Monotonicity of Interleukin-1 Receptor-Ligand Binding with respect to Antagonist in the presence of Decoy Receptor

Gilles Gnacadja, Alex Shoshitaishvili, Michael J. Gresser, Brian Varnum, David Balaban, Mark Durst, Chris Vezina, Yu Li

Systems Informatics and Inflammation Research Departments, Amgen, Inc.
One Amgen Center Drive, Thousand Oaks, California 91320-1799, USA
gnacadja@amgen.com

Revision G.03 June 20, 2006

Abstract

We consider the interaction between interleukin-1 IL-1, its receptor IL-1RI, the receptor antagonist IL-1Ra and a decoy receptor (or trap) that binds both with the ligand and the antagonist. We study how the interaction between IL-1Ra and the decoy receptor influences the effect of either reagent on reducing the equilibrium concentration of the receptor-ligand complex. We obtain that, given a certain relationship among the equilibrium constants and the total concentrations of solutes, IL-1Ra can reverse the effect of the decoy receptor of decreasing the equilibrium concentration of the receptor-ligand complex. This finding derives from a mathematical result applicable to any reversible chemical reaction system comprising four species arranged in a square such that each species binds its two immediate neighbors. The result gives the monotonicity of the equilibrium concentrations of the complex species as functions of the total concentrations of the simple species.

Keywords: Interleukin-1, Decoy Receptor, Trap, Chemical Reaction Network, Equilibrium, Monotonicity

1 Introduction

Interleukin-1 (IL-1) is produced in response to inflammatory stimuli. IL-1 binds with the IL-1 type I receptor (IL-1RI), and this triggers a signal which

causes various physiological responses leading to inflammation, cartilage degradation and bone resorption [1, 6, 16]. The human IL-1 receptor antagonist (IL-1Ra) binds with the receptor and hence inhibits the receptor-ligand binding. Biotechnology-produced antagonists such as Amgen's Kineret® are administered as therapeutic agents to subjects with diseases in which IL-1 is a causative factor, e.g. rheumatoid arthritis patients. In an effort to obtain even stronger receptor-ligand binding inhibition, a decoy receptor or trap that binds to the ligand has also been used [7]. However the trap also binds the antagonist. It is hence not obvious to what extent this interaction between trap and antagonist will influence the effectiveness of either agent at blocking IL-1 signaling. In fact, in vitro experiments conducted at Amgen indicated that the antagonist can reverse trap-induced inhibition. The mathematical modeling work we present in this paper has been motivated by these experimental observations and instrumental in their interpretation.

The problem we study is how equilibrium concentration of the receptor-ligand complex depends on initial concentrations of antagonist and trap, specifically the monotonicity of these dependences. We show that as the total concentration of IL-1Ra is increased, depending on the total concentration of trap present, the equilibrium concentration of the receptor-ligand complex either decreases, or increases to a peak then decreases. A set of conditions on the dissociation constants and the initial concentrations controls which situation applies. The various monotonicity results taken together lead to the conclusion that the trap is beneficial in inhibiting the receptor-ligand binding, and that this effect can be partially reversed by IL-1Ra under certain conditions.

These results are obtained by investigating the polynomial system which relates initial and equilibrium concentrations through conservation and equilibrium equations. Specifically, we calculate the derivatives of equilibrium concentrations with respect to initial concentrations and study their signs. The apparent simplicity of the four component chemical system is deceptive; studying the signs of the partial derivatives was an interesting and challenging mathematical undertaking.

Other examples of four component systems with the same interactions exist in biology. One such example is the system comprising the tumor necrosis factor TNF, the p55 TNF-R receptor, an antagonist, and the p55 sTNF-R shed/soluble receptor. Another one comprises the stem cell factor SCF, the c-kit receptor, an antagonist of the receptor, and the s-c-kit soluble receptor.

Closer to the particular system we focus on in this paper is the one with the same components, except that soluble IL-1 receptor sIL-1RI is in lieu of trap. As has been reported in [2], this is also a case of IL-1Ra and an IL-1 sequestering agent having antagonistic effects on each other's inhibitory activity against IL-1/ IL-1RI binding. Both because the mathematics involved is challenging and interesting, and a number relevant instances exist in biology, we wish to offer the analysis and solution to other mathematicians and biologists.

2 The Interleukin-1 Chemical Reaction Network and Applicable Mathematical Equations

We present in this section the reaction network we are interested in and the equations to analyze it. Dinarello [6] is a comprehensive review of Interleukin-1. There are a number of references that extensively cover the modeling of receptor-ligand interactions, for instance Limbird [19], Cornish-Bowden [5], Lauffenburger and Linderman [18], and Matthews [20]. Bywater, Sorensen, Rogen and Hjorth [3], and Chaves, Sontag and Dinerstein [4] are two examples where particular receptor-ligand reaction systems are studied through mathematical analysis. They offer evidence that modeling even simple reaction systems can result in substantial levels algebraic complexity. While our work is concerned with the monotonicity of equilibrium concentrations with respect to initial concentrations, we note that one could also study the monotonicity of time-dependent concentrations with respect to initial concentrations; see for example Siegel [22] and Siegel and Lozinski [24].

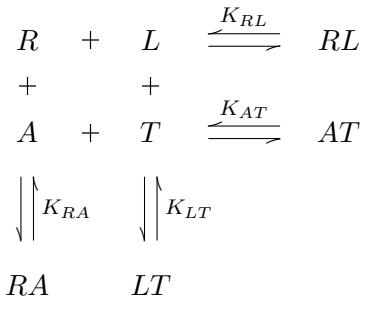

Figure 1: Reaction network for IL-1 (L), IL-1RI (R), IL-1Ra (A) and trap (T).

Our reaction network, shown on Figure 1, describes the interaction of the ligand IL-1, the ligand receptor IL-1RI, the receptor antagonist IL-1Ra and the decoy receptor or trap, denoted L, R, A and T respectively. Arrow inscriptions K_{RL}, K_{RA}, K_{LT}, K_{AT} denote the dissociation constants.

This network is a simple model of a more complex piece of biochemistry. For instance, signal transduction that follows the binding of IL-1 and IL-1RI and precedes the formation of IL-6 is not represented. The model has nevertheless been adequate to explain the experimental observations that motivated this theoretical work. For the model to be applicable, all four components L, R, A and T must be able to interact together in the same biological compartment. This constraint would be violated if the antagonist A is generated locally and remains largely sequestered in the tissue or compartment containing the receptor R, and the ligand L and trap T circulate systematically throughout the organism or system.

If S is a species, then $[S]_0$ and $[S]$ denote the initial and equilibrium concentrations of S respectively. Furthermore, if S is one of the simple species L, R, A, T, then $[S]_{\mathrm{T}}$ denotes the total concentration of S, i.e. the time-independent sum of concentrations of species that have S as a component. We have the conservation equations

$$\begin{array}{rcccl} [R]_0 + [RL]_0 + [RA]_0 & = & [R] + [RL] + [RA] & = & [R]_{\mathrm{T}} \\ [L]_0 + [RL]_0 + [LT]_0 & = & [L] + [RL] + [LT] & = & [L]_{\mathrm{T}} \\ [A]_0 + [RA]_0 + [AT]_0 & = & [A] + [RA] + [AT] & = & [A]_{\mathrm{T}} \\ [T]_0 + [LT]_0 + [AT]_0 & = & [T] + [LT] + [AT] & = & [T]_{\mathrm{T}} \end{array} \tag{1}$$

and the equilibrium equations

$$\frac{[R]\,[L]}{[RL]} = K_{RL}\,, \quad \frac{[R]\,[A]}{[RA]} = K_{RA}\,, \quad \frac{[L]\,[T]}{[LT]} = K_{LT}\,, \quad \frac{[A]\,[T]}{[AT]} = K_{AT}\,. \tag{2}$$

By combining systems (1) and (2), we obtain the following system.

$$\left\{ \begin{array}{rcrcrcl} [R] & + & \dfrac{1}{K_{RL}}\,[R]\,[L] & + & \dfrac{1}{K_{RA}}\,[R]\,[A] & = & [R]_{\mathrm{T}} \\ [L] & + & \dfrac{1}{K_{RL}}\,[R]\,[L] & + & \dfrac{1}{K_{LT}}\,[L]\,[T] & = & [L]_{\mathrm{T}} \\ [A] & + & \dfrac{1}{K_{RA}}\,[R]\,[A] & + & \dfrac{1}{K_{AT}}\,[A]\,[T] & = & [A]_{\mathrm{T}} \\ [T] & + & \dfrac{1}{K_{LT}}\,[L]\,[T] & + & \dfrac{1}{K_{AT}}\,[A]\,[T] & = & [T]_{\mathrm{T}} \end{array} \right. \tag{3}$$

System (3) is a system of four polynomial equations where the unknowns are the equilibrium concentrations $[L]$, $[R]$, $[A]$, $[T]$ of the simple species. There are eight parameters: the four dissociation constants K_{RL}, K_{RA}, K_{LT}, K_{AT}, which depend only on temperature and have been determined experimentally, and the four total concentrations $[L]_{\mathrm{T}}$, $[R]_{\mathrm{T}}$, $[A]_{\mathrm{T}}$, $[T]_{\mathrm{T}}$, which represent the initial conditions. The equilibrium concentrations $[RL]$, $[RA]$, $[LT]$, $[AT]$ of the complex species derive immediately from equilibrium equations (2). Polynomial system (3) is the main subject of our attention in the rest of the paper.

3 Monotonicity

The main issue that motivated this work is the monotonicity of $[RL]$ as a function of $[A]_0$. The answer is in the following Theorem.

Theorem 1. *Consider the ligand-receptor-antagonist-trap reaction network of Figure* 1 *and assume* $[R]_{\mathrm{T}} > 0$ *and* $[L]_{\mathrm{T}} > 0$ *(otherwise* $[RL] = 0$*).*

- *If* $K_{RA} \leqslant K_{AT}$*, then the function* $[A]_{\mathrm{T}} \mapsto [RL]$ *is strictly monotonically decreasing to zero.*
- *Suppose* $K_{AT} < K_{RA}$.
  Let $\tau = \dfrac{K_{LT}\, K_{AT}}{K_{RA} - K_{AT}}$*, and let the function* φ *be defined by*

$$\varphi(t) = t^2 + \frac{K_{RA}\, K_{LT}}{K\,\sqrt{K_{AT}}}\, t + \frac{[R]_{\mathrm{T}}\, K\, t}{K_{RL}\,\sqrt{K_{AT}} + K\, t}\,,$$

  where $K = \sqrt{K_{LT}(K_{RA} - K_{AT})}$.
  φ *is a monotonically increasing bijection* $\mathbb{R}_{\geqslant 0} \to \mathbb{R}_{\geqslant 0}$.
  - *If* $[T]_{\mathrm{T}} \leqslant \tau$*, or if* $[T]_{\mathrm{T}} > \tau$ *and* $[L]_{\mathrm{T}} \geqslant \varphi\left(\sqrt{[T]_{\mathrm{T}}} - \sqrt{\tau}\right)$*, then the function* $[A]_{\mathrm{T}} \mapsto [RL]$ *is strictly monotonically decreasing to zero.*
  - *If* $[T]_{\mathrm{T}} > \tau$ *and* $[L]_{\mathrm{T}} < \varphi\left(\sqrt{[T]_{\mathrm{T}}} - \sqrt{\tau}\right)$*, then the function* $[A]_{\mathrm{T}} \mapsto [RL]$ *first is strictly monotonically increasing, reaches an absolute maximum, then is strictly monotonically decreasing to zero.*

  Equivalently:
  - *If* $[T]_{\mathrm{T}} \leqslant \left(\sqrt{\tau} + \varphi^{-1}([L]_{\mathrm{T}})\right)^2$*, then the function* $[A]_{\mathrm{T}} \mapsto [RL]$ *is strictly monotonically decreasing to zero.*

– *If* $[T]_{\mathrm{T}} > \left(\sqrt{\tau} + \varphi^{-1}([L]_{\mathrm{T}})\right)^2$, *then the function* $[A]_{\mathrm{T}} \mapsto [RL]$ *first is strictly monotonically increasing, reaches an absolute maximum, then is strictly monotonically decreasing to zero.*

The above statements hold with the function $[A]_0 \mapsto [RL]$ *in lieu of the function* $[A]_{\mathrm{T}} \mapsto [RL]$.

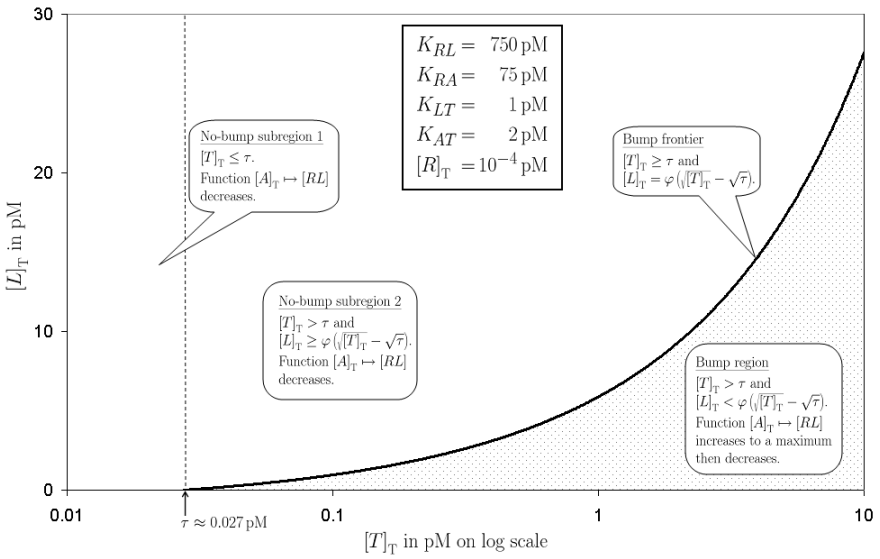

Figure 2: The bump frontier for the function $[A]_{\mathrm{T}} \mapsto [RL]$ with fixed $[R]_{\mathrm{T}}$. The bump frontier, defined by $[T]_{\mathrm{T}} \geqslant \tau$ and $[L]_{\mathrm{T}} = \varphi\left(\sqrt{[T]_{\mathrm{T}}} - \sqrt{\tau}\right)$, partitions the $([T]_{\mathrm{T}}, [L]_{\mathrm{T}})$-plane into two regions according to the monotonicity of the function $[A]_{\mathrm{T}} \mapsto [RL]$: the bump region is the shaded area, the right-hand side of the frontier; the no-bump region is the frontier and its left-hand side. When $[T]_{\mathrm{T}} > \tau$ and $[L]_{\mathrm{T}} < \varphi\left(\sqrt{[T]_{\mathrm{T}}} - \sqrt{\tau}\right)$, the point $([T]_{\mathrm{T}}, [L]_{\mathrm{T}})$ is in the bump region: the function $[A]_{\mathrm{T}} \mapsto [RL]$ strictly increases to a maximum then strictly decreases to zero. When $[T]_{\mathrm{T}} \leqslant \tau$ (and $[L]_{\mathrm{T}}$ is unrestricted), the point $([T]_{\mathrm{T}}, [L]_{\mathrm{T}})$ is in the first no-bump subregion. When $[T]_{\mathrm{T}} > \tau$ and $[L]_{\mathrm{T}} \geqslant \varphi\left(\sqrt{[T]_{\mathrm{T}}} - \sqrt{\tau}\right)$, the point $([T]_{\mathrm{T}}, [L]_{\mathrm{T}})$ is in the second no-bump subregion. In both cases, the function $[A]_{\mathrm{T}} \mapsto [RL]$ strictly decreases to zero.

The assertions in Theorem 1 are proved in section 4. Work there is carried out on an abstract form of the chemical network of interest (see Figure 5).

Theorem 1 is obtained by applying Theorem 6 with X_1, X_2, X_3, X_4 standing for R, L, T, A respectively, and $a_1 = \dfrac{1}{K_{RL}}$, $a_2 = \dfrac{1}{K_{LT}}$, $a_3 = \dfrac{1}{K_{AT}}$, $a_4 = \dfrac{1}{K_{RA}}$.

We illustrate the conclusions of Theorem 1 on Figure 3 and the related notion of bump frontier on Figure 2. The values of dissociation constants K_{RA} and K_{RL} are the midpoints of the ranges (50 to 100 pM and 500 to 1000 pM respectively) reported in Dinarello [6, page 2108, first column, lines 18 and 28]. K_{LT} and K_{AT} were determined at Amgen. The concentrations of IL-1 and IL-1RI and the ranges of concentrations of IL-1Ra and trap are as used in experiments conducted at Amgen.

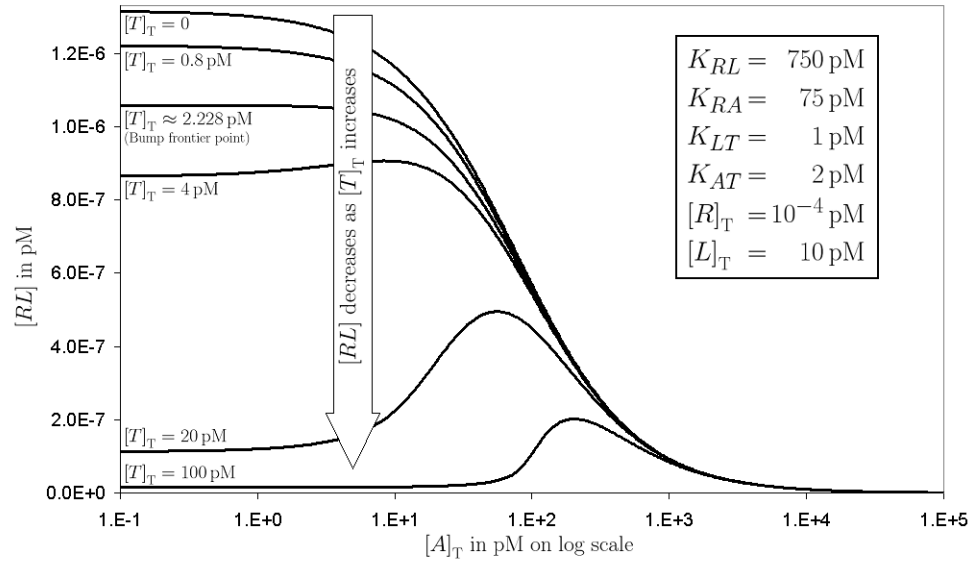

Figure 3: $[RL]$ as function of $[A]_T$ at fixed $[R]_T$ and $[L]_T$ and varying $[T]_T$. For $[R]_T$ and $[L]_T$ as fixed, as $[T]_T$ increases, the function $[A]_T \mapsto [RL]$ first is in the "no-bump regime": the effects of IL-1Ra and trap combine to reduce the binding of IL-1 and IL-1RI. Then the function crosses into the "bump regime": IL-1Ra can reverse trap-induced inhibition of IL-1/IL-1RI binding. On another hand, for any fixed values of $[R]_T$, $[L]_T$ and $[A]_T$, the function $[T]_T \mapsto [RL]$ is strictly decreasing, whence the graphs globally get lower.

We introduced the concept of "bump frontier" in Remark 7 for the abstract network. This is illustrated for the IL-1 network on Figure 2, where required condition $K_{AT} < K_{RA}$ is satisfied and the function φ is as defined in Theorem 1. For fixed $[R]_{\mathrm{T}}$, the bump frontier separates the $([T]_{\mathrm{T}}, [L]_{\mathrm{T}})$-plane into two regions. When the point $([T]_{\mathrm{T}}, [L]_{\mathrm{T}})$ is in the "bump region", i.e. the right-hand side of the bump frontier, the function $[A]_{\mathrm{T}} \mapsto [RL]$ increases to a maximum then decreases; IL-1Ra can reverse trap-induced inhibition of IL-1/IL-1RI binding. When the point $([T]_{\mathrm{T}}, [L]_{\mathrm{T}})$ is in the "no-bump region", comprised of the bump frontier and the left-hand side thereof, the function $[A]_{\mathrm{T}} \mapsto [RL]$ decreases; IL-1Ra and trap combine their effects to reduce IL-1/IL-1RI binding. The "no-bump region" is partitioned into two subregions. The first subregion is defined by the condition $[T]_{\mathrm{T}} \leqslant \tau$ and no condition on $[L]_{\mathrm{T}}$; there is not enough trap to cause a bump. The second subregion is characterized by the conditions $[T]_{\mathrm{T}} > \tau$ and $[L]_{\mathrm{T}} \geqslant \varphi\left(\sqrt{[T]_{\mathrm{T}}} - \sqrt{\tau}\right)$; there is enough trap to potentially cause a bump, but for this to actually happen, there must also be enough IL-1.

The dissociation constants are only approximately known. In particular, we have $K_{AT} \approx 2$ pM and $K_{RA} = 75 \pm 25$ pM. So even if the constants are let to assume other sensible values, the condition $K_{AT} < K_{RA}$ remains valid, and therefore the notions of bump frontier and bump region persist.

Consider the condition $K_{LT} \leqslant K_{AT} < K_{RA}$. The condition $K_{LT} \leqslant K_{AT}$ used in Theorem 6 with X_1, X_2, X_3, X_4 standing for L, R, A, T and $a_1 = \dfrac{1}{K_{RL}}$, $a_2 = \dfrac{1}{K_{RA}}$, $a_3 = \dfrac{1}{K_{AT}}$, $a_4 = \dfrac{1}{K_{LT}}$ implies that the functions $[T]_{\mathrm{T}} \mapsto [RL]$ is strictly decreasing. On another hand, with the condition $K_{AT} < K_{RA}$, Theorem 1 tells us the monotonicity of the function $[A]_{\mathrm{T}} \mapsto [RL]$. By combining both facts, we can predict the behavior of the family of graphs of the functions $[A]_{\mathrm{T}} \mapsto [RL]$ as $[R]_{\mathrm{T}}$ and $[L]_{\mathrm{T}}$ are fixed and $[T]_{\mathrm{T}}$ ranges from 0 to ∞. First the graphs are monotonically decreasing; then as $[T]_{\mathrm{T}}$ reaches the bump frontier, the graphs present an increasing phase followed by a decreasing phase. At the same time, the graphs globally get lower. Figure 3 illustrates these predictions.

As explained earlier, we chose the values of dissociation constants and ranges of concentrations to reflect the reality of actual experiments. Figure 4 shows the results of a representative experiment. Calculation and experimental results are in good agreement with regard to the monotonicity behavior with respect to concentration of IL-1Ra. For the monotonicity behavior

with respect to concentration of IL-1TRAP, experimental curves cross over whereas the model predicts that the curves globally get lower. As we said in section 2, the model does not comprehensively represent all biochemical processes involved, and we may be seeing a consequence of that limitation. Within the scope of the model, the crossover phenomenon can be observed if $K_{LT} > K_{AT}$. This in fact is not an impossibility considering that the values of K_{LT} and K_{AT} are close ($K_{LT} \approx 1$ pM and $K_{AT} \approx 2$ pM) and subject to errors. Note that in contrast, we are certain on the validity of the condition $K_{AT} < K_{RA}$ ($K_{AT} \approx 2$ pM and $K_{RA} = 75 \pm 25$ pM) which makes possible the monotonicity behavior with respect to concentration of IL-1Ra.

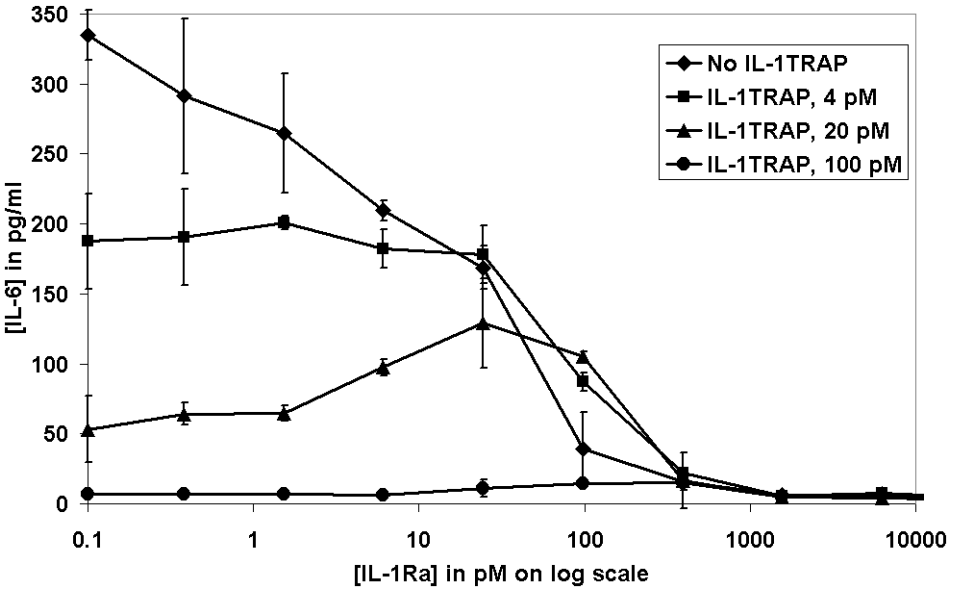

Figure 4: IL-1-induced IL-6 in human chondrocytes treated with IL-1TRAP and IL-1Ra. The 0.1 pM concentration on the horizontal axis actually indicates no IL-1Ra.

> Serially diluted IL-1Ra was mixed with fixed amounts of IL-1TRAP and incubated for one hour. The binding reactions and 10 pM IL-1 were then added to primary human chondrocytes. Supernatants were collected 18 hours later and the amount of IL-6 measured by ELISA. IL-6 is a proxy for IL-1RI-bound IL-1.

4 Mathematical Abstraction and Proofs

Theorem 1 is in fact a particular instance of Theorem 6. Theorem 6 is the main result in this section, but we will reach it only after a series of intermediate steps: Proposition 2 ensures that we can legitimately talk about "the" equilibrium of the system, and Lemmas 3, 4 and 5 are technical results whose purpose is their use in the proof of Theorem 6.

We will work with a form of system (3) that is more amenable to mathematical manipulations (system (4) below). To that end, we first consider the reaction network of Figure 5, an abstracted form of the one of Figure 1. While working with this network, we use several notations with integer index i with $1 \leqslant i \leqslant 4$. Incrementing or decrementing the index could cause it to be outside the bounds. In such situations, the out-of-range value is to be interpreted as its modulo 4 equivalent within the bounds.

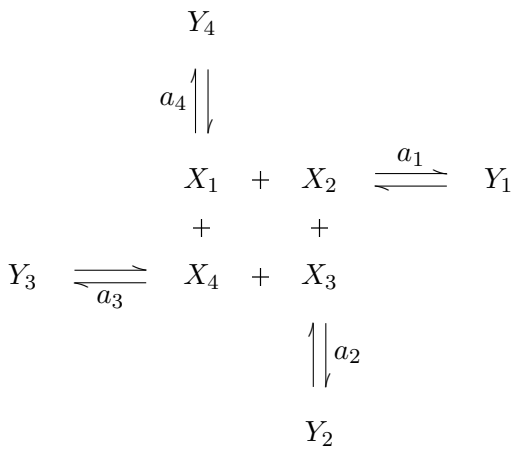

Figure 5: An Abstract Ligand-Receptor-Antagonist-Trap Reaction Network.

We introduce the following notations:

a_i :	Binding constant of the reaction $X_i + X_{i+1} \rightleftharpoons Y_i$.
b_i :	Total concentration of X_i.
x_i°, y_i° :	Initial concentrations of X_i, Y_i.
x_i, y_i :	Equilibrium concentrations of X_i, Y_i.

Our concern is to understand the monotonicity of equilibrium concentra-

tions x_i and y_i as functions of initial concentrations x_j°. We will do this by studying the sign of the partial derivatives $\dfrac{\partial x_i}{\partial x_j^\circ}$ and $\dfrac{\partial y_i}{\partial x_j^\circ}$. But first, we verify the existence and uniqueness of equilibrium. We will use Chemical Reaction Network Theory, a mathematical treatise of chemical reaction networks which has developed through the work of Horn and Jackson [17] and Feinberg [8, 9, 10, 11, 12]. Further developments include Siegel and Chen [23], Siegel and MacLean [25], and Gatermann and Huber [13]. Gunawardena [15] presents the theory with a interesting change of emphasis in the mathematical approach. We will mainly use the Deficiency-Zero Theorem; see for instance Feinberg [11, Theorem 4.1]. This is a key result in the theory which provides sufficient conditions for the existence and uniqueness of equilibrium.

Proposition 2. *Consider the reaction network of Figure* 5*, with binding constants* $a_1, a_2, a_3, a_4 \in \mathbb{R}_{>0}$*. For any choice of total concentration vector* $b = (b_1, b_2, b_3, b_4) \in \mathbb{R}^4_{\geqslant 0}$*, the network admits a unique equilibrium.*

Proof. The result is trivially true if $b_1 = b_3 = 0$, or if $b_2 = b_4 = 0$, because no reactions occur. Because of the circular symmetry in the network, it suffices to prove the result for $b \in \mathbb{R}_{>0} \times \mathbb{R}_{>0} \times \{0\} \times \{0\}$, for $b \in \mathbb{R}_{>0} \times \mathbb{R}_{>0} \times \mathbb{R}_{>0} \times \{0\}$, and for $b \in \mathbb{R}^4_{>0}$. The first and second cases are equivalent to the existence and uniqueness of equilibrium given any choice of positive total concentrations for the reaction networks of Figures 6 and 7 respectively.

$$X_1 \quad + \quad X_2 \quad \underset{}{\overset{a_1}{\rightleftharpoons}} \quad Y_1$$

Figure 6: Reaction Network of Figure 5 when $b_3 = b_4 = 0$.

We now analyze the network of Figure 5 with the aim of applying the Deficiency-Zero Theorem.

- The network is reversible, and hence is weakly reversible.
- There are 8 species X_i, Y_i $(i = 1, 2, 3, 4)$. They form the set $\mathscr{S}$. For every $s \in \mathscr{S}$, let the function $s^* : \mathscr{S} \to \mathbb{R}$ be defined by $s^*(s) = 1$ and $s^*(s') = 0$ for $s' \in \mathscr{S} \backslash \{s\}$. Then $\mathscr{S}^* = \{s^* : s \in \mathscr{S}\}$ is a basis for the vector space $\mathbb{R}^{\mathscr{S}}$ of functions $\mathscr{S} \to \mathbb{R}$. A composition is an element γ of $\mathbb{R}^{\mathscr{S}}$ with values in $\mathbb{R}_{\geqslant 0}$; $\gamma(s)$ is the concentration of species s.

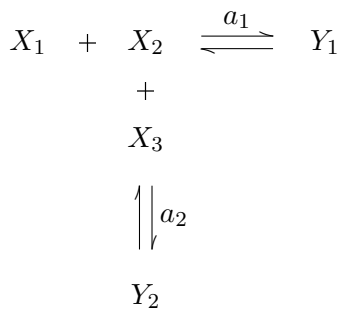

Figure 7: Reaction Network of Figure 5 when $b_4 = 0$.

- There are $\nu = 8$ complexes $X_i + X_{i+1}, Y_i$ $(i = 1, 2, 3, 4)$. They form the set $\mathscr{C}$, which is partitioned into the $\lambda = 4$ linkage classes $\{X_i + X_{i+1}, Y_i\}$.

- There are 8 reaction vectors in $\mathbb{R}^{\mathscr{S}}$, $r_i^+ = Y_i^* - X_i^* - X_{i+1}^*$ and $r_i^- = -r_i^+$, corresponding to the reactions $X_i + X_{i+1} \to Y_i$ and $Y_i \to X_i + X_{i+1}$. They form the set $\mathscr{R}$, which spans the stoichiometric subspace S in $\mathbb{R}^{\mathscr{S}}$. The set $\mathscr{R}^+ = \{r_i^+ : i = 1, 2, 3, 4\}$ is a basis for S, so the rank of the network is $\sigma = 4$.

- The deficiency of the network is $\delta = \nu - \lambda - \sigma = 0$.

- We now prove that choosing a positive stoichiometric compatibility class amounts to choosing a vector of positive total concentrations $b = (b_1, b_2, b_3, b_4)$. We equip $\mathbb{R}^{\mathscr{S}}$ with the Euclidean structure for which $\mathscr{S}^*$ is an orthonormal basis. Let $t_i = X_i^* + Y_{i-1}^* + Y_i^*$. If $\gamma \in \mathbb{R}^{\mathscr{S}}$ is a composition, then $\gamma \cdot t_i = \gamma(X_i) + \gamma(Y_{i-1}) + \gamma(Y_i)$ is the total (free and bound) concentration of X_i. Let T be the (four-dimensional) subspace of $\mathbb{R}^{\mathscr{S}}$ spanned by t_1, t_2, t_3, t_4. Then $\mathbb{R}^{\mathscr{S}} = S \oplus_\perp T$, orthogonal direct sum. This gives rise to a bijection between the quotient space $\mathbb{R}^{\mathscr{S}}/S$ and $\mathbb{R}^4$ such that an equivalence class $\Gamma \in \mathbb{R}^{\mathscr{S}}/S$ and a vector $b = (b_1, b_2, b_3, b_4) \in \mathbb{R}^4$ that map to each other are related by $\Gamma = \left\{\gamma \in \mathbb{R}^{\mathscr{S}} : \gamma \cdot t_i = b_i, \forall\, i = 1, 2, 3, 4\right\}$. We claim that $\Gamma \cap \mathbb{R}^{\mathscr{S}}_{>0} \neq \varnothing \Leftrightarrow b \in \mathbb{R}^4_{>0}$. Indeed, if $\gamma \in \Gamma \cap \mathbb{R}^{\mathscr{S}}_{>0}$, then $b_i = \gamma \cdot t_i = \gamma(X_i) + \gamma(Y_{i-1}) + \gamma(Y_i) > 0$ for $i = 1, 2, 3, 4$, hence $b \in \mathbb{R}^4_{>0}$. And conversely, if $b \in \mathbb{R}^4_{>0}$, with $\gamma = \sum_{j=1}^4 b_j X_j^*$, we have $\gamma \in \mathbb{R}^{\mathscr{S}}_{>0}$ and $\gamma \cdot t_i = b_i$ for $i = 1, 2, 3, 4$, whence $\gamma \in \Gamma$, $\gamma \in \Gamma \cap \mathbb{R}^{\mathscr{S}}_{>0}$.

So we have an induced bijection between the set of positive stoichiometric compatibility classes, which we denote $\mathbb{R}^{\mathscr{S}}_{>0}/S$, and $\mathbb{R}^4_{>0}$ such that corresponding $\Gamma \in \mathbb{R}^{\mathscr{S}}_{>0}/S$ and $b = (b_1, b_2, b_3, b_4) \in \mathbb{R}^4_{>0}$ are related by $\Gamma = \left\{\gamma \in \mathbb{R}^{\mathscr{S}}_{>0} : \gamma \cdot t_i = b_i, \forall\, i = 1, 2, 3, 4\right\}$; Γ is the set of positive compositions for which the total concentrations of X_1, X_2, X_3, X_4 are b_1, b_2, b_3, b_4 respectively.

We conclude with the Deficiency-Zero Theorem (Feinberg [11, Theorem 4.1]) that for any choice of total concentration vector $b = (b_1, b_2, b_3, b_4) \in \mathbb{R}^4_{>0}$, the network has a unique equilibrium.

The networks of Figures 6 and 7 are (weakly) reversible, and one can also show that they have deficiency zero: in the first case, we have $\nu = 2$ complexes, $\lambda = 1$ linkage class, and rank $\sigma = 1$; in the second case, we have $\nu = 4$ complexes, $\lambda = 2$ linkage classes, and rank $\sigma = 2$. So we can apply the Deficiency-Zero Theorem to these networks as well. The interpretation of positive stoichiometric compatibility classes in terms of total concentrations of X_1, X_2 and of X_1, X_2, X_3 respectively can also be verified in an analogous manner. □

The binding constants a_1, a_2, a_3, a_4 are fixed in $\mathbb{R}_{>0}$ for the rest of this section. The equations at our disposal are in the following three systems.

$$\left\{\begin{array}{rcrcrcl} x_1 & + & a_4\,x_4\,x_1 & + & a_1\,x_1\,x_2 & = & b_1 \\ x_2 & + & a_1\,x_1\,x_2 & + & a_2\,x_2\,x_3 & = & b_2 \\ x_3 & + & a_2\,x_2\,x_3 & + & a_3\,x_3\,x_4 & = & b_3 \\ x_4 & + & a_3\,x_3\,x_4 & + & a_4\,x_4\,x_1 & = & b_4 \end{array}\right. \tag{4}$$

$$\left\{\begin{array}{l} y_1 = a_1\,x_1\,x_2 \\ y_2 = a_2\,x_2\,x_3 \\ y_3 = a_3\,x_3\,x_4 \\ y_4 = a_4\,x_4\,x_1 \end{array}\right. \tag{5}$$

$$\left\{\begin{array}{rcrcrcl} x_1^\circ & + & y_4^\circ & + & y_1^\circ & = & b_1 \\ x_2^\circ & + & y_1^\circ & + & y_2^\circ & = & b_2 \\ x_3^\circ & + & y_2^\circ & + & y_3^\circ & = & b_3 \\ x_4^\circ & + & y_3^\circ & + & y_4^\circ & = & b_4 \end{array}\right. \tag{6}$$

In order to properly state the problem, we introduce the functions $f = (f_1, f_2, f_3, f_4)$, $g = (g_1, g_2, g_3, g_4)$, and $h = (h_1, h_2, h_3, h_4)$ defined by

$$\begin{array}{lcl} f_i(x) & = & x_i + a_{i-1}x_{i-1}x_i + a_i x_i x_{i+1}\,, \\ g_i(x) & = & a_i x_i x_{i+1}\,, \\ h_i(x,y) & = & x_i + y_{i-1} + y_i\,, \end{array}$$

for $x = (x_1, x_2, x_3, x_4), y = (y_1, y_2, y_3, y_4) \in \mathbb{R}^4_{\geqslant 0}$. We note that systems (4), (5), (6) are the equations

$$f(x) = b\,, \tag{7}$$
$$y = g(x)\,, \tag{8}$$
$$h(x^\circ, y^\circ) = b \tag{9}$$

respectively, where $b = (b_1, b_2, b_3, b_4) \in \mathbb{R}^4_{\geqslant 0}$.

By Proposition 2, f is a bijection $\mathbb{R}^4_{\geqslant 0} \to \mathbb{R}^4_{\geqslant 0}$. In fact, f is a C^∞-diffeomorphism $\mathbb{R}^4_{\geqslant 0} \to \mathbb{R}^4_{\geqslant 0}$. So we can define functions $F = (F_1, F_2, F_3, F_4)$ and $G = (G_1, G_2, G_3, G_4)$ for $(x^\circ, y^\circ) \in \mathbb{R}^4_{\geqslant 0} \times \mathbb{R}^4_{\geqslant 0}$ by

$$F(x^\circ, y^\circ) = f^{-1}(h(x^\circ, y^\circ)) \text{ and } G(x^\circ, y^\circ) = g(F(x^\circ, y^\circ))\ .$$

Given initial concentration vectors x° and y°, we have the vector of total concentrations $b = h(x^\circ, y^\circ)$, and the vectors of equilibrium concentrations $x = f^{-1}(b) = F(x^\circ, y^\circ)$ and $y = g(x) = G(x^\circ, y^\circ)$. What we are after are the signs of the partial derivatives of $x^\circ \mapsto F(x^\circ, y^\circ)$ and $x^\circ \mapsto G(x^\circ, y^\circ)$. Consider the following Jacobian matrices:

$$J_1 = \left[\frac{\partial F_i}{\partial x^\circ_j}(x^\circ, y^\circ)\right], J_2 = \left[\frac{\partial G_i}{\partial x^\circ_j}(x^\circ, y^\circ)\right], \tilde{J}_1 = \left[\frac{\partial f_i}{\partial x_j}(x)\right], \tilde{J}_2 = \left[\frac{\partial g_i}{\partial x_j}(x)\right],$$

where row and column indices i and j range between 1 and 4. Since the Jacobian matrix of $x^\circ \mapsto h(x^\circ, y^\circ)$ is uniformly the identity matrix, we have $J_1 = \tilde{J}_1^{-1}$ and $J_2 = \tilde{J}_2 J_1 = \tilde{J}_2 \tilde{J}_1^{-1}$. In concise notations, we have:

$$\tilde{J}_1 = \left[\frac{\partial b_i}{\partial x_j}\right]\ ,\ J_1 = \left[\frac{\partial x_i}{\partial b_j}\right] = \left[\frac{\partial x_i}{\partial x^\circ_j}\right]\ ,\ \tilde{J}_2 = \left[\frac{\partial y_i}{\partial x_j}\right]\ ,\ J_2 = \left[\frac{\partial y_i}{\partial b_j}\right] = \left[\frac{\partial y_i}{\partial x^\circ_j}\right]\ .$$

Lemma 3. *Consider the quantities of systems* (4) *and* (5) *and assume that* b_1*,* b_2*,* b_3 *are fixed in* $\mathbb{R}_{\geqslant 0}$*. We have:*

$$\lim_{b_4\to\infty} x_1 = 0 \qquad \lim_{b_4\to\infty} x_2 = b_2 \qquad \lim_{b_4\to\infty} x_3 = 0 \qquad \lim_{b_4\to\infty} x_4 = \infty$$
$$\lim_{b_4\to\infty} y_1 = 0 \qquad \lim_{b_4\to\infty} y_2 = 0 \qquad \lim_{b_4\to\infty} y_3 = b_3 \qquad \lim_{b_4\to\infty} y_4 = b_1$$

Proof. Suppose $b_4 \to \infty$. We have $(1+a_3x_3+a_4x_1)x_4 = b_4$. Since $(1+a_3x_3+a_4x_1)$ is bounded, we have $x_4 \to \infty$. Then we have $(1 + a_4x_4 + a_1x_2)x_1 = b_1$ and $(1+a_4x_4+a_1x_2) \to \infty$. So $x_1 \to 0$. Likewise, $(1+a_2x_2+a_3x_4)x_3 = b_3$ and

$(1 + a_2x_2 + a_3x_4) \to \infty$, and $x_3 \to 0$. Then we have $(1 + a_1x_1 + a_2x_3)x_2 = b_2$ with $(1 + a_1x_1 + a_2x_3) \to 1$, so $x_2 \to b_2$. And then, that $x_1 \to 0$ and $x_3 \to 0$ imply $y_1 = a_1x_1x_2 \to 0$ and $y_2 = a_2x_2x_3 \to 0$ respectively. Finally, $y_3 = b_3 - x_3 - y_2 \to b_3$ and $y_4 = b_1 - x_1 - y_1 \to b_1$. □

We have

$$\tilde{J}_1 = \begin{bmatrix} 1 + a_4x_4 + a_1x_2 & a_1x_1 & 0 & a_4x_1 \\ a_1x_2 & 1 + a_1x_1 + a_2x_3 & a_2x_2 & 0 \\ 0 & a_2x_3 & 1 + a_2x_2 + a_3x_4 & a_3x_3 \\ a_4x_4 & 0 & a_3x_4 & 1 + a_3x_3 + a_4x_1 \end{bmatrix}$$

Let $D = \det\tilde{J}_1$ and $U = DJ_1$. The expressions of D and of the entries of matrix U are provided in the Appendix (section 7). We see that $D > 0$, and so $\text{sign}(J_1) = \text{sign}(U)$. We observe the following sign pattern.

$$\text{sign}(J_1) = \text{sign}(U) = \begin{bmatrix} + & - & + & - \\ - & + & - & + \\ + & - & + & - \\ - & + & - & + \end{bmatrix} \tag{10}$$

(We note that in fact, $D \geqslant 1$. Also, that $D > 0$ can be obtained without complex calculations: the matrix $\tilde{J}_1$ has positive diagonal entries and is diagonally dominant by column.) Obtaining $\text{sign}(J_2)$ will not be as direct. Let

$$A = \begin{bmatrix} a_1 & 0 & 0 & 0 \\ 0 & a_2 & 0 & 0 \\ 0 & 0 & a_3 & 0 \\ 0 & 0 & 0 & a_4 \end{bmatrix}, \; V = \begin{bmatrix} x_2 & x_1 & 0 & 0 \\ 0 & x_3 & x_2 & 0 \\ 0 & 0 & x_4 & x_3 \\ x_4 & 0 & 0 & x_1 \end{bmatrix}, \text{ and } W = VU\,.$$

We have $\tilde{J}_2 = AV$, and so

$$DJ_2 = AW\,. \tag{11}$$

It follows that $\text{sign}(J_2) = \text{sign}(W)$. The entries of W are provided in the Appendix (section 7). We have the following sign pattern.

$$\text{sign}(J_2) = \text{sign}(W) = \begin{bmatrix} + & + & \star & \star \\ \star & + & + & \star \\ \star & \star & + & + \\ + & \star & \star & + \end{bmatrix} \tag{12}$$

where $\star$ indicates a possibly changing sign. Because of the circular symmetry in the reaction network, it is enough to study one case of possibly changing

sign. We choose to investigate the sign of $\dfrac{\partial y_1}{\partial b_4}$. Note that if $b_1 = 0$ or $b_2 = 0$, then $y_1 = 0$ constantly.

Lemma 4. *For $b_1, b_2, b_3 \geqslant 0$, let (ξ_1, ξ_2, ξ_3) be the nonnegative solution of the system*

$$\left\{\begin{array}{lllllll} x_1 & & & + & a_1\,x_1\,x_2 & = & b_1 \\ x_2 & + & a_1\,x_1\,x_2 & + & a_2\,x_2\,x_3 & = & b_2 \\ x_3 & + & a_2\,x_2\,x_3 & & & = & b_3 \end{array}\right. \tag{13}$$

Suppose $a_3 > a_4$, let $\beta = \dfrac{a_4}{a_2(a_3 - a_4)}$, and fix $b_1 \geqslant 0$ and $b_3 \geqslant \beta$.

- *The function $b_2 \mapsto \xi_3$ is a monotonically decreasing bijection $[0, \infty[\to]0, b_3]$.*
- *The value of b_2 for which $\xi_3 = \sqrt{\beta\, b_3}$ is $b_2 = \varphi\left(\sqrt{b_3} - \sqrt{\beta}\right)$, where*

$$\varphi(t) = t^2 + \frac{a_3\, t}{\sqrt{a_2 a_4 (a_3 - a_4)}} + \frac{b_1 a_1 \sqrt{a_3 - a_4}\, t}{\sqrt{a_2 a_4} + a_1 \sqrt{a_3 - a_4}\, t}\,. \tag{14}$$

- *φ is a monotonically increasing bijection $\mathbb{R}_{\geqslant 0} \to \mathbb{R}_{\geqslant 0}$.*

Proof. System (13) is system (4) with $b_4 = 0$. So, by Proposition 2, it does have a unique nonnegative solution. We can use equation (10) and argue as in the proof of Lemma 3. We obtain that the function $b_2 \mapsto \xi_3$ decreases from b_3 to 0 as b_2 ranges from 0 to ∞.
Let $s = \sqrt{b_3}$, $t_0 = \sqrt{\beta}$, and $t = s - t_0 = \sqrt{b_3} - \sqrt{\beta}$. Suppose $\xi_3 = \sqrt{\beta\, b_3}$. Then $\xi_3 = t_0 s = t_0(t + t_0)$. From the equation $\xi_3 + a_2\xi_2\xi_3 = b_3$, we have

$$\xi_2 = \frac{b_3 - \xi_3}{a_2\xi_3} = \frac{s^2 - t_0 s}{a_2 t_0 s} = \frac{t}{a_2 t_0}\,.$$

From the equation $\xi_1 + a_1\xi_1\xi_2 = b_1$, we have

$$\xi_1 = \frac{b_1}{1 + a_1\xi_2} = \frac{b_1}{1 + \dfrac{a_1 t}{a_2 t_0}} = \frac{a_2 b_1 t_0}{a_2 t_0 + a_1 t}\,.$$

With these expressions of ξ_1, ξ_2, ξ_3 we obtain

$$\begin{aligned} b_2 &= \xi_2 + a_1\xi_1\xi_2 + a_2\xi_2\xi_3 \\ &= \frac{t}{a_2 t_0} + \frac{a_1 b_1 t}{a_2 t_0 + a_1 t} + t(t + t_0) \\ &= t^2 + \left(t_0 + \frac{1}{a_2 t_0}\right) t + \frac{a_1 b_1 t}{a_2 t_0 + a_1 t}\,. \end{aligned}$$

Set

$$\varphi(t) = t^2 + \left(t_0 + \frac{1}{a_2 t_0}\right) t + \frac{a_1 b_1 t}{a_2 t_0 + a_1 t}\,.$$

Then $\varphi(0) = 0$, $\lim\limits_{t\to\infty} \varphi(t) = \infty$, and

$$\varphi'(t) = 2t + \left(t_0 + \frac{1}{a_2 t_0}\right) + \frac{a_1 a_2 b_1 t_0}{(a_2 t_0 + a_1 t)^2} > 0\,.$$

So φ is a monotonically increasing bijection from $[0, \infty[$ onto itself. It remains to show that $\varphi(t)$ has the form of equation (14). And we do have

$$\begin{aligned} t_0 + \frac{1}{a_2 t_0} &= \sqrt{\frac{a_4}{a_2(a_3 - a_4)}} + \frac{1}{a_2}\sqrt{\frac{a_2(a_3 - a_4)}{a_4}} \\ &= \frac{a_4}{\sqrt{a_2 a_4 (a_3 - a_4)}} + \frac{a_3 - a_4}{\sqrt{a_2 a_4 (a_3 - a_4)}} \\ &= \frac{a_3}{\sqrt{a_2 a_4 (a_3 - a_4)}} \end{aligned}$$

and

$$\frac{1}{a_2 t_0 + a_1 t} = \frac{1}{a_2\sqrt{\dfrac{a_4}{a_2(a_3 - a_4)}} + a_1 t} = \frac{\sqrt{a_3 - a_4}}{\sqrt{a_2 a_4} + a_1\sqrt{a_3 - a_4}\, t}\,.$$

□

Equation (11) gives the following equation involving the partial derivative we want to study.

$$D\,\frac{\partial y_1}{\partial b_4} = a_1 w_{14}\,. \tag{15}$$

From the Appendix (section 7), we have

$$w_{14} = x_1 x_2\left(a_2(a_3 - a_4)x_3 - a_4(1 + a_2 x_2 + a_3 x_4)\right)\,. \tag{16}$$

If $b_3 > 0$, we have $x_3 > 0$, and

$$w_{14} = x_1 x_2\left(a_2(a_3 - a_4)x_3 - \frac{a_4 b_3}{x_3}\right)\,. \tag{17}$$

If in addition $a_3 > a_4$, with $\beta = \dfrac{a_4}{a_2(a_3 - a_4)}$, we have

$$w_{14} = a_2(a_3 - a_4)\frac{x_1 x_2}{x_3}\left({x_3}^2 - \beta\, b_3\right)\,, \tag{18}$$

and if $b_1, b_2 > 0$, then

$$\frac{1}{a_1 a_2 (a_3 - a_4)} \frac{x_3 D}{x_1 x_2} \frac{\partial y_1}{\partial b_4} = {x_3}^2 - \beta\, b_3\,. \tag{19}$$

Lemma 5. *Suppose* $a_3 > a_4$, *let* $\beta = \dfrac{a_4}{a_2(a_3 - a_4)}$, *and suppose* $b_1 > 0$, $b_2 > 0$, $b_3 \geqslant \beta$. *Let* (ξ_1, ξ_2, ξ_3) *be as in Lemma* 4. *Then the following are equivalent:*

(i) $\forall\, b_4 > 0, \dfrac{\partial y_1}{\partial b_4} < 0$;

(ii) $\xi_3 \leqslant \sqrt{\beta\, b_3}$.

Proof. Suppose condition (i) of the Lemma is satisfied. Then, $\left.\dfrac{\partial y_1}{\partial b_4}\right|_{b_4=0} \leqslant 0$. By evaluating both sides of equation (19) for $b_4 = 0$, we obtain ${\xi_3}^2 - \beta\, b_3 \leqslant 0$, i.e. condition (ii). Now suppose that condition (ii) is satisfied. By equation (10) and Lemma 3, x_3 decreases strictly from ξ_3 to 0 as b_4 ranges from 0 to ∞. So for every $b_4 > 0$, we have $x_3 < \xi_3 \leqslant b_3$, then ${x_3}^2 - \beta\, b_3 < {\xi_3}^2 - \beta\, b_3 \leqslant 0$, and by equation (19), $\dfrac{\partial y_1}{\partial b_4} < 0$. Thus, condition (i) is satisfied. □

Theorem 6. *Consider the systems of equations* (4) *and* (5), *and suppose* $b_1, b_2 > 0$ *(otherwise* $y_1 = 0$*).*

- *If* $a_3 \leqslant a_4$, *then the function* $b_4 \mapsto y_1$ *is strictly monotonically decreasing to zero.*
- *Suppose* $a_3 > a_4$. *Let* $\beta = \dfrac{a_4}{a_2(a_3 - a_4)}$ *and consider the function* $\varphi : \mathbb{R}_{\geqslant 0} \to \mathbb{R}_{\geqslant 0}$ *be given by*

$$\varphi(t) = t^2 + \frac{a_3\, t}{\sqrt{a_2 a_4 (a_3 - a_4)}} + \frac{b_1 a_1 \sqrt{a_3 - a_4}\, t}{\sqrt{a_2 a_4} + a_1 \sqrt{a_3 - a_4}\, t}\,.$$

  - *If* $b_3 \leqslant \beta$, *or if* $b_3 > \beta$ *and* $b_2 \geqslant \varphi\left(\sqrt{b_3} - \sqrt{\beta}\right)$, *then the function* $b_4 \mapsto y_1$ *is strictly monotonically decreasing to zero.*
  - *If* $b_3 > \beta$ *and* $b_2 < \varphi\left(\sqrt{b_3} - \sqrt{\beta}\right)$, *then the function* $b_4 \mapsto y_1$ *first is strictly monotonically increasing, reaches an absolute maximum, then is strictly monotonically decreasing to zero.*

Equivalently:

- *If* $b_3 \leqslant \left(\sqrt{\beta} + \varphi^{-1}(b_2)\right)^2$, *then the function* $b_4 \mapsto y_1$ *is strictly monotonically decreasing to zero.*
- *If* $b_3 > \left(\sqrt{\beta} + \varphi^{-1}(b_2)\right)^2$, *then the function* $b_4 \mapsto y_1$ *first is strictly monotonically increasing, reaches an absolute maximum, then is strictly monotonically decreasing to zero.*

The above statements hold with the function $x_4^\circ \mapsto y_1$ *in lieu of the function* $b_4 \mapsto y_1$.

Proof. We know from Lemma 3 that $\lim_{b_4 \to \infty} y_1 = 0$.

If $b_3 = 0$, then $x_3 = 0$, and from equation (16), we have $w_{14} = -a_4 x_1 x_2 (1 + a_2 x_2 + a_3 x_4) \leqslant -a_4 x_1 x_2 < 0$. Then by equation (15), $\dfrac{\partial y_1}{\partial b_4} < 0$.

➢ Suppose $a_3 \leqslant a_4$. We already have $\dfrac{\partial y_1}{\partial b_4} < 0$ for $b_3 = 0$. And for $b_3 > 0$, from equation (17) we have $w_{14} \leqslant -\dfrac{a_4 b_3 x_1 x_2}{x_3} < 0$, whence $\dfrac{\partial y_1}{\partial b_4} < 0$ by equation (15). Thus $\dfrac{\partial y_1}{\partial b_4} < 0$ for any $b_3 \geqslant 0$; $b_4 \mapsto y_1$ is strictly decreasing.

➢ Suppose $a_3 > a_4$ and $b_3 \leqslant \beta$. We already have $\dfrac{\partial y_1}{\partial b_4} < 0$ for $b_3 = 0$. If $b_3 > 0$, then $0 < x_3 < b_3$, ${x_3}^2 < \beta\, b_3$ and, by equation (19), $\dfrac{\partial y_1}{\partial b_4} < 0$. Thus $\dfrac{\partial y_1}{\partial b_4} < 0$ for any $b_3 \geqslant 0$; $b_4 \mapsto y_1$ is strictly decreasing.

➢ Suppose $a_3 > a_4$, $b_3 > \beta$ and $b_2 \geqslant \varphi\left(\sqrt{b_3} - \sqrt{\beta}\right)$. By Lemma 4, $\xi_3 \leqslant \sqrt{\beta\, b_3}$. Then by Lemma 5, $\dfrac{\partial y_1}{\partial b_4} < 0$ for $b_4 > 0$. Thus, $b_4 \mapsto y_1$ is strictly decreasing.

➢ Suppose $a_3 > a_4$, $b_3 > \beta$ and $b_2 < \varphi\left(\sqrt{b_3} - \sqrt{\beta}\right)$. From equation (19), we obtain

$$\frac{1}{a_1 a_2 (a_3 - a_4)} \frac{\partial}{\partial b_4}\left(\frac{x_3 D}{x_1 x_2} \frac{\partial y_1}{\partial b_4}\right) = 2\, x_3 \frac{\partial x_3}{\partial b_4}\,. \tag{20}$$

By equation (10), $\dfrac{\partial x_3}{\partial b_4} < 0$. So by equation (20), the function $b_4 \mapsto \dfrac{x_3 D}{x_1 x_2} \dfrac{\partial y_1}{\partial b_4}$ is strictly decreasing. Hence, this function is either positive, or negative, or has a unique zero and is positive before the zero and negative after the

zero. Since $\dfrac{x_3 D}{x_1 x_2} > 0$, the function $b_4 \mapsto \dfrac{\partial y_1}{\partial b_4}$ has the same property. So the function $b_4 \mapsto y_1$ either is strictly increasing, or is strictly decreasing, or strictly increases to an absolute maximum then strictly decreases. The first alternative is impossible because $\lim\limits_{b_4 \to \infty} y_1 = 0$ by Lemma 3. We have $b_2 < \varphi\left(\sqrt{b_3} - \sqrt{\beta}\right)$, so by Lemma 4, $\xi_3 > \sqrt{\beta\, b_3}$. Then Lemma 5 excludes the second alternative. So $b_4 \mapsto y_1$ strictly increases to an absolute maximum then strictly decreases. □

Theorem 1 is obtained from Theorem 6 with X_1, X_2, X_3, X_4 standing for R, L, T, A respectively, and $a_1 = \dfrac{1}{K_{RL}}$, $a_2 = \dfrac{1}{K_{LT}}$, $a_3 = \dfrac{1}{K_{AT}}$, $a_4 = \dfrac{1}{K_{RA}}$.

Remark 7. Suppose $b_1, b_2 > 0$ and $a_3 > a_4$. The equivalent conditions $b_2 = \varphi\left(\sqrt{b_3} - \sqrt{\beta}\right)$ and $b_3 = \left(\sqrt{\beta} + \varphi^{-1}(b_2)\right)^2$ define the "bump frontier." This is the curve in the plane with coordinates b_2, b_3 that separates the "bump region" and the "no-bump region." The "bump region" is characterized by $b_2 < \varphi\left(\sqrt{b_3} - \sqrt{\beta}\right)$, or equivalently $b_3 > \left(\sqrt{\beta} + \varphi^{-1}(b_2)\right)^2$. The "no-bump region" is characterized by $b_3 \leqslant \left(\sqrt{\beta} + \varphi^{-1}(b_2)\right)^2$. It is the union of two subregions: one is defined by the condition $b_3 \leqslant \beta$ and the other one by the conditions $b_3 > \beta$ and $b_2 \geqslant \varphi\left(\sqrt{b_3} - \sqrt{\beta}\right)$. The function $b_4 \mapsto y_1$ has or does not have a bump depending on whether the point represented by b_2, b_3 is in the bump region or in the no-bump region. See Figure 2 for an illustration.

There is more to understand about this reaction system (Figure 5) with regard to monotonicity. For instance, what is the monotonicity of the equilibrium concentration y_1 of Y_1 when both the total concentrations b_3 and b_4 of X_3 and X_4 vary in a constrained fashion, say $b_3 = b_4$? (This could model an experiment designed to investigate a cocktail of compounds.) Can we have several bumps? While the approach employed here very opportunistically exploits the particular structure of the system, we intend to study the monotonicity problem with other methods for more insight into this and more complex reaction systems.

5 Conclusion

The four component system shown in Figure 1 is sufficiently complex that mathematical analysis was necessary to understand under what conditions of relative concentrations and equilibrium constants the trap and the antagonist might negatively affect each others potency at blocking IL-1 signaling.

This is of practical interest, because a very high affinity IL-1 trap has been administered to rheumatoid arthritis patients, resulting in only modest improvement in signs and symptoms of the disease [7]. At concentrations of trap achieved in the blood of the patients, trap is expected, based on in vitro human whole blood experiments, to provide significantly greater blockade of IL-1 than the marketed IL-1 receptor antagonist Kineret® at blood levels reached at clinical doses; see [14] and [26]. One could conclude from these observations that stronger blockade of IL-1 signaling by trap is not more effective than the blockade provided by Kineret® because IL-1 is not a major driver of signs and symptoms of the disease in most patients. However, when one takes into account that in rheumatoid arthritis patients, high levels of the naturally occurring IL-1Ra are achieved [21], and that trap binds IL-1Ra in addition to IL-1, one must ask whether the high levels of IL-1Ra might occlude trap sufficiently to reduce its ability to bind IL-1 in patients. Obviously, such an effect is possible, and the work presented here defines the relationships among concentrations and equilibrium constants which must exist in order for IL-1Ra to effectively reduce the ability of trap to bind IL-1. These conditions fall within a physiologically realistic range, although the concentrations of IL-1 and IL-1Ra within the inflamed rheumatoid arthritis joints are not known. An IL-1 receptor antagonist currently being studied by Amgen, Inc. does not compete with IL-1Ra, and thus its effectiveness should not be influenced by IL-1Ra.
From a mathematical standpoint, we have shown that efforts to understand monotonicity in chemical reaction networks with competing bindings need not be limited to chemical or numerical experiments, but can be approached comprehensively through mathematical analysis.

6 Acknowledgments

We thank the anonymous reviewers for comments that helped improved the article.

7 Appendix: Algebraic Expressions

This section details the algebraic expressions of certain entities used in section 3. We used Computer Algebra System Maple™ to obtain and verify these expressions.

The determinant D is given by

$$D = 1 + (a_1a_3 + a_2a_4)(x_1x_3 + x_2x_4) + D_1 + D_2 + D_3 + D_4$$

where

$$\begin{aligned} D_i &= (a_i + a_{i+3})x_i + a_ia_{i+3}{x_i}^2 + (a_ia_{i+1} + a_ia_{i+3} + a_{i+1}a_{i+3})x_ix_{i+1} \\ &\quad + a_ia_{i+1}(a_{i+2} + a_{i+3})x_ix_{i+1}x_{i+2} + a_ia_{i+3}(a_{i+1}x_{i+1} + a_{i+2}x_{i+3}){x_i}^2 . \end{aligned}$$

The entries of matrix U are as follows.

$$\begin{aligned} u_{i,i} &= 1 + (a_i + a_{i+3})x_i + a_{i+1}x_{i+1} + (a_{i+1} + a_{i+2})x_{i+2} + a_{i+2}x_{i+3} \\ &\quad + a_ia_{i+3}{x_i}^2 + a_{i+1}a_{i+2}{x_{i+2}}^2 + (a_i + a_{i+3})(a_{i+1}x_{i+1} + a_{i+2}x_{i+3})x_i \\ &\quad + (a_ia_{i+2} + a_{i+1}a_{i+3})x_ix_{i+2} + a_{i+1}a_{i+2}(x_{i+1} + x_{i+3})x_{i+2} \\ &\quad + a_{i+1}a_{i+2}(a_ix_{i+1} + a_{i+3}x_{i+3})x_ix_{i+2} + a_ia_{i+3}(a_{i+1}x_{i+1} + a_{i+2}x_{i+3}){x_i}^2 \\ u_{i,i+2} &= a_ia_{i+1}x_ix_{i+1} + a_{i+2}a_{i+3}x_ix_{i+3} + a_{i+1}a_{i+2}(a_ix_{i+1} + a_{i+3}x_{i+3})x_ix_{i+2} \\ &\quad + a_ia_{i+3}(a_{i+1}x_{i+1} + a_{i+2}x_{i+3}){x_i}^2 \\ -u_{i,i+1} &= a_ix_i(1 + a_{i+3}x_i + a_{i+1}x_{i+1} + a_{i+2}x_{i+2} + a_{i+2}x_{i+3}) \\ &\quad + a_{i+1}a_{i+2}(a_ix_{i+1} + a_{i+3}x_{i+3})x_ix_{i+2} + a_ia_{i+3}(a_{i+1}x_{i+1} + a_{i+2}x_{i+3}){x_i}^2 \\ -u_{i+1,i} &= a_ix_{i+1}(1 + a_{i+3}x_i + a_{i+1}x_{i+1} + a_{i+2}x_{i+2} + a_{i+2}x_{i+3}) \\ &\quad + a_{i+2}a_{i+3}(a_ix_i + a_{i+1}x_{i+2})x_{i+1}x_{i+3} + a_ia_{i+1}(a_{i+3}x_i + a_{i+2}x_{i+2}){x_{i+1}}^2 \end{aligned}$$

The entries of matrix W are as follows.

$$\begin{aligned} w_{i,i} &= x_{i+1} + a_{i+1}{x_{i+1}}^2 + (a_{i+1} + a_{i+2})x_{i+1}x_{i+2} + a_{i+3}x_ix_{i+1} + a_{i+2}x_{i+1}x_{i+3} \\ &\quad + a_{i+2}a_{i+3}x_ix_{i+1}x_{i+3} + a_{i+1}a_{i+3}(x_{i+1} + x_{i+2})x_ix_{i+1} \\ &\quad + a_{i+1}a_{i+2}(x_{i+1} + x_{i+2} + x_{i+3})x_{i+1}x_{i+2} \\ w_{i,i+1} &= x_i + a_{i+3}{x_i}^2 + (a_{i+2} + a_{i+3})x_ix_{i+3} + a_{i+1}x_ix_{i+1} + a_{i+2}x_ix_{i+2} \\ &\quad + a_{i+1}a_{i+2}x_ix_{i+1}x_{i+2} + a_{i+1}a_{i+3}(x_i + x_{i+3})x_ix_{i+1} \\ &\quad + a_{i+2}a_{i+3}(x_i + x_{i+2} + x_{i+3})x_ix_{i+3} \\ w_{i,i+2} &= x_ix_{i+1}(a_{i+3}(a_{i+2} - a_{i+1})x_{i+3} - a_{i+1}(1 + a_{i+3}x_i + a_{i+2}x_{i+2})) \\ w_{i,i+3} &= x_ix_{i+1}(a_{i+1}(a_{i+2} - a_{i+3})x_{i+2} - a_{i+3}(1 + a_{i+1}x_{i+1} + a_{i+2}x_{i+3})) \end{aligned}$$

With

$$b_i = x_i(1 + a_i x_{i+1} + a_{i+3} x_{i+3})$$

we have

$$\begin{aligned} w_{i,i+2} &= x_i x_{i+1} \left(a_{i+3}(a_{i+2} - a_{i+1}) x_{i+3} - \frac{a_{i+1} b_{i+3}}{x_{i+3}} \right) \\ w_{i,i+3} &= x_i x_{i+1} \left(a_{i+1}(a_{i+2} - a_{i+3}) x_{i+2} - \frac{a_{i+3} b_{i+2}}{x_{i+2}} \right) \end{aligned}$$

References

[1] B. Bresnihan, J. M. Alvaro-Gracia, M. Cobby, M. Doherty, Z. Domljan, P. Emery, G. Nuki, K. Pavelka, R. Rau, B. Rozman, I. Watt, B. Williams, R. Aitchison, D. McCabe, and P. Musikic, *Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist*, Arthritis Rheum. **41** (1998), no. 12, 2196–2204.

[2] D. Burger, R Chicheportiche, J. G. Giri, and J.-M. Dayer, *The Inhibitory Activity of Human Interleukin-1 Receptor Antagonist is Enhanced by Type II Interleukin-1 Soluble Receptor and Hindered by Type I Interleukin-1 Soluble Receptor*, J. Clin. Invest. **96** (1995), 38–41.

[3] R. P. Bywater, A. Sorensen, P. Rogen, and P. G. Hjorth, *Construction of the simplest model to explain complex receptor activation kinetics*, J. Theor. Biol. **218** (2002), 139–147.

[4] M. Chaves, E. D. Sontag, and R. J. Dinerstein, *Steady-states of receptor-ligand dynamics: a theoretical framework*, J. Theor. Biol. **227** (2004), 413–428.

[5] A. Cornish-Bowden, *Fundamentals of Enzyme Kinetics*, 3rd ed., Portland Press, 2004.

[6] C. A. Dinarello, *Biologic Basis for Interleukin-1 in Disease*, Blood **87** (1996), no. 6, 2095–2147.

[7] A. N. Economides, L. R. Carpenter, J. S. Rudge, V. Wong, E. M. Koehler-Stec, C. Hartnett, E. A. Pyles, X. Xu, T. Daly, M. R. Young, J. P. Fandl, F. Lee, S. Carver, J. McNay, K. Bailey, S. Ramakanth,

R. Hutabarat, T. T. Huang, C. Radziejewski, G. D. Yancopoulos, and N. Stahl, *Cytokine traps: multi-component high-affinity blockers of cytokine action*, Nat. Med. **9** (2003), no. 1, 47–52.

[8] M. Feinberg, *Lectures on Chemical Reaction Networks*, `http://www.che.eng.ohio-state.edu/~Feinberg/LecturesOnReactionNetworks/`, 1980.

[9] ———, *Chemical Reaction Network Structure and the Stability of Complex Isothermal Reactors - I. The Deficiency Zero and Deficiency One Theorems*, Chemical Engineering Science **42** (1987), no. 10, 2229–2268.

[10] ———, *Chemical Reaction Network Structure and the Stability of Complex Isothermal Reactors - II. Multiple Steady States for Networks of Deficiency One*, Chemical Engineering Science **43** (1988), no. 1, 1–25.

[11] ———, *The Existence and Uniqueness of Steady States for a Class of Chemical Reaction Networks*, Arch. Rational Mech. Anal. **132** (1995), 311–370.

[12] ———, *Multiple Steady States for Chemical Reaction Networks of Deficiency One*, Arch. Rational Mech. Anal. **132** (1995), 371–406.

[13] K. Gatermann and B. Huber, *A Family of Sparse Polynomial Systems Arising in Chemical Reaction Systems*, J. Symbolic Computation **33** (2002), 275–305.

[14] H. P. Guler, J. R. Caldwell, R. M. Fleischmann, S. B. Cohen, A. J. Kivitz, R. M. Levy, T. Littlejohn, H. McIlwain, H. Offenberg, J. Papadopoulos, and N. Stahl, *Weekly Treatment with IL-1 Trap is Well Tolerated and Improves ACR Criteria in Patients with Active Rheumatoid Arthritis*, Ann. Rheum. Dis. **61 suppl 1** (2002).

[15] J. Gunawardena, *Chemical Reaction Network Theory for in-silico Biologists*, `http://www.jeremy-gunawardena.com/papers/crnt.pdf`, 2003.

[16] C. H. Hannum, C. J. Wilcox, W. P. Arend, F. G. Joslin, D. J. Dripps, P. L. Heimdal, L. G. Armes, A. Sommer, S. P. Eisenberg, and R. C. Thompson, *Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor*, Nature **343** (1990), 336–340.

[17] F. Horn and R. Jackson, *General Mass Action Kinetics*, Arch. Rational Mech. Anal. **47** (1972), 81–116.

[18] D. A. Lauffenburger and J. J. Linderman, *Receptors: Models for Binding, Trafficking, and Signaling*, Oxford University Press, 1996.

[19] L. E. Limbird, *Cell Surface Receptors: A Short Course on Theory and Methods*, 3rd ed., Springer, 2004.

[20] J. C. Matthews, *Fundamentals of Receptor, Enzyme, and Transport Kinetics*, CRC, 1993.

[21] W. L. Olszewski, J. Pazdur, E. Kubasiewicz, M. Zaleska, C. J. Cooke, and N. E. Miller, *Lymph Draining From Foot Joints in Rheumatoid Arthritis Provides Insight into Local Cytokine and Chemokine Production and Transport to Lymph Nodes*, Arthritis Rheum. **44** (2001), no. 3, 541549.

[22] D. Siegel, *Monotonicity Properties of Solutions to Systems of Differential Equations Arising in Chemical Kinetics*, Differential Equations (C. M. Dafermos, G. Ladas, and G. Papanicolaou, eds.), Marcel Dekker, 1989, pp. 637–646.

[23] D. Siegel and Y. F. Chen, *Global Stability of Deficiency Zero Chemical Networks*, Canadian Applied Math. Quarterly **2** (1994), no. 3, 413–434.

[24] D. Siegel and D. W. Lozinski, *Monotonicity Properties of the Michaelis-Menten Reactions of Enzyme Kinetics*, Rocky Mountain J. Math. **20** (1990), no. 4, 1157–1172, G. J. Butler Memorial Conference, Edmonton, Alberta, 1988.

[25] D. Siegel and D. MacLean, *Global Stability of Complex Balanced Mechanisms*, J. Mathematical Chemistry **27** (2000), 89–110.

[26] B.-B. Yang, S. Baughman, and J. T. Sullivan, *Pharmacokinetics of Anakinra in Subjects with Different Levels of Renal Function*, Clin. Pharmacol. Ther. **74** (2003), no. 1, 85–94.