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The effect of receptor site nonuniformity on the measurement of rate constants

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Abstract

The BIAcore is an instrument for measuring rate constants in real time by using a surface–volume geometry. Though current models for the resulting reaction include transport effects for the reactant in solution, they do not account for spatial nonuniformities in the reactant attached to the wall. This work accounts for such nonuniformities and establishes that in the limit of small Damköhler number, such effects are negligible due to the averaging characteristics of the instrumentation.
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1. Introduction

Obtaining accurate quantitative estimates for the rate constants for biochemical reactions is a pressing experimental need. These rate constants yield valuable insights into the chemical processes that occur inside living organisms, such as those that occur on the surface of a cell [1]. One popular and accurate way of obtaining reaction data is through the use of the BIAcore, a surface plasmon resonance device.

The BIAcore device consists of a channel through which one of the reactants (the analyte) is convected in standard two-dimensional Poiseuille flow from $x = 0$, the inlet position. The other reactant, called the receptor, is coupled to a dextran layer on the ceiling of the channel (see Fig. 1). Reactant binding

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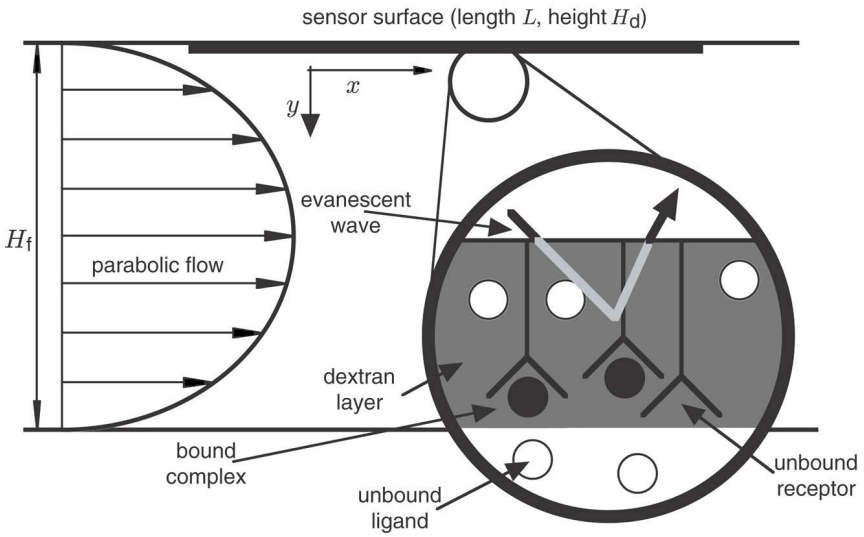

Fig. 1. Schematic diagram of the BIAcore device.

causes changes in the refractive index of the dextran layer. These changes are measured by a polarized light beam, and averaged over the length of the ceiling to provide real-time measurement of the bound-state concentration [2,3]. The beam has a finite *penetration depth*, so reactions occurring near the ceiling provide a stronger signal than those further away.

Because of fluid dynamic effects in the BIAcore, the mathematical model is more complicated than simple kinetics. Contemporary models of the BIAcore include the effects of convective transport (and associated downstream depletion) [4–7], the effects of diffusive transport in the dextran [8,9], and the effect of the finite penetration depth of the evanescent wave [10]. However, to date they have always assumed a constant density R_T of receptors in the layer. But the receptors are embedded in the dextran layer using a convective flow process similar to the one used in the experiment itself [11,12]. Thus we expect that the true receptor density will have nonuniformities induced by depletion as does the bound state. In this paper we will analyze these nonuniformities, and we conclude that due to the averaging process in the BIAcore, these nonuniformities do not affect rate constant measurements to the order now considered to be the standard.

2. Receptors on a surface

Because the ratio of the width of the receptor layer to the width of the channel is so small [8], it is often instructive initially to treat the receptor layer as a surface, and we do so here. When calculating its output, the BIAcore averages the dimensionless bound state B to produce a sensogram reading $\bar{B}$; in the surface case, the averaging is given by

$$\bar{B}(t) = \frac{1}{x_{\max} - x_{\min}} \int_{x_{\min}}^{x_{\max}} B(x,t)\,\mathrm{d}x,$$

where $x_{\min}$ and $x_{\max}$ are the limits of the scanning range of the device. Thus any useful mathematical model for the BIAcore must eventually be expressed in terms of $\bar{B}$, not B. On the other hand, any

data input to our model must also be expressed in terms of averages. In particular, upon introducing a variable receptor density $R(x)$, we realize that the BIAcore will measure only $\bar{R}$. Thus we may set $\bar{R} = R_T$, replacing the uniform density of previous models with the average density. But what is the size of the oscillations?

It can be shown [4] that the relevant dimensionless parameter characterizing the effects of convection is given by the *Damköhler number*

$$Da = \frac{\tilde{k}_a R_T L^{1/3} H_f^{1/3}}{V^{1/3} \tilde{D}_f^{2/3}} = \frac{\text{reaction rate}}{\text{diffusion rate in unstirred layer}}.$$

The "unstirred layer" refers to the boundary layer near the surface of width $Pe^{-1/3}$, where Pe is the Peclét number. In this layer, diffusion and convection balance. Here L and H_f are the dimensions of the channel, V is proportional to the flow rate, $\tilde{k}_a$ is the association constant for the analyte, and $\tilde{D}_f$ is the diffusion coefficient for the analyte in the fluid buffer.

Experimentalists strive to work in the regime where Da is small and transport effects are limited. Focusing on this case, we use Da as a small perturbation parameter. In the case of small Da, previous studies of association [4] and dissociation [5] experiments show that transport effects introduce an $O(Da)$ correction to the well-mixed case where transport is infinitely fast.

The receptors are often bound to the dextran by an injection (flow) process similar to that for the analyte in the experiment [11,12]. The immobilization process is allowed to run for a long time in an attempt to reach a constant steady state. Nevertheless, because of our previous analyses, we expect that there will always be a small deviation at any time. Thus it is reasonable to write the density of receptors R as

$$R(x) = R_T[1 + DaR_\Delta(x)], \qquad R_\Delta = O(1). \tag{1a}$$

Note that Da is the Damköhler number for the ligand–receptor reaction (the one we choose as our perturbation parameter), not the receptor–dextran reaction. $\tilde{k}_a$ and $\tilde{D}_f$ are similar for the analyte and receptor, so the two Damköhler numbers are of similar order. (The term *immobilization* arises from keeping the dissociation constant $\tilde{k}_d$ low.) Moreover, we require that

$$\overline{R_\Delta} = 0, \tag{1b}$$

so that $\bar{R} = R_T$.

The governing equations for B in the case of large Pe (as achieved in the BIAcore) have previously been derived [4]. With (1a) replacing the uniform condition $R = R_T$, these equations become

$$\frac{\partial B}{\partial t} = [1 - DaC(x,t)][1 + DaR_\Delta(x) - B] - KB, \qquad x \in [0,1], \tag{2a}$$

$$C(x,t) = \frac{1}{3^{1/3}\Gamma(2/3)} \int_0^x \frac{\partial B}{\partial t}(x - \xi, t) \frac{d\xi}{\xi^{2/3}}. \tag{2b}$$

Here C represents the deviation of the analyte concentration from its upstream value 1, and K is the *affinity constant*, which is a ratio of the rate constants. Note from (2b) that as expected, the analyte depleted at x is an integral of the differential changes upstream ($0 < \xi < x$).

The second bracketed term in (2a) represents the number of receptor sites available for binding. In our model, we consider "available sites" to be equivalent to "empty sites", so the term in question is just the total number of receptors less the number bound. In actuality, due to the mismatch in size between the

large ligand molecules and the more tightly packed receptor sites, it is possible for a molecule to bind to the surface and occlude nearby reacting sites [13]. For the model under consideration, we neglect these *steric hindrance* effects in (2a), which would introduce a C dependence into the second bracketed term in (2a), and hence additional nonlinearities into our problem.

Now that we have chosen our small parameter Da, we may let $B = B_0 + o(1)$ in (2a) to obtain an ODE for B_0. In an association experiment, B_0 is zero initially, so B_0 is a function of t only:

$$\frac{\mathrm{d}B_0}{\mathrm{d}t} = 1 - (1+K)B_0, \qquad B_0(0) = 0, \tag{3}$$

which is the operator for the standard dimensionless perfectly mixed case, which considers kinetics only. From the form of (2b) we see that to leading order $C = h(x)\mathrm{d}B_0/\mathrm{d}t$. (For reasons that will become clear, we leave $h(x)$ arbitrary for now.) Making this substitution into (2a), we obtain

$$\frac{\partial B}{\partial t} + Da(1-B)\frac{\mathrm{d}B_0}{\mathrm{d}t}h(x) = 1 - (1+K)B + DaR_{\Delta}(x) + O(Da^2). \tag{4}$$

As remarked above, the BIAcore returns measurements of $\bar{B}$. Thus we average (4) to obtain

$$\frac{\mathrm{d}\bar{B}}{\mathrm{d}t}\left[1 + Da(1-\bar{B})\bar{h}\right] = 1 - (1+K)\bar{B} + O(Da^2). \tag{5}$$

In deriving (5), we note that due to the uniformity of B_0, the errors we make in replacing $\mathrm{d}B_0/\mathrm{d}t$ by $\mathrm{d}\bar{B}/\mathrm{d}t$ and replacing $\overline{Bh}$ by $\bar{B}\bar{h}$ are both $O(Da^2)$. Note also that the R_{Δ} term vanishes because $\overline{R_{\Delta}} = 0$.

In order to fit the $\bar{B}$ data using a curve fitting program in Matlab or a data-analysis program, the following form is usually more convenient:

$$\frac{\mathrm{d}\bar{B}}{\mathrm{d}t} = \frac{1-(1+K)\bar{B}}{1+Da(1-\bar{B})\bar{h}} + O(Da^2), \qquad \bar{h} = \frac{3^{5/3}(x_{\max}^{4/3} - x_{\min}^{4/3})}{4\Gamma(2/3)(x_{\max} - x_{\min})}, \tag{6}$$

where in calculating $\bar{h}$ we have used (2b). Note from (6) that if we are in the perfectly mixed case where $Da = 0$, (6) reduces to the standard kinetic equation (3). If we were (mistakenly) using the standard kinetic model, the effect of the transport term in the denominator would be to seem to make the affinity constant K vary with time. Hence (6) is an example of an *effective rate constant* (ERC) model, which is quite generic. Note that all the effects of transport, geometry, etc. are lumped into the parameter $\bar{h}$. Thus we can use (6) for a variety of different devices [14] and geometries [15], with the only change being a new value for $\bar{h}$.

With the $\overline{R_{\Delta}}$ term gone, (6) is exactly the result for *uniform* R [5]. Thus, even though the nonuniformity appears at $O(Da)$, the sensogram averaging keeps it from affecting the solution to the leading two orders. In other words, since B_0 is uniform, the first troublesome term to average is CR_{Δ}, which appears at $O(Da^2)$, beyond the accuracy of the ERC model.

For completeness, we mention that the BIAcore can also be used for a dissociation experiment. Once the association experiment has reached a steady state, the injected ligand is removed, so Eq. (2a) is replaced by

$$\frac{\partial B}{\partial t} = -DaC(x,t)[1 + DaR_{\Delta}(x) - B] - KB, \qquad x \in [0,1],$$

where the minus sign is retained since by (2b) we see that $C < 0$. Since the steady state for the association experiment is uniform [4], the same argument about B_0 and the form of C applies, so (6) is replaced by

$$\frac{\mathrm{d}\bar{B}}{\mathrm{d}t} = -\frac{K\bar{B}}{1 + Da(1-\bar{B})\bar{h}} + O(Da^2), \tag{7}$$

with the same value of $\bar{h}$. Again, this is the same result as in the uniform case [5].

3. Receptors in a layer

If the receptors are treated more realistically—namely in a layer—the results are similar. The averaging is now given by [10]

$$\bar{B}(t) = \frac{\delta}{(1-\mathrm{e}^{-\delta})(x_{\max} - x_{\min})} \int_{x_{\min}}^{x_{\max}} \int_{-1}^{0} \mathrm{e}^{-\delta(y+1)} B(x, y, t)\, \mathrm{d}y\, \mathrm{d}x.$$

Here $y \in [-1, 0]$ represents the dextran layer and δ is the ratio of the dextran thickness H_d to the penetration depth of the measuring wave. (Hence $\delta = 0$ corresponds to an infinite penetration depth and perfect measurements.)

Eqs. (1a) and (2a) are unchanged except that all nonuniform functions must now depend on the additional independent variable y. The equation for $C(x, y, t)$ is now given by [8]

$$C(x, y, t) = \frac{1}{3^{1/3}\Gamma(2/3)} \int_0^x \frac{\partial F}{\partial y}(x - \xi, 0, t) \frac{\mathrm{d}\xi}{\xi^{2/3}} - DF, \tag{8a}$$

$$\frac{\partial^2 F}{\partial y^2} = \frac{\partial B}{\partial t}, \qquad \frac{\partial F}{\partial y}(x, -1, t) = 0, \qquad F(x, 0, t) = 0. \tag{8b}$$

Note the similarity between the integrals in (2b) and (8a). In each case, the integral represents the depletion effects. Here

$$D = \frac{\tilde{D}_\mathrm{f}/(H_\mathrm{f} Pe^{-1/3})}{\phi \tilde{D}_\mathrm{d}/H_\mathrm{d}} = \frac{\text{diffusion “velocity” in diffusive boundary layer}}{\text{diffusion “velocity” in dextran}}$$

is a parameter that measures the resistance to transport caused by the presence of the dextran layer. Here ϕ is the partition coefficient and $\tilde{D}_\mathrm{d}$ is the diffusion coefficient for the analyte in the dextran layer. Note that the effects in the two directions separate: the effects of convection in the x-direction are totally contained within the integral in (8a), while the effects of diffusion in the y-direction are totally contained within the second term.

None of these additional complications affects our arguments of Section 2, however. As long as Da is small, B_0 is still a function of t only, so from (8b) we have that $F = g(y)\mathrm{d}B_0/\mathrm{d}t$. This in turn implies that $C = h(x, y)\mathrm{d}B_0/\mathrm{d}t$, and making this substitution into (2a), we obtain

$$\frac{\partial B}{\partial t} + Da(1-B)\frac{\mathrm{d}B_0}{\mathrm{d}t} h(x, y) = 1 - (1+K)B + DaR_\Delta(x, y) + O(Da^2),$$

which of course is the analog of (4) in two dimensions. Thus the averaging proceeds as before, and (6) still holds, this time with [10]

$$\bar{h} = D\frac{\delta^2 + 2[(\delta+1)\mathrm{e}^{-\delta} - 1]}{2\delta^2(1-\mathrm{e}^{-\delta})} + \frac{3^{5/3}(x_{\max}^{4/3} - x_{\min}^{4/3})}{4\Gamma(2/3)(x_{\max} - x_{\min})}. \tag{9}$$

As before, Eq. (6) (with our new definition of $\bar{h}$) is exactly the result for uniform R [10].

For the dissociation case, the same arguments still hold. Thus Eq. (7) with $\bar{h}$ defined in (9) is the correct ERC equation, and it is the same equation as the one with uniform R [10].

4. Conclusions and further research

Given the current state of the art in SPR technology, simple models for surface–volume reactions are needed to obtain accurate constants for the reactions. Most of the different models in use, which so carefully track transport effects in the ligand–receptor reaction, treat the initial receptor density R as uniform. This is odd, since the same transport effects are in play when the receptors are initially laid down in the dextran. Thus one would expect $R(x)$ to have the form (1a), since the sizes of those transport effects are characterized by Da. This, in turn, would lead one to believe that the sensogram data would be affected at $O(Da)$ by these nonuniformities.

Fortunately, the BIAcore measures the spatial average of the data it collects. Thus some of the more negative effects of nonuniformity in R are averaged away, as they are with B. In particular, since the initial sensor signal is $\bar{R}$, the nonuniformities we examine must have mean zero. This fact, coupled with the fact that the leading order of B is uniform, yields the result that up to $O(Da)$, the nonuniformity in R does not affect the solution. Thus, we see that even though the *nonuniformities* are $O(Da)$, their *contribution* to the sensogram reading does not appear until $O(Da^2)$.

Because of this, the nonuniformity does not affect the form of $\bar{B}$ in the ERC model, which is accurate to $O(Da^2)$. Since the ERC model is quite convenient for data fitting, we are in the happy position of reporting that one can ignore the nonuniformities in R when calculating rate constants—as long as Da is small.

Though experimentalists strive to remain in this regime, there are times when it is impossible to do so, for instance when $\tilde{k}_a$ is large. In previous numerical studies with a uniform R_T, the ERC approximation (6) has been shown to be good even in the case when Da is moderate [10,16], even though there is no reason asymptotically why it should be so. With this positive outcome, we are hopeful that (6) will also hold for the case of moderate Da with nonuniform R. Numerical simulations will test this hypothesis.

Clearly the analysis herein will also fail if R_Δ is large, i.e., if the nonuniformities in the receptor sites are moderate. Such a problem can normally be avoided by extending the injection time of the receptor in the immobilization step. Regardless of this, since the mean of these larger nonuniformities would still be zero, we expect that (6) should hold in this regime as well. Numerical simulations can also test this hypothesis.

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