A MATHEMATICAL FEASIBILITY ARGUMENT FOR THE USE OF APTAMERS IN CHEMOTHERAPY

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ABSTRACT. The primary purpose of this note is to give a proof in principle of the following: Suppose \( I \) denotes a drug (inhibitor) which must be distributed spatially throughout a cell, but prefers to remain outside the cell or at most be concentrated in a thin region just inside the cell membrane due to the transport properties of the cell membrane. Suppose that \( E \) is a a compound (protein, aptamer) that binds to \( I \), is expressed by the cell and remains in the cell.

Then it is possible for the rate constants \( k_1, k_{-1} \) in the mechanism:

\[
E + I \xrightarrow{k_1} P \xrightarrow{k_{-1}} E
\]

to be such that free drug will be distributed throughout the cell.

We show how this idea may be used to choose aptamer rate constants to aid in intra cellular enzyme inhibition via an extra cellular supplied drug.

1. INTRODUCTION

Living cells have evolved to have a lipid outer membrane that impedes the movement of most compounds into the cell cytoplasm and further into the nucleus. Proteins integral to the outer membranes of these cells facilitate the movement of necessary nutrients such as sugars into the cell. But, the membrane generally provides a barrier to the entry of unnatural or toxic compounds are generally kept out of the cell. Consequently, drugs used to treat illnesses that have a mechanistic basis inside cells must be designed to penetrate the cell membrane. The efficiency by which the drug can accumulate in the cell is an important aspect of the effectiveness of the drug.

Cancer drugs that inhibit cell division are one type of compound that needs to penetrate the cell membrane and accumulate intracellularly. Cancer cells in which the drug does not accumulate to sufficiently high a concentration are likely to survive. It is also frequently found that cancer cells that do survive a drug treatment have either increased their expression of a membrane protein that pumps the drug out of the cell or have increased the expression of the target protein of the drug. Thus, increased amounts of these drug pumps called Pgp (P-glycoprotein) or MDR (multi drug resistance protein) decreases the intracellular concentration of the drug [1, 2, 3].

Similarly, increased amounts of the target protein of the drug results in the drug being soaked up by the excess target protein with a consequent low, but functional portion of the target protein population free of drug and capable of activity. This process of increasing gene expression (amplification) with the resulting resistance to drugs is a common means by which cancer cells evade medical treatments. Thus, a means of combating the resistant condition would have useful medical applications. Here we propose that the intracellular concentration of drug might be increased by the presence, inside the cell, of an attractant for that drug.

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As the drug attractant we shall use an aptamer. Aptamers are small single stranded nucleic acid molecules that have been selected for tight and specific binding to another molecule; in this case the other molecule is the drug. Thus it is proposed that the presence of an aptamer with the appropriate kinetic parameters in the cell cytoplasm will draw its target drug into the cell and create a condition of higher intracellular free drug concentration than in the absence of the aptamer. Although pumps and target proteins are often increased 10-100-fold in resistant compared with drug-sensitive cells, there are reports of increases in the range of 2-5-fold resulting in resistance [4, 5, 6]. This observation suggests that cells can acquire resistance with a small increment in their content of certain proteins. It also suggests that the intracellular concentration of some drugs do not rise much above that needed for inhibiting the cellular event to which it is targeted. An aptamer that is specific for a particular drug and that is expressed in a particular cell could also be engineered to concentrate variants of the drug. The drug variant can be one that has attached to it a signaling moiety such as a radioisotope, say $^{18}$fluorine, for which the emitted signal penetrates solid tissues such as in the human body. Thus, the aptamer has the potential of providing a means of obtaining an image of the cells in which it is located.

2. Part 1: The chemical kinetics (Protein-Inhibitor)

In the first go around, we have an protein, an inhibitor, and a product denoted as $E, I, P$ according to the usual chemical conventions. We shall also use the notation $e(x,t) = [E](t)$ to underscore the fact that these species are distributed in space as well as time.

The mechanism is

$$E + I \xrightarrow{k_{1}} P \xrightarrow{k_{-1}} E$$

which we do not assume to be in equilibrium. We make the following assumptions,

1. The reaction takes place in a bounded region $\Omega$ of three space (to be viewed as the cytoplasm of a cell).
2. The proteins $P, E$ decay with the same rate constant $\mu > 0$.
3. The inhibitor species, $I$, is the only one of the three capable of molecular diffusion, which, for simplicity we take to be linear and isotropic.
4. The inhibitor may or may not decay. Let $\nu \geq 0$ denote this decay constant.
5. The cell functions as a source for the protein, i.e. there is a nonnegative function $S(x,t)$ defined on the region $\Omega$ for all nonnegative times such that $S$ denotes the cellular rate of production of the protein.

These assumptions and the law of mass action lead us to the following rate equations:

$$\frac{\partial e(x,t)}{\partial t} = k_{-1}p - k_{1}c_i - \mu e + S(x,t),$$

$$\frac{\partial p(x,t)}{\partial t} = -k_{-1}p + k_{1}c_i - \mu p,$$

$$\frac{\partial i(x,t)}{\partial t} = D \Delta i + k_{-1}p - k_{1}c_i - \nu i$$

where $\Delta$ denotes the usual Laplace operator. To these must be appended the initial conditions:

$$e(x,0) = e_0(x) \geq 0,$$

$$c(x,0) = c_0(x) \geq 0,$$

$$p(x,0) = p_0(x) \geq 0.$$
On the other hand, only one boundary condition is needed. These can be shown to be of Michaelis-Menten form, namely,

\[ D \partial_t i(x, t) = \frac{-K_c(x, t) - t_b}{K_m + i(x, t)} \]

where \( i_b \) is the background concentration of drug in the cell due solely to membrane transport, where \( K_c, K_m \) may be thought of Michaelis-Menten constants for transport of inhibitor through the cell membrane, \( \partial_n \) and where \( \partial_n \) denotes the usual outward normal derivative.\(^1\) Here \( c \) is the mean surface area concentration of transfer protein in the cell wall. (The units of \( c \) can be expressed in micro-moles per square micron for example. In this way the cell wall thickness is eliminated from the discussion below)

\(^1\)Imagine the cell wall of thickness \( \delta_w \) to be situated so that the \( x \)-axis is normal to the wall. The interior of the wall is then the set of points \( \{(x, y, z)| 0 < x < \delta_w \} \). Suppose \( p \) is the transfer protein in the cell wall and that \( I(x, y, z) \) is the concentration of inhibitor inside the cell or in the wall interior \( (x \leq \delta_w) \) while \( J(x, y, z) \) is the concentration of inhibitor exterior to the cell or interior to the cell \( (x > 0) \). Then we have the chemical reactions within the wall:

\[ I + p \overset{\kappa_1}{\underset{\kappa_2}{\rightleftharpoons}} \{I\} + \{p\} \overset{\kappa_3}{\underset{\kappa_4}{\rightleftharpoons}} J + p. \]

Regarding the concentrations of the intermediates \( \{I\}, \{J\} \) as being nearly constant, we obtain from mass action that

\[ [I] \left[ \begin{array}{c} \kappa_1 [I] \\ \kappa_2 [J] \end{array} \right] = \left[ \begin{array}{cc} \kappa_1 - \kappa_3 & -\kappa_3 \\ -\kappa_3 & \kappa_2 + \kappa_3 \end{array} \right] \left[ \begin{array}{c} [I] \\ [J] \end{array} \right] \]

or, upon solving, that

\[ \left[ \begin{array}{c} [I] \\ [J] \end{array} \right] = \left[ \begin{array}{c} [I] \\ [J] \end{array} \right] \frac{\kappa_1 [I]}{\kappa_2 [J]} \frac{\kappa_1 + \kappa_3 - \kappa_2}{\kappa_1 + \kappa_3} \]

where \( \kappa_2 = \kappa_1 + \kappa_3 \). Since the total concentration of transfer protein is fixed, \( P_0 \) say, we have, in the cell wall, that

\[ [I] + [I] + [J] = P_0 \]

and consequently:

\[ [I] = \frac{P_0}{1 + \kappa_2 [I] + \kappa_1 [J]} \]

and for \( j = -1, 2 \)

\[ \kappa_j = \frac{\kappa_1 + \kappa_3 + \kappa_2}{\kappa_0}. \]

Thus, from mass action we obtain:

\[ \frac{\partial [I]}{\partial t} = -\kappa_1 [I][P] + \kappa_1 \{I\} \]

\[ = -\left( \kappa_1 \kappa_3 [I] - \kappa_2 \kappa_3 \frac{[I]}{1 + \kappa_2 [I] + \kappa_1 [J]} \right). \]

Consider now a small control volume \( dv = \delta_v \delta_x \delta_z \) in the cell wall. The rate of accumulation of \( I \) (in micro moles per unit time) in this control volume is

\[ \delta_v \delta_x \frac{\partial [I]}{\partial t} \]

However, by Pick's first law, the flux of \( I, J \), out of the cell is \( -D \frac{\partial [I]}{\partial x} \). Therefore, into this control volume must flow

\[ -\delta_v \delta_x \delta_z \frac{\partial [I]}{\partial x} \]

micro moles per unit time. Thus, in order to have mass balance at the interface, we must have

\[ D \frac{\partial [I]}{\partial x} = \frac{\kappa_1 \kappa_3 [I] - \kappa_2 \kappa_3 [J]}{1 + \kappa_2 [I] + \kappa_1 [J]} = \frac{[I]}{[J]} \]

If, as is the situation here, \( [J] = J_0 \) is essentially constant and \( \kappa_1 \kappa_2 \kappa_3 \) is very small (i.e., most of the inhibitor prefers to be concentrated in the exterior of the cell in the absence of diffusion), there will be a little inhibitor in the cell at equilibrium. Its value is \( i_b = \frac{\kappa_2 \kappa_3 J_0}{(\kappa_1 + \kappa_3)} \). If we set \( K_c = \frac{\kappa_1 \kappa_2}{K_0} \) and \( K_m = \frac{1}{(1 + \kappa_1 J_0)} \), we obtain the boundary condition (2.4) if we agree to write \( c = \delta_v P_0 \). The quantity \( c \) may be viewed as the concentration of transfer protein in micromoles per unit area.
3. A SIMPLE "CONSERVATION" LAW AND STEADY STATE EQUATIONS

It is easy to see by adding the first two equations in (2.2) and a quadrature that

\[ \epsilon(x, t) + p(x, t) = [\epsilon_0(x) + p_0(x)]e^{-\mu t} + \int_0^t e^{-\mu(t-s)}S(x, s) \, ds, \]

(3.1)

The import of this is that we may eliminate one of the variables, \( p, \epsilon \) from further consideration in (2.2).

If \( S(x, t) \) is time independent, it is reasonable to entertain the possibility of steady states for (2.2). Let us set \( \varphi(x) = S(x, t)/\mu \) in this case. Let \( \psi(x, t) = \int_0^t e^{-\mu(t-s)}S(x, s) \, ds + [\epsilon_0(x) + p_0(x)]e^{-\mu t}. \) Then

\[ \lim_{t \to \infty} \psi(x, t) = \varphi(x). \]

If there were no source term, the conservation law tells us that the concentrations of \( \epsilon, p \) would vanish in the limit as \( t \to \infty. \) In this case, the concentration of inhibitor remaining in the system, call it \( I_r, \) would satisfy the steady state problem

\[ 0 = D\Delta I_r - \nu I_r \quad \text{in } \Omega, \]

(3.2)

\[ D\partial_n I_r(x) = \frac{cK_r[I(x) - i_b]}{K_m + I_r(x)} \quad \text{in } \partial\Omega. \]

If \( D = 0, \) then either \( I_r \) is identically zero (if \( \nu > 0 \)) or indeterminant if \( \nu = 0. \) On the other hand, if \( D > 0, \) this steady state problem may or may not support positive solutions. When \( D > 0 \) and \( \nu = 0, \) one sees from Green's identity that

\[ 0 = D \int_\Omega \Delta I_r \, dx = \int_{\partial\Omega} cK_r[I(x) - i_b] \frac{\partial I_r}{\partial n} \, ds \]

and consequently \( I_r = 0. \) Thus, in the case of long lived inhibitor, there can be no inhibitor present in the cell.

When there is a competition between decay and diffusion and such positive solutions exist, they must be regarded as arising from the effect of molecular transport of inhibitor across the cell membrane which would naturally occur in the absence of any source of protein.

Throughout the remainder of this discussion, we shall consider that \( \epsilon(., 0) = p(., 0) = \dot{i}(., 0) = 0. \) The system of interest then becomes:

\[ \frac{\partial \epsilon(x, t)}{\partial t} = (k_{-1} + \mu)(\psi - \epsilon) - k_1 \epsilon i, \]

\[ \frac{\partial \dot{i}(x, t)}{\partial t} = D\Delta \dot{i} + k_{-1}(\psi - \epsilon) - k_1 \epsilon i - \nu i. \]

(3.3)

Let \( E, P, I \) denote the steady state limits (assuming they exist) as \( t \to \infty \) of the variables \( \epsilon, p, i. \) The steady state boundary value problem becomes

\[ 0 = D\Delta I \quad I \left( \nu + \mu \frac{k_1 \varphi}{k_{-1} + k_1 \mu} \right) \quad \text{in } \Omega, \]

(3.4)

\[ D\partial_n I(x) = \frac{cK_r[I(x) - i_b]}{K_m + I(x)} \quad \text{in } \partial\Omega. \]

The question of solvability of this problem is not easily resolved. However, if there is a solution which is positive on the boundary, the minimum must occur in the interior and be positive there. (If the minimum were negative and larger than \( -k_{-1} - \mu)/k_1 \) (which must always hold), we would have \( I' < 0 \) at a negative minimum, an impossibility.)

Once this problem is solved, we can write:

\[ E(x) = \frac{(\mu + k_{-1})\varphi(x)}{k_1 I(x) + \mu + k_{-1}}, \]

(3.5)

\[ P(x) = \varphi(x) - E(x). \]

(Notice that \( P \geq 0 \) since \( E \leq \varphi. \) Since the boundary condition of Neumann type and nonlinear, the solution set may be empty or have several values depending on the range of the parameters.)
In order to obtain more or less explicit solutions of (3.5) and to discuss the stability of the solutions, we turn next to the one dimensional problem. The problem (3.4) is highly nonlinear and not solvable by elementary methods. In order that the solution be meaningful physically, we must require as a side condition that
\[ I(x) > 0 \text{ in } \Omega. \]

4. An Analysis of steady states in one dimension with constant source term \( \varphi(x) = \varphi_0. \)

Here we take the source to be a positive constant and ask the following question:
Suppose we have a solution of the problem\(^2\)
\[
0 = D I''(x) - I \left( \nu + \mu \frac{k_1 \varphi}{k_1 I(x) + k_{-1} + \mu} \right) \quad \text{in } [0, x_0],
\]
\[
D I'(0) = -\frac{c K_c (I(0) - i_b)}{K_m + I(0)},
\]
\[
D I'(0L) = \frac{c K_c (I(L) - i_b)}{K_m + I(L)},
\]
\[
I(0) = I(L) > 0.
\]

The maximum principle assures us that \( I(x) \geq 0 \text{ on } (0, L). \) It is easy to see that there are no constant solutions of this problem.

We pose the following question. Suppose we wish to have a minimum concentration of the inhibitor in the cell of concentration, \( I_m \) say. Given \( I(0) = I(L) \), how large must the diffusion coefficient be in order to insure that \( I(x) \geq I_m \text{ on } [0, L]? \)

The simplest way to answer this question is to observe that the minimum of the solution must occur at \( x = L/2 \) in view of the symmetry of the problem and the sign of the derivatives at the boundary conditions. Define
\[
H(z) = \int_0^2 w \left( \nu + \mu \frac{k_1 \varphi_0}{k_1 w + k_{-1} + \mu} \right) dw,
\]
\[
= \frac{\nu}{2} z^2 + \mu \varphi_0 \left[ z - \frac{k_{-1} + \mu}{k_1} \ln \left( 1 + z \frac{k_1}{k_{-1} + \mu} \right) \right],
\]
\[
G(z) = \frac{c K_c (z - i_b)}{K_m + z}.
\]

The function \( H(z) \) is only defined for \( 1 \leq z \), but in this problem \( z \) is further restricted to be nonnegative. In this range, \( H \) is strictly increasing and positive except at \( z = 0. \)

In view of the assumption that \( I(\tau_0) = I_m \), we obtain, after multiplying the differential equation by \( I' \) and integrating both sides of the resulting equation over the interval \([0, x_0]): \)
\[
0 < (I'(\tau_0))^2 - 2D H(I_m) + (1/D^2) [G^2(I(0)) - 2DH(I(0))].
\]

Consequently, \( D \) cannot be too small, i.e., if
\[
2D < \frac{G^2(I(0))}{H(I(0)) - H(I_m)},
\]

\(^2\)The existence and uniqueness of the solution is not in doubt. For \( x \in [0, L/2] \) it is given implicitly by:
\[
x = D \int_{r(0)}^{I(0)} \frac{dr}{\sqrt{G^2(I(0)) + 2DH(r)/H(I(0))}}
\]
and for \( x \in [L/2, L] \) by \( I(x) = I(L - x) \).

\(^3\)Similar issues can be addressed when \( \varphi \) is not constant, but the analysis is a bit trickier. We will discuss this case in the section on aptamers.
the diffusion gradient at \( x = 0 \) will not be strong enough to drive sufficient inhibitor across the entire half interval \([0, L/2]\) for this value of \( I(0) \) to achieve the value of \( I_m \). On the other hand, for all values of \( D \) such that

\[
2D \geq \frac{G^2(I(0))}{H(I(0)) - H(I_m)},
\]

The diffusion gradient at \( x = 0 \) will be strong enough to achieve a minimum value \( I_{m'} \geq I_m \) at \( x = L/2 \)

where

\[
I_{m'} = H^{-1}(H(I(0)) - G^2(I(0))/2D)).
\]

When \( I_m = I_{m'} \) it is more convenient to write

\[
H(I_m) = H(I(0)) \left(1 - \frac{G^2(I(0))}{2DH(I(0))}\right) = H(I(0)) \left(1 - \frac{F(I(0))}{2D}\right)
\]

where we have set

\[
F(z) = \frac{G^2(z)}{H(z)}.
\]

We need to know something about the behavior of the graph of \( F(z) \) for \( z > 0 \). It is not hard to check that

\[
\lim_{z \to +\infty} F(z) = 0
\]

and that

\[
\lim_{z \to 0} F(z) = +\infty.
\]

Moreover, \( F'(i_b) = F''(i_b) = 0 \).

Finally, it is not too hard to see that \( F(z) \) has a critical point \( (a point where \( F'(z) = 0 \) where \( G(z)H(z) - G(z)H'(z) = 0) \). However, at such critical points, one finds \( F''(z) = (G(z)/H(z))[2G''(z) - G(z)H''(z)/H(z)] \). Clearly \( H'' > 0 \) while \( G''(z) < 0 \). Consequently, at such points, \( F''(z) < 0 \). This means that \( F \) has precisely one maximum (at \( z_1 \)) and a minimum at \( z = i_b \). Since \( F \) has a vertical asymptote at \( z = 0 \) we must have \( z_1 > i_b \).

It is a logical consequence of this these observations that there are two non-degenerate cases:

a. Suppose \( 2D > F'(Z_1) \). Then the equation \( 2D = F'(z) \) has a unique positive solution, \( z_1 < i_b \). From (4.7) we draw the following conclusions. If \( I(L) = I(0) = z_1 \), then the minimum of the solution is zero. If \( I(L) - I(0) < z_1 \), there is no solution since then the right hand side of (4.7) will be negative. If \( z = I(L) - I(0) > z_1 \) then the minimum first increases to \( i_b \) since \( F'(i_b) = 0 \), then decreases until \( z = z_1 \), after which it increases with \( z \).

b. Suppose \( 2D < F'(Z_1) \). Then the equation \( 2D = F'(z) \) has three positive solutions, \( z_1 < i_b, i_b < z_2 < z_3 \). \( z_1 > Z_1 \). Again, the minimum of the solution will be zero and this occurs at the \( z_2 \). There is no solution if \( z < z_1 \) or \( z_1 < z < z_3 \). \( I_{m}(z) \) has a maximum of \( i_b \) on \((z_1, z_2)\) and increases without bound as \( z \) increases beyond \( z_3 \).

\[\text{The functional relationship between } I_m, D \text{ and } L \text{ is given implicitly by the formula}\]

\[
L = 2D \int_{I_m}^{I(0)} \frac{dz}{\sqrt{G^2(I(0)) + 2DH(z) - H(I(0))}}.
\]

Since

\[
2D = \frac{G^2(I(0))}{H(I(0)) - H(I_m)}
\]

This simplifies to

\[
L = \sqrt{\frac{2}{D}} \int_{I_m}^{I(0)} \frac{dz}{\sqrt{H(z) - H(I_m)}}
\]

The singularity is integrable if \( I_m > 0 \) since then \( H'(I_m) > 0 \).
c. If we fix $I(0)$, then as $D \to +\infty$, $I_m \uparrow I(0)$. Thus, the larger the diffusion constant, the more efficacious is the delivery of the inhibitor to the cell center for given concentration of drug just inside the cell wall.

**Remark 1.** In the case that one may actually calculate the maximum value of $P$ occurs when $k_1 = +\infty$, i.e., when the reaction $I + E \rightleftharpoons P$ lies entirely to the right. Then

$$F'(z) = \frac{2|z-K_m|}{(K_m + z)^2(\nu z^2 + 2\nu \varphi z)}.$$  

This function has a double root at $z = i_0$, behaves like $C/z$ for small positive $z$ and decays to zero as $z \to +\infty$. The equation $2D = F(z)$ may have as many as four roots, but one of them will be in the interval $(-K_m, 0)$ and thus not relevant to this discussion.

From (3.5) it follows that for this choice of source function, the maximum value of the protein concentration occurs at the center of the cell while the product, like the inhibitor, has minimum concentration at the cell center.

5. Efficiency of the Mechanism

The efficiency of the mechanism is defined as the ratio of product to protein in the limit of large time. There are a couple of ways to define this. We could do it locally, i.e., $E(x) = P(x)/E(x) = \varphi(x)/E(x) - 1 = k_1 I(x)/(\mu + k_-)$. This says that if there is no decay of protein or product and if the reaction is irreversible, the efficiency is infinite wherever $I$ is not zero. However it is probably better to give a non local or global definition to assign a single number to the process, i.e.

$$E = \frac{1}{V(\Omega)} \int_{\Omega} P(x)/E(x) \, dx.$$  

(Here $V(\Omega)$ is the volume of the cell.) The local efficiency for the solution given in (3.4) is $E(x) = k_1 I(x)/(\mu + k_-)$. The mean efficiency is

$$E = \frac{1}{L} \int_{0}^{L} I(x) \, dx$$

which is necessarily positive since $I$ is nonnegative and somewhere positive. The integral may be calculated directly from the solution of (3.4).

6. Part 2: The Chemical Kinetics (Enzyme-Inhibitor-Aptamer)

In this section, we consider the effect of an aptamer on the efficiency of the reaction:

$$E + I \xrightarrow{k_1} IE.$$  

where now we have used $IE$ to denote the protein-inhibitor product. The protein is presumed to have a separate binding site for the aptamer (a small molecule of DNA). The aptamer also has a binding site for the inhibitor. Therefore, the aptamer, $A$, interacts with both the protein and the inhibitor via

$$A + I \xrightarrow{i_1} AI,$$

$$A + E \xrightarrow{m_1} EA.$$  

(6.2)
Furthermore these three binary complexes (IE, EA, AI) interact to form two distinct types of trinary complexes

\[ AI + E \xrightarrow{k_2} \xrightarrow{k_{-2}} EAI, \]

\[ EA + I \xrightarrow{l_2} \xrightarrow{l_{-2}} IEA, \]

(6.3)

\[ EA + I \xrightarrow{m_2} \xrightarrow{m_{-2}} EAI, \]

\[ IE + A \xrightarrow{n_2} \xrightarrow{n_{-2}} IEA. \]

The open site on the trinary complex EAI binds with the inhibitor

\[ I + EAI \xrightarrow{k_{-3}} \xrightarrow{k_3} IEAI, \]

(6.4)

\[ IEA + I \xrightarrow{l_{-3}} \xrightarrow{l_3} IEAI \]

We must not overlook the possibility that

\[ IE + AI \xrightarrow{k_{-4}} \xrightarrow{k_4} IEAI. \]

Some further reduction in the number of available rate constants can be made if we assume:

1. The E–A bond formation is irreversible (m_{-2} = k_{-2} = k_{-4} = n_{-2} = 0) and the rate of formation of the bond does not depend on whether or not I is bound to either E or A so that \( m_{-2} = k_{-2} = k_{-4} = n_{-2} = 0 \).

2. The rate of formation and disassociation of the A–I bond does not depend on whether or not either is bound to E (l_{-3} = l_{2} = m_{3} = 0 \Rightarrow \lambda_{3} > 0).

3. The rate of formation and disassociation of the E–I bond does not depend on whether or not either is bound to A. (k_{1} = l_{2} = k_{3} = 0 \Rightarrow k_{1} = k_{3} = 0). 

In order to set the stage for the rate equations to follow, we make the assumption that, in terms of molecular weights, \( M_I << M_E << M_A \) where \( M_j \) denotes the molecular weight of species \( J \). We have nine molecular species \{ I, A, E, AI, AE, IE, EAI, IEA, IEAI \}. Six of these involve E and, in view of size considerations will be considered incapable of diffusion in the cell cytoplasm. Two involve A but not E and will be considered to be somewhat diffusible. The smallest molecule, I, will correspondingly be the most diffusible of the nine species. Therefore, in terms of diffusion coefficients, we take \( D = D_I > D_A = D_{AI} = D_E \Rightarrow > 0 \) while the diffusion coefficient of any molecule with E as a subunit will be taken to be zero. We will also assume that, in the absence of diffusion, all of the reactions above are reversible so that no rate constant vanishes. We will also need a more compact notation. We write \[ [EA] = e_0(x,t), \quad [EI] = \phi(x,t), \quad [AI] = a_0(x,t), \quad [IEA] = \phi_0(x,t), \quad [EAI] = e_{00}(x,t), \quad [IEAI] = \phi_{00}(x,t) \]

where the notation is intended to suggest the largest member of the complex. The steady state versions of these functions will be denoted with a superscript, i.e., \( \phi^*(x) = \lim_{t\to\infty} \phi(x,t) \) if it. In order to determine whether or not the addition of the aptamer inhibitor, we need to compute the efficiency ratio

\[ \phi^* = \frac{1}{V(\Omega)} \int_{\Omega} \frac{|\phi(x) + e_0^* + \phi_0^*|}{|e^* + e_0^* + e_{00}^*|} \, dx \]

for the steady state solution of the system which replaces (2.2). The ratio must be computed in this manner since some of the protein is bound up with the aptamer and not inhibitor. Thus it is not a priori clear whether or not addition of aptamer increases the draw of inhibitor from the cell exterior. We assume the cell expresses
enzyme and aptamer at steady rates exponentially decreasing rate $S_0(x)$, $S_e(x)$ respectively. Suppose also the this inhibitor decays at a rate $\nu$. Assume furthermore that the compounds $E, EI, EA, EAI, IEA, IEAI$ have roughly the same half life $\ln 2/\mu e$. The decay rate for the aptamer will be denoted by $\nu_a$. We assume that the diffusion constants satisfy $D > D_a \geq D_c = 0$. Mass action applied to

\begin{align*}
\partial_t a &- D \Delta a + k_{-1} v + e^{-\nu} a + \nu_a + l_{-1} \omega_i + (k_{-1} + l_{-1}) \omega_a \\
\partial_t e  &- (k_{1} e + l_{1} a + (k_{1} + l_{1}) e_{a} + k_{1} e_{ai} + l_{1} i e_{ai}) \partial e_i - \nu e \partial e \\
\partial_t a_i &- D_a \Delta a_i + l_{ai} - (l_{-1} - \nu_a + m_{1} e + i e) a + S_a(x), \\
\partial_t c &- D \Delta c + l_{1} c - (k_{1} i + m_{1} (a + a_i)) c - \nu c + S_e(x), \\
\partial_t i &- k_{1} i e - (k_{1} + m_{1} (a + a_i)) i e - \nu i e, \\
\partial_t e_{a} &- m_{1} e_{a} + l_{1} i e_{ai} - (k_{1} + l_{1}) i e_{a} - \nu e_{a} \\
\partial_t e_{ai} &- m_{1} e_{ai} + l_{1} i e_{ai} - (k_{1} + l_{1}) i e_{ai} - \nu e_{ai} \\
\partial_t i e_{ai} &- k_{1} i e_{ai} + l_{1} i e_{ai} + (k_{1} + l_{1}) i e_{ai} - \nu i e_{ai}.
\end{align*}

(6.7)

Because the species $I, A, A_I$ are diffusing, we must supply boundary conditions for them. Those for $I$ are as above while for $A, A_I$, when $D_a > 0$ we take flux zero conditions:

\begin{align*}
\partial_t c(0, t) = \partial_x c(L, t) = \partial_x c_I(0, t) = \partial_x c_I(L, t) = 0.
\end{align*}

(6.8)

The conservation law for the non diffusing species is not obvious, but one can convince oneself that if we set

\begin{align*}
\Sigma = [e + i e + e_a + e_{ai} + i e_{a} + i e_{ai}],
\end{align*}

(6.9)

then we have the conservation law

\begin{align*}
\partial_t \Sigma + \nu \Sigma = S_e(x).
\end{align*}

(6.10)

From this, in turn

\begin{align*}
\Sigma(x, t) = \Sigma(x, 0) e^{\nu t} + S_e(x)(-e^{\nu t})/\mu.
\end{align*}

(6.11)

We can use the conservation law to eliminate $i \omega_i$ from the nine dynamic equations 6.7 and disregard the last of these in the sequel.

Two further useful identities can be gleaned from (6.5). First, if we add the last four equations, we find

\begin{align*}
\partial_t [e_a + e_{ai} + i e_a + i e_{ai}] = m_{1} (a + a_i)(e + i e) - \nu(e_a + e_{ai} + i e_a + i e_{ai}).
\end{align*}

(6.11)

Moreover, if we add the second and third equations and subtract from them the sum of the fourth and fifth equations we have that

\begin{align*}
\partial_t [a + a_i] - (e + i e) = D_a \Delta (a + a_i) + S_a(x) - S_e(x) - [\nu_a(a + a_i) - \mu(e + i e)].
\end{align*}

(6.12)

Integrating this over the domain and taking note of the boundary conditions for $a, a_i$ we obtain:

\begin{align*}
\partial_t \int_0^L (a + a_i) dx = \partial_t \int_0^L (e + i e) dx + \int_0^L (S_a(x) - S_e(x)) dx - \int_0^L [\nu_a(a + a_i) - \mu(e + i e)] dx.
\end{align*}

(6.13)

Now we are in a position to discuss stationary solutions. Suppose there is a stationary solution. Then, at steady state, the preceding equation tells us that

\begin{align*}
\int_0^L (S_a(x) - S_e(x)) dx - \int_0^L [\nu_a(a^* + a_i^*) - \mu(e^* + i e^*)] dx,
\end{align*}

(6.14)
an equation that simplifies considerably if $S_0 = S_e = S$. In this case we see that

$$
\nu_0 \int_0^L (e^s + \kappa e^s) \, dx - \mu \int_0^L \kappa (e^s + \kappa e^s) \, dx
$$

Assuming that initially there is no inhibitor, aptamer or protein in the cell,

$$
0 = D_0 \Delta a^s + l_1 l_0 e^s - (b_2 l_2 + b_1 a^s + b_1 e^s + b_1 e^s + b_1 e^s + b_1 e^s + b_1 e^s) a^s - l_1 e^s,
$$

$$
0 = D_0 \Delta a^s + l_1 l_0 e^s - (b_2 l_2 + b_1 a^s + b_1 e^s + b_1 e^s + b_1 e^s + b_1 e^s + b_1 e^s) a^s,
$$

$$
0 = -l_1 e^s + [b_2 l_2 + b_1 a^s + b_1 e^s + b_1 e^s + b_1 e^s + b_1 e^s + b_1 e^s] a^s + b_1 e^s.
$$

We have, in addition to these eight equations, the stationary limit of the conservation law:

$$
e^s + \kappa e^s + e^s + e^s + e^s + e^s + e^s + e^s = \varphi(x) = S(x)/\mu = \psi(x).
$$

If we add the fourth and fifth equations in (6.16) we find that

$$
e^s + \kappa e^s = \frac{\mu \psi}{\mu + m_1 (a^s + \kappa)}.
$$

If we add the second and third equations in (6.16) and use (6.20) we find

$$
\mu \psi \varphi + m_1 (a^s + \kappa) = \mu + m_1 (a^s + \kappa) \varphi a^s + \kappa.
$$

This equation, together with the boundary conditions

$$(a^s + \kappa)(0) = (a^s + \kappa)(L) = 0
$$

will have a unique positive solution $(a^s + \kappa)$ since the function $F(z) = \frac{\mu \psi}{\mu + m_1} - \nu \varphi z$ is a decreasing function of $z$. If the flux is prescribed, i.e.

$$(a^s + \kappa)'(0) = (a^s + \kappa)'(L) = 0
$$

so that the aptamer forms are constrained to stay in the cell, there will be a non trivial constant if $\varphi$ is constant.\footnote{As above, when $D_0 = 0$, the boundary conditions may be abandoned and the unique solution is}

$$
(a^s + \kappa) = \psi = \frac{2 \mu \psi}{(\mu/m_1 + \sqrt{\mu/m_1})^2} + 4 \mu \psi(x)/(\mu m_1 \nu_0)
$$

regardless of whether or not $\varphi$ is constant.

If $D_0 \neq 0$ and the zero flux conditions are employed, this expression will also be a (nontrivial) constant solution if $\varphi$ is constant and the zero flux condition is satisfied since then the zero flux conditions are automatically satisfied. We shall have more to say about equation (6.22) later.
We will have need of each term in the sums $A, E$. The ratio $e^s / i e^s$ is easily determined from the fifth equation in (6.16). This permits us to determine both $e^s$ and $i e^s$ in terms of $i^s$ and $A$.

Now things get 'etucky.' The second and third equations must be solved for one or the other of $a^s, a_i^s$ in terms of $A$. If we write $a_i^s = A - a^s$ then the second equation in (6.16) may be written as

$$0 = D_a \Delta a^s - [l_{-1} + \nu_{-1} + l_1 i^s + m_1 \mathbf{E}] a^s + [l_{-1} A + \nu \varphi(x)].$$

Again, in principle, one can find a solution of this equation, but only in terms of $i^s$. In order to attempt solution of the remaining three stationary equations, beginning with the conservation law, we write them as follows:

$$e_i^s + i e_i a_i^s + i \omega_i^s = m_1 \left( \frac{\varphi A}{\mu + m_1 A} \right),$$

$$[(k_1 + l_1) i^s + \mu] e_i^s - k_{-1} i e_i a_i^s - l_{-1} e_i^s = m_1 e_i^s a_i^s,$$

$$-l_1 e_i^s - k_{-1} \omega_i^s + (l_{-1} + \mu + k_1) e_i a_i^s = m_1 e_i a_i^s,$$

$$-k_1 i e_i^s - l_1 \omega_i^s + (k_{-1} + \mu + l_1 i^s) e_i^s = m_1 i e_i a_i^s.$$

The system of equations can be written in the form: $\mathfrak{A} X = B$ where

$$\mathfrak{A} = \begin{bmatrix} [ (k_1 + l_1) i^s + \mu] & -k_{-1} & -l_{-1} & 0 \\ -l_1 i^s & 0 & (l_{-1} + \mu + k_1 i^s) & -k_{-1} \\ -k_1 i^s & (k_{-1} + \mu + l_1 i^s) & 0 & -l_{-1} \end{bmatrix}, \quad X = \begin{bmatrix} e_i^s \\ e_i a_i^s \\ \omega_i^s \\ i \omega_i^s \end{bmatrix}, \quad B = \begin{bmatrix} m_1 \frac{\varphi A}{\mu + m_1 A} \\ m_1 e_i^s a_i^s \\ m_1 e_i a_i^s \\ m_1 i e_i a_i^s \end{bmatrix}.$$}

A somewhat tedious, but routine, hand calculation yields

$$\det \mathfrak{A} = -(k_{-1} + l_{-1})(k_1 i^s + k_{-1} + \mu)(l_1 i^s + l_{-1} + \mu).$$

This determinant is necessarily not zero provided $k_{-1}, l_{-1} > 0$. Thus we can express $e_i^s, i e_i^s, e_i^s, i e_i^s$ uniquely in terms of $e^s, \alpha^s, i^s, i e^s, \alpha^s$. (We have not proved that these variables will be non negative if the latter set of variables and the entries of $B$ are nonnegative.)

7. THE CASE WHEN EITHER THE APTAMER DIFFUSION ($D_a = 0$) OR $\varphi$ CONSTANT.

In this section we confine our attention to the case for which $D_a$ vanishes. However, we will not explicitly employ this restriction except to say that either this restriction holds or else $\varphi$ is constant. In either case, we will have a solution of (6.22) together with no flux boundary conditions.

Consider first the problem of efficiency. We are concerned with the local efficiency here. The system is more efficient at $x$ with the aptamer than without it if and only if

$$(7.1) \quad \mathfrak{E}(a) - \frac{\nu e^s + \nu a_i^s + i \omega_i^s}{e^s + a_i^s + i \omega_i^s} \geq \mathfrak{E}(0) - \frac{k_1 i^s}{k_{-1} + \mu},$$

where the value of $\mathfrak{E}(0)$ on the right is taken from Section 5 and the value of the inhibitor is assumed to be the same in both computations.

Equivalently

$$(7.2) \quad \mathfrak{R} = \frac{\mathfrak{E}(a)}{\mathfrak{E}(0)} = \frac{k_{-1} + \mu}{k_1 i^s} \frac{\nu e^s + \nu a_i^s + i \omega_i^s}{e^s + a_i^s + i \omega_i^s}.$$
in view of the conservation law. If we add the second equation to the third equation in (6.23) and then add \( k_{-1} \) times the first equation to the resulting third equation we obtain

\[
e^{\ast} + e_{o\ast} = \frac{m_1 A}{k_{1\ast} + k_{-1} + \mu} \left( \frac{k_{-1} \varphi + e^{\ast} (\mu + m_1 A)}{\mu + m_1 A} \right)
\]

From \( e^\ast + e^{\ast} = \mathcal{E} \) and the fifth equation (6.23) we find

\[
e^{\ast} = \frac{\mu \varphi}{\mu + m_1 A} \frac{k_{1\ast} + \mu + m_1 A}{(k_{-1} + \mu + k_{1\ast} + m_1 A)^\gamma},
\]

\[
e^{\ast} = \frac{\mu \varphi}{k_{1\ast} + m_1 A} \frac{k_{1\ast} + \mu + m_1 A}{(k_{-1} + \mu + k_{1\ast} + m_1 A)^\gamma}
\]

The relevant parameter here is \( \eta \equiv m_1 A \). We wish to calculate \( \mathfrak{R} \) when \( \eta \gg \mu \approx \eta \) which corresponds to having a large \( E - A \) binding constant. We find that

\[
e^{\ast} + e_{o\ast} \approx \frac{\varphi (k_{-1} + \mu)}{k_{1\ast} + \mu + k_{1\ast}},
\]

\[
e^{\ast} \approx \frac{\mu \varphi}{m_1 A} \frac{k_{1\ast} + \mu + k_{1\ast}}{m_1 A},
\]

\[
e^{\ast} \approx \frac{k_{1\ast} \mu \varphi}{(m_1 A)^2}
\]

Substituting these into the definition of \( \mathfrak{R} \) yields

\[
\mathfrak{R} = \frac{\mu (k_{-1} + \mu)/(m_1 A)^2 + (k_{-1} + \mu)/(k_{-1} + \mu + k_{1\ast})}{\mu/(m_1 A) + (k_{-1} + \mu)/(k_{-1} + \mu + k_{1\ast})}
\]

Therefore this ratio will exceed unity if and only if \( \mu < m_1 A < \mu + k_{-1} \).

**Remark 2.** We can get an idea of how large \( \eta \) must be for \( \mathfrak{R} \) to be large. We know that

\[
A = \frac{2(\mu/\nu_a) \varphi(x)}{1 + \sqrt{1 + 4m_1 \varphi/\nu_a}}
\]

From this we see that when \( m_1 \) is large, \( m_1 A \approx \mu \sqrt{m_1 \varphi/\nu_a} \). Then \( \mu + m_1 A \approx \mu (1 + \sqrt{m_1 \varphi/\nu_a}) \). We take \( \nu_a = \mu \) for want of better information. Then \( \mu < m_1 A < \mu + k_{-1} \) if and only if \( \mu < m_1 \varphi < (k_{-1}/\mu + 1)^2 \).

For typical numerical values we use, with \( M \) as molarity,

\[
m_1 = 0.33 \times 10^4 / \text{M-sec},
\]

\[
k_{1\ast} = 10^4 / \text{M-sec},
\]

\[
k_{-1} = 10^{-9} \text{sec},
\]

\[
\mu = 10^{-5} \text{sec} = \nu_a,
\]

\[
\varphi(x) \approx 3 \times 10^{-8} M.
\]

The density \( \varphi \approx 10^3 \) molecules per cell. Using a cell volume of \( 4\pi \times (2.5 \text{microns})^3 / 3 \) and Avogadro’s number \( 6.3 \times 10^{23} \) molecules/mole, this density converts to the above value. This gives \( m_1 \varphi \approx 10^{-4} \) and \( k_{-1} / \mu \approx k_{-1} \).

Substituting these values in the expression for \( \mathfrak{R} \) at infinite dilution \( (c = 0) \) yields

\[
\mathfrak{R} = \frac{1 + 10^9 10^6}{1 + 10^9 10^4} \approx 10.0
\]

Notice that \( m_1 A = \sqrt{\mu m_1 \varphi} \approx 10^{-4.5} > \mu \).
Once we have determined \(e_i, \ldots, e_n\), we need to solve the first equation in (6.16) for \(i^*\). Careful inspection of this equation shows us that it has the form:

\[
0 = D \Delta i^* - \nu i^* = (\Delta_{i^*} + \Delta_{i^*} + \Delta_{i^*} + \Delta_{i^*})
\]

where \(\Delta_{i^*} = k_{i^*} - k_{i^*} \delta e - k_{i^*} \delta e\) etc. That is, each \(\Delta_i\) is the difference between the reverse and forward rates for (6.1)-(6.3). As we have seen above, the critical part of the problem is to solve the partial differential equations for \(a, a_t, i_t\) in terms of \(i\). Suppose that this has been done and we wish to find an integro-differential equation for \(i\) alone. For notational convenience define the following quantities:

\[
S(i^*) = (k_1 + l_1)i^* + \mu, \\
K(i^*) = \frac{k_{i^*}}{S(i^*)}, \\
L(i^*) = \frac{l_{i^*}}{S(i^*)}, \\
\kappa_a(i^*) = k_{i^*} + k_{i^*} + \mu, \\
\lambda_a(i^*) = l_{i^*} + l_{i^*} + \mu, \\
\kappa_a(i^*) = k_{i^*} + k_{i^*} + \nu, \\
\lambda_a(i^*) = l_{i^*} + l_{i^*} + \nu.
\]

Then we see from the linear equations that

\[
e_a^* + e_{ai}^* = \frac{m_1}{\kappa_a(i^*)} \left( \frac{k_{i^*} \varphi A}{\mu + m_1 A} + e_i^* A \right), \\
e_a^* + e_{ai}^* = \frac{m_1}{\lambda_a(i^*)} \left( \frac{l_{i^*} \varphi A}{\mu + m_1 A} + a^* E \right), \\
e_a^* - K(i^*) e_a^* + L(i^*) e_{ai}^* + m_1 e_a^* = \frac{e_a^*}{S(i^*)}, \\
i_{ai}^* = \frac{m_1 \varphi A}{\mu + m_1 A} - (e_a^* + e_{ai}^* + e_{ai}^*)
\]

Consequently,

\[
e_a^* = \frac{m_1}{K(i^*) + L(i^*) + 1} \left[ K(i^*) \left( \frac{k_{i^*} \varphi A}{\mu + m_1 A} + e_i^* A \right) + L(i^*) \left( \frac{l_{i^*} \varphi A}{\lambda_a(i^*)} + a^* E \right) + e_a^* a^* \right] + \left( l_{i^*} A - (l_{i^*} + l_{i^*}) \right)
\]

From this equation, it is an easy matter to find \(t_{ai}^*, e_{ai}^*, i_{ai}^*\) from (7.10). From these expressions, it can be shown after some calculation that:

\[
0 = D \Delta i^* - \nu i^* + [(k_{i^*} - (k_{i^*} + k_{i^*}) e^*) + [(l_{i^*} A - (l_{i^*} + l_{i^*}) a^*)
\]

\[
+ m_1 \left\{ \left( k_{i^*} + k_{i^*} \right) \frac{e^* A}{\kappa_a(i^*)} + \left( l_{i^*} + l_{i^*} \right) \frac{a^* E}{\lambda_a(i^*)} \right\}
\]

\[
+ m_1 \mu \left\{ \left( \frac{k_{i^*}}{\kappa_a(i^*)} + \frac{l_{i^*}}{\lambda_a(i^*)} \right) \frac{\varphi A}{\mu + m_1 A} \right\}, \\
- D \Delta i^* - \nu i^* + [(k_{i^*} e^* - k_{i^*} e^*) + [(l_{i^*} A - l_{i^*} a^*)
\]

\[
- m_1 \left\{ \left( k_{i^*} e^* - k_{i^*} e^* \right) \frac{A}{\kappa_a(i^*)} + \left( l_{i^*} a^* - l_{i^*} a^* \right) \frac{E}{\lambda_a(i^*)} \right\}
\]

or finally

\[
0 = D \Delta i^* - \nu i^* - (k_{i^*} e^* - k_{i^*} e^*) \left\{ 1 + \frac{m_1 \varphi A}{\kappa_a(i^*)} \right\} - (l_{i^*} a^* - l_{i^*} a^*) \left\{ 1 + \frac{m_1 E}{\lambda_a(i^*)} \right\}.
\]
When \( A = 0 \) we recover the equation for \( I \) in Section 2 as we should. Secondly, if \( E = 0 \), we see that the aptamer alone will bind to the inhibitor and influence the concentration of free inhibitor in much the same way as the enzyme. Even when the aptamer does not bind to the enzyme, both the enzyme and the aptamer work to raise the concentration of free inhibitor. Finally, the larger the binding constant, \( m_1 \), the larger will be the effect on the increase of free inhibitor in the cell.

We next evaluate the right hand side of this equation when \( m_1 \varphi \) (which is data) is large and \( \nu_\alpha = \mu \) in the case \( D_\alpha = 0 \). From (7.4) and the observation that \( m_1A \approx \sqrt{m_1 \mu \varphi} \),

\[
\begin{align*}
A & \approx \sqrt{\mu \varphi / m_1}, & E & \approx \sqrt{\mu \varphi / m_1}, \\
e^\varphi & \approx \sqrt{\mu \varphi / m_1}, & \rho e & \approx k_1 i^\varphi / m_1, \\
\alpha^\varphi & \approx \sqrt{\mu \varphi / m_1}, & \alpha^\varphi & \approx l_1 i^\varphi / m_1.
\end{align*}
\]

Under these circumstances,

\[
k_1 i^\varphi e^\varphi - k_{-1} i e^\varphi \approx [\sqrt{m_1 \mu \varphi} - k_{-1}] [k_1 i^\varphi / m_1] \approx [\sqrt{\mu \varphi} / m_1] (k_1 i^\varphi)
\]

with a similar approximation for \( l_1 i^\varphi e^{-l_{-1} i} \). Then (7.12) reduces to

\[
0 - D \Delta i^\varphi - \nu i^\varphi = (k_{-1} + l_1) i^\varphi \sqrt{\frac{\mu \varphi}{m_1}} - \mu \varphi \left( \frac{k_1 i^\varphi}{\kappa_\alpha(i^\varphi)} + \frac{l_1 i^\varphi}{\kappa_\alpha(i^\varphi)} \right)
\]

This equation, in view of the dependence of \( \lambda_2 \) and \( \kappa_\alpha \) on \( i^\varphi \), is highly nonlinear. When \( \max \{k_{-1} + \mu, l_{-1} + \nu_\alpha\} \ll \min \{k_1, l_1\} i^\varphi \) this equation reduces to

\[
0 - D \Delta i^\varphi - \nu i^\varphi = (k_1 + l_1) i^\varphi \sqrt{\frac{\mu \varphi}{m_1}} - 2\mu \varphi.
\]

Notice that if we set \( m_1 = +\infty = 1/\nu \), the above equation reduces to

\[
0 = D \Delta i^\varphi - 2\mu \varphi.
\]

In the case that \( D_\alpha = 0 \) and \( \varphi << 1 \), we have, in addition to (7.6), that

\[
[L_{-1} + \nu_\alpha + l_1 i^\varphi + m_1 E] \alpha^\varphi = [l_{-1} A + \mu \varphi(x)].
\]

Thus,

\[
\begin{align*}
\alpha^\varphi(x) & = \frac{(L_{-1} + \nu_\alpha)(\mu \varphi(x))}{\nu_\alpha \lambda_2(i^\varphi(x))}, \\
\alpha^\varphi(x) & = \frac{k_1 i^\varphi(x) \mu \varphi(x)}{\nu_\alpha \lambda_2(i^\varphi(x))}, \\
e^\varphi(x) & = \frac{(k_{-1} + \mu)(\varphi(x))}{\kappa_\alpha(i^\varphi(x))}, \\
e^\varphi(x) & = \frac{k_1 i^\varphi(x) \varphi(x)}{\kappa_\alpha(i^\varphi(x))}.
\end{align*}
\]

The first of these derives from the approximation that \( \lambda_2(i^\varphi(x)) + m_1 E = \lambda_2(i^\varphi(x)) + m_1 \varphi \approx \lambda_2(i^\varphi(x)) \). The third follows from the hypothesis that \( k_{-1} + \mu + m_1 A \approx k_{-1} + \mu \) and the fifth equation of (6.16) when \( \varphi \) is very small. We then use (7.6) and (7.16) to evaluate right hand side of (7.12). Substitution of these in (7.12) further reveals the highly nonlinear nature of the boundary value problem for the determination of the inhibitor. We obtain

\[
0 - D \Delta i^\varphi - \nu i^\varphi = \frac{k_1 i^\varphi \varphi(x)}{\kappa_\alpha(i^\varphi(x))} \left( 1 + \frac{m_1 \mu \varphi}{\kappa_\alpha(i^\varphi(x))} \right) \frac{l_1 i^\varphi \varphi(x)}{\kappa_\alpha(i^\varphi(x))} \left( 1 + \frac{m_1 \mu \varphi}{\kappa_\alpha(i^\varphi(x))} \right)
\]

This equation also contains some useful qualitative information. First, when \( m_1 = l_1 = 0 \), we recover the stationary equation for \( I \) in the absence of aptamer. Secondly, even if \( l_1 = 0 \), a very large binding constant
will work to absorb free inhibitor into the region at a higher rate than would be the case if there were no aptamer present. If we use the above formulas to calculate the relative increase in efficiency, we find that

$$\Re = \frac{1 + (k_e(i^*) + \mu) \frac{m_1 \varphi}{\mu k_e(i^*)}}{1 + \frac{k_{-1}k_1i^* + (k_{-1} + \mu) \frac{m_1 \varphi}{\mu k_e(i^*)}}{k_{-1} + \mu}}$$

which is always larger than unity. The asymptotic formula (7.5) tells us that if \(m_1A > k_{-1} + \mu\), then \(\Re < 1\).

8. The case that \(D_a \neq 0\) and \(\varphi \neq \text{constant}\).

The reader is reminded that equation (7.12) holds even when \(D_a \neq 0\). However, it is not possible to recover the individual concentrations \(a^*, a_1^*, b^*\) without first solving (7.12) together with the second and third equations of (6.16). From (7.3) we obtain \(e^*, ie^*\) in terms of \(a^*, a_1^*, b^*\). Substituting these into (8.1) and rewriting the equations for \(a^*, A = a^* + a_1^*\), we obtain the following system:

$$0 = D\Delta i^* - \nu_i^* - \frac{m_1 i^* \varphi}{k_e(i^*)} - a^*(i_1 + L_{-1}) \left( 1 + \frac{m_1 \varphi}{\lambda_e(i^*)(\mu + m_1A)} \right)$$

$$+ l_{-1}A \left( 1 + \frac{m_1 \varphi}{\lambda_e(i^*)(\mu + m_1A)} \right),$$

$$0 = D\Delta a^* - \left( \frac{m_1 \mu \varphi}{\mu + m_1A} + \frac{\lambda_e(i^*)}{\mu + m_1A} \right) a^* + \mu \varphi + L_{-1}A,$$

$$0 = \partial_a \Delta A + \frac{\mu \varphi}{\mu + m_1A} - \nu_o A$$

As we remarked earlier, the last of these can be solved, at least in principle, as we shall see below when we impose the no flux conditions. Assuming \(A\) is known, then the first two of these become a coupled system of differential equations which cannot be solved except by numerical methods. The boundary conditions for \(i^*\) are of the form given in (3.4) while for the concentration of free aptamer, \(A^*\), one uses the zero flux conditions.

From now on we will restrict our attention to the one-dimensional case. We write \(\Delta w = w''\) in this case.

We claim the following:

1. When \(\nu_o > 0\), equation (8.1), together with the zero flux boundary conditions \(A'(0) = A'(L) = 0\), has a unique solution. This follows from the standard theory of partial differential equations and the fact zero is not in the spectrum of the operator \(D_a A'' - \nu_o A\) with no flux boundary conditions. Consequently when \(\varphi = \text{constant}\) the only solution of the third equation with the zero flux boundary condition is the constant solution given earlier.

A second useful consequence of the theory is that even when \(\varphi\) is not constant, the function \(\hat{A}\) which satisfies

$$0 = \frac{\mu^2 \varphi}{\mu + m_1A} - \nu_o \hat{A}$$

when \(D_a = 0\), will be the limit, as \(D_a \rightarrow 0\), of the solution if \(\varphi\) also satisfies the zero flux boundary conditions.

2. At low concentrations of inhibitor, it is possible to chose \(i_1\) so large that inhibitor must reach every point of the cell.

To see this, we note that, in view of the last item, we can write

$$\varphi \approx \frac{\nu_o A(\mu + m_1A)}{\mu^2}.$$
Recall that there is a one-one correspondence between $A$ and $\varphi$, i.e.,

$$A(\varphi) = \frac{2\mu^2\varphi(x)/(m_1\nu_a)}{(\mu/m_1) + \sqrt{(\mu/m_1)^2 + 4\mu^2\varphi(x)/(m_1\nu_a)}}$$

Using the expression for $\varphi$ in the second equation of (8.1), assuming that $i^a \approx 0$, we find

$$0 = D_A a^{\nu''} - \left\{ \frac{m_1 \mu \varphi}{\mu + m_1 A} + \lambda_a(0) \right\} a^s + \mu \varphi + l_{-1} A$$

and consequently, neglecting diffusion of $A$,

$$a^s \approx \frac{\mu \varphi + l_{-1} A}{m_1 \mu \varphi + (\nu_a + l_{-1})(\mu + m_1 A)}$$

since $\lambda_a(0) = \nu_a + l_{-1}$. It follows that, to zero order in $\nu_a$,

$$0 = D_A a^{\nu''} - i^s \left\{ \nu + \frac{\nu_a \varphi}{\mu + m_1 A} \right\} + l_1 a^s \left[ 1 + \frac{\nu_a A}{\mu^2(\mu + k_{-1})} \right] + l_{-1} (A - a^s) \left[ 1 + \frac{\nu_a A}{\mu^2(\mu + k_{-1})} \right].$$

When one neglects the diffusion of $A$ and takes $i^s \approx 0$, then one finds, from direct calculation with the formulas above, that $a^s = A$. The exact expression for $a^s$ is

$$a^s = \frac{D_A D_a a^{\nu''} + (\mu \varphi + l_{-1} A)(\mu + m_1 A)}{m_1 \mu \varphi + (\nu_a + l_{-1})(\mu + m_1 A)},$$

which gives us an idea of how the diffusion of $A$ affects the concentration of $A$ when $i^s \approx 0$. Thus, a reasonable approximation to this last differential equation for $i^s$ is:

$$0 = D_a a^{\nu''} - i^s \left\{ \nu + \frac{\nu_a \varphi}{\mu + m_1 A} \right\} + l_1 A \left[ 1 + \frac{\nu_a A}{\mu^2(\mu + k_{-1})} \right].$$

The important observation here is to note the coefficient of $l_1$ is positive and independent of $l_1$ since it only depends on $A$ as one sees from the third equation of (8.1). Define, for convenience, the functions

$$\mathcal{R}(A) = \frac{\nu_a A}{\mu + m_1 A},$$

$$\mathcal{L}(A) = \frac{1 + \frac{\nu_a A}{\mu^2(\mu + k_{-1})}}{\mu^2(\mu + k_{-1})}.$$

If we assume for the moment that $A$ is constant, then a quadrature yields,

$$\frac{1}{2D} \left( \frac{\varepsilon K_c i^s(0)}{K_m} \right)^2 - [i^s(0)]^2 \left[ \nu + k_1 \mathcal{R}(A) + l_1 \mathcal{L}(A) \right] = (D/2)[i^{\nu''}(x)]^2 - [i^s(x)]^2 [\nu + k_1 \mathcal{R}(A) + l_1 \mathcal{L}(A)]$$

over any interval $[0, x]$ on which $i^s \geq 0$. It follows from this that if

$$l_1 > \frac{(1/2D)(\varepsilon K_c/K_m)^2 - \nu - k_1 \mathcal{R}(A)}{\mathcal{L}(A)}$$

we cannot have $i^s(x_0) = 0$ for any point $x_0$ in the interval.

The argument of the last paragraph can be extended to the case for which $A$ is not constant on $[0, L]$, provided $A$ is increasing on $(0, L/2)$ and decreasing on $(L/2, L)$ if one notes that on an interval $[0, x_0]$ where $i^s(x_0) = 0$, one has that $0 \leq i^s(x) \leq I_a(0)$. The reason for this is that when one derives (8.2) in this case, one picks up an extra term of the form

$$\frac{1}{2} \int_0^{x_0} [i^s(x)]^2 [k_1 \mathcal{R}'(A) + l_1 \mathcal{L}'(A)] A'(x) \, dx.$$
after integration by parts, which since $\mathcal{R}^2, \mathcal{L}'$ are increasing, is non-negative and hence may be neglected and still preserve the sense of (8.3).

References


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