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# *Analysis of a Mathematical Model of Real-Time Competitive Binding on a Microarray*

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**Keywords:** Law of mass action, conservation laws, kinetic equations, competitive binding, planar systems, linearization

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**Abstract:** A mathematical model of competitive binding on a microarray in real-time yields a planar system of nonlinear ordinary differential equations. This model can be used to explore dimensionless formulation, linear approximation, and reduction. Real-time competitive binding is proposed as an uncommon approach to advance the study of planar systems of differential equations.

## 1 Introduction

We suggest an uncommon approach to investigate the planar autonomous system of differential equations

$$\begin{aligned}\dot{x} &= f(x, y), \\ \dot{y} &= g(x, y).\end{aligned}\tag{1.1}$$

In practice, the model system used to introduce systems of the form (1.1) often relies on a physical example. For instance, many approaches rely on the physical intuition gained from the study of a linear, second order, scalar differential equation (e.g. mass-spring-dashpot or RLC circuit) and use the equivalent first order system of equations as an introductory example. Other classical approaches use examples like Romeo and Juliet (see Strogatz [12, p 139]) or predator-prey interactions (see Borrelli and Coleman [3, pp 466-471]) to explore stability of equilibria, nullclines, trajectories, limit cycles, etc., in the phase plane of the system (1.1). These examples all have the attractive quality that relative simplicity of the modeling equations allow for analytic or geometric arguments (often both!) matching intuition gained from considering the actual behavior of the physical system.

In particular, many biological systems also can be modeled through a system of equations of the form (1.1). For instance, such modeling equations are used better understand cellular action potential, relating an excitation variable ( $x$ ) to a recovery variable ( $y$ ) in many contexts, including works by FitzHugh [6] and Mitchell and Schaeffer [10]. Other

examples relate susceptible individuals ( $x$ ) to infected individuals ( $y$ ) in a disease epidemic (the well-known SI model) [8], or the concentration of bromous acid ( $x$ ) to concentration of oxidized catalyst metal ( $y$ ) in the classic Belousov-Zhabotinsky reaction [13]. Similarly, a planar system of the form (1.1) is used to establish existence of traveling waves in the Fisher equation [5], describing advance of an advantageous gene.

We propose examination of two-species competitive binding as an additional canonical model to study of planar systems of differential equations. In doing so, we emphasize kinetic reaction schemes, the law of mass action, conservation laws, linearization, and dimensionless formulation; all of which are central to applied mathematics but often uncommon in the classical approaches described previously.

In section 2 we give background appropriate to the model system, including an example from the literature as motivation. In section 3 we discuss the modeling equations, the reduction to a planar system, and approximate solutions via linearization. These modeling techniques generate a discussion of the ways in which the linearized system matches the intuition gained by examining the physical system. We close in section 4 with sample simulations and exercises which might be appropriate for incorporation into a course covering a planar autonomous system of differential equations.

## 2 Background

Kinetic reaction schemes are ubiquitous in biological systems, many similar to the classical example of Michaelis and Menten [7]. Modeling of such schemes relies on the law of mass action [11], which states that the rate of a reaction is proportional to the concentrations of the species involved in the reaction. For instance, application of the law of mass action to the simple reaction



yields differential equations describing the rate of change of the concentrations involved in the reaction,

$$\begin{aligned} \frac{d[A]}{dt} &= -k[A][B], \\ \frac{d[B]}{dt} &= -k[A][B], \\ \frac{d[C]}{dt} &= k[A][B], \end{aligned} \tag{2.2}$$

where  $k$  is a constant describing the rate of the reaction. It is quick to note that the rate at which A and B decrease is equal to the rate at which C increases, which is typical of such reaction schemes. Moreover, the equations illustrate two conservation laws, which are also typical of such reaction schemes. In this case, we see that

$$\frac{d}{dt}([A] + [C]) = 0 \tag{2.3}$$

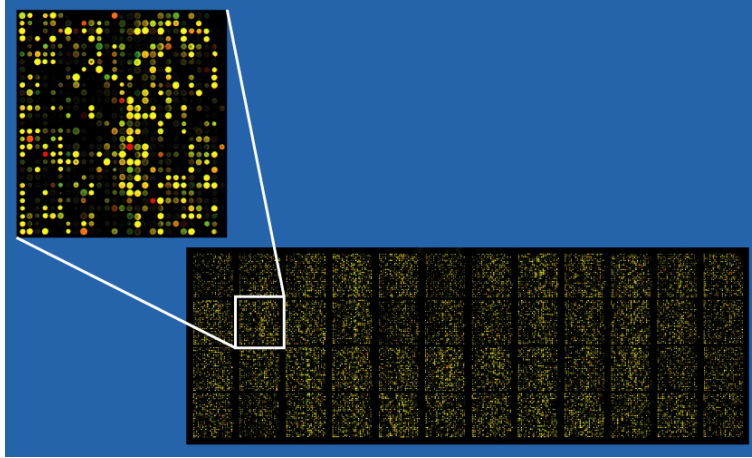


Figure 1: The microarray contains approximately 40,000 probes. Used according to a Creative Commons license [4].

so that  $[A] + [C]$  is a constant quantity. In many experimental schemes, the initial amount of complex,  $[C]$  is zero, so that the conserved quantity depends only on the initial amount of  $[A]$ , given by  $a_0$ , as  $[A] + [C] = a_0$ . Similarly, we see that the sum  $[B] + [C] = b_0$ . Below, both the law of mass action and corresponding conservation laws will be used to derive a mathematical model of the model system.

## 2.1 Model System

As a model system, we consider the example of competitive binding on a microarray in real-time. Since coming toward the forefront in the mid 1990s, microarrays have been seminal in advancement of research to better understand expression of many genes in a single experiment. In particular, one cannot overemphasize the impact of the microarray on the advancement of cancer research, for instance, in the work by Khan et al. [9].

A microarray is a chip, containing many spots, used to measure the expression of a specific gene (or genes). **Figure 1** shows an example of a microarray containing approximately 40,000 probes. A typical use of a microarray is the detection of single nucleotide polymorphism (SNP). In this case, we examine a mathematical model of a two-species experiment, described by Blair et al. [2]. In the experimental design, a spot contains probes, which are specifically designed to correspond to a target DNA sequence. In real time, a hybridization process containing a target and competitor DNA (where the competitor DNA mismatch differs by a SNP) bind to the probes. Measurement of bound target is characterized through collection of fluorescent signal. A typical signal gives fluorescent intensity as a function of time. As shown in **Figure 2**, fluorescent signal serves as a proxy for concentration of target DNA bound to a probe. Although Zhang et al. [14, equations (9)–(10)] consider a single soluble species on two spots, we use a similar approach for two soluble species on a single spot. Following Blair et al. [2, system (12.6)], we assume that two different species (a target and mismatch) bind in competition for the same binding

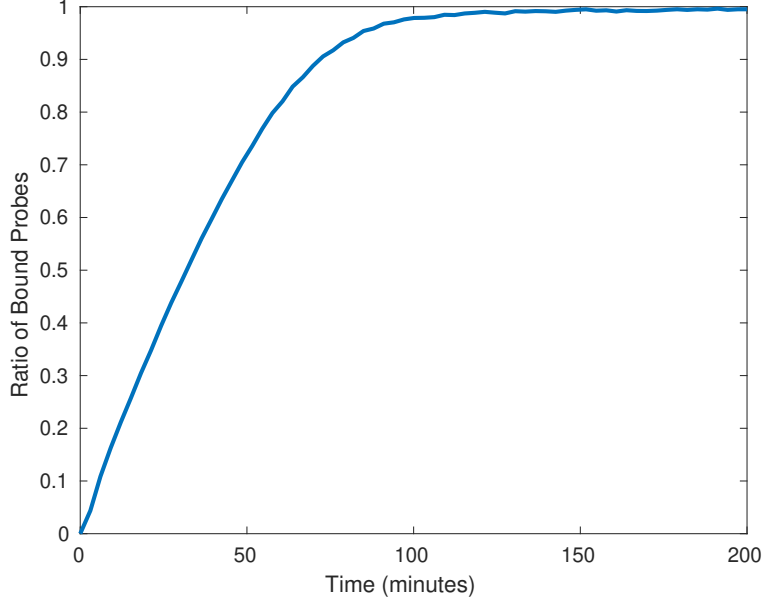


Figure 2: The fluorescent signal from a real time microarray experiment serves as a proxy for the ratio of probes bound by the target DNA. Adapted from Blair et al. [2].

sites in the reactions



where  $C^T$  and  $C^C$  represent the unbound target and competitor,  $B^T$  and  $B^C$  represent bound target and competitor, and  $S$  represents the binding site on the spot. We seek to model an experiment in which a fixed initial amount of target competes with a variety of initial amounts of mismatched competitor.

### 3 Modeling Equations

Applying the law of mass action and corresponding conservation laws to the reactions given by (2.4)–(2.5), we obtain the following differential equations for concentrations of bound target  $u$  and bound competitor  $v$ ,

$$\begin{aligned} \frac{du}{dt} &= k_+^T(C_0^T - u)(p_0 - u - v) - k_-^T u, \\ \frac{dv}{dt} &= k_+^C(C_0^C - v)(p_0 - u - v) - k_-^C v, \end{aligned} \quad (3.1)$$

where  $C_0^T$  and  $C_0^C$  are initial amounts of unbound target and competitor, and  $p_0$  is the number of probe sites. In order to better understand the coupled, nonlinear system of

differential equations, we consider two different nondimensional scalings. The scalings are based on the known characteristic concentrations in the experiment, namely the initial amount of unbound target ( $C_0^T$ ) and the initial amount of available probe sites ( $p_0$ ).

### 3.1 Target Scaling

In the so-called target scaling, we imagine a scenario where the initial amount of available probes is larger than the initial amount of unbound target ( $p_0 > C_0^T$ ) and scale the bound concentrations  $u$  and  $v$  by the initial amount of unbound target as  $x = u/C_0^T$  and  $y = v/C_0^T$ . Scaling time by the characteristic forward reaction rate for the match,  $\tau = k_+^T p_0 t$  and taking  $[ \dot{\phantom{x}} ] = \frac{d}{d\tau}$ , we find that

$$\begin{aligned}\dot{x} &= (1 - x)(1 - \varepsilon_p x - \varepsilon_p y) - K_p x, \\ \dot{y} &= k_a(\gamma - y)(1 - \varepsilon_p x - \varepsilon_p y) - k_d K_p y,\end{aligned}\tag{3.2}$$

where the parameters are  $K_p = \frac{k_-^T}{k_+^T p_0}$ ,  $k_a = k_+^C/k_+^T$ ,  $k_d = k_-^C/k_-^T$ ,  $\gamma = C_0^C/C_0^T$ , and  $\varepsilon_p = C_0^T/p_0$ .

We immediately see the implication of such a scenario, namely that  $p_0 > C_0^T$  implies that the constant  $\varepsilon_p < 1$ . In the case where  $p_0 \gg C_0^T$  (so that  $\varepsilon_p \ll 1$ ), we formally set  $\varepsilon_p = 0$  and find that the coupled, nonlinear system of differential equations completely decouples,

$$\begin{aligned}\dot{x} &= 1 - (1 + K_p)x, \\ \dot{y} &= k_a \gamma - (k_a + k_d K_p)y,\end{aligned}\tag{3.3}$$

to a linear, nonhomogeneous system. It is quick to interpret this mathematical limit as a complete misunderstanding of the model system. In order to guarantee that the target and mismatched DNA compete for binding sites on the probe, there may not be more probe available than unbound DNA. If so, the competitive model system is not actually competitive at all, as illustrated by the decoupled system.

### 3.2 Probe Scaling

In the so-called probe scaling, we imagine the experimentally interesting scenario where the initial amount of unbound target is greater than the initial amount of available probe ( $C_0^T > p_0$ ) and scale the bound concentrations  $u$  and  $v$  by the initial amount of available probe as  $x = u/p_0$  and  $y = v/p_0$ . Scaling time by the characteristic forward reaction rate for the match,  $\tau = k_+^T C_0^T$  and again taking  $[ \dot{\phantom{x}} ] = \frac{d}{d\tau}$ , we find that

$$\begin{aligned}\dot{x} &= (1 - \varepsilon_T x)(1 - x - y) - K_T x, \\ \dot{y} &= k_a(\gamma - \varepsilon_T y)(1 - x - y) - k_d K_T y,\end{aligned}\tag{3.4}$$

where  $K_T = \frac{k_-^T}{k_+^T C_0^T}$ , and the constants are as above, except that  $\varepsilon_T = p_0/C_0^T$ .

In analogy to the previous subsection, we can examine the case  $C_0^T \gg p_0$  (so that  $\varepsilon_T \ll 1$ ) by formally setting  $\varepsilon_T = 0$ . In this case, the system maintains its competitive

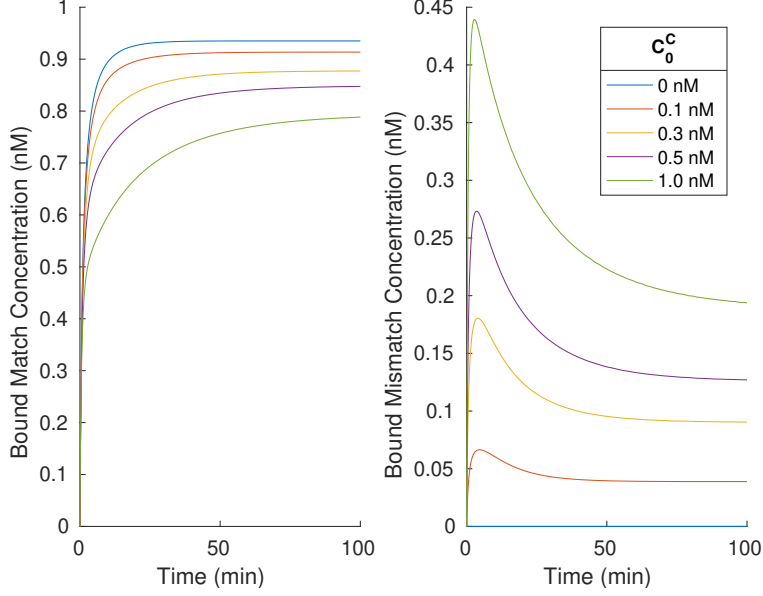


Figure 3: Curves show numerical approximation to the mathematical model of a typical hybridization experiment with varying initial concentration of unbound mismatch,  $C_0^C$ .

nature,

$$\begin{aligned} \dot{x} &= 1 - (1 + K_T)x - y, \\ \dot{y} &= k_a\gamma - k_a\gamma x - (k_a\gamma + k_dK_T)y, \end{aligned} \quad (3.5)$$

as the coupled nonlinear system of differential equations reduces to a linear, nonhomogeneous system, maintaining competition through the coupling.

## 4 Model Exploration

Parameter values for the forward and backward reactions are described by Zhang et al. [14] as  $k_+^T = \mathcal{O}(10^{-5}) \text{ M}^{-1}\text{s}^{-1}$  and  $k_-^T = \mathcal{O}(10^5) \text{ s}^{-1}$ . In an experiment where the mismatched competitor differs by a SNP, it is common to assume that the forward reaction rates are approximately equal, so that  $k_+^C \approx k_+^T$  and that the backward reaction rates differ approximately by at least an order of magnitude, so that  $k_-^C = \mathcal{O}(10k_-^T)$  (or larger).

To guarantee competition, we may not have  $C_0^T < p_0$ , however, it is typically not the case that  $C_0^T \gg p_0$ . In the first of two experiments described by Blair et al. [2, Figure 12.4], the microarray surface was spotted with probes matching the target (wild type), and the competitor (SNP) concentration was varied. These values correspond to  $C_0^T = 1 \text{ nM}$ ,  $p_0 = 1 \text{ nM}$  and  $C_0^C$  varying between 0 and 1 nM. We find a numerical approximation to the solution of the system (3.4) using the parameter values  $k_+^T = 1 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ ,  $k_-^T = 4.5 \times 10^{-6} \text{ s}^{-1}$ ,  $k_+^C = 1 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ ,  $k_-^C = 7.5 \times 10^{-4} \text{ s}^{-1}$ , as described by Bishop et al. [1]. Solution curves for bound target (match) and bound competitor (mismatch) are shown in Figure 3.

## 4.1 Exercises

The following exercises might be useful to better investigate this topic.

**Problem 4.1.** Apply the law of mass action to the reaction scheme given by (2.4)–(2.5) to find differential equations for the concentrations of bound target and competitor. What are the conservation laws? Use those conservation laws to derive the coupled system of nonlinear differential equation given by (3.1).

**Problem 4.2.** Explore numerical solutions of the system (3.1) with the file `Competitive-Binding.ode` for a variety of parameter values.

**Problem 4.3.** Use the target scaling  $x = u/C_0^T$  and  $y = v/C_0^T$ , and scale time by the characteristic forward reaction rate for the match,  $\tau = k_+^T p_0 t$  to show that the system (3.1) can be converted into the system (3.2). Why does this scaling make sense in the case  $C_0^T < p_0$ ?

**Problem 4.4.** Consider the limiting case  $C_0^T \ll p_0$ . Find the analytical solution of the system (3.3). Compare values of the analytical solution to values obtained from `CompetitiveBinding.ode` for a variety of value of  $p_0$ . For what values do the graphs appear similar? At what value of  $p_0$  do they begin to differ?

**Problem 4.5.** Use the probe scaling  $x = u/p_0$  and  $y = v/p_0$ , and scale time by the characteristic forward reaction rate for the match,  $\tau = k_+^T C_0^T t$  to show that the system (3.1) can be converted into the system (3.4). Why does this scaling make sense in the case  $C_0^T > p_0$ ?

**Problem 4.6.** Consider the limiting case  $p_0 \ll C_0^T$ . Find the analytical solution of the system (3.5). Compare values of the analytical solution to values obtained from `CompetitiveBinding.ode` for a variety of value of  $C_0^T$ . For what values do the graphs appear similar? At what value of  $C_0^T$  do they begin to differ?

**Problem 4.7.** Consider the behavior (so-called competitive displacement [2]) observed in Figure 3, where the bound competitor concentration peaks then declines toward its equilibrium value. Explain how the parameter values lead to these two phases of behavior.

**Problem 4.8.** In the second experiment described by Blair et al. [2, Figure 12.4], the microarray surface was spotted with probes matching the SNP DNA instead. The hybridization experiment then proceeded as with the first case, namely that a fixed initial amount of wild type DNA was placed with varying initial amounts of SNP DNA. How would the mathematical model change in this case? Derive a system in analogy to system (3.1) and use a probe scaling to find a dimensionless version in analogy to system (3.4). How would the parameter values change? Experiment numerically using another version of `Competitive-Binding.ode`.



## 5 Conclusion

We have presented a mathematical model of a biophysical experiment that can be used to illustrate nondimensional scalings, and linear approximation of nonlinear dynamics. To the extent that many investigations of planar systems use physical intuition and phase plane behavior to contextualize solution trajectories of model systems, our model is an uncommon approach to the study of such planar systems. Exercises are intended to further study of planar systems by exploring scaling of parameters, numerical approximation to solutions of nonlinear differential equations and limitations of linearization as an approximate solution.

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