An Invitation to Pharmacostatics

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Pharmacology, the study of interactions between biological processes and therapeutic agents, is traditionally presented as consisting of two subdisciplines: pharmacokinetics, which is about the distribution and metabolism of drugs in organisms; and pharmacodynamics, which is about the organisms' response to drugs. In discovery-stage pharmacology however, one primary concern is what we call pharmacostatics, the characterization of equilibrium parameters and states of core interactions of physiologic and therapeutic interest. This usually takes the form of studying dose-response curves, without consideration for the relevant qualitative properties of the underlying reaction networks, e.g. the existence, multiplicity and asymptotic stability of steady states. Furthermore, steady state calculations customarily employ manually derived closed-form expressions based on approximating assumptions. While these formulas may seem adequate most of the time, the assumptions need not apply, and there are genuine though seemingly uncommon cases where this approach is not feasible and/or fails to explain non-monotone dose-response curves. It is this paper's aim to stimulate interest in mathematical problems arising in pharmacostatics. We specifically pose two problems about a particular relevant class of networks of reversible binding reactions. The first problem is to exploit a certain fixed-point formulation of the equilibrium equation to devise an algorithmic method that would be compellingly preferable to current practice in the pharmacostatics context. The second problem is to explicitly anticipate the possibility of non-monotone dose-response curves from network topology. Addressing these problems would positively impact biopharmaceutical research, and they have inherent mathematical interest.

Keywords

Pharmacostatics; Receptor Pharmacology; Non-Monotone Dose-Response Curve; Equilibrium Calculation; Polynomial System Solving; Fixed-Point Algorithm

Mathematics Subject Classification (2010)

13P15; 47H10; 47J25; 92C42; 92C45

1 Introduction

This paper features two mathematical problems originating in pharmacology. Broadly speaking, pharmacology is the study of interactions between biological processes and therapeutic agents. Here we are primarily interested in receptor-mediated molecular mechanisms investigated in discovery-stage drug research. Subsequent stages of the research pipeline include the two most prominent branches of pharmacology: pharmacokinetics, which studies the distribution and metabolism of drugs in biological systems; and pharmacodynamics, which studies how biological systems respond to drugs. By etymological analogy, we propose to call *pharmacostatics* the area of interest herein, because it is concerned with understanding the equilibrium states and parameters of biochemical networks that model core disease-causing and drug-action mechanisms. A drug candidate that does not have satisfactory pharmacostatic attributes does not advance further in the research and development pipeline.

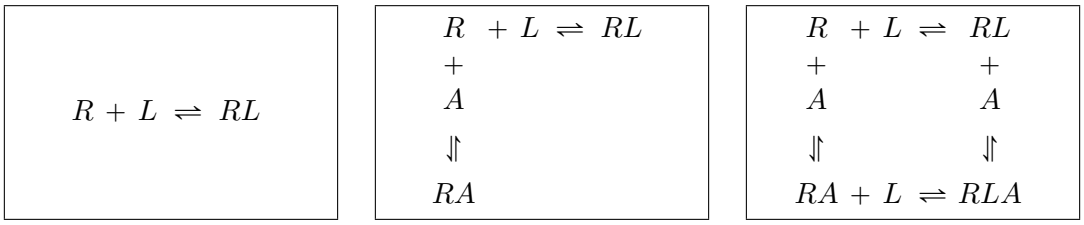
At the center of the mathematical problems we will discuss is a particular polynomial system which needs to be solved to calculate equilibrium concentrations of molecular species and produce dose-response curves. In this context, tacit assumptions of existence, uniqueness and global asymptotic stability of equilibria usually underlie the notion of equilibrium state, and calculations usually employ manually derived formulas based on approximating assumptions. Problems with this custom include:

- New manual derivations have to be performed for every new network or variation of an existing network;
- The condition used to justify this approach, the *no ligand depletion assumption*, is not rigorously defined and need not apply;
- The method may be used only on *receptor-centric networks* (explained later); and
- These formulas produce monotone (increasing or decreasing sigmoid) dose-response curves, but though infrequent, there are genuine instances of non-monotone responses.

The paper's content spans three sections. Section 2 provides background material on the class of reaction networks upon which we pose problems. We strive to summarize, through examples and intuition, theoretical constructs we developed in prior work. In Section 3, we describe the equilibrium equation and make a case for how we wish to have it solved. The system can be approached with standard methods, e.g., by converting it into a minimization problem. The method we envisage, however, should offer the simplicity, performance and a priori certainty of convergence of the kind afforded by the Banach Contraction Principle to the problem of finding the fixed point of a contraction map. A method with such qualities should foster its adoption for the intended application. But beyond that aim, this problem has inherent mathematical interest. Finally Section 4 discusses the monotonicity of dose-response curves arising from solving the equilibrium equation. By way of motivation, we describe, through Figures 4.1 and 4.2 and accompanying comments, an actual case of non-monotone response from prior work. We then formulate the problem of identifying mechanisms capable of such behavior and characterizing how it arises, and draw attention to a few publications on monotonicity in general settings which may provide paths toward addressing the problem herein.

2 Background: Networks of Reversible Binding Reactions

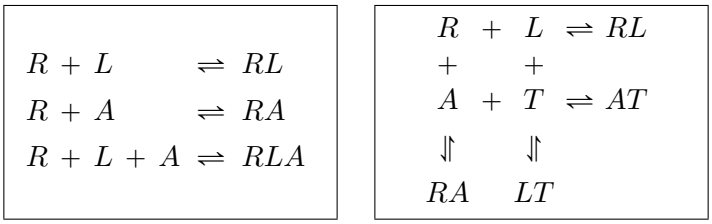
Reaction networks used in pharmacostatic models of receptor-mediated mechanisms belong to what we termed *complete networks of reversible binding reactions* in Gnacadja (2009). Three basic examples are the reversible binding of a ligand to a receptor (Figure 2.1a), the reversible orthosteric (i.e., on the same site) binding of a ligand and an antagonist to a receptor (Figure 2.1b), and the reversible allosteric (i.e., on different sites) binding of a ligand and modulator to a receptor (Figure 2.1c). A less frequent but important example (Figure 2.1e) has a ligand and an antagonist both competing for the same site on a receptor and likewise on a decoy receptor. More elaborate examples can be found in Kenakin (2009) and Durroux (2005), for instance. We developed in Gnacadja (2009) a rigorous framework for these networks grounded on Chemical Reaction Network Theory. (The framework does not natively accommodate isomerism, e.g., molecules existing in several conformations, but can be extended to do so.) We summarize here in an intuitive manner assisted by the examples of Figure 2.1.



(a) Reversible binding of a ligand L to a receptor R

(b) Reversible orthosteric binding of a ligand L and an antagonist A to a receptor R

(c) Reversible allosteric binding of a ligand L and a modulator A to a receptor R



(d) Normal network corresponding to the network of Figure 2.1c

(e) Ligand L and antagonist A competing for the same site on receptor R and likewise on decoy receptor (or trap) T

Figure 2.1: Some reaction networks used to model receptor-mediated mechanisms

From observing these networks, one may form an intuition of elementary species, composite species, and compositions of the latter with respect to the former. For an example, consider the networks of Figures 2.1c and 2.1d:

- The elementary species are R, L, A;
- The composite species are RL, RA, RLA; and
- With both sets of species ordered as listed, the compositions are $(1, 1, 0)$, $(1, 0, 1)$, $(1, 1, 1)$.

For another example, consider the network of Figure 2.1e:

- The elementary species are R, L, A, T;
- The composite species are RL, RA, LT, AT; and

- With both sets of species ordered as listed, the compositions are $(1,1,0,0)$, $(1,0,1,0)$, $(0,1,0,1)$, $(0,0,1,1)$.

Remark: It is certainly helpful that elementary and composite species are denoted here (and often in general) with single and multiple letters, respectively. However, these are notational conveniences, and they do not define the concepts of elementary and composite species.

In general, a *normal network of reversible binding reactions* has:

- Elementary species $X_1, \ldots, X_n$, where n is a positive integer;
- Composite species Y_α of composition α with respect to $(X_1, \ldots, X_n)$, where $\alpha \in I$ and I is a nonempty finite set of nonnegative integer n-tuples other than $0_n, e_{n,1}, \ldots, e_{n,n}$; 0_n is the zero n-tuple, $e_{n,i}$ is the n-tuple with 1 in position i and 0 elsewhere; and
- For each $\alpha = (\alpha_1, \ldots, \alpha_n) \in I$, the binding-dissociation reaction pair

$$\sum_{i=1}^{n} \alpha_i X_i \quad \rightleftharpoons \quad Y_\alpha$$

  governed by the Law of Mass Action, and its equilibrium binding constant $a_\alpha \in \mathbb{R}_{>0}$.

The more general notion of *complete network of reversible binding reactions* extends that of normal network by sensibly supporting composite species among the reactants of binding reactions, specifically by ensuring the conservation of composition and detailed-balanced equilibrium. Reaction networks in pharmacostatics are indeed assumed to be detailed-balanced. This means that at equilibrium, each subnetwork consisting of a binding reaction and the reverse dissociation reaction is also at equilibrium. As a result, a complete network can be *normalized* for the purpose of equilibrium calculation. For illustration, the networks of Figures 2.1a, 2.1b and 2.1e are already normal, whereas the normal network corresponding to the complete network of Figure 2.1c is given on Figure 2.1d. Each binding-dissociation reaction pair is endowed with an equilibrium binding constant. The assumption of detailed balance on complete networks is equivalent to a particular set of relations among the equilibrium binding constants, and these relations give rise to the equilibrium binding constants in the corresponding normal network. In pharmacostatics, these relations are usually expressed in terms of cooperativity factors. Also, one usually works with equilibrium dissociation constants, rather than equilibrium binding constants; they are inverses of each other. We use binding constants only because we find it more convenient to do so in most algebraic manipulations.

3 Polynomial and Fixed-Point Formulations of the Equilibrium Problem

Polynomial Equation for Equilibrium

Employing notations used in pharmacology, we denote $[S]$ the concentration of a species S as a function of time, $[S]_{\text{Eql}}$ the concentration of S at equilibrium, and $[S]_{\text{Ttl}}$ the total (free and bound) concentration of S if S is an elementary species. Note that, for all $i = 1, \ldots, n$,

$$[X_i]_{\text{Ttl}} \quad = \quad [X_i] + \sum_{\alpha \in I} \alpha_i [Y_\alpha] \quad = \quad [X_i]_{\text{Eql}} + \sum_{\alpha \in I} \alpha_i [Y_\alpha]_{\text{Eql}}.$$

Let the polynomial map $f = (f_1, \ldots, f_n) : \mathbb{R}^n \to \mathbb{R}^n$ be defined by

$$f_i(x) \quad = \quad x_i + \sum_{\alpha \in I} \alpha_i \, a_\alpha \, x^\alpha .$$

(We employed the multi-index power notation $x^\alpha = (x_1, \ldots, x_n)^{(\alpha_1, \ldots, \alpha_n)} = x_1^{\alpha_1} \cdots x_n^{\alpha_n}$.) Theorem 3.4 in Gnacadja (2009) states that f is an infinitely smooth endo-diffeomorphism of the nonnegative quadrant $\mathbb{R}^n_{\geqslant 0}$ of $\mathbb{R}^n$. Let $g = (g_1, \ldots, g_n) : \mathbb{R}^n_{\geqslant 0} \to \mathbb{R}^n_{\geqslant 0}$ be the inverse map, and let $h = (h_\alpha)_{\alpha \in I}$, where for each $\alpha \in I$, h_α is the map $\mathbb{R}^n_{\geqslant 0} \to \mathbb{R}_{\geqslant 0}$ given by $h_\alpha(b) = a_\alpha \big(g(b)\big)^\alpha$. The equilibrium equation is

$$f(x) \quad = \quad b$$

in the sense that if $b = (b_1, \ldots, b_n)$ is a vector of total concentrations, i.e. $[X_i]_{\mathrm{Ttl}} = b_i$ for $i = 1, \ldots, n$, then the solution $x = g(b)$ is the vector of equilibrium concentrations of the elementary species, i.e. $[X_i]_{\mathrm{Eql}} = g_i(b) = x_i$ for $i = 1, \ldots, n$; and for the composite species we have $[Y_\alpha]_{\mathrm{Eql}} = h_\alpha(b) = a_\alpha x^\alpha$ for $\alpha \in I$. For illustration, for the network of Figure 2.1c, the map f is given as follows.

$$\left\{ \begin{array}{lcl} f_1(x) & = & x_1 + a_{(1,1,0)}\, x_1\, x_2 + a_{(1,0,1)}\, x_1\, x_3 + a_{(1,1,1)}\, x_1\, x_2\, x_3 \\ f_2(x) & = & x_2 + a_{(1,1,0)}\, x_1\, x_2 \qquad\qquad + a_{(1,1,1)}\, x_1\, x_2\, x_3 \\ f_3(x) & = & x_3 \qquad\qquad + a_{(1,0,1)}\, x_1\, x_3 + a_{(1,1,1)}\, x_1\, x_2\, x_3 \end{array} \right.$$

This network, often referred to as the Allosteric Ternary Complex Model, is particularly important in receptor pharmacology, and we devoted a paper, Gnacadja (2011), to explicitly solving its equilibrium equation.

Equilibrium Equation Formulated as a Fixed-Point Problem

The way in which the surjectivity of f was established in Gnacadja (2009) is pertinent to how we seek to reformulate the equilibrium problem. Let the map $F = (F_1, \ldots, F_n) : \mathbb{R}^n_{\geqslant 0} \times \mathbb{R}^n_{\geqslant 0} \to \mathbb{R}^n_{\geqslant 0}$ be defined by

$$F_i(b, x) \quad = \quad \frac{b_i}{1 + \displaystyle\sum_{\alpha \in I,\, \alpha_i \geqslant 1} \alpha_i \, a_\alpha \, x^{\alpha - e_{n,i}}} .$$

Then the solutions of the equation $f(x) = b$ are the fixed points of the map $F(b, \cdot)$. And the map $F(b, \cdot)$ does have fixed points by the Brouwer Fixed Point Theorem. Our proposal seeks to further exploit this fixed-point formulation.

Problem I. *Reformulate the fixed-point problem $F(b, x) = x$, e.g., through a judicious transformation of the map $F(b, \cdot)$, so as to calculate the (unique) fixed point by convergent fixed-point iteration.* ∎

Because the map $F(b, \cdot)$ is order-reversing (with respect to the componentwise order in $\mathbb{R}^n$), $b = F(b, 0_n)$ is the maximum value of $F(b, \cdot)$ on $\mathbb{R}^n_{\geqslant 0}$, and iterating $F(b, \cdot)$ starting at b yields two monotone subsequences, one descending from b and the other ascending from $F(b, b)$. They enclose the fixed point, and they converge either to it or to a 2-orbit of $F(b, \cdot)$ (which some call coupled fixed points, though they are not fixed points of $F(b, \cdot)$, but of $F(b, \cdot)^2$). For illustration, for the network of Figure 2.1c, the map F is

given as follows.

$$\begin{cases} F_1(b,x) &= \dfrac{b_1}{1 + a_{(1,1,0)}\, x_2 + a_{(1,0,1)}\, x_3 + a_{(1,1,1)}\, x_2\, x_3} \\ F_2(b,x) &= \dfrac{b_2}{1 + a_{(1,1,0)}\, x_1 + a_{(1,1,1)}\, x_1\, x_3} \\ F_3(b,x) &= \dfrac{b_3}{1 + a_{(1,0,1)}\, x_1 + a_{(1,1,1)}\, x_1\, x_2} \end{cases}$$

Rationale for the Fixed-Point Reformulation

The fixed-point formulation is successful under particular restrictions on two network attributes that reflect a sense of the size of the network: the number n of elementary species and the arity of the composite species. With regard to arity, we established the following in prior work.

Theorem 3.1 (Theorem 5.4 in Gnacadja (2007)). *Let A be a real nonnegative $n \times n$ matrix, and let $b = (b_1, \ldots, b_n) \in \mathbb{R}^n_{>0}$. Consider the map $\varphi = (\varphi_1, \ldots, \varphi_n) : \mathbb{R}^n_{\geqslant 0} \to \mathbb{R}^n_{>0}$ given by*

$$\varphi_i(x) \;=\; \frac{b_i}{1 + (A{\cdot}x^\top)_i}.$$

Let $c = \|A{\cdot}b^\top\|_\infty$ and $k = \dfrac{c}{1+c}$. With respect to the metric d on $\mathbb{R}^n_{>0}$ given by

$$d(u,v) \;=\; \max_{1 \leqslant i \leqslant n} \big|\ln(u_i/v_i)\big|,$$

the map φ is k-Lipschitz, and thus is a contraction, on $]0, b_1] \times \cdots \times]0, b_n]$. ∎

A few notes are in order regarding Theorem 3.1.

- $x^\top$ is the transpose of x and $(A{\cdot}x^\top)_i$ is the component of index i of the column-vector $A{\cdot}x^\top$.
- The map d is indeed a metric on $\mathbb{R}^n_{>0}$ and it is equivalent as such to the more ordinary metrics (L^1, L^2, L^∞, etc.) on compact subsets. The metric d measures distances on the natural-logarithmic scale.
- The map φ need not be a contraction with respect to the ordinary metrics. For example, with $n = 1$, $A > 1$, and $b = A$, the map φ is strictly expansive on a subinterval of $[0, b]$ with respect to the ordinary metric on $\mathbb{R}$. Indeed, with $r = 1 - 1/A$, we have $|\varphi(u) - \varphi(v)| > |u - v|$ if $u, v \in [0, r]$ and $u \neq v$.

Coming back to the motivating problem, it results from Theorem 3.1 that fixed-point iteration of $F(b, \cdot)$ converges if all composite species are binary, as is the case for the networks of Figures 2.1a and 2.1e. In other words, Problem I is solved in this case without any further transformation of $F(b, \cdot)$. As to the number n of elementary species, in the limit case $n = 1$, the map $F(b, \cdot)$ is covered by the following observation.

Proposition 3.2. *Let D be an interval in $\mathbb{R}$, let ψ a differentiable self-map of D, and suppose there exist $m, M \geqslant 0$ such that $-M \leqslant \psi' \leqslant -m$. (In particular, ψ is monotone-decreasing.) For any $\lambda \in [0, 1]$, let*

$$\psi_\lambda \;=\; \lambda\psi + (1-\lambda)\mathrm{Id} \;:\; x \mapsto \lambda\psi(x) + (1-\lambda)x$$

and

$$k_\lambda \quad = \quad \max(\,|1-(1+m)\lambda|\,,\,|1-(1+M)\lambda|\,).$$

The map ψ_λ is k_λ-Lipschitz with respect to the ordinary norm of the real line. If (and only if) $0 < \lambda < \dfrac{2}{1+M}$, then $k_\lambda < 1$, and iteration of ψ_λ converges to the (unique) fixed point of ψ. We have $\arg\min\limits_{\lambda\in[0,1]}(k_\lambda) = \lambda_0 = \dfrac{2}{2+m+M}$ and $k_{\lambda_0} = \dfrac{M-m}{2+m+M} < 1$.■

The map transformation used in Proposition 3.2 (convex combination with the identity map) preserves fixed points in any dimension. However in dimension two or higher, it can in general only improve the Lipschitz constant of a map that already is a contraction (and thereby accelerate the convergence of fixed-point iteration). The one-dimensional case in Proposition 3.2 exploits the fact that the real line is totally ordered. We expect that there would be transformations taking advantage of the particular properties of the map $F(b,\cdot)$, including the uniqueness of the fixed point and the fact that the map is order-reversing. Enclosure algorithms based on cell division and discarding work but are not computationally efficient.

The customary approach in pharmacostatics to solve the polynomial system $f(x) = b$ is to substitute selected instances of x_i for b_i when X_i is a ligand, in a way that is reminiscent of the derivation of the classical Michaelis-Menten equation in enzyme kinetics. This is done under the *no ligand depletion assumption*, which posits that ligands are in excess amounts compared to receptors, and consequently are not bound to the receptor in significant amounts at equilibrium. There are several publications on recognizing and addressing the errors associated with this assumption for particular networks, e.g., Wells et al. (1980), Goldstein and Barrett (1987), Horovitz and Levitzki (1987), Wang and Jiang (1996), Martin et al. (1991), Swillens (1995), Scaramellini-Carter et al. (2007), Avlani et al. (2008), Raccor et al. (2008), Zhen et al. (2010), and Gnacadja (2011). But the counter-arguments to this method are not simply about the repetitive labor of manual derivations and whether the margins of error are acceptable. There is also that the customary manual algebraic procedure may be conducted only on *receptor-centric networks*, whereby we mean networks in which there is one single species that is designated as the receptor and is a constituent (possibly in various conformations) in all composite species. The network of Figure 2.1e, for instance, is not receptor-centric and, perhaps non-coincidentally, is an example for the non-monotonicity problem in the next section.

Older literature on the fixed-point formulation of the equilibrium problem includes Perrin (1965), Perrin and Sayce (1967), Storer and Cornish-Bowden (1976) and Kuzmič (1998). More recently, the result in Theorem 3.1 (Theorem 5.4 in Gnacadja (2007)) was rediscovered in Dorp et al. (2011) with respect to another metric. Through Problem I, we seek an algorithmic method that would be compellingly preferable to manual derivations, be they approximate or exact. A definite resolution would be a valuable contribution to pharmacostatics in drug discovery and the various areas that motivated the cited publications. Of course, the ultimate objective is to solve the polynomial system $f(x) = b$ with speed and a priori certainty of success. (Success with probability one over some non-finite probability space would not be sufficient, for example.) So solutions that do not use the fixed-point formulation would certainly be welcome.

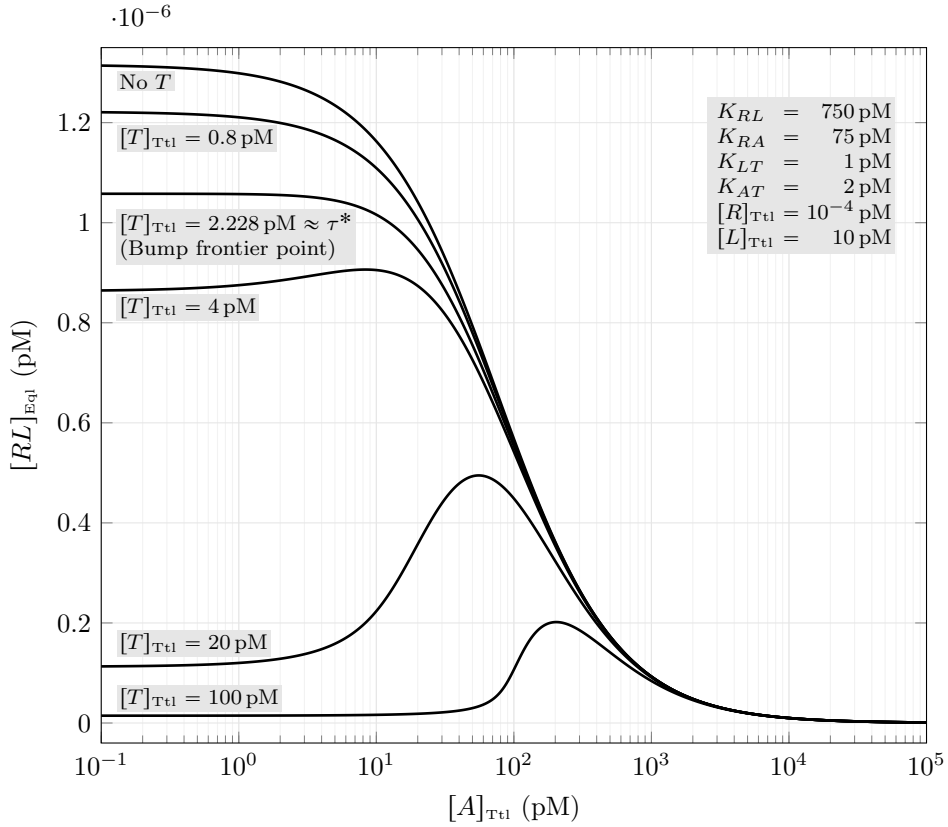


Figure 4.1: The dose-response function $[A]_{\text{Ttl}} \mapsto [RL]_{\text{Eql}}$ for the network of Figure 2.1e. The four equilibrium dissociation constants and the total concentrations of the receptor R and the ligand L are fixed, and several values of the total concentration of trap T are selected. The function has the familiar decreasing-sigmoid profile when $[T]_{\text{Ttl}} \leqslant \tau^*$ but possesses a bump when $[T]_{\text{Ttl}} > \tau^*$. The bump frontier point τ^* is further illustrated on Figure 4.2.

4 Monotonicity of Dose-Response Functions

Pharmacostatic studies seek to understand how the dose of a drug candidate affects the equilibrium state of the network under consideration. A dose-response function is a standard tool used to capture and evaluate some important aspects of this relation. Usually the dose is the total concentration of the drug and the response is the equilibrium concentration of a particular (usually composite) species, or the total concentration at equilibrium of the bound forms of a particular elementary species. For the networks of Figures 2.1e and 2.1c for instance, interesting dose-response functions are $[A]_{\text{Ttl}} \mapsto [RL]_{\text{Eql}}$ and $[A]_{\text{Ttl}} \mapsto [RL]_{\text{Eql}} + [RLA]_{\text{Eql}}$, respectively. The response is chosen because it is either an indicator of disease that one seeks to decrease or of health that one seeks to increase. Thus, dose-response functions are expected to be monotone, and indeed, they most often have a decreasing or increasing sigmoid profile.

Dose-response functions can, however, be non-monotone. Non-monotonicity is not often mentioned in the pharmacology literature, but instances include Tuček et al. (2002) and Di Veroli et al. (2015). There appears to be greater awareness in the toxicology area; see, for instance, Calabrese and Baldwin (2001), Conolly and Lutz (2004), United States National Academy of Sciences (2014), and references therein. We studied an actual pharmacology case in Gnacadja et al. (2007) and provided the conditions under which non-monotonicity does occur. The network was as on Figure 2.1e, and the dose

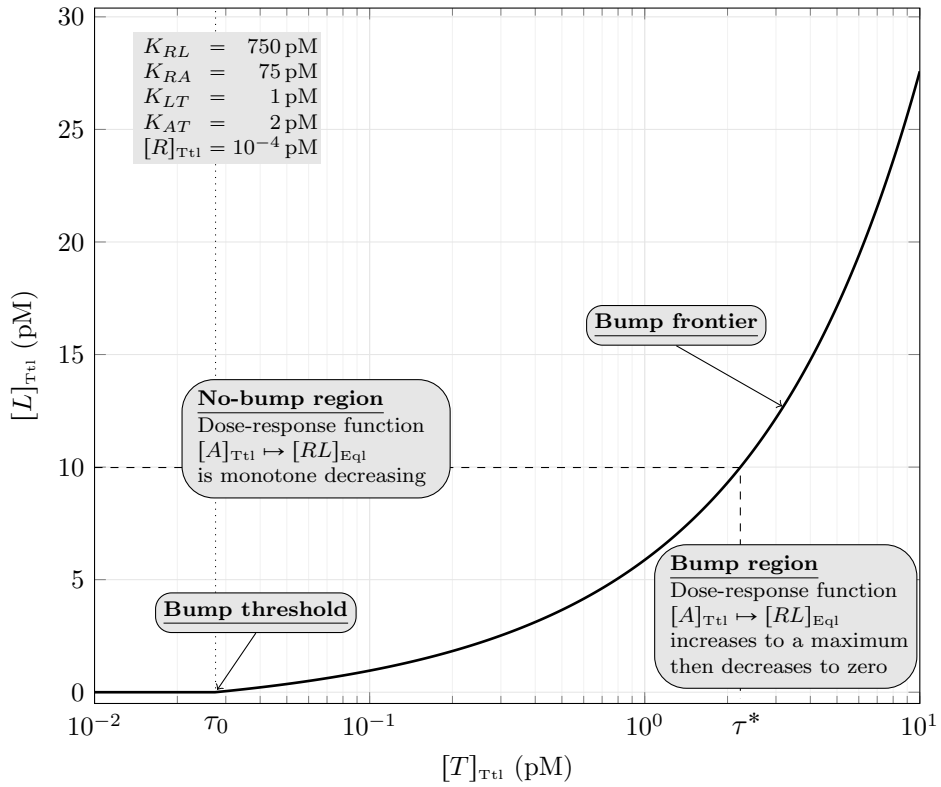


Figure 4.2: The bump frontier for the dose-response function $[A]_{\text{Ttl}} \mapsto [RL]_{\text{Eql}}$ for the network of Figure 2.1e. The bump frontier is a hypersurface in the 7-dimensional space with coordinate system $(K_{RL}, K_{RA}, K_{LT}, K_{AT}, [R]_{\text{Ttl}}, [L]_{\text{Ttl}}, [T]_{\text{Ttl}})$. It partitions the space according to the monotonicity behavior of the dose-response function and is part of the no-bump region. Shown here is the 2-dimensional slice resulting from fixing K_{RL}, K_{RA}, K_{LT}, K_{AT} and $[R]_{\text{Ttl}}$ as specified. Figure 4.1 effectively was generated by sampling the horizontal line $[L]_{\text{Ttl}} = 10$ pM at the specified values of $[T]_{\text{Ttl}}$. We see in particular the bump frontier point $[T]_{\text{Ttl}} = \tau^*$. Also noteworthy is the bump threshold τ_0. For $[T]_{\text{Ttl}} \leqslant \tau_0$, the bump frontier coincides with the hyperplane $[L]_{\text{Ttl}} = 0$, i.e. there is no bump regardless of how much ligand L there is. We have $\tau_0 = K_{LT}K_{AT}/(K_{RA} - K_{AT})$; if $K_{RA} \leqslant K_{AT}$, there never is a bump.

and response species were A and RL, respectively; see Figure 4.1. In general terms, non-monotonicity may occur in this setting because of two competing competitions, the one in which L and A compete for binding with R, and the other in which the same two species L and A compete for binding with T. We have anecdotal reports of drug candidates having been eliminated because of unexplainable dose-response curves. In the case studied in Gnacadja et al. (2007), the program did continue thanks in part to the theoretical explanations provided. And this brings us to the following quest.

Problem II. *Characterize the complete networks of reversible binding reactions that are capable of producing non-monotone dose-response functions, and the precise conditions under which non-monotonicity does occur.* ■

Problem II requires a precise definition of a dose-response function. Let $d \in \{1, \ldots, n\}$ and $b_1, \ldots, b_{d-1}, b_{d+1}, \ldots, b_n \in \mathbb{R}_{\geqslant 0}$ be fixed. A dose-response function for the dose species X_d is any of the following maps $\mathbb{R}_{\geqslant 0} \to \mathbb{R}_{\geqslant 0}$.

- The map u_i with $u_i(b_d) = g_i(b_1, \ldots, b_n)$ for some $i \in \{1, \ldots, n\}$, when the response is the equilibrium concentration of elementary species X_i.

- The map v_α with $v_\alpha(b_d) = h_\alpha(b_1, \ldots, b_n)$ for some $\alpha \in I$, when the response is the equilibrium concentration of composite species Y_α.

- The map w_i with $w_i(b_d) = \sum_{\alpha \in I} \alpha_i h_\alpha(b_1, \ldots, b_n)$ for some $i \in \{1, \ldots, n\}$, when the response is the concentration at equilibrium of all bound forms of elementary species X_i.

We noted earlier that the maps $[A]_{\text{Ttl}} \mapsto [RL]_{\text{Eql}}$ and $[A]_{\text{Ttl}} \mapsto [RL]_{\text{Eql}} + [RLA]_{\text{Eql}}$ are interesting dose-response functions for the networks of Figures 2.1e and 2.1c, respectively. The former is the map v_α for $d = 3$ and $\alpha = (1, 1, 0, 0)$, because $A = X_3$ and $RL = Y_{(1,1,0,0)}$. The latter is the map w_i for $d = 3$ and $i = 2$, because $A = X_3$ and $L = X_2$. (In both cases we used the species ordering stipulated in Section 3.) Note that a dose-response function in practice may be some canonical multiple of a dose-response function as defined here. In the two examples noted here for instance, one may instead consider the receptor occupancy ratios, i.e., the dose-response functions $[A]_{\text{Ttl}} \mapsto [RL]_{\text{Eql}}/[R]_{\text{Ttl}}$ and $[A]_{\text{Ttl}} \mapsto ([RL]_{\text{Eql}} + [RLA]_{\text{Eql}})/[R]_{\text{Ttl}}$.

Sontag (2014) developed an algebraic-combinatorial technique that is able to reveal whether any dose-response function of the u_i or v_α kind is capable of being non-monotone. Earlier Fishtik et al. (1995) developed a method based on multivariate analysis for similar goals. Pérez Millàn and Dickenstein (2015) provide systematic means to derive implicit polynomial equations relating dose and response, and thereby a possibility to study the monotonicity of dose-response curves via implicit differentiation. With Problem II, we seek to foster a more proactive posture with regard to the monotonicity of dose-response curves. We not only ask what networks can give rise to non-monotone dose-response functions. We also ask under what circumstances non-monotonicity does occur, i.e., a concept akin to what we called the *bump frontier* in Gnacadja et al. (2007) for the special case of the network of Figure 2.1e; see Figure 4.2.

The class of complete networks of reversible binding reactions is already relatively small in the reaction network universe, yet not all its members are of pharmacological interest. Also not all species are interesting dose species (though for the network of Figure 2.1e, that would not matter much because of the circular symmetry in the network structure). Accordingly answers to Problem II under further pertinent restrictions on the network class would still be valuable. Furthermore short of a fully characterized bump frontier, a *bump threshold* (see Figure 4.2) would still be of interest.

5 Epilogue

Much like pharmacokinetics can be described from a mathematical perspective as the study of the kinetics of reaction networks of interest in preclinical and clinical pharmacology, pharmacostatics would be the study of the equilibria of reaction networks of interest in discovery-stage pharmacology. In this paper, we sought to motivate and pose two mathematical problems in pharmacostatics: a fast and fail-proof algorithm to compute (unique) equilibria in a particular class of networks, and a method to explicitly anticipate non-monotone dose-response functions based on network topology. There are undoubtedly more interesting and applicable problems. For instance, while multistability was not discussed in this paper, it is very important, for example, in enzymatic networks. A valuable result in this context would an explicit partitioning of the state space (or more specifically, for the reaction network theory aware reader, the stoichiometric compatibility classes) into basins of attraction (or otherwise). Explicitness and

relevance will need to be a general theme for contributions in this area. Existence results, or explicit results on toy networks, while tangible and valuable, should seldom be endpoints.

References

Avlani, V. A., McLoughlin, D. J., Sexton, P. M., and Christopoulos, A. (2008). The Impact of Orthosteric Radioligand Depletion on the Quantification of Allosteric Modulator Interactions. *The Journal of Pharmacology and Experimental Therapeutics*, 325(3):927–934. DOI 10.1124/jpet.108.136978.

Calabrese, E. J. and Baldwin, L. A. (2001). The Frequency of U-Shaped Dose Responses in the Toxicological Literature. *Toxicological Sciences*, 62(2):330–338. DOI 10.1093/toxsci/62.2.330.

Conolly, R. B. and Lutz, W. K. (2004). Nonmonotonic Dose-Response Relationships: Mechanistic Basis, Kinetic Modeling, and Implications for Risk Assessment. *Toxicological Sciences*, 77(1):151157. DOI 10.1093/toxsci/kfh007.

Di Veroli, G. Y., Fornari, C., Goldlust, I., Mills, G., Koh, S. B., Bramhall, J. L., Richards, F. M., and Jodrell, D. I. (2015). An Automated Fitting Procedure and Software for Dose-Response Curves with Multiphasic Features. *Scientific Reports*, 5(14701). DOI 10.1038/srep14701.

Dorp, M. G. A. v., Berger, F., and Carlon, E. (2011). Computing Equilibrium Concentrations for Large Heterodimerization Networks. *Physical Review E*, 84(3):036114:1–8. DOI 10.1103/PhysRevE.84.036114.

Durroux, T. (2005). Principles: A Model for the Allosteric Interactions between Ligand Binding Sites within a Dimeric GPCR. *Trends in Pharmacological Sciences*, 26(7):376–384. DOI 10.1016/j.tips.2005.05.006.

Fishtik, I., Nagypàl, I., and Gutman, I. (1995). Response Reactions: A Way to Explain the Unusual Behaviour of Multiple Equilibrium Systems. *Journal of the Chemical Society, Faraday Transactions*, 91(2):259–267. DOI 10.1039/FT9959100259.

Gnacadja, G. (2007). Fixed Points of Order-Reversing Maps in $\mathbb{R}^n_{>0}$ and Chemical Equilibrium. *Mathematical Methods in the Applied Sciences*, 30(2):201–211. DOI 10.1002/mma.782.

Gnacadja, G. (2009). Univalent Positive Polynomial Maps and the Equilibrium State of Chemical Networks of Reversible Binding Reactions. *Advances in Applied Mathematics*, 43(4):394–414. DOI 10.1016/j.aam.2009.05.001.

Gnacadja, G. (2011). A Method to Calculate Binding Equilibrium Concentrations in the Allosteric Ternary Complex Model that Supports Ligand Depletion. *Mathematical Biosciences*, 232(2):135–141. DOI 10.1016/j.mbs.2011.05.003.

Gnacadja, G., Shoshitaishvili, A., Gresser, M. J., Varnum, B., Balaban, D., Durst, M., Vezina, C., and Li, Y. (2007). Monotonicity of Interleukin-1 Receptor-Ligand Binding with respect to Antagonist in the presence of Decoy Receptor. *Journal of Theoretical Biology*, 244(3):478–488. DOI 10.1016/j.jtbi.2006.07.023.

Goldstein, A. and Barrett, R. W. (1987). Ligand Dissociation Constants from Competition Binding Assays: Errors Associated with Ligand Depletion. *Molecular Pharmacology*, 31(6):603–609. http://molpharm.aspetjournals.org/content/31/6/603.abstract (Erratum: vol. 33 (1988), no. 6, page 720).

Horovitz, A. and Levitzki, A. (1987). An Accurate Method for Determination of Receptor-Ligand and Enzyme-Inhibitor Dissociation Constants from Displacement Curves. *Proceedings of the National Academy of Sciences USA*, 84(19):6654–6658. http://www.pnas.org/content/84/19/6654.short.

Kenakin, T. (2009). *A Pharmacology Primer: Theory, Applications, and Methods*. Academic Press, 3rd edition. ISBN 9780123745859.

Kuzmič, P. (1998). Fixed-Point Methods for Computing the Equilibrium Composition of Complex Biochemical Mixtures. *Biochemical Journal*, 331:571–575.

Martin, R. L., Renosto, F., and Segel, I. H. (1991). A Simple Method for Calculating the Dissociation Constant of a Receptor (or Enzyme) · Unlabeled Ligand Complex from Radioligand Displacement Measurements. *Proceedings of the National Academy of Sciences USA*, 284(1):26–29. DOI 10.1016/0003-9861(91)90257-J.

Pérez Millàn, M. and Dickenstein, A. (2015). Implicit Dose-Response Curves. *Journal of Mathematical Biology*, 70(7):1669–1684. DOI 10.1007/s00285-014-0809-4.

Perrin, D. D. (1965). Multiple Equilibria in Assemblages of Metal Ions and Complexing Species: A Model for Biological Systems. *Nature*, 206:170–171.

Perrin, D. D. and Sayce, I. G. (1967). Computer Calculation of Equilibrium Calculations in Mixtures of Metal Ions and Complexing Species. *Talanta*, 14:833–842.

Raccor, B. S., Vogt, A., Sikorski, R. P., Madiraju, C., Balachandran, R., Montgomery, K., Shin, Y., Fukui, Y., Jung, W.-H., Curran, D. P., and Day, B. W. (2008). Cell-Based and Biochemical Structure-Activity Analyses of Analogs of the Microtubule Stabilizer Dictyostatin. *Molecular Pharmacology*, 73(3):718–726. DOI 10.1124/mol.107.042598.

Scaramellini-Carter, C. M., Leighton-Davies, J. R., and Charlton, S. J. (2007). Miniaturized Receptor Binding Assays: Complications Arising from Ligand Depletion. *Journal of Biomolecular Screening*, 12(2):255–266. DOI 10.1177/1087057106297788.

Sontag, E. D. (2014). A Technique for Determining the Signs of Sensitivities of Steady States in Chemical Reaction Networks. *IET Systems Biology*, 8(6):251–267. DOI 10.1049/iet-syb.2014.0025.

Storer, A. C. and Cornish-Bowden, A. (1976). Concentration of $MgATP^{2-}$ and Other Ions in Solution. Calculation of the True Concentrations of Species Present in Mixtures of Associating Ions. *Biochemical Journal*, 159:1–5.

Swillens, S. (1995). Interpretation of Binding Curves Obtained with High Receptor Concentrations: Practical Aid for Computer Analysis. *Molecular Pharmacology*, 47(6):1197–1203. http://molpharm.aspetjournals.org/content/47/6/1197.short.

Tuček, S., Michal, P., and Vlachovà, V. (2002). Modelling the Consequences of Receptor–G-Protein Promiscuity. *Trends in Pharmacological Sciences*, 23(4):171–176. DOI 10.1016/S0165-6147(00)01996-9.

United States National Academy of Sciences (2014). *Review of the Environmental Protection Agency's State-of-the-Science Evaluation of Nonmonotonic Dose-Response Relationships as they Apply to Endocrine Disruptors*. The National Academies Press. ISBN 9780309297547 http://www.nap.edu/catalog.php?record_id=18608.

Wang, Z. and Jiang, R.-F. (1996). A Novel Two-Site Binding Equation presented in terms of the Total Ligand Concentration. *FEBS Letters*, 392(3):245–249. DOI 10.1016/0014-5793(96)00818-6.

Wells, J., Birdsall, N., Burgen, A., and Hulme, E. (1980). Competitive Binding Studies with Multiple Sites: Effects Arising from Depletion of the Free Radioligand. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 632(3):464–469. DOI 10.1016/0304-4165(80)90242-1.

Zhen, J., Antonio, T., Dutta, A. K., and Reith, M. E. A. (2010). Concentration of Receptor and Ligand Revisited in a Modified Receptor Binding Protocol for High-Affinity Radioligands: [^{3}H]Spiperone Binding to D_2 and D_3 Dopamine Receptors. *Journal of Neuroscience Methods*, 188(1):32–38. DOI 10.1016/j.jneumeth.2010.01.031.