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A mathematical feasibility argument for the use of aptamers in chemotherapy and imaging ★

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Abstract

A challenge for drug design is to create molecules with optimal functions that also partition efficiently into the appropriate *in vivo* compartment(s). This is particularly true in cancer treatments because cancer cells upregulate their expression of multidrug resistance transporters, which necessitates a higher concentration of extracellular drug to promote sufficiently high intracellular concentrations for cell killing. Pharmacokinetics can be improved by ancillary molecules, such as cyclodextrins, that increase the effective concentrations of hydrophobic drugs in the blood by providing hydrophobic binding pockets. However, the extent to which the extracellular concentration of drug can be increased is limited. A second approach, different from the "push" mechanism just discussed, is a "pull" mechanism by which the effective intracellular concentrations of a drug is increased by a molecule with an affinity for the drug that is located inside the cell. Here we propose and give a proof in principle that intracellular RNA aptamers might perform this function.

The mathematical model considers the following: Suppose I denotes a drug (inhibitor) that must be distributed spatially throughout a cell, but that tends to remain outside the cell due the transport properties of the cell membrane. Suppose that E, a deleterious enzyme that binds to I, is expressed

by the cell and remains in the cell. It may be that the equilibrium $E + I \xrightleftharpoons[k_{-1}]{k_1} P$ is not sufficiently far enough to the right to drive enough free inhibitor into the cell to completely inhibit the enzyme.

Here we evaluate the use of an intracellular aptamer with affinity for the inhibitor (I) to increase the efficiency of inhibitor transport across the cell membrane and thus drive the above equilibrium further to the right than would ordinarily be the case. We show that this outcome will occur if (1) the aptamer neither binds too tightly nor too weakly to the inhibitor than the enzyme and (2) the aptamer is much more diffusible in the cell cytoplasm than the enzyme. Thus, we propose and show by simulation that an intracellular aptamer can be enlisted for an integrated approach to increasing inhibitor effectiveness and imaging aptamer-expressing cells.

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1. Introduction

Living cells have evolved to be surrounded by a lipid membrane that impedes the movement of most compounds into the cell cytoplasm. Proteins integral to the cell membrane facilitate the movement of necessary nutrients such as sugars into the cell. But, the membrane provides a barrier to the entry of many unnatural or toxic compounds. 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 its effectiveness.

Many anti-cancer drugs need to penetrate the cell membrane to perform their functions. Cancer cells in which the drug does not accumulate to a sufficiently high concentration are likely to survive and proliferate. Those that survive a drug treatment frequently have either increased expression of a membrane protein that pumps the drug out of the cell or have increased expression of the target protein of the drug. Increased amounts of these drug pumps called Pgp (P-glycoprotein) or MDR (multi drug resistance protein), of which there are many, decreases the intracellular concentration of the drug [5,1]. The level of pump activity can also be increased without cell division in response to certain drugs. When the level of activity of the efflux pumps is increased by one of several mechanisms, the surviving population tends to become increasingly drug resistant with continued drug treatment.

Another mechanism by which cells can become resistant to a drug is to increase the intracellular concentration of the drug's target protein. In this case, the drug is soaked up by the excess target protein, leaving a small fraction of the target protein population free of drug and capable of activity. Cells that can increase expression of the target protein by mechanisms such as gene amplification are more likely to survive.

Medical science has tried many approaches to combating drug resistance [14]. These approaches include drugs or antibodies that inhibit the pumps, antisense DNAs to decrease pump mRNA levels, and transcriptional regulators to decrease pump mRNA production. With better knowledge of the three-dimensional structures of specific protein pumps and the use of computational approaches to drug design, new anti-pump drugs continue to be developed and some small molecule drugs may eventually be found to solve the problem of multi drug resistance. However, none have yet been identified and neither have other approaches been very successful in combating multi drug resistance. Thus, a new 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 a receptor for that drug.

As the drug receptor we 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 and capable of diffusion through 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 the concentrations of drug pumps and target proteins are often increased 10–100-fold in resistant compared with drug-sensitive cells, these increases may be the result of many smaller sequential increases in the expression of these proteins as the cells proliferate that protect the cells from death in the presence of the drug. There are also reports of increases in protein pump expression in the range of 2–5-fold resulting in resistance [18,21,8]. These observations suggest that cells can acquire resistance with a small increment in their content of protective proteins. They also suggest that the intracellular concentrations of some drugs do

not reach levels much above that necessary for inhibiting the cellular event to which they are targeted.

As well as protecting the cell from drug damage, an aptamer that is used to concentrate a drug in a cell could also be used to image that cell by using a drug variant with an attached signaling moiety (e.g. a radioisotope) for which the emitted signal penetrates solid tissues such as in the human body. Thus, the aptamer could provide a means of imaging the cells in which it is located while simultaneously increasing the intracellular concentration of the drug for more effective therapy.

The efficiency of the drug (heretofore referred to as inhibitor) is defined by the ability of a fixed extracellular concentration of inhibitor to elevate the concentration of the intracellular inhibitor bound enzyme at the expense of unbound enzyme at infinite enzyme dilution.

We show that without diffusion the aptamer can have no effect on the inhibitor efficiency. Then we show that when the aptamer is diffusible, within limits that depend upon the aptamer-inhibitor on and off rates, the efficiency of the inhibitor is increased.

We have not considered the effect of internal cell convection on our model for the following reasons. Internal convection away from the cell cytoplasm and toward the enzyme also acts as a facilitator of inhibitor enhancement within the cell. However, estimates on the directionality as well as the magnitude of these convective effects are likely to be highly cell type dependent. Also, studies of mammalian cells show that convection currents are not created with cell motility such as muscle contraction [15]. Therefore we have tried to take a more conservative approach and only consider molecular diffusion.

In what follows, we build the model from the ground up in order to illustrate the effects of introducing the aptamer into the protein-inhibitor system, first without aptamer diffusion and then with it.

OUTLINE AND SUMMARY OF THE PAPER

- Section 2. The chemical kinetics (protein-inhibitor). In this section we give the assumptions to be made on the enzyme-inhibitor system, record the reaction diffusion equations for the system, and state the boundary conditions that describe the transport of the inhibitor across the cell membrane.
- Section 3. A steady state boundary value problem for the inhibitor when the enzyme does not diffuse is formulated.
- Section 4. Definition of the efficiency of the mechanism. Here we define the notion of inhibitor efficiency *at infinite enzyme dilution*. We take some pains to do this because there are various notions of efficiency of the reaction $E+I \rightleftharpoons \{E:I\}$ and it is important to distinguish clearly among them in order to avoid confusion.
- Section 5. The chemical kinetics (enzyme-inhibitor-aptamer). In this section, the additional terms in the kinetics due to the aptamer are introduced into the enzyme-inhibitor dynamics along with the assumption that the aptamer is expressed by the cell only in the (idealized) cell nucleus. (See Figure 1.)
- Section 6 The case of no aptamer diffusion ($D_a = 0$). Here we argue, using the Maximum Principle for parabolic equations, [17], that without aptamer diffusion, one cannot increase the local efficiency of the inhibitor.

- Section 7. The case when the aptamer is diffusible ($D_a > 0$). In contrast to the preceding section, we show that it is possible for a *diffusible* aptamer to increase the inhibitor efficiency without increasing the external concentration of the inhibitor.
- Section 8. Simulations. Here we present simulations that illustrate the conclusions of the preceding sections. They show that there is a range of aptamer-inhibitor affinities (on-off rate ratios) for which the effect on the inhibitor efficiency is most pronounced in the case of diffusible aptamers. An overall summary of the figures is given in this section while a more detailed discussion of each figure is included in its figure caption.
- Section 9. A summary of the salient results is given.

2. The chemical kinetics (Protein-Inhibitor)

Here we give the assumptions to be made on the enzyme-inhibitor system, record the reaction diffusion equations for the system for both the dynamic and the steady state cases and state the boundary conditions that describe the transport of the inhibitor across the cell wall.

We are given a protein, an inhibitor, and a product of their interaction $\{E : I\}$ denoted as E, I, P according to the usual chemical conventions. We also use the notation $e(\mathbf{x}, t) = [E](t)$ to underscore the fact that these species are distributed in space as well as time.

The mechanism is

which we do **not** assume to be in equilibrium. We assume:

1. The reaction takes place in the cytoplasm of a cell; in mathematical terms, a bounded region Ω of three space.
2. The protein E decays with rate (decay) constant $\mu_e > 0$. The bound enzyme-inhibitor complex $P = \{EI\}$ decays with rate constant $\mu_{ei} \geq 0$.
3. Neither the enzyme nor the product P is diffusible relative to the the inhibitor and (later) the aptamer.
4. The inhibitor species, I , is the only one of I, E, P capable of molecular diffusion.
5. The inhibitor may or may not decay. Let $v \geq 0$ denote this rate constant.
6. The cell functions as a source for the protein, i. e. there is a nonnegative function $S_e(\mathbf{x})$ defined on the region Ω for all nonnegative times that defines the cellular rate of production of the protein. It is assumed that this function is positive (has positive support) on a subset Ω_E of Ω .
7. The cell also functions as a source for the aptamer, i. e. there is a nonnegative function $S_a(\mathbf{x})$ defined on the region Ω that defines the cellular rate of production of the aptamer. It has support set $\Omega_a \subset \Omega$. We think of Ω_a as the cell nucleus.

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

$$\begin{aligned} \frac{\partial e(\mathbf{x}, t)}{\partial t} &= \kappa_{-1} p - \kappa_1 e i - \mu_e e + S_e(\mathbf{x}), \\ \frac{\partial p(\mathbf{x}, t)}{\partial t} &= -\kappa_{-1} p + \kappa_1 e i - \mu_{ei} p, \\ \frac{\partial i(\mathbf{x}, t)}{\partial t} &= D \Delta i + \kappa_{-1} p - \kappa_1 e i - v i \end{aligned} \quad (2.2)$$

where Δ denotes the usual Laplace operator. To these must be appended the initial conditions:

$$i(\mathbf{x}, 0) = i_0(\mathbf{x}) \geq 0, \quad e(\mathbf{x}, 0) = e_0(\mathbf{x}) \geq 0, \quad p(\mathbf{x}, 0) = p_0(\mathbf{x}) \geq 0. \quad (2.3)$$

On the other hand, only one boundary condition is needed. This can be shown to be of Michealis-Menten form, namely:

$$-D\partial_n i(\mathbf{x}, t) = \frac{cK_c(i(\mathbf{x}, t) - i_b)}{K + i(\mathbf{x}, t)} + P_e(i - J) \quad (2.4)$$

where P_e is the membrane permeability of the inhibitor, J is the concentration of the inhibitor on the extracellular side of the cell membrane and is assumed to be constant, i_b is the background concentration of the inhibitor in the membrane due solely to membrane transport at equilibrium in the absence of diffusive transport, where K_c and K are the transport constants for the active transport of inhibitor through the cell membrane, $\partial\Omega$, and where ∂_n denotes the usual outward normal derivative. This boundary condition is the statement that the diffusive flux into the cell is balanced by the sum of the active transport from the inside to the outside and what little passive transport there is into the cell. Here i_b is small relative to the concentration of the drug exterior to the cell (see Appendix 10).

The meaning of (2.4) is this: If the concentration of I is larger than the threshold value i_b , the contribution to the flux out of the cell by the active transport will be positive that is, I will leave the cell. If the concentration is smaller than the threshold value, this contribution to the flux out will be negative. Likewise if the concentration of I exceeds J , passive diffusion will contribute positively to the flux out of the cell while if the concentration of I is smaller than J , passive diffusion will contribute to positively to the flux into the cell.

Remark 1

We have neglected the diffusion of the enzyme and its product in this section because the enzyme is a much larger molecule than the inhibitor by several orders of magnitude. However, it is perhaps worth mentioning the effect the diffusion of the enzyme can have on the concentration of inhibitor. With enzyme diffusion, the system (2.2) becomes:

$$\begin{aligned} \frac{\partial e(\mathbf{x}, t)}{\partial t} &= D_e \Delta e + \kappa_{-1} p - \kappa_1 e i - \mu_e e + S_e(\mathbf{x}), \\ \frac{\partial p(\mathbf{x}, t)}{\partial t} &= D_e \Delta p - \kappa_{-1} p + \kappa_1 e i - \mu_{ei} p, \\ \frac{\partial i(\mathbf{x}, t)}{\partial t} &= D \Delta i + \kappa_{-1} p - \kappa_1 e i - \nu i \end{aligned} \quad (2.5)$$

where we assume that the enzyme and the product have the same diffusivity. Typically the boundary conditions will be of the no flux type:

$$-D_e \partial_n e = -D_e \partial_n p = 0 \quad \text{in } \partial\Omega.$$

These can easily be modified if there is enzyme or enzyme product leakage from the cell.

The steady state system with steady source then becomes:

$$\begin{aligned} 0 &= D_e \Delta E^s + \kappa_{-1} P^s - \kappa_1 E^s I^s - \mu_e E^s + S_e(x), \\ 0 &= D_e \Delta P^s - \kappa_{-1} P^s + \kappa_1 E^s I^s - \mu_{ei} P^s, \\ 0 &= D \Delta I^s + \kappa_{-1} P^s - \kappa_1 E^s I^s - \nu I^s \end{aligned} \quad (2.6)$$

where we have used upper case letters with the superscript s to denote the steady state solution in the absence of the aptamer. (In the presence of the aptamer, we will use p^s , i^s , e^s to denote the steady states for the product, the inhibitor and the enzyme respectively.)

3. A steady state problem for the inhibitor when the enzyme does not diffuse

In this section, the long time behavior of the dynamical system is discussed. A boundary value problem for the free inhibitor in the cell is given when the enzyme is not diffusible in the cytoplasm.

When $D_e = 0$, a number of simple observations can be made to aid us in understanding the effects of diffusion on the dynamics.

It is easy to see by adding the first two equations in (2.2) that the following inequalities hold:

$$\begin{aligned} \min \{\mu_e, \mu_{ei}\} (e(\mathbf{x}, s) + p(\mathbf{x}, t)) &\leq S_e(\mathbf{x}) - \frac{\partial e(\mathbf{x}, t)}{\partial t} - \frac{\partial p(\mathbf{x}, t)}{\partial t}, \\ \max \{\mu_e, \mu_{ei}\} (e(\mathbf{x}, s) + p(\mathbf{x}, t)) &\geq S_e(\mathbf{x}) - \frac{\partial e(\mathbf{x}, t)}{\partial t} - \frac{\partial p(\mathbf{x}, t)}{\partial t}. \end{aligned} \quad (3.1)$$

From these inequalities, we may deduce from Gronwall's inequality, [9], that

$$S_e(\mathbf{x}) \frac{(1 - e^{-\max\{\mu_e, \mu_{ei}\}t})}{\max\{\mu_e, \mu_{ei}\}} + e^{-\max\{\mu_e, \mu_{ei}\}t} [e_0(\mathbf{x}) + p_0(\mathbf{x})] \leq e(\mathbf{x}, t) + p(\mathbf{x}, t) \quad (3.2)$$

and

$$S_e(\mathbf{x}) \frac{(1 - e^{-\min\{\mu_e, \mu_{ei}\}t})}{\min\{\mu_e, \mu_{ei}\}} + e^{-\min\{\mu_e, \mu_{ei}\}t} [e_0(\mathbf{x}) + p_0(\mathbf{x})] \geq e(\mathbf{x}, t) + p(\mathbf{x}, t) \quad (3.3)$$

These tell us that the growth and decay of total enzyme is ultimately tightly regulated by the source function and the half lives of each species. They also tell us that when the enzyme cannot diffuse, it is eventually concentrated on the support set of the source, i. e. on the set of points in the cell where $S_e(\mathbf{x}) > 0$.

On the other hand, because inhibitor is entering from the boundary, it is reasonable to entertain the possibility of steady states for (2.2). Then we can see how the influx of the inhibitor from the boundary influences the production of bound enzyme inside the cell. Let I^s , E^s , P^s be the steady state solutions of (2.2). From the second of these equations, $KP^s = E^s I^s$, where $K = (k_{-1} + \mu_{ei})/k_1$ is the effective disassociation constant for the binding. Now set $K_{m,e} = (k_{-1} + \mu_{ie})/k_1\mu_{ie} = K/\mu_{ei}$ and note that $k_{-1}P^s - k_1I^s E^s = \mu_e E^s - S_e(\mathbf{x})$. A short calculation shows that the steady value for e is

$$E^s = \frac{S_e(\mathbf{x})}{\mu_e + K_1\mu_{ie}I^s / (K_{-1} + \mu_{ie})} = \frac{K_{m,e}S_e(\mathbf{x})}{\mu_e K_{m,e} + I^s}. \quad (3.4)$$

Thus, in the absence of enzyme diffusion, the free enzyme in the cell is positive, when and only when the source of enzyme is positive.

We see that

$$-\mu_e E^s + S_e = \frac{\kappa_1 \mu_{ie} I^s / (\kappa_{-1} + \mu_{ie}) S_e}{\mu_e + \kappa_1 \mu_{ie} I^s / (\kappa_{-1} + \mu_{ie})} = \frac{I^s S_e}{\mu_e \mathcal{K}_{m,e} + I^s}.$$

Then I^s satisfies the steady state problem

$$\begin{aligned} 0 &= D \Delta I^s - \nu I^s - \frac{I^s S_e(\mathbf{x})}{\mu_e \mathcal{K}_{m,e} + I^s} \quad \text{in } \Omega, \\ -D \partial_n I^s(\mathbf{x}) &= \frac{c K_e (I^s(\mathbf{x}) - i_b)}{K_m + I^s(\mathbf{x})} + T_e (I^s - J) \quad \text{in } \partial \Omega. \end{aligned} \quad (3.5)$$

Once this problem is solved, we can write for the total inhibitor, in the cell:

$$I_T = P^s + I^s = I^s (E^s + \mathcal{K}) / \mathcal{K}.$$

4. Definition of the efficiency of the mechanism at infinite enzyme dilution

Here we define the notion of inhibitor efficiency *at infinite enzyme dilution*. There are various notions of efficiency of the reaction $E + I \rightleftharpoons EI$ and it is important to distinguish clearly among them in order to avoid confusion.

We consider two of these notions here. For the first of these, the ratio of the product to the total enzyme is a reasonable choice. That is:

$$\mathfrak{E} = \frac{[EI]}{[E] + [EI]}.$$

When the concentrations are independent of time but not necessarily independent of cell location, we write this locally as:

$$\mathfrak{E}(\mathbf{x}) = \frac{P^s(\mathbf{x})}{E^s(\mathbf{x}) + P^s(\mathbf{x})}.$$

A second definition can be based on a combinatorial argument. We define an efficiency as the mean number of collisions of enzyme with inhibitor resulting in product divided by the mean number of possible collisions of enzyme with inhibitor. That is, if $[E]_T$ and $[I]_T$ denote the total number of enzyme and inhibitor molecules (per unit volume) respectively, the total number of interactions on average is $[E]_T [I]_T = ([E] + [EI])([I] + [EI]) = [I][E] + [I][EI] + [E][EI] + [EI]^2$. At equilibrium, the term $[I][E] (= K_d [IE])$ represents the the total number of $I - E$ interactions, on average, that do *not* result in a molecule of product. Therefore it is reasonable to define an alternative efficiency as

$$\mathfrak{E}_{alt} = 1 - \frac{[E][I]}{([E]_{tot})([I]_{tot})} = \frac{[EI]}{[E] + [EI]} \left(1 + \frac{[E]}{[I] + [EI]} \right) = \mathfrak{E} \left(1 + \frac{[E]}{[I] + [EI]} \right). \quad (4.1)$$

We can think of the expression on the right as the sum of two terms, one $\mathfrak{E}$ due solely to the presence of the inhibitor, while the second, $\mathfrak{E}\{[E]/([I] + [EI])\}$, can be viewed as representing

the contribution of the enzyme to this form of the efficiency, because, as the concentration, $[E]$, of free enzyme decreases, this term goes to zero. Indeed as $[E] \rightarrow 0$,

$$\frac{\mathfrak{E}_{alt} - \mathfrak{E}}{\mathfrak{E}} = \frac{[E]}{[I] + [EI]} \rightarrow 0$$

so that **these two notions agree at infinite enzyme dilution.**

Because we are interested in having a measure of how the presence of the aptamer increases the efficiency of the inhibitor in the cell, we want to have a measure of efficiency that does not involve the enzyme, i. e., we want an average measure of how much enzyme-inhibitor binding there is when there are very few molecules of enzyme around, both when the aptamer is not present to when it is present. **Therefore, for our purposes, we will use the ratio $\mathfrak{E}$ as our definition of efficiency although the reader should understand that we are really talking about the efficiency of the inhibitor at infinite enzyme dilution.**

The definition in (4.1) masks the effect of the inhibitor on the efficiency because it includes the enzyme concentration.

Now consider the efficiency at infinite enzyme dilution (efficiency at IED) when there is no diffusion of the enzyme. In view of the equation for the steady state inhibitor, we must calculate this efficiency pointwise. Then, as we saw above, $[EI] = P^s(\mathbf{x}) = E^e(\mathbf{x})I^s(\mathbf{x})/K$. We find that

$$\mathfrak{E}(\mathbf{x}) = \frac{I^s(\mathbf{x})}{K + I^s(\mathbf{x})}$$

where we have used the earlier steady state definitions. In this definition, the efficiency at infinite enzyme dilution depends only upon the inhibitor as it should.

From the point of view of measurable quantities, we assign a single global efficiency to the process, i. e.

$$\mathfrak{E} = \frac{1}{V(\Omega_E)} \int_{\Omega} \mathfrak{E}(\mathbf{x}) \, d\mathbf{x}. \quad (4.2)$$

(Here $V(\Omega_E)$ is the volume of Ω_E where the enzyme concentration is not zero.)

If we are not in a steady state situation, there are only two possibilities for the support set of the enzyme, i.e. the set where the enzyme concentration is positive depending on whether or not the enzyme is diffusible. If the enzyme is not diffusible, then it is positive only where the source function is positive, on the set Ω_E .

When the enzyme is diffusible, thanks to the principle of infinite speed of propagation for parabolic equations, (see e.g. [?]), the support set is Ω . Letting Ω' be one of these two sets, we can follow the efficiency in time viz:

$$\mathfrak{E}(t) = \frac{1}{V(\Omega')} \int_{\Omega'} \mathfrak{E}(\mathbf{x}, t) \, d\mathbf{x} = \frac{1}{V(\Omega')} \int_{\Omega'} \frac{p(\mathbf{x}, t)}{e(\mathbf{x}, t) + p\mathbf{x}, t} \, d\mathbf{x}. \quad (4.3)$$

Throughout most of the remainder of this paper we take $D_e = 0$ and hence $\Omega' = \Omega_E$.

5. The chemical kinetics (enzyme-inhibitor-aptamer)

In this section, the additional terms in the kinetics due to the aptamer are introduced into the enzyme-inhibitor dynamics along with the assumption that the aptamer is expressed by the cell only in the (idealized) cell nucleus. We show that the efficiency of the inhibitor depends implicitly on the concentration of the aptamer.

We consider the effect of an aptamer on the efficiency of the reaction: $E + I \xrightleftharpoons[\kappa_{-1}]{\kappa_1} IE$ where now we have used IE to denote the protein-inhibitor product. The aptamer has a binding site for the inhibitor.

The aptamer, A , interacts with the inhibitor via

To set the stage for the rate equations to follow, we assume that, in terms of molecular weights, $M_I \ll M_A \ll M_E$ where M_J denotes the molecular weight of species J . We have five molecular species $\{I, A, E, AI, IE, \}$. The smallest molecule, I , will be the most diffusible. Therefore, we take $D = D_I > D_A = D_{AI} = D_a \Rightarrow 0$ while the diffusion coefficient of E is set to zero. We also assume that, in the absence of diffusion, all of the reactions above are reversible so that no rate constant vanishes. The notation for the steady state versions of the aptamer dependent concentrations is given Table 2. In order to determine whether or not the addition of the aptamer affects the efficiency of the inhibitor, we need to compute the efficiency ($D_e = 0$ here so $\Omega' = \Omega_E$):

$$\mathfrak{E}(a, t) = \frac{1}{V(\Omega_E)} \int_{\Omega_E} \frac{p(\mathbf{x}, t)}{e(\mathbf{x}, t) + p(\mathbf{x}, t)} d\mathbf{x} \quad (5.2)$$

for the solution of the system (5.3) below that replaces (2.2). We assume the cell expresses enzyme and aptamer at steady rates $S_e(\mathbf{x}), S_a(\mathbf{x})$ respectively. Suppose that this inhibitor decays at a rate ν . Assume furthermore that the compounds E, IE have roughly the same half life, $\ln 2/\mu_e$ say. The decay rate for the aptamer will be denoted by ν_a . We assume that the diffusion constants satisfy $D > D_a \geq D_e = 0$. Mass action applied to the above chemical equations leads to:

$$\begin{aligned} \partial_t i &= D\Delta i + \kappa_{-1}p - \kappa_1 ei + l_{-1}p_a - l_1 ai - \nu i, \\ \partial_t a &= D_a\Delta a + l_{-1}p_a - (l_1 i + \nu_a)a + S_a(\mathbf{x}), \\ \partial_t p_a &= D_a\Delta p_a + l_1 ai - (l_{-1} + \nu_{ai})p_a, \\ \partial_t e &= \kappa_{-1}p - (\kappa_1 i + \mu_e)e + S_e(\mathbf{x}), \\ \partial_t p &= \kappa_1 ie - (\kappa_{-1} + \mu_{ei})p \end{aligned} \quad (5.3)$$

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

$$\partial_x a(0, t) = \partial_x a(L, t) = \partial_x p_a(0, t) = \partial_x p_a(L, t) = 0 \quad (5.4)$$

so that the aptamer forms are constrained to stay in the cell.

Assuming a steady state solution, there results

$$\begin{aligned}
0 &= D\Delta i^s + \kappa_{-1} p^s - \kappa_1 e^s i^s + l_{-1} p_a^s - l_1 a^s i^s - v i^s, \\
0 &= D_a \Delta a^s + l_{-1} p_a^s - (l_1 i^s + v_a) a^s + S_a(\mathbf{x}), \\
0 &= D_a \Delta p_a^s + l_1 a^s i^s - (l_{-1} + v_{ai}) p_a^s, \\
0 &= \kappa_{-1} p^s - (\kappa_1 i^s + \mu_e) e^s + S_e(\mathbf{x}), \\
0 &= \kappa_1 i^s e^s - (\kappa_{-1} + \mu_{ei}) p^s.
\end{aligned} \tag{5.5}$$

Because the last two of these equations do not depend on the aptamer explicitly, we still have $Kp^s = Kp^s = e^s i^s$ and $e^s(\mathbf{x}) = K_{m,e} S_e(\mathbf{x}) / (\mu_e K_{m,e} + i^s(\mathbf{x}))$. **However the quantity i^s now depends on a_s .**

6. The case of no aptamer diffusion ($D_a = 0$)

We show that without aptamer diffusion, one cannot increase the local efficiency of the inhibitor, i.e. the diffusibility of an aptamer is necessary for it to improve the efficiency of an inhibitor at infinite enzyme dilution.

Because we can obtain explicit formulas for the concentrations of the enzyme and the product in terms of the inhibitor in the steady state, we can study the problem analytically in this case. Thus we examine solutions of (5.5) together with no flux boundary conditions.

Consider the problem of efficiency. We are concerned with the local efficiency here. Suppose that $e(\mathbf{x}) > 0$. The system is more efficient at $\mathbf{x}$ with the aptamer than without it if and only if

$$\mathfrak{E}(a)(\mathbf{x}) = \frac{p^s(\mathbf{x})}{e^s(\mathbf{x}) + p^s(\mathbf{x})} > \mathfrak{E}(0) = \frac{I^s(\mathbf{x})}{I^s(\mathbf{x}) + K} \tag{6.1}$$

where the value of $\mathfrak{E}(0)$ on the right is taken from Section 4 **and** the value of the inhibitor concentration is assumed to be i^s when the aptamer is present and I^s when it is not.

In view of the fact that the function $\omega/(1 + \omega)$ is strictly increasing in ω , (6.1) will hold on Ω_E if and only if $(\mathfrak{R}(\mathbf{x}) = (K/I^s(\mathbf{x})) \cdot (p^s(\mathbf{x})/e^s(\mathbf{x}))) > 1$ holds there. We see that $\mathfrak{R}(\mathbf{x}) = i^s(\mathbf{x})/I^s(\mathbf{x})$. Thus, the efficiency is increased by the aptamer at a point of Ω' if and only if the free inhibitor is increased at the corresponding point.

This leads us to the next part of our mission, to determine how the free inhibitor concentration with aptamer behaves relative to the free inhibitor concentration without it. In order to understand this relationship, we need to examine the diffusion equations for inhibitor and aptamer.

Once we have determined e , p , p_a and a , we need to solve the first equation in (5.5) for i^s . This equation has the form

$$0 = D\Delta i^s - v i^s + (\Delta p^s + \Delta p_a^s) \tag{6.2}$$

where $\Delta p^s = \kappa_{-1} p^s - \kappa_1 e i^s$ etc. From the stationary equations when $D_a = 0$, these become,

$$l_1 a^s i^s - l_{-1} p_a^s = \Delta p_a^s = v_{ai} p_a^s, \Rightarrow p_a^s = \frac{l_1 a^s i^s}{l_{-1} + v_{ai}}, l_1 a^s i^s + v_a a^s = S_a(\mathbf{x}) + l_{-1} p_a^s, \Rightarrow a^s = \frac{l_{-1} p_a^s + S_a(\mathbf{x})}{l_1 i^s + v_a}$$

$$l_1 a^s i^s + v_a a^s = S_a(\mathbf{x}) + l_{-1} p_a^s \Rightarrow a^s = \frac{l_{-1} p_a^s + S_a(\mathbf{x})}{l_1 i^s + v_a}$$

$$\kappa_1 i^s e^s - \kappa_{-1} p^s = \Delta p^s = \mu_e p^s = \mu_e e^s - S_e(\mathbf{x}) = -\frac{i^s S_e(\mathbf{x})}{\mu_e \mathcal{K}_{m,e} + i^s}.$$

Therefore after a little algebra,

$$\begin{aligned} 0 &= D\Delta i^s - v i^s - v_{ai} P_a^s - v_{ei} p^s \\ &= D\Delta i^s - v i^s - \frac{i^s S_a(\mathbf{x})}{v_a \mathcal{K}_{m,a} + i^s} - \frac{i^s S_e(\mathbf{x})}{\mu_e \mathcal{K}_{m,e} + i^s}, \end{aligned} \quad (6.3)$$

where we have set $K_{m,a} = (l_{-1} + v_{ia})/l_1 v_{ia}$.

When $S_a(\mathbf{x}) = 0$ we recover the equation for the steady concentration of the inhibitor in Section 2 as we should. Secondly, if $e^s = 0$, the aptamer alone will bind to the inhibitor and influence the concentration of free inhibitor in much the same way as the enzyme by lowering it over what it would be if there were neither aptamer nor enzyme in the system. *However, because all of the terms on the right hand side of (6.3) are negative, the level of free inhibitor inside the cell cannot be raised by the presence of a non diffusible aptamer. That is, one cannot decrease the free or active enzyme concentration with a non diffusible aptamer.* This is a consequence of the Maximum Principle. That is, in any region where $\Delta i^s \geq 0$, the maximum of I must occur on the boundary of the region or else I^s is a constant.¹ See [17].

7. The case when the aptamer is diffusible ($D_a > 0$)

In contrast to the preceding section, we show that it is possible for a *diffusible* aptamer to increase the inhibitor efficiency if the external concentration of the inhibitor is constant.

The boundary conditions for (6.3) are the same as for the case for which there is no aptamer present. Thus, no additional free inhibitor can come from outside the cell beyond that dictated by boundary transport.

If the aptamer is diffusible, then $l_1 a^s i^s - l_{-1} p_a^s = D_a \Delta a^s - v_a a^s + S_a(\mathbf{x})$. The boundary conditions will be those for the operator $D_a \Delta - v_a I$ with no flux boundary conditions. Then the inhibitor equation becomes

$$0 = D\Delta i^s - v i^s - \frac{i^s S_e(\mathbf{x})}{\mu_e \mathcal{K}_{m,e} + i^s} - (D_a \Delta a^s - v_a a^s + S_a(\mathbf{x})) \quad (7.1)$$

and we can no longer invoke (6.3). Consequently we cannot rule out the possibility that diffusion of the aptamer can raise the level of inhibitor in Ω_E if the aptamer can diffuse within the cell. That is, it may be possible to raise the free inhibitor concentration in Ω_E above the zero aptamer level if the sum of all the terms following $D\Delta i^s - v i^s$ in (7.1) is positive. Equation (7.1) suggests that this is quite possible in Ω_E if the local concentration of free aptamer and

¹In its simplest form, the Maximum Principle for functions having a continuous second derivative on an interval (a, b) says that if the second derivative is nonnegative on (a, b) then the function cannot have a maximum at a point c , $a < c < b$ unless the function is constant on (a, b) .

free inhibitor are both small relative to the bound aptamer, i. e., $l_1 i^s a^s \leq l_{-1} p_a^s$ or equivalently $0 > D_a \Delta a^s - v_a a^s + S_a(\mathbf{x})$ in Ω_E . Hence, if this inequality holds in Ω_E , we have

$$D \Delta I^s - v I^s - \frac{I^s S_e(\mathbf{x})}{\mu_e \mathcal{K}_{m,e} + I^s} = 0 > D \Delta i^s - v i^s - \frac{i^s S_e(\mathbf{x})}{\mu_e \mathcal{K}_{m,e} + i^s}$$

or

$$D \Delta (I^s - i^s) \geq v (I^s - i^s) + \frac{I^s S_e(\mathbf{x})}{\mu_e \mathcal{K}_{m,e} + I^s} - \frac{i^s S_e(\mathbf{x})}{\mu_e \mathcal{K}_{m,e} + i^s} \quad (7.2)$$

As a function of I^s , the function $(I^s S_e(\mathbf{x})) / (\mu_e \mathcal{K}_{m,e} + I^s)$ is strictly increasing. Thus, if the aptamer failed to increase the inhibitor concentration on Ω_E or at its boundary, then $I^s - i^s \geq 0$ there and hence would have a positive maximum on Ω_E or at its boundary. But then the right hand side of this last equation is non negative on Ω_E and hence so is $\Delta(I^s - i^s)$. But the strong form of the Maximum Principle tells us that at an interior positive maximum, $\Delta(I^s - i^s) < 0$. The positive maximum occurred on the boundary of Ω_E or in $\Omega - \Omega_E$. It cannot occur on the former set because then the outer normal would be zero at such a point and the second statement of the Maximum Principle states that the normal derivative must be strictly positive at such a point. Thus $I^s - i^s < 0$ on Ω_E and the inhibitor efficiency *must* increase on the set Ω_E .

That is, we have demonstrated that if $D_a \Delta a^s - v_a a^s + S_a(\mathbf{x}) < 0$ in Ω_E and if $i^s - I^s \geq 0$ on $\Omega - \Omega_E$, diffusion of the aptamer will increase the inhibitor efficiency. Moreover, we have not ruled out the possibility that $i^s - I^s$ can have both signs on $\Omega - \Omega_E$ and still be positive on Ω_E . We illustrate this possibility in the simulations.

Remark 2

Because, in general, the aptamer is synthesized by the cell in the nucleus (it is a short chain RNA) while the enzyme is synthesized in the cytoplasm, $S_a(\mathbf{x}) = 0$ on Ω_E . Thus the condition that the free inhibitor is larger in Ω_E with the aptamer than without it reduces to condition that $D_a \Delta a^s - v_a a^s < 0$ on Ω_E .

Remark 3

It is perhaps worth mentioning that the off rate plays a critical role in this. For example, if the off rate, $l_{-1} = 0$, then the capture of inhibitor by aptamer will not influence the inhibitor efficiency. The first three equations of system (5.3) become

$$\begin{aligned} \partial_t i &= D \Delta i + \kappa_{-1} p - \kappa_1 e i - l_1 a i - v i, \\ \partial_t a &= D_a \Delta a - (l_1 i + v_a) a + S_a(\mathbf{x}), \\ \partial_t p_a &= D_a \Delta p_a + l_1 a i - v_{ai} p_a, \end{aligned} \quad (7.3)$$

The first of these tells us that when $l_{-1} = 0$ less free inhibitor is available to the cell than there would be if no aptamer with this property were present. Consequently, we would expect a decrease in efficiency beyond that in which there is no aptamer present.

When l_{-1} is large, it is better to set $\tau = l_{-1} t$ in (5.3) and then pass to the limit as $l_{-1} \rightarrow \infty$. The resulting equations become

$$\partial_\tau i = p_a, \quad \partial_\tau a = p_a, \quad \partial_\tau p_a = -p_a, \quad \partial_\tau e = 0, \quad \partial_\tau p = 0 \quad (7.4)$$

where we have assumed that the sources S_e and S_a are uniformly bounded. These tell us that $p_a(\tau) \approx p_a(\tau_0)e^{(\tau-\tau_0)}$, $i(\tau) \approx i(\tau_0) + p_a(\tau_0)(1 - e^{(\tau-\tau_0)})$. Thus, as $\tau \rightarrow \infty$, $i(\tau) \rightarrow i(\tau_0) + p_a(\tau_0)$. Assuming that at $\tau = \infty$ the enzyme and the inhibitor are in equilibrium with the product, we can write for large τ

$$\mathfrak{E} = \frac{p(\tau)}{p(\tau) + e(\tau)} = \frac{i(\tau)}{i(\tau) + K} \xrightarrow{\tau \rightarrow \infty} \frac{i(\tau_0) + p_a(\tau_0)}{i(\tau_0) + p_a(\tau_0) + K} > \frac{i(\tau_0)}{i(\tau_0) + K}$$

where the last expression is the efficiency if there were no aptamer present in the system. This inequality is confirmed in the simulations. See Figure 10.

8. Simulations

As a test of the ideas above, we considered a three dimensional rectilinear cell in which the spatial distribution of the reactants was uniform in two dimensions. Thus the nucleus is viewed as a “slab” located in the middle third of this box as indicated by the horizontal axis in Figure 1.

In order to test the claims of the preceding section numerically, we calculate the values for a and i as the limit as time becomes large. That is, we consider the initial-boundary value problem given by (5.3), (5.4) and (2.4) for $0 < x < L$ and $t > 0$.

The same system can be used to determine the inhibitor distribution when there is no aptamer in the cell by setting $S_a = 0$ in the second and third equations (5.3). The solution of these two equations will then be $a = p_a = 0$. The solution of the first, fourth, and fifth equations will give the concentration of free inhibitor in the (one dimensional) cell when only the enzyme is present.

The effects of aptamer diffusion on the total concentration of inhibitor in the cell, the mean total inhibitor, the concentration of bound enzyme, the concentration of free enzyme and the mean efficiency of inhibition are illustrated in Figure 2, Figure 3, Figure 4, Figure 5 respectively.

In Figure 6 the effect of aptamer diffusion on the internal concentration of the free inhibitor is shown in regions where the hypothesis of the Maximum Principle holds ($Q < 0$). In Figure 7 the effect of aptamer diffusion on the free inhibitor concentration is shown in regions where this hypothesis fails ($Q \geq 0$).

The computations demonstrate that there is a range of aptamer-inhibitor affinities (on/off rates) for which the effect on the inhibitor efficiency is most pronounced. (Figure 10, Figure 12).

In Figure 8, Figure 9 and Figure 11 one sees the effect on the local concentration of free enzyme when the aptamer diffusion coefficient, the aptamer off rate and the magnitude of the cellular rate of aptamer synthesis are separately varied. These figures can be viewed as constituting a sensitivity analysis.

9. Discussion

Our mathematical model suggests that it may be possible to raise the level of free enzyme inhibitor in a cell by means of a cell-expressed **diffusible** aptamer that binds to the inhibitor with a binding constant that is within a prescribed range. If the binding is not tight enough (the off rate is large relative to the on rate), the efficiency is not appreciably increased over that without the aptamer present. Whereas if the binding of the aptamer to the drug is too tight (the on rate is large relative to the off rate), the efficiency is somewhat increased over what we

might expect when the aptamer is absent. In these simulations, the range of off rates for which the efficiency was appreciably raised was biochemically realistic as was the time during which the efficiency is raised.

On the other hand, we have shown that it is impossible for a nondiffusible aptamer to increase the overall inhibitor efficiency in a cell over the inhibitor efficiency when no aptamer is present.

Cell populations often become more resistant to drugs because the cells amplify genes encoding drug efflux pumps. Cells can also respond to certain drugs with increased expression of genes that encode drug efflux pumps. [4]. However, studies have shown that it takes 2–7 days for the drugs to increase the pump activity after adding an inducing concentration of drug [6,12,13, 19]. This time course is much longer than our model predicts for the time course of increase in drug due to aptamer. Therefore, our results suggest that the aptamer will increase the drug concentration sufficiently to kill the cell before the cell can increase the concentration of efflux pumps sufficiently to oppose the action of the aptamer.

Appendix

We present a continuum model analog of the compartment model discussed in [20]. Our model also allows for some back flow from the apical region to the cell interior. Imagine the cell membrane of thickness δ_x to be situated so that the x -axis is normal to the membrane. The interior of the membrane is then the set of points $\{(x, y, z) | 0 < x < \delta_x\}$. Suppose T is the transfer protein in the cell membrane and that $I(x, y, z)$ is the concentration of inhibitor inside the cell ($x \leq 0$) while $[J](x, y, z)$ is the concentration of inhibitor exterior to the cell ($x \geq \delta_x$). Let $I'(x, y, z)$, $J'(x, y, z)$ be the concentrations in the membrane near $x = 0$ and $x = \delta_x$ respectively. Then we have the chemical reactions within the membrane:

Regarding the concentrations of the intermediates $\{I'T\}$, $\{J'T\}$ as being nearly constant, we obtain from mass action that

$$[T] \begin{bmatrix} \kappa_m [I] \\ \kappa_{-2} [J] \end{bmatrix} = \begin{bmatrix} \kappa_{-m} + \kappa_3 & -\kappa_{-3} \\ -\kappa_3 & \kappa_2 + \kappa_{-3} \end{bmatrix} \begin{bmatrix} [\{I'T\}] \\ [\{J'T\}] \end{bmatrix} \quad (10.3)$$

or, upon solving, that

$$\begin{bmatrix} [\{I'T\}] \\ [\{J'T\}] \end{bmatrix} = \frac{[T]}{\mathfrak{d}_e} \begin{bmatrix} \kappa_2 + \kappa_{-3} & \kappa_{-3} \\ \kappa_3 & \kappa_{-m} + \kappa_3 \end{bmatrix} \begin{bmatrix} \kappa_m [I] \\ \kappa_{-2} [J] \end{bmatrix} \quad (10.4)$$

where $\mathfrak{d}_e = (\kappa_{-m} + \kappa_3)(\kappa_2 + \kappa_{-3}) - \kappa_{-3}\kappa_3 > 0$. Since the total concentration of transfer protein is fixed, T_0 say, we have, in the cell membrane, that $[T] + [\{I'T\}] + [\{J'T\}] = T_0$ and consequently: $[T] = T_0/(1 + \mathfrak{R}_2[I] + \mathfrak{R}_{-1}[J])$ where $\mathfrak{R}'_2 = (\kappa_2 + \kappa_3 + \kappa_{-3})\kappa_m$ and $\mathfrak{R}'_{-1} = (\kappa_{-m} + \kappa_3 + \kappa_{-3})\kappa_{-2}$. Thus, from mass-action we obtain:

$$\begin{aligned} \frac{\partial [I]}{\partial t} &= -\kappa_m [I] [T] + \kappa_{-m} [\{I'T\}] \\ &= -(\kappa_m \kappa_2 \kappa_3 [I] - \kappa_{-m} \kappa_{-2} \kappa_{-3} [J]) [T] / \mathfrak{d}_e \\ &= \frac{-(\kappa_m \kappa_2 \kappa_3 [I] - \kappa_{-m} \kappa_{-2} \kappa_{-3} [J]) [T_0]}{\mathfrak{d}_e + \mathfrak{R}'_2 [I] + \mathfrak{R}'_{-1} [J]}. \end{aligned} \quad (10.5)$$

In order to relate the concentrations inside the membrane to those outside the membrane we write $[I'] = K_I [I]$ and $[J'] = K_J [J]$ where the K 's are the partition coefficients. Then with the understanding that $\mathfrak{R}_2 = K_I \mathfrak{R}'_2$ and $\mathfrak{R}_{-1} = K_J \mathfrak{R}'_{-1}$, at $x = 0$ we must have

$$\frac{\partial [I]}{\partial t} = \frac{-(\kappa_m \kappa_2 \kappa_3 [I] - \kappa_{-m} \kappa_{-2} \kappa_{-3} (K_J/K_I) [J]) T_0}{\mathfrak{D}_e + \mathfrak{R}_2 [I] + \mathfrak{R}_{-1} [J]}.$$

Finally, to take into account passive diffusion across the membrane, we add the term $-P_e([I] - [J])/\delta_x$ to the right hand side of this equation. Here P_e is the membrane permeability constant. We must also include the decay of the inhibitor, by adding an additional term $-\nu[I]$ to this side of the equation. Thus $\mathfrak{R}_{-1} = K_J \mathfrak{R}'_{-1}$, at $x = 0$ we must have

$$\frac{\partial [I]}{\partial t} = \frac{-(\kappa_m \kappa_2 \kappa_3 [I] - \kappa_{-m} \kappa_{-2} \kappa_{-3} (K_J/K_I) [J]) T_0}{\mathfrak{D}_e + \mathfrak{R}_2 [I] + \mathfrak{R}_{-1} [J]} - \frac{P_e ([I] - [J])}{\delta_x} - \nu [I]$$

Consider now a small control volume $\delta v = \delta_x \delta_y \delta_z$ in the cell membrane. The rate of accumulation of I (in micro moles per unit time) in this control volume is $\delta v \partial [I]/\partial t$. (If $[I]_x(0, t) > 0$, mass flows out of the membrane and into the cell.) However, by Fick's first law,

the flux of I out of the cell in micromoles per square micron per unit time is $-D \frac{\partial [I]}{\partial x}$. Therefore, into this control volume must flow $\delta_y \delta_z D \partial [I]/\partial x$ in units of micro moles per unit time. Thus, in order to have mass balance at the interface, we must have, at $x = 0$

$$\frac{D}{\delta_x} \frac{\partial [I]}{\partial x} (0, t) = - \frac{(\kappa_m \kappa_2 \kappa_3 [I] - \kappa_{-m} \kappa_{-2} \kappa_{-3} (K_J/K_I) [J]) T_0}{\mathfrak{D}_e + \mathfrak{R}_2 [I] + \mathfrak{R}_{-1} [J]} - \frac{P_e ([I] - [J])}{\delta_x} - \nu [I].$$

If, as is the situation here, $[J] = J_0$ is essentially constant and $\kappa_{-m} \kappa_{-2} \kappa_{-3}$ is very small, (i. e., most of the inhibitor is concentrated in the exterior of the cell in the absence of diffusion), there will be a little inhibitor in the cell at equilibrium. When the permeability can be neglected, its concentration is $i_b = \kappa_{-m} \kappa_{-2} \kappa_{-3} (K_J/K_I) [J] J_0 / (\kappa_m \kappa_2 \kappa_3)$. Multiplying both sides of this by δ_x and assuming that $\nu \delta_x$ is very small, we obtain

$$-D \frac{\partial [I]}{\partial x} (0, t) = \frac{(\kappa_m \kappa_2 \kappa_3 [I] - \kappa_{-m} \kappa_{-2} \kappa_{-3} (K_J/K_I) [J]) T_0}{\mathfrak{D}_e + \mathfrak{R}_2 [I] + \mathfrak{R}_{-1} [J]} + P_e (I - J). \quad (10.6)$$

If we set $K_c = \delta_x T_0 \kappa_m \kappa_2 \kappa_3 / \mathfrak{R}_2$ and $K = (\mathfrak{D}_e + \mathfrak{R}_{-1} J_0) / \mathfrak{R}_2$ we obtain the boundary condition (2.4). The quantity $\delta_x T_0$ may be viewed as the concentration of transfer protein in micromoles per unit area. That is, it is the mean surface area concentration of transfer protein in the cell membrane. The left hand side of this equation is the flux out of the cell interior, into the membrane. Notice that if the concentration of J exceeds that of I the permeability term contributes a negative contribution to the flux out of the cell, i.e. a positive contribution to the flux into the cell.

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Figure 1. The geometry of the problem

In this figure, the support of the enzyme is two intervals $[L/6, L/3]$ and $[23L/30, 14L/15]$ while the support of the aptamer source, S_a is presumed to be the interval $[L/3, 2L/3]$. Thus part of the enzyme support is close to the "nucleus" and part of the support is close to the cell membrane. The figure can also be viewed as a three dimensional figure in which the y, z variation of the concentration densities are uniform in x .

Figure 2. The effect of the aptamer on total intracellular inhibitor concentration

The panels (a), (b) are plots of the total inhibitor ($[I] + [EI]$) in the absence of the aptamer and with the aptamer respectively. Notice how much the total inhibitor concentration has increased in passing from panel (a) to panel (b).

Figure 3. The mean total inhibitor as a function of time
The mean total inhibitor,

$$(1/L) \int_0^L i(x, t) dx$$

is plotted (in the absence enzyme) both in the presence of the aptamer and without the aptamer. Notice that after an hour, there is a more than 10 fold increase in the total inhibitor in the cell in presence of aptamer compared with in its absence.

Figure 4. The effect of the aptamer on bound enzyme concentration

Panels (a), (b) are plots of the bound enzyme ($[EI]$) in the absence of the aptamer and with the aptamer respectively. Notice that the total bound enzyme increases significantly in passing from panel (a) to panel (b).

Figure 5. The effect of the aptamer on the free enzyme concentration and on the mean efficiency at infinite enzyme dilution

Here panels (a), (b) are plots of free $[E]$ without and with the mobile aptamer present. Notice that after a short time, almost all of the free (active) enzyme is gone in the latter case. In panel (c), we track the mean efficiency of the inhibitor over time with the aptamer absent while in panel (d) we track the mean efficiency of the inhibitor over time with the aptamer present. In the presence of the aptamer there is a very short delay until the efficiency reaches nearly 100%. Without the aptamer the efficiency stays well below this number even after several hours (not shown).

Figure 6. Numerical illustration of the sufficiency of the hypothesis of the Maximum Principle
 The horizontal axis in these panels as well as those in Figure 7 denotes cell position in units of microns. In panels (a,c) we have plotted the quantity $Q(x, T) = D_a a''(x, T) - v_a(x, T) + S_a(x, T)$ in the left and right enzyme regions respectively for a large time T for which the solution appears to be nearly in steady state. In panels (b) (d) we have plotted the ratio of free inhibitor concentration in the absence of the aptamer to the free inhibitor concentration when in the presence of the aptamer in these same regions. Notice that this ratio is smaller than unity meaning that the reciprocal ratios are quite large. That is, the diffusion of the aptamer greatly increases the concentration of free aptamer in the enzyme region.

Figure 7. Numerical illustration of the lack of necessity of the hypothesis of the Maximum Principle
 In panels (a,c,e) we have plotted the quantity $Q(x, T) = D_a a''(x, T) - v_a(x, T) + S_a(x, T)$ in the exterior of the enzyme regions for a large time T for which the solution appears to be nearly at steady state. In panels (b), (d) we have plotted the ratio of free inhibitor concentration in the absence of the aptamer to the free inhibitor concentration in the presence of the aptamer in these same regions.

Figure 8. Sensitivity of the free enzyme concentration to the diffusivity of the aptamer
 In this figure, we plot the free enzyme concentration for four values of the diffusion coefficient D_a . Panel (a), $D_a = 0$; Panel (b), $D_a = 7.92(10^{-7})\text{cm}^2\text{h}^{-1}$; Panel (c), $D_a = 7.92(10^{-4})\text{cm}^2\text{h}^{-1}$; Panel (d), $D_a = 3.96(10^{-3})\text{cm}^2\text{h}^{-1}$.

Figure 9. Sensitivity of the free enzyme concentration to the aptamer-inhibitor off rate
 We plot the free enzyme concentration for four values of the off constant L_{-1} . Panel (a), $L_{-1} = 0 \text{ h}^{-1}$; Panel (b), $L_{-1} = 1.44 \text{ h}^{-1}$; Panel (c), $L_{-1} = 1.44(10^3) \text{ h}^{-1}$; Panel (d), $L_{-1} = 1.44(10^7) \text{ h}^{-1}$.

Figure 10. Sensitivity of the mean efficiency to the aptamer-inhibitor off rate

Here we consider the variation of the overall efficiency as a function of the off rate l_{-1} and the time up to time $T = 1$ hr. The off rate tick points correspond to $n = -32, \dots, -1, 0, 1, \dots, 32$ where $l_{-1}(n) = 1.44(10^3)2^n = l_{-1}(0)2^n$. That is $3.35(10^{-8})\text{h}^{-1} \leq l_{-1} \leq 6.18(10^{12})\text{h}^{-1}$, a range of twenty orders of magnitude. Notice that the terminal efficiencies near the upper end of this range ($n = 32$) are larger than those near the lower end of the range ($n = -32$). The meaning of this is that even aptamers that tightly bind the inhibitor, thus interfering with the inhibitor's ability to bind the enzyme, increase the efficiency over those aptamers that only loosely bind the inhibitor.

Figure 11. Sensitivity of the free enzyme concentration to the mean rate of aptamer expression
 In this figure, we plot the free enzyme concentration for four values of the mean strength of $S_a, \overline{S_a}$. Panel (a), $\overline{S_a} = 0$; Panel (b), $\overline{S_a} = 8.44(10^{-1}) \mu\text{Mh}^{-1}$; Panel (c), $\overline{S_a} = 8.44(10^2) \mu\text{Mh}^{-1}$; Panel (d), $\overline{S_a} = 8.44(10^5) \mu\text{Mh}^{-1}$.

Figure 12. The mean efficiency at two particular times

Snapshots of mean efficiency as a function of off rate after 0.5, one and two hours. Notice that when the off rate is very large, the mean efficiency is much larger than when the off rate is very small.

Table 1

Notation

Species	symbol	time dependent concentration	steady state without aptamer	steady state with aptamer
free enzyme	E	$[E], e(\mathbf{x}, t)$	$E^s(\mathbf{x})$	$e^s(\mathbf{x})$
free inhibitor	I	$[I], i(\mathbf{x}, t)$	$I^s(\mathbf{x})$	$i^s(\mathbf{x})$
bound enzyme	$E : I(EI)$	$[EI], p(\mathbf{x}, t)$	$P^s(\mathbf{x})$	$p^s(\mathbf{x})$
free aptamer	A	$[A], a(\mathbf{x}, t)$		$a^s(\mathbf{x})$
bound aptamer	$A : I(AI)$	$[AI], p_a(\mathbf{x}, t)$		$p_a^s(\mathbf{x})$

Table 2

Numerical values used in simulations

Equation	constants	values	Notes and Comments
	T	1.0 hour	time scale
(2.2)	D	$1.85(10^{-3})\text{cm}^2\text{h}^{-1}$	(See notes.)
"	k_{-1}	36h^{-1}	
"	k_1	$k_{-1} * 10^8 = 3.6 * 10^9 (\mu\text{M h})^{-1}$	
"	v	0h^{-1}	(stable complex)
"	μ_e	$1.6(10^{-2})\text{h}^{-1}$	See notes. [20].
"	μ_{ie}	$1.6(10^{-2})\text{h}^{-1}$	
(2.4)	K_c	$0.576\mu\text{M} \cdot \text{cm}(\text{h})^{-1}$	See notes. [20].
"	K	$2.19\mu\text{M}$	See notes. [20].
"	i_b	0.0	$k_{22} = 0$. [20].
"	J	$1.0 - 10^2 \mu\text{M}$	(We took $J = 1.0\mu\text{M}$.)
"	Pe	$3.6 \times 10^{-2} \text{cm/h}$	permeability [20].
(5.3)	D_a	$2.33(10^{-4})\text{cm}^2\text{h}^{-1}$	(See notes.)
"	v_a	$2.31(10^{-2})\text{h}^{-1} - 1.39\text{h}^{-1}$	(See notes)
"	v_{ai}	$2.31(10^{-2})\text{h}^{-1} - 1.39\text{h}^{-1}$	(See notes.)
"	L_{-1}	$1.44(10^3)\text{h}^{-1}$	(See notes.)
"	l_1	$1.03(10^4)(\mu\text{M})^{-1}\text{h}^{-1}$	(See notes.)
"	S_e	$0.03381(\mu\text{M})\text{h}^{-1}$	(See notes.)
"	S_a	$4.616(\mu\text{M})\text{h}^{-1}$	(See notes.)

¹The diffusion constants of aptamer and inhibitor were computed from the Stokes-Einstein equation and a knowledge of the molecular weights of aptamer and inhibitor and using the known diffusion coefficient $DT_f = 2.664(10^{-3})\text{cm}^2\text{h}^{-1}$ for tirapazamine (3-amino-1,2,4-benzotriazine-1,4 dioxide) [10]. This molecule has a molecular weight of 168 daltons as one sees from its structure [2]. Thus $D_a = 2.664(10^{-3})(1.68/2500)^{1/3} = 2.3299 * 10^{-4} \text{cm}^2\text{h}^{-1}$ for an RNA molecule consisting of a string of 40 aptamers.

²In order to find the values of K_C , K used related values from [20] by first modifying equation (9) of that paper to include a back reaction from the apical side of the cell. The mechanism becomes:

where the back reaction constant k_{22} will be small to correspond to drug moving out of the cell exterior and back to the membrane. This leads to the addition of the terms $-k_{22}C_A T_0 V_{AO}$ and $-k_{22}C_A T_0$ to the right hand side of the third and fourth of equations (10) of [20]. Then, at steady state, these two equations can be rearranged to permit us to make the following identifications where the variables on the right with the exception of k_{22} are found in [20]:

$$K_1 \equiv \frac{k_{11}}{k_{r1} + k_{21}}, \quad K_2 \equiv \frac{k_{22}}{k_{r1} + k_{21}}, \quad I = C_C, \quad J = C_A, \\ i_b = \frac{K_{AO} k_{21} k_{22}}{K_{PC} k_{r1} k_{11}} C_A, \quad K = \frac{K_2 K_{AO} C_A + 1}{K_1 K_{PC}}, \quad K_C = L k_{21} T(0). \quad (9.1)$$

A glossary of the constants taken from [20] is given on page 736 of that article. Here L is the thickness of the membrane. When $k_{22} = 0$, $i_b = K_2 = 0$. In [20] three drugs were tested: quinidine, amprenavir, and loperamide. We used the data for aprenavir in Table 3.

³On and off rates (l_1 , l_{-1} values) were determined by Tianjiao Wang, in one of the authors (MNH) laboratory, for the interaction of the malachite green aptamer with its target malachite green. (The original values were $3 \times 10^6/\text{M} - \text{sec}$ for the on rate and $0.4/\text{sec}$ for the off rate).

⁴The value of S_a was set in order to ensure a final concentration of approximately 2×10^6 aptamers per cell. This number of aptamers was calculated from the assumptions of a 40 nucleotides (nt) long aptamer, 20 aptamers, each consisting of a string of 40 nucleotides encoded in an 800 nt long RNA and 10^5 RNA molecules per cell expressed from an exogenous promoter. For this and v_a see, [16]. In figure 11 we see the effect of changing the aptamer source to yield a final concentration of from no aptamers per cell up to 10^6 aptamers per cell. There is variability because the aptamer decay rate lies in the range of 0.02 – 2 hours. One micromole per liter corresponds to about 10^{-10} to 10^{-12} micromoles or between 10^5 to 10^7 molecules per cell depending on the cell volume. Thus, with a decay rate of .01/h and 10^6 molecules per cell, $S_a = 0.01\mu\text{M/h}$. With a decay rate of 2/h, $S_a = 2.0\mu\text{M/h}$. Figure 11 gives

the decay of free enzyme concentration in the cell for representative values of S_a between $0\mu M/h$ and $20\mu M/h$. With 10^5 molecules per cell, the maximum value of S_a over this range of aptamer decay rates is $2.0\mu M/h$.

⁵ The value for S_e was set in order to ensure a final concentration of 1 uM enzyme. For this and μ_e see [7].

Table 3

Numerical values taken from [20], Table 4.

constant	range	notes
k_{11}	$10^3(\mu M \cdot sec)^{-1}$	(From [20].)
k_{21}	16/sec	(From [20].)
k_{r1}	$5 \times 10^5/sec$	(From [20].)
C_A	1 – 100 μ M	(Figure 11([20]), amprenavir)
$K_{PC} = C_{PC}/C_C$	228 ± 17	(Table 2([20]), amprenavir)
$K_{AO} = C_{AO}/C_A$	228 ± 17	(Table 2([20]), amprenavir)
$T(0)$	50 μ M	
L	2×10^{-3} microns	(membrane thickness)