On the validity and errors of the pseudo-first-order kinetics in ligand–receptor binding

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Abstract

The simple bimolecular ligand–receptor binding interaction is often linearized by assuming pseudo-first-order kinetics when one species is present in excess. Here, a phase-plane analysis allows the derivation of a new condition for the validity of pseudo-first-order kinetics that is independent of the initial receptor concentration. The validity of the derived condition is analyzed from two viewpoints. In the first, time courses of the exact and approximate solutions to the ligand–receptor rate equations are compared when all rate constants are known. The second viewpoint assess the validity through the error induced when the approximate equation is used to estimate kinetic constants from data. Although these two interpretations of validity are often assumed to coincide, we show that they are distinct, and that large errors are possible in estimated kinetic constants, even when the linearized and exact rate equations provide nearly identical solutions.

Keywords: pseudo-first-order kinetics, ligand–receptor binding, experimental design, approximation validity, rate constant estimation, fitting procedure.

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1. Introduction

In biochemical kinetics, simplifying assumptions that decouple or reduce the order of rate equations for complex reaction mechanisms are ubiquitous. Aside from making theoretical analysis of complex reactions more tractable, order-reducing approximations can greatly simplify the interpretation of experimental data [1, 2]. Experiments performed under conditions that allow for linearization have historically been the preferred method for estimating equilibrium and rate constants because they allow for the isolation of a subset of the interactions [3, 4, 5]. For this reason, when designing an experiment, it is essential to know the necessary conditions for the simplifying assumptions to be valid. Significant theoretical work has been directed at deriving rigorous bounds for the validity of simplifying assumptions [6, 7, 8, 9, 10, 11], but this work often overlooks the manner in which the reduced models are used to interpret experimental results. In many cases, the simplified models are used to estimate equilibrium and rate constants from experimental data [12, 13, 14, for example]. Rarely is the validity of a simplifying assumption analyzed with this utility in mind. To examine how this viewpoint can affect the conditions for validity, we consider the simplest model for ligand–receptor binding with 1:1 stoichiometry [15].

In the simplest case, the binding of a ligand L to a receptor R is a bimolecular reversible association reaction with 1:1 stoichiometry yielding a ligand–receptor intermediate complex C :

where k_1 and k_{-1} are, respectively, the association and dissociation rate constants of the ligand–receptor complex. This reaction scheme is mathematically described by a system of coupled nonlinear second-order differential equations. By applying the law of mass action to reaction (1), we obtain

$$\frac{d[C]}{dt} = -\frac{d[R]}{dt} = -\frac{d[L]}{dt} = k_1([R][L] - K_S[C]). \quad (2)$$

In this system the parameter $K_S = k_{-1}/k_1$ is the equilibrium constant [15, 4] and the square brackets denote concentration. Since no catalytic processes are involved, the reaction is subject to the following conservation laws:

$$[R_0] = [R](t) + [C](t) \quad (3)$$

$$[L_0] = [L](t) + [C](t), \quad (4)$$

where $[R_0]$ and $[L_0]$ are the initial receptor and initial ligand concentrations. If the bimolecular reaction (1) is initiated far from the equilibrium and in the absence of ligand–receptor complex, the system (2) has the initial conditions at $t = 0$:

$$([L], [R], [C]) = ([L_0], [R_0], 0) . \quad (5)$$

We have expressed quantities in terms of concentration of species. These equations are frequently given in terms of binding site number, using the identity [15]

$$[C] = \left(\frac{n}{N_{AV}} \right) C , \quad (6)$$

where n is the cell density, N_{AV} is Avogadro’s number, and C denotes the number of ligand-bound receptors per cell. We use the concentration formulation here for clarity and without loss of generality.

The system (2) can be solved, subject to the conservation laws [16]. Substituting (3) and (4) into (2) we obtain:

$$\frac{d[C]}{dt} = k_1 \left(([R_0] - [C])([L_0] - [C]) - K_S [C] \right) . \quad (7)$$

We can rewrite this expression by factoring as follows:

$$\frac{d[C]}{dt} = k_1 ((\lambda_+ - [C])(\lambda_- - [C])) , \quad (8)$$

where

$$\lambda_{\pm} = \frac{(K_S + [R_0] + [L_0]) \pm \sqrt{(K_S + [R_0] + [L_0])^2 - 4[R_0][L_0]}}{2} . \quad (9)$$

This ordinary differential equation is readily solved subject to the initial conditions (5) as

$$[C](t) = \lambda_- \left(\frac{1 - \exp(-\frac{t}{t_C})}{1 - \frac{\lambda_-}{\lambda_+} \exp(-\frac{t}{t_C})} \right) , \quad (10)$$

with

$$t_C = \left[k_1 \sqrt{(K_S + [R_0] + [L_0])^2 - 4[R_0][L_0]} \right]^{-1} . \quad (11)$$

The quantity t_C is the timescale for significant change in $[C]$. In this particular case, t_C can be considered as the time required for the reaction to

reach steady-state. Solutions for $[R](t)$ and $[L](t)$ can now be constructed by substituting (10) into conservation laws (3) and (4).

Although there is a closed form solution for the reacting species of the simple bimolecular ligand–receptor interaction, experimental biochemists prefer to determine the kinetic parameters of the ligand–receptor binding using graphical methods [15]. One of the graphical methods commonly used consists of plotting the solution of the ligand association assuming no ligand depletion on a logarithmic scale with respect to time. Both the association and dissociation rate constants can be determined using this linear graphical method [17]. Similarly, if one seeks to avoid inaccuracies due to logarithmic fitting, nonlinear regression can be used to fit the kinetic data to a single exponential. However, the use of both of these methods has the disadvantage of making an assumption with respect to the relative concentrations of ligand and binding sites [16].

In the ligand–receptor interaction with 1:1 stoichiometry and no ligand depletion it is generally thought that, if the initial ligand concentration is much higher than the initial receptor concentration, i.e.

$$[L_0] \gg [R_0] , \quad (12)$$

the ligand concentration $[L]$ remains effectively constant during the course of the reaction, and only the receptor concentration $[R]$ changes appreciably with time [18, 19, 3, 4]. Since kinetic order with respect to time is the same as with respect to $[R]$, reaction (1) is said to follow *pseudo-first-order* (PFO) kinetics if the $[R]$ dependence is of first order. The rates of second-order reactions in chemistry are frequently studied within PFO kinetics [20, 21]. In the present case, the second-order reaction (1) becomes mathematically equivalent to a first-order reaction, reducing to

where $k_{\varphi} \equiv k_1[L_0]$ is the pseudo rate constant. This procedure is also known as the method of flooding [5]. The solution of the governing equations for a reaction linearized by PFO kinetics (or flooding) is straightforward, and is widely employed to characterize kinetics and fit parameters with the aid of progress curves. An error is however present due to the fact that, in actuality, the concentration of the excess reactant does not remain constant [20].

80 In 1961, Silicio and Peterson [20] made numerical estimates for the frac-
81 tional error in the observed PFO constant for second-order reactions. They
82 found that the the fractional error is less than 10% if the reactant concentra-
83 tion ratio, $[R_0] : [L_0]$ say, is tenfold. On the other hand, Corbett [22] found
84 that simplified expressions with the PFO kinetics can yield more accurate
85 data than is generally realized, even if only a twofold excess of one the reac-
86 tants is employed. For ligand–receptor dynamics, Weiland and Molinoff [16]
87 claim that the PFO simplification is acceptable if experimental conditions are
88 such that less than 10% of the ligand is bound. These results indicate that
89 the conditions whereby a second-order ligand–receptor reaction is reduced to
90 first order remain to be well-established.

91 It is widely believed that second-order reactions can be studied by PFO
92 kinetics using progress curves only when the excess concentration of one of
93 the reactants is large [21, 5, for example]. However, contrary to the widely
94 established knowledge, Schnell and Mendoza [10] have found that the con-
95 dition for the validity of the PFO in the single substrate, single enzyme
96 reaction does not require an excess concentration of one of the reactant with
97 respect to the other. In the present work, we derive the conditions for the
98 validity of the PFO approximation in the simple ligand–receptor interaction.
99 Additionally, we show two fundamentally different methods of assessing the
100 validity of the approximation. The first compares the exact and approximate
101 solutions to the rate equations under identical conditions. The second mea-
102 sures the veracity of parameters estimated by fitting the approximate model
103 to data. Although these two measures of validity are generally assumed to
104 coincide, we show that they are quantitatively and qualitatively distinct. In
105 Section 2 the reduction of the ligand–receptor association by PFO kinetics is
106 summarized followed by its dynamical analysis in Section 3. The new valid-
107 ity condition is derived in Section 4, and an analysis of the errors observed
108 with the PFO kinetics is presented in Section 5. This is followed by a brief
109 discussion (Section 6).

110 2. The governing equations of the ligand–receptor dynamics with 111 no ligand depletion

112 In ligand–receptor dynamics with 1:1 stoichiometry and no ligand deple-
113 tion, the second-order ligand–receptor interaction in reaction (1) is neglected
114 when condition (12) holds; the reaction effectively becomes first order since
115 the concentration of the reactant in excess is negligibly affected. This is

equivalent to assuming that

$$[L_0] - [C] \approx [L_0] . \quad (14)$$

The alternative case, in which the depletion of the receptor is assumed to be negligible, is shown to be symmetric in Appendix A. By substituting (14) into (7), the equation can be simplified as follows:

$$\frac{d[C]}{dt} = k_1 \left([R_0][L_0] - (K_S + [L_0])[C] \right) . \quad (15)$$

Note that this equation can also be obtained by applying the law of mass action to reaction scheme (13). The solution for (15) with the conservation laws (4) and (5) can be obtained by direct integration [23]:

$$[C](t) = \frac{[R_0][L_0]}{K_S + [L_0]} \left(1 - \exp \left(-(k_\varphi + k_{-1})t \right) \right) . \quad (16)$$

Note that expressions for $[R](t)$ and $[L](t)$ can again be obtained by substituting (16) into (3) and (4), respectively.

Experimentally, the clear advantage of applying the pseudo-first-order kinetics to the ligand–receptor reaction is that, as shown by equation (16), it provides solutions that can be linearized by using a logarithmic scale to fit progress curves of the interacting species and thus it could lead to complete reaction characterization, namely the rate constants k_1 and k_{-1} .

As we have previously pointed out, it has been assumed that the condition $[L_0] \gg [R_0]$ implies

$$[L](t) = [L_0] - [C](t) \approx [L_0] \quad \Rightarrow \quad \left| \frac{[C]}{[L_0]} \right|_{\max} \ll 1 . \quad (17)$$

Up to this point, most of the scientists using the PFO kinetics assume that it is reasonable to overestimate the maximum complex concentration when there is a ligand excess, because all receptor molecules could instantaneously combine with ligand molecules, i.e. $[C]_{\max} = [R_0]$. However, this simplification is unrealistic from the biophysical chemistry point of view as it will assume that in the conservation law (4) all ligand molecules are only in one form: the free ligand [see, equation (17)]. In the next section, we obtain a more a reliable estimate of $[C]_{\max}$ by studying the geometry of the phase plane of system (2). This will permit us to make a better estimate of the conditions for the validity of the PFO ligand–receptor dynamics (1).

142 3. Phase-plane analysis leads to conditions for the validity of the 143 pseudo-first-order kinetics

144 The phase-plane trajectories of system (2) are determined by the ratio of
145 $d[C]/dt$ to $d[L]/dt$:

$$\frac{d[C]}{d[L]} = -1 . \quad (18)$$

146 This expression is integrated to obtain the family of solution curves:

$$[C]([L]) = -[L] + m , \quad (19)$$

where

$$m = \begin{cases} [C_0] & \text{for } ([C], [L]) = (0, [L_0]) \text{ at } t = 0 \\ [L_0] & \text{for } ([C], [L]) = ([C_0], 0) \text{ at } t = 0 . \end{cases}$$

147 The use of $[L_0]$ as the constant in (19) for initial conditions on the horizontal
148 axis follows from the relation $d[C]/dt = -d[L]/dt$.

149 The phase plane is divided into two regions by the nullcline

$$[C]([L]) = \frac{[R_0][L]}{K_S + [L]} , \quad (20)$$

150 obtained by setting $d[C]/dt = 0$ or $d[L]/dt = 0$ in (2). Note that for $[L] = K_S$
151 (equilibrium constant), $[C] = [R_0]/2$. The nullcline converges at large ligand
152 concentrations because

$$\lim_{[L] \rightarrow \infty} [C]([L]) = [R_0] . \quad (21)$$

153 The phase plane trajectories (19) and its nullcline (20) are show in Fig. 1.
154 The trajectory flow is attracted by a unique curve, which is a stable manifold
155 and is equivalent to the nullcline for this case. All trajectories tend to this
156 manifold as they approach the steady state as $t \rightarrow \infty$ [24].

157 Binding of ligand to cell surface receptors has been amenable to *in vitro*
158 experimental investigation for the past four decades [25]. In the typical exper-
159 imental approach, isolated membranes possessing free receptors are studied
160 using ligands as pharmaceutical agents [26]. The reaction mixture is free
161 of ligand-receptor complex at the beginning of the experiment, that is the
162 initial conditions are like those stated in (5). It is important to note that
163 for a ligand-receptor interaction with trajectories departing from the positive

Figure 1: **Phase-plane behavior of the ligand-receptor reaction (1).** The solid curves with arrows are the trajectories in the phase planes, which are described by (19). The trajectories tend to a stable manifold as they approach to the steady-state. In this case, the manifold is the nullcline (20) of the system, which converges to $[R_0]$ for large ligand concentrations.

horizontal axis, i.e. with initial conditions (5), the trajectories are bounded
by

$$0 \leq [C](L) \leq [C]^* \quad \text{for} \quad ([L], [C])(t=0) = ([L_0], 0), \quad (22)$$

where $[C]^*$ is the ligand-receptor complex concentration at the steady-state, and is equivalent to the maximum ligand-receptor complex concentration ($[C]_{\max}$) that the trajectories can reach if they depart from the positive horizontal axis. $[C]^*$ can be estimated from the intersection of (19) and (20) or by estimating the steady-state value of the ligand-receptor complex concentration in (10), that is

$$[C]^* = \lim_{t \rightarrow \infty} [C](t) = \lim_{t \rightarrow \infty} \left[\lambda_- \left(\frac{1 - \exp(-\frac{t}{t_C})}{1 - \frac{\lambda_-}{\lambda_+} \exp(-\frac{t}{t_C})} \right) \right] = \lambda_- , \quad (23)$$

where λ_- is given by (9).

It suffices therefore to investigate the behavior of the ratio of the solution (10) at steady-state to $[L_0]$, which we do in the next section.

175 4. Derivation of a new sufficient condition for the validity of the 176 pseudo-first-order kinetics

177 To derive a mathematical expression in terms of the kinetic parameters
178 for condition (17), we will use the fact that, for initial conditions given by
179 (22), $[C]_{max}$ is the concentration given by allowing the reaction described
180 by (10) to go to steady-state. We can now formulate (17) as follows:

$$\frac{(K_S + [R_0] + [L_0]) - \sqrt{(K_S + [R_0] + [L_0])^2 - 4[R_0][L_0]}}{2[L_0]} \ll 1 . \quad (24)$$

181 This can be rewritten as

$$\left(\frac{(K_S + [R_0] + [L_0])}{2[L_0]} \right) \left(1 - \sqrt{1 - r} \right) \ll 1 , \quad (25)$$

with

$$r = \frac{4[R_0][L_0]}{(K_S + [R_0] + [L_0])^2} .$$

182 At this point it is convenient to nondimensionalize the above expression
183 by using reduced concentrations. Scaling with respect to K_S , equation (25)
184 becomes

$$\left(\frac{(1 + [R'_0] + [L'_0])}{2[L'_0]} \right) \left(1 - \sqrt{1 - r'} \right) \ll 1 , \quad (26)$$

185 where

$$r' = \frac{4[R'_0][L'_0]}{(1 + [R'_0] + [L'_0])^2} , \quad \text{with} \quad [R'_0] = \frac{[R_0]}{K_S} \quad \text{and} \quad [L'_0] = \frac{[L_0]}{K_S} . \quad (27)$$

186 Quadratic expressions similar to (26) are common in chemical kinetics. For
187 practical use in the analysis of chemical kinetics experiments, quadratic ex-
188 pressions are generally replaced with simpler expressions. Noting that

$$r' = \frac{4[R'_0][L'_0]}{(1 + [R'_0] + [L'_0])^2} \ll 1 \quad (28)$$

189 for any value of $[R'_0]$ and $[L'_0]$ (for more details, see Appendix B), we can then
190 calculate a Taylor series expansion of (26) to obtain right-hand factor of

$$\left(1 - \sqrt{1 - r'} \right) = \frac{1}{2}r' + \frac{1}{8}r'^2 + O(r'^3) \approx \frac{1}{2}r' , \quad (29)$$

191 which simplifies (26) to

$$\frac{[R'_0]}{1 + [R'_0] + [L'_0]} \ll 1 . \quad (30)$$

192 This is a simple analytical expression for the condition for the validity of
193 PFO kinetics. Note that condition (30) is valid for

$$\begin{aligned} \text{(a)} \quad & [L'_0] \gg [R'_0] , \text{ or} \\ \text{(b)} \quad & [L'_0] \leq [R'_0] , \text{ if } [R'_0] \ll 1 . \end{aligned} \quad (31)$$

194 Interestingly, we have a new condition for the use of the PFO approx-
195 imation in ligand–receptor binding with 1:1 stoichiometry. Equation (31a)
196 is the constraint (12) already in widespread use. However, equation (31b)
197 extends the range of conditions under which PFO dynamics may be applied.
198 The regions of validity of the PFO approximation are illustrated graphically
199 in Fig. 2 by plotting conditions (30) and (31) in the space of initial ligand
200 concentrations, $[L_0]$, and equilibrium constant, K_S , normalized by $[R_0]$. Typ-
201 ically, PFO kinetics are assumed valid when the ratio of $[L_0]:[R_0]$ is greater
202 than 10:1 [20, 3, 27] Applying this same “rule of thumb”, we set the left-hand
203 side of (30) equal to 0.1 to separate valid from non-valid regions in Fig. 2.
204 Note that the plane is divided into three regions by lines corresponding to
205 condition (30) and the generally used condition (12). Region B comprises
206 a portion of the space where PFO kinetics were previously assumed to be
207 invalid, but in fact the errors introduced by the approximation in this region
208 are expected to be small, even for initial conditions such that $[L_0] \approx [R_0]$.

209 5. There are two types of errors observed with the application of 210 approximations in reaction kinetics

211 In reaction kinetics, there are two type of errors that can be committed
212 when applying an approximation to the governing equations of a complex
213 reaction. Research in mathematical chemistry and biology primarily focuses
214 on estimates of errors in the concentrations of reacting and product species.
215 This error – which we name *concentration error* – is commonly evaluated
216 by calculating the difference between the solution of the approximate equa-
217 tion (16) with that of the exact equation (10) (see, for example, [28]). The
218 concentration error provides a measure of how closely the approximate solu-
219 tion matches the exact solution. However, experimentally, PFO approxima-
220 tions are often used to derive expressions that facilitate estimating kinetic

Figure 2: **Validity regions in the $[L_0]/[R_0]$ – $K_S/[R_0]$ log-log plane for the use of pseudo-first-order kinetics to model ligand–receptor reaction (1).** The dashed line indicates $10[R_0] = [L_0]$, the lower line is $9[R_0] = [L_0] + K_S$. In region A, $[R_0] \gg [L_0]$, where PFO kinetics has here been shown to be valid, as previously thought. In region B, $[R_0]$ does not greatly exceed $[L_0]$, but $K_S \gg [R_0]$. Here we have shown that PFO kinetics holds, even for $[L_0] \approx [R_0]$. In region C, where $[R_0]$ does not greatly exceed $[L_0]$ and $K_S/[R_0]$ is not much greater than 1, PFO kinetics is not valid.

221 constants using nonlinear regression methods. Therefore, of particular utility
 222 are error estimates from fitting kinetic parameters using the mathematical
 223 expression derived with the PFO approximation. We called these *estimation*
 224 *errors*.

225 Naively, it would seem that if the difference between the complex concen-
 226 tration, $C(t)$, is small between the PFO approximation and exact solution,
 227 then the PFO equation should provide accurate estimates of the rate con-

228 stants when used to model experimental data. This, however, is not neces-
 229 sarily true in general. To better understand why the concentration and esti-
 230 mation errors are not the same, and to show where they diverge, we compare
 231 errors based on the numerical difference between the exact and PFO models
 232 with those based on estimating rates constants using the PFO model.

Figure 3: Time course of species concentrations and concentration errors of pseudo-first-order approximation for the ligand-receptor reaction (1). Panels (a)-(c) show the concentration of the complex as a function of time for the cases: (a) $[L_0] = 10[R_0]$ and $K_S = [R_0]$, (b) $[L_0] = [R_0]$ and $K_S = 30[R_0]$, (c) $[L_0] = [R_0]$ and $K_S = [R_0]$. The dashed lines correspond to calculations assuming pseudo-first-order kinetics, while solid lines are exact solutions. (d)-(f) show the errors induced by assuming pseudo-first-order kinetics for case (a)-(c), respectively.

233 5.1. Analysis of the concentration error

234 Theoretically we define a concentration error measure as

$$\text{CE}(t) = \left| \frac{[C]_{\text{exact}}(t) - [C]_{\text{PFO}}(t)}{C_{\text{exact}}(t)} \right|. \quad (32)$$

235 For the bimolecular ligand-receptor binding (1), we can calculate analytically
 236 the above expression by replacing $[C]_{\text{exact}}(t)$ with (10), and $[C]_{\text{PFO}}(t)$ with
 237 (16). However, this expression is too cumbersome, and hence we present a

numerical analysis of the concentration error. Fig. 3 presents the time course of the exact and approximate complex concentration [Fig. 3(a)–(c)], and a calculation of the percentage concentration error ($100 \times \text{CE}$) [Fig. 3(d)–(f)] introduced by the PFO approximation for initial conditions lying in region A, B and C of Fig. 2, respectively. The error remains less than 3% over the course of the reaction for the cases satisfying condition (30), and approaches 30% for the point in region C.

Figure 4: **Maximum and steady-state concentration errors for the ligand–receptor reaction (1).** Panel (a) shows a heat map of the maximum concentration error incurred by assuming pseudo-first-order approximation for different initial conditions. Coloring corresponds to the error as defined in (32). Similarly, Panel (b) shows the steady-state concentration error between the exact and pseudo-first-order solutions. The black lines correspond to condition (30) when the left-hand side is equal to 10.

It is useful to define a scalar measure based on (32). For this there are many options, yet in order to remain as conservative as possible, we choose the maximum value of concentration error over the time course of the reaction, which we call the *maximum concentration error*. Additionally, we calculate the *steady-state concentration error*, defined as $\lim_{t \rightarrow \infty} \text{CE}(t)$. Fig. 4 shows the maximum and steady-state concentration error contours for initial conditions in the $[L_0]$ – K_S plane. In general, the maximum concentration error is well-described by the newly-derived condition. The error contours allow for a quantification of what is meant by “much less than”. The commonly used requirement that $[L_0] \geq 10[R_0]$ produces errors always

less than 5%. In fact, when K_S is less than $[R_0]$, a ratio of ligand to receptor of approximately 4:1 is enough to constrain the error to below 5%. Although the condition for the validity is symmetric in $[L_0]$ and $[K_S]$, the errors are not. The ratio of $K_S:[R_0]$ must be approximately 20:1 for the error to remain below 5%. This is not surprising since the exact solution is not symmetric in $[L_0]$ and K_S , so we should expect that the two quantities would have different effects. Nevertheless, the notion that PFO kinetics can rightly be assumed even when the ligand is not present in excess holds true.

5.2. Analysis of the estimation error

Next, we calculate the error in estimated rate constants by generating sample data using the exact solution. The frequency of the sampling, ω_{obs} , and the time span of the sampling window, t_{obs} , are varied. Values of ω_{obs} range from t_c^{-1} to $4t_c^{-1}$. Higher sampling frequencies were also tested, but results are not presented as they did not show appreciable difference from the case of $\omega_{obs} = 4t_c^{-1}$. The sampling windows tested begin at $t = 0$ and continue for $t_{obs} = 3t_c, 10t_c, 100t_c$. An “experimental protocol” for a numerical experiment then consists of choosing specific ω_{obs} and t_{obs} , and using (10) to calculate $[C](n\omega_{obs}^{-1})$ for integers $n \in [0, t_{obs}\omega_{obs}]$. For each simulated data set (for which there is no experimental error), the rate constants k_1 and k_{-1} are estimated by fitting the data with equation (16) using the Levenberg–Marquardt algorithm as implemented in SciPy (version 0.17.0, <http://www.scipy.org>) with initial estimates for k_1 and k_{-1} equal to the values used to generate the data. We then define the *estimation error* of a parameter k_i as

$$EE(k_i) = \left| \frac{k_i - k_i^*}{k_i} \right|, \quad (33)$$

where k_i^* is the estimate of k_i calculated from fitting the PFO solution to the generated data. Additionally, for the ligand–receptor interaction, we calculate the *mean estimation error* as an aggregate measure of the parameter estimation

$$MEE = \text{mean} \{EE(k_1), EE(k_{-1})\}. \quad (34)$$

Concentration error measures, such as the maximum or steady-state concentration errors, that compare exact and approximate solutions to the ligand–receptor complex concentration, are fundamentally different from those incurred by using an approximate model to fit experimental data. To illustrate this point, Fig. 5 shows contours for the mean estimation error when different

Figure 5: **Mean estimation error in the rate constants when applying the pseudo-first-order approximation to the ligand-receptor reaction (1).** Heat maps of the mean estimation error of the rate constants are shown for different experimental protocols. Columns, from left to right, correspond to increasing length of observation time. Rows, from top to bottom, correspond to increasing frequency of sampling. For short observation windows, large errors occur even when $[L_0] \gg [R_0]$. Also, counter-intuitively, the error at some initial conditions increases as the sampling frequency increases (e.g. down column 1). The black lines correspond to condition (30) when the left-hand side is equal to 10.

288 “experimental protocols” are used to generate data. For a given sampling fre-
 289 quency, the mean estimation error contours increasing conform to the contour
 290 derived from condition (30) as t_{obs} increases. However, for initial conditions

with $[L_0] \geq 10[R_0]$ and $K_S \leq [R_0]$, significant estimation errors can occur if the observation time is not sufficiently long. Even after $10t_c$ of observation, at which point $[C](t) > 0.999[C]^*$, the mean error in the estimated parameters can exceed 10% when K_S is small. Only after nearly $100t_c$ the mean estimation error contours closely mimic the theoretical condition for the validity of the PFO kinetics. This highlights the difference between the errors calculated by comparing the exact and approximate solutions of the concentration equations, and those errors due to fitting an approximate model to data. Additionally, it should caution experimentalists from applying PFO approximations whenever one species is in excess. The values of the rate constants must be considered as well.

Interestingly, increasing the frequency of sampling does not necessarily improve the estimation of the rate constants. In fact, the first column in Fig. 5 shows, that the mean estimation error actually increases as more sample points are used. This effect saturates quickly as the frequency is increased, but nevertheless, using fewer *exact* data points can lead to improved predictions. One major benefit of numerous data points is that it reduces error due to measurement noise, and this likely will outweigh the gains from using fewer data points when fitting. Yet, in cases where accurate measurements are possible, fitting more data to an approximate model can have deleterious effects on the accuracy of parameter estimates made from such a fit. It may be possible to take advantage of both of these effects by recording data at a high frequency, say using an optical assay [29], then performing a number of fits on subsets of the data sampled at lower frequency, thereby reducing both the experimental noise and the estimation error incurred by fitting to an approximate model.

5.3. The error in the estimated parameters for high ligand concentration is due to inaccuracies in k_{-1}

Greater understanding of the estimation errors found at high ligand concentrations and low K_S can be gained by comparing the estimation errors of k_1 and k_{-1} individually. Fig. 6 shows contours of $EE(k_1)$ [panel (a)] and $EE(k_{-1})$ [panel (b)] for numerical data generated with $\omega_{obs} = 2t_c^{-1}$ and $t_{obs} = 3t_c$. From this, it is clear that the inaccuracies lie in predictions of the dissociation rate constant k_{-1} . The reason k_{-1} errors are large for cases in which $[L_0] \gg [R_0]$ and $K_S \ll [R_0]$ can be understood by examining the PFO solution (16) in the limit $K_S/[L_0] \rightarrow 0$. Keeping terms up to linear order in

Figure 6: **Comparison of estimation errors for k_1 and k_{-1} .** Heat maps of the EE(k_1) and EE(k_{-1}) are presented in (a) and (b), respectively. The sampling frequency used to generate data was $\omega_{obs} = 2t_c^{-1}$, and the observation window was $t_{obs} = 3t_c$. The black lines correspond to condition (30) when the left-hand side is equal to 10. The error contour for k_{-1} estimation shows clear deviations from the analytical conditions, whereas k_1 estimations are accurate where PFO kinetics is shown to be theoretically valid.

$K_S/[L_0]$, we obtain

$$[C](t) \approx [R_0] (1 - \exp(-k_1[L_0]t)) \left[1 - \frac{K_S}{[L_0]} \left(1 - \frac{k_1[L_0]t}{\exp(k_1[L_0]t) - 1} \right) \right]. \quad (35)$$

319 The zeroth-order term has no dependence on k_{-1} . Hence, in this limit there
 320 is no unique mapping between the parameters ($[R_0], [L_0], k_1, k_{-1}$) and time
 321 onto the concentration $[C]$, since $[C]$ is completely described by the param-
 322 eters ($[R_0], [L_0], k_1$) and time. This implies that estimates of k_{-1} from progress
 323 curve experiments performed under conditions of high ligand concentration
 324 and low disassociation constant are unreliable. However, estimates of the
 325 association rate constant k_1 from such experiments should be valid. Unfor-
 326 tunately, since K_S is not generally known a priori, a different measurement,
 327 such as an equilibrium binding assay, is required to estimate its value. Then,
 328 with knowledge of K_S , k_{-1} can be calculated.

Figure 7: **Comparison of approximate model with exact and fitted parameters for the ligand-receptor reaction (1).** The blue line represents the exact solution and blue squares are simulated data points. The green dashed line is the pseudo-first-order approximation using the same rate constants used to in the exact solution. The red dot-dashed line is the pseudo-first-order approximation using rate constants found by fitting the pseudo-first-order model to the data generated using the exact solution.

329 5.4. *The pseudo-first-order kinetics can lead to significant estimation errors*
 330 *when the conditions of the pseudo-first-order approximation are valid*

331 Taken together, Fig. 4 and Fig. 5 illustrate an important, yet often over-
 332 looked, distinction between methods by which to assess the validity of an ap-
 333 proximation in chemical kinetics. The first method, popular among theorist,
 334 attempts to answer the following question: Given a set of known parameters,
 335 how well does the approximate model represent the exact model? This com-
 336 parison can be made by calculating a maximum or steady-state concentration
 337 error, as we do here, or through other measures such as a mean-squared dif-
 338 ference over the time course. The second method, which is of greatest impor-
 339 tance to experimentalists, answers a subtly different question: Given data,
 340 how well do parameters estimated by fitting the data with an approximate
 341 model represent the actual parameters? This distinction, between a forward
 342 and an inverse problem, is not generally considered when deriving conditions
 343 for the validity of reduced kinetic models [30]. To illustrate this, Fig. 7 shows

344 data points generated with exact solution to the governing equations of the
 345 ligand–receptor reaction (1), the PFO solution using the same rate constants
 346 as those used to generate the exact data points, and the PFO solution us-
 347 ing rate constants calculated through nonlinear regression of the simulated
 348 data. The PFO solution using the exact rate constants captures the kinetics
 349 much more closely than the PFO solution using estimated rate constants,
 350 especially as steady-state is approached. Often, it is the former case that is
 351 used by theorists to determine valid ranges for an approximation, while the
 352 latter case is where the approximation is actually used to interpret data. As
 353 we have shown, these two cases are distinct. Hence, when providing ranges
 354 for the validity of a simplifying approximations to theory, it is crucial that
 355 the application of that theory be kept in mind.

356 6. Discussion

357 We have investigated the application of the PFO approximation to ligand–
 358 receptor binding dynamics. PFO kinetics are used to linearize the solutions to
 359 the differential equations that describe the concentration of ligand, receptor
 360 and ligand–receptor complex over time, allowing them to be fit by a single
 361 exponential [16, 15]. This approximation is known to introduce errors that
 362 are acceptably small under certain conditions, which have generally been
 363 described by $[L_0] \gg [R_0]$. In this paper, we show that this condition is
 364 somewhat more stringent than necessary, specifically when $[R_0] \ll K_s$. In
 365 fact the condition $[R_0] \ll K_s$ provides another sufficient condition under
 366 which one may safely use the PFO approximation, with little regard to the
 367 concentration $[L_0]$.

368 Although it is possible to derive closed-form solutions that describe the
 369 kinetics of simple ligand–receptor binding [see, equation (10)], this equation
 370 is cumbersome. The PFO approximation gives a much simpler solution [see,
 371 equation (16)], which can be linearized by use of logarithmic plots to facilitate
 372 data fitting [17]. With this simpler form a linear fit suffices to determine
 373 all of the relevant rate constants leading to a complete description of the
 374 reaction kinetics. The new condition developed here extends the validity
 375 of this method into new territory, increasing its usefulness. Specifically, in
 376 cases where reagents are either expensive or difficult to isolate in significant
 377 quantities, the new condition suggests far more economical usage (in some
 378 cases, orders of magnitude lower concentration) of reagents is possible.

379 Additionally, we have shown that there is often an inconsistency between
380 the derivation of conditions for validity of an approximation, and the relevant
381 measure of error for applications of that approximation. Approximations to a
382 theory are generally taken as valid if, using identical input parameters, exact
383 and approximate solutions for species concentration are sufficiently similar.
384 This requires minimizing the concentration error introduced in equation (32).
385 However, approximate models are often used to estimate kinetic parameters
386 through fitting to experimental data. We have demonstrated that estimation
387 errors may be significant, even for conditions in which the approximate and
388 exact models are nearly identical. This effect is particularly apparent when
389 one reactant is in excess, the disassociation constant is small, and the length
390 of the observation is not sufficiently long.

391 Commonly, experimental protocols for kinetic binding assays call for mea-
392 surements to be made until concentrations “plateau” [27]. However, the def-
393 inition of “plateau” is often left to the judgment of the investigator. Our
394 analysis shows that stopping measurement prematurely can lead to signif-
395 icant errors in the rate constants estimated by such experiments. A more
396 rigorous definition of the necessary experimental time to reach plateau should
397 involve the inherent timescale, t_c . The error contours in Fig. 5 show that for
398 many initial conditions, specifically for those with large enough K_S , mea-
399 surements over $3t_c$ are sufficient. It should be possible, however, to test if
400 the experimental conditions are problematic without prior knowledge of K_S ,
401 so long as the initial concentrations of receptor is known. If, at steady-state,
402 $[C]$ is very near $[R_0]$, then the affinity of the ligand for the receptor is high
403 enough (and K_S will be small enough) to make the value of k_{-1} from regres-
404 sion analysis unreliable. Since affinities between ligands and receptors are
405 typically quite high, this will often be the case. Hence this suggest that it is
406 necessary to estimate the equilibrium constant using a separate assay. Then,
407 with knowledge of K_S , the rate constants can be unambiguously estimated
408 from kinetic data.

409 Lastly, we emphasize that the difference between the concentration error
410 and estimation error are not specific to the case of ligand–receptor binding.
411 The problem of estimating parameter values for models is well known in the
412 model calibration field [31], and has also received attention from mathemat-
413 ical and systems biologists [30, 32]. The essential questions are whether or
414 not a parameter in a model actually corresponds to the underlying physical
415 property it is meant to represent, and whether the value of the parameter can
416 be uniquely determined from data. Frequently, the value of the parameter

417 that provides the best fit to data differs from the most accurate assessment
 418 of the underlying physical property, estimated through some other means. In
 419 the case of ligand–receptor binding, the rate constants estimated using PFO
 420 kinetics correspond to a best-fit of experimental data to an approximate
 421 model. In many cases, these estimates will not coincide with the actual rate
 422 constants for the second-order reaction. In fact, this difference is quite general
 423 and future studies should investigate how the validity of approximations in,
 424 for example, Michaelis–Menten kinetics, or inhibited ligand–receptor binding
 425 might change when their ability to accurately predict parameters from data
 426 is considered.

427 **Appendix A. Symmetry of case with no receptor depletion**

428 The second case referred to in the text (Section 2) applies when the
429 concentration of the receptor $[R_0]$ is much greater than that of the ligand
430 $[L_0]$, which implies that

$$[R_0] - [C] \approx [R_0] . \quad (\text{A.1})$$

431 Substituting (A.1) into equation (7) gives

$$\frac{d[C]}{dt} = k_1 \left([R_0][L_0] - (K_S + [R_0])[C] \right) . \quad (\text{A.2})$$

432 [Compare with (15)]. Solving this equation yields

$$[C](t) = \frac{[R_0][L_0]}{K_S + [R_0]} \left(1 - \exp(-(k_\varphi + k_{-1})t) \right) . \quad (\text{A.3})$$

433 This solution is symmetrical with (16). Condition (A.1) gives us the following
434 implication parallel with (17)

$$[R](t) = [R_0] - [C](t) \approx [R_0] \quad \Rightarrow \quad \left| \frac{[C]}{[R_0]} \right|_{\max} \ll 1 . \quad (\text{A.4})$$

435 Similar to the case with no ligand depletion, the maximum concentration
436 is equal to λ_- . Hence, following the same procedure as in Section 4, a
437 condition, symmetric to (30), for the case with negligible receptor depletion
438 is found to be

$$\frac{[L'_0]}{1 + [L'_0] + [R'_0]} \ll 1 . \quad (\text{A.5})$$

439 **Appendix B. Validity of approximation (28)**

440 The derivation of the conditions given by (30) requires that equation (28)
441 be satisfied, which we reiterate as

$$r' \ll 1 \quad (\text{B.1})$$

$$\frac{4[R'_0][L'_0]}{(1 + [R'_0] + [L'_0])^2} \ll 1 . \quad (\text{B.2})$$

442 The above inequality can be written as

$$\frac{1}{1 + [R'_0] + [L'_0]} \ll \frac{1}{2\sqrt{[R'_0][L'_0]}} . \quad (\text{B.3})$$

443 Since the denominators are both positive, we can rearrange this as

$$-\left([R'_0] - 2\sqrt{[R'_0][L'_0]} + [L'_0]\right) \ll 1 \quad (\text{B.4})$$

444 and factoring the left side then gives

$$-\left(\sqrt{[R'_0]} - \sqrt{[L'_0]}\right)^2 \ll 1. \quad (\text{B.5})$$

445 In the above inequalities, the left side is always negative and the right side
446 is clearly positive. Therefore, it is appropriate to assume that $r' \ll 1$.

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