# No oscillations in the Michaelis-Menten approximation of the dual futile cycle under a sequential and distributive mechanism

Luna Bozeman[1] and Adriana Morales[2]

[1]Department of Mathematical Sciences, Clemson University, Clemson, SC 29634
[2]Department of Mathematics, University of Puerto Rico, Río Piedras Campus, San Juan, PR 00931

July 21, 2016

**Abstract**

Protein phosphorylation and dephosphorylation are important intracellular processes. The main object of study in this paper is the dual futile cycle, a phosphorylation system that describes the dual-site phosphorylation of a protein by a kinase/phosphatase pair in a sequential and distributive mechanism. Here, we analyze the 2D Michaelis-Menten (M-M) approximation of this system. It has been previously shown that this system is bistable and that it converges to a steady state. Here we show that the 2D M-M version of the dual futile cycle has a non-oscillatory behavior. Understanding its behavior could help us understand the mitogen-activated protein kinases (MAPK) system which has recently been shown to have oscillations.

## 1 Introduction

Protein phosphorylation and dephosphorylation are important intracellular processes that play a role in signal transduction, cell-cycle control, and nuclear signal integration [9]. Phosphorylation is the enzyme-mediated addition of a phosphate group to a protein substrate, which often modifies the function of the substrate. A *multiple futile cycle* (or *multisite phosphorylation system*) refers to when a substrate has multiple sites at which phosphate groups can be attached. Multisite phosphorylation systems can either be *processive* or *distributive*. Here, we focus on distributive systems, which occur when each enzyme-substrate binding results in one addition or removal of a phosphate group, with 2 sites. The phosphorylation system is also assumed to be *sequential*, which occurs when the phosphate groups are added at binding sites in a certain order.

More specifically, the main object of study is the dual futile cycle, a phosphorylation system that describes the 2-site phosphorylation of a protein by a kinase/phosphatase pair in a distributive and sequential mechanism. For this system, Sontag and Wang showed that within restricted parameter ranges, the system exhibits generic convergence to steady states but no more complicated behavior [11]. Angeli *et al.* show that no species tend to be eliminated for any possible parameter values [1].

Central features of phosphorylation systems that are often analyzed are bistability and oscillations. Most recently, Hell and Rendall showed that the dual futile cycle exhibits bistability for certain values of the parameters, meaning that there exist two distinct stable stationary solutions [3]. But what about the oscillatory behavior of the system? Errami *et al.* have shown that this system has oscillatory behavior by using an algorithm to detect Hopf bifurcation fixed points in chemical reaction networks with symbolic rate constants [2]. Jolley *et al.* considered a variant of the dual-site network, in which the two phosphate groups are added in the same order as they are removed (rather than the reverse order), thus, there are four phosphoforms rather than three. Here, they showed that sustained oscillations could be observed in certain parameter regions [5]. This motivates the question: Do oscillations exist in the 2D M-M approximation of the dual futile cycle under a sequential and distributive mechanism?

In this paper we reduce the dual futile cycle into two dimensions, using Michaelis-Menten (M-M) theory [7]. For this reduction, Sontag and Wang present a proof of its non-oscillatory behavior using monotone

systems theory [11]. Here, we present a simpler proof of the non-oscillatory behavior of this system by using Bendixson's criterion.

The motivation of studying the behavior of the dual futile cycle is to gain more insight on the mitogen-activated protein kinases (MAPK) cascade system. The dual futile cycle is one of the layers that form the MAPK system. Numerous authors have shown numerically that the MAPK system has oscillatory behavior [2, 6, 8]. An analytical proof of these results does not exist. Understanding the dual futile cycle can help us understand the MAPK cascade system.

The paper is organized as follows. In Section 2 we introduce the dual futile cycle. Section 3 is a detailed explanation of the reduction of the dual futile cycle into 2 dimensions. The core of this paper is the proof of the non-oscillatory behavior of the 2D version of the futile cycle presented in Section 4. Finally, in Section 5 we discuss some future directions for the MAPK cascade system.

## 2 The dual futile cycle

The following chemical reaction network is called the *dual futile cycle* which describes 2-site phosphorylation that follows a distributive and sequential mechanism:

$$\begin{aligned} S_0 + E \underset{k_2}{\overset{k_1}{\rightleftarrows}} S_0E \xrightarrow{k_3} S_1 + E \underset{k_5}{\overset{k_4}{\rightleftarrows}} S_1E \xrightarrow{k_6} S_2 + E \\ S_2 + F \underset{\ell_2}{\overset{\ell_1}{\rightleftarrows}} S_2F \xrightarrow{\ell_3} S_1 + F \underset{\ell_5}{\overset{\ell_4}{\rightleftarrows}} S_1F \xrightarrow{\ell_6} S_0 + F \end{aligned} \tag{1}$$

A substrate $S_0$ is converted into a product $S_2$ in an *activation reaction* facilitated by an enzyme $E$. Conversely, $S_2$ is transformed back or *deactivated* into the original substrate $S_0$, by the action of a second enzyme $F$. The structure of the dual futile cycle can be seen in Figure 1.

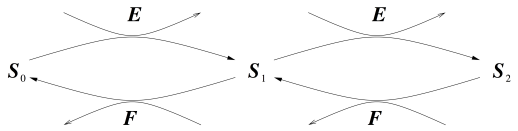


Figure 1: The dual futile cycle

The species in this chemical reaction network include a kinase $E$, a phosphate $F$, and substrates (phosphoforms) $S_0$, $S_1$, and $S_2$. The intermediate complexes, which are $S_0E$, $S_1E$, $S_2F$, and $S_1F$, are the *bound enzyme-substrate complexes*.

For simplicity we will change our notation. The intermediate complexes, $S_0E$, $S_1E$, $S_2F$, and $S_1F$ will now be $C_1$, $C_2$, $C_3$, and $C_4$, respectively. In this system the reaction constants $k_i$ and $\ell_i$, $i \in \{1, 2, \ldots, 6\}$ are positive numbers and the brackets indicate concentrations. According to mass-action kinetics the chemical reaction system defined by the 2-site phosphorylation network (1) can be modeled by the following ODEs:

$$\frac{d[S_0]}{dt} = \ell_6[C_4] - k_1[S_0][E] + k_2[C_1], \tag{2}$$

$$\frac{d[S_2]}{dt} = k_6[S_1][E] - \ell_1[S_2][F] + \ell_2[C_3], \tag{3}$$

$$\frac{d[C_1]}{dt} = k_1[S_0][E] - (k_2 + k_3)[C_1], \tag{4}$$

$$\frac{d[C_2]}{dt} = k_4[S_1][E] - (k_5 + k_6)[C_2], \tag{5}$$

$$\frac{d[C_4]}{dt} = \ell_4[S_1][F] - (\ell_5 + \ell_6)[C_4], \tag{6}$$

$$\frac{d[C_3]}{dt} = \ell_1[S_2][F] - (\ell_2 + \ell_3)[C_3], \tag{7}$$
$$\frac{d[S_1]}{dt} = k_3[C_1] - k_4[S_1][E] + k_5[C_2] + \ell_3[C_3] + \ell_5[C_4] - \ell_4[S_1][F], \tag{8}$$
$$\frac{d[E]}{dt} = (k_2 + k_3)[C_1] + (k_5 + k_6)[C_2] - K_1[S_0][E] - k_4[S_1][E], \tag{9}$$
$$\frac{d[F]}{dt} = (\ell_2 + \ell_3)[C_3] + (\ell_5 + \ell_6)[C_4] - \ell_1[S_2][F] - \ell_4[S_1][F], \tag{10}$$

together with the following *conservation laws*:

$$\begin{aligned} S_T &= [S_0] + [S_2] + [S_1] + [C_1] + [C_2] + [C_4] + [C_3], \\ E_T &= [E] + [C_1] + [C_2], \\ F_T &= [F] + [C_4] + [C_3]. \end{aligned} \tag{11}$$

The quantities in (11) are the total concentrations of the enzymes and the substrates that are conserved under evolution. Note that the concentrations of $[E]$ and $[F]$ can be expressed in terms of $E_T$ and $F_T$ and the concentrations of their respective complexes. For example, the concentration of $[E]$ can be written as follows:

$$\frac{d[E]}{dt} = -\frac{d[C_1]}{dt} - \frac{d[C_2]}{dt}.$$

In a similar way, equation (8) can be expressed in terms of $S_T$ and equations (2) to (6). Thus it is possible to discard the equations (8) to (10), and reduce the number of equations in the system from nine to six and the resulting system is equivalent to the original nine-equation one. This six-equation system matches with the one found in [3, 10, 11].

# 3 The 2D M-M reduction of the dual futile cycle

Using Michaelis-Menten (M-M) theory [7] we now reduce the network (1) to 2 dimensions. It should be noted that apart from the slightly different notation this reduction is identical to the one in [3]. A similar reduction can be found in [11].

After rescaling the concentrations and time, the new system of equations becomes

$$\begin{aligned} \frac{d[S_0]}{d\tau} &= \ell_6[\widetilde{C}_4] - k_1[S_0][\widetilde{E}] + k_2[\widetilde{C}_1], \\ \frac{d[S_2]}{d\tau} &= k_6[S_1][\widetilde{E}] - \ell_1[S_2][F] + \ell_2[\widetilde{C}_3], \\ \varepsilon\frac{d[\widetilde{C}_1]}{d\tau} &= k_1[S_0][\widetilde{E}] - (k_2 + k_3)[\widetilde{C}_1], \\ \varepsilon\frac{d[\widetilde{C}_2]}{d\tau} &= k_4[S_1][\widetilde{E}] - (k_5 + k_6)[\widetilde{C}_2], \\ \varepsilon\frac{d[\widetilde{C}_4]}{d\tau} &= \ell_4[S_1][\widetilde{F}] - (\ell_5 + \ell_6)[\widetilde{C}_4], \\ \varepsilon\frac{d[\widetilde{C}_3]}{d\tau} &= \ell_1[S_2][\widetilde{F}] - (\ell_2 + \ell_3)[\widetilde{C}_3], \end{aligned} \tag{12}$$

where

$$\begin{array}{llllll} E_T = \varepsilon\widetilde{E}_T, & [C_1] = \varepsilon[\widetilde{C}_1], & [C_4] = \varepsilon[\widetilde{C}_4], & [E] = \varepsilon[\widetilde{E}], & \tau = \varepsilon t, \\ F_T = \varepsilon\widetilde{F}_T, & [C_2] = \varepsilon[\widetilde{C}_2], & [C_3] = \varepsilon[\widetilde{C}_3], & [F] = \varepsilon[\widetilde{F}], & \end{array}$$

for a small $\varepsilon > 0$. Since the enzyme concentrations are small compared to the concentrations of the substrates then $\varepsilon > 0$ is also chosen to be small.

Setting $\varepsilon = 0$ in the system (12) gives

$$\begin{aligned}
[\widetilde{C}_1] &= \frac{k_1}{k_2 + k_3}[S_0][\widetilde{E}], \\
[\widetilde{C}_2] &= \frac{k_4}{k_5 + k_6}[S_1][\widetilde{E}], \\
[\widetilde{C}_4] &= \frac{\ell_4}{\ell_5 + \ell_6}[S_1][\widetilde{F}], \\
[\widetilde{C}_3] &= \frac{\ell_1}{\ell_2 + \ell_3}[S_2][\widetilde{F}].
\end{aligned} \tag{13}$$

Adding these equations in pairs gives

$$\begin{aligned}
\widetilde{E}_T &= \left[1 + \frac{k_1}{k_2 + k_3}[S_0] + \frac{k_4}{k_5 + k_6}[S_1]\right][\widetilde{E}], \\
\widetilde{F}_T &= \left[1 + \frac{\ell_4}{\ell_5 + \ell_6}[S_1] + \frac{\ell_1}{\ell_2 + k_3}[S_2]\right][\widetilde{F}].
\end{aligned} \tag{14}$$

Using (13), the rescaled equations (12) can be written as

$$\begin{aligned}
\frac{d[S_0]}{d\tau} &= -\frac{k_1 k_3}{k_2 + k_3}[S_0][\widetilde{E}] + \frac{\ell_4 \ell_6}{\ell_5 + \ell_6}[S_1][\widetilde{F}], \\
\frac{d[S_2]}{d\tau} &= \frac{k_4 k_6}{k_5 + k_6}[S_1][\widetilde{E}] - \frac{\ell_1 \ell_3}{\ell_2 + \ell_3}[S_2][\widetilde{F}].
\end{aligned} \tag{15}$$

Note that $S_T = [S_0] + [S_1] + [S_2] + \varepsilon([\widetilde{C}_1] + [\widetilde{C}_2] + [\widetilde{C}_4] + [\widetilde{C}_3])$. Let $\widetilde{S}_T = S_T(0)$ depend on $\varepsilon$, then for $\varepsilon = 0$ the relation

$$\widetilde{S}_T = [S_0] + [S_1] + [S_2]$$

holds so that $[S_1] = \widetilde{S}_T - [S_0] - [S_2]$. Thus the equations in (15) are of the form

$$\begin{aligned}
\frac{d[S_0]}{d\tau} &= -\frac{a_1[S_0]}{1 + b_1[S_0] + c_1[S_1]} + \frac{a_2[S_1]}{1 + c_2[S_1] + d_2[S_2]} \\
\frac{d[S_2]}{d\tau} &= \frac{a_3[S_1]}{1 + b_1[S_0] + c_1[S_1]} - \frac{a_4[S_2]}{1 + c_2[S_1] + d_2[S_2]},
\end{aligned} \tag{16}$$

where

$$\begin{aligned}
a_1 &= \frac{k_1 k_3 \widetilde{E}_T}{k_2 + k_3}, \quad & a_2 &= \frac{\ell_4 \ell_6 \widetilde{F}_T}{\ell_5 + \ell_6}, \\
a_3 &= \frac{k_4 k_6 \widetilde{E}_T}{k_5 + k_6}, \quad & a_4 &= \frac{\ell_1 \ell_3 \widetilde{F}_T}{\ell_2 + \ell_3}, \\
b_1 &= \frac{k_1}{k_2 + k_3}, \quad & c_1 &= \frac{k_4}{k_5 + k_6}, \\
c_2 &= \frac{\ell_4}{\ell_5 + \ell_6}, \quad & d_2 &= \frac{\ell_1}{\ell_2 + \ell_3},
\end{aligned}$$

by using (14).

The condition for a stationary solution is obtained by setting the equations in (16) to zero. To simplify we assume that the ratios $\frac{k_i}{k_{i+1}+k_{i+2}}$, $i = 1, 4, 7, 10$, between the constants of the reactions producing and consuming the intermediate complexes during phosphorylation are all equal. That is, from now on

$$b_1 = c_1 = c_2 = d_2,$$

which will be denoted as $b$. Therefore our Michaelis-Menten system (16) can be written as

$$\begin{aligned}\frac{d[S_0]}{d\tau} &= -\frac{a_1[S_0]}{1+b(\widetilde{S}_T-[S_2])}+\frac{a_2[S_1]}{1+b(\widetilde{S}_T-[S_0])},\\ \frac{d[S_2]}{d\tau} &= \frac{a_3[S_1]}{1+b(\widetilde{S}_T-[S_2])}-\frac{a_4[S_2]}{1+b(\widetilde{S}_T-[S_0])}.\end{aligned} \tag{17}$$

Finally, we substitute $[S_1]=\widetilde{S}_T-[S_0]-[S_2]$ in (17), which gives us our final M-M system:

$$\begin{aligned}\frac{d[S_0]}{d\tau} &= -\frac{a_1[S_0]}{1+b(\widetilde{S}_T-[S_2])}+\frac{a_2(\widetilde{S}_T-[S_0]-[S_2])}{1+b(\widetilde{S}_T-[S_0])} &:= f([S_0],[S_2]),\\ \frac{d[S_2]}{d\tau} &= \frac{a_3(\widetilde{S}_T-[S_0]-[S_2])}{1+b(\widetilde{S}_T-[S_2])}-\frac{a_4[S_2]}{1+b(\widetilde{S}_T-[S_0])} &:= g([S_0],[S_2]).\end{aligned} \tag{18}$$

# 4 Proof of non-oscillatory behavior

Central features of many biological and biochemical systems that are analyzed are oscillations and bistability. The bistability of the dual futile cycle has been shown by Hell and Rendall in [3]. In this section we concentrate on the oscillatory behavior of our 2D M-M reduction of the dual futile cycle (18), and we prove that it does not have periodic solutions. To do so, we will use the well-known Bendixson's criterion.

**Theorem 4.1** (Bendixson's Criterion)**.** *Suppose $D$ is a simply connected open subset of $\mathbb{R}^2$. If*

$$\frac{\partial f}{\partial x}+\frac{\partial g}{\partial y}\neq 0$$

*and does not change sign in $D$, then there are no periodic orbits of the autonomous system*

$$\frac{dx}{dt}=f(x,y) \quad \textit{and} \quad \frac{dy}{dy}=g(x,y)$$

*in $D$.*

**Theorem 4.2.** *The M-M 2D version of the dual futile cycle in* (18) *does not have periodic solutions for all choices of parameters.*

*Proof.* We prove this by using Theorem 4.1. Consider the system (18), for some choice of $\widetilde{S}_T, a_1, a_2, a_3, a_4, b, \widetilde{E}_T, \widetilde{F}_T \gneq 0$. The set $D=\{0\lneq[S_0],[S_2]\lneq\widetilde{S}_T,[S_0]+[S_2]\lneq\widetilde{S}_T\}$ is a simply connected open subset of $\mathbb{R}^2$. The partial derivatives of system (18) are given by

$$\begin{aligned}\frac{\partial f}{\partial \tau} &= -\frac{a_1[S_0]}{1+b(\widetilde{S}_T-[S_2])}+\frac{a_2([\widetilde{S}_T]-[S_0]-[S_2])}{1+b(\widetilde{S}_T-[S_0])}\\ \frac{\partial g}{\partial \tau} &= \frac{a_3(\widetilde{S}_T-[S_0]-[S_2])}{1+b(\widetilde{S}_T-[S_2])}-\frac{a_4[S_2]}{1+b(\widetilde{S}_T-[S_0]-[S_2])}.\end{aligned}$$

Now, we must verify that

$$\frac{\partial f}{\partial \tau}+\frac{\partial g}{\partial \tau}\neq 0$$

on $D$. By adding and simplifying, we get that

$$\frac{\partial f}{\partial \tau}+\frac{\partial g}{\partial \tau}=-\frac{a_4}{1+b(\widetilde{S}_T-[S_0])}-\frac{a_2(1+b[S_2])}{(1+b(\widetilde{S}_T-[S_0]))^2}-\frac{a_1(1+b(\widetilde{S}_T-[S_2]))+a_3(b[S_0]+1)}{(1+b(\widetilde{S}_T-[S_2]))^2}. \tag{19}$$

Notice that the sum of the partial derivatives in (19) never changes signs because of the bounds that are set for $[S_0]$ and $[S_2]$, thus proving that system (18) has no periodic orbits.

□

*Remark.* We can use the Poincaré-Bendixson theorem to prove that every solution converges to some steady state. This has been previously proved by Sontag and Wang using monotone system theory [11].

# 5 Discussion and future directions

The proof of non-oscillatory behavior for the dual futile cycle helps us gain a better understanding of large reaction networks. A well-studied model in cell biology is the MAPK cascade which describes the activity of mitogen-activated protein kinase. It contains three layers, each of which is a multiple phosphorylation loop of the type of the multiple futile cycle. More precisely, one of these layers is the dual futile cycle. The layers are linked by the fact that the fully phosphorylated form of the protein which is the substrate in one layer is the kinase for the next layer. This system has been modeled by a system of ODE using mass action kinetics by Huang and Ferrell [4]. Numerical and heuristic evidence has been found indicating that this system has periodic solutions [2, 6, 8]. Even though this evidence exists, there does not exist an analytical proof of oscillatory behavior in the MAPK cascade. Can Michaelis-Menten reduction help? Does the non-oscillatory behavior of the dual futile cycle contradict the fact that MAPK cascade has been said to have oscillations?

# Acknowledgments

We would like to first and foremost thank our mentor, Dr. Anne Shiu. This research experience would not have been possible if not for her mentorship and guidance. We would also like to thank Ola Sobieska for her assistance. This research was conducted as part of the NSF-funded REU program in Mathematics at Texas A&M University (DMS-1460766), Summer 2016.

# References

[1] D. Angeli, P. de Leenheer, and E.D. Sontag. A Petri net approach to the study of persistence in chemical reaction networks. *Mathematical Biosciences*, 2007.

[2] Hassan Errami, Markus Eiswirth, Dima Grigoriev, Werner M. Seiler, Thomas Sturm, and Andreas Weber. Detection of Hopf bifurcations in chemical reaction networks using convex coordinates. *Journal of Computational Physics*, 291:279 – 302, 2015.

[3] Juliette Hell and Alan D. Rendall. A proof of bistability for the dual futile cycle. *Nonlinear Analysis: Real World Applications*, 24:175–189, 2015.

[4] C.Y. Huang and J.E. Jr. Ferrell. Ultrasensitivity in the mitogen-activated protein kinase cascade. *PNAS*, 93(19):10078–10083, 1996.

[5] CraigC. Jolley, KojiL. Ode, and HirokiR. Ueda. A design principle for a posttranslational biochemical oscillator. *Cell Reports*, 2(4):938 – 950, 2012.

[6] Boris N. Kholodenko. Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades. *European Journal of Biochemistry*, 267(6):1583–1588, 2000.

[7] J.D. Murray. Mathematical biology, 3rd edn. 2002.

[8] L. Quiao, R.B. Nachbar, I.G. Kevrekidis, and S.Y. Shvartsman. Bistability and oscillations in the Huang-Ferrell model of MAPK signaling. 3:1819–1826, 2007.

[9] C. Salazar and T. Höfer. Multisite protein phosphorylation - from molecular mechanisms to kinetic models. *FEBS Journal*, 276(12):31773198, 2009.

[10] L. Wang and E.D. Sontag. On the number of steady states in a multiple futile cycle. *Journal of Mathematical Biology*, 57:29–52, 2008.

[11] L. Wang and E.D. Sontag. Singularly perturbed monotone systems and an application to double phosphorylation cycles. *J. Nonlinear Science*, 18:527–550, 2008.