A MATHEMATICAL JOURNEY THROUGH OPTICAL BIOSENSORS

by

Ryan Murray Evans

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Applied Mathematics

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by

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ABSTRACT

Many chemical reactions in nature involve a stream of reactants (ligand molecules) flowing through a fluid-filled volume, over a surface to which other reactants (receptors) are confined. Such surface-volume reactions occur during processes like blood platelet adhesion, drug absorption, and DNA damage repair. To measure rate constants associated with such reactions, scientists use optical biosensors. Correct interpretation of biosensor data requires having an accurate mathematical model, and models have been successfully proposed and progressively refined throughout the years. Although such models are typically limited to situations involving only a single type of reaction on the surface (e.g., bimolecular), many chemical reactions involve more than one step or molecule. One example is the polymerase switch which occurs during DNA translesion synthesis, which is a reaction thought to be critical to DNA damage repair. We formulate and study a mathematical model for this multiple-component process. We derive physically relevant numerical and analytic results, including a nonlinear set of Ordinary Differential Equations (i.e., Effective Rate Constant Equations) which may be used to estimate reaction rate constants from raw data. We also demonstrate that our mathematical model can aid in eliminating ambiguity from sensogram data.

We extend the theory for bimolecular reactions as well. Modeling these reactions results in a nonlinear differential equation, and we can find an approximate solution to this equation using traditional perturbation methods in the reaction-limited parameter regime. However, outside of this parameter regime these methods fail. By coupling the strained coordinates technique with the Homotopy Perturbation Method, a method motivated by concepts from topology, we are able to find an expression for the approximate solution of our equation despite the presence of a strong nonlinearity.
Many biochemical reactions in nature involve a stream of chemical reactants (ligand molecules) flowing through a fluid-filled volume, and another reactant (receptors) confined to a surface. Such surface-volume reactions occur during blood platelet adhesion [2], drug absorption [3], antigen-antibody interactions [21], and DNA damage repair [28]. Fundamental to understanding these reactions is getting accurate quantitative measurements of the underlying reaction rate constants.

To measure rate constants associated with surface-volume reactions, scientists use optical biosensors: see Figure 1.1 for a schematic of one such instrument. Biosensor technology has become extremely popular in recent years; 10,000 papers have cited the use of an optical biosensor as of 2009 alone [23]. There are two phases to any optical-biosensor experiment: an injection phase, and a wash phase. During the injection phase, ligand molecules are injected into the biosensor at concentration \( \tilde{C}(\tilde{x}, \tilde{y}, \tilde{t}) \) in a buffer fluid—the tilde variables denote dimensional quantities throughout. These

Figure 1.1: Cross-sectional schematic of binding/unbinding in an optical biosensor.
molecules then diffuse through the buffer fluid onto the channel floor, where they bind with receptors. Mass changes on the floor due to ligand binding are averaged over a portion of the channel floor $[\bar{x}_{\text{min}}, \bar{x}_{\text{max}}]$ to produce measurements of the form

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

(1.1)

where $B(\bar{x}, \bar{t})$ is the concentration of bound ligand molecules. After the bound state concentration reaches a chemical equilibrium, scientists prepare the device for another experiment by washing it with the buffer fluid—this is the wash phase of the experiment. During the wash phase, only the buffer fluid is flowing through the biosensor, not the fluid containing the ligand molecules. This has the effect of causing all of the bound ligand molecules at the surface to dissociate, and get swept out of the biosensor. We remark that the injection and wash phases are sometimes referred to as the association and dissociation phase respectively; however it should be highlighted that both association and dissociation occur during each phase of the experiment. Hence, we will refer to the two phases as the injection phase and the wash phase.

Measuring rate constants with biosensor data requires having an accurate mathematical model of this process; mathematical models have been successfully proposed and progressively refined throughout the years: [6], [7], [8], [9], [10], [11], [16], [30], [31]. Such mathematical models are limited to reactions involving only a single step, or a single molecule. However, chemists are currently using biosensor technology to measure reaction rates associated with reactions involving multiple steps and molecules. One example of such a multiple-component process is the polymerase switch which occurs during DNA translesion synthesis; this reaction is thought to be critical to DNA damage repair. This reaction involves the two polymerase molecules polδ and polη, which we denote by $L_1$ and $L_2$ respectively.

When running biosensor experiments to measure rate constants associated with this multiple-component process, scientists inject $L_1$ and $L_2$ (concentrations $\bar{C}_1(\bar{x}, \bar{y}, \bar{t})$ and $\bar{C}_2(\bar{x}, \bar{y}, \bar{t})$) into the biosensor in a buffer fluid at the uniform inflow concentrations $\bar{C}_1(0, \bar{y}, \bar{t}) = \bar{C}_{1,u}$ and $\bar{C}_2(0, \bar{y}, \bar{t}) = \bar{C}_{2,u}$. Once these ligand molecules diffuse to the
surface of the biosensor, they may interact with a PCNA receptor (denoted by $E$) to
form the three different reacting species $EL_1$, $EL_2$, and $EL_1L_2$:

$$(P_1) : E + L_2 \xrightleftharpoons{\tilde{k}_a}{\tilde{k}_d} EL_2,$$  \quad (1.2)

$$(P_2) : E + L_1 \xrightleftharpoons{\tilde{k}_a}{\tilde{k}_d} EL_1, \quad EL_1 + L_2 \xrightleftharpoons{\tilde{k}_a}{\tilde{k}_d} EL_1L_2 \xrightleftharpoons{\tilde{k}_d}{\tilde{k}_a} EL_2 + L_1,$$  \quad (1.3)

see Figure 1.2; $E$ denotes our empty receptor. In our notation $\tilde{k}_a$ denotes the rate at
which a ligand molecule $L_i$ associates with a receptor $E$, and $\tilde{k}_d$ denotes the rate at
which ligand $L_i$ dissociates from the product $EL_i$. In addition, $\tilde{j}_k$ denotes the rate
at which ligand molecule $L_i$ binds with the product $EL_j$. Similarly, $\tilde{j}_i$ represents the
rate at which molecule $L_i$ dissociates from the product $EL_iL_j$.

The end product of this reaction is $EL_2$, and can form via two different path-
ways, depicted in Figure 1.2 as $(P_1)$ and $(P_2)$. The first pathway (labeled $(P_1)$) involves
direct binding, i.e. a ligand $L_2$ molecule diffuses to the surface and directly binds with
an available receptor to form $EL_2$. In the second pathway (labeled $(P_2)$) a ligand $L_1$
molecule first diffuses to the surface to bind with an available receptor site to form
$EL_1$. Then, a ligand $L_2$ molecule diffuses to the surface to bind with $EL_1$, forming
$EL_1L_2$. Finally the ligand $L_1$ dissociates from the product $EL_1L_2$ (or “switches out”),
thereby leaving us with $EL_2$.

To simplify the problem of measuring these eight rate constants, one may think
to run two separate experiments: a first by injecting ligand $L_2$ alone, and a second by
injecting ligand $L_1$ alone. Since the second pathway is completely eliminated during

![Diagram](image)

Figure 1.2: Different pathways in our multiple-component reaction.
the first experiment, the system has been reduced to one involving only the single step reaction in pathway one. Because the single-step system has been well studied, this would allow us to calculate the rate constants $\tilde{k}_a$ and $\tilde{k}_d$. By running the second experiment we could calculate $\tilde{k}_a$ and $\tilde{k}_d$ in a similar way. Unfortunately, these experiments alone will tell us nothing about the rate constants $\tilde{k}_a$, $\tilde{k}_d$, $\tilde{k}_a$, $\tilde{k}_d$ present in pathway two. Thus to determine the other four rate constants, we must construct an experiment involving both $L_1$ and $L_2$. This may be done by either injecting $L_1$ and $L_2$ at the same time, or injecting the two sequentially. However, most optical biosensors measure only mass changes at the surface due to ligand binding, and produce lumped measurements of the form

$$\tilde{S}(t) = \tilde{s}_1B_1(t) + (\tilde{s}_1 + \tilde{s}_2)\frac{\partial}{\partial \tilde{t}} B_{12}(\tilde{t}) + \tilde{s}_2B_2(\tilde{t}).$$

Here $\tilde{B}_i(\tilde{x}, \tilde{t})$, $i = 1, 12, 2$ represent the concentration of the reacting species $EL_1$, $EL_1L_2$, $EL_2$; $\tilde{B}_i$ is defined analogously to (1.1); and the $\tilde{s}_i$ are proportional to the molecular weights. Thus we are faced with the problem of fitting four rate constants to one signal, which immediately raises a question of uniqueness—more than one set of rate constants may be fit to the same signal (1.4). We address this question in Chapter 9. We demonstrate that in situations where our inverse problem lacks a unique solution, it is possible to identify the true set of rate constants. We propose two methods for identifying the correct set of rate constants: one based on displacing the signal (1.4), and another based on adjusting the inflow concentrations $\tilde{C}_{i,u}$.

Lack of uniqueness applies even to well-stirred systems, but transport effects on ligand binding render the well-stirred approximation inappropriate in our case. These effects are observed during the injection phase through a phenomenon known as upstream ligand depletion, whereby ligand molecules diffuse to the surface to bind with receptor sites upstream, before they bind with receptor sites downstream. Additionally, ligand molecules dissociating upstream may rebind to other receptor sites downstream.

To account for such transport effects, we develop a partial differential equation (PDE) model for this advection-diffusion-reaction system in Chapter 2. Rather than
treat this system numerically, we undertake an analytic study. Following the existing literature [6], in Chapter 2 we demonstrate that transport effects play a role only in a thin unstirred region near the surface. We study this unstirred layer in Chapter 3 for various parameter regimes; in Chapter 4 we focus on the reaction-limited and transport-dominant parameter regime. In particular, we reduce the full PDE model, to a nonlinear set of integrodifferential equations (IDEs) describing the evolution of the reacting species concentration. We find a two-term approximation to this set of IDEs, and also further reduce this set of IDEs to a nonlinear set of ordinary differential equations (ODEs) describing the evolution of the average reacting species concentration. We refer to this set of ODEs as our Effective Eate Constant (ERC) equations, and we shall see that a key feature of these equations is their simplicity.

To verify the accuracy of this set of ODEs, in Chapter 5 we develop a semi-implicit finite difference algorithm to approximate the solution to the full IDE system. We also study the convergence and stability of our method; for simplicity we will limit our convergence and stability studies to the bimolecular case, involving only a single ligand molecule. We then verify the accuracy of our ERC equations in Chapter 6.

To prepare the biosensor for experiments, scientists must place PCNA rings on the receptors confined to the surface. This placement is itself a surface-volume reaction, and thus subject to transport effects. Not every receptor site is paired with a PCNA ring, which results in multiple receptor types. Therefore in Chapter 2, we also formulate a multiple-receptor model. In Chapter 7, we present and verify a set of ERC equations for our multiple-receptor model. Additionally, we study the sensogram reading associated with our multiple-receptor model in Chapter 10.

In addition to studying multiple-component reactions, we also extend the theory for bimolecular reactions. As we shall see, modeling surface-volume reactions in optical biosensors results in a nonlinear differential equation for $\overline{B}$. In the reaction-limited and transport-dominant parameter regime, the nonlinearity is weak, and one may find an expression for $\overline{B}$ using traditional perturbation techniques. However, when reaction and diffusion balance, the nonlinearity is strong and traditional perturbation techniques
fail. In Chapter 8 we couple the Homotopy Perturbation Method with a traditional strained coordinates technique to find an approximate expression for $\overline{B}$ which holds for a wide parameter range.
Chapter 2
MULTIPLE-COMPONENT MODELS

We now turn our attention to deriving our multiple-component models by first deriving the velocity profile. We then present the dimensional advection-diffusion-reaction system. Herein, we will keep the multiple-ligand model in mind, and present results for our multiple-receptor model when appropriate.

2.1 Velocity Profile Derivation

Flow through the channel is governed by the two-dimensional incompressible Navier-Stokes equations:

\[ \tilde{\rho} \left( \frac{\partial \tilde{v}_i}{\partial t} + (\tilde{v}_i \cdot \tilde{\nabla}) \tilde{v}_i \right) = -\tilde{\nabla} \tilde{p}_i + \tilde{\mu}_i \tilde{\nabla}^2 \tilde{v}_i, \]
\[ \tilde{\nabla} \cdot \tilde{v}_i = 0. \]

Here \( \tilde{v}_i, \tilde{p}_i, \tilde{\mu}_i, \tilde{\rho}_i \) denote dimensional velocity, pressure, viscosity, and density. Additionally, the operators \( \frac{\partial}{\partial t}, \tilde{\nabla}, \tilde{\nabla}^2 \) denote dimensional time derivative, gradient, and Laplacian operators respectively. The subscript \( i = 1, 2 \) denotes the velocities for the two unbound ligand concentrations \( \tilde{C}_i \). The buffer that contains \( \tilde{C}_1 \) will be the same buffer that will contain \( \tilde{C}_2 \), therefore the densities \( \tilde{\rho}_i \), viscosities \( \tilde{\mu}_i \), pressure gradients \( \nabla \tilde{p}_i \), and velocities \( \tilde{v}_i \) will be the same. Hence deriving the velocity profile for each of the ligands is equivalent to deriving the velocities for the flow of a single ligand, so we write:

\[ \tilde{\rho} \left( \frac{\partial \tilde{v}}{\partial t} + (\tilde{v} \cdot \tilde{\nabla}) \tilde{v} \right) = -\tilde{\nabla} \tilde{p} + \tilde{\mu} \tilde{\nabla}^2 \tilde{v}, \]  
\[ \tilde{\nabla} \cdot \tilde{v} = 0. \]
In addition, we impose no-slip conditions at the floor and ceiling of the channel:

\[
\tilde{v}_x(\tilde{x}, 0, \tilde{t}) = 0, \quad (2.1c)
\]

\[
\tilde{v}_x(\tilde{x}, \tilde{H}, \tilde{t}) = 0. \quad (2.1d)
\]

We introduce the dimensionless variables:

\[
x = \frac{\tilde{x}}{\tilde{L}}, \quad y = \frac{\tilde{y}}{\tilde{H}}, \quad v = \frac{\tilde{v}}{\tilde{V}}, \quad t_c = \frac{\tilde{V}}{\tilde{L}}. \quad (2.2)
\]

Here \(\tilde{H}\) is the height of the channel, which is much less than the length \(\tilde{L}\), and \(\tilde{V}\) is the characteristic velocity associated with the flow, yet to be defined. For dimensions of the device, as well as other parameters, see Appendix A. The variable \(t_c\) represents the convective time scale; as we shall see later, there are multiple time scales associated with this problem. Upon introducing the dimensional variables (2.2), equations (2.1a) and (2.1b) become

\[
\frac{\tilde{\rho} \tilde{V}^2}{\mu L} \left( \frac{\partial v}{\partial t_c} + v_x \frac{\partial v}{\partial x} + \epsilon^{-1} v_y \frac{\partial v}{\partial y} \right) = -\tilde{\nabla} \tilde{p} + \frac{\tilde{V}}{\tilde{H}^2} \left( \epsilon^2 v_{xx} + \frac{\partial^2 v}{\partial y^2} \right),
\]

(2.3)

\[
\epsilon \frac{\partial v_x}{\partial x} + \frac{\partial v_y}{\partial y} = 0, \quad (2.4)
\]

where \(\epsilon = \frac{\tilde{H}}{\tilde{L}} \ll 1\) is our aspect ratio. For simplicity and to obtain useful analytic results we assume that our flow is unidirectional. Aside from being mathematically useful this assumption has been to been shown to be physically reasonable as well [29]. Under this assumption \(v_y = 0\); using this and our small aspect ratio \(\epsilon \ll 1\) to neglect \(\epsilon^2 \frac{\partial^2 v}{\partial x^2}\) (diffusion in the \(x\)-direction) results in the leading order component-wise equations:

\[
\left( \frac{\tilde{\rho} \tilde{V} \tilde{H}^2}{\mu L} \right) \left( \frac{\partial v_x}{\partial t_c} + v_x \frac{\partial v_x}{\partial x} \right) = -\frac{\tilde{H}^2}{\mu \tilde{V}} \frac{\partial \tilde{p}}{\partial x} + \frac{\partial^2 v_x}{\partial y^2},
\]

(2.5a)

\[
\frac{\partial \tilde{p}}{\partial y} = 0. \quad (2.5b)
\]

Thus there is no pressure gradient in the vertical direction, which is consistent with the unidirectional flow assumption. The factor of \(\frac{\tilde{\rho} \tilde{V} \tilde{H}^2}{\mu L}\) on the left hand side of (2.5a) suggests the appropriate Reynolds number for our flow is

\[
\text{Re} = \frac{\tilde{\rho} \tilde{V} \tilde{H}^2}{\mu \tilde{L}}. \quad (2.6)
\]
This is also consistent with literature on surface-volume reactions in optical biosensors [10], [29]. Taking (2.6) to be our Reynolds number, one may calculate \( \text{Re} \ll 1 \) for physically realistic flows (see Appendix A). Hence, we must have

\[
\frac{\tilde{H}^2}{\tilde{V} \mu \tilde{x}} \frac{\partial \tilde{p}}{\partial \tilde{x}} = \frac{\partial^2 v_x}{\partial y^2},
\]

(2.7)

Since \( v_y = 0 \), our continuity equation (2.4) implies that \( v_x \) is a function of \( y \) alone, and (2.5b) implies that \( \tilde{p} \) is a function of \( x \) alone. Hence (2.7) implies

\[
\frac{\tilde{H}^2}{\tilde{V} \mu} \frac{d \tilde{p}}{d \tilde{x}} = \frac{d^2 v_x}{d y^2} \equiv \lambda.
\]

for some constant \( \lambda \). Therefore the pressure gradient is constant in the \( x \)-direction (and in the \( y \)-direction too, from (2.5b)), so we may write

\[
\left( \frac{\tilde{H}^2}{\tilde{V} \mu} \right) \frac{-\Delta p}{L} = \frac{d^2 v_x}{d y^2} \equiv \lambda,
\]

where \( \Delta p > 0 \) and the pressure gradient is negative because pressure is higher where the buffer enters than where it exits. To simplify our calculations, we take the characteristic velocity to be

\[
\tilde{V} = \frac{\tilde{H}^2 \Delta p}{2 \tilde{V} \mu L}.
\]

This may lead one to believe that experimentally one may calculate the characteristic velocity from the pressure gradient. In reality neither are known; however we may relate the velocity to the flow rate, which is known. Once the velocity is known, the pressure completely drops out of the analysis and is not needed. For more details on how this is done we refer the reader to [10]. This transforms the velocity equation into the ODE

\[
\frac{d^2 v_x}{d y^2} = -2.
\]

Integrating this equation and applying the no-slip conditions \( v_x(0) = v_x(1) = 0 \), we find the velocity profile to be

\[
v_x(y) = y(1 - y),
\]

(2.8)
as expected for a two-dimensional Poiseuille flow.
2.2 Multiple-Ligand Model

In this section we present the governing equations for the evolution of the multiple ligand concentrations in the biosensor. We first derive the governing equations for the injection phase, and then modify our approach to give the equations in the wash phase.

2.2.1 Injection Phase

We write the concentration of the ligands as:

\[ L_1(\tilde{x}, \tilde{y}, \tilde{t}) = \tilde{C}_1(\tilde{x}, \tilde{y}, \tilde{t}), \]
\[ L_2(\tilde{x}, \tilde{y}, \tilde{t}) = \tilde{C}_2(\tilde{x}, \tilde{y}, \tilde{t}). \]

The evolution of the unbound ligand concentration in the channel obeys an advection-diffusion equation

\[
\frac{\partial \tilde{C}_1}{\partial \tilde{t}} = \tilde{D}_1 \nabla^2 \tilde{C}_1 - \tilde{v} \cdot \nabla \tilde{C}_1, \tag{2.9a}
\]
\[
\frac{\partial \tilde{C}_2}{\partial \tilde{t}} = \tilde{D}_2 \nabla^2 \tilde{C}_2 - \tilde{v} \cdot \nabla \tilde{C}_2, \tag{2.9b}
\]

for \((\tilde{x}, \tilde{y}) \in (0, \tilde{L}) \times (0, \tilde{H})\) and \(\tilde{t} \in (0, \infty)\). Initially there is no unbound ligand in the channel:

\[
\tilde{C}_i(\tilde{x}, \tilde{y}, 0) = 0. \tag{2.10a}
\]

There is a prescribed inflow concentration for each of the ligands:

\[
\tilde{C}_i(0, \tilde{y}, \tilde{t}) = \tilde{C}_{i,u}. \tag{2.10b}
\]

There is no change in the unbound ligand concentration as the fluid exits the channel:

\[
\frac{\partial \tilde{C}_i}{\partial \tilde{x}} (\tilde{L}, \tilde{y}, \tilde{t}) = 0. \tag{2.10c}
\]

Also, there is no flux through the ceiling:

\[
\frac{\partial \tilde{C}_i}{\partial \tilde{y}} (\tilde{x}, \tilde{H}, \tilde{t}) = 0. \tag{2.10d}
\]
With equations describing the evolution of the unbound ligand concentrations $\tilde{C}_i$ in hand, we now discuss the surface-reaction kinetics, given by reactions (1.2) and (1.3) described in Chapter 1. We first fix our notation by letting: 

\[ [EL_1] (\tilde{x}, \tilde{t}) = \tilde{B}_1 (\tilde{x}, \tilde{t}), \quad [EL_1L_2] (\tilde{x}, \tilde{t}) = \tilde{B}_{12} (\tilde{x}, \tilde{t}), \quad [EL_2] (\tilde{x}, \tilde{t}) = \tilde{B}_2 (\tilde{x}, \tilde{t}) \]

denote the concentrations of the reacting species $EL_1$, $EL_1L_2$, and $EL_2$ respectively; 

\[ \tilde{B}_1 (\tilde{x}, \tilde{t}), \tilde{B}_{12} (\tilde{x}, \tilde{t}), \tilde{B}_2 (\tilde{x}, \tilde{t}) \]

denote a vector in $\mathbb{R}^3$ whose components are the three bound state concentrations; 

\[ \tilde{B}_\Sigma = \tilde{B}_1 + \tilde{B}_{12} + \tilde{B}_2 \]

and $\tilde{R}_T$ denote the initial empty receptor concentration. We note the total concentration of empty receptors at any point during the experiment is given by 

\[ [E] (\tilde{x}, \tilde{t}) = \tilde{R}_T - \tilde{B}_\Sigma (\tilde{x}, \tilde{t}). \]

The kinetics system is governed by the law of mass action; however, before presenting the full set of PDEs, we will derive the equation for $\tilde{B}_1 (\tilde{x}, \tilde{t})$. Change in $\tilde{B}_1 (\tilde{x}, \tilde{t})$ can be due only to association or dissociation. Mathematically, we can write this as 

\[ \frac{\partial \tilde{B}_1}{\partial \tilde{t}} = \tilde{S}_0 (\tilde{x}, \tilde{t}) - \tilde{S}_i (\tilde{x}, \tilde{t}). \] (2.11)

Here $\tilde{S}_0 (\tilde{x}, \tilde{t})$ denotes the sources and $\tilde{S}_i (\tilde{x}, \tilde{t})$ denotes the sinks. The sources for $EL_1$ will be: direct binding of an $L_1$ molecule with an empty receptor, or an $L_2$ molecule dissociating from the product $EL_1L_2$. These are the reactions:

\[ E + L_1 \xrightarrow{k_a} EL_1, \]

\[ EL_1L_2 \xrightarrow{1/k_d} EL_1 + L_2. \]

Therefore we may conclude

\[ \tilde{S}_0 (\tilde{x}, \tilde{t}) = \underbrace{1k_a(\tilde{R}_T - \tilde{B}_\Sigma)\tilde{C}_1 (\tilde{x}, 0, \tilde{t})}_{\text{Gain due to binding with empty receptor}} + \underbrace{1/2k_d \tilde{B}_{12}}_{\text{Gain to dissociation event}}. \] (2.12)

Sinks for $\tilde{B}_1$ may be found in a similar way. The concentration $\tilde{B}_1$ will decrease if: a $L_2$ molecule binds with the product $EL_1$, or if a ligand $L_1$ molecule dissociates from the product $EL_1$. These are the reactions:

\[ EL_1 + L_2 \xrightarrow{1/k_a} EL_1L_2, \]

\[ EL_1 \xrightarrow{1/k_d} E + L_1. \]
Therefore we may write
\[
\tilde{S}_i(\tilde{x}, \tilde{t}) = \frac{1}{2} k_a \tilde{B}_1 \tilde{C}_2 \left( \tilde{x}, 0, \tilde{t} \right) + \frac{1}{2} k_d \tilde{B}_1 .
\] (2.13)

Substituting (2.12) and (2.13) into (2.11) we find
\[
\frac{\partial \tilde{B}_1}{\partial \tilde{t}} = \tilde{k}_a (\tilde{R}_T - \tilde{B}_\Sigma) \tilde{C}_1 \left( \tilde{x}, 0, \tilde{t} \right) + \frac{1}{2} \tilde{k}_d \tilde{B}_{12} - \frac{1}{2} \tilde{k}_a \tilde{B}_1 \tilde{C}_2 \left( \tilde{x}, 0, \tilde{t} \right) + \frac{1}{2} \tilde{k}_d \tilde{B}_{12} - \frac{1}{2} \tilde{k}_a \tilde{B}_1 \tilde{C}_2 \left( \tilde{x}, 0, \tilde{t} \right) .
\]

One may go through this argument to derive the fully reversible kinetics system:
\[
\frac{\partial \tilde{B}_1}{\partial \tilde{t}} = \tilde{k}_a (\tilde{R}_T - \tilde{B}_\Sigma) \tilde{C}_1 + \frac{1}{2} \tilde{k}_d \tilde{B}_{12} - \frac{1}{2} \tilde{k}_a \tilde{B}_1 \tilde{C}_2 ,
\] (2.14a)
\[
\frac{\partial \tilde{B}_{12}}{\partial \tilde{t}} = \frac{1}{2} \tilde{k}_a \tilde{B}_1 \tilde{C}_2 + \frac{1}{2} \tilde{k}_d \tilde{B}_{12} - \frac{1}{2} \tilde{k}_a \tilde{B}_1 \tilde{C}_2 ,
\] (2.14b)
\[
\frac{\partial \tilde{B}_2}{\partial \tilde{t}} = \frac{1}{2} \tilde{k}_a \tilde{B}_1 \tilde{C}_2 + \frac{1}{2} \tilde{k}_d \tilde{B}_{12} - \frac{1}{2} \tilde{k}_a \tilde{B}_1 \tilde{C}_2 .
\] (2.14c)
\[
\tilde{B}(\tilde{x}, 0) = 0 .
\] (2.14d)

We emphasize that (2.14) is simply a statement of the law of mass action. Furthermore, in writing the above equations we have omitted the arguments of \( \tilde{C}_1(\tilde{x}, 0, \tilde{t}) \) – for simplicity we will adopt the custom of omitting the arguments when writing the kinetics system and understand that the kinetics system (2.14) holds only on the reacting surface of the biosensor at \( \tilde{y} = 0 \).

It remains to give the bottom boundary condition to (2.9a)–(2.10d), which will couple the unbound and bound ligand concentrations. Since there are no other sources or sinks at the surface, the diffusive flux into the surface must equal the rate of change of the bound state concentrations. Thus, on the surface at \( \tilde{y} = 0 \):
\[
\tilde{D}_1 \frac{\partial \tilde{C}_1}{\partial \tilde{y}} (\tilde{x}, 0, \tilde{t}) = \frac{\partial \tilde{B}_1}{\partial \tilde{t}} (\tilde{x}, 0, \tilde{t}) + \frac{\partial \tilde{B}_{12}}{\partial \tilde{t}} (\tilde{x}, 0, \tilde{t}) ,
\] (2.15a)
\[
\tilde{D}_2 \frac{\partial \tilde{C}_2}{\partial \tilde{y}} (\tilde{x}, 0, \tilde{t}) = \frac{\partial \tilde{B}_{12}}{\partial \tilde{t}} (\tilde{x}, 0, \tilde{t}) + \frac{\partial \tilde{B}_2}{\partial \tilde{t}} (\tilde{x}, 0, \tilde{t}) .
\] (2.15b)

One may notice that we are treating the receptors as though they are on a surface, rather than embedded in a dextran gel layer. In [8], Edwards constructs (from first
principles) equations for the evolution of the unbound and bound ligand concentrations in a dextran gel layer, and shows the bimolecular analog to (2.15) results as a physically limiting case (i.e., as the height of the dextran gel layer $H_d$ approaches zero) of equations constructed from first principles. We do not include a receptor layer in this work, however this may be a subject of future research.

We now non-dimensionalize our model using the scalings:

$$x = \tilde{x} \frac{L}{\tilde{L}}, \quad y = \tilde{y} \frac{H}{\tilde{H}}, \quad v = \tilde{v} \frac{\tilde{V}}{V}, \quad t_c = \tilde{t} \frac{\tilde{L}}{\tilde{t}}, \quad C_i = \frac{C_i}{C_{i,u}}, \quad B_i = \frac{B_i}{R_T}.$$  

The nondimensional advection-diffusion equations for the evolution of the unbound ligand concentrations then takes the form:

$$\frac{\partial C_1}{\partial t_c} = \left( D_1 Pe^{-1} \left( \epsilon^2 \frac{\partial^2 C_1}{\partial x^2} + \frac{\partial^2 C_1}{\partial y^2} \right) - y(1 - y) \frac{\partial C_1}{\partial x} \right), \quad (2.16a)$$

$$\frac{\partial C_2}{\partial t_c} = Pe^{-1} \left( \epsilon^2 \frac{\partial^2 C_2}{\partial x^2} + \frac{\partial C_2}{\partial y^2} \right) - y(1 - y) \frac{\partial C_2}{\partial x}, \quad (2.16b)$$

subject to the initial and boundary data:

$$C_i(x, y, 0) = 0, \quad (2.17a)$$

$$C_i(0, y, t) = 1, \quad (2.17b)$$

$$\frac{\partial C_i}{\partial x}(1, y, t) = 0, \quad (2.17c)$$

$$\frac{\partial C_i}{\partial y}(x, 1, t) = 0, \quad (2.17d)$$

$$F_i D \frac{\partial C_1}{\partial y}(x, 0, t_c) = \frac{\partial B_1(x, t_c)}{\partial t_c} + \frac{\partial B_{12}(x, t_c)}{\partial t_c}, \quad (2.17e)$$

$$D \frac{\partial C_2}{\partial y}(x, 0, t_c) = \frac{\partial B_{12}(x, t_c)}{\partial t_c} + \frac{\partial B_2(x, t_c)}{\partial t_c}. \quad (2.17f)$$

The small parameter $\epsilon$ is our aspect ratio, defined in Section 2.1. We consider the parameter

$$Pe = \frac{\tilde{V} \tilde{H}^2}{D_2 \tilde{L}} \quad (2.18)$$

to be the Péclet number associated with our system. The flow rate will be much greater than either of the diffusion rates $\tilde{D}_i$, thus $Pe \gg 1$. We remark that another choice is

$$Pe^* = \frac{\tilde{V} \tilde{H}}{D_2}. \quad (2.19)$$
Since $\text{Pe} \gg 1$ and
\[ \text{Pe} = \text{Pe}^* \epsilon, \]
this implies $\frac{\bar{V} \bar{H}}{D_2} \gg \epsilon^{-1}$ (see Appendix A). The normalized diffusion coefficient $D$ is
\[ D = \frac{\bar{D}_2 \bar{C}_{2,u}}{(\bar{R}_T \bar{H})} = \text{diffusion rate to reacting surface}, \]
\[ \bar{V} / L = \text{convection transport in channel}, \]
and for physically realistic systems $D \ll 1$. We have also defined $D_r = \bar{D}_1 \bar{C}_{1,u}$, $C_r = \bar{C}_{1,u} / \bar{C}_{2,u}$, and $F_r = D_r C_r$. The dimensionless parameter $F_r$ which appeared during our non-dimensionalization is not (nor is it related to) the Froude number $F_r$; rather it represents the ratio of the diffusion rates of our ligand molecules times the ratio of the inflow concentrations. The parameter $D_r$ is on the order of one, and since $\bar{C}_{i,u}$ are at our disposal, we will treat $C_r$ as an $O(1)$ parameter unless specified otherwise. On the $t_c$ time scale the dimensionless bound state system is:
\[
\frac{\partial B_1}{\partial t_c} = k_a (1 - B_2) C_1 + \frac{1}{2} k_d B_{12} - \frac{1}{2} k_a B_1 C_2, \quad (2.20a)
\]
\[
\frac{\partial B_{12}}{\partial t_c} = k_a B_1 C_2 + \frac{1}{2} k_a B_2 C_1 - \frac{1}{2} k_d B_{12} - \frac{1}{2} k_d B_{12}, \quad (2.20b)
\]
\[
\frac{\partial B_2}{\partial t_c} = \frac{1}{2} k_d B_{12} + 2 k_a (1 - B_2) C_2 - \frac{1}{2} k_a B_2 C_1 - 2 k_d B_2, \quad (2.20c)
\]
\[
B(x, 0) = 0, \quad (2.20d)
\]
on the surface at $y = 0$, where
\[
1 k_a = \frac{\bar{L} \bar{C}_{1,u}}{\bar{V}} \bar{k}_a, \quad 1 k_d = \frac{\bar{L} \bar{k}_d}{\bar{V}}, \quad 2 k_a = \frac{\bar{L} \bar{k}_a}{\bar{V}}, \quad 2 k_d = \frac{\bar{L} \bar{k}_d}{\bar{V}},
\]
\[
2 k_a = \frac{\bar{L} \bar{C}_{2,u}}{\bar{V}} \bar{k}_a, \quad 2 k_d = \frac{\bar{L} \bar{k}_d}{\bar{V}}, \quad 2 k_a = \frac{\bar{L} \bar{k}_a}{\bar{V}}, \quad 2 k_d = \frac{\bar{L} \bar{k}_d}{\bar{V}}.
\]
In addition, the dimensionless sensogram reading is given by
\[
S(t) = \bar{B}_1(t) + \left(1 + \frac{\bar{s}_2}{\bar{s}_1}\right) \bar{B}_{12}(t) + \frac{\bar{s}_2}{\bar{s}_1} \bar{B}_2(t). \quad (2.21)
\]
Notice we have normalized the reading by setting
\[
S(t) = \frac{\bar{S}(t)}{\bar{s}_1 \bar{R}_T}.
\]
2.2.2 Wash Phase

The equations in the wash phase are very similar to the injection phase. Once the buffer fluid is injected, ligand flow throughout the channel will be governed by (2.16a) and (2.16b). We assume the biosensor will be saturated with unbound ligand by the time the wash phase begins, so \( C_1(x, y, 0) = 1 \) and \( C_2(x, y, 0) = 1 \). This also implies uniform unbound ligand concentration at the surface \( C_1(x, 0, 0) = 1 \) and \( C_2(x, 0, 0) = 1 \). The assumption that the biosensor will be saturated with unbound ligand by the time the wash phase begins is physically reasonable. As we show in Section 2.4 the bound state evolves on a slower time scale: thus by the time the bound state concentration reaches a chemical equilibrium the unbound ligand concentration in the biosensor will be nearly uniform. Although it is possible that small nonuniformities will exist in the unbound ligand concentration at the end of the injection phase, we expect such nonuniformities will be negligible and we neglect them herein.

The boundary conditions (2.17c)–(2.17f) will still hold. Since no ligand is being injected into the biosensor during the wash phase, (2.17b) is replaced by

\[
C_i(0, y, t_c) = 0.
\]

During the wash phase, the kinetics system will still be given by (2.20a)–(2.20c). We assume that binding has proceeded long enough during the injection phase for the bound state concentration to reach a chemical equilibrium; thus the initial conditions will be given by the equilibrium solution to (2.20a)–(2.20c). A solution to the resulting linear system will be guaranteed to exist if and only if the matrix

\[
A_c = \begin{pmatrix}
(1k_a + 1k_d + \frac{1}{2}k_a) & 1k_a - \frac{1}{2}k_d & 1k_a \\
-\frac{1}{2}k_a & (\frac{1}{2}k_d + \frac{2}{1}k_d) & -\frac{3}{2}k_a \\
2k_a & 2k_a - \frac{2}{1}k_d & (2k_a + 2k_d + \frac{2}{1}k_a)
\end{pmatrix}
\]

is invertible. Its determinant

\[
\det(A_c) = 2k_a[\frac{1}{2}k_d(1k_d + \frac{1}{2}k_a) + 2k_a(\frac{1}{2}k_d + 1k_a + \frac{1}{2}k_d)] + (2k_a + 2k_d)[\frac{1}{2}k_a2k_d
+ 1k_d(\frac{1}{2}k_d + \frac{1}{2}k_d)] + 1k_a[\frac{1}{2}k_a(\frac{1}{2}k_d + 2k_d) + 2k_a(\frac{1}{2}k_d + \frac{1}{2}k_a) + 2k_d(\frac{1}{2}k_d + \frac{1}{2}k_a)]
\]

(2.23)
will be positive if each of the rate constants are positive. This assumption is certainly physically reasonable, as we are studying the fully reversible kinetics system (1.2) and (1.3). The notation \( A_c \) denotes the rate constants on the convective time-scale; we will rescale them later. Thus writing \( B_\infty \) to be the steady-state solution to (2.20a)–(2.20c), we have

\[
B_\infty = A_c^{-1}f_c, \quad (2.24)
\]
\[
f_c = (1k_a, 0, 2k_a)^T. \quad (2.25)
\]

So our initial condition for the wash phase is

\[
B(x, 0) = A_c^{-1}f_c. \quad (2.26)
\]

### 2.3 Multiple-Receptor Model

In this section we derive the multiple-receptor model. Since the derivation is analogous to the multiple-ligand model, we present only the dimensionless equations.

#### 2.3.1 Injection Phase

Since we are considering only a single ligand in this case, the unbound ligand flow will be given by

\[
\frac{\partial C}{\partial t_c} = (D_r \text{Pe}^{-1}) \left( \epsilon^2 \frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} \right) - y(1 - y) \frac{\partial C}{\partial x} \quad (2.27)
\]

Equations similar to (2.17a) and (2.17d) will hold. Now we have two bound receptor types at the boundary. Let \( B_f(x, t_c) \) be the concentration of ligand bound to a free DNA receptor, and \( B_p(x, t_c) \) be the concentration of ligand bound to a receptor with a PCNA ring attached. Therefore the boundary condition at \( y = 0 \) will take the form of the diffusive flux condition

\[
D \frac{\partial C(x, 0, t_c)}{\partial y} = \frac{\partial B_f}{\partial t_c} + \frac{\partial B_p}{\partial t_c}. \quad (2.28)
\]

Let \( R_f = \frac{\bar{R}_f}{R_f} \), \( R_p = \frac{\bar{R}_p}{R_f} \) denote the ratio of free DNA receptors to the total number of receptors, and the ratio of receptors with PCNA rings attached to the total number of
receptors. Hence, \( \bar{R}_T = \bar{R}_f + \bar{R}_p \), or in dimensionless terms \( 1 = R_f + R_p \). We note that in a typical experiment, we expect \( R_f \ll 1 \). The bound ligand concentrations \( B_f \) and \( B_p \) are then given by

\[
\frac{\partial B_f}{\partial t_c} = t k_a (R_f - B_f) C(x, 0, t_c) - t k_d B_f, \quad (2.29a) \\
\frac{\partial B_p}{\partial t_c} = p k_a (R_p - B_p) C(x, 0, t_c) - p k_d B_p. \quad (2.29b)
\]

Here \( t k_a \) represents the rate at which a ligand molecule associates with a free DNA receptor, and \( t k_d \) represents the rate at which a ligand molecule dissociates from a free DNA receptor. Additionally, \( p k_a \) represents the rate at which a ligand molecule associates to a receptor with a PCNA ring, and \( p k_d \) represents the rate at which a ligand molecule dissociates from a receptor with a PCNA ring. Although equations (2.29a) and (2.29b) appear to decouple, we shall see that coupling is introduced through \( C(x, 0, t_c) \). In writing these equations, we have used the scalings

\[
B_f = \frac{\bar{B}_f}{\bar{R}_T}, \quad B_p = \frac{\bar{B}_p}{\bar{R}_T}, \quad \bar{t} k_a = \frac{\bar{L} C_u (\bar{t} k_a)}{V}, \\
\bar{t} k_d = \frac{\bar{L} (\bar{t} k_d)}{V}, \quad \bar{p} k_a = \frac{\bar{L} C_u (\bar{p} k_a)}{V}, \quad \bar{p} k_d = \frac{\bar{L} (\bar{p} k_d)}{V}.
\]

These equations are also subject to the initial conditions \( B_f(x, 0) = B_p(x, 0) = 0 \). The dimensional sensogram signal is given in this case as

\[
\bar{S}(\bar{t}) = \bar{B}_f(\bar{t}) + \bar{B}_p(\bar{t}), \quad (2.30)
\]

and the dimensionless version is given by

\[
S(t_c) = \frac{\bar{S}}{\bar{s} \bar{R}_T} = \bar{B}_f(t_c) + \bar{B}_p(t_c). \quad (2.31)
\]

As in our multiple-ligand model, \( \bar{s} \) is proportional molecular weight of our ligand.

2.3.2 Wash Phase

We briefly summarize the equations for the wash phase for the multiple-receptor model. The unbound ligand concentrations inside the channel will be governed by
No ligand is flowing into the biosensor, thus \( C(0, y, t_c) = 0 \). A no-flux condition for \( C \) will hold on the ceiling, and as the ligand exits the channel on the right. In addition, equation (2.28) will still hold. By the time the wash phase begins, the channel will be saturated with ligand; thus \( C(x, y, 0) = 1 \). This also implies the unbound ligand concentration at the surface of the biosensor will be uniform; hence \( C(x, 0, 0) = 1 \). The initial conditions will be given by the steady state solution to (2.29a)–(2.29b), which is

\[
B_f(x, 0) = \frac{(R_f)_f \cdot k_a}{k_a + k_d}, \tag{2.32a}
\]

\[
B_p(x, 0) = \frac{(R_p)_p \cdot k_a}{k_a + k_d}. \tag{2.32b}
\]

### 2.4 Outer Solution

In each case we have a high Péclet number convection-diffusion system coupled to a set of PDEs describing reaction on the surface (as we shall demonstrate in Chapter 4, this is where the nonlinearity enters). We shall see below that high Péclet number flow results in a boundary layer near the surface of the biosensor—a fine degree of resolution would be required to capture this effect numerically.

To simplify our PDE system we apply perturbation techniques, by treating \( P_e^{-1} \) as a small perturbation parameter. We then look for the leading order term, in an approximation of the form:

\[
C_i(x, y, t_c; P_e) = C_i(x, y, t_c) + o(1), \tag{2.33}
\]

\[
B_i(x, t_c; P_e) = B_i(x, t_c) + o(1). \tag{2.34}
\]

Note that since we are only looking for the leading order term, we retain the same notation. Substituting (2.33)–(2.34) into (2.16a)–(2.17f), we find

\[
\frac{\partial C_i}{\partial t_c} = -y(1 - y) \frac{\partial C_i}{\partial x}, \tag{2.35}
\]

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and:

\[ C_i(x, y, 0) = 0, \quad (2.36a) \]
\[ C_i(0, y, t_c) = 1, \quad (2.36b) \]
\[ 0 = \frac{\partial B_1}{\partial t_c} + \frac{\partial B_{12}}{\partial t_c}, \quad (2.36c) \]
\[ 0 = \frac{\partial B_{12}}{\partial t_c} + \frac{\partial B_2}{\partial t_c}, \quad (2.36d) \]

where \((2.36c)\) and \((2.36d)\) hold on the reacting surface at \(y = 0\). We have also made use of the fact that \(D \ll 1\). Right away we see a difficulty—our parabolic system has been reduced to a hyperbolic one. Thus, we cannot satisfy all of the boundary conditions, which signals a singular perturbation problem. The solution to \((2.35)–(2.36a)\) is given by

\[ C_i(x, y, t_c) = \begin{cases} 
0 & \text{if } x - y(1 - y)t_c > 0, \\
1 & \text{else.} 
\end{cases} \quad (2.37) \]

This may be interpreted as the uniform inflow concentration propagating via the convective wave front. Equations \((2.36c)\) and \((2.36d)\) imply that evolution of the \(B_i\) is constant with respect to the convective time scale during the injection phase, which may be seen as follows: integrating \((2.36c)\) and \((2.36d)\) we get

\[ K_1(x) = B_1 + B_{12}, \]
\[ K_2(x) = B_{12} + B_2. \]

The initial conditions imply the arbitrary functions of integration \(K_1(x), K_2(x)\) are identically zero. Since the concentrations \(B_i\) are nonnegative, this implies \(B_i = 0\). A similar argument can be made for the multiple-receptor system as well.

2.5 Diffusive Layers

The above convective layer solution may have a discontinuity about the convective wave front. In addition the boundary conditions \((2.17c)–(2.17f)\) may not be satisfied, as our outer solution fails to be differentiable at the convective wave front.
\[ t_c = x - y(1 - y). \] To address these issues, one could introduce boundary layers on this time scale. Since the dynamics of interest occur on a slower time scale, we do not concern ourselves with the construction of such layers on this time scale.
Chapter 3
BOUNDARY LAYER WITH REACTION

We saw that to leading order, the bound state evolution is constant with respect to the convective time scale. This suggests we compress time by introducing the scaling
\[ t_D = \frac{t_c}{Pe^{1/3}}. \]  
(3.1a)
The ligand molecules will diffuse to the surface over this longer *diffusive* time scale. In addition, our outer solution (2.35) approaches 1 everywhere except the boundaries. To investigate the dynamics in this layer we stretch \( y \) by setting
\[ \eta = Pe^{1/3}y. \]  
(3.1b)
We will refer to this layer as the *unstirred layer*. The unusual \( Pe^{1/3} \) scaling can be found through a balancing argument, and is typical for surface-volume reactions in optical biosensors: see [6], [10], or [11]. We also remark that our \( Pe^{1/3} \) scaling is not related to the \( Pe^{1/3} \) scaling found by Rines and Young in their article “How Rapidly is a Passive Scalar Mixed Within Closed Streamlines?” [22]. We will use these scalings to study the injection phase of the multiple-ligand system. Since these arguments also apply to the wash phase and the multiple-receptor model, we simply summarize the relevant equations at the end of this chapter.

3.1 Governing Equations in the Layer

Substituting (3.1a) and (3.1b) into our advection-diffusion equations (2.16a) and (2.16b), we find:

\[ Pe^{-1/3} \frac{\partial C_1}{\partial t_D} = (D_r Pe^{-1}) \left( \epsilon^2 \frac{\partial^2 C_1}{\partial x^2} + Pe^{2/3} \frac{\partial^2 C_1}{\partial \eta^2} \right) - Pe^{-1/3} \eta (1 - Pe^{-1/3} \eta) \frac{\partial C_1}{\partial x}, \]

\[ Pe^{-1/3} \frac{\partial C_2}{\partial t_D} = Pe^{-1} \left( \epsilon^2 \frac{\partial^2 C_2}{\partial x^2} + Pe^{2/3} \frac{\partial^2 C_2}{\partial \eta^2} \right) - Pe^{-1/3} \eta (1 - Pe^{-1/3} \eta) \frac{\partial C_2}{\partial x}. \]
The leading order $O(\text{Pe}^{-1/3})$ equations are:

$$\frac{\partial C_1}{\partial t_D} = D_D \frac{\partial^2 C_1}{\partial \eta^2} - \eta \frac{\partial C_1}{\partial x},$$  (3.2a)

$$\frac{\partial C_2}{\partial t_D} = D_D \frac{\partial^2 C_2}{\partial \eta^2} - \eta \frac{\partial C_2}{\partial x},$$  (3.2b)

for $0 < x < 1$, $0 < \eta < \infty$, and $t_D > 0$. Note that diffusion in the $x$-direction is $O(\text{Pe}^{-1/3}) \ll O(\text{Pe}^{-1/3})$, so the $\frac{\partial^2 C_i}{\partial x^2}$ terms drop out. This leaves us with pure advection in the $x$-direction and diffusion in the $\eta$-direction. Hence, the solutions to (3.2a) and (3.2b) may not satisfy (2.17c), the no-flux condition at $x = 1$. To address this issue one would introduce a boundary layer at $x = 1$: however, measurement stops at $x_{\text{max}}$. Since this is an $O(1)$ distance away from the right boundary, the layer won’t affect our measurements, and we omit its construction from our analysis. When convenient we treat the $x$-axis as semi-infinite, and not concern ourselves with satisfying the right boundary condition (2.17c).

The initial and boundary data is then:

$$C_i(x, \eta, 0) = 0,$$  (3.3a)

$$C_i(0, \eta, t_D) = 1,$$  (3.3b)

$$F_D \frac{\partial C_1(x, 0, t_D)}{\partial \eta} = \frac{\partial B_1(x, t_D)}{\partial t_D} + \frac{\partial B_{12}(x, t_c)}{\partial t_D},$$  (3.3c)

$$D_D \frac{\partial C_2(x, 0, t_D)}{\partial \eta} = \frac{\partial B_{12}(x, t_D)}{\partial t_D} + \frac{\partial B_2(x, t_D)}{\partial t_D}.$$  (3.3d)

We have defined the scaled diffusion coefficient

$$D_D = \text{Pe}^{2/3} D,$$

which can be $o(1)$ or $O(1)$; however we limit ourselves to the physically relevant case, when $D_D \ll 1$. The larger diffusion coefficient $D < D_D$, and the wider boundary layer, both reflect the effects of diffusion on our $t_D$ timescale.

It remains to construct a matching condition for the PDE system (3.2a)–(3.3d). To construct our matching condition let $C_{i,\text{outer}}(x, y, t_c)$ denote our outer solution (2.35) for $i = 1, 2$ and let $C_{i,\text{layer}}(x, \eta, t_D)$ denote the solution to (3.2a)–(3.3d) for $i = 1, 2$. We
only introduce this notation to construct the matching conditions, here and in Sections
3.2 and 3.3. Recasting the outer solution in terms of our layer variables, we require
that the value of \( C_{i,\text{outer}}(x, \eta, t_D; \text{Pe}) \) as one comes into the layer agrees with the value
of \( C_{i,\text{layer}}(x, \eta, t_D) \) as one comes out of the layer. Mathematically, we express this as:

\[
\lim_{t_D \to 0} \lim_{\eta \to \infty} C_{i,\text{layer}}(x, \eta, t_D) = \lim_{\text{Pe} \to \infty} C_{i,\text{outer}}(x, \eta, t_D; \text{Pe}). \quad (3.4)
\]

Using the definition of \( C_{i,\text{outer}}(x, \eta, t_D; \text{Pe}) \) (given by (2.35)), equation (3.4) becomes

\[
\lim_{t_D \to 0} \lim_{\eta \to \infty} C_{i,\text{layer}}(x, \eta, t_D) = \lim_{\text{Pe} \to \infty} \begin{cases} 
0 & \text{if } x - \eta(1 - \eta \text{Pe}^{-1/3}) t_D > 0, \\
1 & \text{else}.
\end{cases} \quad (3.5)
\]

or simply

\[
\lim_{t_D \to 0} \lim_{\eta \to \infty} C_{i,\text{layer}}(x, \eta, t_D) = \begin{cases} 
0 & \text{if } x - \eta t_D > 0, \\
1 & \text{else}.
\end{cases} \quad (3.5)
\]

Since the outer solution is the same for each of the ligands, the matching condition
is also the same. The matching condition (3.5) reflects the behavior of \( C_i \) only as we
exit the diffusive layer, not the full behavior in the layer which combines the effects of
convection and diffusion.

The kinetics system on the \( t_D \) time scale is given by:

\[
\frac{\partial B_1}{\partial t_D} = k_a \text{Pe}^{1/3} \left[ (1 - B_\Sigma) C_1 - K_d B_1 - \frac{1}{2} K_d B_1 C_2 + \frac{1}{2} K_d B_{12} \right], \quad (3.6a)
\]

\[
\frac{\partial B_{12}}{\partial t_D} = k_a \text{Pe}^{1/3} \left[ \frac{1}{2} K_d B_1 C_2 - \frac{1}{2} K_d B_{12} + \frac{2}{1} K_d B_2 C_1 - \frac{2}{1} K_d B_{12} \right], \quad (3.6b)
\]

\[
\frac{\partial B_2}{\partial t_D} = k_a \text{Pe}^{1/3} \left[ \frac{1}{2} K_d B_{12} - \frac{2}{1} K_d B_2 C_1 + 2 K_a (1 - B_\Sigma) C_2 - 2 K_d B_2 \right], \quad (3.6c)
\]

\[
B(x, 0) = 0, \quad (3.6d)
\]

for \( \eta = 0 \). Here we have defined

\[
K_d = \frac{1}{k_d}, \quad K_a = \frac{1}{2 k_a}, \quad \frac{1}{2} K_d = \frac{k_d}{2 k_a}, \quad \frac{1}{2} K_a = \frac{k_a}{2 k_d}, \quad K_d = \frac{2}{1} k_a, \quad K_a = \frac{2}{1} k_d. \quad (3.7)
\]
The two most important effects in the layer are reaction and diffusion. The relative balance of the two will determine the spatiotemporal evolution of the reacting species $B_i$. The Damköhler number, denoted $Da$, is a key dimensionless group which measures the relative strength of reaction to diffusion, and its size plays a pivotal role in the behavior of the bound state system. To obtain this key parameter we substitute (3.6a), (3.6b) into (3.3c); and (3.6b), (3.6c) into (3.3d), to get:

\[
\begin{align*}
F_r \frac{\partial C_1}{\partial \eta} &= Da[(1 - B_\Sigma)C_1 - 1K_d B_1 + 2K_a B_2 C_1 - 2K_d B_12], \\
\frac{\partial C_2}{\partial \eta} &= Da[\frac{1}{2}K_a B_1 C_2 - \frac{1}{2}K_d B_12 + 2K_a (1 - B_\Sigma) C_2 - 2K_d B_2].
\end{align*}
\]

The Damköhler number

\[
Da = \frac{1k_a Pe^{1/3}}{D_D} = \frac{\text{Reaction Rate}}{\text{Rate of Diffusion in the layer}}
\]

can be very small, moderate, or very large. We now investigate each possibility.

### 3.2 Case 1: $Da \ll 1$

We first study our PDE system when $Da \ll 1$. Since

\[
\frac{1k_a Pe^{1/3}}{D_D} = Da \ll 1,
\]

we conclude that $1k_a Pe^{1/3} \ll 1$ (since $D_D \ll 1$). In fact, because $D_D \ll 1$ this will always be the case. Since $Da \ll 1$ the right hand side of (3.6a)–(3.6c) is zero to leading order. This indicates that the bound state does not evolve on the diffusive time scale, and we need to introduce a slower time scale to study $B$. A small Damköhler number thus corresponds to the *reaction-limited* regime, where reaction kinetics are much slower than transport. We shall show this in Chapter 4, when $Da \ll 1$ reaction kinetics decouple from transport effects to leading order. Since reaction and transport occur on two disparate time scales (in this case), one is in the best possible position to measure rate constants. Although in practice $Da$ can be smaller than or on the order of one, the more common case is $Da = O(1)$; see [11] for a discussion of experimental design in optical biosensors and its relation to the size of $Da$. 

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Our new time scale for reaction takes the form

\[ t = k_a Pe^{1/3} t_D. \] (3.8)

Substituting (3.8) into (3.2a)–(3.2b), and using the fact that \( k_a Pe^{1/3} \ll 1 \), we have:

\[
D r \frac{\partial^2 C_1}{\partial \eta^2} = \eta \frac{\partial C_1}{\partial x},
\] (3.9a)

\[
\frac{\partial^2 C_2}{\partial \eta^2} = \eta \frac{\partial C_2}{\partial x},
\] (3.9b)

with the boundary data:

\[
C_i(0, \eta, t) = 1, \quad (3.10a)
\]

\[
\frac{\partial C_1}{\partial \eta}(x, 0, t) = \frac{Da}{F_r} \left( \frac{\partial B_1}{\partial t} + \frac{\partial B_{12}}{\partial t} \right), \quad (3.10b)
\]

\[
\frac{\partial C_2}{\partial \eta}(x, 0, t) = Da \left( \frac{\partial B_{12}}{\partial t} + \frac{\partial B_2}{\partial t} \right). \quad (3.10c)
\]

Notice the time derivative has completely dropped out of equations (3.2a) and (3.2b). In (3.9a) and (3.9b) we have diffusion in the \( \eta \)-direction equal to linear advection. The flow is in steady state, and changes in the \( C_i \) are driven by reaction on the surface (3.10b)–(3.10c).

To construct our matching condition, let \( C_i, \text{outer}(x, y, t_c) \) denote our outer solution (2.35) and let \( C_i, \text{layer}(x, \eta, t) \) denote the layer solution, this time governed by (3.9a)–(3.10c). The unbound ligand in the bulk compartment reaches equilibrium when \( t_c = \mathcal{O}(1) \), while the form of (3.2a) and (3.2b) implies that as we exit the unstirred layer (i.e., \( \eta \to \infty \)) \( C_i, \text{layer}(x, \eta, t_D) \) will reach equilibrium when \( t_D = \mathcal{O}(1) \). Hence, for large \( t_D \) the unbound ligand concentration \( C_i, \text{layer}(x, \eta, t) \) will be uniform as we exit the layer. However, the \( t \) time scale is much slower than the \( t_D \) scale (when \( Da \ll 1 \)); therefore \( C_i, \text{layer}(x, \eta, t) \) will be uniform as we exit the layer—even for very small \( t \). Hence, in order to match the uniform outer concentration in the bulk compartment we require

\[
\lim_{\eta \to \infty} C(x, \eta, t) = 1. \quad (3.11)
\]

This will arise naturally from the form of equations (3.9a)–(3.10c), since any change in the unbound ligand concentrations \( C_i, \text{layer}(x, \eta, t) \) will be a result of diffusive flux into
the reacting surface—not convection. Condition (3.11) may also be derived by recasting the outer solution (2.35) in terms of our layer coordinates

\[
C_{i,\text{outer}}(x, \eta, t) = \begin{cases} 
0 & \text{if } x - \eta(1 - \text{Pe}^{-1/3}\eta)(1^{1/3})^{-1}t > 0, \\
1 & \text{else},
\end{cases}
\]

(3.12)

and noticing that because \(1k\alpha Pe^{1/3} \ll 1\), we will always be in the latter case of (3.12). That is, the unbound ligand concentration will be uniform in the bulk compartment on the \(t\) time scale, which results in the matching condition (3.11).

The kinetics system is given on the \(t\) time scale by:

\[
\frac{\partial B_1}{\partial t} = (1 - B_\Sigma)C_1 - \frac{1}{2}K_aB_1C_2 + \frac{1}{2}K_dB_{12},
\]

(3.13a)

\[
\frac{\partial B_{12}}{\partial t} = \frac{1}{2}K_aB_1C_2 - \frac{1}{2}K_dB_{12} + \frac{1}{2}K_aB_2C_1 - \frac{1}{2}K_dB_{12},
\]

(3.13b)

\[
\frac{\partial B_2}{\partial t} = K_dB_{12} - \frac{1}{2}K_aB_2C_1 + 2K_a(1 - B_\Sigma)C_2 - 2K_dB_2.
\]

(3.13c)

\[
B(x, 0) = 0,
\]

(3.13d)

when \(\eta = 0\).

### 3.3 Case 2: \(Da \gg 1\)

When \(Da \gg 1\) equations (3.10b) and (3.10c) become:

\[
0 = \frac{\partial B_1}{\partial t_w} + \frac{\partial B_{12}}{\partial t_w},
\]

\[
0 = \frac{\partial B_{12}}{\partial t_w} + \frac{\partial B_2}{\partial t_w}.
\]

This implies the bound state evolution is constant on this time scale during the injection phase, as in Subsection 2.4. Since the unbound ligand concentration is governed by (3.9a) and (3.9b), the change in \(C_{i,\text{layer}}(x, \eta, t)\) will again be due to diffusive flux into the reacting surface, although this time the limiting process is not reaction—it is diffusion; ligand molecules bind with receptor sites as soon as they reach the reacting surface. We are therefore in the transport-limited regime, and we must introduce a new time
scale to study the effects of diffusion near the wall into the reacting surface. Equations (3.10b) and (3.10c) imply that our new time scale takes the form

$$t_w = \frac{t}{Da}. \quad (3.14)$$

which is also equivalent to

$$t_w = D_D Pe^{-1/3} t_c.$$  

We remark that this fortunately does not occur much in practice [6], because in this case the biosensor is measuring diffusion rather than reaction.

Equations (3.9a)–(3.10a) all hold on the $t_w$ time scale, and the boundary conditions (3.10b) and (3.10c) become:

$$\frac{\partial C_1}{\partial \eta}(x,0,t_w) = \frac{1}{F_r} \left( \frac{\partial B_1}{\partial t_w} + \frac{\partial B_{12}}{\partial t_w} \right), \quad (3.15a)$$

$$\frac{\partial C_2}{\partial \eta}(x,0,t_w) = \frac{\partial B_{12}}{\partial t_w} + \frac{\partial B_2}{\partial t_w}. \quad (3.15b)$$

Now the diffusive flux into the surface balances with reaction.

To construct our matching condition on the $t_w$ scale, we again let $C_{i,\text{outer}}(x,y,t_c)$ denote our outer solution (2.35) and let $C_{i,\text{layer}}(x,\eta,t_w)$ denote the layer solution, governed by (3.9a)–(3.10a) on the $t_w$ scale, and (3.15). The derivation of our matching condition is analogous to the one given in Section 3.2 to construct (3.11). The unbound ligand concentration in the bulk compartment $C_{i,\text{outer}}(x,y,t_c)$ reaches steady state when $t_c = \mathcal{O}(1)$, while the form of (3.2a) and (3.2b) implies that $C_{i,\text{layer}}(x,\eta,t_D)$ reaches steady state as we exit the layer when $t_D = \mathcal{O}(1)$. Therefore, the unbound ligand concentration $C_{i,\text{layer}}(x,\eta,t_D)$ will be uniform for large $t_D$ as we exit the layer. Since the $t_w$ scale is much slower than the $t_D$ time scale (i.e. diffusion into the surface is much slower than diffusion into the layer), $C_{i,\text{layer}}(x,\eta,t_w)$ will be uniform as we exit the layer. This amounts to imposing the condition

$$\lim_{\eta \to \infty} C(x,\eta,t_w) = 1. \quad (3.16)$$

Again, this behavior will arise naturally from the form of (3.9a)–(3.10a) (on the $t_w$ scale), and (3.15). Change in $C_{i,\text{layer}}(x,\eta,t_w)$ is driven by diffusion into the reacting
surface—not convection. We see derive (3.16) mathematically by recasting the outer solution $C_{i,\text{outer}}(x, y, t_c)$ in terms of our layer variables $C_{i,\text{layer}}(x, \eta, t_w)$

$$C_{i,\text{layer}}(x, \eta, t_w) = \lim_{D_D \to 0} \begin{cases} 0 & \text{if } x - \eta(1 - \text{Pe}^{-1/3})t_w > 0, \\ 1 & \text{else.} \end{cases}$$

(3.17)

Since $D_D \ll 1$, as we exit the layer we will always be in the latter case of (3.17).

Finally, the kinetics system on the $t_w$ time scale is:

$$\frac{\partial B_1}{\partial t_w} = Da \left[ (1 - B_2)C_1 - K_d B_1 - \frac{1}{2} K_a B_1 C_2 + \frac{1}{2} K_d B_{12} \right],$$

(3.18a)

$$\frac{\partial B_{12}}{\partial t_w} = Da \left[ \frac{1}{2} K_a B_1 C_2 - \frac{1}{2} K_d B_{12} + \frac{1}{2} K_a B_2 C_1 - K_d B_{12} \right],$$

(3.18b)

$$\frac{\partial B_2}{\partial t_w} = Da \left[ K_d B_{12} - \frac{2}{3} K_a B_2 C_1 + 2 K_a (1 - B_2) C_2 - 2 K_d B_2 \right],$$

(3.18c)

$$B(x, 0) = 0,$$

(3.18d)

at $\eta = 0$.

### 3.4 Case 3: $Da = \mathcal{O}(1)$

We now study the system when $Da = \mathcal{O}(1)$. If $D_D \ll 1$, in order for $Da = \mathcal{O}(1)$, we must also have $1 k_a \text{Pe}^{1/3} \ll 1$. This is because

$$\frac{1 k_a \text{Pe}^{1/3}}{D_D} = Da = \mathcal{O}(1).$$

In this case, $t$ and $t_w = \frac{t}{Da}$ are of the same order of magnitude. This reflects the balance between diffusion into the surface and reaction in this parameter regime. In this case, we may proceed on the $t$ time scale as in Section 3.2, except with $Da = O(1)$. Specifically, evolution of the system will be governed by (3.9a)–(3.10c), (3.11), and (3.13a)–(3.13c).

### 3.5 Summary, Wash Phase, and Multiple-Receptor Model

We have seen that there are four distinct time scales. The flow in the bulk compartment reaches equilibrium on the convective $t_c$ time scale, and ligand molecules
diffuse to the unstirred layer over the longer diffusive $t_D$ time scale. Depending on the balance between reaction and transport, evolution of the reacting species concentration occurs on either the $t$ time scale, or the $t_w$ time scale. In the reaction-limited and transport-dominant parameter regime, the reacting species evolves on the $t$ time scale. On the other hand, in the reaction-dominant and transport-limited parameter regime, evolution of the reacting species occurs on the slowest $t_w$ time scale. In the case that reaction and diffusion balance, $t$ and $t_w$ are the same order.

All of the previous arguments go through for the wash phase as well, so instead of repeating the analysis, we only summarize the differences between the two phases. During the wash phase there is no ligand being injected into the biosensor, thus the inflow condition (3.3b) will change. On the $t_c$ time scale (3.3b) will be replaced by

$$C_i(0, \eta, t_c) = 0,$$  \hspace{1cm} (3.19)

with similar replacements on the $t_D, t, t_w$ time scales. Because the biosensor will be saturated with unbound ligand when the wash phase begins, the convective layer solution (2.37) now takes the form

$$C_i(x, y, t_c) = \begin{cases} 
1 & \text{if } x - y(1 - y)t_c > 0, \\
0 & \text{else.}
\end{cases}$$

This will subsequently change each of our matching conditions in an straightforward manner. For example, in the wash phase, (3.11) becomes

$$\lim_{\eta \to \infty} C(x, \eta, t) = 0.$$ 

We could repeat the analysis of Sections 3.1 through 3.4, to derive governing equations for our multiple-receptor model on each of the four time scales. However, we will instead simply summarize the relevant equations for the wash phase in Chapter 7.
Chapter 4

EFFECTIVE RATE CONSTANT EQUATIONS

The reaction dynamics in the unstirred layer depend upon the balance between reaction and diffusion—i.e., the size of the Damköhler number. We first study the bound state system in the reaction-limited and transport-dominant parameter regime by finding a two-term approximation to $B$ when $Da \ll 1$.

4.1 Unbound Ligand Concentration at the Surface

To derive a two-term expansion for (3.13), we need a closed form for $C_1(x, 0, t)$ and $C_2(x, 0, t)$. Physically, we expect the unbound ligand concentration at the surface to be a perturbation away from the uniform inflow concentration; thus we propose

$$C_i(x, \eta, t) = 1 + Da_c(x, \eta, t),$$  \hspace{1cm} (4.1)

for some function $c_i$ to be determined. Substituting (4.1) into (3.9a), (3.10a), (3.10b), and (3.11) when $i = 1$ we find

$$D_r \frac{\partial^2 c_1}{\partial \eta^2} = \eta \frac{\partial c_1}{\partial x},$$  \hspace{1cm} (4.2)

subject to

$$c_1(0, \eta, t) = 0,$$  \hspace{1cm} (4.3a)

$$\frac{\partial c_1}{\partial \eta}(x, 0, t) = \frac{1}{Fr} \left( \frac{\partial B_1}{\partial t} + \frac{\partial B_{12}}{\partial t} \right),$$  \hspace{1cm} (4.3b)

$$\lim_{\eta \to \infty} c_1(x, \eta, t) = 0.$$  \hspace{1cm} (4.3c)

Treating the $x$-axis as semi-infinite, we take a Laplace transform in $x$ of (4.2) to arrive at

$$D_r \frac{\partial^2 \hat{c}_1}{\partial \eta^2} = \eta s \hat{c}_1,$$  \hspace{1cm} (4.3d)
which is an Airy’s equation, whose solution is a linear combination of Airy functions:

\[ \hat{c}_1(\eta, t; s) = K_1(t; s) \text{Ai}\left(\left(\frac{s}{D_t}\right)^{1/3} \eta\right) + K_2(t; s) \text{Bi}\left(\left(\frac{s}{D_t}\right)^{1/3} \eta\right). \]  

(4.4)

The asymptotic behavior of \( \text{Ai}(x) \) and \( \text{Bi}(x) \) is given by [15]:

\[ \lim_{x \to \infty} \text{Ai}(x) = \frac{1}{2\sqrt{\pi x}^{1/4}} \exp\left( -\frac{2}{3} x^{3/2} \right) \left( 1 - \frac{5}{48 x^{2/3}} \right), \]  

(4.5)

\[ \lim_{x \to \infty} \text{Bi}(x) = \frac{1}{2\sqrt{\pi x}^{1/4}} \exp\left( \frac{2}{3} x^{3/2} \right) \left( 1 + \frac{5}{48 x^{2/3}} \right). \]  

(4.6)

Hence, for our solution to stay finite as we exit the layer we require that \( K_2(t; s) \equiv 0. \)

Computing \( \frac{\partial \hat{c}_1}{\partial \eta} \), evaluating at zero, and using (4.3b) we have

\[ K_1(t; s) \left( \frac{s}{D_t} \right)^{1/3} \text{Ai}'(0) = \frac{1}{F} \left( \frac{\partial \hat{B}_1}{\partial t} + \frac{\partial \hat{B}_{12}}{\partial t} \right). \]  

(4.7)

Rearranging and using \( \text{Ai}'(0) = -\frac{3^{1/6}\Gamma(2/3)}{2\pi} \), we find

\[ K_1(t; s) = -\frac{2\pi D_t^{1/3}}{s^{1/3} F_3^{1/6} \Gamma(2/3)} \left( \frac{\partial \hat{B}_1}{\partial t} + \frac{\partial \hat{B}_{12}}{\partial t} \right). \]  

(4.8)

Substituting (4.8) into (4.4), we have

\[ \hat{c}_1(\eta, t; s) = -\frac{2\pi D_t^{1/3}}{s^{1/3} F_3^{1/6} \Gamma(2/3)} \left( \frac{\partial \hat{B}_1}{\partial t} + \frac{\partial \hat{B}_{12}}{\partial t} \right) \text{Ai}\left(\left(\frac{s}{F}\right)^{1/3} \eta\right), \]  

(4.9)

which satisfies our matching condition (4.3c) due to the asymptotic behavior of \( \text{Ai}(x) \) (4.5); using (4.5) we have

\[ \lim_{\eta \to \infty} \hat{c}_1(\eta, t; s) = 0, \]  

(4.10)

which formally implies (4.3c) is satisfied. We need the value of (4.9) only at the surface, so evaluating (4.9) at \( \eta = 0 \), we have

\[ \hat{c}_1(0, t; s) = -\frac{2\pi D_t^{1/3}}{s^{1/3} F_3^{1/6} \Gamma(2/3)} \left( \frac{\partial \hat{B}_1}{\partial t} + \frac{\partial \hat{B}_{12}}{\partial t} \right) \text{Ai}(0) \]  

\[ = -\frac{2\pi D_t^{1/3}}{s^{1/3} F_3^{1/6} \Gamma(2/3)} \left( \frac{\partial \hat{B}_1}{\partial t} + \frac{\partial \hat{B}_{12}}{\partial t} \right) \left( \frac{\Gamma(1/3)}{2\pi^{3/6}} \right). \]  

(4.11)

Applying the convolution theorem, and substituting (4.11) into (4.1) we find

\[ C_1(x, 0, t) = 1 - \frac{D_t^{1/3} Da}{F_1 \Gamma(2/3) 3^{1/6}} \int_0^x \left( \frac{\partial B_1}{\partial t} + \frac{\partial B_{12}}{\partial t} \right) (\nu, t) \frac{d\nu}{(x - \nu)^{2/3}}. \]  

(4.12a)
We may derive a similar form for $C_2$:
\[
C_2(x, 0, t) = 1 - \frac{Da}{\Gamma(2/3)3^{1/3}} \int_0^x \left( \frac{\partial B_{12}}{\partial t} + \frac{\partial B_2}{\partial t} \right)(\nu, t) \frac{d\nu}{(x - \nu)^{2/3}}. \tag{4.12b}
\]
Since the fractional Riemann-Liouville integral is defined as [19, page 65]
\[
\mathcal{J}^\beta f = \int_0^x f(\nu) \frac{d\nu}{(x - \nu)^{1-\beta}}, \tag{4.13}
\]
we may recognize the integral terms in (4.12a) and (4.12b) as fractional Riemann-Liouville integrals of order $\beta = \frac{1}{3}$. This fractional integral accounts for the ligand depletion effect in the injection phase. One would expect, especially early on in the experiment, that ligand molecules will diffuse to the surface upstream before they will diffuse to bind with receptor sites downstream. This is precisely what (4.12a) and (4.12b) tell us. Additionally, during the injection phase $\frac{\partial B_i}{\partial t}$ will be monotonically increasing with time; therefore (4.12a) and (4.12b) will never be greater than the uniform inflow concentration.

### 4.2 A Two-Term Expansion

Substituting (4.12a) and (4.12b) into (3.13a)–(3.13c) we get a nonlinear system of fractional integrodifferential equations (IDE). We are now in a position to search for a two-term approximation for the bound state concentrations. In doing so, we will write (3.13a)–(3.13c) in terms of (4.1) to simplify notation. Substituting (4.1) into (3.13a)–(3.13c), we have:

\[
\frac{\partial B_1}{\partial t} = (1 - B_\Sigma)(1 + Da_1) - 1K_dB_1 - \frac{1}{2}K_aB_1(1 + Da_2) + \frac{1}{2}K_dB_{12}, \tag{4.14a}
\]
\[
\frac{\partial B_{12}}{\partial t} = \frac{1}{2}K_aB_1(1 + Da_2) - \frac{1}{2}K_dB_{12} + \frac{1}{2}K_aB_2(1 + Da_1) - 1K_dB_{12}, \tag{4.14b}
\]
\[
\frac{\partial B_2}{\partial t} = 1K_dB_{12} - \frac{1}{2}K_aB_2(1 + Da_1) + 2K_a(1 - B_\Sigma)(1 + Da_2) - 2K_dB_2, \tag{4.14c}
\]
which holds at $\eta = 0$. We now search for an approximation of the form
\[
B_i = \alpha^0 B_i + Da^1 B_i + O(Da^2), \tag{4.15}
\]
and substituting this into (4.14a) through (4.14c), the leading order equations are:

$$\frac{\partial^0 B}{\partial t} = -A^0 B + f,$$

$$0^0 B(x, 0) = 0.$$  \hspace{1cm} (4.16)

We have defined

$$A = \begin{pmatrix} (1 + 1K_d + \frac{1}{2}K_a) & 1 - \frac{1}{2}K_d & 1 \\ -\frac{1}{2}K_a & (\frac{1}{2}K_d + \frac{1}{2}K_a) & -\frac{2}{1}K_a \\ 2K_a & 2K_a - \frac{2}{1}K_d & (2K_a + 2K_d + \frac{2}{1}K_a) \end{pmatrix},$$  \hspace{1cm} (4.17)

$$f = \begin{pmatrix} 1 \\ 0 \\ 2K_a \end{pmatrix}.$$  \hspace{1cm} (4.18)

This matrix-vector pair is simply (2.22) and (2.25) scaled by \(1k_a\). The system (4.16) is easily solved via an integrating factor:

$$\frac{\partial}{\partial t} [(e^{At})^0 B] = e^{At} f$$

$$\Rightarrow 0^0 B = e^{-At} A^{-1} e^{At} f + e^{-At} K(x),$$

for some arbitrary function \(K(x)\). Using our initial condition, the fact that \(A^{-1}\) commutes with \(e^{At}\), and the identity \(e^{-At} e^{At} = I\) we arrive at

$$0^0 B(x, t) = A^{-1}(I - e^{-At}) f.$$  \hspace{1cm} (4.19)

Thus the leading order approximation is the well-stirred approximation. To quantify the effects of transport, we find the correction term. The \(O(Da)\) problem is:

$$\frac{\partial^1 B}{\partial t} = -A^1 B - L_1(x, t)0^1 B + g(x, t),$$

$$1^1 B(0) = 0,$$  \hspace{1cm} (4.20)

where

$$L_1(x, t) = \begin{pmatrix} 0 c_1 + \frac{1}{2}K_a 0 c_2 & 0 c_1 & 0 c_1 \\ -\frac{1}{2}K_a 0 c_2 & 0 & -\frac{2}{1}K_a 0 c_1 \\ 2K_a 0 c_2 & 2K_a 0 c_2 & 2K_a 0 c_2 + \frac{2}{1}K_a 0 c_1 \end{pmatrix},$$

$$g(x, t) = \begin{pmatrix} 0 c_1 \\ 0 \\ 2K_a 0 c_2 \end{pmatrix}. \hspace{1cm} (4.21)$$
The functions $^0c_1$ and $^0c_2$ are the $\mathcal{O}(Da)$ terms obtained upon substituting (4.15) into (4.12a) and (4.12b); they take the form

$$^0c_1(x,0,t) = -\frac{D_1^{1/3}}{F_1\Gamma(2/3)3^{1/3}} \int_0^x \left( \frac{\partial^0 B_1}{\partial t} + \frac{\partial^0 B_{12}}{\partial t} \right) \frac{d\nu}{(x-\nu)^{2/3}}, \quad (4.22)$$

$$^0c_2(x,0,t) = -\frac{1}{3^{1/3}\Gamma(2/3)} \int_0^x \left( \frac{\partial^0 B_{12}}{\partial t} + \frac{\partial^0 B_2}{\partial t} \right) \frac{d\nu}{(x-\nu)^{2/3}}. \quad (4.23)$$

Since the leading order approximation is independent of space, we may rewrite the $^0c_i$ terms as:

$$^0c_1(x,0,t) = -\frac{3^{2/3}D_1^{1/3}x^{1/3}}{F_1\Gamma(2/3)} \left( \frac{\partial^0 B_1}{\partial t} + \frac{\partial^0 B_{12}}{\partial t} \right), \quad (4.24)$$

$$^0c_2(x,0,t) = -\frac{3^{2/3}x^{1/3}}{\Gamma(2/3)} \left( \frac{\partial^0 B_{12}}{\partial t} + \frac{\partial^0 B_2}{\partial t} \right). \quad (4.25)$$

For convenience we define

$$h(x) = \frac{3^{2/3}x^{1/3}}{\Gamma(2/3)}, \quad (4.26)$$

and write (4.24) and (4.25) as:

$$^0c_1(x,0,t) = -\frac{D_1^{1/3}h(x)}{F_1} \left( \frac{\partial^0 B_1}{\partial t} + \frac{\partial^0 B_{12}}{\partial t} \right), \quad (4.27)$$

$$^0c_2(x,0,t) = -h(x) \left( \frac{\partial^0 B_{12}}{\partial t} + \frac{\partial^0 B_2}{\partial t} \right). \quad (4.28)$$

After some algebra, we may then express $g(x,t)$ as

$$g(x,t) = hL_2e^{-At}f, \quad (4.29)$$

where

$$L_2 = \begin{pmatrix} \frac{1}{F_1^{1/3}} & \frac{1}{F_1^{1/3}} & 0 \\ 0 & 0 & 0 \\ 0 & 2K_a & 2K_a \end{pmatrix}. \quad (4.30)$$

We will use this form to find an explicit solution for (4.20). To this end, we use an integrating factor:

$$\frac{\partial^1 B}{\partial t} + A^1 B = L_1(x,t)^0 B + hL_2e^{-At}f$$

$$\Rightarrow \frac{\partial}{\partial t}(e^{At} \ 1^1 B) = e^{At} L_1(x,t)^0 B + he^{At} L_2 e^{-At},$$

to give us

$$1^1 B = \int_0^t e^{-A(t-s)} L_1(x,t)^0 B \ ds + he^{-At} \int_0^t e^{As} L_2 e^{-As} f \ ds. \quad (4.31)$$
4.2.1 Secular Term

While our expansion is mathematically sound, it is unwieldy and difficult to deal with in practice. Additionally, the term

\[ he^{-At} \int_0^t e^{As} L_2 e^{-As} f \, ds \quad (4.32) \]

may be secular, depending on the values of the rate constants. This would be easy to see if \( e^{-As} \) and \( L_2 \) were to commute, but this is not the case.

Before showing that (4.32) may contain a secular term, we make two remarks. First, since \( \det(A_c) > 0 \) (see equation (2.23)), this implies that \( \det(A) > 0 \). Therefore, none of the eigenvalues of \( A \) are zero. Additionally, we know that \( e^{-As} f \) is not in the null space of \( L_2 \), which implies (4.32) is nonzero. To see why, first note that because \( e^{\pm As} \) are both nonsingular operators, the operator \( L_2 \) is the only one that could possibly render (4.32) zero. Additionally, \( e^{-As} f \in \text{Null}(L_2) \) only if

\[ L_2 e^{-As} f = \sum_{n=0}^{\infty} \frac{(-As)^n}{n!} f = 0, \]

which will be true only if

\[ L_2 (-As)^n f = 0 \quad (4.33) \]

for each \( n = 0, 1, 2, \ldots \). By equating coefficients of \( s^n \) on each side of (4.33), we must have

\[ L_2 If = 0. \quad (4.34) \]

However, since

\[ f \not\in \text{span}\{(1, -1, 1)^T\} = \text{null}(L_2), \]

we see that (4.34) doesn’t hold.

We now show how a secular term may arise in (4.32); in general, this will depend upon the rate constants. Let \( \{v_1, v_2, v_3\} \) be three eigenvectors of the matrix \(-A\), with associated eigenvalues \(-\lambda_1, -\lambda_2, -\lambda_3\). The eigenvalues may or may not be distinct; this will not affect the argument. Physically, we expect \( \text{Re}(\lambda_i) > 0 \), although we do not impose this restriction. Additionally, our eigenvectors may be generalized eigenvectors,
but this modifies the argument only slightly. Since our eigenvectors form a basis for \( \mathbb{R}^3 \), we may write

\[
\mathbf{f} = c_1 \mathbf{v}_1 + c_2 \mathbf{v}_2 + c_3 \mathbf{v}_3,
\]

so that

\[
e^{-\mathbf{A}s} \mathbf{f} = c_1 e^{-\lambda_1 s} \mathbf{v}_1 + c_2 e^{-\lambda_2 s} \mathbf{v}_2 + c_3 e^{-\lambda_3 s} \mathbf{v}_3.
\]

Not all of the \( c_i \) can be zero, since (4.32) is nonzero. Applying \( L_2 \) to the above expression yields

\[
e^{-\mathbf{A}s} \mathbf{f} = c_1 e^{-\lambda_1 s} L_2 \mathbf{v}_1 + c_2 e^{-\lambda_2 s} L_2 \mathbf{v}_2 + c_3 e^{-\lambda_3 s} L_2 \mathbf{v}_3.
\]

The vectors comprising \( L_2 \mathbf{f} \in \mathbb{R}^3 \); therefore they may also be expressed as a linear combination of eigenvectors. Hence,

\[
L_2 e^{-\mathbf{A}s} \mathbf{f} = c_1 e^{-\lambda_1 s} \left( \sum_{i=1}^{3} d_i \mathbf{v}_i \right) + c_2 e^{-\lambda_2 s} \left( \sum_{i=1}^{3} d'_i \mathbf{v}_i \right) + c_3 e^{-\lambda_3 s} \left( \sum_{i=1}^{3} d''_i \mathbf{v}_i \right). \tag{4.35}
\]

We may then compute the integrand of (4.32) by multiplying the above expression by \( e^{\mathbf{A}s} \). In writing the resulting expression, we write only the terms which contribute to the secularity. Multiplying (4.35) by \( e^{\mathbf{A}s} \), we see

\[
e^{\mathbf{A}s} L_2 e^{-\mathbf{A}s} \mathbf{f} = c_1 d_1 e^{-\lambda_1 s} e^{\mathbf{A}s} \mathbf{v}_1 + c_2 d'_2 e^{-\lambda_2 s} e^{\mathbf{A}s} \mathbf{v}_2 + c_3 d''_3 e^{-\lambda_3 s} e^{\mathbf{A}s} \mathbf{v}_3 + \cdots
\]

\[
= c_1 d_1 e^{-\lambda_1 s} e^{\lambda_1 s} \mathbf{v}_1 + c_2 d'_2 e^{-\lambda_2 s} e^{\lambda_2 s} \mathbf{v}_2 + c_3 d''_3 e^{-\lambda_3 s} e^{\lambda_3 s} \mathbf{v}_3 + \cdots
\]

\[
= c_1 d_1 \mathbf{v}_1 + c_2 d'_2 \mathbf{v}_2 + c_3 d''_3 \mathbf{v}_3 + \cdots
\]

Thus one can now see that integrating the above from 0 to \( t \) produces a term of the form

\[
e^{-\mathbf{A}t}(c_1 d_1 t \mathbf{v}_1 + c_2 d'_2 t \mathbf{v}_2 + c_3 d''_3 t \mathbf{v}_3), \tag{4.36}
\]

which is secular. This will be true as long as the rate constants are such that at least one of the coefficients in (4.36) is nonzero. This argument also carries through if the \( \mathbf{v}_i \) are generalized eigenvectors; there will just be more terms which appear upon applying the matrix exponential to our eigenvectors.
Since we expect \( \lambda_i > 0 \), the term (4.36) should decay to zero. Unfortunately, this term is still troubling, because for \( t = \mathcal{O}(\text{Da}^{-1}) \), we have

\[
\mathcal{O}(B_1) = \mathcal{O}(B_0 - B_\infty).
\]

To derive a more accurate expansion, one may think to propose a multiple-scale expansion; however such an expansion would be even more unwieldy and difficult to deal with. In Subsection 4.3 we propose another approach, motivated by [12], in which we derive a nonlinear set of ODE’s for \( \overline{B} \). Before deriving this set of ODE’s, we first study the wash phase of the experiment.

### 4.2.2 Wash Phase

During the wash phase, the kinetics system is still given by (3.13a)-(3.13c), but this time with the initial condition

\[
B(x, 0) = A^{-1}f.
\]

(4.37)

We have yet to find a closed form for \( C_i(x, 0, t) \) for the wash phase. Since there is no ligand flowing into the biosensor, equations (3.10a) and (3.11) are replaced by:

\[
C_i(0, \eta, t) = 0,
\]

(4.38)

\[
\lim_{\eta \to \infty} C_i(x, \eta, t) = 0.
\]

(4.39)

One may show, as in Section 4.1, that \( C_i(x, 0, t) \) now takes the form:

\[
C_1(x, 0, t) = -\frac{D_1^{1/3}\text{Da}}{F_1\Gamma(2/3)3^{1/3}x} \int_0^x \left( \frac{\partial B_1}{\partial t} + \frac{\partial B_{12}}{\partial t} \right) \frac{d\nu}{(x - \nu)^{2/3}},
\]

(4.40a)

\[
C_2(x, 0, t) = -\frac{\text{Da}}{\Gamma(2/3)3^{1/3}x} \int_0^x \left( \frac{\partial B_{12}}{\partial t} + \frac{\partial B_2}{\partial t} \right) \frac{d\nu}{(x - \nu)^{2/3}}.
\]

(4.40b)

In the reaction-limited and transport-dominant parameter regime, we expect only trace amounts of unbound ligand to be available for binding at the surface. In addition, during the wash phase, ligand molecules dissociating upstream can rebind to receptor sites further downstream. This ligand rebinding effect results in having more unbound
ligand at the surface for binding downstream than upstream, and is reflected in the fractional integrals (4.44) and (4.45). During the wash phase $B_i$ will be monotonically decreasing with respect to time: thus (4.44) and (4.45) are nonnegative.

During the wash phase, the kinetics system is then given by (3.13a)-(3.13c), using (4.44) and (4.45). We now derive a two-term approximation, in the same manner as the injection phase. Proposing an expansion of the form (4.15), and substituting this expansion into our kinetics system, we arrive at the leading order problem

$$\frac{\partial^0 B}{\partial t} = -D^0 B, \quad 0^0 B(x, 0) = A^{-1} f.$$ (4.41)

Here $D$ is the dissociation operator given by

$$D = \begin{pmatrix} K_d & -\frac{1}{2} K_d & 0 \\ 0 & (\frac{1}{2} K_d + \frac{1}{2} K_d) & 0 \\ 0 & -\frac{1}{2} K_d & 2K_d \end{pmatrix}. \quad (4.42)$$

The solution to this problem is

$$0^0 B(x, t) = e^{-Dt} A^{-1} f,$$

which corresponds to the standard exponential decay solution, as obtained in the well-stirred limit. Indeed, one may easily compute the eigenvalues of $D$ to see that they will be positive as long as the rate constants are positive. The $O(Da)$ problem is

$$\frac{\partial^1 B}{\partial t} = -D^1 B - L_1(x, t) 0^0 B + g(x, t). \quad (4.43)$$

Here $L, g$ are as in (4.21), except

$$0^1 c_1(x, 0, t) = - \frac{D^{1/3} Da}{F \Gamma(2/3) 3^{1/3}} \int_0^x \left( \frac{\partial^0 B_1}{\partial t} + \frac{\partial^0 B_{12}}{\partial t} \right) \frac{d\nu}{(x - \nu)^{2/3}}, \quad (4.44)$$

$$0^1 c_2(x, 0, t) = - \frac{Da \Gamma(2/3) 3^{1/3}}{\Gamma(2/3) 3^{1/3}} \int_0^x \left( \frac{\partial^0 B_{12}}{\partial t} + \frac{\partial^0 B_2}{\partial t} \right) \frac{d\nu}{(x - \nu)^{2/3}}, \quad (4.45)$$

This implies that $g$ no longer reduces to (4.29), but instead reduces to

$$h L_2 D e^{-Dt} A^{-1} f.$$
The solution to (4.43) is given by

\[ \mathbf{1} B = \int_0^t e^{(t-s)D} L_1(x, t)^0 B \; ds + h e^{-D t} \int_0^t e^{D s} L_2 D e^{-D s} A^{-1} f \; ds. \]

Like our two-term approximation to \( B \) during the wash phase, our approximation to \( B \) is also unwieldy and difficult to deal with. Additionally, the term

\[ h e^{-D t} \int_0^t e^{D s} L_2 D e^{-D s} A^{-1} f \; ds \quad (4.46) \]

may be secular. Recalling \( B_\infty = A^{-1} f \), and setting \( B_\infty' = D A^{-1} f \), we can write (4.46) as

\[ h e^{-D t} \int_0^t e^{D s} L_2 e^{-D s} B_\infty' \; ds. \quad (4.47) \]

We may then apply the argument of Subsection 4.2.1 to show (4.47) may be secular. Actually, this case is a bit simpler, since the form of (4.42) allows us to easily compute the eigenvalues. As in Subsection 4.2.1, whether (4.47) is secular will ultimately depend upon the values of the eigenvalues of \( D \), and thus the rate constants. As in the injection phase, the potential secularity calls for a multiple-scale expansion. Unfortunately, a multiple-scale expansion would be even more unwieldy and difficult to deal with. This, with our results from the injection phase, motivates us to adopt the approach of Edwards and Jackson in [12].

**4.3 ERC Equations**

We briefly recall [12]. In the presence of only a single ligand, equations (3.13a)–(3.13c), using (4.12a) and (4.12b), reduce to

\[ \frac{\partial B}{\partial t} = (1 - B) C(x, 0, t) - K B, \quad (4.48a) \]

\[ C(x, 0, t) = 1 - \frac{D a}{3^{1/3} \Gamma(2/3)} \int_0^x \frac{\partial B}{\partial t} \frac{d \nu}{(x - \nu)^{2/3}}, \quad (4.48b) \]

\[ B(x, 0) = 0. \quad (4.48c) \]

One may also see [6]. We derive a differential equation, directly in terms of \( \overline{B} \), as follows. In a regular expansion

\[ B = ^0 B + D a^1 B + \mathcal{O}(D a^2), \quad (4.49) \]
the leading order approximation to $B$ is

$$0B(t) = \alpha^{-1}(1 - e^{-\alpha t}), \quad (4.50)$$

$$\alpha = 1 + K. \quad (4.51)$$

Note the leading-order approximation is independent of space. We now substitute (4.49) into (4.48b) to write

$$C(x, 0, t) = 1 - \text{Dah}(x) \frac{dB_0}{dt} + \mathcal{O}(Da^2), \quad (4.52)$$

where $h$ is given by (4.26). However, since $\text{Da}B = \text{Da}B_0 + \mathcal{O}(Da^2)$, this is equivalent to

$$C(x, 0, t) = 1 - \text{Dah}(x) \frac{\partial B}{\partial t} + \mathcal{O}(Da^2); \quad (4.53)$$

hence

$$\frac{\partial B}{\partial t} = (1 - B) \left( 1 - \text{Dah}(x) \frac{\partial B}{\partial t} \right) - KB + \mathcal{O}(Da^2). \quad (4.54)$$

With the fractional integral out of the equation, we would now like to average each side of the equation to obtain a differential equation for $B$. This would be very straightforward, if it were not for the terms

$$\text{Dah}(x)B \frac{\partial B}{\partial t}, \quad \text{Dah}(x) \frac{\partial B}{\partial t}.$$

We would like to write

$$\text{Da} \left( h(x)B \frac{\partial B}{\partial t} \right) = \text{Da} \left( \overline{h} \cdot \overline{B} \frac{dB}{dt} \right),$$

but this is sadly not true. However, we can use the relation

$$\text{Da}B = \text{Da}^0B + \mathcal{O}(Da^2),$$

to compute

$$\text{Da} \left( h(x)B \frac{\partial B}{\partial t} \right) = \text{Da} \left( h(x) \left( \text{Da}^0B + \text{Da}^1B + \cdots \right) \frac{\partial}{\partial t} \left( \text{Da}^0B + \text{Da}^1B + \cdots \right) \right)$$

$$= \text{Dah}^0 \frac{d^1B}{dt} + \mathcal{O}(Da^2)$$

$$= \text{Dah} \cdot \overline{B} \frac{dB}{dt} + \mathcal{O}(Da^2).$$
Similarly, one can show
\[ \text{Da}(x) \frac{\partial B}{\partial t} = \text{Da} \frac{d\bar{B}}{dt} + O(Da^2). \]

Therefore we may average (4.54) to obtain
\[ \frac{d\bar{B}}{dt} = (1 - \bar{B}) \left( 1 - \text{Da} \frac{d\bar{B}}{dt} \right) - K\bar{B} + O(Da^2), \quad (4.55) \]

which we may rearrange to obtain the nonlinear ODE:
\[ \frac{d\bar{B}}{dt} = \left[ (1 - \bar{B}) - K\bar{B} \right] (1 - p) + O(Da^2), \quad (4.56a) \]
\[ p = \frac{\text{Da}(1 - \bar{B})h}{1 + \text{Da}(1 + \bar{B})h}, \quad (4.56b) \]

with the initial condition \( \bar{B}(0) = 0 \). Equation (4.56) was presented first in [12]; in this work Edwards and Jackson refer to (4.56) as an Effective Rate Constant equation (ERC equation). This ERC equation has numerous advantages, and the first is its simplicity. It is a simple nonlinear ODE, which can be easily solved using a standard ODE package, like ODE45 in MATLAB. A numerical solution to (4.56) is far simpler than a complicated multiple-scale approximation to (4.48). Furthermore, although (4.56) is formally valid only when \( Da \ll 1 \), its solution agrees with a numerical approximation of the IDE (4.48) for moderate and large Da as well. Hence, we seek to derive a set of ERC equations to find an approximation to \( \bar{B} \) as given in (3.13a)–(3.13d).

The strategy employed in deriving (4.56) extends quite naturally to our multiple-ligand model; we will sketch the derivation. Instead of writing (4.48b) as (4.53), we write (4.12a) and (4.12b) as:
\[ C_1(x, 0, t) = 1 - \frac{D_1^{1/3} \text{Da}}{F_1} \left( \frac{\partial B_1}{\partial t} + \frac{\partial B_{12}}{\partial t} \right) + O(Da^2), \quad (4.57) \]
\[ C_2(x, 0, t) = 1 - \text{Da} h \left( \frac{\partial B_{12}}{\partial t} + \frac{\partial B_2}{\partial t} \right) + O(Da^2). \quad (4.58) \]
Substituting (4.57) and (4.58) into (3.13a)–(3.13c), we arrive at:

\[
\frac{\partial B_1}{\partial t} = (1 - B_1) \left( 1 - \frac{D_1^{1/3}D_{th}}{F_r} \left( \frac{\partial B_1}{\partial t} + \frac{\partial B_{12}}{\partial t} \right) \right) - 1 K_d B_1 \tag{4.59a}
\]

\[
- \frac{1}{2} K_a B_1 \left( 1 - D_{th} \left( \frac{\partial B_{12}}{\partial t} + \frac{\partial B_2}{\partial t} \right) \right) + \frac{1}{2} K_d B_{12} + O(Da^2),
\]

\[
\frac{\partial B_{12}}{\partial t} = \frac{1}{2} K_a B_1 \left( 1 - D_{th} \left( \frac{\partial B_1}{\partial t} + \frac{\partial B_2}{\partial t} \right) \right) - \frac{1}{2} K_d B_{12} \tag{4.59b}
\]

\[
+ \frac{1}{2} K_a B_2 \left( 1 - \frac{D_1^{1/3}D_{th}}{F_r} \left( \frac{\partial B_1}{\partial t} + \frac{\partial B_{12}}{\partial t} \right) \right) - \frac{1}{2} K_d B_{12} + O(Da^2),
\]

\[
\frac{\partial B_2}{\partial t} = \frac{1}{2} K_a B_1 \left( 1 - D_{th} \left( \frac{\partial B_1}{\partial t} + \frac{\partial B_{12}}{\partial t} \right) \right) - \frac{1}{2} K_d B_2 \tag{4.59c}
\]

By employing the same strategy used to derive (4.55) from (4.54), we average and manipulate (4.59a)–(4.59c) to get:

\[
\frac{d\bar{B}_1}{dt} = (1 - \bar{B}_1) \left( 1 - \frac{D_1^{1/3}D_{th}}{F_r} \left( \frac{d\bar{B}_1}{dt} + \frac{d\bar{B}_{12}}{dt} \right) \right) - 1 K_d \bar{B}_1 \tag{4.60a}
\]

\[
- \frac{1}{2} K_a \bar{B}_1 \left( 1 - D_{th} \left( \frac{d\bar{B}_{12}}{dt} + \frac{d\bar{B}_2}{dt} \right) \right) + \frac{1}{2} K_d \bar{B}_{12} + O(Da^2),
\]

\[
\frac{d\bar{B}_{12}}{dt} = \frac{1}{2} K_a \bar{B}_1 \left( 1 - D_{th} \left( \frac{d\bar{B}_1}{dt} + \frac{d\bar{B}_{12}}{dt} \right) \right) - \frac{1}{2} K_d \bar{B}_{12} \tag{4.60b}
\]

\[
+ \frac{1}{2} K_a \bar{B}_2 \left( 1 - \frac{D_1^{1/3}D_{th}}{F_r} \left( \frac{d\bar{B}_1}{dt} + \frac{d\bar{B}_{12}}{dt} \right) \right) - \frac{1}{2} K_d \bar{B}_{12} + O(Da^2),
\]

\[
\frac{d\bar{B}_2}{dt} = \frac{1}{2} K_a \bar{B}_1 \left( 1 - D_{th} \left( \frac{d\bar{B}_1}{dt} + \frac{d\bar{B}_{12}}{dt} \right) \right) - \frac{1}{2} K_d \bar{B}_2 \tag{4.60c}
\]

These equations are written more compactly as:

\[
\frac{d\bar{B}}{dt} = M^{-1}(\bar{B})(-A\bar{B} + f) + O(Da^2), \tag{4.61a}
\]

\[
\bar{B}(0) = 0, \tag{4.61b}
\]
where

\[ M^{-1}(B) = I + DaN(B), \]

\[
N(B) = \begin{pmatrix}
\frac{D_1^{1/3}}{Fr} (1 - B_\Sigma) & \frac{D_1^{1/3}}{Fr} (1 - B_\Sigma) - \frac{1}{2} K_a \bar{h} \cdot B_1 & -\frac{1}{2} K_a \bar{h} \cdot B_1 \\
\frac{1}{2} K_a \bar{h} \cdot B_2 & \frac{1}{2} K_a \bar{h} \cdot B_1 + \frac{2}{1} K_a \left( \frac{D_1^{1/3}}{Fr} \right) B_2 & \frac{2}{1} K_a \left( \frac{D_1^{1/3}}{Fr} \right) B_2 \\
-\frac{1}{2} K_a \left( \frac{D_1^{1/3}}{Fr} \right) B_2 & -\frac{1}{2} K_a \left( \frac{D_1^{1/3}}{Fr} \right) B_2 + 2 K_a \bar{h}(1 - B_\Sigma) & 2 K_a \bar{h}(1 - B_\Sigma)
\end{pmatrix}.
\]

We note the state-dependent mass matrix \( M(B) \) is a function of time, so it is not possible to simplify the numerically problem by writing (4.61a) as

\[
\dot{z} = -AM^{-1}z + f,
\]

\[ z = M(B)B. \]

The ERC equations for the wash phase are:

\[
\frac{dB}{dt} = M^{-1}(B)(-DB) + O(Da^2), \quad B(0) = A^{-1}f. \tag{4.62}
\]

These ERC equations inherit all of the advantages of using (4.56) over (4.48); they are simple, and are easily solved using a standard ODE solver, like ODE45 in MATLAB. This provides a convenient way of reconstructing the sensogram signal, and estimating rate constants from raw data. To investigate the accuracy of our ERC equations for our multiple-component system, we develop a numerical approximation to (3.13), (4.12a), (4.12b).
Chapter 5
NUMERICS

We now develop a semi-implicit finite difference method to approximate the solution to (3.13), (4.12a), (4.12b). We discuss our numerical method for the injection phase of the multiple-ligand experiment; our numerical method for the wash phase and the multiple-receptor experiment both take the same form.

5.1 Semi-Implicit Finite Difference Algorithm

In this Section we discuss our numerical method: for the full details of our derivation see Appendix B. We index space by \( i \) and time by \( n \), to write \( B^j_{i,n} = B_j(i\Delta x, n\Delta t) \). Our numerical method takes the form:

\[
\frac{\partial B_{1,n+1}^1}{\partial t} = (1 - B^\Sigma_{i,n}) C_{i,n+1}^1 - 1 K_d B_{i,n}^1 - 2 K_a B_{i,n}^1 C_{i,n+1}^2 + 2 K_d B_{i,n}^{12},
\]

\[
\frac{\partial B_{1,n+1}^{12}}{\partial t} = \frac{1}{2} K_a B_{i,n}^2 C_{i,n+1}^2 - 1 K_d B_{i,n}^{12} + 2 K_a B_{i,n}^2 C_{i,n+1}^1 - 2 K_d B_{i,n}^{12},
\]

\[
\frac{\partial B_{2,n+1}^2}{\partial t} = 2 K_d B_{i,n}^{12} - 2 K_a B_{i,n}^2 C_{i,n+1}^1 + 2 K_a (1 - B^\Sigma_{i,n}) C_{i,n+1}^2 - 2 K_d B_{i,n}^2,
\]

where:

\[
C_{i,n+1}^1 = 1 - \frac{D_{i}^1}{F_i 3^{1/3} \Gamma(\frac{2}{3})} \int_{x_i}^{x_i} \left\{ \frac{\partial B_{1}^1}{\partial t} (x_i - \nu, t_{n+1}) + \frac{\partial B_{12}^1}{\partial t} (x_i - \nu, t_{n+1}) \right\} \frac{d\nu}{\nu^{2/3}},
\]

\[
C_{i,n+1}^2 = 1 - \frac{Da}{3^{1/3} \Gamma(\frac{2}{3})} \int_{x_i}^{x_i} \left\{ \frac{\partial B_{12}^1}{\partial t} (x_i - \nu, t_{n+1}) + \frac{\partial B_{2}^2}{\partial t} (x_i - \nu, t_{n+1}) \right\} \frac{d\nu}{\nu^{2/3}}.
\]

We also impose the initial condition (3.13d)

\[ B_{i,0} = 0 \]

at each of our \( N + 1 \) equally spaced discretization nodes \( x_i \), for \( i = 0, \ldots, N \). Notice the form of (5.2) and (5.3) renders our algorithm semi-implicit. We start upstream at
$x = 0$, and march our way downstream to $x = 1$ at each timestep. In this way, we are able to use updated information at time step $n + 1$ in (5.2) and (5.3), rather than the older value $\frac{\partial B_j}{\partial t}$. See Figure 5.1 for an illustration of our time stepping algorithm. In Figure 5.1 the first row represents our initial condition at $t = 0$, the second row represents the first time step at $t = \Delta t$, and the third row represents the second time step at $t = 2\Delta t$. In addition, the first column represents the node at $x = 0$, the second column represents the node at $x = \Delta x$, the third column represents the node at $x = 2\Delta x$, and so on. To march forward in time we first use our initial condition when $t = 0$ at the node $x = 0$ (denoted by $(0, 0)$), and march forward one time step to $t = \Delta t$ at the node $x = 0$ (that is, to $(0, \Delta t)$). Then, we use the values at $(0, \Delta t)$ and $(\Delta x, 0)$ to find the value at $(\Delta t, \Delta x)$. Similarly, we can use our initial condition and the values at $(0, \Delta t)$, and $(\Delta x, \Delta t)$, to find the value at $(2\Delta x, \Delta t)$. We let the information flow downstream from left to right at each time step, and repeat as many time steps as necessary.
Figure 5.1: Time stepping algorithm.

Turning our attention back to equations (5.1a)-(5.3), we observe that they are not yet fully discretized; we still need to discretize the time derivatives and the convolution integral. We seek to discretize the convolution integrals using the trapezoidal rule; however (5.2) and (5.3) both have singularities at the origin when \( \nu = 0 \). We
subtract out the singularity to write (5.2) and (5.3) as:

\[
C_{1,i,n+1}^1 = 1 - \frac{D_1^{1/3}Da}{F^{3/4}} \int_0^{x_i} \left\{ \frac{\partial B_1^1(x_i - \nu, t_{n+1}) - \partial B_{1,i,n+1}^1}{\nu^{1/3}} + \frac{\partial B_{1,i,n+1}^1}{\nu^{1/3}} \right\} \frac{\nu}{\nu^2} + 3x_i^{1/3} \left( \frac{\partial B_{1,i,n+1}^1}{\nu^{1/3}} + \frac{\partial B_{1,i,n+1}^1}{\nu^{1/3}} \right),
\]

(5.4)

\[
C_{2,i,n+1}^2 = 1 - \frac{Da}{3^{1/3}F^{1/3}(\frac{x_i}{2})} \int_0^{x_i} \left\{ \frac{\partial B_{12}^1(x_i - \nu, t_{n+1}) - \partial B_{1,i,n+1}^{12}}{\nu^{2/3}} + 3x_i^{1/3} \left( \frac{\partial B_{1,i,n+1}^{12}}{\nu^{2/3}} + \frac{\partial B_{2,i,n+1}^{12}}{\nu^{2/3}} \right) \right\} d\nu + \frac{\partial B_2^1(x_i - \nu, t_{n+1}) - \partial B_{2,i,n+1}^2}{\nu^{2/3}} + 3x_i^{1/3} \left( \frac{\partial B_{2,i,n+1}^2}{\nu^{2/3}} + \frac{\partial B_{2,i,n+1}^2}{\nu^{2/3}} \right).
\]

(5.5)

We are implicitly assuming

\[
\lim_{\nu \to 0} \left( \frac{\partial B_j^1(i\Delta x - \nu, n\Delta t) - \partial B_j^2(i\Delta x, n\Delta t)}{\partial t} \right) \frac{1}{\nu^{2/3}} < \infty,
\]

(5.6)

and will justify this when Da \ll 1 in Subsection 5.2. With (5.2) and (5.3) written as (5.4) and (5.5), we may then apply the trapezoidal rule to discretize our fractional integrals. We approximate the time derivative as

\[
\frac{\partial B_{1,i,n+1}^j}{\partial t} \approx \frac{d B_{1,i,n}^j}{\Delta t} := \frac{B_{1,i,n}^j - B_{1,i,n-1}^j}{\Delta t},
\]

(5.7)

and take \( \Delta t = t_n - t_{n-1} \) constant. The resulting fully discretized IDE system will be implicit, as the variables

\[ dB_{1,i,n+1}^j, \ j = 1, 12, 2, \]

are on each side of the fully discretized versions of (5.1a)–(5.1c), with (5.4) and (5.5).

We can solve this system for \( dB_{1,i,n+1}^j \), and write the resulting equations in matrix-vector form as

\[
\frac{dB_{1,i,n+1}^j}{\Delta t} = M_{1,i,n}^{-1}(B_{1,i,n})(-A_{i,n+1}B_{1,i,n} + f_{1,n+1}).
\]

(5.8)

We have written \( M_{1,i,n}^{-1}(B_{1,i,n}) \) to emphasize the fact that our mass matrix \( M(B_{1,i,n}) \) is a function of \( B_{1,i,n} \). We march forward in time by using an second order Adams-Bashforth method of the form

\[
B_{1,i,n+1} = B_{1,i,n} + \frac{1}{2}(3dB_{1,i,n+1} - dB_{1,i,n}),
\]

(5.9)
for \( n \geq 1 \). We move from the first time step at \( n = 0 \) to \( n = 1 \) via Euler’s method. After solving for \( B_{i,n} \) at all desired time steps, we can compute \( \overline{B}_n \) by applying the trapezoidal rule to \( B_{i,n} \) at each time step \( n \).

We briefly comment on \( A_{l,n+1} \) and \( f_{l,n+1} \) in (5.8). The entries of this matrix-vector pair are dependent upon the function values \( B_{j,l,n+1} \), only for \( l \) such that \( 0 \leq l < i \). Since these values are known, the system (5.8) is not implicit. The dependence of \( A_{t,n+1}, f_{t,n+1} \) on function values upstream is a consequence of the fact that we are starting upstream at \( x = 0 \), and marching our way downstream at each time step.

In addition, we remark that our decision to use a second-order Adams-Bashforth, rather than third or fourth-order, was based off the fact that second-order convergence of the method is preserved when used in conjunction with our semi-implicit time stepping scheme. This was not the case with a third order Adams-Bashforth, likely due to the fact that our method is semi-implicit. Although one may be concerned that using a second-order time stepping scheme limits the accuracy of our numerical method, we will show in the next section that the largest reduction in accuracy may be attributed to the singular integrand in our convolution integral. We shall see that, due to our to singular integrand and the functional form of \( B \), our solution converges at a rate of \( O(\Delta x^{2/3}) \) for when \( Da \) is small or moderate (\( i.e., \) the physically relevant cases), and \( O(\Delta x^{1/3}) \) when \( Da \gg 1 \). Fortunately, we are able to achieve faster convergence when computing \( \overline{B} \), which we discuss the next Section 5.2. For a discussion of stability, we refer the reader to Section 5.3.

5.2 Convergence Study

We now examine the convergence rate of our algorithm. The singularity in (5.4) and (5.5) affects the spatial convergence of our method. How strongly the singularity affects convergence depends upon the size of the Damköhler number, and whether the quantity of interest is computed over an interval containing the singularity at \( x = 0 \). Hence, the convergence may be different for the quantities \( B_i, B_i|_s, \overline{B}_i, \) or \( \overline{B_i}|_s \), where
$B_i |_s$ denotes the restriction of $B_i$ to the scanning range $[x_{\text{min}}, x_{\text{max}}]$, and

\[
\mathcal{B}_i(t) = \int_0^1 B_i(x, t) \, dx, \tag{5.10}
\]

\[
\mathcal{B}_i |_s(t) = \frac{1}{x_{\text{max}} - x_{\text{min}}} \int_{x_{\text{min}}}^{x_{\text{max}}} B_i(x, t) \, dx. \tag{5.11}
\]

Notice in (5.10) and (5.11) we have drawn a distinction between averaging over $[0, 1]$ and the scanning range $[x_{\text{min}}, x_{\text{max}}]$. Mathematically we are interested in the convergence rates of both (5.10) and (5.11), but (5.11) is the only quantity of physical relevance. One is not concerned with (5.10) in practice, since measurements are taken over $[x_{\text{min}}, x_{\text{max}}]$. Thus when presenting results, we will write $\mathcal{B}_i |_s$ to emphasize that our results were computed over the scanning range $[x_{\text{min}}, x_{\text{max}}]$. Writing $\mathcal{B}_i |_s$ in each of our calculations would be cumbersome, therefore in this chapter we will write $\mathcal{B}$ to refer both to $\mathcal{B}$ and $\mathcal{B}_i |_s$; it will be clear from the context which quantity we are discussing. In every other chapter we will limit our average to the scanning range $[x_{\text{min}}, x_{\text{max}}]$. In addition, the quantities $B_i, B_i |_s, \mathcal{B}_i, \mathcal{B}_i |_s$ are defined in an analogous manner for the bimolecular case, involving only a single ligand.

When studying convergence, we limit ourselves to the presence of only a single reacting species. Numerical evidence in Tables 5.1–5.3 suggests our analysis generalizes to our multiple-ligand model. Furthermore, the heart of our analysis relies on the $x^{1/3}$ spatial dependence in the perturbation expansion

\[
B(x, t) = 0^B(t) + x^{1/3} Da^1 B(t) + \mathcal{O}(Da^2), \tag{5.12}
\]

for $Da \ll 1$. After some algebra, one can write our expansion (4.15) as

\[
B(x, t) = 0^B(t) + x^{1/3} Da^1 B(t) + \mathcal{O}(Da^2). \tag{5.13}
\]

Thus, although time-dependent functions $0^B(t), 1^B(t)$ are significantly more complicated than $0^B(t), 1^B(t)$, the $x^{1/3}$ spatial dependence is the same.

The rest of this chapter is organized as follows. We derive estimates for convergence rates of $B$ and $B|_s$ in Subsection 5.2.1, and derive error estimates for the convergence rates of $\mathcal{B}$ and $\mathcal{B}|_s$ in Subsection 5.2.2. We present temporal convergence
results in Subsection 5.2.3. We tabulate our results for spatial convergence, as well as provide additional details about our simulations, in Subsection 5.2.4. In Section 5.3 we study the stability of our method.

### 5.2.1 Analysis of $B$

As noted, we provide error estimates for the bound state concentration in the presence of a single ligand $B$, not the multiple-ligand model. The IDE system (3.13a)–(3.13c), (4.12a), (4.12b) reduces to (4.48), and our numerical method (5.1a)–(5.1c), (5.4), (5.5) reduces to

\[
\frac{\partial B_{i,n+1}}{\partial t} = (1 - B_{i,n})C_{i,n+1} - KB_{i,n},
\]

(5.14a)

\[
\frac{B_{i,n+1}}{1} = 1 - \frac{Da}{\Gamma(\frac{2}{3})} \int_{0}^{x_i} \left\{ \frac{\partial B}{\partial t}(x_i - \nu, t_{n+1}) - \frac{\partial B_{i,n+1}}{\partial t} \right\} d\nu + 3x_i^{1/3} \frac{\partial B_{i,n+1}}{\partial t},
\]

(5.14b)

\[
B_{i,0} = 0.
\]

(5.14c)

We first study the spatial discretization error for $Da \ll 1$ and $Da = O(1)$. For numerical evidence in support of the estimates in this Subsection see Figures 5.2 and 5.3 at the end of this Subsection and Tables 5.1–5.3 in Subsection 5.2.4.

The only spatial discretization error we incur will be due to applying the trapezoidal rule to discretize (5.14b). To investigate the effect of the singularity in (5.14b) we will need an analytic form for $B$ when $x \ll 1$. One can show that

\[
B(x,t) = 0B(t) + x^{1/3}DaB(t) + O(x^{2/3}Da^2),
\]

(5.15)

for $x \ll 1$. Note, this is true even when $Da = O(1)$. Interestingly, one can also show (5.15) holds when $Da \ll 1$ by proposing an expansion of the form

\[
B(x,t) = 0B(t) + DaB(x,t) + O(Da^2)
\]
to solve (4.48). Thus we will use (5.15) for $Da \ll 1$ when needed. We first compute the dominant error contribution at the node $x_1 = \Delta x$:

$$
\text{err}(\Delta x) = \left| \frac{Da}{3^{1/3} \Gamma(2/3)} \int_0^{\Delta x} \left( \frac{\partial B}{\partial t}(\Delta x - \nu, t) - \frac{\partial B}{\partial t}(\Delta x, t) \right) \nu^{-2/3} d\nu \right|
$$

\begin{equation}
- \left( g(\Delta x) + g(0) \right) \Delta x,
\end{equation}

where

$$
g(\nu) = \frac{Da}{3^{1/3} \Gamma(2/3)} \left( \frac{\partial B}{\partial t}(\Delta x - \nu, t) - \frac{\partial B}{\partial t}(\Delta x, t) \right) \nu^{-2/3}.
\end{equation}

The function $g(\nu)$ is discontinuous at $\nu = 0$, but since $\lim_{\nu \to 0} g(\nu) = 0$, we take $g(0) = 0$. To justify this, we use (5.15) when $x \ll 1$ to calculate:

\begin{equation}
\lim_{\nu \to 0} \left[ \left( \frac{\partial B}{\partial t}(\Delta x - \nu, t) - \frac{\partial B}{\partial t}(\Delta x, t) \right) \nu^{-2/3} \times \right.
\end{equation}

\begin{equation}
\left. \left( \frac{\partial B}{\partial t}(t) + Da(\Delta x)^{1/3} \frac{\partial^1 B}{\partial t}(t) \right) \right] = 0,
\end{equation}

\begin{equation}
= \lim_{\nu \to 0} - Da \frac{(\Delta x^{1/3} - (\Delta x - \nu)^{1/3})}{v} \cdot \left( \nu^{1/3} \frac{\partial^1 B}{\partial t}(t) \right) + \ldots.
\end{equation}

We have omitted higher-order terms in our asymptotic expansion which will not contribute to the leading-order error. Therefore

$$
\lim_{\nu \to 0} \left( \frac{\partial B}{\partial t}(\Delta x - \nu, t) - \frac{\partial B}{\partial t}(\Delta x, t) \right) \nu^{-2/3} = \lim_{\nu \to 0} - \frac{Da}{3\Delta x^{2/3}} \cdot \left( \nu^{1/3} \frac{\partial^1 B}{\partial t}(t) \right) = 0,
$$

which implies $\lim_{\nu \to 0} g(\nu) = 0$. Moreover, this also formally justifies (5.6). To compute $g(\Delta x)$, we note that a calculation analogous to (5.18) gives

$$
g(\nu) = \frac{Da^2}{3^{1/3} \Gamma(2/3)} \left( \frac{\Delta x^{1/3} - (\Delta x - \nu)^{1/3}}{v^{2/3}} \right) \cdot \left( \frac{\partial^1 B}{\partial t}(t) \right) + \ldots,
$$

to leading-order; thus, $g(\Delta x) = O(Da^2 \Delta x^{-1/3})$. Using our values for $g(0)$ and $g(\Delta x)$ we find

$$
\frac{(g(0) + g(\Delta x))\Delta x}{2} = O(Da^2 \Delta x^{2/3}).
$$
The integral term in (5.16) is $O(Da^2 \Delta x^{2/3})$, which is seen using (5.15):

$$
\frac{Da}{3^{1/3} \Gamma(2/3)} \int_0^{\Delta x} \left( \frac{\partial B}{\partial t} (\Delta x - \nu, t) - \frac{\partial B}{\partial t} (\Delta x, t) \right) \nu^{-2/3} \, d\nu
\approx \frac{Da}{3^{1/3} \Gamma(2/3)} \int_0^{\Delta x} \left( (\partial^0 B)^{(t)} + (\Delta x - \nu)^{1/3} Da \frac{\partial^1 B}{\partial t} (t) + O(\nu^{2/3}) \right)
- \left( \frac{\partial^0 B}{\partial t} (t) + \Delta x^{1/3} Da \frac{\partial^1 B}{\partial t} (t) + O(\Delta x^{2/3}) \right) \nu^{-2/3} \, d\nu
= \frac{Da^2 \Delta x^{2/3}}{3^{1/3} \Gamma(2/3)} \left( \frac{\sqrt{\pi} \Gamma(1/3)}{2^{2/3} \Gamma(1 + 2/3)} - 3 \right) \frac{\partial^1 B}{\partial t} (t) + O(Da^3 \Delta x)
= O(Da^2 \Delta x^{2/3}).
$$

From (5.16), we see that at $x_1 = \Delta x$ we commit an $O(Da^2 \Delta x^{2/3})$ error, so the convergence of our algorithm is bounded above by $\text{err}(\Delta x) = O(Da^2 \Delta x^{2/3})$. Figure 5.2 supports this conclusion when $Da = .01 \ll 1$ and $Da = 1$.

We see from Tables 5.1–5.3 and Figure 5.2 when $Da = 10 \gg 1$ convergence is much slower. From the above computations we see that the largest term in (5.16) is $O(Da^2 \Delta x^{2/3})$, and when $Da = 10$ we have

$$
Da^2 = 100 > 8 \approx \Delta x_{\text{ref}}^{-1/3},
$$

where $\Delta x_{\text{ref}}^{1/3}$ denotes the value of $\Delta x$ for our reference solution. Thus, it follows that

$$
\Delta x^{1/3} = \Delta x^{-1/3} \Delta x^{2/3} < Da^2 \Delta x^{2/3},
$$

which implies that in this case we can achieve a rate of convergence no better that $O(\Delta x^{1/3})$. The data in Subsection 5.2.4, along with Figures 5.2 and 5.3, both support this conclusion. Fortunately, in practice the Damköhler number is no larger than $O(1)$, and this is not the quantity of physical relevance.

We now turn our attention to the discretization error which results from computing $B|_{\beta}$. Subsection 5.2.4 and Figures 5.2 and 5.3 indicate that we achieve a spatial
convergence rate of $\mathcal{O}(\Delta x^{4/3})$. We prove this when $Da \ll 1$. Letting $x \in [x_{\min}, x_{\max}]$, one may use (5.15) to show
\[
\frac{Da}{3^{1/3} \Gamma(2/3)} \int_0^x \left( \frac{\partial B}{\partial t} (x - \nu, t) - \frac{\partial B}{\partial t} (x, t) \right) \nu^{-2/3} \, d\nu
\]
\[
= \frac{Da^2}{3^{1/3} \Gamma(2/3)} \frac{\partial^1 B}{\partial t} (t) \int_0^x [(x - \nu)^{1/3} - x^{1/3}] \nu^{-2/3} \, d\nu + \cdots. \tag{5.19}
\]
This equation may be written as
\[
\frac{Da^2}{3^{1/3} \Gamma(2/3)} \frac{\partial^1 B}{\partial t} (t) \left\{ \int_0^{\Delta x} [(x - \nu)^{1/3} - x^{1/3}] \nu^{-2/3} \, d\nu + \int_{\Delta x}^x [(x - \nu)^{1/3} - x^{1/3}] \nu^{-2/3} \, d\nu \right\}.
\]
The singularity dominates the error, since one may approximate the term on the right to $\mathcal{O}(\Delta x^2)$ using the trapezoidal rule. Next, we expand $(x - \nu)^{1/3}$ about $\nu = 0$ to write the left term in the above equation as
\[
\int_0^{\Delta x} \left[ \left( x^{1/3} - \frac{\nu}{3x^{2/3}} + \cdots \right) - x^{1/3} \right] \nu^{-2/3} \, d\nu \approx - \int_0^{\Delta x} \frac{\nu^{1/3}}{3x^{2/3}} \, d\nu. \tag{5.20}
\]
The result will follow once we show that applying the trapezoidal rule to the right hand side of (5.20) results in an error of $\mathcal{O}(\Delta x^{4/3})$. We actually prove a slightly more general result, which we formulate as a theorem.

**Theorem 1.** Applying the trapezoidal rule to an integral of the form
\[
\int_0^x \nu^\beta \, d\nu, \quad \beta \in (0, 1), \tag{5.21}
\]
results in a convergence rate of $\mathcal{O}(\Delta x^{\beta+1})$.

**Proof.** Denoting the trapezoidal rule over $[a, b]$ as $\mathcal{T}(\cdot, [a, b])$, the error in approximating (5.21) is
\[
\text{err}(\Delta x) = \left| \int_0^x \nu^\beta \, d\nu - \mathcal{T}(\nu^\beta, [0, x]) \right| \tag{5.22}
\]
\[
= \left| \left( \int_0^{\Delta x} \nu^\beta \, d\nu - \mathcal{T}(\nu^\beta, [0, \Delta x]) \right) + \left( \int_{\Delta x}^1 \nu^\beta \, d\nu - \mathcal{T}(\nu^\beta, [\Delta x, 1]) \right) \right|. \tag{5.23}
\]
It is well known that the term on the right is $\mathcal{O}(\Delta x^2)$; we compute the term on the left:
\[
\left| \int_0^{\Delta x} \nu^\beta \, d\nu - \mathcal{T}(\nu^\beta, [0, \Delta x]) \right| = \left| \Delta x^{\beta+1} \frac{\beta + 1}{\beta + 1} - \frac{(\Delta x^\beta - 0) \Delta x}{2} \right|
\]
\[
= \left| \Delta x^{\beta+1} \left( \frac{1}{\beta + 1} - \frac{1}{2} \right) \right|.
\]
Therefore (5.22) is $O(\Delta x^{3+1})$, as desired.

This also completes our proof that the spatial discretization error when computing $B|_s$ is $O(\Delta x^{4/3})$, when $Da \ll 1$. The data in Subsection 5.2.4 supports this conclusion. Interestingly, the error is $O(\Delta x^{4/3})$ when $Da = O(1)$ and larger as well.

Figure 5.2: Convergence for $B_1$, $Da = .01, 1, 10$, from left to right.
5.2.2 Analysis of $\overline{B}_i$

The convergence of $\overline{B}$ will depend upon the averaging interval and the size of the Damköhler number. For numerical evidence in support of these estimates see Figures 5.5 and 5.6 at the end of this Subsection and Tables 5.1–5.3 in Subsection 5.2.4. The analysis for $\overline{B}$, $\overline{B}_{i | s}$ is more slightly involved, and we limit ourselves to the case that $\text{Da} \ll 1$. Previously the only source of spatial error was the convolution integral. However, since the averaged equation is given by

$$\frac{dB}{dt} = (1 - B) \left( 1 - \frac{\text{Da}}{3^{1/3} \Gamma(2/3)} \int_0^x \left( \frac{dB}{dt} (x - \nu, t) - \frac{dB}{dt} (x, t) \right) \nu^{-2/3} \, d\nu \right)$$

$$+ \frac{\text{Da}(1 - B)}{3^{1/3} \Gamma(2/3)} \cdot \left( 3x^{1/3} \frac{dB}{dt} (x, t) \right) - K \overline{B}_i,$$

$$\overline{B}_i(0) = 0,$$  \hspace{1cm} (5.24a)  

$$\overline{B}(0) = 0,$$  \hspace{1cm} (5.24b)

Figure 5.3: Convergence for $B_{1 | s}$, Da = .01, 1, 10, from left to right.
we will incur error from each of the terms:

\begin{align}
K\bar{B}, \\
\text{Da}x^{1/3}B\frac{\partial B}{\partial t}, \\
\text{Da}x^{1/3}\frac{\partial B}{\partial t}, \\
\frac{\text{Da}}{3^{1/3}\Gamma(2/3)}\int_0^2 \left( \frac{\partial B}{\partial t}(x - \nu, t) - \frac{\partial B}{\partial t}(x, t) \right) \nu^{-2/3} \, d\nu, \\
\frac{\text{Da}B}{3^{1/3}\Gamma(2/3)}\int_0^x \left( \frac{\partial B}{\partial t}(x - \nu, t) - \frac{\partial B}{\partial t}(x, t) \right) \nu^{-2/3} \, d\nu.
\end{align}

We must consider each one separately. The term $K\bar{B}$ will converge like $O(\text{Da}\Delta x^{4/3})$ when averaging over $[0, 1]$, and $O(\text{Da}\Delta x^2)$ otherwise. This can be seen by substituting (5.15) into (5.25), and appealing to Theorem 1 and the fact that $T(x^{1/3}, [x_{\min}, x_{\max}])$ converges at a rate of $\Delta x^2$. Notice this term grows linearly with the Damköhler number; we shall see the convolution integral grows quadratically with the Damköhler number, which plays a role in convergence.

The term (5.26) will converge at a rate of $O(\text{Da}\Delta x^{4/3})$ when averaging over the singularity, and $O(\text{Da}\Delta x^2)$ when averaging over the scanning range. To see this, observe that upon substituting (5.15) into (5.26), to leading order we have

\[
\text{Da}x^{1/3}\left(0B(t)\frac{\partial 0B}{\partial t}(t)\right).
\]

Again, applying Theorem 1 and using the fact that $T(x^{1/3}, [x_{\min}, x_{\max}])$ converges at a rate of $O(\Delta x^2)$ gives the desired result. The term (5.27) also has a convergence rate of $O(\text{Da}\Delta x^{4/3})$ when averaging over $[0, 1]$, and a convergence rate of $O(\text{Da}\Delta x^2)$ over $[x_{\min}, x_{\max}]$, for analogous reasons.

We now turn our attention to the term (5.28). We will first derive an error estimate when averaging over the whole interval, and then discuss the convergence results when averaging over the scanning range. Term (5.28) may be written fully as

\[
\frac{\text{Da}}{3^{1/3}\Gamma(2/3)}\int_0^1 \int_0^x \left( \frac{\partial B}{\partial t}(x - \nu, t) - \frac{\partial B}{\partial t}(x, t) \right) \nu^{-2/3} \, d\nu \, dx.
\]
Using (5.15), to leading order, this reduces to
\[
\frac{\text{Da}^2}{3^{1/3} \Gamma(2/3)} \frac{\partial^1 B}{\partial t}(t) \int_0^1 \int_0^x ((x - \nu)^{1/3} - x^{1/3}) \nu^{-2/3} \, d\nu \, dx,
\]
where we have omitted higher-order terms. We wish to compute the error incurred by approximating the above double integral using the trapezoidal rule. To this end we treat the inner integral as a function of \(x\), defining
\[
f(x) = \int_0^x ((x - \nu)^{1/3} - x^{1/3}) \nu^{-2/3} \, d\nu,
\]
making note of its closed form
\[
f(x) = \frac{x^{2/3}}{2} \left( \frac{2^{1/3} \sqrt{\pi} \Gamma\left(\frac{1}{3}\right)}{\Gamma\left(\frac{5}{6}\right)} - 6 \right).
\]
In Subsection 5.2.1 we saw if \(x \in [x_{\text{min}}, x_{\text{max}}]\), then the error incurred by approximating \(f\) by the trapezoidal rule is \(O(\Delta x^{4/3})\); this is true for any \(x_i \neq \Delta x\). In this case the error will be \(O(\Delta x^{2/3})\). To see this observe
\[
f(\Delta x) = \frac{1}{2} \Delta x^{2/3} \left( \frac{2^{1/3} \sqrt{\pi} \Gamma\left(\frac{1}{3}\right)}{\Gamma\left(\frac{5}{6}\right)} - 6 \right),
\]
\[
T(f(x), [0, \Delta x]) = -\frac{\Delta x^{2/3}}{2};
\]
hence the error in computing (5.31) using the trapezoidal rule at \(x = \Delta x\) is
\[
\text{err}(\Delta x) = |f(\Delta x) - T(f(x), [0, \Delta x])] = O(\Delta x^{2/3}).
\]
Now we apply the trapezoidal rule to the iterated integral (omitting the constants):
\[
\int_0^1 \int_0^x ((x - \nu)^{1/3} - x^{1/3}) \nu^{-2/3} \, d\nu \, dx
\]
\[
= \frac{\Delta x}{2} f(x_0) + \sum_{i=1}^{N-1} \Delta x f(x_i) + \frac{\Delta x}{2} f(x_N) + O(\Delta x^{5/3}).
\]
Typically, when applying the trapezoidal rule we get an error of \(O(\Delta x^2)\). However, Theorem 1 implies that applying the trapezoidal rule to (5.32) results in an error of \(O(\Delta x^{2/3+1}) = O(\Delta x^{5/3})\). If we were not approximating \(f\) with a quadrature rule,
then this would be the only error; however, we must consider the fact that we are actually approximating $f$ via a quadrature formula. We know that we commit an error of $O(\Delta x^{2/3})$ at $x_1 = \Delta x$ and an error on the order of $O(\Delta x^{4/3})$ everywhere else. We compute

$$
\int_0^1 \int_0^x \left( (x - \nu)^{1/3} - x^{1/3} \right) \nu^{-2/3} d\nu \, dx
$$

$$
= \frac{\Delta x}{2} T(f(x), [0, x_0]) + \sum_{i=1}^{N-1} \Delta x T(f(x), [0, x_i]) + \frac{\Delta x}{2} T(f(x), [0, x_N]) + O(\Delta x^{5/3}).
$$

The right hand side of the above is

$$
\frac{\Delta x}{2} (f(x_1) + O(\Delta x^{2/3})) + \sum_{i=2}^{N-1} \Delta x f(x_i) + O(\Delta x^{4/3}) + \frac{\Delta x}{2} (f(x_N) + O(\Delta x^{4/3}))
$$

$$
+ O(\Delta x^{5/3}),
$$

or

$$
\frac{\Delta x}{2} f(x_1) + O(\Delta x^{5/3}) + \sum_{i=2}^{N-1} \Delta x f(x_i) + O(\Delta x^{7/3}) + \left( \frac{\Delta x}{2} f(x_N) + O(\Delta x^{7/3}) \right)
$$

$$
+ O(\Delta x^{5/3}).
$$

In the above sum, there are $N = \Delta x^{-1}$ terms of order $O(\Delta x^{7/3})$. Thus, upon applying the trapezoidal rule twice, (5.30) is equal to

$$
\frac{\text{Da}^2}{3^{1/3} \Gamma(2/3)} \frac{\partial B_1}{\partial t}(t) \left( \frac{\Delta x}{2} f(x_1) + \sum_{n=1}^{N-1} \Delta x f(x_i) + \frac{\Delta x}{2} f(x_N) \right) + O(\text{Da}^2 \Delta x^{4/3})
$$

$$
+ O(\text{Da}^2 \Delta x^{5/3}).
$$

Thus the largest error associated with this term is $O(\text{Da}^2 \Delta x^{4/3})$. This argument may be modified to show that the convergence rate is also $O(\text{Da}^2 \Delta x^{4/3})$ if we average over $[x_{\text{min}}, x_{\text{max}}]$. If we are averaging over $[x_{\text{min}}, x_{\text{max}}]$, then instead of summing $N$ terms which are $O(\Delta x^{7/3})$, we will be summing approximately $.5N$ terms which are $O(\Delta x^{7/3})$. Since

$$
.5N \mathcal{O}(\Delta x^{7/3}) = \mathcal{O}(\Delta x^{4/3}),
$$

the above error estimate still holds if we are averaging over $[x_{\text{min}}, x_{\text{max}}]$. 

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We now study the nonlinear term, and first consider averaging over \([0, 1]\). By definition we may write (5.29) as

\[
\frac{\text{Da}}{3^{1/3} \Gamma(2/3)} \int_0^1 B(x, t) \int_0^x \left( \frac{\partial B}{\partial t}(x - \nu, t) - \frac{\partial B}{\partial t}(x, t) \right) \nu^{-2/3} \, d\nu \, dx.
\]

Upon using (5.15) and simplifying, we obtain

\[
\frac{\text{Da}}{3^{1/3} \Gamma(2/3)} \int_0^1 (0 B(t) + x^{1/3} \text{Da} B(t))^1 B(t) \int_0^x \left( (x - \nu)^{1/3} - x^{1/3} \right) \nu^{-2/3} \, d\nu + \cdots.
\]

We have omitted higher-order terms that won’t contribute to the dominant error. The convergence of the \(0 B(t)\) term will be identical to the convergence of the linear one. Thus, we focus our attention on the \(x^{1/3}(1 B(t))\) term

\[
\frac{(\text{Da}^2)(1 B(t))^2}{3^{1/3} \Gamma(2/3)} \int_0^1 x^{1/3} \int_0^x \left( (x - \nu)^{1/3} - x^{1/3} \right) \nu^{-2/3} \, d\nu \, dx.
\]

The closed form of the inner term is given as

\[
x^{1/3} f(x) = x^2 \left( \frac{2^{1/3} \sqrt{\pi} \Gamma(1/3)}{\Gamma(5/6)} - 6 \right), \tag{5.34}
\]

using (5.32). Because the trapezoidal rule integrates linear functions exactly, there will no longer be the \(O(\Delta x^{5/3})\) term. Instead we have

\[
\int_0^1 x^{1/3} \int_0^x \left( (x - \nu)^{1/3} - x^{1/3} \right) \nu^{-2/3} \, d\nu \, dx
\]

\[
= \frac{\Delta x}{2} x_0^{1/3} \mathcal{T}(f(x), [0, x_0]) + \sum_{i=1}^{N-1} \Delta x x_i^{1/3} \mathcal{T}(f(x), [0, x_i]) + \frac{\Delta x}{2} x_N^{1/3} \mathcal{T}(f(x), [0, x_N])
\]

\[
= \sum_{i=1}^{N-1} \Delta x x_i^{1/3} (f(x_i)) + \mathcal{O}(\Delta x^{4/3}) + \frac{\Delta x}{2} x_N^{1/3} (f(x_N)) + \mathcal{O}(\Delta x^{4/3}).
\]

To simplify the above sum, we can use (5.34) and the definition of the trapezoidal rule to write

\[
\sum_{i=1}^{N-1} \Delta x x_i^{1/3} f(x_i) + \frac{\Delta x}{2} x_N^{1/3} f(x_N)
\]

as

\[
\kappa \mathcal{T}(x, [0, 1]), \quad \kappa = \frac{1}{2} \left( \frac{2^{1/3} \sqrt{\pi} \Gamma \left( \frac{1}{3} \right)}{\Gamma \left( \frac{5}{6} \right)} - 6 \right).
\]
Also

\[ x_i^{1/3} \Delta x \mathcal{O}(\Delta x^{4/3}) = \mathcal{O}(\Delta x^{7/3}), \]

and the sum of all such terms is \( \mathcal{O}(\Delta x^{4/3}) \). Hence

\[
\int_0^1 x^{1/3} \int_0^x \left( (x - \nu)^{1/3} - x^{1/3} \right) \nu^{-2/3} \, d\nu \, dx = \kappa \Delta x \mathcal{T}(x, [0, 1]) + \mathcal{O}(\Delta x^{4/3}),
\]

so the error associated with (5.29) is \( \mathcal{O}(\Delta x^{4/3}) \). Again, this does not change if we average over the scanning range \([x_{\min}, x_{\max}]\).

The \( \mathcal{O}(\Delta x^{4/3}) \) error associated with both (5.28) and (5.29) explains the reduction in convergence in \( B_i \) seen in Tables 5.1–5.3 and Figures 5.2 and 5.3, when \( \text{Da} \) increases from small to moderate. When averaging over \([x_{\min}, x_{\max}]\) there are two competing sources of error in (5.24a): one of \( \mathcal{O}(\Delta x^2) \) (from terms (5.25)–(5.27)), and one of \( \mathcal{O}(\Delta x^{4/3}) \) (from terms (5.28)–(5.29)). When \( \Delta x^{4/3} < \text{Da} \Delta x^2 \), or \( \text{Da} < \Delta x^{2/3} \), the former is larger. Conversely, when \( \Delta x^{2/3} < \text{Da} \) the latter is larger. Below is a series of simulations we ran for the single ligand case for different Damköhler numbers.

Figure 5.4: \( \text{Da} = .001 \) to 150, \( \text{K} = 1 \), Ref Sol: \( N = 200 \), Trial Solution: \( N = 20 \).

To generate Figure 5.4, we increased \( \text{Da} \) from \( \text{Da} = .001 \) to \( \text{Da} = 150 \), and computed two solutions for each value of \( \text{Da} \): a reference solution with \( N = 200 \) nodes in space, and another trial solution with \( N = 20 \). We computed the absolute difference between the two, and created the logarithmic plot in Figure 5.4.
As one can see, when the Damköhler number is small, the error starts off small and increases linearly due to contributions from (5.25)–(5.27). As the Damköhler number increases, the $O(Da^2\Delta x^{4/3})$ contributions form (5.28) and (5.29) kick in, and cause the error to increase quadratically. Note, the slope changes at approximately

$$\log(Da) \approx -2 \approx \log(\Delta x^{2/3}) = \log \left( \frac{1}{20^{2/3}} \right),$$

which agrees with our discussion in the above paragraph. The error then asymptotes for large Damköhler number. As discussed in Section 3.3 the correct time scale for the bound state system is the $t_w = \frac{t}{Da}$ time scale (also see [6]). For surface reaction kinetics involving only a single ligand, this transforms (5.24a) into

$$Da^{-1} \frac{dB}{dt_w} = (1 - B) \left( 1 - \frac{1}{3^{1/3} \Gamma(2/3)} \int_0^x \left( \frac{\partial B}{\partial t_w}(x - \nu, t_w) - \frac{\partial B}{\partial t_w}(x, t_w) \right) \nu^{-2/3} d\nu \right)$$

$$+ \frac{(1 - B)}{3^{1/3} \Gamma(2/3)} \left( 3x^{1/3} \frac{\partial B}{\partial t_w}(x, t_w) \right) - KB,$$

$B(0) = 0.$

When $Da \gg 1$, the above equation is nonlinear to leading order and hopeless to solve in closed form. Although we do not have an analytic expression for $B$ for $Da \gg 1$, there is nothing about the above equation that suggests the error should continue to grow quadratically. The fact that $Da$ is not a coefficient of the convolution integral in the above equation, along with our numerical evidence in Figure 5.4, gives us confidence that $Da$ has a negligible effect on the error in this parameter regime.
Figure 5.5: Convergence for $B_1$, $Da = .01, 1, 10$, from left to right.
Figure 5.6: Convergence for $B_1|_s$, Da = .01, 1, 10.

5.2.3 Temporal Convergence Results

Our results for temporal convergence are depicted below. As expected, the results below indicate that we are achieving second-order convergence in time.
Figure 5.7: Temporal convergence: $Da = .01$

### 5.2.4 Convergence Results and Details

Our numerical results for the rates of convergence for $B_i$, $B_i|_s$, $\overline{B}_i$, and $\overline{B}_i|_s$ are tabulated below.

<table>
<thead>
<tr>
<th></th>
<th>$Da \ll 1$</th>
<th>$Da = O(1)$</th>
<th>$Da \gg 1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B_1$</td>
<td>$O(\Delta x^{71})$</td>
<td>$O(\Delta x^{67})$</td>
<td>$O(\Delta x^{34})$</td>
</tr>
<tr>
<td>$B_1</td>
<td>_s$</td>
<td>$O(\Delta x^{1.32})$</td>
<td>$O(\Delta x^{1.38})$</td>
</tr>
<tr>
<td>$\overline{B}_1$</td>
<td>$O(\Delta x^{1.36})$</td>
<td>$O(\Delta x^{1.39})$</td>
<td>$O(\Delta x^{1.07})$</td>
</tr>
<tr>
<td>$\overline{B}_1</td>
<td>_s$</td>
<td>$O(\Delta x^{2.09})$</td>
<td>$O(\Delta x^{1.33})$</td>
</tr>
</tbody>
</table>

Table 5.1: Convergence results for $B_1$. Here $Da = .01, 1, 10$
We now provide the details on how we computed these results. All of our results were generated during the injection phase, although similar results hold for the wash phase as well. When measuring spatial error we computed our reference solution on a mesh with $\Delta x = \Delta t = \frac{1}{512}$. The temporal error of $O(\Delta t^2)$ is always on the same order of or smaller than the spatial error. Thus there is no problem taking $\Delta x = \Delta t$ to compute our reference solution. To measure convergence, we then computed solutions on grids of size $\Delta x = \frac{1}{2^j}$, for $1 < j < 7$, keeping $\Delta t = \frac{1}{512}$ constant throughout all of our simulations. To measure the convergence rates of $B_i$ and $B_i|_s$, we project our reference solution onto the others and measure pointwise error at each time step. We can then compute the maximum error at each time step, and then take the maximum over all of our time steps. To measure convergence rates of $\overline{B}_i$ and $\overline{B}_i|_s$, we can simply compare values with our reference solution at each time step, and take the maximum error over all time.
When computing $B_i|s$ we took $x_{\text{min}} = .25$ and $x_{\text{max}} = .75$. This is because solutions successively computed on meshes of width $\Delta x = \frac{1}{2^i}$, with $2 < i < 8$, will always contain these points. Thus there is no problem in comparing $B_i|s$ with our reference solution, because in both cases we are averaging over $[.25,.75]$. This would not be possible if our averaging points were $x_{\text{min}} = .2$ and $x_{\text{max}} = .8$, for a mesh of width $\Delta x = \frac{1}{2^i}$ may not contain these points. For example, a mesh of width $\Delta x = \frac{1}{2^3}$ does not contain the points $x_{\text{min}} = .2$ and $x_{\text{max}} = .8$. Since this mesh does not contain $x_{\text{min}} = .2$ and $x_{\text{max}} = .8$, we are unable to compute $B|s$ and compare this solution to our reference solution.

On the other hand, meshes of width $\Delta x = \frac{1}{2^i}$ will always contain the points $x_{\text{min}} = .25$ and $x_{\text{max}} = .75$. Thus when measuring convergence in this chapter we have taken $x_{\text{min}} = .25$ and $x_{\text{max}} = .75$. This also makes projecting our reference solution onto the others simple. It should be noted that in every other chapter, we have taken $x_{\text{min}} = .2$ and $x_{\text{max}} = .8$. These choices are closer to that in the existing literature [12].

We measured temporal convergence in a similar manner to spatial convergence. We generated a reference solution with $\Delta t = \Delta x = \frac{1}{512}$, and computed other solutions on grids of size $\Delta t = \frac{1}{2^i}$, for $i = 3,\ldots,8$. We kept $\Delta x = \frac{1}{512}$ fixed throughout our simulations. In addition, we measured $B_i|s$ to compute the temporal rate of convergence. In addition, to generate our convergence results we took $K_1 = 1$, $2K_a = 1$, $2K_d = 1$, $\frac{2}{3}K_a = 1/2$, $\frac{2}{3}K_d = 2$, $\frac{1}{2}K_a = 2$, and $\frac{1}{2}K_d = 1/2$; see Figure 5.8 for a visualization of this. Observe that in Figure 5.8 we are using the scaled rate constants as in (3.7); hence the rate constant $K_a$ has dropped out in the scaling. These rate constants were used in measuring both spatial and temporal convergence. The eigenvalues for the matrix $A$ are $\lambda_1 \approx 4.5$, $\lambda_2 \approx 2.25 + .66i$, $\lambda_3 \approx 2.25 - .66i$; thus there are no repeated eigenvalues, and $\text{Re}(\lambda_i) > 0$ as expected. Further, $EL_2$ will tend to form through the second pathway (P$_2$).
5.3 Stability Analysis

It is of practical interest for us to investigate the absolute stability of our method; that is, we seek to determine when errors accrued in one time step will be magnified in the next. As in Section 5.2, we limit ourselves to the presence of a single ligand.

As outlined in Subsection 5.1, we start upstream and march downstream at each time step. Thus if there are instabilities when computing $B$ at the boundary $B_{0,j}$ for any time step $j$, these instabilities will flow downstream, and will be magnified at the node $B_{0,j+1}$. Thus (5.14a) is stable only if our time stepping algorithm for solving

$$\frac{dB}{dt} = (1 - B) - KB$$  \hspace{1cm} (5.35)

is stable. Thus we study the stability of (5.35) when computed via second-order Adams-Bashforth:

$$B_{n+1} = B_n + \frac{\Delta t}{2}(3dB_n - dB_{n-1}).$$  \hspace{1cm} (5.36)

Here $B_n$ denotes the approximated value of $B(t_n)$. The value $B(t_{n+1})$ will satisfy the above equation up to some truncation error $\tau_n$:

$$B(t_{n+1}) = B(t_n) + \frac{\Delta t}{2}(3B'(t_n) - B'(t_{n-1})) + \tau_n.$$  \hspace{1cm} (5.37)
By substituting the definition of $B'$ (5.35) into (5.36) and (5.37), and then taking the difference of the resulting equations, one can derive a recurrence relation for the error $E_n$ at each time step:

$$E_{n+1} = \left(1 - \frac{3}{2} \alpha \Delta t\right) E_n + z \frac{\alpha \Delta t}{2} E_{n-1} + \Delta t + \tau_n,$$

(5.38)

where $\alpha$ is as in (4.51). Letting $z = \alpha \Delta t$, we write the associated homogenous recurrence relation as

$$E_{n+1} = \left(1 - \frac{3z}{2}\right) E_n + \frac{z}{2} E_{n-1},$$

(5.39)

whose solution is

$$E_n = c_1 4^{-n} \left(2 - 3z - \sqrt{4 - 4z + 9z^2}\right)^n + c_2 4^{-n} \left(2 - 3z + \sqrt{4 - 4z + 9z^2}\right)^n,$$

(5.40)

for constants $c_1, c_2$ that will depend upon the initial data and the particular solution of (5.39). Thus our algorithm will be stable only if

$$\left|\left(2 - 3z \pm \sqrt{4 - 4z + 9z^2}\right)\right| \leq 4,$$

(5.41)

otherwise there will be exponential growth in our errors. The region of stability in the complex plane is plotted below.

Figure 5.9: Stability region of (5.35) with an AB2.

Using the fact that $\alpha \Delta t \in \mathbb{R}$ and (5.41) we may determine that our algorithm will produce physically meaningful results only if

$$|\alpha \Delta t| \leq 1.$$
To test this hypothesis we run two simulations with $\Delta t = \frac{1}{25}$: with $\alpha = 23$, and another with $\alpha = 26$. Plotted below is a figure of $B|_s$ when $\alpha = 23$.

![Figure 5.10: Solution converging when $\alpha \Delta t < 1$.](image)

When $\alpha = 23$, we have $\alpha \Delta t = .92$, and although the error is large, the solution does not blow up. The error dampens out as we march forward in time, just as we expect from (5.40). However, when $\alpha = 26$ we have $\alpha \Delta t = 1.08$. In this case errors grow exponentially from one time step to the next, and the solution blows up, reaching a maximum value on the order of $10^{153}$.

![Figure 5.11: $B|_s$ blowing up when $\alpha \Delta t > 1$.](image)

Although we have discussed stability only for the bimolecular case, this approach could be generalized to our multiple-component system. Since we are starting upstream and working our way downstream, error incurred when computing $B_{0,n}$ will affect the
accuracy of $B_{i,n}$. Therefore our method (5.8) will be stable only if a second order Adams-Bashforth applied to

$$\frac{dB}{dt} = -AB + f \quad B(0) = 0,$$

is stable, and this depends on the eigenvalues of the matrix $A$ (defined as in (4.17)). The eigenvalues of this $3 \times 3$ symbolic matrix are very unwieldy, however they could provide insight into the stability of our method; future work could include finding rigorous bounds on these eigenvalues. Moreover, eigenvalues given in Subsection 5.2.4 correspond to a representative set of rate constants for our simulations, and we do not suspect that stability was a concern in generating any of our results. Even if it were found that certain physically relevant combinations of rate constants rendered our system stiff, then we could make our method fully implicit, and use a Backwards Differentiation Formula in tandem with a Newton’s method to solve the resulting nonlinear system at each time step.
Chapter 6

ERC VERIFICATION

We now show the ERC equations provide an accurate approximation to the sensogram average. Below in Figures 6.1 and 6.2 are plots of the absolute error between the solution to the ERC equations and the averaged exact solution (as computed by our FD scheme) for all time, when Da = .45. In addition, we have also depicted $B_i(x,t)$, as given by our finite difference algorithm in Figures 6.3 and 6.4. All simulations in this chapter were run until steady state.

![Graphs showing error comparison between ERC equations and exact solution](image)

Figure 6.1: Injection phase, Da = .45. Notice the sign change in the error at approximately $t = 2$ seconds in $B_{12}|s$. Similar sign changes may be noted in the other reacting species as well.
As one can see the ERC equations serve as an excellent approximation, although the Damköhler number is moderate. In generating the above figures we needed to tighten up the default tolerences in ODE45 (in particular the relative error), and use the mass matrix command. Failure to do so results in non-physical oscillations.
Figure 6.3: Injection phase, all rate constant taken equal to one, and $Da = 2$. Notice the upstream ligand depletion effect near $x = 0$ in each of the reacting species; this is especially evident in $B_{12}$.

Figure 6.4: Injection phase together with wash phase, for $B_1$. All rate constants taken equal to one, and $Da = 2$. Notice the ligand rebinding effect during the wash phase. Ligand molecules dissociating upstream rebind to receptor sites further downstream.
We now ask: can we extend this approximation, which is formally valid only for small Damköhler number, to large Damköhler number? Edwards and Jackson have found similar results in the bimolecular case [12]. To test our hypothesis, we computed the maximum absolute error over all time for various Damköhler numbers, and obtained the following results.

Figure 6.5: Injection phase: Absolute error over all time for various Da.

Evidently our ERC equations do not only do well for small Damköhler, but also do well for large Damköhler as well. We see that for Da ≪ 1 the error is small, as expected. Then the error grows at rates of p = 1.92, p = 1.88, and 1.85. This agrees favorably with the theoretical $O(Da^2)$ and previous results [12]. The error then reaches an asymptote corresponding roughly to a two percent absolute error, which is good considering that there will be error in any laboratory experiment. As the Damköhler number increases, we gradually transition to the transport-limited and reaction-dominant parameter regime. Because transport is slow and reaction is fast, ligand molecules bind with receptor sites near the beginning of the channel, which...
results in a lack of unbound ligand available for binding downstream.

Similar results hold for the wash phase. One can see that the error starts off small, and then reaches an asymptote corresponding to approximately two percent absolute error. The error increases at a rate that compares favorably with our theoretical predictions. This time, as reaction gets faster relative to transport, there is a higher probability of a ligand molecule rebinding downstream. As the Damköhler number gets very large, transport becomes inefficient, and ligand molecules rebind quickly.

For both the injection and wash phase of the experiment, we used the values $1K_d = 1/2, 2K_a = 1, _2^2K_d = 1, _1^2K_a = 1, _2^2K_d = 2, _1^2K_a = 2$, and $1K_d = 1/2$. This is the same as the injection phase; see Figure 6.7 for a visualization of the dominant pathways. We remark that in generating the above figures, it was necessary to use the mass-matrix command in MATLAB for solving the ERC equation, and to tighten up the ODE tolerances. We also had to reduce the relative error in our finite difference

Figure 6.6: Wash phase: Absolute error over all time for various Da.

For both the injection and wash phase of the experiment, we used the values $1K_d = 1/2, 2K_a = 1, _2^2K_d = 1, _1^2K_a = 1, _2^2K_d = 2, _1^2K_a = 2$, and $1K_d = 1/2$. This is the same as the injection phase; see Figure 6.7 for a visualization of the dominant pathways. We remark that in generating the above figures, it was necessary to use the mass-matrix command in MATLAB for solving the ERC equation, and to tighten up the ODE tolerances. We also had to reduce the relative error in our finite difference
algorithm, which we did by taking time steps of size $\Delta t = \frac{\Delta x}{6} = \left(\frac{1}{6}\right)\left(\frac{1}{100}\right)$ in order to drive the relative error down.

In addition, upon inspecting the error for $B_1$ in Figure 6.1 and the error for $B_{12}$ and $B_2$ in Figure 6.2, we see that the error reaches a maximum very quickly after injection begins. This is also indicative of fact that the relative error is higher at the beginning of our numerical simulation, a problem that could potentially be resolved by implementing an adaptive time-stepping procedure.

![Figure 6.7: Pathway visualization for simulations.](image-url)
In this chapter we present a set of ERC equations for the multiple-receptor model. As a first step towards deriving the ERC equations, we first need the governing equations for the multiple-receptor model in the unstirred layer. We consider the bound state system in the reaction-limited and transport-dominant parameter regime, i.e., when \( Da \ll 1 \). Thus following Section 3.2, we introduce the scaling:

\[
t = f_k a t_c
\]  

(7.1)

to transform (2.29a) and (2.29b) into

\[
\frac{\partial B_f}{\partial t} = (R_f - B_f)C(x,0,t) - t K_d B_f,
\]

(7.2a)

\[
\frac{\partial B_p}{\partial t} = p K_a (R_p - B_p)C(x,0,t) - p K_d B_p.
\]

(7.2b)

Notice, this time we are scaling by the rate constant \( f_k a \), rather than \( 1/k_a \). As discussed in Section 3.2, this is the correct time scale for reaction when \( Da \ll 1 \). In introducing the \( t \) time scale we have scaled the rate constants:

\[
t K_d = \frac{f k_d}{f k_a}, \quad p K_a = \frac{p k_a}{p k_a}, \quad p K_d = \frac{p k_d}{p k_a}.
\]

It remains to relate \( C(x,0,t) \) to the bound state concentrations \( B_f \) and \( B_p \). Using an argument analogous to the one given in Section 4.1, one may show:

\[
C(x,0,t) = 1 - \frac{Da}{3^{1/3} \Gamma(2/3)} \int_0^x \left( \frac{\partial B_f}{\partial t} + \frac{\partial B_p}{\partial t} \right) \frac{d\nu}{(x - \nu)^{2/3}},
\]

(7.3)

\[
C(x,0,t) = -\frac{Da}{3^{1/3} \Gamma(2/3)} \int_0^x \left( \frac{\partial B_f}{\partial t} + \frac{\partial B_p}{\partial t} \right) \frac{d\nu}{(x - \nu)^{2/3}}.
\]

(7.4)
for the injection and wash phases respectively. Equations (7.3) and (7.4) have analogous physical interpretations to the ones given in Section 4.1 and Subsection 4.2.2. Given the governing equations, we can write the ERC equation for the injection phase as

\[
\frac{d\mathbf{B}}{dt} = M^{-1}(\mathbf{B})(-A\mathbf{B} + \mathbf{f}) + \mathcal{O}(Da^2),
\]  

(7.5)

where

\[
M = I + DaN(\mathbf{B}),
\]

\[
N(\mathbf{B}) = \bar{h} \begin{pmatrix}
(R_f - \mathbf{B}_f) & (R_f - \mathbf{B}_f) \\
(pK_a(R_p - \mathbf{B}_p)) & pK_a(R_p - \mathbf{B}_p)
\end{pmatrix},
\]

\[
A = \begin{pmatrix}
(1 + fK_d) & 0 \\
0 & (pK_a + pK_d)
\end{pmatrix},
\]

\[
f = \begin{pmatrix}
R_f \\
pK_aR_p
\end{pmatrix}.
\]

The initial conditions are given by \(\mathbf{B}(0) = 0\). The ERC equations for the wash phase are given by

\[
\frac{d\mathbf{B}}{dt} = -M^{-1}(\mathbf{B})D\mathbf{B} + \mathcal{O}(Da^2),
\]  

(7.6)

where

\[
D = \begin{pmatrix}
(K_d) & 0 \\
0 & pK_d
\end{pmatrix},
\]

and \(M, N\) are as before. The initial conditions are given by

\[
\mathbf{B}_f(0) = \frac{R_f}{1 + fK_d},
\]

\[
\mathbf{B}_p(0) = \frac{(R_p)pK_a}{pK_a + pK_d},
\]
which we obtained from (2.32a) and (2.32b). To test the accuracy of our ERC equations we employ a semi-implicit finite difference algorithm as in Chapter 5; during the injection phase our method takes the form

\[
\frac{\partial B_{f,i,n+1}}{\partial t} = (R_i - B_{f,i,n})C_{i,n+1} - K_d B_{f,i,n}, \tag{7.7a}
\]

\[
\frac{\partial B_{p,i,n+1}}{\partial t} = p K_a (R_p - B_{p,i,n})C_{i,n+1} - p K_d B_{p,i,n}, \tag{7.7b}
\]

and

\[
C_{i,n+1} = 1 - \frac{Da}{3^{1/3} \Gamma(\frac{2}{3})} \int_0^{x_i} \left\{ \frac{\partial B_{f}}{\partial t}(x_i - \nu, t_{n+1}) + \frac{\partial B_{p}}{\partial t}(x_i - \nu, t_{n+1}) \right\} \frac{d\nu}{\nu^{2/3}}, \tag{7.8}
\]

\[
C_{i,n+1} = -\frac{Da}{3^{1/3} \Gamma(\frac{2}{3})} \int_0^{x_i} \left\{ \frac{\partial B_{f}}{\partial t}(x_i - \nu, t_{n+1}) + \frac{\partial B_{p}}{\partial t}(x_i - \nu, t_{n+1}) \right\} \frac{d\nu}{\nu^{2/3}} \tag{7.9}
\]
during the injection and wash phases respectively. Our discretization and time stepping procedures are the same as given in Chapter 5; for the details see Appendix B. Below, we have depicted a comparison of our ERC approximation with our numerical approximation as computed by our semi-implicit finite difference method.

![Figure 7.1: Absolute error of ERC approximation for injection phase, Da = .45.](image)

As in Chapter 6, although Da = .45 is by no means moderate, our ERC approximation matches up very well with our numerical results. We also investigated the accuracy of our ERC equations for a range of Da; below are our results.
Again, as with Chapter 6, we see that the error starts off small, increases at a rate which compares favorably to our theoretical predictions, and then reaches an asymptote corresponding to a small absolute error. Upon inspecting Figures 7.1 and 7.2, one may notice that in both cases, the absolute error seems to be slightly larger for $B_p$. Due to the relatively small number of free DNA receptors, $B_p$ is an order of magnitude larger than $B_f$; this is seen in the figure below.

Thus, through our ERC equations, we have found a simple, quick, and accurate approximation to the bound state concentration for the multiple-receptor experiment. We note that in generating these results we took $fK_d = 1, pK_a = 2, pK_d = \frac{1}{2}$.
Chapter 8

HOMOTOPY PERTURBATION METHOD APPROXIMATION

Deriving our ERC equations in Chapters 4 and 7 relied on the assumption that we were operating in the reaction-limited and transport-dominant parameter regime. Although these equations are formally valid when $\text{Da} \ll 1$, their solutions compare very favorably to our numerical simulations for moderate and large $\text{Da}$ as well. Furthermore, our ERC equations have the advantage of being simple, and give a tool to for estimating rate constants from raw data.

Given these results, a natural question arises: can we derive an accurate expression for $B$ that also holds for $\text{Da} = \mathcal{O}(1)$ and $\text{Da} \gg 1$? When $\text{Da} = \mathcal{O}(1)$, equation (4.48) has $\mathcal{O}(1)$ nonlinearities, and is hopeless to solve in closed form. In addition, Edwards has shown [6] that when $\text{Da} \gg 1$, the bound state $B$ evolves on the $t_w = \frac{1}{\text{Da}}$ time scale (one may also see Section 3.3). On this time scale (4.48) takes the form

$$\text{Da}^{-1} \frac{\partial B}{\partial t_w} = (1 - B) \left(1 - \frac{1}{3^{1/3} \Gamma(2/3)} \int_0^x \frac{\partial B}{\partial t_w} (x - \nu)^{2/3} \frac{d\nu}{(x - \nu)^{2/3}}\right) - KB, \quad (8.1a)$$

$$B(x, 0) = 0. \quad (8.1b)$$

Proposing an expansion of the form

$$B(x, t_w) = 0^B(x, t_w) + (\text{Da}^{-1})^1 B(x, t_w) + \mathcal{O}(\text{Da}^{-2})$$

results in the leading order equation

$$0 = (1 - 0^B) \left(1 - \frac{1}{3^{1/3} \Gamma(2/3)} \int_0^x \frac{\partial 0^B}{\partial t_w} (x - \nu)^{2/3} \frac{d\nu}{(x - \nu)^{2/3}}\right) - K 0^B,$$

which is again unwieldy and hopeless to solve in closed form.

Since $\mathcal{B}$ is the quantity of physical relevance, in what follows we search for an analytic approximation for $\mathcal{B}$ which holds for a wide range of $\text{Da}$. One may think of three possible approaches.
Approach 1. Find an approximation to

\[
\frac{d\bar{B}}{dt} + \alpha \bar{B} = 1 - (1 - B) \frac{Da}{3^{1/3} \Gamma(2/3)} \int_0^x \frac{\partial \bar{B}}{\partial t} (x - \nu)^{-2/3} \, d\nu, \tag{8.2a}
\]

\[
\bar{B}(0) = 0. \tag{8.2b}
\]

Approach 2. Directly find an approximation to (4.48), and average the result.

Approach 3. Find an approximation to the bimolecular ERC equations, given by equation (4.55) or (4.56).

The first approach actually complicates the problem by introducing two dependent variables into the problem, \(B\) and \(\bar{B}\). In order for this approach to work we would need another equation, and we will not consider this approach any further. The second approach may be the first to come to mind, but it is also the most difficult. The spatial dependence, different time scales for reaction and transport, and \(O(1)\) nonlinearities on the \(t_w\) time scale make the problem of finding a single approximation that works for all parameter regimes a formidable one.

On the other hand, we have seen that the ERC approximation retains of a high degree accuracy for both our multiple ligand model and the bimolecular model [12]). Thus, to find an approximate expression for \(\bar{B}\), we will adopt the third approach by finding an analytic expression for the solution of the bimolecular ERC equation (4.56). However, when \(Da = O(1)\) or larger there are no small parameters in the problem, and traditional perturbation methods fail.

Thus, our approach will be based on the Homotopy Perturbation Method (HPM)—a method which doesn’t rely on the existence of a small parameter in the equation of interest. Motivated by topology, this method was first developed in 1999 by Ji-Huan He [14]. This technique has been applied to a number of problems such as: the KdV equation [20], Helmholtz equation [20], Blasius boundary layer flow [1], Ricatti equations of both integer [25] and fractional order [18], and the Van der Pol Oscillator [26]. Many more examples could be cited.
In this chapter, we couple the Homotopy Perturbation Method with a traditional strained coordinates technique to find an analytic expression for $\mathcal{B}$. We limit ourselves to the bimolecular case (4.48). Through this coupling, we will be able to find an approximate expression for $\mathcal{B}$, which is accurate regardless of the size of Da. The rest of this chapter is organized as follows: in Section 8.1, we outline the Homotopy Perturbation Method; in Subsection 8.2.1, we find an approximate expression for $\mathcal{B}$ during the injection phase of the experiment; and in Subsection 8.2.2 we find an approximate expression for $\mathcal{B}$ during the wash phase.

8.1 Homotopy Perturbation Method

A homotopy is a continuous deformation of one curve into another. Consider two real valued functions $f$ and $g$. We may construct a convex homotopy between $f$ and $g$ by setting

$$
\mathcal{H}(x;p) = (1 - p)f + pg, \quad p \in [0, 1].
$$

By varying $p$ from 0 to 1, we may continuously deform $f$ into $g$. We apply this idea to differential operators, by considering the equation

$$
\mathcal{A}(B) = f,
$$

which we will write as

$$
\mathcal{A}(B) - f = 0. \quad (8.3)
$$

First observe that we may resolve the differential operator $\mathcal{A}$ into its linear and non-linear components $\mathcal{L}, \mathcal{N}$ respectively:

$$
\mathcal{A}(B) = \mathcal{L}(B) + \mathcal{N}(B).
$$

We can construct a homotopy between $\mathcal{L}$ and $\mathcal{A} - f$ by setting

$$
\mathcal{H}(B;p) := (1 - p)(\mathcal{L}(B) - \mathcal{L}(0)) + p(\mathcal{A}(B) - f), \quad (8.4)
$$

$$
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$$
where $^0b$ is an initial guess that satisfies the initial and boundary data. By varying $p$ from 0 to 1, we continuously deform the linearized operator into the full nonlinear left hand side of (8.3). We then propose a series solution to

$$\mathcal{H}(B; p) = 0$$

(8.5)

of the form

$$B(x, t; p) = ^0B(x, t) + (p)^1 B(x, t) + (p^2)^2 B(x, t) + \cdots.$$  

(8.6)

Since $B(x, t; p)$ solves (8.5), we have

$$(1 - p)(\mathcal{L}(B(x, t; p)) - \mathcal{L}(^0b)) + p(\mathcal{A}(B(x, t; p) - f) = 0$$

$$\Rightarrow \lim_{p \to 1}(1 - p)(\mathcal{L}(B(x, t; p)) - \mathcal{L}(^0b)) + p(\mathcal{A}(B(x, t; p) - f) = 0$$

$$\Rightarrow \lim_{p \to 1} \mathcal{A}(B(x, t; p) - f = 0$$

$$\Rightarrow \mathcal{A}(B(x, t; 1)) = f,$$

provided $\lim_{p \to 1} \mathcal{L}(B(x, t; p))$ and $\lim_{p \to 1} \mathcal{A}(B(x, t; p))$ exist, and we can interchange the limiting operation. Although it may not be the case that $\lim_{p \to 1} \mathcal{A}(B(x, t; p) = \mathcal{A}(\lim_{p \to 1} B(x, t; p))$, this does not concern us, since finding an accurate approximation is our goal—not finding an exact solution. Thus, to obtain our approximation we retain the first few terms of (8.6) and let $p$ approach 1, to give

$$B(x, t; 1) = ^0B + ^1B + ^2B + \cdots.$$  

(8.7)

However, there is no guarantee that the above series converges. Thus following [17], instead of defining our homotopy in terms of (8.5), we introduce a parameter $q$, referred to as the convergence-control parameter. Rather than using (8.5), we define our homotopy as

$$\mathcal{H}(B; p; q) := (1 - p)(\mathcal{L}(B) - \mathcal{L}(^0b)) + qp(\mathcal{A}(B) - f).$$

(8.8)

Hence, (8.7) will always be a function of our parameter $q$; that is

$$B(x, t; 1; q) = ^0B(x, t) + ^1B(x, t; q) + ^2B(x, t; q) + \cdots.$$  

(8.9)
To choose \( q \), we numerically minimize

\[
R(q) := \| A(B) - f \|_2^2 = \int_{\Omega} (A(B) - f)^2 \, dx \, dt. \quad (8.10)
\]

Here \( \Omega \) denotes the region in which our approximation \( B \) holds. Clearly the smaller \( (8.10) \) is, the closer \( (8.9) \) will be to the exact solution. It may seem inconsistent that we are numerically minimizing \( (8.10) \). We first remark that, in our specific case,

\[
\frac{dR}{dq} = 0 \quad (8.11)
\]

is a transcendental equation which does not have an algebraic solution. Additionally, the solution to \( (8.10) \) is not the only value of \( q \) which renders \( (8.9) \) convergent. If so desired, we could use bisection to analytically approximate the solution to \( (8.11) \), to the desired degree of accuracy. For convenience we used \texttt{NMinimize[]} in Mathematica.

### 8.2 Perturbation Approximation

#### 8.2.1 Injection Phase

We now recall the bimolecular ERC equation (4.55). Omitting the \( \mathcal{O}(\text{Da}^2) \) terms this equation may be written as:

\[
\frac{dB}{dt} = (1 - B) \left( 1 - \bar{h} \text{Da} \frac{dB}{dt} \right) - KB
\]

\[
B(0) = 0,
\]

\[
h(x) = \frac{3^{2/3} x^{1/3}}{\Gamma(2/3)},
\]

\[
\bar{h} = \frac{1}{x_{\text{max}} - x_{\text{min}}} \int_{x_{\text{min}}}^{x_{\text{max}}} h(x) \, dx = \frac{3^{5/3} \left( x_{\text{max}}^{4/3} - x_{\text{min}}^{4/3} \right)}{4 \Gamma(2/3) (x_{\text{max}} - x_{\text{min}})},
\]

where \( h \) is defined as in (4.26). In order to a construct our homotopy we must choose \( ^0b, \mathcal{L}, \mathcal{N}, \) and \( f \). We choose \( ^0b \) to be the function which satisfies the initial condition \( B(x,0) = 0 \) and the concentration at the boundary \( x = 0 \), given by taking \( x = 0 \) in (4.48a). That is, we choose our initial guess \( ^0b \) to be the function that satisfies

\[
\frac{d^0b}{dt} + \alpha ^0b = 1, \quad ^0b(0) = 0
\]

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which is

\[ 0^b(t) = \alpha^{-1}(1 - e^{-\alpha t}), \]  

(8.12a)

where \( \alpha = 1 + K \) as in (4.51). To construct our homotopy we then take:

\[ \mathcal{L}(B) = \frac{dB}{dt} + \alpha B, \]  

(8.12b)

\[ \mathcal{N}(B) = \mathcal{N}(1 - B) \frac{dB}{dt}, \]  

(8.12c)

\[ f = 1. \]  

(8.12d)

We then search for solutions of the form

\[ B(t; p; q) = 0^B(t; q) + (p)^1B(t; q) + (p^2)^2B(t; q) + \cdots \]  

(8.13)

to

\[ \mathcal{H}(B; p; q) := (1 - p) \left( \mathcal{L}(B) - \mathcal{L}(0^B) \right) + q p(\mathcal{A}(B) - f) = 0; \]  

(8.14)

we also enforce the initial conditions

\[ ^iB(0; p, q) = 0, \quad \forall i. \]  

(8.15)

Equating like powers of \( p \) in (8.14) we obtain the following equations:

\[ [1] : \mathcal{L}(0^B) = 1, \]  

(8.16a)

\[ [p] : \mathcal{L}(1^B) = -q Da\hbar \frac{d^{0^B}}{dt} (1 - 0^B), \]  

(8.16b)

\[ [p^2] : \mathcal{L}(2^B) = (1 - q)\mathcal{L}(1^B) - q Da\hbar \left[ (1 - 0^B) \frac{d^{1^B}}{dt} - 1^B \frac{d^{0^B}}{dt} \right], \]  

(8.16c)

subject to the initial conditions

\[ ^0B(0) = ^1B(0; q) = ^2B(0; q) = 0. \]  

(8.16d)

Here, \([p^i]\) denotes the equation found upon equating coefficients of \( p^i \) in the equation obtained by substituting (8.13) into (8.14). Notice the nonlinearity completely drops out of the leading order [1] equation, and is relegated to a forcing term in the rest.
Also, observe that we have written \( i^B \) as \( iB \), since the \( iB \) are independent of space. The solutions to (8.16a) and (8.16b) are given by:

\[
\begin{align*}
0^B(t) &= \alpha^{-1}(1 - e^{-\alpha t}), \\
1^B(t; q) &= -qDa\alpha e^{2\alpha t}(-1 + e^{\alpha t} - \alpha t e^{\alpha t} + \alpha^2 t e^{\alpha t})/\alpha^2;
\end{align*}
\]

the solution to (8.16c) is unwieldy and we do not list it here; we have simply written equation (8.16c) for completeness. Observe that \( 0^B \) is the solution obtained in the well-stirred limit—this plays a role in the accuracy of our expansion.

To this end, we now investigate how accurate our solution is, both with and without the convergence control parameter \( q \). By taking \( q = 1 \) in (8.14), we may reduce this equation to one of the form (8.4), without the convergence control parameter. In this way, we obtain the following results for a five-term approximation to \( B \) when \( Da = 2 \).

![Figure 8.1: Left: Five-term approximation when Da = 2. Right: the four correction terms.](image)

In Figure 8.1, \( B_s \) denotes the approximate solution as given by our semi-implicit finite difference algorithm. Observe that the correction terms are growing in magnitude, which causes our expansion to actually get worse as we add more terms. Clearly, in order to ensure that our expansion is convergent and accurate, we must include the
convergence control parameter $q$ in our expansion. On the left in Figure 8.2, we have depicted both two-term and five-term approximations, together with our numerical solution. On the right, we have depicted the first four correction terms, along with the steady state solution $\alpha^{-1}$ subtracted from the first term $^0B$. We choose $q$ by numerically minimizing

$$\mathcal{R}(q) = \|A(B) - f\|^2 = \int_0^T (A(B) - f)^2 \, dt,$$

(8.18) using a standard minimization algorithm in Mathematica. In particular, we choose $T = 5$, which resulted in a minimum value of $\mathcal{R} \approx 0.0106541$. This minimum value corresponded to a value of $q \approx 0.424987$ for our five-term approximation.

Although our approximation now appears to converge, it is still inadequate, as (8.17b) contains secular terms of the form $te^{-\alpha t}$. This term vanishes as $t \to \infty$, but it hinders the accuracy of our expansion because

$$|te^{-\alpha t}| > |\alpha^{-1}e^{-\alpha t}| = |^0B - \alpha^{-1}|,$$

for $t$ sufficiently large. This is evident on the right in Figure 8.2. Since each $^iB$ contains a term of the form $^i t e^{-\alpha t}$, we see that there is a $t$ such that $|^0B - \alpha^{-1}| < ^iB$ for $i = 1, \ldots, 4$. This is depicted in the right graph of Figure 8.2.

Figure 8.2: Left: two-term and five-term approximations, plotted together with numerical solution. Right: $^0B - \alpha^{-1}$ plotted together with first four correction terms. Here $Da = 2$. 

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As noted, we are seeking an accurate expression for $\overline{B}$, regardless of the size of $Da$. Taking $Da = 100$, the left graph in Figure 8.3 demonstrates that our current approach is unable to meet this criteria. To generate this approximation we took $T = 200$ in (8.18), which yielded a *minimum* residual $R \approx 854.686$, corresponding to $q \approx .00568$. Upon seeing the left graph in Figure 8.3, the problem is quite obvious: the time scale is off. While our approximation reaches equilibrium after about seven seconds, it takes our numerical solution about 175. Mathematically, the need for another time scale is reflected through both a secular term of the form $te^{-\alpha t}$, and the fact that our leading order term (8.17a) is within $e^{-3\alpha} = e^{-3 \cdot 2} \approx .00248$ of equilibrium after three seconds. The reason the bound state concentration takes longer to reach chemical equilibrium when $Da = 100$ is due to the upstream ligand depletion effect. As we transition to the transport-limited parameter regime, ligand molecules take longer to get to the surface: thus binding takes longer.

One may think to solve the issue by finding an approximation to $\overline{B}$ on the $t_w$ time scale. The right graph in Figure 8.3 demonstrates that although this improves our approximation, the expansion is still inaccurate. Our well-stirred leading order term $^0\overline{B}$ does not respond to changes in $Da$ (thus transport effects), and the time scale is

![Graphs showing numerical solution compared to approximation on $t$ and $t_w$ time scales.](image)

Figure 8.3: Left: Numerical solution plotted with a five-term approximation on $t$ time scale. Right: Numerical solution plotted with a five-term approximation on the $t_w$ time scale. Here $Da = 100$. 

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off.

The issues with our expansion (secular terms and the wrong time scale) are not overcome by simply adding more terms; a more sophisticated approach is required. Hence, we couple the HPM with a traditional strained coordinates technique by proposing a time scale of the form

$$\tau = (1 + \sum_{n=1}^{\infty} p^n \omega_n) t,$$  \hspace{1cm} (8.19)

where the $\omega_n$ are chosen to eliminate the secular terms.

The coupling leaves (8.16a) unchanged, so $^0B$ is still given by (8.17a). Thus our leading order term is still the well-stirred approximation, but on the strained time scale $\tau$. Equation (8.16b) becomes

$$\omega_1 \frac{d^0B}{d\tau} + \frac{d^1B}{d\tau} + \alpha^1B = -q Da \tilde{h} \frac{d^0B}{d\tau} (1 - ^0B)$$

$$\Rightarrow \omega_1 e^{-\alpha \tau} + \frac{d^1B}{d\tau} + \alpha^1B = -q Da \tilde{h} (1 - \alpha^{-1}) e^{-\alpha \tau} + q \alpha^{-1} Da \tilde{h} e^{-2\alpha \tau}.$$ \hspace{1cm} (8.20)

To suppress the secularity we set

$$\omega_1 = -Da \tilde{h} q (1 - \alpha^{-1}),$$ \hspace{1cm} (8.21)

which renders our time scale (8.19) a function of Da. Hence, the time to equilibrium for a leading order approximation will now be a function of how efficient transport is. In addition, (8.19) is a function of $q$—if this were not the case (8.19) could diverge for $Da \gg 1$. Using (8.21), equation (8.20) becomes:

$$\frac{d^1B}{d\tau} + \alpha^1B = q \alpha^{-1} Da \tilde{h} e^{-2\alpha \tau}$$

$$\Rightarrow ^1B(\tau; q) = -\frac{Da \tilde{h} q e^{-2\alpha \tau} (e^{\alpha \tau} - 1)}{\alpha^2}.$$  

One may repeat this procedure to calculate $\omega_2$ and $^2B$:

\[
\omega_2 = Da \tilde{h} q \alpha^{-2}(\alpha - 1)(-Da \tilde{h} q - \alpha + q \alpha + Da \tilde{h} q \alpha),
\]

\[
^2B(\tau; q) = \frac{(Da \tilde{h} q e^{-3\alpha \tau})(e^{\alpha \tau} - 1)[3Da \tilde{h} q + e^{\alpha \tau}(-3Da \tilde{h} q - 2\alpha + 2q + 2Da \tilde{h} q \alpha)]}{2\alpha^3}.
\]
We may continue in this manner to calculate as many terms as we wish.

To determine the accuracy of our HPM and strained coordinates coupling, we compared our numerical solution to our series expansion for different values of Da. Our results are depicted in Figures 8.4–8.6, and tabulated in Table 8.1.

Figure 8.4: Two-term and five-term approximations, Da = 1/2.

Figure 8.5: Two-term and five-term approximation, Da = 2.
Figure 8.6: Two-term and five-term approximation, Da = 100.

| Da  | Terms | q     | $||\mathcal{A}(B) - f||^2_2$ | $T$ in (8.18) | Time to equilibrium |
|-----|-------|-------|-------------------------------|----------------|---------------------|
| $\frac{1}{2}$ | 2     | 0.75039 | 0.00015                       | 10             | 10                  |
| $\frac{1}{2}$ | 5     | 0.70729 | $4.31290 \times 10^{-10}$     | 10             | 10                  |
| 2    | 2     | 0.43915 | 0.00897                       | 10             | 10                  |
| 2    | 5     | 0.37967 | $2.29489 \times 10^{-6}$      | 10             | 10                  |
| 100  | 3     | 0.01591 | 1.69195                       | 200            | 200                 |
| 100  | 5     | 0.01391 | 0.08497                       | 200            | 200                 |

Table 8.1: Summary of results for the injection phase.

On a brief technical note, we came into difficulties minimizing (8.18). To compute (8.18) we used \texttt{Integrate[]} in Mathematica, and incurred significant roundoff error, especially when Da = 100. To minimize the effects of roundoff error, we took advantage of Mathematica’s symbolic computing capabilities by defining all irrational parameters \textit{after} using the \texttt{Integrate[]} command. In this way, \texttt{Integrate[]} was able to do much of the computation symbolically, thus eliminating roundoff error during this step of our calculations. This discussion applies to our results for the wash phase as well.
Figure 8.5 and Table 8.1 show that our new two-term expansion when $Da = 2$ does better than a five-term expansion without the strained time scale. Furthermore, we see that our five-term approximation is orders of magnitude better than our previous expansion. When $Da = 100$, we are able to find a reasonable approximation to $\overline{B}$—something that was out of reach with our previous approach. As $Da$ increases we gradually transition from the reaction-limited and transport-dominant regime, to the transport-limited and reaction-dominant parameter regime; our expansion now captures this behavior.

We now have a reasonable approximation to $\overline{B}$ when $Da = 100$, but from Figure 8.6 one may be concerned that our HPM approximation is not converging when $Da = 100$. First, we observe that the data in Table 8.1 shows the residual is decreasing as we add more terms. Moreover, we observe that our series was not constructed to converge to $\overline{B}$—it was constructed to converge to the solution of the bimolecular ERC equation (4.55). While the ERC equation is a good approximation to $\overline{B}$, it does not coincide exactly with $\overline{B}$. Below in Figure 8.7 we have depicted the difference between the solution to the bimolecular ERC equation (4.55), and our HPM approximation. The results in Figure 8.7 indicate that our series does indeed converge to the function it was intended to.
Figure 8.7: Left: Absolute difference between three, four, and five-term approximation and the solution to the bimolecular ERC equation (4.56) when Da = 2 on the t time scale. Right: Same, but here Da = 100 (also on the t time scale).

8.2.2 Wash Phase

To develop an expression for \( \overline{B} \) during the wash phase, we first summarize the relevant equations. In the presence of a single ligand molecule, (3.13a)–(3.13c), (4.44), (4.45), and (4.37) reduce to

\[
\begin{align*}
\frac{\partial B}{\partial t} &= (1 - B)C(x, 0, t) - KB, \quad (8.22a) \\
C(x, 0, t) &= -\frac{Da}{3^{1/3}\Gamma(2/3)} \int_0^x \frac{\partial B}{\partial t} \frac{d\nu}{(x - \nu)^{2/3}}, \quad (8.22b) \\
B(x, 0) &= \alpha^{-1}; \quad (8.22c)
\end{align*}
\]

one may also see [11]. The ERC equation in this case is given by (omitting the \( O(Da^2) \) terms):

\[
\begin{align*}
\frac{d\overline{B}}{dt} &= -hDa(1 - \overline{B})\frac{d\overline{B}}{dt} - K\overline{B}, \quad (8.23a) \\
\overline{B}(0) &= 0. \quad (8.23b)
\end{align*}
\]
To construct a homotopy of the form (8.8), we set:

\[ \mathcal{L} = \frac{dB}{d\tau} + KB, \]  
\[ \mathcal{N} = \tilde{h}Da(1 - B)\frac{Da}{3^{1/3}\Gamma(2/3)}, \]  
\[ f = 0. \]

(8.24a)  
(8.24b)  
(8.24c)

Here \( \tau \) is the strained time scale, defined as (8.19). We require our initial guess \( 0b \) to satisfy the initial condition (8.23a) and the concentration at the boundary at \( x = 0 \):

\[ \frac{d0b}{d\tau} = -K0b, \]

\[ 0b(0) = \alpha^{-1}, \]

or

\[ 0b = \alpha^{-1}e^{-K\tau}. \]  
(8.24d)

We then define our homotopy \( \mathcal{H} \) via (8.8), using (8.24). We propose an expansion of the form (8.13) subject to \( 0B(0; q) = \alpha^{-1} \), and \( iB(0, q) = 0 \) for \( i \geq 1 \). The first three terms of our expansion are:

\[ 0B(\tau) = \frac{e^{-K\tau}}{\alpha}, \]

\[ 1B(\tau; q) = -\frac{Da\tilde{h}q e^{-2K\tau}(-1 + e^{K\tau})}{\alpha^2}, \]

\[ 2B(\tau; q) = \frac{Da\tilde{h}q e^{-3K\tau}(-1 + e^{K\tau})[-3Da\tilde{h}q + e^{Kt}(Da\tilde{h}q - 2\alpha + 2q\alpha + 2Da\tilde{h}q\alpha)]}{2\alpha^3}. \]

Also, \( \omega_1 \), and \( \omega_2 \) take the form:

\[ \omega_1 = -Da\tilde{h}q, \]

\[ \omega_2 = Da\tilde{h}q(-1 + q + Da\tilde{h}q). \]

Again, \( q \) is choosen to minimize (8.18). Our results for the wash phase are depicted below in Figures 8.8–8.9, and tabulated in Table 8.2.
Figure 8.8: Two-term and five-term approximation, Da = 1/2.

Figure 8.9: Three-term and five-term approximation, Da = 100

<table>
<thead>
<tr>
<th>Da</th>
<th>Terms</th>
<th>q</th>
<th>$|A(B) - f|_2^2$</th>
<th>$T$</th>
<th>Time to equilibrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\frac{1}{2}$</td>
<td>2</td>
<td>0.23782</td>
<td>0.00010</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>$\frac{1}{2}$</td>
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<td>0.64453</td>
<td>$6.79536 \times 10^{-7}$</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>0.00832</td>
<td>0.02366</td>
<td>800</td>
<td>800</td>
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<td>0.00053</td>
<td>800</td>
<td>800</td>
</tr>
</tbody>
</table>

Table 8.2: Summary of results for the wash phase.
As with the injection phase, our expansion matches our numerical solution quite well—we have obtained an accurate expansion using only two terms.

Physically, as Da grows, transport becomes less efficient as we gradually transition to the reaction-dominant and transport-limited parameter regime. Ligand molecules dissociating upstream quickly rebind to receptor sites downstream; hence, the evolution of $\bar{B}$ takes longer for larger Da. As in the injection phase, our expansion is able to account for this transition. Additionally, remarks analogous to the ones at the end of Subsection 8.2.1 concerning Figure 8.6 also apply Figure 8.9. That is, our HPM approximation is ultimately converging to (8.23), not the averaged solution of (8.22).
Chapter 9

SENSOGRAM STUDY: MULTIPLE-LIGAND MODEL

The sensogram reading (2.21) lumps data from all of the reacting species into one signal, which raises two key questions.

1. Given a sensogram reading, what is the behavior of the reacting species?

2. When can more than one set of rate constants generate a given sensogram reading?

Our mathematical model can aid in answering both of these questions. We will address question 1 by performing a case-by-case analysis, i.e. studying (2.21) for different sets of rate constants, to explain the behavior of the sensogram reading (2.21) in terms of the reacting species concentrations $B_i$. An exhaustive case-by-case analysis would take volumes; thus we limit ourselves to certain physically-limiting parameter regimes, e.g., $1K_d = 1$ and $2K_a \gg 1$; see Table 9.1. Through this case-by-case analysis, we will discover a sensogram reading which corresponds to two different sets of reaction rate constants. We propose two methods for determining the true set of rate constants: one based off of a manipulation of the signal (2.21), and another based upon adjusting the uniform inflow concentrations.

For simplicity, we will consider the bound state system in the well-stirred limit, rendering $C_1(x,0,t)$ and $C_2(x,0,t)$ both constant. Additionally, we will be interested in how the sensogram signal responds to adjusting the inflow concentrations. To keep the bound state system in terms of dimensionless variables, this requires adjusting the
inflow concentrations relative to our reference concentrations $\tilde{C}_{1,u}$ and $\tilde{C}_{2,u}$. Thus, we formulate the bound state system as

\[
\frac{dB_1}{dt} = (1 - B_1)C_1 - K_dB_1 - \frac{1}{2}K_aB_1C_2 + \frac{1}{2}K_dB_{12}, \tag{9.1a}
\]

\[
\frac{dB_{12}}{dt} = \frac{1}{2}K_aB_1C_2 - \frac{1}{2}K_dB_{12} + \frac{2}{1}K_aB_2C_1 - K_dB_{12}, \tag{9.1b}
\]

\[
\frac{dB_2}{dt} = \frac{2}{1}K_dB_{12} - \frac{2}{1}K_aB_2C_1 + 2K_a(1 - B_1)C_2 - 2K_dB_2. \tag{9.1c}
\]

\[B(0) = 0. \tag{9.1d}\]

Here $C_1$ and $C_2$ are dimensionless parameters which represent the relative uniform inflow concentrations. For example, the values $C_1 = C_2 = .9$ represent injection of both ligands at 90% of our reference concentrations $\tilde{C}_{1,u}$ and $\tilde{C}_{2,u}$. Unless otherwise noted, we take $C_1 = C_2 = 1$.

To perform our case-by-case analysis, we must determine how to simulate the sensogram reading (2.21); in practice there are different ways to do this, depending on the order in which we inject the two ligands. One can imagine injecting the two ligands $L_1$ and $L_2$ simultaneously, or in sequence. In our simulations, we first inject ligand $L_1$ until $B_1$ reaches chemical equilibrium, then we will stop injecting $L_1$, and begin to inject ligand $L_2$ until the bound state system reaches a chemical equilibrium.

As we shall see, the behavior of (2.21) is dependent upon the value of $K_d$. Thus we organize our case-by-case analysis into three cases, depending on value of $K_d$. We then break each case into various subcases, as organized in Table 9.1. In this Table, we list only the parameters which are not set equal to one.
In our sensogram study, we shall see that for a given set of rate constants, the product $EL_2$ may tend to form via direct binding (pathway $P_1$) or the ligand switching process (pathway $P_2$); this information is critical to scientists studying DNA damage repair. Therefore, we have identified whether $EL_2$ forms primarily through direct binding or the ligand switching process in Table 9.1 in the dominant pathways column. If $P_1$ is the dominant pathway, then $EL_2$ forms primarily due to direct binding; conversely, if $P_2$ is the dominant pathway, then $EL_2$ forms primarily through the ligand

<table>
<thead>
<tr>
<th>Case</th>
<th>Parameter Values</th>
<th>Dominant Pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(a)</td>
<td>$K_d = 100$</td>
<td></td>
</tr>
<tr>
<td>1(b)</td>
<td>$K_d = 100$</td>
<td>$2K_d = \frac{1}{100}$</td>
</tr>
<tr>
<td>2 (a)</td>
<td>$K_d = 1$</td>
<td>Balanced</td>
</tr>
<tr>
<td>2(b)</td>
<td>$K_d = 1$</td>
<td>$K_a = 100$</td>
</tr>
<tr>
<td>2(b)</td>
<td>$K_d = 1$</td>
<td>$2K_a = 100$</td>
</tr>
<tr>
<td>2(b)</td>
<td>$K_d = 1$</td>
<td>$2K_a = 100$</td>
</tr>
<tr>
<td>2(b)</td>
<td>$K_d = 1$</td>
<td>$2K_a = 100$</td>
</tr>
<tr>
<td>2(b)</td>
<td>$K_d = 1$</td>
<td>$2K_a = 100$</td>
</tr>
<tr>
<td>2(b)</td>
<td>$K_d = 1$</td>
<td>$2K_a = 100$</td>
</tr>
<tr>
<td>2(b)</td>
<td>$K_d = 1$</td>
<td>$2K_a = 100$</td>
</tr>
<tr>
<td>3(a)</td>
<td>$K_d = \frac{1}{100}$</td>
<td></td>
</tr>
<tr>
<td>3(b)</td>
<td>$K_d = \frac{1}{100}$</td>
<td>$2K_a = 100$</td>
</tr>
<tr>
<td>3(b)</td>
<td>$K_d = \frac{1}{100}$</td>
<td>$2K_a = 100$</td>
</tr>
<tr>
<td>3(c)</td>
<td>$K_d = \frac{1}{100}$</td>
<td>$2K_a = 100$</td>
</tr>
<tr>
<td>3(c)</td>
<td>$K_d = \frac{1}{100}$</td>
<td>$2K_a = 100$</td>
</tr>
</tbody>
</table>

Table 9.1: Parameter values for different cases. Parameters not listed have a value of one.
switching process. If $P_2$ is the dominant pathway, we have identified the most critical step; as labeled below.

Figure 9.1: Reaction kinetics diagram, with steps identified in $P_2$.

Above, $(P_{2,a})$ refers to the first step in $P_2$, $(P_{2,b})$ refers to the second step, and $(P_{2,c})$ refers to the third step. As a visual aid, we will denote a weak pathway through a dashed arrow, and a strong pathway through a bold arrow.

Figure 9.2: Reaction kinetics diagram, $P_2$ is dominant.

In Figure 9.2 the step $(P_{2,b})$ is weak, and the step $(P_{2,c})$ is strong. We will now proceed with our sensogram study by explaining the behavior of (2.21) in terms of $B_i$, and will start by discussing the reading when $K_d \gg 1$. 

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9.1 Case 1: \( K_d = 100 \)

9.1.1 Subcase 1(a)

Observe the equilibrium value of \( B_1 \) after the first injection is

\[
B_{1,\infty} = \frac{C_1}{C_1 + K_d}
\]  
(9.2)

(see 9.1a with \( C_1 = 1 \) and \( C_2 \)) so this case corresponds to a very low concentration of \( B_1 \). Taking all of the other parameters equal to one, we obtain the following sensogram reading:

![Sensogram Average and Bound Complexes](image)

Figure 9.3: Subcase 1(a): \( K_d = 100 \).

The sensogram reads as expected: because of the high dissociation rate the sensogram reading of the mass remains very close to zero for the duration of the first stage. Then as ligand two is injected, it binds with all of the empty receptors,
eventually reaching equilibrium. We make note of the relatively small mass reading on the sensogram, compared to the amount of $B_2$. This is due to the fact that $B_2$ is the lightest of the three bound complexes. Also with such a high dissociation the model is insensitive to the parameters $\frac{2}{1}K_a, \frac{2}{1}K_d, \frac{1}{2}K_a, \frac{1}{2}K_d$; thus the experiment will be inconclusive.

9.1.2 Subcase 1(b)

We now examine the reading when $2K_d = \frac{1}{100}$.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{sensogram_average.png}
\caption{Subcase 1(b) : $1K_d = 100$, $2K_d = \frac{1}{100}$.}
\end{figure}

Notice that almost all of the $L_2$ that binds, stays there. This is a result of the small dissociation constant $1K_d$. 

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9.2 Case 2: $1K_d = 1$

We now examine the sensogram reading when $1K_d = 1$, and consider many different subcases, depending on the value of the rate constants.

9.2.1 Subcase 2(a)

In this subcase, we take all the rate constants equal to 1. In this case we obtain the following sensogram reading and bound state concentrations:

![Sensogram and Bound Complexes](image)

Figure 9.5: Subcase 2(a), all reaction rates equal.

Here, the sensogram reading drops off immediately after injection two begins. This is because $EL_2$ is much lighter than $EL_1$, and although some $EL_1L_2$ is formed, it is not heavy enough to compensate for the amount of $EL_2$ being formed. One may notice that the $B_{12}$ reaches its maximum relatively early after the second injection, and then steadily drops off. This is due to the fact that there is only one source and
two sinks. In the second stage the complex $EL_1L_2$ can be formed through only the reaction

$$EL_1 + L_2 \xrightleftharpoons{1 \frac{1}{2} K_a} EL_1L_2,$$

but $B_1$ decays due the reactions

$$EL_1L_2 \xrightleftharpoons{1 \frac{1}{2} K_d} EL_1 + L_2,$$

$$EL_1L_2 \xrightleftharpoons{2 \frac{1}{2} K_d} EL_2 + L_1;$$

thus, only $EL_2$ will be left after the second stage of the experiment.

### 9.2.2 Subcase 2(b)

In this case, we examine the pull between pathway $(P_{1,b})$ and pathway $(P_2)$. This requires examining the subcases 2(b)$_i$–2(b)$_iv$; thus we will consider each of these subcases in the order outlined in Table 9.1. We first examine when $(P_{1,b})$ is dominant, taking $1 \frac{1}{2} K_a = Da^{-1}$. Doing so we obtain the following sensogram reading:
We see at the start of the second injection there is a sharp increase in the mass due to the high association rate. That is because \( \frac{1}{2} K_a \gg 1 \), and all of the unbound ligand \( L_2 \) very quickly binds with \( EL_1 \). This explains the sharp increase. Then after the initial influx of the second ligand the dissociation process kicks in, and \( B_{12} \) decays to zero as \( B_2 \) reaches equilibrium. The mass reading drops off because the \( EL_1L_2 \) is being converted to the lighter complex \( B_2 \).

As before, taking a small value of \( 2K_d \) such as \( 2K_d = Da \), it will be easier for the second ligand to bind with the receptors. Taking \( 2K_d = \frac{1}{100} \) gives the following behavior:
Generally, a smaller value of $2K_d$ results in a larger value of $B_2$ at the end of the second stage. Conversely, a larger value of $2K_d$ results in a smaller value of $B_2$ at the end of the second stage of the experiment. The spike in the sensogram reading exists for the same reasons as described above.

We now study the sensogram when $2K_a = 100$, and all of the other rate constants are 1. In this case we get nearly the same sensogram reading as case 2(b), despite a different set of rate constants.

Figure 9.7: Subcase 2(b)ii: $\frac{1}{2}K_a = 100$, $2K_d = \frac{1}{100}$, $C_1 = 1.$
We see the sudden influx of ligand $L_2$, coupled with the high rate of association, drives up the sensogram reading. As time increases, $B_1$ decays to zero while $B_2$ increases to a chemical equilibrium. Some $EL_1L_2$ forms, but then the sinks kick in and drive $B_{12}$ to zero. We note that running the same computation, but with $\frac{1}{2}K_a = \frac{1}{100}$, gives nearly identical behavior. The only difference is that $B_{12}$ remains near zero for the duration of the experiment. This is to be expected, as in this case it is quite difficult for $EL_1L_2$ to form.

Observe that the the sensogram signal, if not displaced, reads nearly identically to the one in subcase 2(b)$_{iii}$ (see Figure 9.6). However, by subtracting the value

$$\left(1 - \frac{C_1}{C_1 + \frac{1}{2}K_d}\right) B_2$$

(9.3)
from (2.21) we are able to distinguish the two readings. The displaced signal is depicted in red in Figures 9.6 and 9.8. The coefficient in (9.3) is the concentration of empty receptors immediately after the first stage is over, and at the start of the second stage. By subtracting this amount off, we can see the amount of $B_{12}$ forming at the beginning of the second stage of the experiment.

On the other hand, the signals can also be delineated by adjusting the inflow concentrations, as seen in the figures below.

![Graphs showing sensogram average and bound complexes](image)

*Figure 9.9: Subcase: 2(b)iv: $\frac{1}{2}K_a = 100$, $C_1 = .1.$*
In Figure 9.9 we have depicted subcase 2(b)_i (see Figure 9.6) with $C_1 = .1$, and in Figure 9.10 we have depicted subcase 2(b)_iii (see Figure 9.6) with $C_1 = .1$. We first comment on Figure 9.9: in this case there is not much $EL_1$ for $L_2$ to bind with during the second stage, and whatever $EL_1$ is present becomes $EL_1L_2$, due to the strong affinity $\frac{1}{2}K_a$. Much of the ligand $L_2$ molecules which have not bound to $EL_1$, bind with an empty receptor.

On the other hand, in Figure 9.10 the large value of $2K_a$ drives all of the ligand $L_2$ molecules to directly bind with an empty receptor. Therefore, we may delineate identical sensogram readings by either: manipulating (2.21), or adjusting the inflow concentrations. In practice, one should attempt to manipulate (2.21) to delineate the cases before adjusting the inflow concentrations; this requires running fewer experiments.

Figure 9.10: Subcase 2(b)_v: $2K_a = 100, C_1 = .1$. 

In Figure 9.9 we have depicted subcase 2(b)_i (see Figure 9.6) with $C_1 = .1$, and in Figure 9.10 we have depicted subcase 2(b)_iii (see Figure 9.6) with $C_1 = .1$. We first comment on Figure 9.9: in this case there is not much $EL_1$ for $L_2$ to bind with during the second stage, and whatever $EL_1$ is present becomes $EL_1L_2$, due to the strong affinity $\frac{1}{2}K_a$. Much of the ligand $L_2$ molecules which have not bound to $EL_1$, bind with an empty receptor.

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Figure 9.10: Subcase 2(b)_v: $2K_a = 100, C_1 = .1$. 

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On the other hand, in Figure 9.10 the large value of $2K_a$ drives all of the ligand $L_2$ molecules to directly bind with an empty receptor. Therefore, we may delineate identical sensogram readings by either: manipulating (2.21), or adjusting the inflow concentrations. In practice, one should attempt to manipulate (2.21) to delineate the cases before adjusting the inflow concentrations; this requires running fewer experiments.
9.2.3 Subcase 2(c)

In this subcase we study the sensogram reading by adjusting the rate constants in the third step of the second pathway, \( P_{2.c} \). Taking \( ^2_1 K_d = 100 \) we obtain the following reading:

We obtain a Sensogram reading that is similar to subcase 2(a), but with a sharper dropoff at the start of the second stage. In case 2(a) the small amount of \( EL_1L_2 \) makes the transition to equilibrium smoother; however in this case the large value of \( ^2_1 K_d \) implies there is very little \( EL_1L_2 \) to drive up the reading. Most of the ligand \( L_1 \) molecules will switch out, once an ligand \( L_2 \) molecule binds with \( EL_1 \).

We now examine when pathway \( (P_2) \) is dominant by taking \( ^2_1 K_d = D_a \). In this case the complex \( B_{12} \) is slower to dissociate. Because of this we will see a slower

Figure 9.11: Subcase 2(c); \( ^2_1 K_d = 100 \).
transition to the equilibrium after the second injection because $B_1$ is slow to dissociate from $B_{12}$.

We now study the sensogram when $2\kappa_{d} = \frac{1}{100}$; the reading is given below.

![Sensogram Average](image1)

![Bound Complexes](image2)

Figure 9.12: Subcase 2(c)ii : $2\kappa_{d} = \frac{1}{100}$.

Notice the equilibrium value for the sensogram reading in this case is the same as the previous case (when $2\kappa_{d} = 100$); however this time the transition is more gradual. This makes sense in light of the small dissociation rate $2\kappa_{d}$.

9.3 Case 3 : $\kappa_{d} = \frac{1}{100}$

We now examine the sensogram reading in the case when $\kappa_{d} = \frac{1}{100}$. Equation (9.2) implies the reacting surface will be nearly saturated with ligand $L_1$ by the end of the first stage of the experiment.
9.3.1 Subcase 3(a)

We first study the sensogram reading when the rest of the rate constants are one.

![Sensogram Average](image)

![Bound Complexes](image)

Figure 9.13: Subcase 3(a) : \( K_d = \frac{1}{100} \).

Because the complex \( EL_1 \) occupies nearly all of the receptors, the ligand \( L_2 \) binds mostly with \( EL_1 \), rather than the empty receptors. This momentarily increases the mass at the surface and the sensogram reading. Since \( EL_1L_2 \) is the heaviest of the three complexes, this explains the blip in the sensogram reading at the start of the second phase.

9.3.2 Subcase 3(b)

In this subcase we study the second step in the second pathway, i.e. \( (P_{2,b}) \). We take \( \frac{1}{2}K_a = 100 \) to produce the reading below.
Since the surface of the biosensor is nearly saturated, the large value of $\frac{1}{2}K_a$ implies that all of the ligand $L_2$ molecules will rush to bind with the $EL_1$ already present. This explains the large spike in the reading at the start of the second phase.

We now study the signal when $2K_a = 100$. 
Although some $EL_1$ forms at the start of the second injection, $B_{12}$ gradually decays to zero. The high affinity $2K_a$ results in most of the ligand $L_2$ molecules sticking to empty receptors, as the empty receptors become available.

### 9.3.3 Subcase 3(c)

In this subcase we study the third step in the second pathway, i.e. $(P_{2,c})$, by first taking $\frac{1}{2}K_d = 100$. The resulting reading is depicted below.
The high value of $\frac{2}{1}K_d$ results in the $EL_1L_2$ formed being very quickly converted to $EL_2$. Hence, it appears to the naked eye as though $B_{12} = 0$ for the duration of the second phase. Additionally, we see $B_1$ decay to zero, and $B_2$ reach its chemical equilibrium.

We now examine the evolution of the sensogram reading when $\frac{2}{1}K_d = \frac{1}{100}$. Doing so gives the reading below.
Observe that the reading takes much longer to reach equilibrium in this subcase than in the other cases. This is a result of the small dissociation rates $K_d$ and $K_d$. Since the floor of the biosensor is nearly saturated with ligand $L_1$, there are not many empty receptor sites for $L_2$ to bind with; thus most ligand $L_2$ molecules bind with the $L_1$ already there. However, $K_d$ is very small, which implies it will take a longer time for $L_2$ to switch out of the complex $EL_1L_2$. Thus we see as $B_{12}$ decays to zero, $B_2$ gradually reaches its chemical equilibrium.
Chapter 10

SENSOGRAM STUDY: MULTIPLE-RECEPTOR MODEL

In this chapter we study the senogram signal (2.31) for our multiple-receptor model. As in Chapter 9, we will be studying the bound state system in the well-stirred limit. This reduces (2.29a) and (2.29b) to:

\[
\frac{\partial B_f}{\partial t_c} = t_k a (R_f - B_f) - t_k d B_f, \tag{10.1}
\]

\[
\frac{\partial B_p}{\partial t_c} = t_k d (R_p - B_p) - t_k d B_p, \tag{10.2}
\]
during the injection phase. The above equations are linear, and can be solved exactly in terms of exponentials. We will not simulate the wash phase during this chapter. Additionally in this case, we will study the equations on the \( t_c \) time scale.

In a typical experiment, \( R_f \) is small (in our simulations we take \( R_f = .1 \)). Therefore, we will not notice the effect of the free DNA receptors if the association rate \( t_k a \) is too small, or the dissociation rate \( t_k d \) is too large; we will not consider these cases. We then organize our study according the values of \( t_k a \) and \( t_k d \), which will break our study into four cases. Each of these cases will then be broken into three subcases, depending on the value of \( p k_a \); see Table 10.1. In our simulations we take \( p k_d = \frac{1}{100} \), in accordance with physical observations that binding with PCNA molecules is tight.
<table>
<thead>
<tr>
<th>Case</th>
<th>Parameter Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(a)</td>
<td>( t_{ka} = 1 )  ( t_{kd} = 1 )  ( p_{ka} = 1 )</td>
</tr>
<tr>
<td>1(b)</td>
<td>( t_{ka} = 1 )  ( t_{kd} = 1 )  ( p_{ka} = \frac{1}{100} )</td>
</tr>
<tr>
<td>1(c)</td>
<td>( t_{ka} = 1 )  ( t_{kd} = 1 )  ( p_{ka} = 100 )</td>
</tr>
<tr>
<td>2(a)</td>
<td>( t_{ka} = 1 )  ( t_{kd} = \frac{1}{100} )  ( p_{ka} = 1 )</td>
</tr>
<tr>
<td>2(b)</td>
<td>( t_{ka} = 1 )  ( t_{kd} = \frac{1}{100} )  ( p_{ka} = \frac{1}{100} )</td>
</tr>
<tr>
<td>2(c)</td>
<td>( t_{ka} = 1 )  ( t_{kd} = \frac{1}{100} )  ( p_{ka} = 100 )</td>
</tr>
<tr>
<td>3(a)</td>
<td>( t_{ka} = 100 )  ( t_{kd} = 1 )  ( p_{ka} = 1 )</td>
</tr>
<tr>
<td>3(b)</td>
<td>( t_{ka} = 100 )  ( t_{kd} = 1 )  ( p_{ka} = \frac{1}{100} )</td>
</tr>
<tr>
<td>3(c)</td>
<td>( t_{ka} = 100 )  ( t_{kd} = 1 )  ( p_{ka} = 100 )</td>
</tr>
<tr>
<td>4(a)</td>
<td>( t_{ka} = 100 )  ( t_{kd} = \frac{1}{100} )  ( p_{ka} = 1 )</td>
</tr>
<tr>
<td>4(b)</td>
<td>( t_{ka} = 100 )  ( t_{kd} = \frac{1}{100} )  ( p_{ka} = \frac{1}{100} )</td>
</tr>
<tr>
<td>4(c)</td>
<td>( t_{ka} = 100 )  ( t_{kd} = \frac{1}{100} )  ( p_{ka} = 100 )</td>
</tr>
</tbody>
</table>

Table 10.1: Parameter values for different cases. We took \( p_{kd} = 1 \) for all cases.

10.1 Case 1

This is when association and dissociation to the free DNA receptors balance.

10.1.1 Case 1(a)

In the case that all parameters balance, we obtain the results below.
10.1.2 1(b)

Since $p_{k_a}$ is small, ligand molecules bind slowly to receptors with a PCNA ring attached. Since $t_{k_a} \gg p_{k_a}$, binding with free DNA receptors occurs faster.

10.1.3 1(c)

In this subcase, the high value of $p_{k_a}$ causes all of the ligand molecules to associate with the free DNA receptors very quickly, while binding the free DNA receptors takes longer.
10.2 Case 2

The low dissociation rate in this case results in most ligand molecules sticking to the free DNA receptors.

10.2.1 2(a)

This case is similar to 1(a), except nearly all ligand molecules which bind to free DNA receptors will stay there. This results in a higher overall mass reading.

Figure 10.3: Subcase 1(c) : \( f_k a = 1, f_k d = 1, p_k a = 100. \)

Figure 10.4: Subcase 2(a) : \( f_k a = 1, f_k d = \frac{1}{100}, p_k a = 1. \)
10.2.2 2(b)

As with case 1(a), the small value of $p k_a$ results in the ligand molecules taking longer to bind to receptors with a PCNA ring attached. The equilibrium concentration is slightly higher, due to ligand molecules sticking to free DNA receptors.

![Figure 10.5: Subcase 2(c) : $r k_a = 1$, $r k_d = \frac{1}{100}$, $p k_a = \frac{1}{100}$](image)

10.2.3 2(c)

We see in this case that the strong association rate $p k_a$ results in ligand molecules binding to PCNA receptors very quickly. The sensogram reading reaches equilibrium after $B_t$ reaches a chemical equilibrium.

![Figure 10.6: Subcase 2(c) : $r k_a = 1$, $r k_d = \frac{1}{100}$, $p k_a = 100$](image)
10.3 Case 3

In this case, the strong association rate drives ligand molecules to associate with the free DNA receptors very quickly. Hence we see a sharp increase in mass at the start of the reading, followed by a steady path to equilibrium. This sharp increase in the mass reading represents the very fast binding of ligand molecules to free DNA receptors.

10.3.1 3(a)

The very fast binding of ligand molecules to free DNA receptors causes a sharp increase in the mass reading as the experiment begins, followed by a steady increase to equilibrium.

![Figure 10.7: Subcase 3(a): $t_1k_a = 100$, $t_1k_d = 1$, $p_k_a = 1$.](image)

10.3.2 3(b)

In this subcase, we see that ligand molecules bind to free DNA receptors very quickly, while binding to receptors with PCNA rings attached takes much longer. This is due to the small value of $p_k_a$. 

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10.3.3 3(c)

Here, we see the strong association rates drive all the binding to happen very quickly.

10.4 Case 4

Since the sensogram readings in this case are so similar to the ones in Case 3, we will not go into the details for each subcase. The difference between this case and the previous one is that nearly all of the ligand molecules which bind to free DNA receptors will stay there, because $k_d$ is very small.
### 10.4.1 4(a)

Figure 10.10: Subcase 4(a) : \( tk_a = Da^{-1}, tk_d = Da, p k_a = 1 \).

### 10.4.2 4(b)

Figure 10.11: Subcase 4(b) : \( tk_a = Da^{-1}, tk_d = Da, p k_a = Da \).
10.4.3 Subcase 4(c)

Figure 10.12: Subcase 4(c): $\tau k_a = Da^{-1}, \tau k_d = Da, p k_a = Da^{-1}$.

10.5 A Note to Experimentalists

Above we have studied the behavior of the sensogram signal (2.31) for various parameter regimes. Below, we outline a simple procedure that one may use to estimate the rate constants: $\tau k_d$, $\tau k_a$, $p k_d$, and $p k_a$. This method does not account for transport effects, and requires that we fit the rate constants in the following order: $\tau k_d$, $\tau k_a$, $p k_d$, $p k_a$, and requires running four experiments.

The first experiment requires that only free DNA receptors are on the surface of the biosensor. To fit $\tau k_d$, we first run the injection phase of the experiment to equilibrium; let $B_{f,\infty}$ denote the equilibrium value of the sensogram reading in this case. Then, we fit the function

$$S(t) = B_{f,\infty} e^{-\tau k_d t}$$

to the resulting sensogram reading in the wash phase. This will give us the constant $\tau k_d$.

Next, we run a second experiment with only free DNA receptors on the surface of the biosensor. To find the constant $\tau k_a$, we fit the function

$$S(t) = \frac{\tau k_a R_t}{\tau k_d + \tau k_a} \left(1 - e^{-(\tau k_a + \tau k_d) t_e}\right)$$

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to the data obtained in the injection phase of the experiment. We then wash the biosensor with the buffer fluid, in preparation for the next phase.

For the third step, we now place the PCNA rings on the surface of the biosensor, and run the injection phase of the experiment to equilibrium. Let us call the equilibrium value of the injection phase in this case $S_{\text{inj,}\infty}$. To fit the constant $p k_d$, we would like to fit the signal in the wash phase

$$S(t) = \frac{t k_a R_f e^{-t k_a t}}{t k_d + t k_a} + \frac{p k_a R_p e^{-p k_d t}}{p k_d + p k_a}$$  \hspace{1cm} (10.3)

to the data in the wash phase. However, we do not know the constant

$$\frac{p k_a R_p}{p k_d + p k_a},$$

but it may be determined from the calculation

$$\frac{p k_a R_p}{p k_d + p k_a} = S_{\text{inj,}\infty} - \frac{t k_a R_f}{t k_d + t k_a}.$$  

Therefore, we may fit the data obtained in the wash phase of the experiment to (10.3), to determine the constant $p k_d$.

Finally, to fit the constant $p k_a$, we run the injection phase of the experiment (with the PCNA rings). Then, we fit the resulting data to the signal

$$S(t) = \frac{p k_a R_p}{p k_d + p k_a} (1 - e^{-(p k_d + p k_a) t}) + \frac{t k_a R_f}{t k_d + t k_a} (1 - e^{-(t k_d + t k_a) t}),$$

thereby giving us the rate constant $t k_a$. We stress that the above method does not account for transport effects, and thus should only be used when $Da \ll 1$. To account for transport effects, one could develop a more sophisticated procedure based upon our ERC equations in Chapter 7.
11.1 Conclusions

In order to keep up with current uses of biosensor technology, mathematical developments are necessary. To interpret biosensor data associated with complicated multiple-component reactions, an accurate mathematical model is needed. Keeping the polymerase switch which occurs during DNA translesion synthesis in mind, we have developed a mathematical model for multiple-component reactions in optical biosensors.

To account for transport mechanisms which affect ligand binding, we first modeled the velocity of the ligand molecules through the channel with the incompressible Navier-Stokes equations. Low Reynolds number and unidirectional flow simplify the full Navier-Stokes equations to a simple Poiseuille flow profile. We then modeled ligand evolution as an advection-diffusion-reaction system. In particular, we presented the kinetics system for both multiple-ligand and a multiple-receptor systems.

We studied this multiple-component advection-diffusion-reaction system analytically. A large Péclet number results in a two-compartment model; flow through the bulk compartment of the channel reaches equilibrium on a faster convective time scale, while the reaction kinetics occur in a thin unstirred region near the surface. The dynamics in this unstirred layer depend upon the relative strength of reaction to transport, \( i.e. \) the size of the Damköhler number. When \( Da \) is small we are operating in the reaction-limited and transport-dominant parameter regime. When \( Da \) is moderate reaction and diffusion balance, and when \( Da \) is large we are in the transport-limited and reaction-dominant regime.
In the reaction-limited and transport-dominant parameter regime, we saw that our multiple-component kinetics system reduces to a nonlinear set of IDEs for the reacting species concentrations. In turn, we saw this system further reduces to a nonlinear set of ODEs—our ERC equations. In addition to deriving a set of ERC equations for the injection phase of our multiple-ligand model, we also presented ERC equations for the wash phase, and for our multiple receptor model. A distinct advantage of our ERC equations is their simplicity, which is highlighted by the complex nature of the IDE system (3.13a)–(3.13d), (4.12a), (4.12b). Our ERC equations for this multiple-component system give scientists a tool to estimate rate constants—one which accounts for physically relevant transport effects. Furthermore, they are quickly, easily, and accurately solved using a standard ODE solver, like ODE45 in MATLAB.

To verify the accuracy of our ERC equations, we developed a semi-implicit finite difference algorithm for the IDE system (3.13a)–(3.13d), (4.12a), (4.12b), as well as the wash phase and our multiple-receptor model. We derived convergence and stability results when our method reduces to the bimolecular case. The singular integrand in the convolution integral and the form of $B$ results in a convergence rate of $O(\Delta x^2)$ when $Da \ll 1$ for $\mathcal{B}$, and $O(\Delta x^{4/3})$ otherwise. Our theoretical predictions for the bimolecular case agree with our numerical results for the multiple-ligand model, suggesting that our analysis generalizes to the multiple-ligand model. In addition, our method converges in time at the expected rate of $O(\Delta t^2)$, thanks to a second-order Adams-Bashforth time stepping scheme.

We found that our ERC equations are accurate not only for $Da \ll 1$, but they agree with our numerical solutions for a wide range of $Da$. Often experimentalists must place a large amount of receptors on the surface of the biosensor to obtain accurate measurements—this drives $Da$ up to $O(1)$ [6]. Our ERC equations are flexible enough to handle such physically relevant scenarios.

We have also studied the sensogram signals (2.21) and (2.31). For our multiple ligand model, we demonstrated that although a given sensogram signal may correspond
to two different sets of rate constants, in certain cases one can delineate the two readings. We proposed two methods for doing this: one based off of displacing (2.21), and another based on adjusting the uniform feed concentrations. For our multiple-receptor model, we proposed a method of fitting the rate constants to data.

We extended the theory for bimolecular reactions as well. In particular, we coupled the HPM with a traditional strained coordinates expansion to find an approximate solution to the bimolecular ERC equation. In finding approximations to (4.55) and (8.23) we have found the presence of secular terms can severely inhibit the performance of the HPM. After eliminating these terms by coupling the HPM with the traditional strained coordinates approach, we see that our approximation does quite well—far outperforming an expansion found using just the HPM alone. Furthermore, nonlinear initial value problems like the ones considered herein often exhibit secularities. Thus, our HPM and strained coordinates coupling may prove useful to others applying the HPM to nonlinear initial value problems.

11.2 Future Work

In addition to advancing knowledge of multiple-component surface volume reactions in optical biosensors, we have paved the way for new avenues of research as well. Cross-diffusion is the phenomenon in which a gradient in the concentration of one species induces a flux of another chemical species. This may affect ligand binding, and may be a subject of future investigation. In practice, receptor sites may be embedded in a dextran gel layer. Although the dextran gel layer has been studied using classical diffusion [9], fractional diffusive effects may be important. Therefore, we will investigate the effects of fractional diffusion in the dextran gel layer, for both single and multiple-component reactions. Additionally, one of the ligand molecules, say ligand $L_1$, may be larger than the other. The larger ligand $L_1$ molecule may obstruct ligand binding at the surface by blocking neighboring receptor sites. This is referred to as the steric hindrance effect, and may be subject to future study.
There are certainly questions left to answer concerning our numerical method, the first of which being stability as discussed in Subsection 5.3. In addition, a method with a higher order of spatial accuracy is desirable. A greater degree of accuracy may be found through implementing the method of lines as described in [29]; in the future we would like to further investigate the convergence of this method, try different basis functions, and extend this approach to our multiple component system.

We would also like to apply the HPM to the full IDEs (4.48) and (8.22). The spatial dependence introduced by the fractional integral adds another dimension of complexity to the problem, especially given the nonlinear effects and multiple time scales.

Current and future use of biosensor technology relies on accurate mathematical analysis. On the other hand, new and exciting applications of mathematics have been found in studying surface-volume reactions in optical biosensors. We hope that the work herein, and continued study of surface-volume reactions in optical biosensors, will spark advancements in both biochemistry and mathematics.
REFERENCES


Appendix A

PARAMETER VALUES

We tabulate physically relevant dimensional parameter values from the literature [5], [13] [24], [27] below.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>( \tilde{D}_1 )</th>
<th>( \tilde{D}_2 )</th>
<th>( \tilde{k}_a )</th>
<th>( \tilde{k}_d )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>( (10^{-7} \text{ cm}^2/\text{s}) )</td>
<td>( (10^{-7} \text{ cm}^2/\text{s}) )</td>
<td>( (10^8 \text{ cm}^3/(\text{mol} \cdot \text{s}) )</td>
<td>( (10^{-3} \text{s}^{-1}) )</td>
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<td>Biacore T200 Data File</td>
<td>( 10^{-5} - 3 \times 10^{-10} )</td>
<td>( 10^{-2} - 10^3 )</td>
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<td></td>
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<tr>
<td>Rich et al. (2008)</td>
<td>( 10^{-4} - 10^{-2} )</td>
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<td>Torre et al. (2000)</td>
<td>4.0</td>
<td>6.88</td>
<td></td>
<td></td>
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<tr>
<td>Yarmush et al. (1996)</td>
<td>(.5 - 5 \times 10^{-1} )</td>
<td>8.9</td>
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Table A.1: Dimensional parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>( \tilde{H} )</th>
<th>( \tilde{L} )</th>
<th>( \tilde{W} )</th>
<th>( \tilde{R}_T )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>( (1 \text{ cm}) )</td>
<td>( (1 \text{ cm}) )</td>
<td>( (1 \text{ cm}) )</td>
<td>( (10^{-12} \text{ mol/cm}^2) )</td>
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<td></td>
<td></td>
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<tr>
<td>Rich et al. (2008)</td>
<td>.05</td>
<td>2</td>
<td>1.3</td>
<td>( 1.11 \times 10^{-1} - 2.33 \times 10^1 )</td>
</tr>
<tr>
<td>Torre et al. (2000)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yarmush et al. (1996)</td>
<td>.25 - 4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table A.2: Dimensional parameter values (continued).
Table A.3: Dimensional parameter values (continued).

We now comment on some of the values in Tables A.1–A.2. Data for the diffusion rates of the polymerase molecules through a typical buffer fluid is unavailable. As an approximation, we obtained the values for $\tilde{D}_1$ and $\tilde{D}_2$ in the study of Torres et al. In particular, we chose the values in this study which are closest to the molecular weights of our ligands. We have neglected the effects of cross-diffusion; this may be a subject of future investigation.

Rich et al. (2008) conducted experiments using a single ligand only. Thus, for our multiple ligand model, throughout we take

$$2.96 \times 10^{-12} \text{mol/cm}^3 \leq \tilde{C}_{u,i} \leq 2 \times 10^{-10} \text{mol/cm}^3. \quad (A.1)$$

Since the dimensional rate constants are certainly not tabulated in the literature, we consider values that fall within the BIAcore T200’s wide detection range, i.e.:

$$10^3 \text{ cm}^3/(\text{mol} \cdot \text{s}) \leq i\tilde{k}_a \leq 3 \times 10^9 \text{ cm}^3/(\text{mol} \cdot \text{s}), \quad (A.2)$$

$$10^3 \text{ cm}^3/(\text{mol} \cdot \text{s}) \leq j\tilde{k}_a \leq 3 \times 10^9 \text{ cm}^3/(\text{mol} \cdot \text{s}), \quad (A.3)$$

$$10^{-5} \text{ s}^{-1} \leq i\tilde{k}_d \leq 10^{-5} \text{ s}^{-1}, \quad (A.4)$$

$$10^{-5} \text{ s}^{-1} \leq j\tilde{k}_d \leq 1 \text{ s}^{-1}. \quad (A.5)$$

Using the dimensional values above, we calculated extremal bounds bounds on the main dimensionless variables in our model.
We now discuss the values in Table A.4. In calculating the Reynolds number we used the kinematic viscositiy of water at 20° Celsius of $\bar{\nu} = \frac{\bar{u}}{\bar{\rho}} = 10^{-2}$ cm²/s [4], rather than the dynamic viscosity $\bar{\mu}$. Our bounds for $C_t$ in Table A.4 are based off of the the bounds for $\bar{C}_u$ given in Table A.3, although in practice this parameter will be adjusted as the experimentalist desires. For convenience, we have taken $C_t = D_t = F_t = 1$ for all of our computations, unless noted otherwise.

Moreover, one may be concerned about the lower bound on the Péclet number, the upper bound on $D_D$, and the upper bound on the Damköhler number. All of these parameters are given in Table A.4. The table below summarizes these parameters:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\epsilon$</td>
<td>.02–.025</td>
</tr>
<tr>
<td>Re</td>
<td>$1.23 \times 10^{-4}$–.361</td>
</tr>
<tr>
<td>Pe</td>
<td>$1.78–5.24 \times 10^4$</td>
</tr>
<tr>
<td>$D$</td>
<td>$1.21 \times 10^{-6}$–.192</td>
</tr>
<tr>
<td>$D_D$</td>
<td>$1.78 \times 10^{-6}$–57.90</td>
</tr>
<tr>
<td>Da</td>
<td>$4.31 \times 10^{-7}$–3.60 $\times 10^4$</td>
</tr>
<tr>
<td>$i K_a$</td>
<td>$2.05 \times 10^{-9}$–780</td>
</tr>
<tr>
<td>$j K_a$</td>
<td>$2.05 \times 10^{-9}$–780</td>
</tr>
<tr>
<td>$i K_d$</td>
<td>$6.94 \times 10^{-6}$–1.3 $\times 10^4$</td>
</tr>
<tr>
<td>$j K_d$</td>
<td>$6.94 \times 10^{-6}$–1.3 $\times 10^4$</td>
</tr>
<tr>
<td>$2 K_a$</td>
<td>$2.63 \times 10^{-12}$–3.80 $\times 10^{11}$</td>
</tr>
<tr>
<td>$j K_a$</td>
<td>$2.63 \times 10^{-12}$–3.80 $\times 10^{11}$</td>
</tr>
<tr>
<td>$i K_d$</td>
<td>$8.89 \times 10^{-9}$–6.33 $\times 10^{11}$</td>
</tr>
<tr>
<td>$j K_d$</td>
<td>$8.89 \times 10^{-9}$–6.33 $\times 10^{11}$</td>
</tr>
<tr>
<td>$C_t$</td>
<td>.0148–67.56</td>
</tr>
<tr>
<td>$D_t$</td>
<td>.5813</td>
</tr>
<tr>
<td>$F_t$</td>
<td>.008–39.28</td>
</tr>
</tbody>
</table>

Table A.4: Dimensionless parameters.
extremal bounds were calculated using a flow rate of 1 $\mu$L/min—the slowest flow rate possible on the BIAcore T200. Even with the fastest reactions, one can still design the experiment in such a way as to minimize transport effects by increasing the flow rate $\tilde{Q}$, decreasing the initial empty receptor concentration $\tilde{R}_T$, and decreasing the inflow concentration. In the case of the fastest reaction $\tilde{k}_a = 3 \times 10^9$ cm$^3$/(mol $\cdot$ s), we can take:

$$\tilde{Q} = 300 \ \mu$L/min
$$
$$\tilde{V} = .576 \ \text{cm/s}
$$
$$\tilde{R}_T = 2 \times 10^{-13} \ \text{mol/cm}^2
$$
$$\tilde{C}_{2,u} = 2.96 \times 10^{-10} \ \text{mol/cm}^3,
$$
$$\tilde{H} = .04 \ \text{cm},
$$

(here we have used the minimum bound on $\tilde{H}$). These choices yield the dimensionless parameters

$$\text{Re} = .072,
$$
$$\text{Pe} = 1048.19,
$$
$$D_D = .091,
$$
$$\tilde{k}_a = .031,
$$
$$\text{Da} = 3.98.
$$

These values are perfectly in line with our analysis, and the validity of our Effective Rate Constant equations.

Furthermore, the lower bound of 1 $\mu$L/min also resulted in larger upper bounds of $\tilde{i}k_a$, $\tilde{j}k_a$, $\tilde{i}k_d$, and $\tilde{j}k_d$. For our simulations, we kept $\tilde{i}K_a$, $\tilde{j}K_a$, $\tilde{i}K_d$, and $\tilde{j}K_d$ between 0 and 100. In addition, the bounds for the rate constants in our multiple-receptor model are the same as the bounds for the rate constants in our multiple ligand model, as given in Tables A.1 and A.4.
Appendix B

FINITE DIFFERENCE ALGORITHM DERIVATION

In Chapter 5 we presented the semi-discretized IDE system (5.1a)–(5.1c) and (5.4)–(5.5). Moreover, we stated that one can write the fully discretized system as (5.8), along with the associated initial conditions. In this Appendix, we go through the mechanics of this process. Although the algebra is a nightmare, the process itself is simple and can be broken up into three steps. The first two are: spatially discretize (5.4) and (5.5) using the trapezoidal rule (at equally spaced nodes $x_i$, for $i = 0, \ldots, N$), and discretize the time derivatives using the standard backwards difference operator (5.7). This results in a linear system in the variables $\frac{d}{dx_{i,n+1}}$, $j = 1, 12, 2$, whose solution is given by (5.8). With (5.8) we are now in a position to march along in both space and time as described in Subsection 5.1. That is, at each time step (i.e. for fixed $n$), we start at the node $x_0 = 0$, and use (5.8) and (5.9) to successively solve for the values $B_{i,n+1}^j$ for $l = 1, \ldots, N$. Then we increase $n$, and march downstream from $x_0 = 0$, to $x_N = 1$ again; we may run as many iterations as we wish. See Figure 5.1 for a schematic of this process.

We have also applied this numerical method to approximate $B$ during the wash phase (see (3.13a)–(3.13c), using (4.44) and (4.45)), and to approximate $B$ as given in our multiple-receptor model (see (7.2a), (7.2b) using (7.3) or (7.4)). We will discuss these cases as well; however we begin with the injection phase of our multiple-ligand model.

As a first step, we apply the trapezoidal rule and the backwards difference
operator (5.7) to discretize (5.2) and (5.3). Hence, we compute:

\[
\int_0^{x_i} \left( \frac{\partial B_i}{\partial t}(x - \nu) - \frac{\partial B_{i,n+1}}{\partial t} \right) d\nu + 3x_i^{1/3} \frac{dB_{i,n+1}}{dt} \Delta t
\]

\[
= \sum_{j=1}^{i-1} \frac{\Delta x}{x_j^{2/3} \Delta t} (d B_{i-j,n+1} - d B_{i,j,n+1}) + \frac{\Delta x}{2x_i^{2/3} \Delta t} (d B_{0,n+1} - d B_{i,n+1}) + 3x_i^{1/3} \frac{dB_{i,n+1}}{dt}
\]

\[
= \Delta x^{1/3} \left[ \sum_{j=1}^{i-1} \frac{1}{2j/3} (d B_{i-j,n+1} - d B_{i,j,n+1}) + \frac{1}{2(i/3)} (d B_{0,n+1} - d B_{i,n+1}) + \frac{3}{2i/3} d B_{i,n+1} \right]
\]

\[
= \Delta x^{1/3} \left( c d B_{i,n+1} + \text{SUM}_2 B_1 + \frac{1}{2i/3} d B_{0,n+1} \right).
\]

Here we have defined:

\[
c = 3i^{1/3} - \frac{1}{2i/3} - \text{SUM}_1, \quad (\text{B.1})
\]

\[
\text{SUM}_1 = \sum_{j=1}^{i-1} \frac{1}{j^{2/3}} \quad (\text{B.2})
\]

\[
\text{SUM}_2 B_1 = \sum_{j=1}^{i-1} \frac{1}{j^{2/3}} d B_{i-j,n+1}. \quad (\text{B.3})
\]

Additionally, we take \(\text{SUM}_1 = \text{SUM}_2 B_1 = 0\) when \(i = 0\). Therefore, the fully discretized convolution integrals \(C_{i,n+1}^1\) and \(C_{i,n+1}^2\) are:

\[
C_{i,n+1}^1 = 1 - \frac{D_i^{1/3}}{F_i^{3/3} \Gamma(2/3) \Delta t} \left[ c \left( d B_{i,n+1} + d B_{i,n+1}^{12} \right) + \text{SUM}_2 B_1 + \text{SUM}_2 B_{12} \right]
\]

\[
+ \frac{1}{2i/3} \left( d B_{0,n+1} + d B_{0,n+1}^{12} \right), \quad (\text{B.4})
\]

\[
C_{i,n+1}^2 = 1 - \frac{\text{Da} \Delta x^{1/3}}{3^{1/3} \Gamma(2/3) \Delta t} \left[ c \left( d B_{i,n+1}^{12} + d B_{i,n+1}^2 \right) + \text{SUM}_2 B_{12} + \text{SUM}_2 B_2 \right]
\]

\[
+ \frac{1}{2i/3} \left( d B_{0,n+1}^{12} + d B_{0,n+1}^2 \right), \quad (\text{B.5})
\]

where \(\text{SUM}_2 B_{12}, \text{SUM}_2 B_2\) are defined analogously to \(\text{SUM}_2 B_1\). These definitions are motivated by our implementation in MATLAB. For convenience we define:

\[
\omega_1 = \frac{D_i^{1/3}}{F_i^{3/3} \Gamma(2/3)}, \quad \omega_2 = \frac{1}{3^{1/3} \Gamma(2/3)}.
\]
We then discretize the left hand side of (5.1a)–(5.1c), and substitute (B.4) and (B.5) into the resulting system:

\[
\frac{dB^1_{i,n+1}}{\Delta t} = (1 - B_{i,n}^\Sigma) \left\{ 1 - \frac{\omega_1 Da \Delta x^{1/3}}{\Delta t} \left[ c \left( dB^1_{i,n+1} + dB^{12}_{i,n+1} \right) + \text{SUM}_2 B_1 + \text{SUM}_2 B_{12} \right.ight.
\]

\[
+ \frac{1}{2\sqrt{3}} \left( dB^1_{0,n+1} + dB^{12}_{0,n+1} \right) \left\} \right. - K_d B^1_{i,n} \]

\[
- \frac{1}{2} K_a B^1_{i,n} \left\{ 1 - \frac{\omega_2 Da \Delta x^{1/3}}{\Delta t} \left[ c \left( dB^{12}_{i,n+1} + dB^2_{i,n+1} \right) + \text{SUM}_2 B_{12} + \text{SUM}_2 B_2 \right. \right.
\]

\[
+ \frac{1}{2\sqrt{3}} \left( dB^{12}_{0,n+1} + dB^2_{0,n+1} \right) \left\} \right. + \frac{1}{2} K_d B^1_{i,n},
\]

\[
\frac{dB^{12}_{i,n+1}}{\Delta t} = \frac{1}{2} K_a B^1_{i,n} \left\{ 1 - \frac{\omega_2 Da \Delta x^{1/3}}{\Delta t} \left[ c \left( dB^1_{i,n+1} + dB^{12}_{i,n+1} \right) + \text{SUM}_2 B_{12} + \text{SUM}_2 B_2 \right. \right.
\]

\[
+ \frac{1}{2\sqrt{3}} \left( dB^1_{0,n+1} + dB^{12}_{0,n+1} \right) \left\} \right. - \frac{1}{2} K_d B^{12}_{i,n},
\]

\[
\frac{dB^2_{i,n+1}}{\Delta t} = \frac{2}{3} K_a B^1_{i,n} - \frac{2}{3} K_a B^2_{i,n} \left\{ 1 - \frac{\omega_1 Da \Delta x^{1/3}}{\Delta t} \left[ c \left( dB^1_{i,n+1} + dB^{12}_{i,n+1} \right) + \text{SUM}_2 B_1 \right. \right.
\]

\[
+ \text{SUM}_2 B_{12} + \frac{1}{2\sqrt{3}} \left( dB^1_{0,n+1} + dB^{12}_{0,n+1} \right) \left\} \right. \left. + K_a \left( 1 - B_{i,n}^\Sigma \right) \right\{ 1 - \frac{\omega_2 Da \Delta x^{1/3}}{\Delta t} \left[ c \left( dB^{12}_{i,n+1} + dB^2_{i,n+1} \right) + \text{SUM}_2 B_{12} \right. \right.
\]

\[
+ \text{SUM}_2 B_2 + \frac{1}{2\sqrt{3}} \left( dB^{12}_{0,n+1} + dB^2_{0,n+1} \right) \left\} \right. - \frac{1}{2} K_d B^2_{i,n}.\]

We now have a linear system for \( dB^j_{i,n+1}, \ j = 1, 12, 2, \) whose solution is given by (5.8). To show this, we simply need to move the terms \( dB^j_{i,n+1} \) to the left hand side, and write the resulting system in matrix-vector form. First, we define the forcing vector:

\[
f_{i,n+1} = \begin{pmatrix}
1 - \frac{\omega_1 Da \Delta x^{1/3}}{\Delta t} \left[ \text{SUM}_2 B_1 + \text{SUM}_2 B_{12} + \frac{1}{2\sqrt{3}} \left( dB^1_{0,n+1} + dB^{12}_{0,n+1} \right) \right] \\
0 \\
2 K_a \left\{ 1 - \frac{\omega_2 Da \Delta x^{1/3}}{\Delta t} \left[ \text{SUM}_2 B_{12} + \text{SUM}_2 B_2 + \frac{1}{2\sqrt{3}} \left( dB^{12}_{0,n+1} + dB^2_{0,n+1} \right) \right] \right\} 
\end{pmatrix}.\]

We employ the notation \( (f_{i,n+1})_j, \) for \( j = 1, 2, 3, \) to refer to the element in the \( j^{th} \) row of this vector. Now, rearranging all the terms containing \( dB^j_{i,n+1} \) to the left hand
side we have:

\[
\begin{align*}
\frac{d^2 B_{i,n+1}}{\Delta t^2} &+ \frac{c_d \Delta x^{1/3}}{\Delta t} \left[ \omega_1 \left( 1 - B_{i,n}^\Sigma \right) \left( d B_{i,n+1}^1 + d B_{i,n+1}^2 \right) - \omega_2 \frac{1}{2} K_a B_{i,n}^1 \left( d B_{i,n+1}^2 + d B_{i,n+1}^2 \right) \right] \\
&= - \left( B_{i,n}^\Sigma \right) \left\{ 1 - D a \omega_1 \Delta x^{1/3} \left[ \text{SUM} B_{i+2}^1 + \text{SUM} B_{i+2} - \frac{1}{2} \Delta \frac{1}{2} \right] \left( d B_{i,n+1}^2 + d B_{i,n+1}^2 \right) \right\} \\
&\quad - \frac{1}{2} K_a B_{i,n}^1 \left\{ 1 - D a \Delta x^{1/3} \left[ \text{SUM} B_{i+2}^1 + \text{SUM} B_{i+2} - \frac{1}{2} \Delta \frac{1}{2} \right] \left( d B_{i,n+1}^2 + d B_{i,n+1}^2 \right) \right\} \\
&\quad - \frac{1}{2} K_d B_{i,n}^1 + \frac{1}{2} K_d B_{i,n}^2 + (f_{i,n+1})_1,
\end{align*}
\]

To write the fully discretized form of (5.1a)–(5.1c) in matrix-vector form (5.8), we now only need to specify the matrices $M_{i,n}$ and $A_{i,n+1}$. For simplicity, when writing the elements of these matrices we write $m_{ij}$ to refer to the element of the $i^{th}$ row and the $j^{th}$ column of $M_{i,n}$, and use a similar notation when listing the elements of $A_{i,n+1}$. The
elements of $M_{i,n}$ are:

\[ m_{11} = 1 + Da \omega_1 c \Delta x^{1/3} \left( 1 - B^\Sigma_{i,n} \right), \]
\[ m_{12} = c Da \Delta x^{1/3} \left[ \omega_1 \left( 1 - B^\Sigma_{i,n} \right) - \omega_2 K_a B^1_{i,n} \right], \]
\[ m_{13} = -\omega_1 c Da \Delta x^{1/3} \frac{1}{2} K_a B^1_{i,n}, \]
\[ m_{21} = \omega_1 c Da \Delta x^{1/3} \frac{1}{2} K_a B^2_{i,n}, \]
\[ m_{22} = 1 + c Da \Delta x^{1/3} \left( \omega_1 K_a B^2_{i,n} + \omega_2 K_a B^1_{i,n} \right), \]
\[ m_{23} = \omega_2 c Da \Delta x^{1/3} \frac{1}{2} K_a B^1_{i,n}, \]
\[ m_{31} = -$$\omega_1 c Da \Delta x^{1/3} \frac{1}{2} K_a B^2_{i,n}, \]
\[ m_{32} = c Da \Delta x^{1/3} \left[ -\omega_1 K_a B^2_{i,n} + \omega_2 K_a \left( 1 - B^\Sigma_{i,n} \right) \right], \]
\[ m_{33} = 1 + \omega_2 c Da \Delta x^{1/3} \frac{1}{2} K_a \left( 1 - B^\Sigma_{i,n} \right). \]
and entries of $A_{i,n+1}$ are given by:

$$a_{11} = \left\{1 - \frac{\omega_1 Da \Delta x^{1/3}}{\Delta t}\left[\text{SUM}2_{B_1} + \text{SUM}2_{B_{12}} + \frac{1}{2i^{2/3}} \left(\text{d}B_{i,n+1}^1 + \text{d}B_{0,n+1}^{12}\right)\right]\right\}$$

$$+ \frac{1}{2} K_a \left\{1 - \frac{\omega_2 Da \Delta x^{1/3}}{\Delta t}\left[\text{SUM}2_{B_{12}} + \text{SUM}2_{B_2} + \frac{1}{2i^{2/3}} \left(\text{d}B_{i,n+1}^{12} + \text{d}B_{0,n+1}^2\right)\right]\right\} + K_d,$$

$$a_{12} = \left\{1 - \frac{\omega_1 Da \Delta x^{1/3}}{\Delta t}\left[\text{SUM}2_{B_1} + \text{SUM}2_{B_{12}} + \frac{1}{2i^{2/3}} \left(\text{d}B_{i,n+1}^1 + \text{d}B_{0,n+1}^{12}\right)\right]\right\} - \frac{1}{2} K_d,$$

$$a_{13} = \left\{1 - \frac{\omega_1 Da \Delta x^{1/3}}{\Delta t}\left[\text{SUM}2_{B_1} + \text{SUM}2_{B_{12}} + \frac{1}{2i^{2/3}} \left(\text{d}B_{i,n+1}^1 + \text{d}B_{0,n+1}^{12}\right)\right]\right\},$$

$$a_{21} = -\frac{1}{2} K_a \left\{1 - \frac{\omega_2 Da \Delta x^{1/3}}{\Delta t}\left[\text{SUM}2_{B_{12}} + \text{SUM}2_{B_2} + \frac{1}{2i^{2/3}} \left(\text{d}B_{i,n+1}^{12} + \text{d}B_{0,n+1}^2\right)\right]\right\},$$

$$a_{22} = \left(\frac{1}{2} K_d + \frac{1}{2} K_d\right),$$

$$a_{23} = -\frac{2}{1} K_a \left\{1 - \frac{\omega_1 Da \Delta x^{1/3}}{\Delta t}\left[\text{SUM}2_{B_1} + \text{SUM}2_{B_{12}} + \frac{1}{2i^{1/3}} \left(\text{d}B_{i,n+1}^1 + \text{d}B_{0,n+1}^{12}\right)\right]\right\},$$

$$a_{31} = 2 K_a \left\{1 - \frac{\omega_2 Da \Delta x^{1/3}}{\Delta t}\left[\text{SUM}2_{B_{12}} + \text{SUM}2_{B_2} + \frac{1}{2i^{2/3}} \left(\text{d}B_{i,n+1}^{12} + \text{d}B_{0,n+1}^2\right)\right]\right\},$$

$$a_{32} = 2 K_a \left\{1 - \frac{\omega_1 Da \Delta x^{1/3}}{\Delta t}\left[\text{SUM}2_{B_{12}} + \text{SUM}2_{B_2} + \frac{1}{2i^{2/3}} \left(\text{d}B_{i,n+1}^{12} + \text{d}B_{0,n+1}^2\right)\right]\right\} - \frac{1}{2} K_d,$$

$$a_{33} = 2 K_a \left\{1 - \frac{\omega_1 Da \Delta x^{1/3}}{\Delta t}\left[\text{SUM}2_{B_{12}} + \text{SUM}2_{B_2} + \frac{1}{2i^{2/3}} \left(\text{d}B_{i,n+1}^{12} + \text{d}B_{0,n+1}^2\right)\right]\right\} + \frac{1}{2} K_a \left\{1 - \frac{\omega_1 Da \Delta x^{1/3}}{\Delta t}\left[\text{SUM}2_{B_1} + \text{SUM}2_{B_{12}} + \frac{1}{2i^{2/3}} \left(\text{d}B_{i,n+1}^1 + \text{d}B_{0,n+1}^{12}\right)\right]\right\} + 2 K_d.$$

In addition, we enforce the initial condition $B_{i,0} = 0$ for $i = 0, \ldots, N$. With the above definitions of $M_{i,n}$, $A_{i,n+1}$, and $f_{i,n+1}$, we may write the fully-discretized IDE system as (5.8), as desired.

We also applied a semi-implicit finite difference algorithm to approximate $B$ during the wash phase, and the derivation for this phase is very similar. During the wash (4.12a) and (4.12b) are replaced by (4.44) and (4.45), thus we simply replace
Observe that this leaves our mass matrix $M_{i,n}$ unchanged; $A_{i,n}$ and $f_{i,n+1}$ become:

$$C^1_{i,n+1} = - \frac{Dax^{1/3}}{D_t^{2/3} \Gamma(2/3) \Delta t} \left[ c \left( dB^1_{i,n+1} + dB^{12}_{i,n+1} \right) + \text{SUM2}_{B_1} + \text{SUM2}_{B_2} \right] + \frac{1}{2 \xi^{2/3}} \left( dB^1_{0,n+1} + dB^{12}_{0,n+1} \right),$$

$$C^2_{i,n+1} = - \frac{Dax^{1/3}}{\Gamma(2/3) \Delta t} \left[ c \left( dB^{12}_{i,n+1} + dB^2_{i,n+1} \right) + \text{SUM2}_{B_12} + \text{SUM2}_{B_2} \right] + \frac{1}{2 \xi^{2/3}} \left( dB^{12}_{0,n+1} + dB^2_{0,n+1} \right).$$

(B.6) and (B.7) by:

$$C^1_{i,n+1} = - \frac{Dax^{1/3}}{D_t^{2/3} \Gamma(2/3) \Delta t} \left[ c \left( dB^1_{i,n+1} + dB^{12}_{i,n+1} \right) + \text{SUM2}_{B_1} + \text{SUM2}_{B_2} \right] + \frac{1}{2 \xi^{2/3}} \left( dB^1_{0,n+1} + dB^{12}_{0,n+1} \right),$$

$$C^2_{i,n+1} = - \frac{Dax^{1/3}}{\Gamma(2/3) \Delta t} \left[ c \left( dB^{12}_{i,n+1} + dB^2_{i,n+1} \right) + \text{SUM2}_{B_12} + \text{SUM2}_{B_2} \right] + \frac{1}{2 \xi^{2/3}} \left( dB^{12}_{0,n+1} + dB^2_{0,n+1} \right).$$

Observe that this leaves our mass matrix $M_{i,n}$ unchanged; $A_{i,n}$ and $f_{i,n+1}$ become:

$$a_{11} = - \frac{\omega_1 Dax^{1/3}}{\Delta t} \left[ \text{SUM2}_{B_1} + \text{SUM2}_{B_12} + \frac{1}{2 \xi^{1/3}} \left( dB^1_{0,n+1} + dB^{12}_{0,n+1} \right) \right],$$

$$a_{12} = - \frac{\omega_1 Dax^{1/3}}{\Delta t} \left[ \text{SUM2}_{B_2} + \text{SUM2}_{B_12} + \frac{1}{2 \xi^{2/3}} \left( dB^1_{0,n+1} + dB^{12}_{0,n+1} \right) \right] - \frac{1}{2} K_d,$n

$$a_{13} = - \frac{\omega_1 Dax^{1/3}}{\Delta t} \left[ \text{SUM2}_{B_1} + \text{SUM2}_{B_12} + \frac{1}{2 \xi^{2/3}} \left( dB^1_{0,n+1} + dB^{12}_{0,n+1} \right) \right],$$

$$a_{21} = \frac{\omega_1 Dax^{1/3}}{\Delta t} \left[ \text{SUM2}_{B_1} + \text{SUM2}_{B_12} + \frac{1}{2 \xi^{2/3}} \left( dB^1_{0,n+1} + dB^{12}_{0,n+1} \right) \right],$$

$$a_{22} = (\frac{1}{2} K_d + \frac{1}{2} K_d),$$

$$a_{23} = - \frac{\omega_1 Dax^{1/3}}{\Delta t} \left[ \text{SUM2}_{B_1} + \text{SUM2}_{B_12} + \frac{1}{2 \xi^{1/3}} \left( dB^1_{0,n+1} + dB^{12}_{0,n+1} \right) \right],$$

$$a_{31} = - \frac{\omega_1 Dax^{1/3}}{\Delta t} \left[ \text{SUM2}_{B_2} + \text{SUM2}_{B_12} + \frac{1}{2 \xi^{2/3}} \left( dB^1_{0,n+1} + dB^{12}_{0,n+1} \right) \right],$$

$$a_{32} = - \frac{\omega_1 Dax^{1/3}}{\Delta t} \left[ \text{SUM2}_{B_12} + \text{SUM2}_{B_2} + \frac{1}{2 \xi^{2/3}} \left( dB^1_{0,n+1} + dB^{12}_{0,n+1} \right) \right] - \frac{1}{2} K_d,$n

$$a_{33} = - \frac{\omega_1 Dax^{1/3}}{\Delta t} \left[ \text{SUM2}_{B_12} + \text{SUM2}_{B_2} + \frac{1}{2 \xi^{2/3}} \left( dB^1_{0,n+1} + dB^{12}_{0,n+1} \right) \right] + \frac{1}{2} K_d,$n

and

$$f_{i,n+1} = \begin{pmatrix}
- \frac{\omega_1 Dax^{1/3}}{\Delta t} \left[ \text{SUM2}_{B_1} + \text{SUM2}_{B_12} + \frac{1}{2 \xi^{2/3}} \left( dB^1_{0,n+1} + dB^{12}_{0,n+1} \right) \right] \\
0 \\
- \frac{2 K_\omega Da x^{1/3}}{\Delta t} \left[ \text{SUM2}_{B_12} + \text{SUM2}_{B_2} + \frac{1}{2 \xi^{2/3}} \left( dB^1_{0,n+1} + dB^{12}_{0,n+1} \right) \right]
\end{pmatrix}.$$
In addition, we enforce the initial condition $B_{i,0} = Af$, for $i = 0, \ldots, N$. We may proceed to march along in space and time as in the injection phase.

It remains to describe our numerical approximation for our multiple receptor model (7.2a)–(7.2b), using (7.3) or (7.4). We discretize these equations using: (7.7a)–(7.7b), (7.8) during the injection phase, and (7.9) during the wash phase. We employ the same strategy to handle the singularity as in the multiple-ligand model: by subtracting out the singularity at $\nu = 0$ in the convolution integral. After writing the fully discretized system in a form similar to (5.8), we march along in time and space as outlined in Subsection 5.1.

We only need to specify the form of $M_{i,n}$, $A_{i,n+1}$, and $f_{l,n+1}$. During the injection phase the fully-discretized version of $C_{i,n+1}$ is given by:

$$
C_{i,n+1} = 1 - \frac{Da\Delta x^{1/3}}{3^{1/3} \Gamma(2/3) \Delta t} \left[ c \left( dB_{i,n+1}^f + dB_{i,n+1}^p \right) + \text{SUM}2_B + \text{SUM}2_B \right]
$$

where $c$, $\text{SUM}2_B$, and $\text{SUM}2_B$ are defined analogously to (B.1)–(B.3). We may then substitute (B.8) into (7.7a) and (7.7b), and rearrange the resulting equations to solve for $\frac{dB_{i,n+1}}{\Delta t}$ (note in our multiple-receptor model $B = (B_f, B_p)^T$), and find $M_{i,n}$, $A_{i,n+1}$, and $f_{l,n+1}$:

$$
m_{11} = 1 + \frac{Da\Delta x^{1/3}}{3^{1/3} \Gamma(2/3)} \left( R_f - B_{i,n}^f \right),$$

$$
m_{12} = \frac{Da\Delta x^{1/3}}{3^{1/3} \Gamma(2/3)} \left( R_f - B_{i,n}^f \right),$$

$$
m_{21} = \frac{pK_a Da\Delta x^{1/3}}{3^{1/3} \Gamma(2/3)} \left( R_p - B_{i,n}^p \right),$$

$$
m_{22} = 1 + \frac{pK_a Da\Delta x^{1/3}}{3^{1/3} \Gamma(2/3)} \left( R_p - B_{i,n}^p \right);$$

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\( A_{t,n+1} \) is given by
\[
\begin{align*}
\alpha_{11} &= 1 - \frac{D \Delta x^{1/3}}{3^{1/3} \Gamma(2/3) \Delta t} \left[ \text{SUM2}_{B_t} + \text{SUM2}_{B_p} + \frac{1}{2^{2/3}} \left( d_{B_0,n+1}^f + d_{B_0,n+1}^p \right) \right] - \tau K_d, \\
\alpha_{12} &= 0, \\
\alpha_{21} &= 0, \\
\alpha_{22} &= p K_a \left[ 1 - \frac{D \Delta x^{1/3}}{3^{1/3} \Gamma(2/3) \Delta t} \left[ \text{SUM2}_{B_t} + \text{SUM2}_{B_p} + \frac{1}{2^{2/3}} \left( d_{B_0,n+1}^f + d_{B_0,n+1}^p \right) \right] \right] - p K_d;
\end{align*}
\]
and \( f_{t,n+1} \) is
\[
\begin{align*}
\begin{cases}
R_f \left[ 1 - \frac{D \Delta x^{1/3}}{3^{1/3} \Gamma(2/3) \Delta t} \left[ \text{SUM2}_{B_t} + \text{SUM2}_{B_p} + \frac{1}{2^{2/3}} \left( d_{B_0,n+1}^f + d_{B_0,n+1}^p \right) \right] \right] \\
(\mathcal{R}_p) p K_a \left[ 1 - \frac{D \Delta x^{1/3}}{3^{1/3} \Gamma(2/3) \Delta t} \left[ \text{SUM2}_{B_t} + \text{SUM2}_{B_p} + \frac{1}{2^{2/3}} \left( d_{B_0,n+1}^f + d_{B_0,n+1}^p \right) \right] \right] 
\end{cases}
\end{align*}
\]
During the injection phase we also enforce our initial condition \( B_{i,0} = 0 \), for \( i = 0, \ldots, N \). Finally, during the wash phase of our multiple-receptor experiment \( M_{i,n} \) is unchanged, and \( A_{t,n+1} \) and \( f_{t,n+1} \) become:
\[
\begin{align*}
\alpha_{11} &= -\frac{D \Delta x^{1/3}}{3^{1/3} \Gamma(2/3) \Delta t} \left[ \text{SUM2}_{B_t} + \text{SUM2}_{B_p} + \frac{1}{2^{2/3}} \left( d_{B_0,n+1}^f + d_{B_0,n+1}^p \right) \right] - \tau K_d, \\
\alpha_{12} &= 0, \\
\alpha_{21} &= 0, \\
\alpha_{22} &= -\frac{p K_a D \Delta x^{1/3}}{3^{1/3} \Gamma(2/3) \Delta t} \left[ \text{SUM2}_{B_t} + \text{SUM2}_{B_p} + \frac{1}{2^{2/3}} \left( d_{B_0,n+1}^f + d_{B_0,n+1}^p \right) \right] - p K_d;
\end{align*}
\]
and
\[
\begin{align*}
\begin{cases}
-\frac{R_f D \Delta x^{1/3}}{3^{1/3} \Gamma(2/3) \Delta t} \left[ \text{SUM2}_{B_t} + \text{SUM2}_{B_p} + \frac{1}{2^{2/3}} \left( d_{B_0,n+1}^f + d_{B_0,n+1}^p \right) \right] \\
(\mathcal{R}_p) p K_a \frac{D \Delta x^{1/3}}{3^{1/3} \Gamma(2/3) \Delta t} \left[ \text{SUM2}_{B_t} + \text{SUM2}_{B_p} + \frac{1}{2^{2/3}} \left( d_{B_0,n+1}^f + d_{B_0,n+1}^p \right) \right] 
\end{cases}
\end{align*}
\]
This concludes our finite-difference algorithm derivation.