A PROOF OF BISTABILITY FOR THE DUAL FUTILE CYCLE

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Abstract. The multiple futile cycle is an important building block in networks of chemical reactions arising in molecular biology. A typical process which it describes is the addition of $n$ phosphate groups to a protein. It can be modelled by a system of ordinary differential equations depending on parameters. The special case $n = 2$ is called the dual futile cycle. The main result of this paper is a proof that there are parameter values for which the system of ODE describing the dual futile cycle has two distinct stable stationary solutions. The proof is based on bifurcation theory and geometric singular perturbation theory. An important entity built of three coupled multiple futile cycles is the MAPK cascade. It is explained how the ideas used to prove bistability for the dual futile cycle might help to prove the existence of periodic solutions for the MAPK cascade.

1. Introduction

A pattern of chemical reactions frequently encountered in cell biology is one where $n$ phosphate groups are attached to a protein by reactions catalysed by one enzyme $E$ (a kinase) and removed again by reactions catalysed by another enzyme $F$ (a phosphatase). This is sometimes called a multiple futile cycle. An introduction to this type of biological system and how it can be modelled mathematically using ordinary differential equations (ODE) depending on parameters can be found in [16]. It is proved in [16] that for a $n$-fold futile cycle, $n \geq 2$, this system of ODE exhibits multistationarity for certain values of the parameters, i.e. that there exist several different stationary solutions. Upper and lower bounds for the number of stationary solutions as a function of $n$ were also proved in [16]. For $n = 1$ there is a unique stationary solution and it is globally asymptotically stable [1]. The results which follow concern the case $n = 2$ of this system, the dual futile cycle. In that case the maximal number of stationary solutions for any values of the parameters is three. Note for comparison that in the case $n = 3$ while the results of [16] only guarantee that the maximal number of stationary solutions is between three and five it was recently shown in [3] that the upper bound is sharp. In other words, for $n = 3$ there are parameter values for which there exist five stationary solutions.

Beyond the question of the number of stationary solutions it is of great interest to obtain information about their stability. This allows conclusions about the significance of the stationary solutions for the dynamics of more general solutions of the system. It is of particular interest to know whether there exist more than one stable stationary solution for fixed values of the parameters. This phenomenon is called bistability. (In ODE describing chemical systems there are often preferred invariant affine subspaces, the stoichiometric compatibility classes. When talking about fixed parameters it is understood that the stoichiometric compatibility class has been fixed.) In [10] it was concluded using numerical and heuristic approaches that there is bistability in the dual futile cycle. To the authors’ knowledge there
is no rigorous and purely analytical proof of this statement in the literature. The main result of the present paper is a proof of this type.

In [16] and [10] the reactions are modelled using a standard Michaelis-Menten scheme for the catalysis of each reaction and mass action kinetics for the elementary reactions involved. The resulting system will be called the MM-MA system (Michaelis-Menten via mass action) in what follows and it is the system of principal interest here. It is possible, via a quasistationarity assumption of Michaelis-Menten type, to pass formally to a smaller system, called the MM system (Michaelis-Menten) in what follows. The question of bistability in the latter system has been studied by methods which are partly numerical and heuristic in [11].

The strategy used in what follows is to first give a rigorous analytical proof of bistability in the MM system using bifurcation theory. It is shown that there is a generic cusp bifurcation and this implies bistability by well-known methods [9]. Then bistability is concluded for the MM-MA system with the help of geometric singular perturbation theory [3] which gives control over the limiting process from the MM-MA system to the MM system and can be thought of as a far-reaching generalization of earlier work of Tikhonov (see [18], Sect. 39) and Hoppensteadt [6]. The relevance of this type of result to the quasistationary approximation was pointed out in [5]. A similar strategy has been used in [17] to prove that generic solutions of the system describing the dual futile cycle converge to stationary solutions.

The paper is organized as follows. In the next section the basic equations of the model studied in the paper are explained. Sect. 3 is a concise introduction to some of the main mathematical tools used. In Sect. 4 bistability is proved for the Michaelis-Menten system and in Sect. 5 this is used to obtain a corresponding result for the full system. In Sect. 6 some directions in which this research could be extended are indicated. These concern the MAPK cascade. In particular it is discussed how Michaelis-Menten reduction can be applied in that case and an explicit reduced system is presented.

2. The basic equations

Consider a chemical system consisting of a protein $Y$ and the substances $YP$ and $YPP$ obtained by attaching one or two phosphate groups to $Y$. The reactions which attach phosphate groups are catalysed by an enzyme $E$ and those which remove phosphate groups by an enzyme $F$. It is assumed that the enzyme $E$ can only add one phosphate group before releasing the substrate. This is what is called distributive phosphorylation in contrast to processive phosphorylation where more than one phosphate is added during one encounter between the enzyme and its substrate. It is also assumed that the phosphate groups are added at binding sites in a certain order. This is called sequential phosphorylation. It is assumed that dephosphorylation by $F$ has corresponding properties and that phosphate groups are removed in the reverse order to that in which they are added. These assumptions about the nature of the phosphorylation and dephosphorylation processes are common in modelling approaches in the literature. Adopting them, the chemical reactions we are modelling can be written in the following form where the label $k_i$
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\[ Y + E \xrightarrow{k_1} YE \xrightarrow{k_3} YP + E \]

\[ YP + E \xrightarrow{k_5} YPE \xrightarrow{k_6} YPP + E \]

\[ YPP + F \xrightarrow{k_8} YPPF \xrightarrow{k_9} YP + F \]

\[ YP + F \xrightarrow{k_{10}} YPF \xrightarrow{k_{12}} Y + F \]

denotes the reaction constant of reaction \( i \).

An important biological example is the case where \( Y \) is the extracellular signal-regulated kinase (ERK), \( E \) is the MAPK/ERK kinase (MEK) and \( F \) is MAPK phosphatase 3 (MKP3). This is part of a mitogen activated protein kinase (MAPK) cascade, a pattern of chemical reactions of key significance in molecular biology. Some experimental results on this system are summarized in [10] and are as follows. Both MEK and MKP3 act in a distributive fashion. Dephosphorylation by MKP3 is sequential but phosphorylation by MEK has a random component - one order of phosphorylation predominates but the other also occurs. There is some discussion in [10] of the effects of this fact on the dynamics. In what follows the analysis is restricted to the case where both \( E \) and \( F \) act sequentially. It is of interest to note that if distributive phosphorylation is replaced by processive phosphorylation for one or both of the enzymes in this model then the existence of more than one stationary solution, and in particular bistability, can be ruled out [2].

The concentration of a substance \( Z \) is denoted by \( x_Z \). If mass action kinetics are imposed then the assumptions made up to this point uniquely determine the evolution equations for the concentrations of the substances taking part in the reactions. These are the free substrates \( Y, YP \) and \( YPP \), the free enzymes \( E \) and \( F \) and the substrate-enzyme complexes \( YE, YPE, YPPF \) and \( YPF \). They satisfy
the following system of nine ordinary differential equations:

\begin{align*}
1. & \quad \frac{dx_Y}{dt} = -k_1x_Yx_E + k_2x_YE + k_{12}x_YPF, \\
2. & \quad \frac{dx_{YP}}{dt} = -k_4x_YPx_E - k_{10}x_YPx_F + k_3x_YE + k_5xYPE + k_9x_{YPF} + k_{11}x_{YPF}, \\
3. & \quad \frac{dx_{YPF}}{dt} = -k_7x_{YPF}x_F + k_6xYPE + k_8x_{YPF}, \\
4. & \quad \frac{dx_E}{dt} = k_1x_Yx_E - (k_2 + k_3)x_YE, \\
5. & \quad \frac{dx_{EP}}{dt} = k_4x_YPx_E - (k_5 + k_6)xYPE, \\
6. & \quad \frac{dx_{PF}}{dt} = k_{10}x_YPx_F - (k_{11} + k_{12})x_{YPF}, \\
7. & \quad \frac{dx_F}{dt} = -k_1x_Yx_F + k_{14}x_Yx_E + (k_2 + k_3)x_YE + k_5xYPE + k_6x_{YPF}, \\
8. & \quad \frac{dx}{dt} = -k_1x_Yx_F - k_{10}x_Yx_E + (k_8 + k_9)x_{YPF} + (k_{11} + k_{12})x_{YPF}.
\end{align*}

The reaction constants \(k_i\) are positive numbers. It should be noted that apart from the differences in notation these equations are identical to those in \([10]\) and \([16]\). Let

\begin{align*}
10. & \quad \tilde{E} = x_E + x_YE + xYPE, \\
11. & \quad \tilde{F} = x_F + x_{YPF} + x_{YPF}, \\
12. & \quad \tilde{Y} = x_Y + x_{YP} + x_{YPF} + x_{YPF} + x_{YPF} + x_{YPF}.
\end{align*}

These quantities, which are the total concentrations of the enzymes and of the substrate, are conserved under the evolution. Thus \(x_E\) and \(x_F\) can be expressed in terms of \(\tilde{E}\) and \(\tilde{F}\) and the concentrations of their complexes. In a similar way, \(x_{YP}\) can be expressed in terms of \(\tilde{Y}\), \(x_Y\), \(x_{YPF}\) and the concentrations of the complexes involving \(Y\). It is thus possible to discard the evolution equations \((2), (8)\) and \((9)\) and reduce the number of equations from nine to six. The notation \(x_{YP}\) should now be thought of as an abbreviation for the expression of this quantity in terms of \(\tilde{Y}\) and the unknowns in the remaining evolution equations. The six equations depend on the three parameters \(\tilde{E}, \tilde{F}\) and \(\tilde{Y}\). If a solution of the system of nine equations is given, a solution of the system of six equations with certain values of the parameters is obtained. Conversely, given a solution of the system of six equations and a choice of the constants \(\tilde{E}, \tilde{F}\) and \(\tilde{Y}\) a solution of the set of nine equations can be obtained. In order to ensure that the latter is positive the constants \(\tilde{E}, \tilde{F}\) and \(\tilde{Y}\) must be chosen sufficiently large.

Next we want to pass to the Michaelis-Menten limit. This can be done by introducing rescaled variables. If \(Z\) is the concentration of a free enzyme or that of a substrate-enzyme complex let \(x_Z = \epsilon \bar{x}_Z\). Let \(\bar{E} = \epsilon \bar{E}\) and similarly for \(F\). The smallness of \(\epsilon > 0\) amounts to the enzyme concentrations being small compared to the concentrations of the substrates. Finally, let \(\tau = \epsilon t\). For \(\epsilon > 0\) small, the
system exhibits fast-slow dynamics. The two different time scales are reflected in the time variables \( t \) and \( \tau \). The transformed equations are

\[
\frac{dx_Y}{d\tau} = -k_1 x_Y \ddot{x}_E + k_2 \ddot{x}_{Y E} + k_12 \ddot{x}_{Y P F},
\]

\[
\frac{dx_{YP}}{d\tau} = -k_7 x_{YP} \ddot{x}_F + k_6 \ddot{x}_{YP E} + k_8 \ddot{x}_{YP F}.
\]

Note that equations (2), (8) and (9) could also be rescaled in a similar way so that in total a system of nine equations depending on \( \epsilon \) is obtained whose solutions for any \( \epsilon > 0 \) are in one to one correspondence with the solutions of the system (1)-(9). Setting \( \epsilon = 0 \) in equations (15)-(18) gives

\[
\ddot{x}_{Y E} = \frac{k_1}{k_2 + k_3} x_Y \ddot{x}_E - \frac{k_2 + k_3}{k_1} \ddot{x}_{Y E},
\]

\[
\ddot{x}_{YP E} = \frac{k_4}{k_5 + k_6} x_{YP} \ddot{x}_E - \frac{k_5 + k_6}{k_4} \ddot{x}_{YP E},
\]

\[
\ddot{x}_{YP F} = \frac{k_10}{k_11 + k_12} x_{YP} \ddot{x}_F - \frac{k_11 + k_12}{k_10} \ddot{x}_{YP F}.
\]

Adding these equations in pairs gives

\[
\ddot{E} = \left[ 1 + \frac{k_1}{k_2 + k_3} x_Y \right] \ddot{x}_E,
\]

\[
\ddot{F} = \frac{k_10}{k_11 + k_12} x_{YP} \ddot{x}_P - \frac{k_11 + k_12}{k_10} \ddot{x}_{YP P}.
\]

Using (19)-(22) the evolution equations (13)-(14) can be rewritten as

\[
\frac{dx_Y}{d\tau} = -\frac{k_1 k_4}{k_2 + k_3} x_Y \ddot{x}_E + \frac{k_10 k_12}{k_11 + k_12} x_{YP} \ddot{x}_F,
\]

\[
\frac{dx_{YP}}{d\tau} = -\frac{k_4 k_6}{k_5 + k_6} x_{YP} \ddot{x}_F + \frac{k_7 k_9}{k_8 + k_9} x_{YP P} \ddot{x}_F.
\]

Note that \( \ddot{Y} = x_Y + x_{YP} + x_{YP P} + \epsilon (\ddot{x}_{Y E} + \ddot{x}_{YP E} + \ddot{x}_{YP F} + \ddot{x}_{YP P}) \). We define \( \ddot{Y} = \bar{Y}(0) \) to emphasize the dependence on \( \epsilon \). Then for \( \epsilon = 0 \) the relation

\[
\ddot{Y} = x_Y + x_{YP} + x_{YP P}
\]
holds so that setting $x_{YP} = \tilde{Y} - x_Y - x_{YP}$, this is a closed system of equations. Summing up, the equations are of the form

$$\frac{dx_Y}{d\tau} = -v_1 + v_2,$$
$$\frac{dx_{YP}}{d\tau} = v_3 - v_4$$

where, using (23) and (24),

$$v_1 = \frac{a_1 x_Y}{1 + b_1 x_Y + c_1 x_{YP}},$$
$$v_2 = \frac{a_2 x_{YP}}{1 + c_2 x_{YP} + d_2 x_{YP}^P},$$
$$v_3 = \frac{a_3 x_{YP}}{1 + b_1 x_Y + c_1 x_{YP}},$$
$$v_4 = \frac{a_4 x_{YP}^P}{1 + c_2 x_{YP} + d_2 x_{YP}^P}$$

and

$$a_1 = \frac{k_1 k_3 \tilde{E}}{k_2 + k_3}, \quad a_2 = \frac{k_{10} k_{12} \tilde{F}}{k_{11} + k_{12}},$$
$$a_3 = \frac{k_4 k_6 \tilde{E}}{k_5 + k_6}, \quad a_4 = \frac{k_7 k_9 \tilde{F}}{k_8 + k_9},$$
$$b_1 = \frac{k_1}{k_2 + k_3}, \quad c_1 = \frac{k_4}{k_5 + k_6},$$
$$c_2 = \frac{k_{10}}{k_{11} + k_{12}}, \quad d_2 = \frac{k_7}{k_8 + k_9}.$$

The conditions for a stationary solution are $v_1 = v_2$ and $v_3 = v_4$. From now on the simplifying assumption will be made that

$$b_1 = c_1 = c_2 = d_2.$$

Call the common value of these quantities $b$. The simplifying assumption (38) means 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 substrate-enzyme complexes during phosphorylation are equal. Note that (38) is equivalent to a set of relations between the $k_i$ and $b$. If $\tilde{E}$ and $\tilde{F}$ are given then $k_3$, $k_6$, $k_9$ and $k_{12}$ are determined by the $a_i$ and $b$. There are then four degrees of freedom in choosing the other $k_i$ so as to satisfy the other relations. Thus if values $a_i$, $b$, $\tilde{E}$ and $\tilde{F}$ are given they can be realized by many choices of $k_i$.

3. REVIEW OF SOME MATHEMATICAL TECHNIQUES

In this section some ideas from bifurcation theory and geometric singular perturbation theory will be reviewed. Bifurcation theory for ordinary differential equations concerns parameter-dependent systems $\dot{x} = f(x, \mu)$ where the variable $x$ and the parameter $\mu$ belong to subsets of Euclidean spaces. Under some circumstances the flows of the equations for different values of $\mu$ are related to each other by a diffeomorphism of the space with coordinates $x$. In that case the qualitative nature of the flow does not change with $\mu$. This is for instance true in a neighbourhood of a point $x_0$ and a parameter value $\mu_0$ for which $f(x_0, \mu_0) \neq 0$ (Flow-box Theorem),
or in a neighbourhood of a hyperbolic equilibrium (Hartman-Grobman Theorem). If the flows cannot be related in this way for \( \mu \) close to some value \( \mu_0 \) then it is said that a bifurcation occurs at \( \mu_0 \). For example at an equilibrium \( x_0 \) (where of course \( f(x_0, \mu_0) = 0 \)), a bifurcation occurs iff the linearisation \( D_x f(x_0, \mu_0) \) admits eigenvalues with vanishing real part. In this case a non-trivial centre manifold exists. The aim of bifurcation theory is to obtain insights into the qualitative changes in the flow in this kind of situation. This can often provide valuable information on the dynamics of the system for parameter values close to \( \mu_0 \), for instance the number and stability of stationary solutions. This information is obtained when certain criteria are satisfied. Typical examples of relevant conditions are whether certain combinations of derivatives of \( f \) at \((x_0, \mu_0)\) are zero or non-zero or the signs of such combinations. When studying a bifurcation at a stationary point \( x_0 \) it is often possible to reduce the dimension of the space of unknowns \( x \) using centre manifold theory. A detailed discussion of this can be found in chapter 5 of [9]. The essential qualitative features occur on the centre manifold whose dimension is equal to the dimension of the space of generalized eigenvectors of the linearization \( D f(x_0, \mu_0) \) corresponding to purely imaginary eigenvalues.

To make this more concrete we consider the example of greatest importance in what follows, the cusp bifurcation. Suppose that the dynamical system is one-dimensional, remembering that this may have resulted from a system of higher dimension by centre manifold reduction. Suppose that \( \mu \) has two components \( \mu_1 \) and \( \mu_2 \). By choosing coordinates appropriately it can be assumed that the bifurcation occurs at the point \((0, 0)\). The system is said to exhibit a generic cusp bifurcation if

\[
(39) \quad f(0, 0) = 0, \quad f_x(0, 0) = 0, \quad f_{xx}(0, 0) = 0, \quad f_{xxx}(0, 0) \neq 0, \quad f_{x \mu_1} f_{\mu_2} - f_{x \mu_2} f_{\mu_1} \neq 0
\]

where the subscripts denote partial derivatives with respect to the corresponding variables. If only the first three of these conditions are satisfied it might be said loosely that there is a cusp bifurcation but this does not exclude the possibility that extra degeneracies might occur. These can be ruled out by imposing the last two conditions and this is what is meant by the term 'generic' here. When the one-dimensional system arises from a system of higher dimension by centre manifold reduction the diagnostic conditions for a cusp bifurcation can be reexpressed in terms of algebraic conditions on derivatives of the original system, as explained in chapter 8 of [9]. This will be seen in more detail in the example of the dual futile cycle in Sect. 4. The significance of the cusp for the results of this paper is that when a bifurcation of this type with \( f_{xxx}(0,0) < 0 \) occurs at some point there are nearby parameter values for which there exist two stable stationary solutions, and one which is unstable. The case \( f_{xxx}(0,0) > 0 \) gives one stable and two unstable stationary solutions and is not relevant for what follows. The name cusp bifurcation comes from the following. Figure 4 shows \( \mathbb{R}^3 \), where the horizontal plane is the plane of parameters \( \mu = (\mu_1, \mu_2) \), and the vertical direction the one dimensional variable \( x \). The set of equilibria forms a surface in this bifurcation diagram. Near a cusp bifurcation, this surface shows a fold. The line delimiting the folding region projected into the horizontal parameter plane forms a cusp, although the surface in \( \mathbb{R}^3 \) is smooth. For a given parameter in the cusp region, there are two stable equilibria and one unstable one. Furthermore there are heteroclinic orbits connecting the unstable equilibrium to the two stable ones.
Next some remarks will be made on geometric singular perturbation theory (GSPT). This theory was developed by Fenichel [3] and section 3 of that paper provides an introduction to some of the main ideas involved. The standard situation in which this theory is applied is for a system of ODE of the form

\[
\begin{align*}
    x' &= f(x, y, \epsilon), \\
    \epsilon y' &= g(x, y, \epsilon),
\end{align*}
\]

where the prime denotes the derivative with respect to a time coordinate \(\tau\). Here \(f\) and \(g\) are smooth functions and \(\epsilon\) is a parameter. The aim is to understand the qualitative behaviour of solutions of this system in the limit \(\epsilon \to 0\) where the system \((40)\) reads

\[
\begin{align*}
    x' &= f(x, y, 0), \\
    0 &= g(x, y, \epsilon).
\end{align*}
\]

The system \((41)\) is called the reduced system. The dynamics of the reduced system may provide useful information about the qualitative behaviour of solutions of the system \((40)\) with \(\epsilon\) small and non-zero. The system \((40)\) is singular at \(\epsilon = 0\) since the time derivative of \(y\) is multiplied by a factor which vanishes there. This means that regular perturbation theory cannot be applied. Note that the system \((13)-(18)\) is of this general form.

Suppose that in the above system \(x\) is a point of \(\mathbb{R}^{n_1}\) and \(y\) a point of \(\mathbb{R}^{n_2}\). Let \(\tau = ct\) for \(\epsilon > 0\) and denote the derivative with respect to \(t\) by a dot. Transforming the equations to the time coordinate \(t\) and adding the equation \(\dot{\epsilon} = 0\) gives the
following system of $n_1 + n_2 + 1$ equations

\[
\begin{align*}
\dot{x} &= \epsilon f(x, y, \epsilon), \\
\dot{y} &= g(x, y, \epsilon), \\
\dot{\epsilon} &= 0,
\end{align*}
\]

Call system (42) the extended system. This extends smoothly to $\epsilon = 0$ to

\[
\begin{align*}
\dot{x} &= 0, \\
\dot{y} &= g(x, y, 0), \\
\dot{\epsilon} &= 0,
\end{align*}
\]

Assume that the set defined by the equation $g(x, y, 0) = 0$ is equivalent to $y = h_0(x)$ for a smooth function $h_0$. In particular it is a manifold $M_0$. This manifold consists of stationary points of the extended system (42) at $\epsilon = 0$. Assume further that the linearization of system (42) at any point of $M_0$ has no purely imaginary eigenvalues other than the zero eigenvalues arising from the fact that $M_0$ consists of stationary solutions and the fact that $\epsilon$ is a conserved quantity. Call these eigenvalues corresponding to eigenvectors transverse to $M_0$ the transverse eigenvalues. They correspond to the eigenvalues of the linearization of the system $\dot{y} = g(x, y, 0)$ with $x$ held fixed. This leads to the following

Definition 3.1. For a point $(x, h_0(x)) \in M_0$, we define the transverse eigenvalues at $x$ as the eigenvalues of the linearisation $D_y g(x, h_0(x), 0)$, under the assumption that none of them is purely imaginary.

A centre manifold $M$ of the extended system (42) at any point of $M_0$ contains $M_0$. It was shown in [3] that there exists a manifold $M$ which is a centre manifold for all points of $M_0$ close to a given point. The manifold $M$ is sometimes called a slow manifold. The restriction of the extended system (42) to the slow manifold $M$ can be interpreted as a system of $n_1$ equations depending on the parameter $\epsilon$. Remarkably, rewriting this system in terms of the time coordinate $\tau$ gives a system which depends on $\epsilon$ in a regular fashion, even at $\epsilon = 0$. For $\epsilon = 0$ it coincides with the reduced system (41). For $\epsilon > 0$ call this the perturbed reduced system.

The reduction theorem of Shoshitaishvili (see [9], Theorem 5.4) shows that the qualitative behaviour of solutions of the extended system (42) near a point of $M_0$ is determined in a simple way by the dynamics on $M$. In fact it is topologically equivalent to the product of the dynamics on $M$ with a standard saddle. What this means in practise is that dynamical features of the reduced system (41) are inherited by the full system (42) for $\epsilon$ small. In the case of the central example of this paper the real parts of the transverse eigenvalues of the linearization at points of $M_0$ defined in (3.1) are all negative. Thus the standard saddle mentioned above is a hyperbolic sink in this case. This implies that bistability of the MM system (which is the reduced system in this case) is inherited by the perturbed reduced system. It then follows from the theorem of Shoshitaishvili that bistability is inherited by the MM-MA system. In other situations, as will be explained further in Sect. 6 the existence of periodic solutions of the reduced system is inherited by the full system.
4. Bistability for the Michaelis-Menten system

The MM system can be written as

\[
\begin{align*}
\frac{dy}{d\tau} &= -\frac{a_1 x \gamma}{1 + b(\gamma - x_{\gamma P})} + \frac{a_2 x \gamma P}{1 + b(\gamma - x)}, \\
\frac{dx_{\gamma P}}{d\tau} &= \frac{a_3 x y P}{1 + b(\gamma - x_{\gamma P})} - \frac{a_4 x y P P}{1 + b(\gamma - x_{\gamma P})}.
\end{align*}
\]

It has been observed in [14] that if certain restrictions are imposed on the parameters it is possible to find an explicit stationary solution of equations (44)-(45) with interesting properties. Consider a stationary solution that we call $B$, with starred coordinates $(x_{\gamma}^*, x_{\gamma P}^*)$ and the corresponding quantity $x_{\gamma P}^* = \gamma - x_{\gamma P}^* - x_{\gamma P}$. We look for an equilibrium $B$ satisfying $x_{\gamma}^* = x_{\gamma P}^*$, a condition which is satisfied by the special solutions considered in [14]. Then the denominators in the expressions for the evolution equations are all equal and the equations for stationary solutions imply that

\[
a_1 x_{\gamma}^* = a_2 x_{\gamma P}^*, \quad a_3 x_{\gamma P}^* = a_4 x_{\gamma P}^*.
\]

It follows that

\[
a_2 a_4 = a_1 a_3 = 1.
\]

Let $N = 1 + b(\gamma - x_{\gamma})$. The linearization of the system at the stationary point $B$ is $N^{-2}$ times

\[
\begin{bmatrix}
-(a_1 + a_2) + b[-a_1(\gamma - x) - a_2 x] & -a_1 b x - a_2 N \\
-a_3 N - b a_4 x & -(a_3 + a_4) + b[-a_4(\gamma - x) - a_3 x]
\end{bmatrix}
\]

where $x = x_{\gamma}^*$. The equations $a_1 x = a_2(\gamma - 2x)$ and $a_4 x = a_3(\gamma - 2x)$ can be used to eliminate $a_1$ and $a_4$ in favour of $a_2$ and $a_3$. After simplification and multiplication by an overall factor $x$ the matrix [15] becomes

\[
\begin{bmatrix}
-(\gamma - x) a_2 - b a_3(\gamma^2 - 3x\gamma + 3x^2) & -a_2 x + a_3 b(-2\gamma x + 3x^2) \\
-a_3 x + a_3 b(-2\gamma x + 3x^2) & -(\gamma - x) a_2 - b a_3(\gamma^2 - 3x\gamma + 3x^2)
\end{bmatrix}
\]

Now we look for a stationary point $B$ where a bifurcation takes place, so that the above matrix should admit a zero eigenvalue. The determinant of this matrix vanishes precisely when

\[
|b(\gamma - x) + b^2(\gamma^2 - 3x\gamma + 3x^2)| = |bx - b^2(-2\gamma x + 3x^2)|.
\]

There are two cases according to the relative signs of the quantities inside the absolute values. Setting $u = b\gamma$ and $v = bx$ we get the alternative conditions $(u - 3v + 1)(u - 2v) = 0$ and $u(u - v + 1) = 0$. The case $u = 0$ can be discarded since it corresponds to a vanishing concentration of substrate. The case $u = 2v$ is also not relevant since it corresponds to a vanishing concentration of $x_{\gamma P}$. The case $u = v - 1$ leads to a negative concentration of $x_{\gamma P}$. Consider now the remaining case $u = 3v - 1$. A computation shows that

\[
x_{\gamma}^* = x_{\gamma P}^* = \frac{b\gamma + 1}{3b}, \quad x_{\gamma P}^* = \frac{b\gamma - 2}{3b}.
\]
Now
\[
\frac{b\bar{Y} - 2}{b\bar{Y} + 1} = \frac{x_{Y \bar{Y} \bar{P}}}{x_{\bar{Y}}} = \frac{a_3}{a_2} = \frac{a_2 a_4}{a_2 a_3}.
\]

We have a bifurcation if and only this last relation is satisfied. A necessary and sufficient condition that it can be satisfied for parameters with \( b\bar{Y} > 2 \) is that \( \frac{a_2 a_4}{a_1 a_3} > 1 \). In that case \( b\bar{Y} > 2 \). We summarize the conditions necessary and sufficient for the existence of the stationary point denoted by \( B \) with coordinates given by (51):

\[
\frac{a_2 a_4}{a_1 a_3} = 1 \quad \text{equilibrium condition}
\]

\[
1 > \frac{b\bar{Y} - 2}{b\bar{Y} + 1} = \frac{a_1 a_3}{a_2 a_3} > 0 \quad \text{bifurcation condition}
\]

Substituting the bifurcation condition into the linearization \( N^{-2} \) times (48) simplifies it to

\[
-\frac{b\bar{Y}}{N^2} \begin{bmatrix} a_2 & a_2 \\ a_3 & a_3 \end{bmatrix}.
\]

This matrix has rank one with the right eigenvector corresponding to the zero eigenvalue being the transpose of \([1, -1] \) and the left eigenvector \([a_3, -a_2] \). The other eigenvalue has the same sign as the trace and is negative.

Next it will be shown that there is a non-degenerate cusp bifurcation at the point \( B \). The defining conditions for a bifurcation of this type are given by (39) in the case of a one-dimensional system. As discussed in Sect. 3 a non-degenerate cusp bifurcation in higher dimensional dynamical systems is defined by relating the given system to the one-dimensional case by means of centre manifold reduction. The essential dynamics is determined by that on the centre manifold which is one-dimensional in this case. The point \( B \) in the MM system has a one-dimensional centre manifold and so these techniques are applicable. The reduction theorem makes transparent what is happening on an abstract level. On the other hand the concrete calculations which are needed to verify the presence of a non-degenerate cusp bifurcation by obtaining a suitable approximation to the centre manifold and analysing the dynamics on that manifold are hard to keep track of. Here we follow an approach to organizing these calculations explained in Section 8.7 of [9].

It is necessary to calculate certain combinations of derivatives of the right hand side of the MM system at the point \( B \) with respect to the unknowns and the parameters. For the cusp bifurcation it is necessary to vary two parameters. We choose these to be \( a_1 \) and \( a_4 \) and hold all other parameters in the system fixed. It is convenient to introduce the notation \( X^i, i = 1, 2 \) for the right hand sides of the equations of the MM system (44)-(45) and \( x_i, i = 1, 2 \) for the coordinates \( x_{Y \bar{Y}} \) and \( x_{Y \bar{Y} \bar{P}} \). The components of the right and left eigenvectors of the linearization will be denoted by \( R^i \) and \( L_i \), respectively. For derivatives we use the following abbreviations

\[
X^i_{,jk} = \frac{\partial X^i}{\partial x_j} \cdot x^i_{,jk} = \frac{\partial^2 X^i}{\partial x_j \partial x_k} \cdot X^i_{,jkl} = \frac{\partial X^i}{\partial x_j \partial x_k \partial x_l}.
\]

Now higher derivatives of the coefficients in the system MM will be examined. For this it is useful to consider derivatives of rational functions more generally. Each \( X^i \) is a sum of rational functions where numerator and denominator are linear in the
arguments. If $f$ and $g$ are smooth functions of two variables with $g$ non-vanishing then

$$D_i \left( \frac{f}{g} \right) = \left( \frac{D_i f}{g} \right) + f D_i \left( \frac{1}{g} \right), i = 1, 2, \tag{57}$$

$$D_i D_j \left( \frac{f}{g} \right) = \left( \frac{D_i D_j f}{g} \right) + D_i f D_j \left( \frac{1}{g} \right) + D_j f D_i \left( \frac{1}{g} \right). \tag{58}$$

When $f$ is linear the term with $D_i D_j f$ vanishes. Now

$$D_i \left( \frac{1}{g} \right) = -\frac{D_i g}{g^2}. \tag{59}$$

When $g$ is linear the first term vanishes. Thus when both $f$ and $g$ are linear we get

$$D_i D_j \left( \frac{1}{g} \right) = -\frac{g D_i f D_j g - g D_i g D_j f + 2 f D_i g D_j g}{g^3}. \tag{60}$$

Contracting with $R^i R^j$ (i.e. multiplying by this expression and summing over repeated indices) gives

$$R^i R^j D_i D_j \left( \frac{f}{g} \right) = 2 \left( -\frac{g (R^i D_i f) (R^j D_j g) + f (R^i D_i g)^2}{g^3} \right). \tag{62}$$

Here and in what follows we use the summation convention - sums over repeated indices are implicitly assumed.

Consider the quantities $X^{i,jk} R^i R^k$, evaluated at $B$. They are given by

$$X^{1,jk} R^i R^k = 2 N^{-3} [a_1 b (-N + b x Y) + a_2 b^2 x Y P], \tag{63}$$

$$X^{2,jk} R^i R^k = 2 N^{-3} [a_3 b^2 x Y P + a_4 b (-N + b x Y P P)]. \tag{64}$$

After some substitutions this gives

$$X^{1,jk} R^i R^k = 2 N^{-3} a_2 [b (b \tilde{Y} - 2) + 3 b^2 x Y P] = 4 N^{-3} a_2 b^2 x Y P, \tag{65}$$

$$X^{2,jk} R^i R^k = 2 N^{-3} a_3 [3 b^2 x Y P + b (b \tilde{Y} - 2)] = 4 N^{-3} a_3 b^2 x Y P. \tag{66}$$

It follows that $L_i X^{i,jk} R^j R^k = 0$ and this is one of the conditions for a cusp bifurcation.

The next step is to examine the third order derivatives. Note first that

$$D_i D_j D_k \left( \frac{1}{g} \right) = -\frac{6 D_i g D_j g D_k g}{g^4}. \tag{67}$$

In the given situation

$$D_i D_j D_k \left( \frac{f}{g} \right) \quad = \quad 2 \left( \frac{(D_i f D_j g D_k g + D_k f D_i g D_j g + D_j f D_k g D_i g)}{g^2} \right) - \frac{6 f D_i g D_j g D_k g}{g^4} \tag{68}$$
and
\[ K^iK^jK^kD_iD_jD_k \left( \frac{f}{g} \right) = \frac{6(K^iD_j g)^2 (gK^iD_j f - fK^iD_j g)}{g^4}. \]
It follows that
\[ K^iK^jK^kD_iD_jD_k \left( \frac{f}{g} \right) = -3K^iD_j g \frac{K^iK^jD_j \left( \frac{f}{g} \right)}{g}. \]
Hence
\[ L_i X^i_{\cdot jkl} R^j R^k R^l = -12N^{-4}(a_2^2 + a_3^2)b^3xy_p. \]
In the one-dimensional case one of the conditions for a cusp is that the third derivative of the right hand side does not vanish. In higher dimensions the analogue of this condition is not just the expression in (71). There is an extra correction given on p. 374 of [9]. To compute this we need a vector whose image under the linearization at \( B \) is the vector with components \( X^i_{\cdot jkl} R^j R^k \). Call it \( Z \). This is \(-4N^{-1}(a_2 + a_3)^{-1}Y^{-1}bxy_p [a_2, a_3]^T \). The crucial quantity is
\[ X^i_{\cdot jkl} R^j R^k R^l = -3X^i_{\cdot jkl} R^j R^k Z^l L_i. \]
To evaluate this we first calculate the second derivatives of \( X^1 \) and \( X^2 \), which are
\[ N^{-3} \left[ \begin{array}{cc} -2Na_2 b + 2a_2 b^2 xy_p & N(a_1 - a_2) b \\ N(a_1 - a_2) b & 2b^2a_1 xy \\ \end{array} \right] \]
and
\[ N^{-3} \left[ \begin{array}{cc} 2b^2a_4 xy_p & N(-a_3 + a_4)b \\ N(-a_3 + a_4)b & -2Na_3 b + 2a_3 b^2 xy_p \\ \end{array} \right]. \]
Contracting with \( R \) corresponds to taking the differences of the columns of these matrices. The results are
\[ N^{-3}[-N(a_1 + a_2)b + 2a_2 b^2 xy_p \quad N(a_1 - a_2)b - 2b^2a_1 xy] \]
and
\[ N^{-3}[2b^2a_4 xy_p + N(a_3 - a_4)b \quad N(a_3 + a_4)b - 2a_3 b^2 xy_p]. \]
Eliminating \( a_1 \) and \( a_4 \) in terms of \( a_2 \) and \( a_3 \) as has been done in previous arguments and expressing everything in terms of \( bY \) gives
\[ N^{-3} \left[ \begin{array}{cc} -2 \frac{2}{3}a_2 b(b\tilde{Y} + 1) & - \frac{2}{3}a_2 b(b\tilde{Y} + 1) \\ - \frac{2}{3}a_2 b(b\tilde{Y} + 1) & - \frac{2}{3}a_2 b(b\tilde{Y} + 1) \\ \end{array} \right], \quad N^{-3} \left[ \begin{array}{cc} \frac{2}{3}a_3 b(b\tilde{Y} + 1) & - \frac{2}{3}a_3 b(b\tilde{Y} + 1) \\ - \frac{2}{3}a_3 b(b\tilde{Y} + 1) & - \frac{2}{3}a_3 b(b\tilde{Y} + 1) \\ \end{array} \right]. \]
Contracting this with \([a_2, a_3]^T\) and \( L \) gives \(-4N^{-3}a_2a_3(a_2 + a_3)b^2xy \). Hence
\[ X^i_{\cdot jkl} R^j R^k Z^l L_i = 16N^{-3}a_2a_3 b^2 xy_p \tilde{Y}^{-1}. \]
The crucial quantity is strictly negative and so another of the conditions for the cusp bifurcation is satisfied.
It remains to examine the derivatives with respect to parameters. Recall that the parameters to be varied are \( \mu_1 = a_1 \) and \( \mu_2 = a_4 \). The derivatives with respect to the two parameters of the right hand sides of the two MM equations are
\[ \left( \frac{-xy}{1 + b(xy + xy_p)}, 0 \right), \quad \left( 0, \frac{-xy_pp}{1 + b(xy_p + xy_pp)} \right). \]
Contracting these with respect to the left eigenvector $L$ gives
\begin{equation}
-\frac{a_2x_Y}{1+b(x_Y+x_{YP})}, \quad \frac{a_3x_{YP}}{1+b(x_{YP}+x_{YP})}.
\end{equation}
Differentiating the vector field in the direction of the right eigenvector $R$ gives
\begin{equation}
\left(-\frac{a_1(1+bx_{YP})-a_2bx_{YP}}{[1+b(x_Y+x_{YP})]^2}, \quad \frac{a_4(1+bx_{YP})+a_3bx_{YP}}{[1+b(x_{YP}+x_{YP})]^2}\right).
\end{equation}
The derivatives of this with respect to the two parameters are
\begin{equation}
\left(-\frac{(1+bx_{YP})}{[1+b(x_Y+x_{YP})]^2}, \quad 0\right), \quad \left(0, \frac{(1+bx_{YP})}{[1+b(x_{YP}+x_{YP})]^2}\right).
\end{equation}
Contracting these with respect to the left eigenvector gives
\begin{equation}
\left(-\frac{a_2(1+bx_{YP})}{[1+b(x_{YP}+x_{YP})]^2}, \quad -\frac{a_3(1+bx_{YP})}{[1+b(x_{YP}+x_{YP})]^2}\right).
\end{equation}
It follows that
\begin{equation}
\left[\frac{\partial X_i}{\partial \mu_1} \frac{\partial X_j}{\partial \mu_2} - \frac{\partial X_i}{\partial \mu_2} \frac{\partial X_j}{\partial \mu_1}\right] L_i L_j R^k
= -(a_2^2 + a_3^2)N^{-3} x_Y (1+bx_{YP}).
\end{equation}
This is the last diagnostic quantity for the cusp bifurcation and it is seen to be non-zero. This means that all conditions needed for the proof that there is a generic cusp bifurcation at $B$ have been verified.

**Theorem 4.1.** There exist values of the parameters $a_i$, $b$ and $Y$ for which the MM system (44)-(45) has three stationary solutions, two of which are locally asymptotically stable and one of which is a saddle of Morse index one. Heteroclinic orbits connect the saddle to both stable equilibria.

**Proof.** It has been shown that when the conditions (47) and (52) are assumed the system exhibits a generic cusp bifurcation. It follows from the analysis in Section 8.7 of [9] that centre manifold reduction leads to a one-dimensional system satisfying the hypotheses of Theorem 8.1 of [9]. This implies that near the bifurcation point the dynamics on the centre manifold is topologically equivalent to the model system for the cusp bifurcation. Combining this with Theorem 5.4 of [9] and using the fact that the non-zero eigenvalue of the linearization at the bifurcation point is negative gives the statement about the qualitative properties of the stationary solutions. □

5. Bistability for the MM-MA system

In this section it is shown that Theorem 4.1, which asserts bistability for the MM system, can be used to show an analogous result for the MM-MA system. The existence of three stationary solutions for the dual futile cycle with suitable parameter values was proved in [16]. The aim here is to obtain information about the stability of these solutions.

**Theorem 5.1.** There exist values of the parameters $k_i$ and values of the conserved quantities $\bar{E}$, $\bar{F}$ and $\bar{Y}$ for which the system (1)-(9) has three stationary solutions, two of which are stable and one of which is a saddle of Morse index one. Their coordinates are compatible with the given values of the conserved quantities. Furthermore heteroclinic orbits connect the saddle to both stable equilibria.
Information about stability is to be propagated from the MM system to the MM-MA system by using a result showing that solutions of the MM-MA system approximate solutions of the MM system for $\epsilon$ small. For this it is useful to introduce a condensed notation for the equations (18)-(18) with fixed values of $\tilde{Y}$, $\tilde{E}$ and $\tilde{F}$. Let $x = (x_Y - x_Y^*, x_{YP} - x_{YP}^*)$ and $y = (\tilde{x}_{YE} - \tilde{x}_{YE}^*, \tilde{x}_{YPP} - \tilde{x}_{YPP}^*, \tilde{x}_{YP} - \tilde{x}_{YP}^*)$. Here the quantities $x_Y^*$ and $x_{YP}^*$ are the coordinates of the stationary solution $B$ given by (51) and the other starred quantities are those obtained from $x_Y^*$ and $x_{YP}^*$ by substituting them into (19)-(24). Then the evolution equations for the variables $x$ and $y$ in the time scale $\tau = \epsilon t$ are of the form (40). The dependence on $\epsilon$ of the right hand sides of these equations arises because $\tilde{Y}$ depends on $\epsilon$. For $\epsilon = 0$ the second equation of the reduced system (41) becomes an algebraic equation which can be solved in the form $y = h_0(x)$ so that $g(x, h_0(x)) = 0$. Substituting this into the first equation of the reduced system (41) gives an equation of the form $x' = f(x, h_0(x))$, which is the MM-system (44)-(45). The conditions $h_0(0) = 0$ and $g(0, 0) = 0$ hold. The limit of these equations for $\epsilon \to 0$ can be analysed using the results of [3] as mentioned in Sect. 3. Recall the form of the extended system in terms of the time variable $t = \epsilon^{-1}\tau$:

\begin{align}
(85) \quad \hat{x} &= \epsilon f(x, y, \epsilon), \\
(86) \quad \hat{y} &= g(x, y, \epsilon), \\
(87) \quad \hat{\epsilon} &= 0. 
\end{align}

At each of the points of the manifold $M_0$ defined by the vanishing of $g(x, y, 0)$ the rank of the matrix which defines the linearization of the system is four. This is equal to the rank of the matrix defining the linearization of the equation $y' = g(x, y, 0)$ for fixed values of the variables $x$. In this case the calculation is particularly simple since the right hand side of the equation is an affine function of the unknowns. The matrix is the direct sum of two diagonal $2 \times 2$ blocks. Each of these blocks has positive determinant and negative trace and thus all eigenvalues have negative real parts. Thus in particular at each of the points of $M_0$ there is a three-dimensional centre manifold. It follows from Theorem 9.1 of [3] that there is a three-dimensional invariant manifold which is a centre manifold for all of these points. It can be chosen to be $C^k$ for any finite $k$ and it can be written in the form $y = h(x, \epsilon)$, where $h(x, 0) = h_0(x)$. This manifold can be coordinatized by $x$ and $\epsilon$ and the restriction of the system to the manifold can be represented in the form

\begin{align}
(88) \quad x' &= f(x, h(x, \epsilon)), \\
(89) \quad \epsilon' &= 0.
\end{align}

Since it has been shown that the system for $\epsilon = 0$ has three hyperbolic stationary solutions it follows that the system for small positive values of $\epsilon$ also has at least three stationary solutions. The heteroclinics connecting the unstable equilibrium to the stable one also persist for small values of $\epsilon$. The three stationary solutions are also equilibria of the full system and it follows from the theorem of Shoshtashvili that two of them are stable and the other is a saddle of Morse index one connecting to both stable equilibria. There are no more stationary solutions due to the results of [16]. For a fixed value of $\epsilon > 0$ and using the values of $\tilde{Y}$, $\tilde{E}$ and $\tilde{F}$ positive quantities $x_{YP}$, $\tilde{x}_{YE}$ and $\tilde{x}_{F}$ can be determined which define positive stationary solutions of the rescaled version of (11)-(19). Undoing the rescaling gives solutions
of the original system which all have the same values of the conserved quantities. This completes the proof of Theorem 5.1.

Since several conditions are imposed on the parameters of the system, we want to summarize here the remaining degrees of freedom. We already saw that the simplifying condition (38), given $a_1, a_2, a_3, a_4, b, \tilde{E}$ and $\tilde{F}$, leaves 4 degrees of freedom for the coefficients $k_i$. The equilibrium condition (53) is a condition on the parameters $a_1, a_2, a_3$ and $a_4$ and leave us three degrees of freedom for them: given $a_1, a_2, a_3$ and $a_4$ we have $a_2 = a_1 a_2 / a_4$. Putting this into the bifurcation condition (54) gives $a_4 < a_3$, which is only a slight restriction to the freedom of choosing $a_3$. Hence, the parameters $b, a_1, a_4 < a_3, \tilde{E}, \tilde{F}$ and for example $k_1, k_4, k_{10}$ and $k_7$ can be chosen freely and then all other parameters in the system (the other $k_i$, $a_2$ and $\tilde{Y}$) are determined uniquely by the conditions (38), (53) and (54). In particular, we can choose $a_1$ and $a_4$ in such a way that the system is indeed in the cusp region where bistability holds.

We can also look at those conditions from the point of view of fixed reaction constants $k_i$. We saw that we can see the simplifying condition (38) as a comparison between reaction constants of production and consumption of substrate-enzyme complexes during phosphorylation. Once the simplifying assumption has been made, the equilibrium condition (53) translates to

$$\left( \frac{\tilde{E}}{\tilde{F}} \right)^2 = \frac{k_{12}}{k_3} \frac{k_9}{k_6}. \tag{90}$$

The quantities of enzymes $\tilde{E}, \tilde{F}$ have to be tuned accordingly. Together with bifurcation condition (54), equation (90) leads to the inequality

$$\frac{k_{12}}{k_3} > \frac{k_9}{k_6}. \tag{91}$$

The quotients $\frac{k_{12}}{k_3}$ and $\frac{k_9}{k_6}$ again compare reaction constants: $\frac{k_{12}}{k_3}$ compares the constants of the reactions

$$Y E \xrightarrow{k_3} Y P + E, \quad Y P F \xrightarrow{k_{12}} Y + F,$$

and the second quotient the analogous reactions for the double phosphorylated form $YPP$. In order to understand the biological meaning of this inequality it may be useful to state its concrete interpretation in terms of the reactions. The relative rate of production of the phosphorylated and dephosphorylated form is greater for the first cycle than for the second one. Furthermore the total quantity of $Y$ has to be tuned accordingly.

6. Further Directions

An important cellular signalling system is the MAPK cascade. It contains three layers, each of which is a multiple phosphorylation loop of the type of the multiple futile cycle. They 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.

Note that we later focus on the two upper layers of this cascade and use notation \( X \) and \( Y \) for the substrates \( MKKK \) and \( MKK \) respectively. A fundamental study, both theoretical and experimental, of this system was carried out by Huang and Ferrell [7]. They modelled it by a system of ODE using mass action kinetics. Numerical and heuristic evidence has been found indicating that this system has periodic solutions [12]. It may be that there is also chaotic behaviour in solutions of this system [19]. Here we will concentrate on the issue of oscillations. To what extent can the ideas used to prove bistability here be applied to give an analytical proof of oscillatory behaviour in the MAPK cascade and can Michaelis-Menten reduction help?

MAPK cascades are often embedded in positive or negative feedback loops and it was suggested in [8] that negative feedback could lead to oscillations in this context. A model was introduced where the MAPK cascade is in a negative feedback loop belonging to a more complex network of chemical reactions taking place in the cell. In this model a feedback loop between a phosphorylated form of one protein and a substrate in a layer above is induced by chemical reactions external to the cascade. These other reactions are not modelled in detail - instead a generic form is chosen for the coupling. In the paper [8] a Michaelis-Menten-like description of the cascade was used but the assumptions leading to the equations used there were not stated. An insightful discussion of the modelling issues involved has been given in [14]. The equations in [8] with feedback loop are of the type called 'phenomenological' in [14]. An equation with mass action kinetics like the MM-MA model considered above is called 'mechanistic'. Finally a model like the MM model considered above is referred to as 'reduced mechanistic'. We will adopt the terminology of [14] in what follows. In a phenomenological model reduced equations for the individual reactions in a network are used and this may fail to capture interactions between the different reactions.

The difference between phenomenological and reduced mechanistic models will now be discussed in some detail in the case of the dual futile cycle. A model for the dual futile cycle can be extracted from the feedback model of [8] as follows. We take the second layer of the cascade and set the concentration of the phosphorylated form of the protein in the first layer, \([MKKK - P]\) in the notation of [8], to a
constant value. This gives equations for the quantities \([MKK]\) and \([MKK - PP]\) corresponding to our \(x_Y\) and \(x_YPP\). For example, the equation for the first of these is of the form

\[
\frac{d[MKK]}{d\tau} = v_0 - v_3
\]

where

\[
v_3 = \frac{(k_3[MKK - P])[MKK]}{K_3 + [MKK]}.
\]

This quantity \(v_3\) corresponds to \(v_1\) in our notation and the key difference is that it does not depend on \([MKK - P]\) while our \(v_1\) does depend on \(x_YPP\). In this case the model extracted from [8] is the phenomenological one while our model is the reduced mechanistic one.

Returning to the question of oscillations in the MAPK cascade, note first that according to [12] oscillations are already found numerically in a truncated version of the Huang-Ferrell model with only two layers, the first involving only one phosphorylation and the second involving two. For simplicity we now concentrate on that truncated model. More precisely, the reactions involved are the following:

1st layer:
\[
X + E \xrightarrow{k_1} XE \xrightleftharpoons{k_2} XP + E
\]

\[
XP + F \xrightleftharpoons{k_1} XP,F \xrightarrow{k_3} X + F
\]

2nd layer:
\[
Y + XP \xrightleftharpoons{k_4} YXP \xrightarrow{k_5} YP + XP
\]

\[
YP + XP \xrightleftharpoons{k_7} YPX \xrightarrow{k_8} YPP + XP
\]

\[
YPP + G \xrightleftharpoons{k_{10}} YPPG \xrightarrow{k_{11}} YP + G
\]

\[
YP + F \xrightleftharpoons{k_{13}} YPG \xrightarrow{k_{14}} Y + G
\]
Michaelis-Menten reduction is more subtle in this case due to the fact that a single substance, \(XP\), is both a substrate in one reaction and an enzyme in others. This problem has been analysed in \[14\] and \[15\]. In fact these papers treat more general sequences of coupled multiple phosphorylation loops. The numerical results of \[15\] lead to the conclusion that the periodic solutions of the mechanistic model seen in \[12\] are also present in the reduced mechanistic model. They also show that the reduced mechanistic model often gives more accurate results than a phenomenological model even when the parameter \(\epsilon\) is not very small.

While the reduction procedure of \[14\] and \[15\] is close to being a direct generalization of the reduction to the MM system done above to the case of the truncated Huang-Ferrell model it is not exactly the same and it needs to be modified slightly to give a limit for \(\epsilon \to 0\) which is well-behaved from the point of view of GSPT. This modification will now be carried out. The notation is chosen to fit that in the rest of the paper. The proteins in the first and second layers are denoted by \(X\) and \(Y\) respectively. The kinase in the first layer is denoted by \(E\) and the phosphatases in the first and second layers by \(F\) and \(G\). The basic unknowns are the concentrations of \(X\), \(XP\), \(Y\), \(YP\), \(XE\), \(XP.F\), \(X.P\), \(YP.XP\), \(Y.P\), \(YP.P\), \(E\), \(F\) and \(G\). The conservation laws for the three enzymes \(E\), \(F\) and \(G\) and the two substrates \(X\) and \(Y\) can be used to eliminate five of the variables. A key idea in \[14\] is to introduce a new variable \(x_\ast = x_{XP} + x_{Y.P}\), which leads to a cancellation in one of the evolution equations. New variables are introduced by rescaling in a way similar to that done for the dual futile cycle above. They are defined by the relations
\[
\begin{align*}
x_\ast &= \epsilon \tilde{x}_\ast, \quad x_{EX} = \epsilon^2 \tilde{x}_{EX}, \quad x_{F, XP} = \epsilon^2 \tilde{x}_{F, XP}, \quad x_{XP.Y} = \epsilon \tilde{x}_{XP.Y}, \quad x_{XP.YP} = \epsilon \tilde{x}_{XP.YP}, \quad x_{G, YP} = \epsilon \tilde{x}_{G, YP}, \quad x_{G, YPP} = \epsilon \tilde{x}_{G, YPP}, \quad x_{E} = \epsilon^2 \tilde{x}_{E}, \quad x_{F} = \epsilon^2 \tilde{x}_{F}, \quad x_{G} = \epsilon \tilde{x}_{G}, \quad \tau = \epsilon t.
\end{align*}
\]

In addition, and this is the difference to what was done in \[14\], two of the reaction constants are rescaled, \(k_1 = k_1 \epsilon\) and \(k_6 = k_4 \epsilon\). These are the reaction constants for the binding of the enzymes \(E\) and \(F\) to their substrates. The result is the system

\[
\begin{align*}
\frac{d \tilde{x}_\ast}{d \tau} &= k_3 \tilde{x}_{EX} - \tilde{k}_1 (\tilde{x}_\ast - \tilde{x}_{XP,Y} - \tilde{x}_{XP,YP}) \tilde{x}_F + k_5 \tilde{x}_{F, XP} \\
\frac{d \tilde{x}_{YP}}{d \tau} &= -k_7 \tilde{x}_Y (\tilde{x}_\ast - \tilde{x}_{XP,Y} - \tilde{x}_{XP,YP}) + k_8 \tilde{x}_{XP.Y} + k_{12} \tilde{x}_{G, YP}, \\
\frac{d \tilde{x}_{YP.P}}{d \tau} &= k_{15} \tilde{x}_{XP.YP} - k_{16} \tilde{x}_{YP.P} \tilde{x}_G + k_{17} \tilde{x}_{G, YPP}, \\
\epsilon \frac{d \tilde{x}_{EX}}{d \tau} &= \tilde{k}_1 \tilde{x}_X \tilde{x}_E - k_2 \tilde{x}_{EX} - k_3 \tilde{x}_{EX}, \\
\epsilon \frac{d \tilde{x}_{F, XP}}{d \tau} &= \tilde{k}_4 (\tilde{x}_\ast - \tilde{x}_{XP,Y} - \tilde{x}_{XP,YP}) \tilde{x}_F - k_5 \tilde{x}_{F, XP} - k_6 \tilde{x}_{F, XP}, \\
\epsilon \frac{d \tilde{x}_{XP.Y}}{d \tau} &= k_7 \tilde{x}_Y (\tilde{x}_\ast - \tilde{x}_{XP,Y} - \tilde{x}_{XP,YP}) - k_8 \tilde{x}_{XP.Y} - k_9 \tilde{x}_{XP,Y}, \\
\epsilon \frac{d \tilde{x}_{XP.YP}}{d \tau} &= k_{13} \tilde{x}_{YP}(\tilde{x}_\ast - \tilde{x}_{XP,Y} - \tilde{x}_{XP,YP}) - k_{14} \tilde{x}_{XP.YP} - k_{15} \tilde{x}_{XP,YP}, \\
\epsilon \frac{d \tilde{x}_{G, YP}}{d \tau} &= k_{10} \tilde{x}_{YP} \tilde{x}_G - k_{11} \tilde{x}_{G, YP} - k_{12} \tilde{x}_{G, YP}, \\
\epsilon \frac{d \tilde{x}_{G, YPP}}{d \tau} &= k_{16} \tilde{x}_{YP.P} \tilde{x}_G - k_{17} \tilde{x}_{G, YPP} - k_{18} \tilde{x}_{G, YPP}.
\end{align*}
\]
It is then possible to pass to the limit $\epsilon \to 0$. Carrying out steps analogous to those done in the case of the dual futile cycle in a routine way gives

\begin{align}
\frac{d\tilde{x}_*}{d\tau} &= \frac{c_1 x_X - c_2 \tilde{x}_*}{1 + d_1 \tilde{x}_X - 1 + d_2 \tilde{x}_* + b_1 \tilde{x}_Y + b_2 \tilde{x}_Y P}, \\
\frac{d\tilde{x}_Y}{d\tau} &= \frac{a_1 \tilde{x}_* \tilde{x}_Y}{1 + b_1 \tilde{x}_Y + b_2 \tilde{x}_Y P - \frac{a_2 \tilde{x}_Y P}{1 + b_3 \tilde{x}_Y P + b_4 \tilde{x}_Y P P}}, \\
\frac{d\tilde{x}_Y PP}{d\tau} &= \frac{a_3 \tilde{x}_* \tilde{x}_Y P}{1 + b_1 \tilde{x}_Y + b_2 \tilde{x}_Y P - \frac{a_4 \tilde{x}_Y PP}{1 + b_3 \tilde{x}_Y P + b_4 \tilde{x}_Y P P}}.
\end{align}

The constants in this system can be expressed explicitly in terms of the parameters in the full system. To get a closed system the conservation laws for the total amounts of the substrates $X$ and $Y$ must be used.

This system may be compared with one extracted from the system of [8] in a way similar to that done for the dual futile cycle above. This time we take the equations for the substances in the first two layers and remove the explicit feedback. This is done by setting the concentrations $[\text{MAPK}]$, $[\text{MAPK} - P]$ and $[\text{MAPK} - \text{PP}]$ to zero. Then the equations for those quantities are satisfied identically and the dependence of the first reaction rate $v_1$ in [8] on $[\text{MAPK} - \text{PP}]$ disappears. In the resulting system the evolution equations for the second layer substrates $[\text{MKK}]$ and $[\text{MKK} - \text{PP}]$ are independent of the variables involving the third layer substrate $[\text{MAPK}]$. This is in contrast to the system (103)-(105) where there is an intrinsic negative feedback loop. For if the right hand sides of these equations are denoted by $f_i$, $i = 1, 2, 3$, then $\partial f_1 / \partial \tilde{x}_Y P P < 0$ while $\partial f_3 / \partial \tilde{x}_* > 0$. Multiplying the two different signs of these derivatives gives the claimed negative feedback. (For background on the definition and significance of feedback loops the reader is referred to [13].) In the equations extracted from the system of [8], on the other hand, we have $\partial f_1 / \partial [\text{MK} - \text{PP}] = 0$ and there is no intrinsic feedback. According to common heuristics the negative feedback loop in (103)-(105) can be seen as a possible source of oscillations. The interaction not captured in the phenomenological description is what is called sequestration. In certain circumstances a lot of the substance $XP$ is bound to its substrates $Y$ and $YP$ and so is not available to take part in the reaction converting it back to $X$ in the first layer.

The equations (103)-(105) are a reduced system for the truncated Huang-Ferrell model in the sense of GSPT. The transverse eigenvalues (as defined in [8]) have negative real parts. In contrast to the system arising for the dual futile cycle the right hand side of the equations entering the computation of the transverse eigenvalues is no longer affine. However the only nonlinear terms are in the equation for $\tilde{x}_{E,XP}$ and the derivatives of these terms do not influence the eigenvalues. All the other terms are either diagonal or belong to $2 \times 2$ blocks which are similar to those already treated in the case of the dual futile cycle. Hence if it were possible to show that (103)-(105) has a hyperbolic stable periodic solution then it would follow immediately that for $\epsilon$ sufficiently small the full system also has a periodic solution. This is because hyperbolic stable periodic solutions persist under regular perturbations of a dynamical system. Thus the task of proving the existence of the periodic solutions of the truncated Huang-Ferrell model found numerically in [12] reduces to proving an analogous statement for the reduced system. Since the former has dimension nine, even after exploiting all conservation laws, and the latter has dimension three, this could be a significant advantage. The question of existence of periodic solutions of the reduced system will be investigated elsewhere.
Another interesting question concerns the dependence of the results of Theorems 4.1 and 5.1 on the modelling by mass-action kinetics. A crucial argument in the present paper consists in verifying the conditions for a cusp bifurcation to take place. It is already technical for the mass-action kinetics where equilibria and derivatives can be computed explicitly. On the one hand, such computations seem difficult with a more general model for the kinetics of the chemical reactions. But on the other hand, the fact that the evolution equations have a polynomial reaction rates is not essential. Models with more general right hand side could lead to similar results. Furthermore, we conjecture that the assumption (38) is not a necessary condition, but only a convenience to simplify the computations.

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