S, E) \), there is at least one real solution greater than \( \hat{E} \) and there is at least one real solution less than \( \hat{E} \). We have already found three solutions of \( E \) when \( S > 0 \), so there are exact three solutions of \( E \) when \( S > 0 \). The explicit form of all these three solutions, denoted by \( E = x_1(S) \), \( E = x_2(S) \) and \( E = x_3(S) \), can be given in mathematics.

The three solutions of the equation \( ax^3 + bx^2 + cx + d = 0 \) are

\[
x_1 = (36abc - 108a^2d - 8b^3 + 12\sqrt[3]{4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d^{1/2}a^{1/3}})/6a - 2(3ac - b^2)/(3a(36abc - 108a^2d - 8b^3 + 12\sqrt[3]{4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d^{1/2}a^{1/3}}) - b/(3a),
\]

\[
x_2 = -(36abc - 108a^2d - 8b^3 + 12\sqrt[3]{4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d^{1/2}a^{1/3}})/12a + (3ac - b^2)/(3a(36abc - 108a^2d - 8b^3 + 12\sqrt[3]{4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d^{1/2}a^{1/3}}) - b/(3a) + (1/2)\sqrt[3]{((36abc - 108a^2d - 8b^3 + 12\sqrt[3]{4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d^{1/2}a^{1/3}})/6a - 2(3ac - b^2)/(3a(36abc - 108a^2d - 8b^3 + 12\sqrt[3]{4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d^{1/2}a^{1/3}}))/6a - 2(3ac - b^2)/(3a(36abc - 108a^2d - 8b^3 + 12\sqrt[3]{4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d^{1/2}a^{1/3}})}/6a - 2(3ac - b^2)/(3a(36abc - 108a^2d - 8b^3 + 12\sqrt[3]{4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d^{1/2}a^{1/3}}))}
\]

and

\[
x_3 = -(36abc - 108a^2d - 8b^3 + 12\sqrt[3]{4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d^{1/2}a^{1/3}})/12a + (3ac - b^2)/(3a(36abc - 108a^2d - 8b^3 + 12\sqrt[3]{4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d^{1/2}a^{1/3}}) - b/(3a) - (1/2)\sqrt[3]{((36abc - 108a^2d - 8b^3 + 12\sqrt[3]{4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d^{1/2}a^{1/3}})/6a - 2(3ac - b^2)/(3a(36abc - 108a^2d - 8b^3 + 12\sqrt[3]{4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d^{1/2}a^{1/3}})}/6a - 2(3ac - b^2)/(3a(36abc - 108a^2d - 8b^3 + 12\sqrt[3]{4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d^{1/2}a^{1/3}}))}
\]
In this problem, \( a = -k_1 S - k_{-1}, \) \( b = (k_1 S + 2k_{-1})E_0, \) \( c = -k_{-1} E_0^2 + (k_1 S + k_{-1} + k_2)E_0S \) and \( d = -(k_{-1} + k_2)E_0^2 S. \)

We have proved that all the three solutions are real, so we should decide which one represent the curve \( L_3. \)

We choose \( k_1 = 1, k_2 = 1, k_{-1} = 1, E_0 = 1 \) and \( S = 1. \) Then, \( x_1 \approx -0.8892, \) \( x_2 \approx 0.6446 \) and \( x_3 \approx 1.7446. \) Therefore, \( x_2 \) is the right one in the region \( R_2. \)

Because \( x_1, x_2 \) and \( x_3 \) are continuous functions of \( k_1, k_2, k_{-1}, E_0 \) and \( S, \) and \( x_1, x_2 \) and \( x_3 \) can not coincide for any \( k_1 > 0, k_2 > 0, k_{-1} > 0, E_0 > 0 \) and \( S > 0, \) we can conclude that \( E = x_2(S) \) is the explicit form of \( L_3, \) i. e. the replacement of the Michaelis-Menten equation.

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Figure 1: The $S$-$E$ phase plane.

Figure 2: $L_3$ gives a good approximation of the solution after the solution enters the region $R_2$.

Table 1: 250 numerical experiments.

Table 2: Rate constants estimated by $A(S,E) = 0$ or $Q(S,E) = 0$. The first column indicates the measured concentrations of the substrate during the reaction process, and the corresponding concentrations of enzyme is determined by $S$, so we do not show them explicitly. $a : h : b$ means that the concentrations are measured from $S = a$ to $S = b$ with step length $h$. $K^Q_M$ denotes the Michaelis constant $K_M$ estimated by $Q(S,E) = 0$, $K^A_M$ denotes the $K_M$ estimated by $A(S,E) = 0$, $k^A_1$ and $k^A_{-1}$ denote $k_1$ and $k_{-1}$ estimated by $A(S,E) = 0$, if $k_2$ is provided. Here, we assume that $k_2 = 0.2000$ is exactly estimated by equation (35).
Figure 1: The $S$-$E$ phase plane.
Figure 2: $L_3$ gives a good approximation of the solution after the solution enters the region $R_2$
| $k_1$ | $k_2$ | $k_{-1}$ | $S_0$ | $E_0$ | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | $S_6$ |
|------|------|---------|------|------|------|------|------|------|------|------|
| 1    | 1    | 40      | 0.5  | 1360.849 | 978.494 | 659.542 | 404.013 | 211.969 | 83.724 |
| 1    | 3    | 40      | 0.5  | 1607.115 | 1190.643 | 837.895 | 549.093 | 324.898 | 168.645 |
| 1    | 5    | 40      | 0.5  | 1880.447 | 1430.567 | 1045.033 | 724.584 | 471.446 | 296.762 |
| 1    | 7    | 40      | 0.5  | 2182.374 | 1700.043 | 1283.124 | 933.340 | 655.858 | 476.536 |
| 1    | 9    | 40      | 0.5  | 2514.348 | 2000.800 | 1554.320 | 1178.212 | 882.388 | 716.419 |
| 3    | 1    | 40      | 0.5  | 532.045  | 393.568  | 276.394  | 180.568  | 106.235  | 54.160  |
| 3    | 3    | 40      | 0.5  | 623.350  | 473.711  | 345.536  | 239.036  | 154.855  | 96.309  |
| 3    | 5    | 40      | 0.5  | 724.132  | 563.671  | 424.992  | 308.626  | 216.180  | 155.887 |
| 3    | 7    | 40      | 0.5  | 834.896  | 664.039  | 515.482  | 390.276  | 291.599  | 235.622 |
| 3    | 9    | 40      | 0.5  | 956.131  | 775.389  | 617.712  | 484.918  | 382.495  | 338.216 |
| 5    | 1    | 40      | 0.5  | 372.294  | 282.761  | 206.082  | 142.360  | 91.916   | 56.441  |
| 5    | 3    | 40      | 0.5  | 432.776  | 336.705  | 253.673  | 183.969  | 128.495  | 91.841  |
| 5    | 5    | 40      | 0.5  | 499.232  | 396.886  | 307.883  | 232.825  | 173.536  | 139.448 |
| 5    | 7    | 40      | 0.5  | 571.961  | 463.651  | 369.141  | 289.489  | 227.906  | 190.686 |
| 5    | 9    | 40      | 0.5  | 651.252  | 537.344  | 437.867  | 354.516  | 294.417  | 277.761 |
| 7    | 1    | 40      | 0.5  | 308.005  | 239.517  | 180.320  | 130.602  | 90.937   | 64.393  |
| 7    | 3    | 40      | 0.5  | 355.468  | 282.472  | 218.981  | 165.402  | 122.974  | 98.218  |
| 7    | 5    | 40      | 0.5  | 407.406  | 330.128  | 262.678  | 205.787  | 161.686  | 142.000 |
| 7    | 7    | 40      | 0.5  | 464.028  | 382.732  | 311.712  | 252.155  | 207.663  | 196.886 |
| 7    | 9    | 40      | 0.5  | 510.510  | 436.827  | 366.432  | 289.489  | 227.906  | 190.686 |
| 9    | 1    | 20      | 0.5  | 632.410  | 458.999  | 313.794  | 196.824  | 108.175  | 48.333  |
| 9    | 3    | 20      | 0.5  | 657.240  | 480.424  | 331.837  | 211.529  | 119.657  | 57.071  |
| 9    | 5    | 20      | 0.5  | 680.427  | 502.940  | 353.752  | 231.503  | 129.348  | 66.295  |
| 9    | 7    | 20      | 0.5  | 703.623  | 525.434  | 375.665  | 251.469  | 139.147  | 75.612  |
| 9    | 9    | 20      | 0.5  | 727.029  | 548.912  | 397.579  | 271.436  | 148.845  | 85.936  |

Table 1: 250 numerical experiments.
Table 2: Rate constants estimated by $A(S, E) = 0$ or $Q(S, E) = 0$. The first column indicates the measured concentrations of the substrate during the reaction process, and the corresponding concentrations of enzyme is determined by $S$, so we do not show them explicitly. $a : h : b$ means that the concentrations are measured from $S = a$ to $S = b$ with step length $h$. $K_Q^Q$ denotes the Michaelis constant $K_M$ estimated by $Q(S, E) = 0$, $K_M^A$ denotes the $K_M$ estimated by $A(S, E) = 0$, $k_{1}^{A}$ and $k_{-1}^{A}$ denote $k_1$ and $k_{-1}$ estimated by $A(S, E) = 0$, if $k_2$ is provided. Here, we assume that $k_2 = 0.2000$ is exactly estimated by equation (35).

| $S$   | $K_Q^Q$ | $K_M^A$ | $k_1^A$ | $k_{-1}^A$ | $k_2$ |
|-------|---------|---------|---------|-----------|-------|
| 3:0.1:19 | 0.996345 | 0.999979 | 0.3052  | 0.1052    | 0.2000 |
| 3:0.5:19 | 0.996243 | 0.999978 | 0.3055  | 0.1055    | 0.2000 |
| 3:1.0:19 | 0.996112 | 0.999977 | 0.3057  | 0.1057    | 0.2000 |
| 3:2.0:19 | 0.995837 | 0.999975 | 0.3063  | 0.1063    | 0.2000 |
| 3:4.0:19 | 0.995253 | 0.999972 | 0.3073  | 0.1072    | 0.2000 |
| 3:8.0:19 | 0.994059 | 0.999972 | 0.3083  | 0.1083    | 0.2000 |
| 3:16:19  | 0.991973 | 0.999978 | 0.3088  | 0.1088    | 0.2000 |
| 10:0.1:19| 0.998506 | 0.999996 | 0.3017  | 0.1017    | 0.2000 |
| 10:0.5:19| 0.998495 | 0.999996 | 0.3017  | 0.1017    | 0.2000 |
| 10:1.0:19| 0.998481 | 0.999996 | 0.3018  | 0.1018    | 0.2000 |
| 10:2.0:19| 0.998374 | 0.999995 | 0.3019  | 0.1019    | 0.2000 |
| 10:4.0:19| 0.998320 | 0.999995 | 0.3019  | 0.1019    | 0.2000 |
| 10:8.0:19| 0.998215 | 0.999996 | 0.3020  | 0.1020    | 0.2000 |
| 16:0.1:19| 0.999024 | 0.999998 | 0.3011  | 0.1011    | 0.2000 |
| 16:0.5:19| 0.999022 | 0.999998 | 0.3011  | 0.1011    | 0.2000 |
| 16:1.0:19| 0.999020 | 0.999998 | 0.3011  | 0.1011    | 0.2000 |
| 16:2.0:19| 0.998967 | 0.999998 | 0.3011  | 0.1011    | 0.2000 |