Available online at www.sciencedirect.com
SCIENCE DIRECT
C. R. Biologies 329 (2006) 51–61
COMPTES RENDUS BIOLOGIES
http://france.elsevier.com/direct/CRASS3/

Biological modelling / Biomodélisation

The mechanism distinguishability problem in biochemical kinetics: The single-enzyme, single-substrate reaction as a case study

Santiago Schnell [a,b,*], Michael J. Chappell [c], Neil D. Evans [c], Marc R. Roussel [d]

[a] *Centre for Mathematical Biology, Mathematical Institute, 24–29 St Giles', Oxford OX1 3LB, UK*
[b] *Christ Church, Oxford OX1 1DP, UK*
[c] *School of Engineering, University of Warwick, Coventry CV4 7AL, UK*
[d] *Department of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, Alberta, T1K 3M4, Canada*

Received 6 June 2005; accepted after revision 20 September 2005

Available online 19 October 2005

Presented by Michel Thellier

Abstract

A theoretical analysis of the distinguishability problem of two rival models of the single enzyme-single substrate reaction, the Michaelis–Menten and Henri mechanisms, is presented. We also outline a general approach for analysing the structural indistinguishability between two mechanisms. The approach involves constructing, if possible, a smooth mapping between the two candidate models. Evans et al. [N.D. Evans, M.J. Chappell, M.J. Chapman, K.R. Godfrey, Structural indistinguishability between uncontrolled (autonomous) nonlinear analytic systems, Automatica 40 (2004) 1947–1953] have shown that if, in addition, either of the mechanisms satisfies a particular criterion then such a transformation always exists when the models are indistinguishable from their experimentally observable outputs. The approach is applied to the single enzyme-single substrate reaction mechanism. In principle, mechanisms can be distinguished using this analysis, but we show that our ability to distinguish mechanistic models depends both on the precise measurements made, and on our knowledge of the system prior to performing the kinetics experiments. ***To cite this article: S. Schnell et al., C. R. Biologies 329 (2006).***

Résumé

Différenciation des mécanismes en cinétique biochimique : étude de la réaction d'un substrat catalysée par une enzyme. Nous présentons une analyse théorique du problème de la différenciation de deux modèles pour la réaction d'un substrat catalysée par une enzyme, soit les mécanismes de Michaelis–Menten et d'Henri, à partir de données expérimentales provenant d'expériences cinétiques. Nous proposons une méthode d'analyse qui permet, le cas échéant, de prouver l'impossibilité de distinguer deux mécanismes par certaines mesures expérimentales. En bref, on tente de construire une application lisse qui relie les deux modèles. Evans et al. [N.D. Evans, M.J. Chappell, M.J. Chapman, K.R. Godfrey, Structural indistinguishability between uncontrolled (autonomous) nonlinear analytic systems, Automatica 40 (2004) 1947–1953] ont, de plus, démontré que si chaque mécanisme satisfait à un certain critère, une telle transformation existe toujours quand les modèles ne peuvent pas être distingués dans le cadre d'une expérience donnée. La méthode est mise en pratique par une étude approfondie des deux mécanismes de catalyse enzymatique nommés précédemment. En principe, on peut distinguer le modèle de Michaelis et Menten de celui d'Henri, mais, en pratique, cette

* Corresponding author. Present address: Indiana University School of Informatics and Biocomplexity Institute, Eigenmann Hall 906, 1900 East 10th Street, Bloomington, IN 47406, USA.
E-mail address: schnell@indiana.edu (S. Schnell).

1631-0691/$ – see front matter
doi:10.1016/j.crvi.2005.09.005

différenciation dépend et des mesures prises et de nos connaissances antérieures du système. ***Pour citer cet article : S. Schnell et al., C. R. Biologies 329 (2006).***

Keywords: Distinguishability problem; Chemical kinetics; Kinetically equivalent mechanisms; Structural indistinguishability

Mots-clés : Différenciation des modèles ; Cinétique chimique ; Mécanismes cinétiquement équivalents ; Indifférenciabilité structurelle

1. Introduction

Chemical kinetics entails the measurement of concentrations as a function of time with the aim of understanding and characterising the reaction mechanism [1]. The term mechanism refers to the complete set of elementary steps that specifies how a reaction takes place. Elementary reaction steps are those that cannot be decomposed to reveal reaction intermediates that might themselves be identified as separate chemical entities on a biochemically relevant timescale.

Constructing a reaction mechanism remains something of an art [2]. One of the major problems is that several different mechanisms are often consistent with the available data, or may even give the same mathematical representation [3,4]. This is sometimes referred to as the fundamental dogma of chemical kinetics [5], that is, it is not possible to prove that a reaction mechanism is correct. We can only disprove mechanisms by showing inconsistency with data, or with theoretical requirements for a model. However, the problem often remains that some models are indistinguishable in the context of a given experiment or set of experiments. This is precisely the problem that Henri raised in 1902 when he considered two different reaction schemes as tentative explanations for the single enzyme–substrate reaction [6].

Victor Henri, a man of extraordinarily diverse genius (see Appendix A for a short biography), submitted his thesis for the degree of 'Docteur ès sciences' in 1902. The topic of his thesis was on the general theory of enzyme action, which was later published in 1903 in his book entitled *Lois générales de l'action des diastases* [7]. In a paper published in a predecessor of this journal, Henri [8] proposes two reaction mechanisms between an enzyme E and the substrate S, forming an enzyme-substrate complex C. Both reactions yield the product P. The first mechanism is known nowadays as the Michaelis–Menten (MM) mechanism of enzyme action [9], although these authors clearly recognised Henri as the originator. In this scheme, E and S combine to form C, which yields P:

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

In the second mechanism, which is known as the Henri (H) mechanism [10] or Nuisance–Complex mechanism [11,12], C is formed but it does not have a catalytic role in the reaction (nonproductive enzyme-substrate complex). The latter is represented schematically as:

$$C \underset{\tilde{k}_1}{\overset{\tilde{k}_{-1}}{\rightleftharpoons}} S + E \xrightarrow{\tilde{k}_2} P + E \tag{2}$$

Henri discovered that both schemes (MM and H) are kinetically indistinguishable after the application of the equilibrium (or alternatively the quasi-steady-state) approximation, because the rate equations for the reaction velocities have the same form. Although he never committed to a particular mechanism, Henri leaned towards the MM mechanism in his 1903 book [6].

We now know (and Henri clearly suspected) that in the vast majority of biochemical reactions, the complex C is on the pathway from reactants to products. Indeed, the H mechanism is rarely even mentioned in chemical and enzyme kinetics books [12]. In the oxidation of p-cresol by horseradish peroxidase however, the variation of the kinetic constants with pH suggests a nonproductive complex, i.e. the H mechanism [14]. Moreover, the MM and H mechanisms are seriously considered as alternatives in solvolysis [15] and in reactions involving a charge-transfer complex [16,17].

Clearly, the MM and H mechanisms have prominent features at the microscopic level. C is an intermediate in the MM mechanism, while in the H mechanism it simply reduces the active mass of enzyme. These mechanisms are certainly distinguishable with the appropriate experimental design. Admittedly, this particular controversy arises rarely in enzymological investigations. In general, however, knowing which experimental designs may be used to distinguish rival mechanisms is a central concern of chemical kinetics. Revisiting Henri's problem is thus an excellent way to sharpen the conceptual tools available for this task.

1.1. Governing equations of the two reaction mechanisms

Before proceeding further with our discussion, let us consider the governing equations for the reaction mech-

anisms. The time evolution of the MM reaction is obtained by applying the law of mass action to yield the set of coupled nonlinear differential equations

$$\frac{ds}{dt} = k_1(-es + K_S c) \tag{3}$$

$$\frac{de}{dt} = k_1(-es + K_M c) \tag{4}$$

$$\frac{dc}{dt} = k_1(es - K_M c) \tag{5}$$

$$\frac{dp}{dt} = k_2 c \tag{6}$$

with initial conditions $(s, e, c, p) = (s_0, e_0, 0, 0)$ at time $t = 0$. In this system, $K_S = k_{-1}/k_1$ is the equilibrium dissociation constant for the enzyme–substrate complex and $K_M = (k_{-1} + k_2)/k_1$ is known as the MM constant (see Schnell and Maini [18] for a review). The lower-case letters represent concentrations and the subscript 0 denotes initial concentration. This mechanism obeys two conservation laws: the enzyme conservation law obtained by adding (4) and (5):

$$e_0 = e(t) + c(t) \tag{7}$$

and the substrate conservation law obtained by adding (3), (5) and (6):

$$s_0 = s(t) + c(t) + p(t) \tag{8}$$

The H mechanism obeys the same mass conservation laws as the MM mechanism, namely (7)–(8). The full set of differential equations for this mechanism is given by:

$$\frac{ds}{dt} = \left(\tilde{k}_1 + \tilde{k}_2\right)\left(-es + \tilde{K}_H c\right) \tag{9}$$

$$\frac{de}{dt} = \tilde{k}_1\left(-es + \tilde{K}_S c\right) \tag{10}$$

$$\frac{dc}{dt} = \tilde{k}_1\left(es - \tilde{K}_S c\right) \tag{11}$$

$$\frac{dp}{dt} = \tilde{k}_2 es \tag{12}$$

with initial conditions $(s, e, c, p) = (\tilde{s}_0, \tilde{e}_0, 0, 0)$ at time $t = 0$. Note that we have added a tilde in both the initial substrate and enzyme concentrations for the H mechanism. In this system, $\tilde{K}_S = \tilde{k}_{-1}/\tilde{k}_1$ is the equilibrium dissociation constant for the enzyme-substrate complex and $\tilde{K}_H = \tilde{k}_{-1}/(\tilde{k}_1 + \tilde{k}_2)$, which we call the H constant.

Mathematical biochemists usually reduce these systems to only two differential equations, for s and c, which together with the conservation laws fully describe the reaction mechanisms. For the MM mechanism, the system of differential equations becomes

$$\frac{ds}{dt} = k_1\left(-(e_0 - c)s + K_S c\right) \tag{13}$$

$$\frac{dc}{dt} = k_1\left((e_0 - c)s - K_M c\right) \tag{14}$$

with initial conditions $(s, c) = (s_0, 0)$ at time $t = 0$. The mass action rate equations for the H mechanism are:

$$\frac{ds}{dt} = \left(\tilde{k}_1 + \tilde{k}_2\right)\left(-(\tilde{e}_0 - c)s + \tilde{K}_H c\right) \tag{15}$$

$$\frac{dc}{dt} = \tilde{k}_1\left((\tilde{e}_0 - c)s - \tilde{K}_S c\right) \tag{16}$$

with the initial conditions $(s, c) = (\tilde{s}_0, 0)$ at time $t = 0$.

1.2. Kinetic indistinguishability of the two reaction mechanisms

These two mechanisms can be considered kinetically equivalent under two criteria.

I. Roussel [12] has shown that they are equivalent under the linear transformation criterion of Prigogine [19]. By noting that the MM mechanism (1) can be written in its elementary steps:

$$S + E \xrightarrow{k_1} C \tag{17}$$

$$C \xrightarrow{k_{-1}} S + E \tag{18}$$

$$C \xrightarrow{k_2} P + E \tag{19}$$

Adding steps (17) and (19) yields the step

$$S + E \xrightarrow{\tilde{k}_2} P + E \tag{20}$$

The H mechanism can therefore be obtained by linear transformation of the MM mechanism. Specifically, the H mechanism consists of MM reactions (17) and (18) along with reaction (20), which is just the sum of reactions (17) and (19). Mechanisms generated from one another by linear transformation preserve the Onsager reciprocity relations [19], a necessary condition for kinetic equivalence.

II. Two mechanisms are said to be homeomorphic [20, p. 18] and kinetically equivalent [21], if the reduced systems of differential equations describing their time evolution are of the same form, but the individual rate constants are different [11]. It can clearly be noticed by comparing the system (13)–(14) for the MM mechanism with the system (15)–(16) for the H mechanism that the two mechanisms are homeomorphic and kinetically equivalent.

In his seminal paper, Henri [8] warned against the dangers of homeomorphism when he derived, for both mechanisms with the aid of the equilibrium approximation, a reaction rate expression of the form

$$v = \frac{v_{\max} s}{K_{1/2} + s}$$

where $v_{\max}$ is the maximum velocity and $K_{1/2}$ is the concentration of the substrate required for half-maximal velocity [10]. Roussel and Fraser [13] and Roussel [12] show that this is not a surprising result as the equilibrium expression is an approximation to the slow manifold in the positive (s, c) phase plane described with the reduced system of differential equations (13)–(14) and (15)–(16). Roussel and Fraser [13] found that the behaviour of the MM and H mechanisms is identical in the (s, c) phase plane. The only notable difference between the phase plane descriptions of the two mechanisms is the identification of the nullclines. In the MM mechanism, the c nullcline corresponds to the quasi-steady-state approximation, while the s nullcline represents the equilibrium approximation. For the H mechanism, the c and s nullclines correspond, respectively, to the equilibrium and quasi-steady-state approximations.

Under these two kinetic criteria, we are led to the impression that the two reaction mechanisms are indistinguishable. While the MM and H mechanisms are kinetically equivalent, they are distinguishable.

One of the earliest efforts to distinguish these two reaction mechanisms was made by Viale [10]. He presented a specific distinguishability criterion by studying the transient kinetics of the reaction. Viale noted that $\mathrm{d}^2 p/\mathrm{d}t^2$ is positive during the initial transient for the MM mechanism, while for the H mechanism it is negative. Hiromi [11] discovered that the increase of product concentration starts with a lag period after the initiation of the MM reaction mechanism. In contrast, the product formation occurs immediately after the beginning of the reaction in the H mechanism. More recently, the distribution of the delay between reactant mixing and product formation in enzyme-catalysed reactions has been suggested [22] as a criterion for generally distinguishing reaction mechanisms. On the other hand, Czerlinski [23] showed that relaxation kinetics studies can also be employed for distinguishing between the MM and H mechanisms.

It is interesting to note that some of the distinguishability criteria presented above involve P, but previous authors have missed the fact that it is necessary to explicitly include the rate of product formation $\mathrm{d}p/\mathrm{d}t$ or the time course of the product formation to distinguish between the MM and H mechanisms [12]. This is evident from the full set of governing differential equations describing both reaction schemes, that is (3)–(6) and (9)–(12). Earlier work of Roussel and Fraser [13] shows that the steady-state kinetics of the two mechanisms can lead to quantitatively different $\mathrm{d}p/\mathrm{d}t$ curves using Eadie–Hofstee plots if higher-order terms are included in the approximations.

While we are able to distinguish between the MM and H mechanisms employing these criteria, they have specifically been developed for distinguishing between these reaction schemes. From time to time an article appears in the literature emphasising the importance of developing general principles for distinguishing reaction mechanisms [3,4]. Most available methods are empirical and rely on establishing a detailed understanding of the dynamical differences between the proposed reaction mechanisms, as is the case for the MM and H schemes.

1.3. Structure of the paper

In the present work, we briefly describe three of the four kinds of experiments commonly used for enzyme kinetic studies (Section 2). In Appendix B, we outline an approach for analysing the structural indistinguishability between two reactions governed by autonomous analytic systems. The approach involves constructing, if possible, a smooth mapping between the trajectories of two candidate model mechanisms. Evans et al. [29] showed that if, in addition, either of the models satisfies an appropriate criterion, then the mechanisms are indistinguishable from their experimentally observable outputs only when this mapping between trajectories exists. The approach is applied to the two rival single enzyme-substrate reaction mechanisms in Section 3. This is followed by a discussion (Section 4).

2. Experimental approaches used to study reaction kinetics

To set the stage, we need first to understand the experimental approaches employed to study reaction mechanisms and measure the kinetic parameters. In typical biochemical experiments, concentrations are measured by the absorbance of light at one or more wavelengths [24,25]. If the molar absorptivities of the measured components at these wavelengths are known, then biochemists use Beer's law to determine the concentrations. Thus, in matrix notation,

$$\boldsymbol{y} = \varepsilon \boldsymbol{x} \tag{21}$$

where $\boldsymbol{y}$ is the vector of observed absorbances at different wavelengths, $\boldsymbol{x}$ is the vector of concentrations of species absorbing at these wavelengths, and ε is a matrix of molar absorptivities. If the wavelengths are appropriately selected, then ε is an invertible square matrix[1] and we can recover $\boldsymbol{x}$ from $\boldsymbol{y}$ by solving the linear system (21).

In favourable cases, E, S, C and P all absorb at wavelengths where the others have negligible absorptivities and the matrix ε is diagonal. Since C is an intermediate in the single-enzyme-catalysed reactions, its concentration (c) is usually quite small and, unless it has large absorptivity, its absorption can often be neglected. To illustrate the use of a formal structural indistinguishability analysis, let us then consider the simplest case, namely that of an absorbance measurement at a single wavelength; we have:

$$y(t, \boldsymbol{p}) = h(\boldsymbol{x}, \boldsymbol{p}) = \varepsilon_{\mathrm{S}} s(t, \boldsymbol{p}) + \varepsilon_{\mathrm{P}} p(t, \boldsymbol{p}) \tag{22}$$

In this equation, h is the corresponding output structure for the reaction and $\boldsymbol{p}$ is a constant parameter vector (see Appendix B for more details). In general, the substrate (ε_{S}) and product (ε_{P}) absorptivities are significantly different.

Biochemists usually study enzyme-catalysed reactions using four types of experiments [24,25]:

(1) *Initial rate experiments*. When an enzyme is mixed with a large excess of the substrate, the enzyme-substrate intermediate builds up in a fast initial transient. Then the reaction achieves a quasi-steady state (QSS) in which c remains approximately constant over time and the reaction rate changes relatively slowly. Rates are measured for a short period after the attainment of the quasi-steady state, typically by monitoring the accumulation of product with time. Because the measurements are carried out for a very short period and because of the large excess of substrate, the approximation $s \approx s_0$ can be made. The initial rate experiment is the simplest to perform and analyze, being relatively free from complications such as back-reaction and enzyme degradation. It is therefore by far the most commonly used type of experiment in enzyme kinetics.

(2) *Progress curve experiments*. In these experiments, the kinetic parameters are determined from expressions for the species concentrations as a function of time. The concentration of the substrate or product is recorded in time after the initial fast transient and for a sufficiently long period to allow the reaction to approach equilibrium. We note in passing that, while they are less common now, progress curve experiments were widely used in the early period of enzyme kinetics when Henri was active in the field.

(3) *Transient kinetics experiments*. In these experiments, reaction behaviour is tracked during the initial fast transient as the intermediate reaches the QSS period. These experiments are more difficult to perform than either of the above two classes because they require rapid mixing and observation techniques.

(4) *Relaxation experiments*. In these experiments, an equilibrium mixture of enzyme, substrate and product is perturbed, for instance by a temperature, pressure or pH jump, and the return to equilibrium is monitored. The analysis of these experiments requires consideration of the fully reversible reaction. Moreover, relaxation experiments are relatively insensitive to mechanistic details and are thus not typically used for mechanism identification, although they can be under appropriate conditions [23]. We therefore do not consider them further in this article.

3. Structural indistinguishability analyses for proposed reaction schemes

The process of deducing a reaction mechanism entails several steps. One of them is to recognise all the possible reaction mechanisms that are consistent with the data and devise experimental tests to rule out certain of the candidate reaction schemes. These are known as falsifying tests. In this section, we explore the experimental procedures that can be employed to distinguish between the two reaction schemes given by (13)–(14) and (15)–(16) using a structural indistinguishability analysis. (See Appendix B for the theoretical underpinnings.)

3.1. Initial rate experiments

The initial rate experiments are carried out in QSS conditions, implying that the intermediates enter a slowly changing regime (see Roussel and Fraser [27], Schnell and Maini [28] for recent studies). For the one-intermediate mechanisms studied here,

$$\frac{\mathrm{d}c}{\mathrm{d}t} \approx 0$$

[1] If there are n chemical species and m observation wavelengths, then ε is an $m \times n$ matrix. If $m > n$, the concentrations can be recovered from the absorbances using the pseudo-inverse of ε, a procedure that is equivalent to least-squares fitting [26].

Applying the QSS approximation to the MM mechanism, yields

$$c \approx \frac{e_0 s}{K_M + s} \tag{23}$$

We can easily obtain an equation for the evolution of the observable y by substituting (13), (6) and (23) into the derivative with respect to time of (22):

$$\dot{y} = \varepsilon_S \dot{s} + \varepsilon_P \dot{p} = (\varepsilon_P - \varepsilon_S)\frac{v_{max} s}{K_M + s} \tag{24}$$

with $v_{max} = k_2 e_0$.

Proceeding similarly for the H mechanism, we get:

$$c \approx \frac{\tilde{e}_0 s}{s + \tilde{K}_S}$$

Therefore

$$\dot{\tilde{y}} = (\varepsilon_P - \varepsilon_S)\frac{\tilde{v}_{max} s}{s + \tilde{K}_M} \tag{25}$$

where $\tilde{v}_{max} = \tilde{k}_2 \tilde{K}_S \tilde{e}_0$.

Eqs. (24) and (25) are of identical form, thus clearly indistinguishable because the outputs of $\tilde{v}_{max} = v_{max}$ and $\tilde{K}_M = K_M$ are indistinguishable[2]. Therefore, our analysis indicates that the underlying evolution of s implied by the two reduced sets of equations is also indistinguishable. Note that this is true even if we know the absorptivities a priori.

3.2. Progress curve experiments

As we mentioned in Section 2, the substrate (ε_S) and product (ε_P) absorptivities in (22) are generally significantly different, but it may be difficult to select a wavelength where they have different orders of magnitude. If a wavelength can be found where one compound absorbs while the other does not, this problem reduces to measurements of quantities proportional to s or to p. Otherwise, it will be necessary to study the case in which both s and p contribute to the absorbance.

3.2.1. Measuring substrate concentration

Suppose that it is only possible to measure the absorbance of substrate in the experiment. Let $\boldsymbol{x}(t, \boldsymbol{p})$ denote the state vector for the reaction scheme given by (13)–(14) and $\tilde{\boldsymbol{x}}(t, \tilde{\boldsymbol{p}})$ that for (15)–(16), so that

$$\boldsymbol{x}(t, \boldsymbol{p}) = \begin{pmatrix} s(t, \boldsymbol{p}) \\ c(t, \boldsymbol{p}) \end{pmatrix} \quad \text{and} \quad \tilde{\boldsymbol{x}}(t, \tilde{\boldsymbol{p}}) = \begin{pmatrix} s(t, \tilde{\boldsymbol{p}}) \\ c(t, \tilde{\boldsymbol{p}}) \end{pmatrix}$$

where $\boldsymbol{p}$ and $\tilde{\boldsymbol{p}}$ are the corresponding parameter vectors. The right-hand sides of the two models are given by:

$$\boldsymbol{f}(\boldsymbol{x}, \boldsymbol{p}) = \begin{pmatrix} k_1(-(e_0 - x_2)x_1 + K_S x_2) \\ k_1((e_0 - x_2)x_1 - K_M x_2) \end{pmatrix} \tag{26}$$

$$\tilde{\boldsymbol{f}}(\tilde{\boldsymbol{x}}, \tilde{\boldsymbol{p}}) = \begin{pmatrix} (\tilde{k}_1 + \tilde{k}_2)(-(\tilde{e}_0 - \tilde{x}_2)\tilde{x}_1 + \tilde{K}_H \tilde{x}_2) \\ \tilde{k}_1((\tilde{e}_0 - \tilde{x}_2)\tilde{x}_1 - \tilde{K}_S \tilde{x}_2) \end{pmatrix} \tag{27}$$

The corresponding output structures for the reaction schemes are given by:

$$h(\boldsymbol{x}, \boldsymbol{p}) = \varepsilon_S x_1 \tag{28}$$

and

$$\tilde{h}(\tilde{\boldsymbol{x}}, \tilde{\boldsymbol{p}}) = \tilde{\varepsilon}_S \tilde{x}_1 \tag{29}$$

so that the observable outputs are given by:

$$y(t, \boldsymbol{p}) = \varepsilon_S s(t, \boldsymbol{p}), \qquad \tilde{y}(t, \tilde{\boldsymbol{p}}) = \tilde{\varepsilon}_S s(t, \tilde{\boldsymbol{p}})$$

The parameter vectors are given by:

$$\boldsymbol{p} = (k_1, k_{-1}, k_2, \varepsilon_S, s_0, e_0)^T \quad \text{and}$$
$$\tilde{\boldsymbol{p}} = \left(\tilde{k}_1, \tilde{k}_{-1}, \tilde{k}_2, \tilde{\varepsilon}_S, \tilde{s}_0, \tilde{e}_0\right)^T$$

Suitable spaces of admissible parameter vectors for the two schemes are $\Omega = \tilde{\Omega} = \mathbb{R}^6_{>0}$. Let $\boldsymbol{\lambda}(\tilde{\boldsymbol{x}}) = (t_{11}\tilde{x}_1 + t_{12}\tilde{x}_2, t_{21}\tilde{x}_1 + t_{22}\tilde{x}_2)^T$ be a general linear map. This map satisfies (B.5) if and only if:

$$h\left(\boldsymbol{\lambda}(\tilde{\boldsymbol{x}}), \boldsymbol{p}\right) = \varepsilon_S\left(t_{11}\tilde{x}_1 + t_{12}\tilde{x}_2\right) = \tilde{\varepsilon}_S \tilde{x}_1 = \tilde{h}(\tilde{\boldsymbol{x}}, \tilde{\boldsymbol{p}})$$

for all $\tilde{\boldsymbol{x}}$, which can only be the case if $t_{11} = \tilde{\varepsilon}_S/\varepsilon_S$ and $t_{12} = 0$. Therefore $\boldsymbol{\lambda}$ satisfies (B.4) if and only if:

$$\begin{aligned}
&\boldsymbol{f}\left(\boldsymbol{\lambda}(\tilde{\boldsymbol{x}}), \boldsymbol{p}\right) \\
&= \begin{pmatrix} k_1(-(e_0 - (t_{21}\tilde{x}_1 + t_{22}\tilde{x}_2))\frac{\tilde{\varepsilon}_S \tilde{x}_1}{\varepsilon_S} + K_S(t_{21}\tilde{x}_1 + t_{22}\tilde{x}_2)) \\ k_1((e_0 - (t_{21}\tilde{x}_1 + t_{22}\tilde{x}_2))\frac{\tilde{\varepsilon}_S \tilde{x}_1}{\varepsilon_S} - K_M(t_{21}\tilde{x}_1 + t_{22}\tilde{x}_2)) \end{pmatrix} \\
&= \begin{pmatrix} \tilde{\varepsilon}_S/\varepsilon_S & 0 \\ t_{21} & t_{22} \end{pmatrix} \begin{pmatrix} (\tilde{k}_1 + \tilde{k}_2)(-(\tilde{e}_0 - \tilde{x}_2)\tilde{x}_1 + \tilde{K}_H \tilde{x}_2) \\ \tilde{k}_1((\tilde{e}_0 - \tilde{x}_2)\tilde{x}_1 - \tilde{K}_S \tilde{x}_2) \end{pmatrix} \\
&= \frac{\partial \boldsymbol{\lambda}}{\partial \tilde{\boldsymbol{x}}}(\tilde{\boldsymbol{x}})\tilde{\boldsymbol{f}}(\tilde{\boldsymbol{x}}, \tilde{\boldsymbol{p}})
\end{aligned} \tag{30}$$

for all $\tilde{\boldsymbol{x}}$. Considering the first component, this is the case only if:

$$\begin{aligned}
&\left(t_{21}k_{-1}\varepsilon_S + (\tilde{k}_1 + \tilde{k}_2)\tilde{\varepsilon}_S \tilde{e}_0 - e_0 k_1 \tilde{\varepsilon}_S\right)\tilde{x}_1 \\
&\quad + \left(t_{22}k_{-1}\varepsilon_S - \tilde{k}_{-1}\tilde{\varepsilon}_S\right)\tilde{x}_2 \\
&\quad + \left(t_{22}k_1\tilde{\varepsilon}_S - (\tilde{k}_1 + \tilde{k}_2)\tilde{\varepsilon}_S\right)\tilde{x}_1\tilde{x}_2 + t_{21}k_1\tilde{\varepsilon}_S\tilde{x}_1^2 = 0
\end{aligned}$$

for all $\tilde{\boldsymbol{x}}$, which means that each of the coefficients of this polynomial must be zero. From the coefficient of $\tilde{x}_1^2$ it follows (since $k_1 \neq 0$ and $\tilde{\varepsilon}_S \neq 0$ by definition of $\Omega = \tilde{\Omega}$) that $t_{21} = 0$, giving the following resulting expressions for the remaining coefficients:

$$\left(\tilde{k}_1 + \tilde{k}_2\right)\tilde{e}_0 = e_0 k_1, \qquad t_{22}k_{-1}\varepsilon_S = \tilde{k}_{-1}\tilde{\varepsilon}_S, \quad \text{and}$$
$$t_{22}k_1 = \left(\tilde{k}_1 + \tilde{k}_2\right)$$

[2] This analysis can also be extended to the individual parameters in $\tilde{v}_{max}$, v_{max}, $\tilde{K}_M$ and K_M.

which together imply that

$$t_{22} = \frac{\tilde{k}_{-1}\tilde{\varepsilon}_S}{k_{-1}\varepsilon_S} = \frac{\tilde{k}_1 + \tilde{k}_2}{k_1} = \frac{e_0}{\tilde{e}_0} \tag{31}$$

For the second component of (30) to be satisfied, in addition, it is necessary that

$$e_0\left(\frac{k_1\tilde{\varepsilon}_S}{\varepsilon_S} - \tilde{k}_1\right)\tilde{x}_1 + \frac{e_0}{\tilde{e}_0}\left(\tilde{k}_{-1} - (k_{-1}+k_2)\right)\tilde{x}_2 + \frac{e_0}{\tilde{e}_0}\left(\tilde{k}_1 - \frac{k_1\tilde{\varepsilon}_S}{\varepsilon_S}\right)\tilde{x}_1\tilde{x}_2 = 0$$

for all $\tilde{\boldsymbol{x}}$, which implies for the corresponding coefficients that

$$\tilde{k}_{-1} = k_{-1} + k_2 \quad \text{and} \quad \tilde{k}_1 = \frac{k_1\tilde{\varepsilon}_S}{\varepsilon_S} \tag{32}$$

For $\boldsymbol{\lambda}$ to satisfy the remaining equation in Theorem 1, namely Eq. (B.3), it is necessary and sufficient that the following be satisfied:

$$\boldsymbol{\lambda}\big(\tilde{\boldsymbol{x}}_0(\tilde{\boldsymbol{p}})\big) = \begin{pmatrix} (\tilde{\varepsilon}_S\tilde{s}_0)/\varepsilon_S \\ 0 \end{pmatrix} = \begin{pmatrix} s_0 \\ 0 \end{pmatrix} = \boldsymbol{x}_0(\boldsymbol{p})$$

which implies that

$$\tilde{s}_0 = \frac{\varepsilon_S s_0}{\tilde{\varepsilon}_S} \tag{33}$$

Combining the relations in (31), (32) and (33) gives the following:

$$\tilde{k}_1 = \frac{k_1\tilde{\varepsilon}_S}{\varepsilon_S}, \qquad \tilde{k}_{-1} = k_{-1} + k_2, \qquad \tilde{k}_2 = \frac{k_1 k_2 \tilde{\varepsilon}_S}{k_{-1}\varepsilon_S}$$

$$\tilde{s}_0 = \frac{\varepsilon_S s_0}{\tilde{\varepsilon}_S}, \quad \text{and} \quad \tilde{e}_0 = \frac{k_{-1}\varepsilon_S e_0}{(k_{-1}+k_2)\tilde{\varepsilon}_S} \tag{34}$$

Thus for generic $\boldsymbol{p} \in \Omega$, any $\tilde{\boldsymbol{p}} \in \tilde{\Omega}$ that satisfies (34) has a pair $(\tilde{V}_{\tilde{\boldsymbol{p}}}, \boldsymbol{\lambda})$ such that Theorem 1 is satisfied, where

$$\tilde{V}_{\tilde{\boldsymbol{p}}} = \mathbb{R}^2_{>0} \quad \text{and} \quad \boldsymbol{\lambda}(\boldsymbol{x}) = \begin{pmatrix} (s_0 x_1)/\tilde{s}_0 \\ (e_0 x_2)/\tilde{e}_0 \end{pmatrix}$$

Conversely, for generic $\tilde{\boldsymbol{p}} \in \tilde{\Omega}$ choose $\varepsilon_S > 0$ arbitrarily and then set

$$k_1 = \frac{\tilde{k}_1\varepsilon_S}{\tilde{\varepsilon}_S} > 0, \qquad s_0 = \frac{\tilde{\varepsilon}_S\tilde{s}_0}{\varepsilon_S} > 0$$

$$k_{-1} = \frac{\tilde{k}_{-1}\tilde{k}_1}{\tilde{k}_1 + \tilde{k}_2} > 0$$

$$k_2 = \frac{\tilde{k}_2\tilde{k}_{-1}}{\tilde{k}_1 + \tilde{k}_2} > 0, \quad \text{and} \quad e_0 = \frac{\tilde{e}_0\tilde{\varepsilon}_S(\tilde{k}_1 + \tilde{k}_2)}{\tilde{k}_1\varepsilon_S} > 0$$

to obtain a $\boldsymbol{p} \in \Omega$ such that there is a pair $(\tilde{V}_{\tilde{\boldsymbol{p}}}, \boldsymbol{\lambda})$ satisfying Theorem 1, where

$$\tilde{V}_{\tilde{\boldsymbol{p}}} = \mathbb{R}^2_{>0} \quad \text{and} \quad \boldsymbol{\lambda}(\boldsymbol{x}) = \begin{pmatrix} (s_0 x_1)/\tilde{s}_0 \\ (e_0 x_2)/\tilde{e}_0 \end{pmatrix}$$

Therefore it is seen from this analysis that the two reaction schemes are structurally indistinguishable from experiments that only measure the concentration of substrate.

3.2.2. *Measuring product concentration*

Suppose that it is only possible to measure the concentration of product in the experiment to be performed. To allow for the corresponding output structure, the differential equation governing the temporal evolution of the concentration of the product is included in both models, (13)–(14) and (15)–(16). The resulting right-hand sides of the two models are given by

$$\boldsymbol{f}(\boldsymbol{x}, \boldsymbol{p}) = \begin{pmatrix} k_1(-(e_0 - x_2)x_1 + K_S x_2) \\ k_1((e_0 - x_2)x_1 - K_M x_2) \\ k_2 x_2 \end{pmatrix} \tag{35}$$

$$\tilde{\boldsymbol{f}}(\tilde{\boldsymbol{x}}, \tilde{\boldsymbol{p}}) = \begin{pmatrix} (\tilde{k}_1 + \tilde{k}_2)(-(\tilde{e}_0 - \tilde{x}_2)\tilde{x}_1 + \tilde{K}_H\tilde{x}_2) \\ \tilde{k}_1((\tilde{e}_0 - \tilde{x}_2)\tilde{x}_1 - \tilde{K}_S\tilde{x}_2) \\ \tilde{k}_2\tilde{x}_1(\tilde{e}_0 - \tilde{x}_2) \end{pmatrix} \tag{36}$$

and the corresponding output structures for the reaction schemes are given by

$$h(\boldsymbol{x}, \boldsymbol{p}) = \varepsilon_P x_3 \quad \text{and} \tag{37}$$

$$\tilde{h}(\tilde{\boldsymbol{x}}, \tilde{\boldsymbol{p}}) = \tilde{\varepsilon}_P\tilde{x}_3 \tag{38}$$

so that the observable outputs are given by

$$y(t, \boldsymbol{p}) = \varepsilon_P p(t, \boldsymbol{p}), \qquad \tilde{y}(t, \tilde{\boldsymbol{p}}) = \tilde{\varepsilon}_P p(t, \tilde{\boldsymbol{p}})$$

For given $\boldsymbol{p} \in \Omega$ and $\tilde{\boldsymbol{p}} \in \tilde{\Omega}$, the observable outputs for the two reaction schemes are identical if and only if

$$y(t, \boldsymbol{p}) = \tilde{y}(t, \tilde{\boldsymbol{p}}) \quad \text{for all } t \geqslant 0$$

Since the observable outputs are analytic functions about $t = 0$, this is equivalent to the following equations:

$$y^{(i)}(0^+, \boldsymbol{p}) = \tilde{y}^{(i)}(0^+, \tilde{\boldsymbol{p}}) \quad \text{for all } i = 0, 1, 2, \ldots \tag{39}$$

where

$$y^{(i)}(0^+, \boldsymbol{p}) = \lim_{\tau \downarrow 0} \frac{\mathrm{d}^i y}{\mathrm{d}t^i}(\tau, \boldsymbol{p}) \quad \text{and}$$

$$y^{(0)}(0^+, \boldsymbol{p}) = y(0, \boldsymbol{p})$$

Eq. (39) is satisfied for $i = 0$ since both observable outputs are zero at $t = 0$ (zero initial concentration of product). For $i = 1$, we have:

$$y^{(1)}(0^+, \boldsymbol{p}) = \varepsilon_P k_2 c(0, \boldsymbol{p}) = 0 \quad \text{and}$$

$$\tilde{y}^{(1)}(0^+, \tilde{\boldsymbol{p}}) = \tilde{\varepsilon}_P\tilde{k}_2 s(0, \tilde{\boldsymbol{p}})\big(\tilde{e}_0 - c(0, \tilde{\boldsymbol{p}})\big) = \tilde{\varepsilon}_P\tilde{k}_2\tilde{s}_0\tilde{e}_0$$

Therefore, since all of the parameters are positive, there are *no* parameter vector pairs $(\boldsymbol{p}, \tilde{\boldsymbol{p}})$, $\boldsymbol{p} \in \Omega$ and $\tilde{\boldsymbol{p}} \in$

$\tilde{\Omega}$, such that $y(t, \boldsymbol{p}) = \tilde{y}(t, \tilde{\boldsymbol{p}})$ for all $t \geqslant 0$. The two reaction schemes are therefore distinguishable from the proposed experiment.

3.2.3. *Measuring both the substrate and product concentrations*

Now let us consider the case in which we are measuring both the substrate and product in the observable output. Typically, progress curve experiments are carried out during the slow transient of the reaction, and relatively slow recording equipment is employed for determining the progress curves. Accordingly, we would record data along the slow manifold [13].

As shown by Roussel and Fraser [13] and Roussel [12], the slow manifold can be determined by solving a functional equation parameterised by e_0, K_M, and the branching probability $\alpha = k_{-1}/(k_{-1} + k_2)$ for the MM mechanism. The corresponding parameters for the H mechanism are $\tilde{e}_0$, $\tilde{K}_\mathrm{S} = \tilde{k}_{-1}/\tilde{k}_1$, and $\tilde{\alpha} = \tilde{k}_1/(\tilde{k}_1 + \tilde{k}_2)$. Because of the homeomorphism mentioned earlier, the two manifolds have equations of identical form, which may be expressed as $c = C_{\mathcal{M}}(s; e_0, K_\mathrm{M}, \alpha)$ or $c = C_{\mathcal{M}}(s; \tilde{e}_0, \tilde{K}_\mathrm{S}, \tilde{\alpha})$. The rate equations of the MM mechanism on the slow manifold reduce to

$$\begin{aligned}\frac{\mathrm{d}s}{\mathrm{d}t} &= -k_1 s\big[e_0 - C_{\mathcal{M}}(s; e_0, K_\mathrm{M}, \alpha)\big] \\ &\quad + k_{-1} C_{\mathcal{M}}(s; e_0, K_\mathrm{M}, \alpha) \\ \frac{\mathrm{d}p}{\mathrm{d}t} &= k_2 C_{\mathcal{M}}(s; e_0, K_\mathrm{M}, \alpha)\end{aligned}$$

Similarly, for the H mechanism,

$$\begin{aligned}\frac{\mathrm{d}s}{\mathrm{d}t} &= -\big(\tilde{k}_1 + \tilde{k}_2\big)\big[\tilde{e}_0 - C_{\mathcal{M}}(s; \tilde{e}_0, \tilde{K}_\mathrm{S}, \tilde{\alpha})\big] \\ &\quad + \tilde{k}_{-1} C_{\mathcal{M}}(s; \tilde{e}_0, \tilde{K}_\mathrm{S}, \tilde{\alpha}) \\ \frac{\mathrm{d}p}{\mathrm{d}t} &= \tilde{k}_2 s\big[\tilde{e}_0 - C_{\mathcal{M}}(s; \tilde{e}_0, \tilde{K}_\mathrm{S}, \tilde{\alpha})\big]\end{aligned}$$

Suppose that we know ε_S and ε_P. Then, substituting into (22), the observable outputs y and $\tilde{y}$ satisfy the expressions

$$\begin{aligned}\frac{\mathrm{d}y}{\mathrm{d}t} &= -\varepsilon_\mathrm{S} k_1 s\big[e_0 - C_{\mathcal{M}}(s; e_0, K_\mathrm{M}, \alpha)\big] \\ &\quad + C_{\mathcal{M}}(s; e_0, K_\mathrm{M}, \alpha)(k_{-1}\varepsilon_\mathrm{S} + k_2\varepsilon_\mathrm{P}) \\ \frac{\mathrm{d}\tilde{y}}{\mathrm{d}t} &= s\big[\tilde{e}_0 - C_{\mathcal{M}}(s; \tilde{e}_0, \tilde{K}_\mathrm{S}, \tilde{\alpha})\big]\big[-\varepsilon_\mathrm{S}(\tilde{k}_1 + \tilde{k}_2) + \varepsilon_\mathrm{P}\tilde{k}_2\big] \\ &\quad + \tilde{k}_{-1}\varepsilon_\mathrm{S} C_{\mathcal{M}}(s; \tilde{e}_0, \tilde{K}_\mathrm{S}, \tilde{\alpha})\end{aligned}$$

If higher-order approximations for the slow manifolds are calculated, matching the mechanisms requires:

$$\begin{aligned}&e_0 = \tilde{e}_0, \quad K_\mathrm{M} = \tilde{K}_\mathrm{S}, \quad \alpha = \tilde{\alpha} \\ &k_1\varepsilon_\mathrm{S} = \varepsilon_\mathrm{S}\big(\tilde{k}_1 + \tilde{k}_2\big) - \tilde{k}_2\varepsilon_\mathrm{P}, \quad \text{and} \\ &k_{-1}\varepsilon_\mathrm{S} + k_2\varepsilon_\mathrm{P} = \tilde{k}_{-1}\varepsilon_\mathrm{S}\end{aligned}$$

Note that there are five linearly independent conditions and only four free parameters (e_0, k_1, k_{-1} and k_2). Accordingly, we cannot match the two observable outputs for all time, and therefore the reactions may be kinetically distinguishable. This is consistent with the earlier work of Roussel and Fraser [13] and Roussel [12], where they found that the MM and H reaction mechanisms are distinguishable if both the substrate and product are measured in the observable output of progress curve experiments.

The above result holds if we know ε_S and ε_P a priori and are thus not allowed to use them as fitting parameters. If ε_S and ε_P are in fact unknown, we instead get the matching conditions

$$\begin{aligned}&e_0 = \tilde{e}_0, \quad K_\mathrm{M} = \tilde{K}_\mathrm{S}, \quad \alpha = \tilde{\alpha} \\ &k_1\varepsilon_\mathrm{S} = \tilde{\varepsilon}_\mathrm{S}\big(\tilde{k}_1 + \tilde{k}_2\big) - \tilde{k}_2\tilde{\varepsilon}_\mathrm{P}, \quad \text{and} \\ &k_{-1}\varepsilon_\mathrm{S} + k_2\varepsilon_\mathrm{P} = \tilde{k}_{-1}\tilde{\varepsilon}_\mathrm{S}\end{aligned}$$

These conditions may be satisfiable with physically realistic positive constants if:

$$\tilde{\varepsilon}_\mathrm{S}\big(\tilde{k}_1 + \tilde{k}_2\big) > \tilde{k}_2\tilde{\varepsilon}_\mathrm{P} \tag{40}$$

If this is not the case at the particular wavelength chosen, then the two models will be distinguishable. If inequality (40) is satisfied, we can choose

$$\begin{aligned}&e_0 = \tilde{e}_0, \quad k_1 = \frac{1}{\varepsilon_\mathrm{S}}\big[\tilde{\varepsilon}_\mathrm{S}(\tilde{k}_1 + \tilde{k}_2) - \tilde{k}_2\tilde{\varepsilon}_\mathrm{P}\big] \\ &k_{-1} = \frac{1}{\varepsilon_\mathrm{S}}\,\frac{\tilde{k}_{-1}}{\tilde{k}_1 + \tilde{k}_2}\big[\tilde{\varepsilon}_\mathrm{S}(\tilde{k}_1 + \tilde{k}_2) - \tilde{k}_2\tilde{\varepsilon}_\mathrm{P}\big] \\ &k_2 = \frac{1}{\varepsilon_\mathrm{S}}\,\frac{\tilde{k}_{-1}\tilde{k}_2}{\tilde{k}_1(\tilde{k}_1 + \tilde{k}_2)}\big[\tilde{\varepsilon}_\mathrm{S}(\tilde{k}_1 + \tilde{k}_2) - \tilde{k}_2\tilde{\varepsilon}_\mathrm{P}\big]\end{aligned}$$

and

$$\varepsilon_\mathrm{P} = \varepsilon_\mathrm{S}\frac{\tilde{k}_1\tilde{\varepsilon}_\mathrm{P}}{\tilde{\varepsilon}_\mathrm{S}(\tilde{k}_1 + \tilde{k}_2) - \tilde{k}_2\tilde{\varepsilon}_\mathrm{P}}$$

with the choice of ε_S being free.

3.3. *Transient kinetics experiments*

In transient kinetics experiments, measurements are made early during the pre-steady-state phase. Under typical in vitro conditions ($s_0 \gg e_0$), the substrate concentration will change little during this phase as the enzyme-substrate complex C accumulates toward its QSS value.

It is possible to show that the observable outputs of transient kinetics experiments are distinguishable, that is y cannot be superimposed on $\tilde{y}$ for all t, even if we are able to control the parameters of the dynamical system. In typical experiments, the initial substrate concentration and molar absorptivities are known. Thus,

$$y(0, \boldsymbol{p}) = \tilde{y}(0, \tilde{\boldsymbol{p}}) = \varepsilon_S s_0$$

For the MM reaction, we can easily obtain an equation for the evolution of the observable y, by substituting (13), (6) and the initial conditions $(s, c, p)(t = 0) = (s_0, 0, 0)$ into the derivative with respect to time of (22):

$$\dot{y}(0, \boldsymbol{p}) = -\varepsilon_S k_1 e_0 s_0 \tag{41}$$

Proceeding similarly for the H mechanism, we get:

$$\dot{\tilde{y}}(0, \tilde{\boldsymbol{p}}) = -\varepsilon_S \tilde{e}_0 s_0 \big[\tilde{k}_1 + \tilde{k}_2(1 - \varepsilon_P/\varepsilon_S)\big] \tag{42}$$

If $\varepsilon_P \lesssim \varepsilon_S$, then $\dot{y}$ and $\dot{\tilde{y}}$ have the same sign and can be matched:

$$k_1 e_0 = \tilde{e}_0 \big[\tilde{k}_1 + \tilde{k}_2(1 - \varepsilon_P/\varepsilon_S)\big] \tag{43}$$

On the other hand, if ε_P is significantly larger than ε_S, $\tilde{y}$ may increase at early times, a behaviour which is not allowed by Eq. (41). Here we have a case of conditional distinguishability: if $\dot{y} > 0$ at early times, then we can exclude the MM mechanism, whereas for $\dot{y} < 0$, we cannot falsify either of the above mechanisms.

This behaviour of course makes sense: if $\varepsilon_P \ll \varepsilon_S$, then essentially we are just measuring the substrate concentration, and distinguishing between the two mechanisms is impossible, as we have shown in Section 3.2.1. On the other hand, if $\varepsilon_P \gg \varepsilon_S$, y is a signal proportional to the product concentration and distinguishability is guaranteed. Intermediate cases may or may not be distinguishable, depending on the sign of $\dot{y}$, which depends on the sizes of both the absorptivities and the rate constants. Obviously, if there are random errors in the measurements, the problem becomes more difficult in these intermediate cases.

4. Discussion

The primary purpose of this paper is to illustrate an approach for determining whether or not two reaction mechanisms are distinguishable in a particular experimental context. The approach involves constructing, if possible, a smooth mapping between the two candidate models. It was shown in [29] that if either of the mechanisms satisfies an observability criterion, then such a transformation always exists when the models are indistinguishable from their experimentally observable outputs.

We focussed our attention on the classical example of the two rival models of simple enzyme catalysis: the MM and the H mechanisms. Using the structural indistinguishability approach outlined in Appendix B, we find that it is possible to distinguish between the MM and H mechanisms if the reaction under consideration is studied using progress curves or initial transient kinetics experiments, and the reaction observable output measures both the substrate and the product concentrations or only the product concentration. In the progress curve experiments, if we know the molar absorptivities of the observable outputs and measure the mixed (substrate and product) signal (22), then we can distinguish between the two models. This is in agreement with previous work by Roussel and Fraser [13] and Roussel [12]. However, if the substrate and product absorptivities are unknown, we may not be able to distinguish between them, depending on whether or not inequality (40) is satisfied. Similarly, for transient kinetics experiments, distinguishability between the two mechanisms depends on the molar absorptivities of the substrate and product. Thus, our ability to distinguish between models will, in many cases, depend critically on details of what is measured and of what we know about an experimental system a priori. Similar comments have been made by Czerlinski [23] with regard to the use of data from relaxation experiments to distinguish between the Henri and MM mechanisms.

In the biological sciences it is becoming increasingly common to collect data in high-throughput experiments on genomic, proteomic, and metabolomic scales. These data hold the promise of identifying the mechanisms of interactions that comprise large-scale regulatory biochemical networks. Most of the work in this area is focussed on the development of mathematical and computational techniques for the reconstruction of the reaction mechanisms [2]. Unfortunately, these sorts of inverse problems do not afford a unique solution, and little attention has been paid to the development of systematic and comprehensive approaches for distinguishing between mechanisms [22]. The method presented in this paper provides a solution to this problem, because it can be applied to more complex reactions to falsify mechanisms by designing specific experiments.

Acknowledgements

The authors would like to thank Prof. Philip K. Maini (University of Oxford) for his critical comments. S.S. was funded by the Research Training Fellowship Programme in Mathematical Biology (Grant No. 069155) of the Wellcome Trust (London). N.D.E. and

M.J.C. gratefully acknowledge the support of the Engineering and Physical Sciences Research Council (UK) under Grant GR/R70354.

Appendix A. Biography of Victor Henri

Victor Henri was born in Marseilles from Russian parents in 1872. He went to school at St Petersburg and later studied at the Universities of Paris, Göttingen, and Leipzig, taking the degree of PhD at Göttingen and later 'Docteur ès sciences' at Paris. He was an academic of extraordinary genius who published over 500 papers in such diverse disciplines as psychology, physiology, biochemistry and physical chemistry. In 1894, he published his first book entitled *Introduction à la psychologie expérimentale* in collaboration with A. Binet, J. Courtier and J. Philippe. His last book was *Chimie générale*. While his contributions have been considered of the first rank in several fields, his name is not better known because he worked in quite different fields and never over self-advertised his work. In 1939, he placed himself at the disposal of the French government to take up war-related scientific research. He died of natural causes at La Rochelle in 1940 after his unit was evacuated from Paris ahead of the advancing German forces. For more details, the readers can consult the book of Boyde [6, Chapter 7].

Appendix B. Structural indistinguishability between reaction schemes

An important question that needs to be addressed in experimental sciences is the structural identifiability problem of whether the unknown parameters of a postulated model are uniquely determined by the output structure of the model corresponding to the experimental design [30]. Structural indistinguishability for nonlinear systems generalises this problem to one of determining the uniqueness between a pair of possible candidates for the model (or mechanism) structure [29]. More formally, suppose that the following two (parameterised) model structures, $\Sigma(\boldsymbol{p})$ and $\tilde{\Sigma}(\tilde{\boldsymbol{p}})$, are proposed for a given chemical process:

$$\Sigma(\boldsymbol{p})\begin{cases}\dot{\boldsymbol{x}}(t,\boldsymbol{p}) = \boldsymbol{f}(\boldsymbol{x}(t,\boldsymbol{p}),\boldsymbol{p})\\ \boldsymbol{x}(0,\boldsymbol{p}) = \boldsymbol{x}_0(\boldsymbol{p})\\ \boldsymbol{y}(t,\boldsymbol{p}) = \boldsymbol{h}(\boldsymbol{x}(t,\boldsymbol{p}),\boldsymbol{p})\end{cases} \tag{B.1}$$

$$\tilde{\Sigma}(\tilde{\boldsymbol{p}})\begin{cases}\dot{\tilde{\boldsymbol{x}}}(t,\tilde{\boldsymbol{p}}) = \tilde{\boldsymbol{f}}(\tilde{\boldsymbol{x}}(t,\tilde{\boldsymbol{p}}),\tilde{\boldsymbol{p}})\\ \tilde{\boldsymbol{x}}(0,\tilde{\boldsymbol{p}}) = \tilde{\boldsymbol{x}}_0(\tilde{\boldsymbol{p}})\\ \tilde{\boldsymbol{y}}(t,\tilde{\boldsymbol{p}}) = \tilde{\boldsymbol{h}}(\tilde{\boldsymbol{x}}(t,\tilde{\boldsymbol{p}}),\tilde{\boldsymbol{p}})\end{cases} \tag{B.2}$$

where $\boldsymbol{p} \in \Omega$, an open subset of $\mathbb{R}^q$, and $\tilde{\boldsymbol{p}} \in \tilde{\Omega}$, an open subset of $\mathbb{R}^{\tilde{q}}$, are constant parameter vectors. The sets Ω and $\tilde{\Omega}$ denote the sets of admissible parameter vectors for the two models (B.1) and (B.2), respectively. The vectors $\boldsymbol{x}(t,\boldsymbol{p})$ and $\tilde{\boldsymbol{x}}(t,\tilde{\boldsymbol{p}})$ are comprised of the state variables for each model, which are the different species concentrations (s, c, etc.) whose values are governed by the system of differential equations comprising the model, (B.1) and (B.2), respectively. These kinetics, and hence the solutions $\boldsymbol{x}(t,\boldsymbol{p})$ and $\tilde{\boldsymbol{x}}(t,\tilde{\boldsymbol{p}})$, are dependent on the particular parameter vectors $\boldsymbol{p} \in \Omega$ and $\tilde{\boldsymbol{p}} \in \tilde{\Omega}$ used in the models. It is assumed that there exists an open connected set M such that, for any $\boldsymbol{p} \in \Omega$, $\boldsymbol{f}(\cdot,\boldsymbol{p})$ is analytic on M and $\boldsymbol{x}(t,\boldsymbol{p}) \in M$ for all $t \geqslant 0$. In addition, given any $\boldsymbol{x} \in M$, $\boldsymbol{f}(\boldsymbol{x},\cdot)$ is analytic on Ω. Similar assumptions are made for $\tilde{\boldsymbol{f}}$ and the reaction scheme $\tilde{\Sigma}$.

The indistinguishability problem arises because, in general, it is not possible to measure all reactants in a given chemical reaction. An experiment that is used to collect measurements of the process gives rise to an output structure for the model. The analytic functions $\boldsymbol{h}(\boldsymbol{x}(t,\boldsymbol{p}),\boldsymbol{p})$ and $\tilde{\boldsymbol{h}}(\tilde{\boldsymbol{x}}(t,\tilde{\boldsymbol{p}}),\tilde{\boldsymbol{p}})$ determine for what combinations of state variables data are to be collected. The resulting output, or measurement, vectors are $\boldsymbol{y}(t,\boldsymbol{p}) = (y_1(t,\boldsymbol{p}),\ldots,y_r(t,\boldsymbol{p}))^{\mathrm{T}}$ and $\tilde{\boldsymbol{y}}(t,\tilde{\boldsymbol{p}}) = (y_1(t,\tilde{\boldsymbol{p}}),\ldots,y_r(t,\tilde{\boldsymbol{p}}))^{\mathrm{T}}$, respectively, and it is these vectors that are compared with the collected experimental data during subsequent parameter estimation.

Suppose that there exists a $\boldsymbol{p} \in \Omega$ and a $\tilde{\boldsymbol{p}} \in \tilde{\Omega}$ such that $\boldsymbol{y}(t,\boldsymbol{p}) = \tilde{\boldsymbol{y}}(t,\tilde{\boldsymbol{p}})$ for all $t \geqslant 0$. Then it is not possible to distinguish between the model given by (B.1) with parameter vector $\boldsymbol{p}$ (i.e., $\Sigma(\boldsymbol{p})$) and the model given by (B.2) with parameter vector $\tilde{\boldsymbol{p}}$ (i.e., $\tilde{\Sigma}(\tilde{\boldsymbol{p}})$) from their outputs. Therefore, even with perfect data – continuous measurements that are noise-free and error-free – it is not possible to distinguish between the reaction schemes modelled by $\Sigma(\boldsymbol{p})$ and $\tilde{\Sigma}(\tilde{\boldsymbol{p}})$ from the proposed experiment. In this case the models $\Sigma(\boldsymbol{p})$ and $\tilde{\Sigma}(\tilde{\boldsymbol{p}})$ are said to be *output indistinguishable*, which is written as $\Sigma(\boldsymbol{p}) \sim_{\mathrm{o}} \tilde{\Sigma}(\tilde{\boldsymbol{p}})$.

Output indistinguishability refers to a specific pair of candidate schemes for a reaction with a corresponding pair of parameter vectors. Of more interest is a *structural* property relating the two model structures for arbitrary parameter vectors, except for some degenerate set, i.e., those belonging to a subset of a closed set of (Lebesgue) measure zero. Therefore, the schemes Σ and $\tilde{\Sigma}$ are said to be *structurally indistinguishable*, written $\Sigma \sim_{\mathrm{s}} \tilde{\Sigma}$, if for generic $\boldsymbol{p} \in \Omega$ there exists a $\tilde{\boldsymbol{p}} \in \tilde{\Omega}$ such that $\Sigma(\boldsymbol{p}) \sim_{\mathrm{o}} \tilde{\Sigma}(\tilde{\boldsymbol{p}})$; and for generic $\tilde{\boldsymbol{p}} \in \tilde{\Omega}$ there exists a $\boldsymbol{p} \in \Omega$ such that $\Sigma(\boldsymbol{p}) \sim_{\mathrm{o}} \tilde{\Sigma}(\tilde{\boldsymbol{p}})$.

Corollary 4 in Evans et al. [29] provides the following result, which allows testing for structural indistinguishability between two candidate reaction schemes.

Theorem 1 [29]. *Let $\boldsymbol{p} \in \Omega$ and $\tilde{\boldsymbol{p}} \in \tilde{\Omega}$. If there exist a neighbourhood $\tilde{V}_{\tilde{\boldsymbol{p}}}$ of $\tilde{\boldsymbol{x}}_0(\tilde{\boldsymbol{p}})$ and a smooth map $\boldsymbol{\lambda}$, defined on $\tilde{V}_{\tilde{\boldsymbol{p}}}$, such that:*

$$\boldsymbol{\lambda}\big(\tilde{\boldsymbol{x}}_0(\tilde{\boldsymbol{p}})\big) = \boldsymbol{x}_0(\boldsymbol{p}) \tag{B.3}$$

$$\boldsymbol{f}\big(\boldsymbol{\lambda}(\tilde{\boldsymbol{x}}), \boldsymbol{p}\big) = \frac{\partial \boldsymbol{\lambda}}{\partial \tilde{\boldsymbol{x}}}(\tilde{\boldsymbol{x}})\tilde{\boldsymbol{f}}(\tilde{\boldsymbol{x}}, \tilde{\boldsymbol{p}}) \tag{B.4}$$

$$\boldsymbol{h}\big(\boldsymbol{\lambda}(\tilde{\boldsymbol{x}}), \boldsymbol{p}\big) = \tilde{\boldsymbol{h}}(\tilde{\boldsymbol{x}}, \tilde{\boldsymbol{p}}) \tag{B.5}$$

for all $\tilde{\boldsymbol{x}} \in \tilde{V}_{\tilde{\boldsymbol{p}}}$, then $\Sigma(\boldsymbol{p}) \sim_{\mathrm{o}} \tilde{\Sigma}(\tilde{\boldsymbol{p}})$.

To show that the two reaction schemes Σ and $\tilde{\Sigma}$ are structurally indistinguishable using this result, it is sufficient to show that the following conditions are satisfied:

(1) for generic $\boldsymbol{p} \in \Omega$, there exists a triple $(\tilde{\boldsymbol{p}}, \tilde{V}_{\tilde{\boldsymbol{p}}}, \boldsymbol{\lambda})$ satisfying Theorem 1;
(2) for generic $\tilde{\boldsymbol{p}} \in \tilde{\Omega}$, there exists a $\boldsymbol{p} \in \Omega$ and a pair $(\tilde{V}_{\tilde{\boldsymbol{p}}}, \boldsymbol{\lambda})$ such that Theorem 1 is satisfied.

Since it is only necessary to show the existence of a smooth map $\boldsymbol{\lambda}$, different classes of functions can be considered in order of increasing complexity. For example, it may be possible to obtain a result restricting $\boldsymbol{\lambda}$ to lie in the class of invertible linear mappings. Failure to obtain a result using Theorem 1 does not necessarily imply that the reaction schemes are distinguishable, and further tests may need to be applied. (One such test is provided by Theorem 3 in [29].)

References

[1] B.G. Cox, Modern Liquid Phase Kinetics, Oxford University Press, Oxford, 1994.
[2] E.J. Crampin, S. Schnell, P.E. McSharry, Mathematical and computational techniques to deduce complex biochemical reaction mechanisms, Prog. Biophys. Mol. Biol. 86 (2004) 77–112.
[3] P. Érdi, J. Tóth, Mathematical Models of Chemical Reactions, Princeton University Press, Princeton, 1989.
[4] J.H. Espenson, Chemical Kinetics and Reaction Mechanisms, McGraw-Hill, Singapore, 1995.
[5] I.R. Epstein, J.A. Pojman, An Introduction to Nonlinear Chemical Dynamics: Oscillations, Waves, Patterns, and Chaos, Oxford University Press, Oxford, 1998.
[6] T.R.C. Boyde, Foundation Stones of Biochemistry, Voile et Aviron, Hong Kong, 1980.
[7] V. Henri, Lois générales de l'action des diastases, Hermann, Paris, 1903.
[8] V. Henri, Théorie générale de l'action de quelques diastases, C. R. H. Acad. Sci. Paris 135 (1902) 916–919; this paper is quoted variously in the literature as 1901 or 1902: the latter is the date appearing on the title page of the volume, but the individual issue concerned was dated 15 December 1901.
[9] L. Michaelis, M.L. Menten, Die kinetik der invertinwirkung, Biochem. Z. 49 (1913) 333–369.
[10] R.O. Viale, Similarities and differences in the kinetics of the Michaelis scheme and the Henri scheme, J. Theor. Biol. 27 (1970) 377–385.
[11] K. Hiromi, Kinetics of Fast Enzyme Reactions: Theory and Practice, Wiley, New York, 1979.
[12] M.R. Roussel, A rigorous approach to steady-state kinetics applied to simple enzyme mechanisms, PhD thesis, Department of Chemistry, University of Toronto, 1994.
[13] M.R. Roussel, S.J. Fraser, Accurate steady-state approximations: Implications for kinetics experiments and mechanism, J. Phys. Chem. 95 (1991) 8762–8770.
[14] J.E. Critchlow, H.B. Dunford, Studies on horseradish peroxidase. IX. Kinetics of the oxidation of *p*-cresol by compound II, J. Biol. Chem. 247 (1972) 3703–3713.
[15] F.M. Menger, Michaelis–Menten kinetics in the catalyzed solvolysis of tetrachlorophthalic anhydride, J. Am. Chem. Soc. 90 (1968) 4387–4389.
[16] D.M. Chipman, R. Yaniv, P. van Eikeren, Models for nicotinamide coenzymes. Isotope effect discrepancies in the reaction of dihydronicotinamides with trifluoroacetophenone are due to adduct formation, J. Am. Chem. Soc. 102 (1980) 3244–3246.
[17] S. Fukuzumi, J.K. Kochi, Transition-state barrier for electrophilic reactions. Solvation of charge-transfer ion pairs as the unifying factor in alkene addition and aromatic substitution with bromine, J. Am. Chem. Soc. 104 (1982) 7599–7609.
[18] S. Schnell, P.K. Maini, A century of enzyme kinetics. Reliability of the K_{M} and $v_{\max}$ estimates, Comments Theor. Biol. 8 (2003) 169–187.
[19] I. Prigogine, Remarque sur le principe de réciprocité d'Onsager et le couplage des réactions chimiques, Bull. Classe Sci. Acad. R. Belg. 32 (1946) 30–35.
[20] C. Walter, Steady-State Applications in Enzyme Kinetics, The Ronald Press Company, New York, 1965.
[21] C. Dutta, A. Dasgupta, J. Das, On thermodynamic and kinetic equivalence of chemical systems, Proc. Indian Acad. Sci. (Chem. Sci.) 93 (1984) 817–830.
[22] R. Hinch, S. Schnell, Mechanism equivalence in enzyme–substrate reactions: Distributed differential delay in enzyme kinetics, J. Math. Chem. 35 (2004) 253–264.
[23] G.H. Czerlinski, Chemical relaxation of cyclic enzyme systems. V. The Michaelis and Henri schemes, J. Theor. Biol. 32 (1971) 373–382.
[24] A.R. Fersht, Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding, Freeman, New York, 1999.
[25] B. Nölting, Protein Folding Kinetics: Biophysical Methods, Springer, Berlin, 1999.
[26] R. Penrose, On best approximation solutions of linear matrix equations, Proc. Camb. Phil. Soc. 51 (1956) 17–19.
[27] M.R. Roussel, S.J. Fraser, Geometry of the steady-state approximation: Perturbation and accelerated convergence methods, J. Chem. Phys. 93 (1990) 1072–1081.
[28] S. Schnell, P.K. Maini, Enzyme kinetics at high enzyme concentration, Bull. Math. Biol. 62 (2000) 483–499.
[29] N.D. Evans, M.J. Chappell, M.J. Chapman, K.R. Godfrey, Structural indistinguishability between uncontrolled (autonomous) nonlinear analytic systems, Automatica 40 (2004) 1947–1953.
[30] R. Bellman, K.J. Åström, On structural identifiability, Math. Biosci. 7 (1970) 329–339.