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The Effect of a Receptor Layer on the Measurement of Rate Constants

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Many cellular reactions involve a reactant in solution binding to or dissociating from a reactant attached to a surface. Most studies assume that the reactions occur *on* this surface, when in actuality the receptors usually lie in a thin layer on top of it. The effect of this layer is considered, particularly as it relates to the BIAcore™ measurement device, though the results are applicable to biological systems. A dimensionless parameter measuring the strength of the effect of the receptor layer is found. Asymptotic and singular perturbation techniques are used to analyse association and dissociation kinetics, though the effect of the receptor layer need not be small. Linear and nonlinear integral equations result from the analysis; explicit and asymptotic solutions are constructed for physically realizable cases. In addition, effective rate constants are derived that illustrate the combined effects of transport and the receptor layer on the measured rate constants. All these expressions provide a direct way to estimate rate constants from BIAcore™ binding data.

1. INTRODUCTION

In biological systems, many chemical reactions of interest occur between two components, one of which is confined to a surface (often receptors on a cell membrane) while the other floats freely in solution (herein called the *analyte*). For instance, newborns receive immunoglobulins from mother's milk through binding to receptors on intestinal epithelial cells (Raghavan *et al*., 1994). Signaling and adapter molecules in the cytoplasm interact with the cytoplasmic tails of receptors embedded in the plasma membrane (Goldstein and Dembo, 1995). Gene expression is significantly influenced by DNA-protein interactions in these geometries (Szabo *et al*., 1995).

With few exceptions (Goldstein and Wofsy, in preparation; Schuck, 1996; Yarmush *et al*., 1996) it has been customary to model such reactions as occurring *on* the biological surface by imposing a reacting boundary condition on a transport equation for the analyte (Davis *et al*., 1995; Myszka *et al*., 1998; Edwards, 1999, 2000; Edwards *et al*., 1999; Mason *et al*., 1999). A truer model would include a

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small *receptor layer* in which the receptor sites reside and through which the ligand must diffuse to reach them. Due to the thinness of such layers, the surface-reaction approximation has been preferred due to its simplicity.

The thinness of the layer is not the key issue, however. Even a thin layer will significantly affect the reaction if it is nearly impermeable to the ligand. The more relevant consideration is the time it takes for ligand molecules to diffuse through the receptor layer and reach a receptor site. If this time scale is small (in some relative sense), a surface-reaction model is appropriate. In this manuscript we derive the key dimensionless group D which determines the effect of the layer.

We specialize to the case of the BIAcore^{TM}, which is a surface plasmon resonance (SPR) device for measuring rate constants; however, the results can easily be extended to biological systems of interest (Edwards, 2000). The configuration of the BIAcore^{TM} is described in great detail elsewhere (Karlsson *et al*., 1994; Szabo *et al*., 1995). For our purposes, it is sufficient to know that the device consists of a rectangular channel through which the analyte flows. The receptor is embedded in a thin dextran matrix attached to the ceiling of the channel, and real-time data can be gathered as the experiment progresses.

The BIAcore^{TM} is modeled as a coupled system between a convection-diffusion equation in the flow and a reaction-diffusion equation in the dextran layer. After scaling, we derive results in the case of small *Damköhler number* Da, which measures the effect of transport. Since including the layer also involves adding a transport effect, we find that the correction from the dextran layer occurs at $O(\mathrm{Da})$. We obtain explicit solutions for the bound state as well as expressions where the rate constants obtained from such a system can be interpreted in terms of the 'true' rate constants (i.e., the ones if both reactants were in solution).

In addition, we consider the case where $\mathrm{Da} = O(1)$ and transport and reaction effects balance. A nonlinear integral equation results, but the rate constants can easily be estimated from a short-time solution for the bound-state concentration. We consider not only association, but also dissociation experiments. We construct explicit solutions in the case where the initial density of empty receptors is uniform. These results should help experimentalists design and interpret the results of their trials, as well as provide insight into how receptor layers affect biological systems.

2. GOVERNING EQUATIONS

We consider the BIAcore^{TM} to be divided into two regions: the open channel (considered to be the the region $0 \leq \tilde{x} \leq L$, $0 \leq \tilde{y} \leq H_{\mathrm{f}}$, where the subscript 'f' stands for 'flow'), and the dextran layer (considered to be the the region $0 \leq \tilde{x} \leq L$, $0 \geq \tilde{y} \geq -H_{\mathrm{d}}$, where the subscript 'd' stands for 'dextran'). For the purposes of this paper, the dextran layer is considered to be a solid in that there is no appreciable flow in it.

This consideration imposes a no-slip condition on the flow at the dextran–fluid

interface $\tilde{y} = 0$. Since there is already a no-slip condition at the wall $\tilde{y} = H_\mathrm{f}$, the flow is standard parabolic Poiseuille flow with maximal velocity $V/4$. Thus, the equation for the concentration $\tilde{C}_\mathrm{f}$ of the ligand in the channel is as follows:

$$\frac{\partial \tilde{C}_\mathrm{f}}{\partial \tilde{t}} = \tilde{D}_\mathrm{f}\left(\frac{\partial^2 \tilde{C}_\mathrm{f}}{\partial \tilde{x}^2} + \frac{\partial^2 \tilde{C}_\mathrm{f}}{\partial \tilde{y}^2}\right) - V\frac{\tilde{y}}{H_\mathrm{f}}\left(1 - \frac{\tilde{y}}{H_\mathrm{f}}\right)\frac{\partial \tilde{C}_\mathrm{f}}{\partial \tilde{x}}, \tag{1}$$

where $\tilde{D}_\mathrm{f}$ is the molecular diffusion coefficient of the ligand in the flow. For now, we take the initial concentration in the open channel to be an arbitrary constant:

$$\tilde{C}_\mathrm{f}(\tilde{x}, \tilde{y}, 0) = C_{\mathrm{f,i}}. \tag{2}$$

In practice, for an association experiment it would be zero, while for a dissociation experiment it would be some equilibrium value. At the upstream end ($\tilde{x} = 0$), we have a prescribed constant concentration C_T:

$$\tilde{C}_\mathrm{f}(0, \tilde{y}, \tilde{t}) = C_\mathrm{T}. \tag{3}$$

In practice, for an association experiment it would be a specified value, while for a dissociation experiment it would be zero. This upstream concentration will change from run to run of the same experiment.

Since there is no convection in the dextran layer, the governing equation for the concentration $\tilde{C}_\mathrm{d}$ of the ligand is as follows:

$$\frac{\partial \tilde{C}_\mathrm{d}}{\partial \tilde{t}} = \tilde{D}_\mathrm{d}\left(\frac{\partial^2 \tilde{C}_\mathrm{d}}{\partial \tilde{x}^2} + \frac{\partial^2 \tilde{C}_\mathrm{d}}{\partial \tilde{y}^2}\right) - \frac{\partial \tilde{B}_\mathrm{d}}{\partial \tilde{t}}, \tag{4}$$

where $\tilde{D}_\mathrm{d}$ is the molecular diffusion coefficient of the ligand in the dextran. Here $\tilde{B}_\mathrm{d}(\tilde{x}, \tilde{y}, \tilde{t})$ is the concentration of the receptor in the dextran.

Across the interface $\tilde{y} = 0$, the fluxes (which arise only from diffusion) must balance:

$$\tilde{D}_\mathrm{f}\frac{\partial \tilde{C}_\mathrm{f}}{\partial \tilde{y}}(\tilde{x}, 0, \tilde{t}) = \tilde{D}_\mathrm{d}\frac{\partial \tilde{C}_\mathrm{d}}{\partial \tilde{y}}(\tilde{x}, 0, \tilde{t}). \tag{5a}$$

We must also have a condition on the concentration itself at $\tilde{y} = 0$. At the interface, the concentration of the ligand *in the fluid* must be continuous. However, in the dextran layer liquid exists only in some volume fraction ϕ (called the *partition coefficient*). Therefore, the *total* volume concentration in the dextran layer must be reduced by that factor:

$$\phi\tilde{C}_\mathrm{f}(\tilde{x}, 0, \tilde{t}) = \tilde{C}_\mathrm{d}(\tilde{x}, 0, \tilde{t}). \tag{5b}$$

There is no flux through the solid surface adjoining the dextran layer:

$$\tilde{D}_\mathrm{d}\frac{\partial \tilde{C}_\mathrm{d}}{\partial \tilde{y}}(\tilde{x}, -H_\mathrm{d}, \tilde{t}) = 0. \tag{6}$$

Finally, we must describe the reaction by specifying $\partial\tilde{B}/\partial\tilde{t}$. The bound state evolves according to a bimolecular reversible reaction. However, the production term must be modified somewhat. First, since the reaction occurs inside the pores only, it is the *fluid* concentration of $\tilde{C}_{\mathrm{d}}$ that is important in the reaction, so we must divide by the partition coefficient. Second, diffusion of the ligand through the dextran may be inhibited by the tortuosity of the pores. This effect is modeled through the *steric hindrance factor* a, which is scaled so that $a = 1$ corresponds to diffusion through a substance with no pores and $a = 0$ corresponds to no diffusion because of the shape or size of the pores. In summary, the governing equation for the reaction is given by

$$\frac{\partial\tilde{B}_{\mathrm{d}}}{\partial\tilde{t}} = \tilde{k}_{\mathrm{on}}\left[\frac{a}{\phi}\left(\frac{\tilde{R}_{\mathrm{T}}}{H_{\mathrm{d}}} - \tilde{B}_{\mathrm{d}}\right)\tilde{C}_{\mathrm{d}} - \tilde{K}\,\tilde{B}_{\mathrm{d}}\right], \qquad \tilde{K} = \frac{\tilde{k}_{\mathrm{off}}}{\tilde{k}_{\mathrm{on}}}, \tag{7}$$

where $\tilde{k}_{\mathrm{on}}$ and $\tilde{k}_{\mathrm{off}}$ are rate constants, and $\tilde{K}$ is the affinity constant. In keeping with the surface-reaction approximation, values for receptor site density $\tilde{R}_{\mathrm{T}}$ are usually given in units of area concentration. To convert to the volume concentrations we use here, we simply divide by the width H_{d} of the dextran layer.

In addition, we need an initial condition for $\tilde{B}_{\mathrm{d}}$:

$$\tilde{B}_{\mathrm{d}}(\tilde{x}, \tilde{y}, 0) = \tilde{B}_{\mathrm{d,i}}(\tilde{x}, \tilde{y}). \tag{8}$$

In practice, for an association experiment it would be zero, while for a dissociation experiment it would be a steady-state value, which we shall calculate later.

2.1. ***Reducing to the previous case.*** We note that we may always relate our work here to the surface-reaction approximation by defining $\tilde{B}$, which has units of area concentration, to be the accumulated bound state integrated across the dextran layer:

$$\tilde{B}(\tilde{x}, \tilde{t}) = \int_{-H_{\mathrm{d}}}^{0} \tilde{B}_{\mathrm{d}}(\tilde{x}, \tilde{y}, \tilde{t})\, d\tilde{y}.$$

If we integrate (4) and (7) across the dextran layer and take the limit as $H_{\mathrm{d}} \to 0$ using the mean value theorem, we obtain

$$\tilde{D}_{\mathrm{f}}\frac{\partial\tilde{C}_{\mathrm{f}}}{\partial\tilde{y}}(\tilde{x}, 0, \tilde{t}) = \frac{\partial\tilde{B}}{\partial\tilde{t}}, \qquad \frac{\partial\tilde{B}}{\partial\tilde{t}} = \tilde{k}_{\mathrm{on}}a[(\tilde{R}_{\mathrm{T}} - \tilde{B})\tilde{C}_{\mathrm{f}}](\tilde{x}, 0, \tilde{t}) - \tilde{k}_{\mathrm{off}}\tilde{B}, \tag{9}$$

where we have used (5a), (b) and (6). Since a surface has no steric hindrance, we take $a = 1$. Equations (9) exactly match the equations in the surface-reaction approximation (Edwards, 1999).

3. SCALING

Next we must choose suitable scales in order to introduce dimensionless variables. Clearly the time scale of interest is the reaction time scale; it can be shown that only the flow equilibrates on the shorter convective and diffusive time scales. We introduce a boundary layer scaling for $\tilde{y}$ in the flow, since it can be shown that ligand transport is relevant only in the unstirred layer near the interface (Edwards, 1999).

We consider an association experiment, so $C_{\mathrm{f,i}} = C_{\mathrm{T}} \neq 0$. In addition, we neglect the steric hindrance factor, so we take $a = 1$. We normalize the ligand concentrations by the upstream concentration and the bound state concentration by the initial number of receptor sites:

$$x = \frac{\tilde{x}}{L}, \qquad y_{\mathrm{f}} = \mathrm{Pe}^{1/3}\frac{\tilde{y}}{H_{\mathrm{f}}}, \qquad t = \tilde{k}_{\mathrm{on}}C_{\mathrm{T}}\tilde{t}, \qquad C_{\mathrm{f}}(x, y_{\mathrm{f}}, t) = \frac{\tilde{C}_{\mathrm{f}}(\tilde{x}, \tilde{y}, \tilde{t})}{C_{\mathrm{T}}}, \tag{10a}$$

$$\mathrm{Pe} = \frac{V/L}{\tilde{D}_{\mathrm{f}}/H_{\mathrm{f}}^2} = \frac{\text{diffusive rate of mass transfer in flow}}{\text{convective rate of mass transfer in flow}}. \tag{10b}$$

Here y_{f} is a boundary-layer variable because $\mathrm{Pe} \gg 1$ (for more details on the sizes of relevant parameters, see the Appendix).

For the dextran layer, we have a given dimension, namely H_{d}, which we use to normalize $\tilde{y}$. In addition, motivated by (5b), we note that the maximum value of $\tilde{C}$ in the dextran layer is ϕC_{T}. Therefore, we choose the following scalings:

$$y_{\mathrm{d}} = \frac{\tilde{y}}{H_{\mathrm{d}}}, \qquad C_{\mathrm{d}}(x, y_{\mathrm{d}}, t) = \frac{\tilde{C}_{\mathrm{d}}(\tilde{x}, \tilde{y}, \tilde{t})}{\phi C_{\mathrm{T}}}, \tag{11a}$$

$$B_{\mathrm{d}}(x, y_{\mathrm{d}}, t) = \frac{H_{\mathrm{d}}}{\tilde{R}_{\mathrm{T}}}\tilde{B}_{\mathrm{d}}(\tilde{x}, \tilde{y}, \tilde{t}), \qquad B_{\mathrm{d,i}}(x, y_{\mathrm{d}}) = \frac{H_{\mathrm{d}}}{\tilde{R}_{\mathrm{T}}}\tilde{B}_{\mathrm{d,i}}(\tilde{x}, \tilde{y}). \tag{11b}$$

Note that we have scaled the y-variable in the flow region with the Peclét number determined by the dynamics, while in the dextran layer we have scaled by the device-determined width.

Substituting (10a), (b), (11a) and (b) into (7) and (1), we obtain, to leading orders,

$$\frac{\partial B_{\mathrm{d}}}{\partial t} = [(1 - B_{\mathrm{d}})C_{\mathrm{d}} - K B_{\mathrm{d}}], \qquad K = \frac{\tilde{K}}{C_{\mathrm{T}}}, \tag{12}$$

$$k_{\mathrm{on}}\mathrm{Pe}^{1/3}\frac{\partial C_{\mathrm{f}}}{\partial t} = \frac{\partial^2 C_{\mathrm{f}}}{\partial y_{\mathrm{f}}^2} - y_{\mathrm{f}}\frac{\partial C_{\mathrm{f}}}{\partial x}, \qquad k_{\mathrm{on}} = \frac{\tilde{k}_{\mathrm{on}}C_{\mathrm{T}}L}{V}, \tag{13}$$

where in (13) we have used the fact that $\mathrm{Pe} \gg 1$ in eliminating the x-diffusion term. From Edwards (1999) we have that $k_{\mathrm{on}}\mathrm{Pe}^{1/3} \ll 1$, and hence we are in the steady state of the flow transport equation.

Substituting (10a) and (10b) into (4) yields the following:

$$\frac{\partial^2 C_\mathrm{d}}{\partial y_\mathrm{d}^2} = \mathrm{Da}D\left(\frac{\partial B_\mathrm{d}}{\partial t} + R^{-1}\frac{\partial C_\mathrm{d}}{\partial t}\right), \tag{14}$$

$$R = \frac{\tilde{R}_\mathrm{T}}{C_\mathrm{T} H_\mathrm{d}\phi} = \frac{\text{concentration of receptors}}{\text{concetration of analyte}}, \tag{15a}$$

$$\mathrm{Da} = \frac{\tilde{k}_\mathrm{on}\tilde{R}_\mathrm{T}}{\tilde{D}_\mathrm{f}/(H_\mathrm{f}\mathrm{Pe}^{-1/3})} = \frac{\text{reaction 'velocity'}}{\text{diffusion 'velocity' in diffusive boundary layer}}, \tag{15b}$$

$$D = \frac{\tilde{D}_\mathrm{f}/(H_\mathrm{f}\mathrm{Pe}^{-1/3})}{\phi\tilde{D}_\mathrm{d}/H_\mathrm{d}} = \frac{\text{diffusion 'velocity' in diffusive boundary layer}}{\text{diffusion 'velocity' in dextran}}, \tag{15c}$$

where we have used the fact that $H_\mathrm{d} \ll L$ to eliminate the x-diffusion term. We see from the Appendix that $R \gg 1$, and hence we are in the steady state for the diffusion part of (14).

Here Da is the *Damköhler number*. Careful readers will note that we could have combined DaD into a single new Damköhler number relating the reaction velocity to the diffusive velocity in the receptor layer. However, the Damköhler number here is the same one that appears in the surface-reaction approximation when there is no receptor layer. Thus, to reproduce the surface-reaction approximation we need take only $D \to 0$ to obtain the limit $H_\mathrm{d} \to 0$. D measures the effect of the dextran layer. Note that it involves not only the ratio of the widths of the regions, but also diffusive effects as well through the ratio $\tilde{D}_\mathrm{d}/\tilde{D}_\mathrm{f}$, which is sometimes called a *drag coefficient* (Yarmush *et al*., 1996).

We conclude by substituting (10a), (b), (11a) and (b) into the relevant boundary data (3), (5a), (b), (6) and (8):

$$C_\mathrm{f}(0, y_\mathrm{f}, t) = 1, \tag{16}$$

$$\frac{\partial C_\mathrm{d}}{\partial y_\mathrm{d}}(x, 0, t) = D\frac{\partial C_\mathrm{f}}{\partial y_\mathrm{f}}(x, 0, t), \tag{17a}$$

$$C_\mathrm{f}(x, 0, t) = C_\mathrm{d}(x, 0, t), \tag{17b}$$

$$\frac{\partial C_\mathrm{d}}{\partial y_d}(x, -1, t) = 0, \tag{18}$$

$$B_\mathrm{d}(x, y_\mathrm{d}, 0) = B_{\mathrm{d,i}}(x, y_\mathrm{d}), \tag{19}$$

where we have used (15c). In addition, since y_f is a boundary layer variable, we must have the condition that C_f matches the saturated value of the bulk flow as we exit the layer:

$$C_\mathrm{f}(x, \infty, t) = 1. \tag{20}$$

Finally, we consider the measurements that are actually taken by the BIAcore^{TM}. Technically, the measurement is a response signal of an evanescent wave that penetrates into the dextran layer. This response is then averaged over the scanning range, which is a subinterval $[x_{\min}, x_{\max}]$ of the channel length. Due to the nature of the instrument, the signal decays with distance away from the surface $y = -1$ (Liedberg *et al.*, 1993; Schuck, 1996). Thus the dimensionless signal $\bar{B}_{\rm d}$ is given by

$$\bar{B}_{\rm d}(t) = \frac{1}{x_{\max} - x_{\min}} \int_{x_{\min}}^{x_{\max}} \left[\int_{-1}^{0} \mathcal{K}(y_{\rm d}) B_{\rm d}(x, y_{\rm d}, t)\, dy_{\rm d} \right] dx,$$

where $\mathcal{K}(y_{\rm d})$ is the decaying kernel. In this manuscript we assume that the kernel decays over a region much wider than the dextran layer, so we may take it to be equal to 1. Therefore, we have

$$\bar{B}_{\rm d}(t) = \frac{1}{x_{\max} - x_{\min}} \int_{x_{\min}}^{x_{\max}} \left[\int_{-1}^{0} B_{\rm d}(x, y_{\rm d}, t)\, dy_{\rm d} \right] dx. \tag{21}$$

4. Small Da, General Results

In order to obtain accurate rate constant measurements from the BIAcore^{TM}, it is desirable to minimize the effects of transport so that the reaction occurs uniformly along the channel length. This corresponds to the case where Da $\to$ 0 (Edwards, 1999; Edwards *et al.*, 1999). Here we shall calculate the first *two* orders of the bound concentration in the limit of small Da, including the effect of the dextran layer. This will allow us to compare and contrast the effects of transport and the layer in the calculation of rate constants.

If we are to calculate the next order in a perturbation expansion, the question immediately arises: what is the perturbation parameter? Equations (13) and (14) give us two choices: $k_{\rm on}{\rm Pe}^{1/3}$ and Da. It can be shown (Edwards, 1999; Edwards *et al.*, 1999) that Da is larger, and hence should be used as the perturbation parameter. Therefore we expand our expressions in the following forms:

$$C_{\rm f}(x, y_{\rm f}, t) = C_{\rm f,0}(x, y_{\rm f}, t) + {\rm Da} C_{\rm f,1}(x, y_{\rm f}, t) + o({\rm Da}), \tag{22a}$$

$$C_{\rm d}(x, y_{\rm d}, t) = C_{\rm d,0}(x, y_{\rm d}, t) + {\rm Da} C_{\rm d,1}(x, y_{\rm d}, t) + o({\rm Da}), \tag{22b}$$

$$B_{\rm d}(x, y_{\rm d}, t) = B_{\rm d,0}(x, y_{\rm d}, t) + {\rm Da} B_{\rm d,1}(x, y_{\rm d}, t) + o({\rm Da}). \tag{23}$$

Substituting (22a), (b) and (23) into (14), (18), (17b), (a), (13), (20), (16), (12) and (19), we have, to leading two orders:

$$\frac{\partial^2 C_{\rm d,0}}{\partial y_{\rm d}^2} = 0, \qquad \frac{\partial C_{\rm d,0}}{\partial y_d}(x, -1, t) = 0, \tag{24a}$$

$$C_{\mathrm{f},0}(x,0,t) = C_{\mathrm{d},0}(x,0,t), \tag{24b}$$

$$\frac{\partial C_{\mathrm{d},0}}{\partial y_{\mathrm{d}}}(x,0,t) = D\frac{\partial C_{\mathrm{f},0}}{\partial y_{\mathrm{f}}}(x,0,t), \tag{25}$$

$$y_{\mathrm{f}}\frac{\partial C_{\mathrm{f},0}}{\partial x} = \frac{\partial^2 C_{\mathrm{f},0}}{\partial y_{\mathrm{f}}^2}, \qquad C_{\mathrm{f},0}(x,\infty,t) = 1, \qquad C_{\mathrm{f},0}(0,y_{\mathrm{f}},t) = 1, \tag{26}$$

$$\frac{\partial B_{\mathrm{d},0}}{\partial t} = (1 - B_{\mathrm{d},0})C_{\mathrm{d},0} - K B_{\mathrm{d},0}, \qquad B_{\mathrm{d},0}(x,y_{\mathrm{d}},0) = B_{\mathrm{d,i}}(x,y_{\mathrm{d}}), \tag{27}$$

$$\frac{\partial^2 C_{\mathrm{d},1}}{\partial y_{\mathrm{d}}^2} = D\frac{\partial B_{\mathrm{d},0}}{\partial t}, \qquad \frac{\partial C_{\mathrm{d},1}}{\partial y_d}(x,-1,t) = 0, \tag{28a}$$

$$C_{\mathrm{f},1}(x,0,t) = C_{\mathrm{d},1}(x,0,t), \tag{28b}$$

$$\frac{\partial C_{\mathrm{d},1}}{\partial y_{\mathrm{d}}}(x,0,t) = D\frac{\partial C_{\mathrm{f},1}}{\partial y_{\mathrm{f}}}(x,0,t), \tag{29}$$

$$y_{\mathrm{f}}\frac{\partial C_{\mathrm{f},1}}{\partial x} = \frac{\partial^2 C_{\mathrm{f},1}}{\partial y_{\mathrm{f}}^2}, \qquad C_{\mathrm{f},1}(x,\infty,t) = 0, \qquad C_{\mathrm{f},1}(0,y_{\mathrm{f}},t) = 0, \tag{30}$$

$$\frac{\partial B_{\mathrm{d},1}}{\partial t} = (1 - B_{\mathrm{d},0})C_{\mathrm{d},1} - B_{\mathrm{d},1}C_{\mathrm{d},0} - K B_{\mathrm{d},1}, \qquad B_{\mathrm{d},1}(x,y_{\mathrm{d}},0) = 0. \tag{31}$$

Note that D appears only at first order, because D arises from essentially another transport effect, and the leading order of our system will reduce to the well-mixed case. Thus, even matrices with high D values can be ignored if the experiment is designed with a small value of Da.

Solving (24a) and (b), we obtain

$$C_{\mathrm{d},0}(x,y_{\mathrm{d}},t) = C_{\mathrm{f},0}(x,0,t). \tag{32}$$

Therefore, we note from (32) that since the reaction time scale is slower than the diffusion time scale, on the reaction time scale the diffusion is instantaneous and to leading order the concentration in the dextran layer is uniform. Thus to leading order the dextran layer behaves like a surface, since there is no variation in the y_{d}-direction.

Substituting (32) into (25), we have the following:

$$\frac{\partial C_{\mathrm{f},0}}{\partial y_{\mathrm{f}}}(x,0,t) = 0. \tag{33}$$

Solving (26) and (33) yields the constant solutions

$$C_{\mathrm{f},0}(x,y_{\mathrm{f}},t) = 1 \qquad \Longrightarrow \qquad C_{\mathrm{d},0}(x,y_{\mathrm{d}},t) = 1, \tag{34}$$

where we have used (32) again. Substituting (34) into (27) and solving, we obtain

$$B_{\mathrm{d},0}(x, y_{\mathrm{d}}, t) = \frac{1 - e^{-\alpha t}}{\alpha} + B_{\mathrm{d,i}}(x, y_{\mathrm{d}})e^{-\alpha t}, \qquad \alpha = K + 1. \tag{35}$$

Therefore, the measured quantity indicated in (21) would be

$$\bar{B}_{\mathrm{d},0}(t) = \frac{1 - e^{-\alpha t}}{\alpha} + \bar{B}_{\mathrm{d,i}}e^{-\alpha t}. \tag{36}$$

The steady state of (36) is α^{-1}, and it can be shown that this is always the steady state, no matter the size of Da. Thus we may always obtain an estimate for K simply by letting the experiment run to completion.

Taking the derivative of (35) with respect to t, we have the following:

$$\frac{\partial B_{\mathrm{d},0}}{\partial t} = e^{-\alpha t}[1 - \alpha B_{\mathrm{d,i}}(x, y_{\mathrm{d}})]. \tag{37}$$

For algebraic simplicity, we define a new function $F_0(x, y_{\mathrm{d}}, t)$:

$$\frac{\partial^2 F_0}{\partial y_{\mathrm{d}}^2} = \frac{\partial B_{\mathrm{d},0}}{\partial t}, \qquad \frac{\partial F_0}{\partial y_{\mathrm{d}}}(x, -1, t) = 0, \qquad F_0(x, 0, t) = 0. \tag{38}$$

Substituting (37) into (28a) and using (38), we may solve to obtain

$$C_{\mathrm{d},1}(x, y_{\mathrm{d}}, t) = DF_0 + g(x, t), \tag{39}$$

where $g(x, t)$ is unknown.

Then evaluating (39) at the interface and using (28b) and (29), we have the following:

$$C_{\mathrm{f},1}(x, 0, t) = g(x, t), \qquad \frac{\partial C_{\mathrm{f},1}}{\partial y_{\mathrm{d}}}(x, 0, t) = \frac{\partial F_0}{\partial y_{\mathrm{d}}}(x, 0, t). \tag{40}$$

To solve (30) and (40), we note that due to the convective nature of the flow, any exit condition we impose will necessitate the insertion of a boundary layer about $x = 1$. However, since this region is outside of the scanning range, it is not of interest. Thus, we may embed the problem in a semi-infinite region in x and use Laplace transforms in x to solve the problem. Since the quantity of interest is $C_{\mathrm{f},1}(x, 0, t)$, the transform can be inverted relatively easily to yield

$$g(x, t) = -\frac{1}{3^{1/3}\Gamma(2/3)} \int_0^x \frac{\partial F_0}{\partial y_{\mathrm{d}}}(x - \xi, 0, t) \frac{d\xi}{\xi^{2/3}}. \tag{41}$$

Note that F_0 is known, so g can be explicitly determined, in theory. Substituting (41) into (39), and using the result along with (34) in (31), we obtain

$$\frac{\partial B_{\mathrm{d},1}}{\partial t}+\alpha B_{\mathrm{d},1}=(1-B_{\mathrm{d},0})\left[DF_0-\frac{1}{3^{1/3}\Gamma(2/3)}\int_0^x\frac{\partial F_0}{\partial y_{\mathrm{d}}}(x-\xi,0,t)\frac{d\xi}{\xi^{2/3}}\right]. \quad (42)$$

However, there is a problem with (42). We note from (37) that F_0 depends on t only through the quantity $e^{-\alpha t}$, which is a solution of the homogeneous operator in (42). Thus we are forcing the operator in (42) with a multiple of the homogeneous solution. This will lead to terms that behave like $te^{-\alpha t}$, which are similar in form to a secular term in a two-timing problem. Of course, due to the fact that $B_{\mathrm{d},0}$ approaches an $O(1)$ steady state, $\mathrm{Da}B_{\mathrm{d},1} \ll B_{\mathrm{d},0}$ for all t, and so technically the expansion does not fail at this order. However, this form should still give us pause, since if we were to subtract off that steady state, then for $t = O(\mathrm{Da}^{-1})$, the second term in our expansion would be the same size as the displacement from the steady state. The standard way to fix such a problem is to introduce a multiple-scale expansion. However, from the work in Edwards (1999) we know that though it is possible to construct such an expansion, such an expansion is not illuminating.

5. MODERATE DA

We next focus on the case where $\mathrm{Da} = O(1)$. From (34) we note that we may simplify our work if we introduce the following transformations:

$$C_{\mathrm{f}}(x,y_{\mathrm{f}},t)=1-\mathrm{Da}C_{\mathrm{f},\Delta}(x,y_{\mathrm{f}},t), \qquad C_{\mathrm{d}}(x,y_{\mathrm{d}},t)=1-\mathrm{Da}C_{\mathrm{d},\Delta}(x,y_{\mathrm{d}},t), \quad (43)$$

where we choose the minus sign since C_{f} and C_{d} must be less than their saturation values. With these substitutions, our system will be exactly in the form of the system in Section 4 for $C_{\mathrm{d},1}$ and $C_{\mathrm{f},1}$, with the subscript '1' replaced by the subscript 'Δ' throughout.

The boundary conditions in (28a) and (b) still hold. However, since there is no longer any expansion for B_{d}, the operator in (28a) becomes

$$\frac{\partial^2 C_{\mathrm{d},\Delta}}{\partial y_{\mathrm{d}}^2}=-D\frac{\partial B_{\mathrm{d}}}{\partial t}, \quad (44)$$

where the right-hand side is now unknown and the negative sign arises from our substitutions in (43). However, our trick in (38) works, with minor modifications. If we now define

$$\frac{\partial^2 F_\Delta}{\partial y_{\mathrm{d}}^2}=\frac{\partial B_{\mathrm{d}}}{\partial t}, \qquad \frac{\partial F_\Delta}{\partial y_{\mathrm{d}}}(x,-1,t)=0, \qquad F_\Delta(x,0,t)=0, \quad (45)$$

then the solution of (44) and the boundary conditions in (28a) and (b) is

$$C_{\mathrm{d},\Delta}(x, y_{\mathrm{d}}, t) = -[DF_{\Delta} + g(x, t)], \tag{46a}$$

$$g(x, t) = -\frac{1}{3^{1/3}\Gamma(2/3)} \int_0^x \frac{\partial F_{\Delta}}{\partial y_{\mathrm{d}}}(x - \xi, 0, t) \frac{d\xi}{\xi^{2/3}}. \tag{46b}$$

Substituting (43), (46a) and (b) into (12), we obtain

$$1 - \frac{1}{1 - B_{\mathrm{d}}} \left(\frac{\partial B_{\mathrm{d}}}{\partial t} + K B_{\mathrm{d}} \right) = \mathrm{Da} \left[\frac{1}{3^{1/3}\Gamma(2/3)} \int_0^x \frac{\partial F_{\Delta}}{\partial y_{\mathrm{d}}}(x - \xi, 0, t) \frac{d\xi}{\xi^{2/3}} - DF_{\Delta} \right]. \tag{47}$$

In contrast to (42), equation (47) is nonlinear. It is difficult to solve explicitly, but we may glean the information we require (namely the rate constants) more easily by obtaining a small-time linear asymptote for the solution B_{d}. We do so by assuming an expansion of the form

$$B_{\mathrm{d}}(x, y_{\mathrm{d}}, t) = B_{\mathrm{d,i}}(x, y_{\mathrm{d}}) + \beta_{\mathrm{d}}(x, y_{\mathrm{d}})t + o(t), \quad t \to 0. \tag{48}$$

With such a substitution, we simply define a new variable $F_1(x, y_{\mathrm{d}})$ in the following manner:

$$\frac{\partial^2 F_1}{\partial y_{\mathrm{d}}^2} = \beta_{\mathrm{d}}, \qquad \frac{\partial F_1}{\partial y_{\mathrm{d}}}(x, -1) = 0, \qquad F_1(x, 0) = 0. \tag{49}$$

Then substituting (48) and (49) into (47), we obtain, to leading order in t,

$$1 - B_{\mathrm{d,i}} - (\beta_{\mathrm{d}} + K B_{\mathrm{d,i}}) = \mathrm{Da}(1 - B_{\mathrm{d,i}}) \left[\frac{1}{3^{1/3}\Gamma(2/3)} \int_0^x \frac{\partial F_1}{\partial y_{\mathrm{d}}}(x - \xi, 0) \frac{d\xi}{\xi^{2/3}} - DF_1 \right], \tag{50}$$

which is again an unwieldy equation, though it is linear. In order to get at least one result, we will consider the simple, but physically most important, case where $B_{\mathrm{d,i}}$ is a constant in Section 6.3.

6. CONSTANT INITIAL STATE

6.1. ***General remarks on effective rate constants.*** If the initial state is constant and $\mathrm{Da} \ll 1$, we may rewrite our solutions in terms of effective rate constants. In such a formulation, the integral equation for B_{d} is replaced by an ODE for $\bar{B}_{\mathrm{d}}$ which is good to $O(\mathrm{Da})$. Such a form is useful because experiments can usually be designed with $\mathrm{Da} \ll 1$, and commercially available data-fitting software can

more easily estimate the rate constants by fitting the data curve to the solution of an ODE.

Before considering the case discussed here, we present some general remarks on systems with receptor layers. We consider a system similar to that in the rest of the manuscript, but more arbitrary in the spatial domain:

$$\mathcal{L}_\mathrm{d} C_\mathrm{d} = \mathrm{Da} D \frac{\partial B_\mathrm{d}}{\partial t}, \qquad \mathbf{x} \in \mathcal{R}_\mathrm{d}; \qquad \mathcal{F}_\mathrm{d} C_\mathrm{d} = 0, \qquad \mathbf{x} \in \partial\mathcal{R}_{\mathrm{d,n}},$$

$$\mathcal{L}_\mathrm{f} C_\mathrm{f} = 0, \qquad \mathbf{x} \in \mathcal{R}_\mathrm{f}; \qquad \mathcal{F}_\mathrm{f} C_\mathrm{f} = 0, \qquad \mathbf{x} \in \partial\mathcal{R}_{\mathrm{f,n}},$$

$$C_\mathrm{f} = C_\mathrm{d}, \qquad \frac{\partial C_\mathrm{d}}{\partial n} = D \frac{\partial C_\mathrm{f}}{\partial n}, \qquad \mathbf{x} \in \partial\mathcal{R}_{\mathrm{d,f}},$$

$$\frac{\partial B_\mathrm{d}}{\partial t} = (1 - B_\mathrm{d}) C_\mathrm{d} - K B_\mathrm{d}, \qquad \mathbf{x} \in \mathcal{R}_\mathrm{d},$$

where $\partial/\partial n$ refers to the normal derivative, $\mathcal{L}$ is a linear operator, $\mathcal{F}$ is an affine operator, the subscript 'n' refers to a nonreacting boundary (in our case, $\tilde{y} = -H_\mathrm{d}$ and $\tilde{y} = H_\mathrm{f}$), and the double subscript 'd, f' means that boundary shared by the flow and the receptor layer (in our case, $\tilde{y} = 0$).

We take the case where $\mathrm{Da} \ll 1$ and assume that $\mathcal{L}$ and $\mathcal{F}$ are independent of Da. Then our previous scalings yield the analogous equations for the leading two orders:

$$\mathcal{L}_\mathrm{f} C_{\mathrm{f},0} = 0, \qquad \mathbf{x} \in \mathcal{R}_\mathrm{f}; \qquad \mathcal{F}_{\mathrm{f},0} C_{\mathrm{f},0} = 0, \qquad \mathbf{x} \in \partial\mathcal{R}_{\mathrm{f,n}}; \tag{51}$$

$$\mathcal{L}_\mathrm{d} C_{\mathrm{d},0} = 0, \qquad \mathbf{x} \in \mathcal{R}_\mathrm{d}; \qquad \mathcal{F}_{\mathrm{d},0} C_{\mathrm{d},0} = 0, \qquad \mathbf{x} \in \partial\mathcal{R}_{\mathrm{d,n}}; \tag{52a}$$

$$C_{\mathrm{f},0} = C_{\mathrm{d},0}, \qquad \frac{\partial C_{\mathrm{d},0}}{\partial n} = D \frac{\partial C_{\mathrm{f},0}}{\partial n}, \qquad \mathbf{x} \in \partial\mathcal{R}_{\mathrm{d,f}}; \tag{52b}$$

$$\mathcal{L}_\mathrm{d} C_{\mathrm{d},1} = D \frac{\partial B_{\mathrm{d},0}}{\partial t}, \qquad \mathbf{x} \in \mathcal{R}_\mathrm{d}; \tag{53a}$$

$$\mathcal{F}_{\mathrm{d},1} C_{\mathrm{d},1} = 0, \qquad \mathbf{x} \in \partial\mathcal{R}_{\mathrm{d,n}}; \tag{53b}$$

$$\mathcal{L}_\mathrm{f} C_{\mathrm{f},1} = 0, \qquad \mathbf{x} \in \mathcal{R}_\mathrm{f}; \qquad \mathcal{F}_{\mathrm{f},1} C_{\mathrm{f},1} = 0, \qquad \mathbf{x} \in \partial\mathcal{R}_{\mathrm{f,n}}, \tag{54a}$$

$$C_{\mathrm{f},1} = C_{\mathrm{d},1}, \qquad \frac{\partial C_{\mathrm{d},1}}{\partial n} = D \frac{\partial C_{\mathrm{f},1}}{\partial n}, \qquad \mathbf{x} \in \partial\mathcal{R}_{\mathrm{d,f}}; \tag{54b}$$

$$\frac{\partial B_\mathrm{d}}{\partial t} = (1 - B_\mathrm{d})(C_{\mathrm{d},0} + \mathrm{Da} C_{\mathrm{d},1}) - K B_\mathrm{d} + O(\mathrm{Da}^2), \qquad \mathbf{x} \in \mathcal{R}_\mathrm{d}, \tag{55}$$

where $\mathcal{F}_0$ and $\mathcal{F}_1$ are the corresponding pieces of the operator $\mathcal{F}$. Since $\mathcal{F}$ is affine and independent of Da, $\mathcal{F}_1$ must be linear.

If the solution $C_{\mathrm{d},0}$ of (52a) and (b) is uniform in $\partial\mathcal{R}_\mathrm{d}$, the leading order of (54a) and (b),

$$\frac{\partial B_{\mathrm{d},0}}{\partial t} = (1 - B_{\mathrm{d},0}) C_{\mathrm{d},0} - K B_{\mathrm{d},0}, \qquad \mathbf{x} \in \mathcal{R}_\mathrm{d},$$

is a function only of time, and hence $B_{\mathrm{d},0}$ is a function of time only. Then, since both $\mathcal{L}_{\mathrm{d},1}$ and $\mathcal{F}_{\mathrm{d},1}$ are linear, we may write

$$C_{\mathrm{d},1}(\mathbf{x},t) = \frac{dB_{\mathrm{d},0}}{dt} h_{\mathrm{d}}(\mathbf{x}), \qquad C_{\mathrm{f},1}(\mathbf{x},t) = \frac{dB_{\mathrm{d},0}}{dt} h_{\mathrm{f}}(\mathbf{x}), \tag{56}$$

where h_{d} and h_{f} satisfy (53b), (54a) and (b), and

$$\mathcal{L}_{\mathrm{d}} h_{\mathrm{d}} = D, \qquad \mathbf{x} \in \mathcal{R}_{\mathrm{d}}. \tag{57}$$

Substituting (56) into (55) and rearranging, we have

$$\frac{\partial B_{\mathrm{d}}}{\partial t} - \mathrm{Da}(1-B_{\mathrm{d},0}) \frac{dB_{\mathrm{d},0}}{dt} h_{\mathrm{d}} = (1-B_{\mathrm{d}})C_{\mathrm{d},0} - K B_{\mathrm{d}} + O(\mathrm{Da}^2), \qquad \mathbf{x} \in \mathcal{R}_{\mathrm{d}}. \tag{58}$$

We define the average of B in the usual way:

$$\bar{B}_{\mathrm{d}}(t) = \frac{1}{|\mathcal{R}_{\mathrm{d}}|} \int B_{\mathrm{d}}(\mathbf{x},t)\, d(\mathcal{R}_{\mathrm{d}}),$$

where $|\mathcal{R}_{\mathrm{d}}|$ is the area of $\mathcal{R}_{\mathrm{d}}$. Averaging (58), we obtain

$$\frac{d\bar{B}_{\mathrm{d}}}{dt} = \frac{(1-\bar{B}_{\mathrm{d}})C_{\mathrm{d},0} - K\bar{B}_{\mathrm{d}}}{1 - \mathrm{Da}(1-\bar{B}_{\mathrm{d}})\bar{h}_{\mathrm{d}}} + O(\mathrm{Da}^2), \qquad \mathbf{x} \in \mathcal{R}_{\mathrm{d}}, \tag{59}$$

which is exactly the form in Edwards *et al.*, 1999. Thus, the effect of the receptor layer (incorporated only in $\bar{h}_{\mathrm{d}}$) decouples from that of the transport (incorporated only in Da). In the absence of transport, we have that $\mathrm{Da} = 0$, and (56) reduces to the standard ordinary differential equation governing the reaction.

When $\mathrm{Da} \neq 0$, transport slows the reaction, the denominator increases, and hence the observed rate constants are smaller than the true values. Since $1 - \bar{B}_{\mathrm{d}}$ term is the average concentration of vacant receptor sites available for rebinding, one may express the effective rate constants in terms of rebinding probabilities (Edwards *et al.*, 1999).

6.2. ***Specific results, small Da.*** Now we return to the BIAcore$^{\mathrm{TM}}$ geometry when $B_{\mathrm{d,i}}(x)$ is a constant $B_{\mathrm{d,i}}$. In this case, (35) becomes

$$B_{\mathrm{d},0}(x, y_{\mathrm{d}}, t) = \frac{1 - \chi e^{-\alpha t}}{\alpha}, \qquad \chi = 1 - \alpha B_{\mathrm{d,i}}. \tag{60}$$

Since $B_{\mathrm{d},0}$ depends only on t (as postulated in Section 6.1), the averaged quantity will be the same as $B_{\mathrm{d},0}$ itself:

$$\bar{B}_{\mathrm{d},0}(t) = \frac{1 - \chi e^{-\alpha t}}{\alpha}. \tag{61}$$

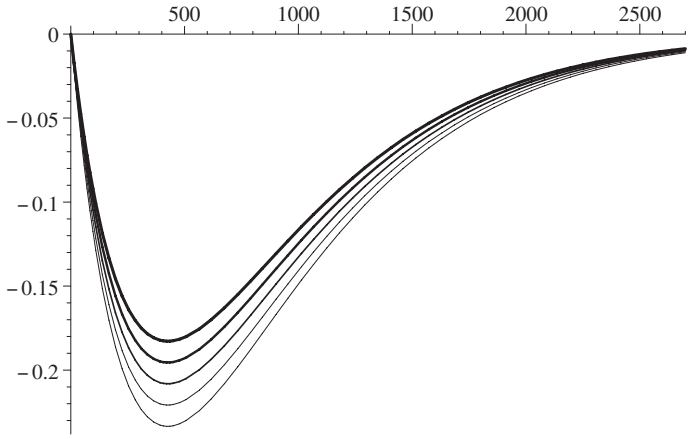

Figure 1. $\bar{B}_{\mathrm{d},1}$ vs $\tilde{t}$ for (in decreasing order of thickness) $D = 0, 1/4, 1/2, 3/4, 1$. Note the true correction to the well-mixed case will be Da times this result.

Substituting our results into (38), we have the following:

$$F_0(x, y_{\mathrm{d}}, t) = \frac{\chi y_{\mathrm{d}}(y_{\mathrm{d}} + 2)e^{-\alpha t}}{2}. \tag{62}$$

Substituting (60) and (62) into (42) and solving subject to the initial condition in (31), we obtain

$$B_{\mathrm{d},1}(x, y_{\mathrm{d}}, t) = -\frac{\chi e^{-\alpha t}}{\alpha}\left[\frac{\chi(e^{-\alpha t} - 1)}{\alpha} - Kt\right]\left\{D\left[\frac{y_{\mathrm{d}}(y_{\mathrm{d}} + 2)}{2}\right] - \frac{3^{2/3}x^{1/3}}{\Gamma(2/3)}\right\}. \tag{63}$$

Substituting (63) into (21) yields

$$\bar{B}_{\mathrm{d},1}(t) = \frac{\chi e^{-\alpha t}}{\alpha}\left[\frac{\chi(e^{-\alpha t} - 1)}{\alpha} - Kt\right]\left[\frac{D}{3} + \frac{3^{5/3}(x_{\max}^{4/3} - x_{\min}^{4/3})}{4\Gamma(2/3)(x_{\max} - x_{\min})}\right]. \tag{64}$$

Note that the last bracketed term is separated into the correction from the layer (the first term) and the correction from transport along the channel (the second term).

Figure 1 shows a graph of $\bar{B}_{\mathrm{d},1}$ [as given by (64)] vs the *dimensional* time $\tilde{t}$ (in seconds), since this is how the constants would be determined in a given experiment. Our parameters listed in Table 1 are from Edwards (1999); the appendix of that work may be consulted for appropriate ranges of the parameters. Each curve represents a different value of D; note that as D increases, the effect of the layer increases, causing a larger deviation from the well-mixed result. However, in all cases the error is small, since the expression $\bar{B}_{\mathrm{d},1}$ must still be multiplied by Da to obtain the true correction.

Table 1. Parameter values for Figs 1 and 2.

Parameter	Value	Parameter	Value
B_i	0	t	$10^{-3}\,\tilde{t}\ \mathrm{s}^{-1}$
C_T (mol cm^{-3})	10^{-11}	x_max	7.92×10^{-1}
Da	10^{-1}	x_min	2.08×10^{-1}
K	1	α	2
$\tilde{k}_\mathrm{on}$ (cm^3 mol^{-1} s^{-1})	10^8	χ	1

Now we turn to the effective rate-constant approach. Substituting (56) into the operator in (28a), we obtain

$$\frac{d^2 h_\mathrm{d}}{dy_\mathrm{d}^2} = D.$$

Thus, using an analogous technique to that used in the end of Section 4 and in deriving (62), we have

$$h_\mathrm{d}(x, y_\mathrm{d}) = \frac{D y_\mathrm{d}(y_\mathrm{d}+2)}{2} + g(x), \qquad h_\mathrm{f}(x,0) = g(x), \qquad \frac{\partial h_\mathrm{f}}{\partial y_\mathrm{d}}(x,0) = 1.$$

Since F_0 and h_d are so similar, we see that in solving the above, we obtain the braced expression in (63):

$$h_\mathrm{d}(x, y_\mathrm{d}) = D\left[\frac{y_\mathrm{d}(y_\mathrm{d}+2)}{2}\right] - \frac{3^{2/3}x^{1/3}}{\Gamma(2/3)},$$

which means that we can use (64) to obtain $\bar{h}_\mathrm{d}$, keeping in mind that we extracted a minus sign:

$$\bar{h}_\mathrm{d} = -\left[\frac{D}{3} + \frac{3^{5/3}(x_\mathrm{max}^{4/3} - x_\mathrm{min}^{4/3})}{4\Gamma(2/3)(x_\mathrm{max} - x_\mathrm{min})}\right]. \tag{65}$$

This is the expression we use in our effective rate constant equation:

$$\frac{d\bar{B}_\mathrm{d}}{dt} = \frac{1 - \alpha\bar{B}_\mathrm{d}}{1 - \mathrm{Da}(1 - \bar{B}_\mathrm{d})\bar{h}_\mathrm{d}} + O(\mathrm{Da}^2). \tag{66}$$

Figure 2 shows the error between the leading-order solution (61) and the solution to the effective rate-constant equation (66) vs time for various values of D. Again, as D increases, the effect of the layer increases, causing a larger deviation from the well-mixed result.

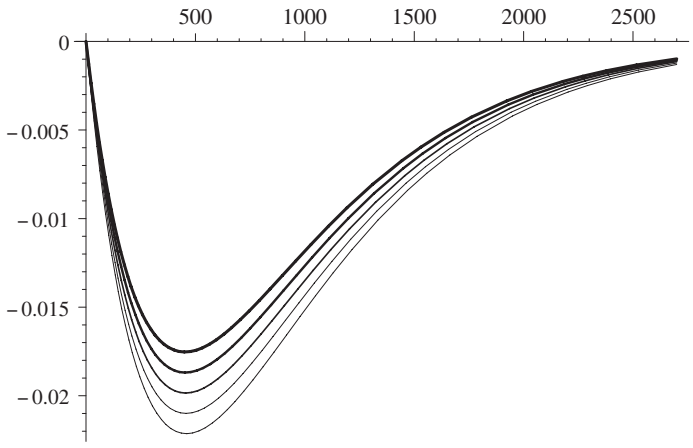

Figure 2. Difference between solution to (66) and (61) vs $\tilde{t}$ for (in decreasing order of thickness) $D = 0, 1/4, 1/2, 3/4, 1$.

6.3. ***Specific results, moderate Da.*** We conclude Section 6 by examining the moderate Da number case. The quantity in which we are truly interested is $\bar{\beta}_{\mathrm{d}}$, which is given by

$$\bar{\beta}_{\mathrm{d}} = \frac{1}{x_{\max} - x_{\min}} \int_{x_{\min}}^{x_{\max}} \frac{\partial F_1}{\partial y_{\mathrm{d}}}(x, 0)\, dx, \tag{67}$$

where we have used (49). Hence it is easiest to work directly with F_1 in (50):

$$1-\alpha B_{\mathrm{d,i}} - \frac{\partial^2 F_1}{\partial y_{\mathrm{d}}^2} = \mathrm{Da}(1-B_{\mathrm{d,i}})\left[\frac{1}{3^{1/3}\Gamma(2/3)} \int_0^x \frac{\partial F_1}{\partial y_{\mathrm{d}}}(x-\xi, 0)\frac{d\xi}{\xi^{2/3}} - DF_1\right]. \tag{68}$$

The solution process for (68) is as follows. By taking Laplace transforms, one can write (68) as an ODE for $\hat{F}_1$, the Laplace transform of F_1, which includes the boundary condition $d\hat{F}_1/dy_{\mathrm{d}}(0)$ as a parameter. Note that the integral of the inverse Laplace transform of this boundary condition is the only piece of information we need. Therefore, the inversion process is relatively straightforward and we obtain

$$\mathcal{I}[\beta_{\mathrm{d}}; x] = \frac{\chi e^{-\mu x}}{\mu} \frac{\tanh\lambda}{\lambda} [e^{\mu x} - 1 - |P(4/3, -\mu x)| + |P(5/3, -\mu x)|], \tag{69}$$

where

$$\mathcal{I}[f; x] = \int_0^x f(\xi)\, d\xi, \qquad \mu = \frac{1}{3}\left[\frac{\mathrm{Da}(1 - B_{\mathrm{d,i}})\Gamma(1/3)\tanh\lambda}{\Gamma(2/3)\lambda}\right]^3,$$
$$\lambda^2 = D\mathrm{Da}(1 - B_{\mathrm{d,i}}), \tag{70a}$$

and P is the normalized incomplete gamma function defined by

$$P(n/3, -\mu x) = \frac{\gamma(n/3, -\mu x)}{\Gamma(n/3)} = \frac{1}{\Gamma(n/3)} \int_0^{-\mu x} e^{-\xi} \xi^{n/3-1}\, d\xi. \tag{70b}$$

Thus we have the following:

$$\bar{\beta}_{\mathrm{d}} = \frac{\mathcal{I}[\beta_{\mathrm{d}}; x_{\max}] - \mathcal{I}[\beta_{\mathrm{d}}; x_{\min}]}{x_{\max} - x_{\min}},$$

and hence it can be shown (Edwards, 1999) that

$$\bar{B}_{\mathrm{d}}(\tilde{t}) \sim B_{\mathrm{i}} + S\tilde{t}, \qquad S = \frac{\tilde{k}_{\mathrm{on}} C_{\mathrm{T}} \{\mathcal{I}[\beta_{\mathrm{d}}; x_{\max}] - \mathcal{I}[\beta_{\mathrm{d}}; x_{\min}]\}}{x_{\max} - x_{\min}}. \tag{71}$$

We use the steady state to obtain an estimate for K. In order to calculate both rate constants, we construct a linear fit to our small-time experimental data. Once we have calculated the slope S of that line, we solve (71) to obtain $\tilde{k}_{\mathrm{on}}$. It is important to note that the relationship between S and $\tilde{k}_{\mathrm{on}}$ is not linear, since β_{d} also depends on $\tilde{k}_{\mathrm{on}}$ through the parameter Da. Then using our value for K, we may calculate $\tilde{k}_{\mathrm{off}}$. We also note that in the limit that $D \to 0$, we obtain

$$\frac{\tanh \lambda}{\lambda} = 1 + O(\lambda^2) = 1 + O(D).$$

Therefore, for small D the size of the correction is the same as that in previous sections.

Using the small- and large-μ behavior of the P function, we can ascertain the large- and small-$\tilde{k}_{\mathrm{on}}$ behavior of S. For small $\tilde{k}_{\mathrm{on}}$, we have

$$S \sim \tilde{k}_{\mathrm{on}} C_{\mathrm{T}} \chi, \qquad \tilde{k}_{\mathrm{on}} \to 0. \tag{72a}$$

As expected, (72a) shows that if there is no forward reaction ($\tilde{k}_{\mathrm{on}} = 0$), the bound concentration will not change ($S = 0$). For large $\tilde{k}_{\mathrm{on}}$, we have the following:

$$S \sim \frac{3^{\frac{4}{3}} \chi C_{\mathrm{T}} V^{\frac{1}{3}} \tilde{D}_{\mathrm{f}}^{\frac{2}{3}} (x_{\max}^{\frac{2}{3}} - x_{\min}^{\frac{2}{3}})}{2^{\frac{1}{3}} (1 - B_{\mathrm{d,i}}) \Gamma\left(\frac{1}{3}\right) R_{\mathrm{T}} L^{\frac{1}{3}} H_{\mathrm{f}}^{\frac{1}{3}} (x_{\max} - x_{\min})}, \qquad \tilde{k}_{\mathrm{on}} \to \infty. \tag{72b}$$

The presence of a finite asymptote for S in the limit of large $\tilde{k}_{\mathrm{on}}$ is physically reasonable, since no matter how fast the reaction proceeds, the mass uptake will be limited by the amount of unbound ligand available for assimilation. Note also that equations (72a) and (b) are independent of D.

Figure 3 shows how the slope S varies with the variable k for various values of D. To plot our results, we again use the parameters from Edwards (1999), which

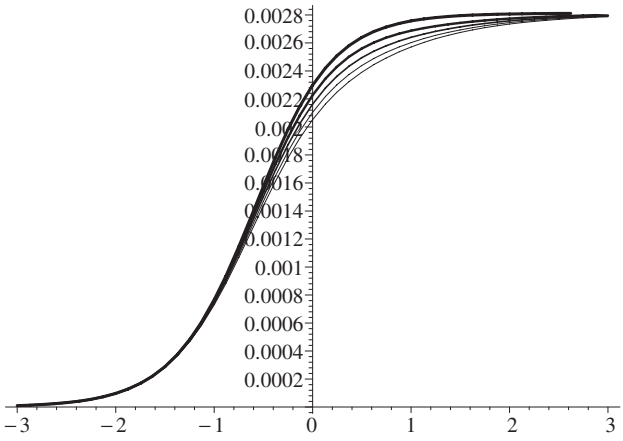

Figure 3. S vs $\log_{10} k$ for (in decreasing order of thickness) $D = 0, 1/4, 1/2, 3/4, 1$.

Table 2. Parameter values for Fig. 3.

Parameter	Value	Parameter	Value
B_{i}	0	R_{T} (mol cm^{-2})	10^{-12}
C_{T} (mol cm^{-3})	10^{-11}	V (cm s^{-1})	1
$\tilde{D}_{\mathrm{f}}$ (cm^2 s^{-1})	2.8×10^{-7}	$x_{\max}$	7.92×10^{-1}
H_{f} (cm)	5×10^{-3}	$x_{\min}$	2.08×10^{-1}
k	$10^{-9}\tilde{k}_{\mathrm{on}}$mol s cm^{-3}	χ	1
L (cm)	2.4×10^{-1}		

are listed in Table 2. Here k is a dimensionless variable introduced to stabilize the numerical calculations. The asymptotes are the same for all graphs since (72a) and (b) are independent of D. Note that as D increases, the slope decreases for moderate values of k. Thus, except for very fast and very slow reactions, the effect of the dextran layer is to slow the initial speed of the reaction. That is to be expected, since the dextran layer introduces another transport barrier. This transport barrier is irrelevant in the cases where the reaction is infinitely fast or infinitely slow.

7. DISSOCIATION EXPERIMENTS

We now make some remarks regarding dissociation experiments. These experiments are usually run after an association experiment has reached steady state. We note from (36) that regardless of the initial data for the association experiment, the steady state is given by $B_{\mathrm{d},0}(x, y_{\mathrm{d}}, \infty) = \alpha^{-1}$, and hence equation (19) is replaced

by

$$B_{\mathrm{d}}(x, y_{\mathrm{d}}, 0) = \frac{1}{\alpha} \tag{73}$$

for the dissociation experiment. Note that this ensures that we are in the uniform case detailed in Section 6.

During the experiment, the external flow is emptied of all ligands, so equations (20) and (16) become

$$C_{\mathrm{f}}(x, \infty, t) = 0, \qquad C_{\mathrm{f}}(0, y_{\mathrm{f}}, t) = 0.$$

Replacing the boundary conditions in (26) with the leading order of the above yields the constant solution

$$C_{\mathrm{f},0}(x, y_{\mathrm{f}}, t) = 0 \qquad \Longrightarrow \qquad C_{\mathrm{d},0}(x, y_{\mathrm{d}}, t) = 0. \tag{74}$$

Since the time scales are not dependent on the initial data, we again see that diffusion will occur fast enough that any ligand that dissociates will diffuse out of the channel and be swept away quickly.

Substituting (74) into (27), we obtain

$$B_{\mathrm{d},0}(x, y_{\mathrm{d}}, t) = \frac{e^{-Kt}}{\alpha}, \tag{75}$$

where we have used (73). Since the definition of F_0 does not depend on the specific form of $B_{\mathrm{d},0}$, equations (39) and (41) still hold. However, using (74) in (28b), we see that the equation analogous to (42) is

$$\frac{\partial B_{\mathrm{d},1}}{\partial t} + K B_{\mathrm{d},1} = (1 - B_{\mathrm{d},0})\left[D F_0 - \frac{1}{3^{1/3}\Gamma(2/3)} \int_0^x \frac{\partial F_0}{\partial y_{\mathrm{d}}}(x - \xi, 0, t) \frac{d\xi}{\xi^{2/3}} \right]. \tag{76}$$

We note that only the left-hand side of (76) is different from (42).

Continuing to simplify (76) using Section 6.2, we note that since

$$\frac{\partial^2 F_0}{\partial y_{\mathrm{d}}^2} = \frac{\partial B_{\mathrm{d},0}}{\partial t} = -K \frac{e^{-Kt}}{\alpha},$$

we have that our derivation proceeds analogously to that before (62) and hence (63) becomes

$$B_{\mathrm{d},1}(x, y_{\mathrm{d}}, t) = \frac{K e^{-Kt}}{\alpha} \left(t + \frac{e^{-Kt} - 1}{K\alpha} \right) \left\{ \frac{3^{2/3} x^{1/3}}{\Gamma(2/3)} - D\left[\frac{y_{\mathrm{d}}(y_{\mathrm{d}} + 2)}{2} \right] \right\}, \tag{77}$$

which again exhibits secular behavior as $t \to \infty$. We may calculate the average as in Section 6.2, since the only change is to the coefficient of the braced quantity. Thus, the equation analogous to (64) is

$$\bar{B}_{\mathrm{d},1}(t) = -\frac{Ke^{-Kt}}{\alpha}\left(t + \frac{e^{-Kt}-1}{K\alpha}\right)\left[\frac{D}{3} + \frac{3^{5/3}(x_{\max}^{4/3} - x_{\min}^{4/3})}{4\Gamma(2/3)(x_{\max} - x_{\min})}\right]. \tag{78}$$

We now focus on the effective rate-constant work. In Section 6.2 the derivation of (65) was independent of the concentration $C_{\mathrm{d},0}$ in the dextran layer. Therefore, (65) still holds in the dissociation phase. The only difference is that we must use (74) instead of (34) in (59), so (66) becomes

$$\frac{d\bar{B}_{\mathrm{d}}}{dt} = \frac{-K\bar{B}_{\mathrm{d}}}{1 - \mathrm{Da}(1-\bar{B}_{\mathrm{d}})\bar{h}_{\mathrm{d}}}. \tag{79}$$

Since the effective rate-constant work is independent of the value of $C_{\mathrm{d},0}$, the combination of (59) and (65) holds in both association and dissociation experiments.

Finally, we focus on the case where $\mathrm{Da} = O(1)$. There are several notational changes, but the general form of the solutions again remains the same. Our transformations (43) become, motivated by (74),

$$C_{\mathrm{f}}(x, y_{\mathrm{f}}, t) = \mathrm{Da}C_{\mathrm{f},\Delta}(x, y_{\mathrm{f}}, t), \qquad C_{\mathrm{d}}(x, y_{\mathrm{d}}, t) = \mathrm{Da}C_{\mathrm{d},\Delta}(x, y_{\mathrm{d}}, t). \tag{80}$$

The change of sign makes the equation analogous to (47)

$$\frac{1}{1-B_{\mathrm{d}}}\left(\frac{\partial B_{\mathrm{d}}}{\partial t} + KB_{\mathrm{d}}\right) = \mathrm{Da}\left[DF_{\Delta} - \frac{1}{3^{1/3}\Gamma(2/3)}\int_0^x \frac{\partial F_{\Delta}}{\partial y_{\mathrm{d}}}(x-\xi, 0, t)\frac{d\xi}{\xi^{2/3}}\right]. \tag{81}$$

Using the β_{d} substitution again, we obtain

$$\beta_{\mathrm{d}} + KB_{\mathrm{d,i}} = \mathrm{Da}(1 - B_{\mathrm{d,i}})\left[DF_1 - \frac{1}{3^{1/3}\Gamma(2/3)}\int_0^x \frac{\partial F_1}{\partial y_{\mathrm{d}}}(x-\xi, 0)\frac{d\xi}{\xi^{2/3}}\right]. \tag{82}$$

Continuing to simplify, we see that the equation analogous to (69) is

$$\mathcal{I}[\beta_1; x] = -\frac{Ke^{-\mu x}}{\alpha\mu}\frac{\tanh\lambda}{\lambda}[e^{\mu x} - 1 - |P(4/3, -\mu x)| + |P(5/3, -\mu x)|]. \tag{83}$$

As in the previous section, we may show that

$$S \sim \frac{\tilde{k}_{\mathrm{off}}}{K+1}, \qquad \tilde{k}_{\mathrm{on}} \to 0, \tag{84a}$$

$$S \sim \frac{3^{4/3}C_{\mathrm{T}}V^{1/3}\tilde{D}_{\mathrm{f}}^{2/3}(x_{\max}^{2/3} - x_{\min}^{2/3})}{2\Gamma(1/3)R_{\mathrm{T}}L^{1/3}H_{\mathrm{f}}^{1/3}(x_{\max} - x_{\min})}, \qquad \tilde{k}_{\mathrm{on}} \to \infty. \tag{84b}$$

8. EXPERIMENTAL CONSIDERATIONS

Now that the mathematical expressions have been calculated, it remains to interpret them in an experimental context. Due to the fact that reactions occur on a time scale slower than transport, the receptor layer in and of itself does not affect measurements. That is, if one could construct an experiment where the flow was infinitely fast, the distribution of ligand along the channel would be uniform. Hence diffusion into the dextran itself would be uniform and adjustment of the calculated rate constants would not be necessary.

Thus, to minimize the effects of the dextran layer, one must first minimize transport effects. This is done by setting the dimensionless parameter Da $\ll 1$, which is equivalent to taking

$$V \gg \frac{\tilde{k}_{\mathrm{on}}^3 \tilde{R}_{\mathrm{T}}^3 H_{\mathrm{f}} L}{\tilde{D}_{\mathrm{f}}^2}. \tag{85}$$

Though the bound for V involves the unknown rate constant $\tilde{k}_{\mathrm{on}}$, one should be able to obtain at least an order-of-magnitude estimate through even unadjusted calculations. The balance is between the upper limit on the flow velocity V achieved in the device vs the lower limit on the receptor density $\tilde{R}_{\mathrm{T}}$ needed to achieve a readout from the BIAcore$^{\mathrm{TM}}$ that is strong enough to be usable.

Once Da has been reduced as much as possible, the next step is to reduce the size of D, which can be achieved by taking

$$H_{\mathrm{d}} \ll \frac{\phi \tilde{D}_{\mathrm{d}} H_{\mathrm{f}}^{1/3} L^{1/3}}{\tilde{D}_{\mathrm{f}}^{2/3} V^{1/3}}. \tag{86}$$

Clearly to achieve this bound one should use as thin a layer as possible. Other considerations are to make the partition coefficient as near to 1 as possible and the diffusion coefficient in the dextran not much smaller than the diffusion coefficient in the flow. An interesting note is that the bound depends inversely on $V^{1/3}$, which in (85) we have tried to make as large as possible. Though clearly a balance must be struck, reducing Da is of primary importance. Since the dependence of D on V is weaker than that of Da, this argues for keeping V as large as possible within device tolerances.

Once the parameters have been adjusted to minimize the effects of the receptor layer, equations (59) and (65) can be used to calculate the rate constants. Equation (59) is simply the standard evolution equation with a denominator that incorporates the effect of transport. Though technically only good in the limit of small Da, a related equation has been shown to be accurate within a few percent in the case of moderate and large Da (Edwards and Jackson, submitted).

In order to be more confident in the case when Da is not small, one can use linear short-time fits of the data to estimate the rate constants. In the association case,

one can use equations (69) and (71). In the dissociation case one uses (83) and the fact that in this case

$$\bar{B}_{\mathrm{d}}(\tilde{t}) \sim \frac{1}{\alpha} + S\tilde{t}. \tag{87}$$

9. CONCLUSIONS

Estimates of rate constants for chemical reactions play a key role in enhancing the understanding of certain biological systems. With the advent of SPR technology and its application in various measurement devices, scientists can now track the evolution of the bound state during a controlled experiment. Unfortunately, such technological advances are useless without the necessary mathematical models to interpret the data.

Previous models for receptor-ligand reactions, both within biological systems and without, have treated the receptors as embedded on a surface, thus reducing the reaction to a boundary condition for a transport equation. In truth, the receptors are embedded in a thin layer above the surface. Hence a more correct mathematical model for the system is a convection-diffusion equation for the analyte coupled across an interface to a diffusion-reaction equation in the receptor layer. By introducing proper dimensionless variables, we noted that the reaction occurs on a time scale slower than that for transport.

There are two key dimensionless groups in the problem: the Damköhler number Da, which measures the strength of transport effects, and the parameter D, which measures the effect of the receptor layer. In the limit that $\mathrm{Da} \to 0$, we reduce to the standard well-mixed kinetic theory. We calculated the first-order correction in the case that $\mathrm{Da} \to 0$, and demonstrated that the correction due to the receptor layer occurs at this order. We calculated general expressions for any initial data, as well as explicit and effective rate constant approximations for uniform initial data. The general expressions are good only for $t = o(\mathrm{Da})$ due to the secular nature of the underlying operator.

In the case where transport is more important and $\mathrm{Da} = O(1)$, we derived a nonlinear integrodifferential equation for the concentration of the bound state. By looking at the small-time asymptotic behavior of the data, we may construct estimates of the rate constants. We indicated how this small-time solution would change as the rate constants varied, and we provided large- and small-$\tilde{k}_{\mathrm{on}}$ behavior of the small-time solution.

Our results indicate that the effects of the receptor layer occur only at $O(\mathrm{Da})$, which is usually small. However, since D need not be small, especially for dense matrices, the effect of the receptor layer can be as large as the effects of transport. By characterizing the relevant properties of the receptor layer in the single parameter D, we provide guidance on how to design trials to minimize the effect of the receptor layer.

NOMENCLATURE

Variables and Parameters. Units are listed in terms of length (L), moles (N), or time (T). If the same letter appears both with and without tildes, the letter with a tilde has dimensions, while the letter without a tilde is dimensionless. The equation where a quantity first appears is listed, if appropriate.

a: steric hindrance factor (7).

$\tilde{B}(\cdot, \tilde{t})$: bound ligand concentration on surface at position $\cdot$ and time $\tilde{t}$, units N/L^2 (4).

$\tilde{C}(\tilde{x}, \tilde{y}, \tilde{t})$: unbound ligand concentration at position $(\tilde{x}, \tilde{y})$ and time $\tilde{t}$, units N/L^3 (1).

$\tilde{D}$: molecular diffusion coefficient, units L^2/T (1).

Da: the Damköhler number, which measures the ratio of reaction and diffusion effects (15b).

$\mathcal{F}$: affine operator.

$F(\cdot)$: arbitrary function, variously defined (38).

$f(\cdot)$: arbitrary function, variously defined.

$g(x, t)$: Dirichlet data, variously defined (39).

H: height of a portion of the channel, units L.

$h(x, \cdot)$: function used in effective rate constant solution (56).

$\mathcal{I}[\cdot; x]$: integration operator, defined in (70a) as

$$\mathcal{I}[f; x] \equiv \int_0^x f(\xi)\, d\xi.$$

$\mathcal{K}(y_d)$: kernel of signal measurement operator.

$\tilde{K}$: affinity constant for system, defined as $\tilde{k}_{\text{off}}/\tilde{k}_{\text{on}}$, units N/L^3 (7).

$\tilde{k}_{\text{off}}$: dissociation rate, units T^{-1} (7).

$\tilde{k}_{\text{on}}$: binding rate, units $L^3/(NT)$ (7).

$\mathcal{L}$: linear operator.

L: length of the channel, units L.

n: arbitrary constant.

$P(n/3, -\beta x)$: normalized incomplete gamma function (70b).

Pe: Peclét number for the system, which measures the ratio of convective to diffusive effects, defined as $VH_{\text{f}}^2/\tilde{D}_{\text{f}}L$ (10b).

$\mathcal{R}$: arbitrary region.

$\tilde{R}_{\text{T}}$: receptor sites, units N/L^2 (7).

S: slope of a line, units T^{-1} (71).

$\tilde{t}$: dimensional time, units T (1).

V: four times the (maximal) velocity of flow at center of channel, units L/T (1).

$\mathbf{x}$: arbitrary position coordinate.

$\tilde{x}$: dimensional measure of length along the channel, units L (1).
$\tilde{y}$: dimensional measure of height from dextran–flow interface, units L (1).
$\mathcal{Z}$: the integers.
α: dimensionless constant, defined as $1 + K$ (35).
$\beta_{\mathrm{d}}(x, y_{\mathrm{d}})$: term in expansion of $B_{\mathrm{d}}(x, y_{\mathrm{d}}, t)$ for small t (48).
λ: dimensionless constant (70a).
μ: dimensionless constant (70a).
ξ: dummy variable.
ϕ: partition coefficient (5b).
χ: dimensionless constant (60).

Other Notation.

d: as a subscript, used to indicate the dextran layer.
f: as a subscript, used to indicate the flow region (1).
i: as a subscript, used to indicate the initial state of a quantity (2).
max: as a subscript, used to indicate the right endpoint of the scanning range.
min: as a subscript, used to indicate the left endpoint of the scanning range.
$n \in \mathcal{Z}$: as a subscript, used to indicate an expansion in Da (22a).
T: as a subscript, used to indicate the total value of a quantity (3).
Δ: as a subscript, used to indicate a displacement (43).
$\bar{}$: used to denote the mean of the bound concentration over a subset of dextran layer.
$\hat{}$: used to indicate the Laplace transform of a quantity.

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APPENDIX

In Table 3 we specifically compile parameter values relevant to the new analysis here; other parameters may be found in the appendix of Edwards (1999). The

Table 3. Parameter values from the literature.

	Parameter				
Reference	C_T (10^{-11} mol/cm^3)	$\tilde{D}_d/\tilde{D}_f$	H_d (10^{-5} cm)	R_T (10^{-12} mol/cm^2)	ϕ
Edwards (1995)			2–5		
Johnsson (1995)			1–2		
Karlsson (1994)			0.3–1		
Löfås and Johnsson (1990)			1		
Parsons and Stockley (1997)			0.3–1		
Schuck (1996)		0.02–0.1	0.1–1		0.3–1
Yarmush *et al.* (1996)	0.25–40	0.04–0.12	1	0.25–4	0.1–0.25

Table 4. Calculated parameters.

Parameter	Range
D	$6.69 \times 10^{-4} \leq D \leq 3.73 \times 10^2$ (see note in text)
Pe	$37.4 \leq \text{Pe} \leq 4.16 \times 10^5$
R	$12.5 \leq R \leq 1.6 \times 10^7$

parameters in Yarmush *et al*. are for a bovine serum albumin/monoclonal antibody system. The varying values of H_d in the literature arise at least partly from the fact that the BIAcore™ comes with various sensor chips, each having different thicknesses of the dextran layer. In addition, the paper by Schuck (1996) contains numerical simulations, and thus various values for $\tilde{D}_d/\tilde{D}_f$, H_d, and ϕ could be employed.

Table 4 shows the ranges of the dimensionless parameters in our analysis given the parameters in Table 3 and Edwards (1999). We note that both $\text{Pe} \gg 1$ and $R \gg 1$, as claimed. We make a brief remark about the upper bound of D in the table, which arises from taking a highly unlikely combination of parameters. If instead of this combination, we take the value $\text{Pe} = 3.71 \times 10^2$ we use to plot our graphs, the upper bound on H_d from Johnsson *et al*. (1995), and the experimental bounds from Yarmush *et al*. (1996), we obtain the upper bound

$$D = 7.19,$$

which is moderate, not large.

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