Dynamical features of the MAPK cascade

Juliette Hell\textsuperscript{1} and Alan D. Rendall\textsuperscript{2}

\textsuperscript{1}Freie Universität Berlin, jhell@zedat.fu-berlin.de.
\textsuperscript{2}Johannes Gutenberg-Universität Mainz, rendall@uni-mainz.de.

Abstract

The MAP kinase cascade is an important signal transduction system in molecular biology for which a lot of mathematical modelling has been done. This paper surveys what has been proved mathematically about the qualitative properties of solutions of the ordinary differential equations arising as models for this biological system. It focusses, in particular, on the issues of multistability and the existence of sustained oscillations. It also gives a concise introduction to the mathematical techniques used in this context, bifurcation theory and geometric singular perturbation theory, as they relate to these specific examples. In addition further directions are presented in which the applications of these techniques could be extended in the future.

1 Introduction

An important process in cell biology is the transmission of information by signalling networks from the cell membrane to the nucleus, where it can influence transcription. This provides the cell with a possibility of reacting to its environment. A common module in many signalling networks is the mitogen activated protein kinase cascade (MAPK cascade). It is the subject of what follows. The MAPK cascade is a pattern of chemical reactions which is widespread in eukaryotes \cite{46}. The individual proteins which make up the cascade differ between different organisms and between different examples within a given organism but what is common is a certain architecture. The cascade consists of three parts which we will call layers. Each layer is a phosphorylation cycle or, as it is sometimes called, a multiple futile cycle \cite{43}.

A multiple futile cycle consists of a protein $X$ which can be phosphorylated by a kinase $E$ at $n$ sites. The resulting phosphoproteins can be dephosphorylated by a phosphatase $F$. The cases of interest for the MAPK cascade are $n = 1$ and $n = 2$. The following sketch shows the MAPK cascade with three layers. Each plain arrow marked with an enzyme represents an enzymatic reaction, i.e. $Y \xrightarrow{G} Z$ stands for the chemical reactions $Y + G \rightleftharpoons YG \rightarrow Z + G$. The dotted arrows between a
phosphorylated protein and an enzyme in the next layer stand for equality, i.e. $Z \rightarrow G$ means $Z = G$. The first layer of the cascade is a simple phosphorylation, i.e. $n = 1$. The next layers are double phosphorylations, i.e. $n = 2$. The species $X_i$ are the proteins in the cascade, or in more detail $X_1 = MAPKKK$ (MAP kinase kinase kinase), $X_2 = MAPKK$ (MAP kinase kinase), $X_3 = MAPK$ (MAP kinase).

It is important that even when there is more than one site where the protein can be phosphorylated there is only one kinase and one phosphatase. Thus different phospho-forms of the protein may compete for binding to one of the enzymes. A MAPK cascade consists of three layers, each of which is a multiple futile cycle with a different protein. The connection between the layers is that the maximally phosphorylated forms of the protein in the first and second layers are the kinases which catalyse the phosphorylations in the second and third layers, respectively. The most extensively studied example in mammals is that where the proteins in the three layers are Raf, MEK and ERK.

The subject of what follows is mathematical modelling of the MAPK cascade. It turns out that this system has a rich dynamics which needs mathematical modelling for its understanding. Pioneering work in studying this question was done by Huang and Ferrell [22]. In that paper the authors presented both theoretical and experimental results and compared them. Their experiments where done in cell extracts from *Xenopus* oocytes. On the theoretical side they wrote down a system of ordinary differential equations describing the time evolution of the concentrations of the substances involved in the MAPK cascade and simulated these equations numerically. The results of the simulations reproduced important qualitative features of the experimental data.

In order to model the reactions taking place it is necessary to make assumptions about the kinetics. In many enzymatic reactions the concentrations of the enzymes are much less than those of the corresponding substrates. This cannot necessarily be assumed in the case of the MAPK cascade. In particular there are substances, for example the dou-
bly phosphorylated form of MEK, which are the substrate for one reaction and the enzyme for others. For this reason the model in [22] uses a Michaelis-Menten scheme for each reaction with a substrate, an enzyme and a substrate-enzyme complex without making an assumption of small enzyme concentration. The elementary reactions involved are assumed to obey mass action kinetics. There are seven conservation laws for the total amounts of the three substrates and the four enzymes which are not also substrates (one kinase, \( E_1 \) and three phosphatases, \( F_i, i = 1, 2, 3 \)). It is assumed that the phosphorylation and dephosphorylation are distributive and sequential. In other words in any one encounter of a substrate with an enzyme only one (de-)phosphorylation takes place, after which the enzyme is released. To add or remove more than one phosphate group more than one encounter is necessary. The phosphorylations take place in a particular order and the dephosphorylations in the reverse order. These assumptions are implemented in the model of [22]. They have also frequently been adopted in other literature concerned with the modelling of this system and we will call them the standard assumptions. The authors of [22] mention that they also did simulations for cases where one or more of the reactions is processive (i.e. more than one phosphate group is added or removed during one encounter). The standard assumptions may not be correct in all biological examples but they are a convenient starting point for modelling which can later be modified if necessary.

In real biological systems the MAPK cascade is part of a larger signalling network and cannot be seen in isolation. Nevertheless, one can hope to obtain insights by first understanding the isolated cascade and later combining it with other reactions. Similarly it can be helpful to approach an understanding of the cascade itself by studying its component parts, the multiple futile cycles. The most frequent approaches to these modelling questions in the literature use simulations and heuristic considerations. An alternative possibility, which is the central theme of this paper, is to prove mathematical theorems about certain aspects of the dynamics with the aim of obtaining insights complementary to those coming from the numerical procedures. The number of mathematically rigorous results on this subject known up to now is rather limited. The aim of this paper is to survey the results of this type which are available and to outline perspectives of how they might be extended. At the same time it gives an introduction to some of the techniques which are useful in this kind of approach. The description starts from the simplest models and proceeds to more complicated ones. We discuss successively the simple futile cycle, the dual futile cycle and the full cascade. The description also proceeds from simple dynamical features to more complicated ones, from multistationarity to multistability and then to sustained oscillations. After this core material has been treated further directions are explored. What happens when the basic cascade is embedded in feedback loops? What happens in systems with other phosphorylation schemes?
2 The simple futile cycle

In this section we look at the case \( n = 1 \) of the multiple futile cycle, in other words we isolate the first layer of the cascade [1]. We omit the index 1 of the chemical species for clarity in this section, since the other layers will play no role here. Modelling this system in a way strictly analogous to that applied to the MAPK cascade in [22] leads to a system of six equations for the substrates \( X \) and \( XP \), the enzymes \( E, F \) and the substrate-enzyme complexes \( X \cdot E \) and \( XP \cdot F \). There are three conserved quantities, which are the total amounts of the enzymes and the substrate, \( E_{\text{tot}} = [E] + [X \cdot E] \), \( F_{\text{tot}} = [F] + [XP \cdot F] \) and \( X_{\text{tot}} = [X] + [XP] + [X \cdot E] + [XP \cdot F] \). where here and in the following \([Z]\) denotes the concentration of the species \( Z \). These can be used to eliminate three of the equations if desired. When the evolution is modelled by mass-action kinetics, these manipulations can be done explicitly.

Suppose we have a system \( \dot{x}_i = f_i(x) \), \( x = (x_1, \ldots, x_n) \in \mathbb{R}^n \) representing the dynamics of a chemical reaction network. The \( x_i(t) \) are the concentrations of the substances involved as functions of time and the dot denotes the time rate of change. A stationary solution (or steady state) is one which satisfies for all \( i \in \{1, \ldots, n\} \), \( x_i(t) \equiv x_{i,0} \) for some fixed concentrations \( x_0 = (x_{1,0}, \ldots, x_{n,0}) \). Thus it satisfies \( \dot{x} = 0 \) or equivalently \( f(x_0) = 0 \). A solution \( x(t) \) is said to converge to the steady state \( x_0 \) if \( \lim_{t \to \infty} x(t) = x_0 \). This is an idealization of the situation where an experimental system settles down to a steady state on a sufficiently long time scale. For instance in the experiments of [22] the system was found to approach a steady state after 100 minutes. Corresponding behaviour was found in the simulations. There is no reason why a chemical system should behave in this simple way for all initial data. The results of [22] indicate that it does so for the data considered there. Even if for a particular system all solutions converge to a steady state, it may be that there exist more than one steady state for fixed values of the total amounts of the substances involved. In the language of chemical reaction network theory [12] there may be more than one steady state in one stoichiometric compatibility class. This is the phenomenon of multistability. It is important for biological processes such as cell differentiation.

In the case of the simple futile cycle it was proved in [3] that there is always exactly one steady state for fixed values of the total amounts \( E_{\text{tot}}, F_{\text{tot}} \) and \( X_{\text{tot}} \) and that all other solutions converge to that steady state. The steady state is globally asymptotically stable and bistability is ruled out in this case. We cannot enter into the details of the proof of this result here but it is appropriate to mention some of the key ideas involved. Suppose that the system \( \dot{x}_i = f_i(x) \) has the property that \( \partial f_j / \partial x_i > 0 \) for all \( i \neq j \) and all \( x \). Then the system is called monotone. If a system does not satisfy this property we may try to make it do so by reversing the signs of some of the variables. In other words we replace the variables \( x_i \) by \( y_i = \epsilon_i x_i \), where each \( \epsilon_i \) is plus or minus one. In general a system is called monotone if there is a mathematical transformation of this kind which makes all partial derivatives of the right hand sides of the equations with \( i \neq j \) positive. There is a graphical criterion to decide whether this is possible. Define a graph which has a vertex for each variable \( x_i \) and which
has an oriented edge connecting node $i$ to node $j$ if $\partial f_j / \partial x_i \neq 0$. Label each edge with the sign of the corresponding derivative. Alternatively we can use the convention that a positive sign is represented by a normal arrow while a negative sign is represented by a blunt-headed arrow. This object is called the species graph. For example the species graph of the simple futile cycle is the following.

Since the use of terms concerning feedback loops is not always consistent between different sources in the literature we specify the terminology which we will use. A feedback loop is a sequence of arrows which starts at one node and ends at the same node, i.e. a cycle. It is called a positive or negative feedback loop according to whether the number of edges with a negative sign it contains is even or odd. More precisely this object may be called a directed feedback loop, while the corresponding definition where the orientation of the edges is ignored is called an undirected feedback loop.

Suppose we have a system of ordinary differential equations for which the sign of the derivative $\partial f_j / \partial x_i$ is independent of $x$ for each fixed $i$ and $j$. Then it can be proved that the system is monotone if and only if the species graph contains no negative undirected feedback loops of length greater than one. In the case of the simple futile cycle the species graph contains at least one negative feedback loop. This is true both for the full six-dimensional system and for the three-dimensional system obtained by eliminating the concentrations of $E$, $F$ and $XP$ using the conservation laws. However, it was shown in [6] that there is a different type of transformation which makes this system monotone. In this transformation the concentrations are replaced as variables by the extents of the reactions. The resulting monotone system has additional good properties and this allows the property of convergence to a unique steady state to be concluded for the original system.

Many chemical systems have interesting limiting cases obtained by letting certain combinations of reaction constants tend to zero. This can lead to a significant reduction in the number of variables in the system and make analytical investigations simpler. Under suitable circumstances solutions of the limiting system approximate solutions of the original system in a certain parameter regime. This can be illustrated by the case of the simple futile cycle. For the species $E, F, X \cdot E, XP \cdot F$ involving the enzymes $E, F$, alone or in a complex, scale the concentrations with a parameter $\epsilon > 0$ while the remaining concentrations $[X]$ and $[XP]$ are not rescaled. In other words, if $y = ([E], [F], [X \cdot E], [XP \cdot F])$ is the vector of their concentrations, define $\tilde{y} = \epsilon y$, where $\epsilon$ is a positive constant. If
\( x = ([X], [XP]) \) is the vector of the remaining concentrations, the original system with mass-action kinetics is of the form
\[
\begin{align*}
\dot{x} &= f(x, y), \\
\dot{y} &= g(x, y),
\end{align*}
\] (3)
where \( f, g \) are linear in each of the concentrations, i.e. entries of the vectors \( x, y \). The smallness of \( \epsilon \) corresponds to the fact that the amount of enzymes is small compared to the amount of the other species. Define a new time coordinate by \( \tau = \epsilon t \) and let a prime denote the derivative with respect to time \( \tau \). The time \( \tau \) is called the slow time scale because its velocity \( \frac{d\tau}{dt} = \epsilon \) is small. This gives rise to a system of the general form
\[
\begin{align*}
x' &= f(x, \tilde{y}), \\
\epsilon \tilde{y}' &= g(x, \tilde{y}).
\end{align*}
\] (4)
In the limit \( \epsilon \to 0 \), and dropping the tildes, the second equation \( \epsilon y' = g(x, y) \) changes from being an ordinary differential equation to being an algebraic equation \( 0 = g(x, y) \). Under favourable conditions this equation can be solved for \( y \) in terms of \( x \) and the result \( y = h(x) \) substituted into the first equation to give an equation for \( x \) alone,
\[
x' = f(x, h(x)).
\] (5)
The result is a system with fewer unknowns. The degeneration to an algebraic equation means that the limit is singular. It may be asked whether the solutions themselves nevertheless behave in a regular way in the limit and indeed this is the case under certain conditions. The appropriate mathematical techniques for studying this are known as geometric singular perturbation theory (GSPT) as introduced by Fenichel, see [13]. This theory will be discussed in more detail below. The particular type of limit just exhibited for the simple futile cycle is sometimes called a Michaelis-Menten limit. In this case it leads to a two-dimensional system. The conservation law for the total amount of substrate survives in the limit in a simplified form and can be used to reduce the system further to a single equation. Let the reaction constants for complex formation, complex dissociation and product formation be denoted by \( k_i, d_i \) and \( a_i \) respectively, with \( i = 1 \) corresponding to phosphorylation and \( i = 2 \) to dephosphorylation.
\[
\begin{align*}
X + E &\xrightarrow{k_1} XE &\xrightarrow{a_1} XP + E \\
XP + F &\xrightarrow{k_2} XP \cdot F &\xrightarrow{a_2} X + F
\end{align*}
\] (6)
The Michaelis constants are defined as usual by $K_{m,i} = \frac{d_i + k_i}{a_i}$. Then the equation can be written as

$$\frac{d}{dt}[XP] = \frac{-k_2 F_{tot}(X_{tot} - [XP])}{K_{m,2} + X_{tot} - [XP]} + \frac{k_1 E_{tot}[XP]}{K_{m,1} + [XP]},$$

(7)

After this reduction it is possible to get an explicit formula for the unique steady state by solving a quadratic equation in the variable $[XP]$. For $\epsilon$ small the total amounts of the enzymes are small compared to the total amount of substrate and this is sometimes described by saying that the enzymes are close to saturation. Varying one of the parameters in the system and monitoring the concentration of phosphorylated protein gives a response function. The main concern of [17] is the form of this function, which corresponds to the property of ultrasensitivity: A small change in the parameter leads to a large change in the value of the response function. This property is quantitative rather than qualitative and not obviously amenable to the application of the analytical techniques to be discussed in this paper. It is an interesting question, whether these techniques can be extended so as to give more information about quantitative properties. This will not be discussed further here except to mention that ultrasensitivity was also the central feature of interest in [22], where the response of the concentration of maximally phosphorylated ERK as a function of that of the first kinase in the cascade was investigated.

3 The dual futile cycle

This section is concerned with the case $n = 2$ of the multiple futile cycle. The second layer of the MAPK cascade is an example of a dual futile cycle. We omit the index indicating the layer in this section, since we consider a single layer of the cascade. The basic system with mass action kinetics can be found, for instance, in [44]. It is possible to do a Michaelis-Menten reduction in a similar way to that done in the last section. We recall that this consists in scaling the concentration $Z$ of the species containing one of the two enzymes $E$, $F$, alone or in a complex, via the transformation $\tilde{y} = \epsilon y$ of their concentration vector $y$, as well as the time by $\tau = \epsilon t$. The limit $\epsilon \to 0$ can be reduced to a lower dimensional ODE by solving an algebraic equation. This leads, after using all conservation laws to eliminate as many variables as possible, to a two-dimensional system, which will be called the MM system (for Michaelis-Menten). More details on this can be found in [20]. The system can be written in the form

$$\frac{d}{dt}[X] = \frac{-k_1 K^{-1}_{m,1} E_{tot}[X]}{1 + K^{-1}_{m,1}[XP] + K^{-1}_{m,3}[XPP]} + \frac{k_2 K^{-2}_{m,2} F_{tot}[XP]}{1 + K^{-1}_{m,2}[XP] + K^{-1}_{m,4}[XPP]},$$

(8)

$$\frac{d}{dt}[XPP] = \frac{k_3 K^{-1}_{m,3} E_{tot}[XP]}{1 + K^{-1}_{m,1}[XP] + K^{-1}_{m,3}[XPP]} - \frac{k_4 K^{-1}_{m,4} F_{tot}[XP]}{1 + K^{-1}_{m,2}[XP] + K^{-1}_{m,4}[XPP]},$$

(9)

When using the conservation law for $X_{tot}$ we have the choice, which of the three concentrations $[X]$, $[XP]$ or $[XPP]$ to eliminate. Here the equation for $[XP]$ has been discarded and on the right hand side of the equations $[XP]$ should be regarded as an abbreviation for $X_{tot} - [X] - [XPP]$. In this
case the MM system is monotone, as defined in the previous section. Since
it is two-dimensional this implies that all solutions converge to steady
states. Moreover it can be shown using GSPT that for $\epsilon$ small but non-
zero almost all initial data give rise to solutions which converge to steady
states [15]. For general $\epsilon$ it was not known until very recently whether
the corresponding statement was true. In [11] the authors used computer-
assisted methods to find periodic solutions of this system, indicating that
the statement is false for general $\epsilon$. They do not only use dynamical
simulations but also use computer algebra to help implement a theoretical
approach to finding Hopf bifurcations. They do not obtain evidence for
the existence of stable periodic solutions, so that it would be consistent
with their findings if periodic solutions, while present, were only relevant
for exceptional initial conditions. Despite the results of [11] the global
behaviour of solutions of the dual futile cycle is much less well understood
than that in the case of the simple futile cycle.

What has been proved is that multistationarity (existence of more than
one steady state) occurs in the dual futile cycle for certain values of the
parameters [44]. In fact it is known that there exist up to three steady
states for given values of the total amounts and that there are never more
than three. The proof of the existence result can be split into two steps.
In the first step the equations for steady states are partly solved explicitly.
This leaves a system of two equations for two unknowns. The second step
involves taking a limit of these equations as a parameter $\epsilon$ tends to zero.
This limit is essentially the Michaelis-Menten limit discussed above. Since
we are dealing with steady states the factor $\epsilon$ in the equation for $y$ plays no
role and the limit is regular. In the limit a single equation for one variable
is obtained and this is relatively easy to analyse. It is possible to find
cases where there are three solutions of the equation $F(x) = 0$ for steady
states of the equation with $\epsilon = 0$, all of which satisfy $dF/dx \neq 0$. This
implies, using the implicit function theorem, that corresponding solutions
exist for $\epsilon$ small but positive. This argument gives no information on the
important issue of stability of these solutions. A steady state $x_0$ will only
be observed in practise if it is stable. This means that if a solution starts
close enough to $x_0$ it will stay close to $x_0$ for all future times. For example
if the linearization at the equilibrium $x_0$ has only eigenvalues with strictly
negative real parts, then the equilibrium $x_0$ is stable.

On the level of simulations multistationarity was already observed for
the system with mass action kinetics in [30] and it was found that two
of the three steady states are stable. Simulations in [32] indicated that
these features are already present in the MM system. On the other hand
until recently there was no mathematical proof of bistability for the dual
futile cycle. A strategy suggested by what has been said up to know for
obtaining such a proof is to first prove bistability for the MM system and
then use GSPT to conclude the corresponding result for the mass action
system. This strategy was carried out in [20] and in that paper we proved
bistability. We now sketch the main lines of the proof.

In the previous section, we saw that the MM reduction is the singular
limit as $\epsilon \to 0$ of the system on the slow time scale [4], where $g(x, \tilde{y}) =
0 \Leftrightarrow \tilde{y} = h(x)$. Now consider the fast time scale, i.e. the same equations
expressed in the original time $t$, augmented by the trivial evolution of the

4
parameter $\epsilon$. This system is called the extended system.

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

(10)

The curve $\{(x, \tilde{y} = h(x), \epsilon = 0)\}$ is a curve of equilibria in the extended system. The linearization of the extended system (10) at such an equilibrium admits two zero eigenvalues with the corresponding eigenvector being $(0, 0, 1)$ pointing in direction of $\epsilon$ orthogonally to the $(x, y)$-plane, and a second vector in the $(x, y)$-plane tangent to the curve $\{(x, h(x))\}$. If the linearization $D_y g(x, h(x), 0)$ admits only eigenvalues with strictly negative real parts, then the center eigenspace (i.e. the eigenspace spanned by the eigenvectors associated to purely imaginary eigenvalues) is exactly two dimensional. The eigenspace is tangential to a manifold called the center manifold: see [41] for details about center manifold theory. The (local) center manifold $M^c(x, h(x), 0)$ of such an equilibrium is an invariant manifold containing all bounded solutions sufficiently near the equilibrium. The center manifold $M^c$ can be written as a graph of a function $\Psi$ over the center eigenspace:

$$M^c(x, h(x), 0) = \{(x, \Psi(x, \epsilon), \epsilon), \epsilon \text{ small}\},$$

(11)

with $\Psi(x, 0) = h(x)$. The manifold $M^c$ is tangent to the center eigenspace of the extended system at each equilibrium $(x, h(x), 0)$. Because of the equation $\dot{\epsilon} = 0$, the invariant 2-dimensional center manifold $M^c$ is foliated by invariant 1-dimensional leaves $\epsilon = \text{constant}$. If the leaf at $\epsilon = 0$ consists of hyperbolic equilibria (i.e. the linearization there admits no purely imaginary eigenvalues) connected by heteroclinic orbits as depicted in figure [11] then this dynamics is preserved in the leaves at $\epsilon$ small. Furthermore, the linearization theorem of Shoshitaishvili (see [27], Theorem 5.4) tells us that, if $D_y g(x, h(x))$ has only stable eigenvalues, the center manifold $M^c$ is attracting. Hence hyperbolic equilibria that are stable in the MM-reduced system give rise to stable equilibria in the extended system. Undoing the scaling $\tilde{y} = \epsilon y$ provides us with stable equilibria for the original mass-action system.

In order to apply GSPT in this context the main property to be checked concerns the eigenvalues of the matrix $A = D_y g(x, h(x))$. By this we mean the matrix of partial derivatives of the function $g$ with respect to the variables $y$ at a point $(x, h(x))$. The condition to be checked for the center manifold $M^c$ to be attracting is that the real parts of these eigenvalues are negative. Fortunately in this case the calculation can be reduced to one for the eigenvalues of two by two matrices, which is relatively simple.

The remaining part of the argument is to prove bistability for the MM system. When this has been done and if the stable steady states are hyperbolic (i.e. the linearization of the system at those points have no eigenvalues with zero real parts) then GSPT tells us that these steady states continue to exist and be stable for $\epsilon$ small but non-zero. For more details we refer to [20]. Bistability for the MM system is proved using bifurcation theory and now some more information will be given concerning that technique. Consider a system of ordinary differential equations
\[ \dot{x} = f(x, \alpha) \] and a steady state \((x_0, \alpha_0)\), i.e. \(f(x_0, \alpha_0) = 0\). Here \(\alpha\) denotes one or more parameters. The linearization of the system at \((x_0, \alpha_0)\) is the matrix of partial derivatives \(A = D_x f(x_0, \alpha_0)\). If no eigenvalue of \(A\) has zero real part the equilibrium \(x_0\) is said to be hyperbolic. Then for \(\alpha\) close to \(\alpha_0\) there is a unique solution of \(f(x_*(\alpha), \alpha) = 0\) with \(x_*\) close to \(x_0\) by the implicit function theorem. The stability properties of the solution are preserved, the dynamics nearby is the same as the dynamics of the linearized system by the Hartman-Grobman Theorem (see [18], Theorem 1.3.1). For instance if \(x_0\) is stable then \(x_*(\alpha)\) is stable. If, on the other hand, \(A\) has an eigenvalue whose real part is zero then \((x_0, \alpha_0)\) is said to be a bifurcation point and the qualitative dynamics of the system may change at that parameter value. For instance as the parameter is varied one steady state may split into several. In other words, new branches of equilibria may come into being at the critical parameter value \(\alpha_0\). Identifying a suitable bifurcation is a way of proving that several steady states
exist for certain parameter values.

For example, consider a system \( \dot{x} = f(x, \alpha) \) with one unknown \( x \) depending on two parameters, \( \alpha = (\alpha_1, \alpha_2) \). Denote by a prime the partial derivative of a function of \( x \) and \( \alpha \) with respect to \( x \). Suppose that \( f(0,0) = 0, \ f'(0,0) = 0, \ f''(0,0) = 0 \) and \( f'''(0,0) \neq 0 \) and that an additional quantity depending on derivatives with respect to the parameters is non-zero. The eigenvalue of the linearization \( A \) that crosses zero depends on the two parameters. In this example there is only one eigenvalue of the Jacobian, which is \( f'(0,0) = 0 \). When the above mentioned quantity is non-zero, it guarantees that the crossing happens at a non-zero velocity with respect to the parameters and transversally to the imaginary axis. This is called a transversality condition. See [27] for details. These are the defining properties of what is called a generic cusp bifurcation. There is a surface of equilibria over the two dimensional parameter space which develops a fold at \( (0,0) \). In a cusp region of the parameter space, three equilibria coexist - two stable ones and an unstable one. See Figure 2. Then there are parameter values near zero for which the system has three steady states close to zero. The case of relevance for the examples considered in this paper is that where \( f'''(0,0) < 0 \) and from now on we will only discuss that case. There two of the steady states close to zero are stable and one unstable. Now suppose there are several variables \( x_i \) and that at the point \( (0,0) \) the derivative \( A = D_x f \) has a zero eigenvalue of multiplicity one. The kernel of \( A \) is of dimension one. The qualitative behaviour of solutions near the steady state is determined by the restriction of the dynamics to a curve, the one dimensional centre manifold, which is tangent to the kernel of \( A \) and invariant under the flow.

Figure 2: Bifurcation diagram for a generic cusp bifurcation: the unstable branch of the surface of equilibria is shaded, as well as the region in the parameter plane where two stable and one unstable equilibria coexist.
While the local stable and unstable manifolds contain all initial conditions for solutions that converge exponentially to the equilibrium in forward or backward time direction respectively, the center manifold contains local bounded dynamics that depends heavily on the nonlinear terms. In this way the general case may be reduced to the one-dimensional case already discussed when the kernel of $A$ is of dimension one. In fact the dynamics in the stable and unstable directions corresponding to eigenvalues with nonzero real parts do not change. These are the main techniques used in the proof of bistability in [20].

It is clear that in order to linearize about a steady state we first have to have that steady state. It is not too difficult to find steady states since there are many parameters in the problem which can be varied. In [20] steady states were considered for which the concentrations of $X$ and $XP$ are equal, since this simplifies the algebra. Moreover it was assumed that all Michaelis constants have a common value $K$. In this case the relation

$$\left(\frac{E_{tot}}{F_{tot}}\right)^2 = \frac{k_3 k_4}{k_1 k_3}$$

holds. A bifurcation point was found under the restriction that $q^2 = (k_1 k_4)/(k_2 k_3) < 1$. The bifurcation occurs when $K X_{tot} = \frac{k_7}{K_7}$. It is important to note that here Michaelis-Menten reduction was carried out for the whole system and not for the two phosphorylation steps separately. The latter alternative leads to a different set of equations. It was used in [25], where the effect of embedding a MAPK cascade in a negative feedback loop was investigated. Consider, for instance, the dual futile cycle which is the third layer of the cascade in [25] and the equation for the unphosphorylated protein. It is of the form

$$\frac{d}{dt}[X] = -\frac{k_7 E_{tot}[X]}{K_7 + [X]} + \frac{k_7 F_{tot}[XP]}{K_{10} + [XP]}$$

which is clearly different from the corresponding equation in the MM system introduced above, even when all Michaelis constants are taken to be equal (which is done in the simulations of [25]).

The results just discussed only give limited information on the region of parameter space in which bistability occurs. In contrast, in [5] rigorous quantitative results on multistationarity were proved. Rather general conditions on the reaction constants were exhibited for which there are one or three steady states.

Bistability is important for the property of being a ‘good switch’: consider our system as an input-output relation where the input consists of the values of the conserved quantities and the output consists of the equilibrium reached by the system after a certain amount of time. For certain values of the input, the stable equilibrium reached by the system is unique and, say, on the lower part of the folded surface of equilibria. When the input enters the cusp region, the output remains on the lower part of the fold until the cusp region is left: at this point, the output switches to the upper part of the folded surface. In fact, the same phenomenon of switching is present when the parameter (input) is one dimensional and there exists a S-shaped curve of equilibria. See figure [40].
Figure 3: The input-output relation follows the lower stable equilibria until it becomes unstable through a fold (also called saddle-node) bifurcation, then jumps to the upper stable branch. Hence such an input-output relation is a good switch.

Sometimes when modelling phosphorylation systems the enzyme concentrations are not included explicitly and instead mass action kinetics is used for the substrates alone. If this is done for the cycle with two phosphorylations, or indeed for a cycle with any number of phosphorylations, a dynamical system is obtained which has a unique stationary solution to which all other solutions converge. This is because it can be shown that the system is a weakly reversible system of deficiency zero and the Deficiency Zero Theorem of chemical reaction network theory (see [12]) can be applied. This type of argument was used to prove corresponding results for the kinetic proofreading model of T cell activation in [39] and for the multiple phosphorylation of the transcription factor NFAT in [35]. These systems only describe small parts of the network involved in T cell activation, which also contains a MAPK cascade. A comprehensive model of this phenomenon presented in [1] is too large to be accessible by direct analytical investigation. Interestingly a very much simplified version of this model introduced in [14] reproduces some of the key features of T cell activation such as specificity, speed and sensitivity. In the simplified model the MAPK cascade is represented by a simple response function.
4 The MAPK cascade

The starting point of this section is the model of the MAPK cascade introduced in [22]. Simulations in [34] revealed the presence of bistability and sustained oscillations in this model. Mathematically the latter correspond to periodic solutions, i.e. solutions which satisfy $x(t+T) = x(t)$ for some time interval $T$ but are not steady states. In [34] similar results were obtained for the truncated cascade consisting of just the first two layers. Michaelis-Menten reduction for the MAPK cascade is made difficult by the fact that there is not a clear division between substrates and enzymes. This issue was studied in [42]. A further development of these ideas in [43] indicated that periodic solutions already occur in the Michaelis-Menten limit. It was shown in [20] that a small modification of these ideas allows a Michaelis-Menten limit of the equations for the truncated cascade to be defined which is well-behaved in the sense of GSPT. Since the truncated system contains a species, $X_1 P$, which is a product in the first layer and an enzyme in the second layer, the $\epsilon$-scaling of the MM-reduction has to be carried out using two different powers of $\epsilon$. We first define a new variable $X_1$ replacing $[X_1 P]$ as follows.

$$X_1 := [X_1 P] + [X_2 \cdot X_1 P] + [X_2 P \cdot X_1 P],$$

The concentration vector is split into three vectors $v_0, v_1, v_2$. The vector $v_2$ is the vector of concentrations of species containing the enzymes of the first layer of the cascade, alone or in a complex, i.e. $v_2 = ([E_1], [F_1], [X_1 \cdot E_1], [X_1 P \cdot F_1])$. This vector is rescaled by $\tilde{v}_2 = \epsilon^2 v_2$ The vector $v_1$ is the vector of concentrations of species containing $X_1$ or the enzymes $X_1 P = E_2$ and $F_2$ of the second layer of the cascade, alone or in a complex, i.e. $v_1 = (X_1, [X_1 P], [E_2], [X_2 \cdot X_1 P], [X_2 P \cdot X_1 P], [X_2 P \cdot F_2], [X_2 P P \cdot F_2])$. This vector is rescaled by $\tilde{v}_1 = \epsilon v_1$. Finally, the vector $v_0$ contains the concentrations of the remaining species, i.e. $x = ([X_2], [X_2 P], [X_2 P P])$ and is not rescaled. Furthermore the reaction constants of the first layer are also rescaled by $\epsilon$. A slow time variable $\tau = \epsilon t$ is introduced: the time derivative w.r.t. $t$ is denoted by an upper dot while the time derivative w.r.t. $\tau$ is denoted by $'$. Using conserved quantities to reduce the dimension of the concentration vectors and the new variable $X_1$, we get a system of the form

$$\begin{cases}
  x' = f(x, y, z), \\
  \epsilon y' = g(x, y, z),
\end{cases} \quad (14)$$

where $x = (X_1, [X_2], [X_2 P P])$ and $y = ([X_1 \cdot E_1], [X_1 P \cdot F_1], [X_2 \cdot X_1 P], [X_2 P \cdot X_1 P], [X_2 P \cdot F_2], [X_2 P P \cdot F_2])$. The limit $\epsilon \to 0$ of this system allows a MM-reduction that is well-behaved in terms of GSPT. For more details see [20], [21]. This result was extended to the full cascade in [21].

The facts just listed indicate that the strategy used to prove bistability in the dual futile cycle might also be used to prove the existence of sustained oscillations in the truncated MAPK cascade, i.e. layers 1 and 2 of cascade [1]. Here the relevant type of bifurcation is a Hopf bifurcation where the linearization at an equilibrium admits a pair of imaginary eigenvalues for a critical parameter. By a classical theorem of Hopf, if under variation of a parameter a pair of complex conjugate eigenvalues
of the linearization passes through the imaginary axis (away from zero) with non-zero velocity then there exist periodic solutions for at least some parameter values. See [24], [18] for details. In [24] it was proved that Hopf bifurcations occur in the MM system for the MAPK cascade. As a consequence periodic solutions occur. If an additional genericity condition (hyperbolicity of the periodic orbit) were satisfied then it could further be concluded using GSPT that periodic solutions also occur in the mass action system for the truncated MAPK cascade. In fact it has not yet been possible to prove hyperbolicity. Instead it was proved that the Hopf bifurcation itself can be lifted to the mass action system and this then gives the existence of periodic solutions of that system. Unfortunately these arguments give no information on the stability of the periodic solutions involved. It is interesting to look at this situation in the light of results on feedback loops. It has been proved that a system can only admit a stable periodic solution if it includes a directed negative feedback loop [5], [36]. It can easily checked directly that this condition holds in the case of the MM system for the truncated MAPK cascade.

The results for the truncated cascade imply analogous results for the full cascade by another application of GSPT. What must be shown is that the truncated cascade can be represented as a limit of the full cascade which is well-behaved in the sense of GSPT. Consider the MM system for the full cascade. Let \( Z \) be the concentration of a protein in the first or second layer and define a new variable by \( \tilde{Z} = \epsilon^{-1}Z \). Let \( c_i \) be any of the rate constants in the third layer and define \( \tilde{c}_i = \epsilon c_i \). The transformed system has a limit for \( \epsilon \to 0 \) which is well-behaved in the sense of GSPT and the limiting system is the MM system for the truncated cascade. Thus in the context of the MM system the Hopf bifurcation can be lifted from the truncated to the full cascade. It can then be further lifted from the MM system for the full cascade to the mass action system.

In [33] an in vitro model of the MAPK cascade was introduced. The substances involved are modified in such a way that certain features of the reaction network are modified. In the first layer Raf is constitutively active which means that for modelling this layer can be ignored. In the third layer ERK can only be phosphorylated once, on tyrosine and not on threonine. (In the wild type system MEK has the unusual property of being a dual specificity kinase which can phosphorylate both threonine and tyrosine.) This leads to a cascade with two layers where the first has
two phosphorylation steps and the second only one:

\[ \text{Raf} \xrightarrow{E_2} X_2 \xrightarrow{P_2} X_2P \xrightarrow{E_2} X_2PP \]

\[ \text{X}_3 \xrightarrow{E_3} X_3 \xrightarrow{P_3} X_3P \]

Here \( X_3 = ERK \). This was modelled mathematically in a certain way in [35] and it was proved that for that system of equations there is a unique steady state and all other solutions converge to it. If, on the other hand, the system is modelled in direct analogy to what was done in [22] a system is obtained which might potentially admit Hopf bifurcations and hence periodic solutions. It was written down in [21] but attempts to prove the existence of Hopf bifurcations using the methods applied to the truncated MAPK cascade have not succeeded. Simulations done in [47] indicate that there may be chaotic behaviour in the MAPK cascade. The approach of the authors was via (numerical) bifurcation theory. They discovered the presence of fold-Hopf bifurcations (where the linearization of the system at the bifurcation point has one zero and a pair of non-zero imaginary eigenvalues) and Hopf-Hopf bifurcations (where the linearization of the system at the bifurcation point has two pairs of non-zero imaginary eigenvalues). See [27] for more details on these types of bifurcations. Bifurcations of these types are often associated with chaos. Simulations for initial data close to the bifurcation points gave pictures consistent with the presence of chaotic behaviour.

In [34] a heuristic explanation for the existence of oscillations in the MAPK cascade was given, involving the embedding of a bistable system in a negative feedback loop. This point of view played no role in the proof of the existence of periodic solutions using bifurcation theory which has just been discussed. It could, however, in principle lead to an alternative proof of that result which could also provide information on the stability of the periodic solution. A corresponding strategy, which makes use of the Conley index (see [7]), has been developed and applied to a system related to that considered in [25]. More details can be found in [4], [10] and [15].
5  Embedding the cascade in feedback loops

Given that modelling predicts oscillatory behaviour in the MAPK cascade it is of great interest to try to observe it experimentally. This has been done in [37]. Oscillations were found in the concentration of activated (i.e. doubly phosphorylated) ERK which had a period of about fifteen minutes and lasted up to ten hours. This effect was monitored by observing the translocation of ERK tagged with GFP between the cytosol and the nucleus. This is relevant because activated ERK is imported into the nucleus and inactive ERK is exported into the cytosol. Mathematical models presented in [37] are more complicated than the basic model of [22] in several ways.

One is that a two-compartment model is used so that transport between cytosol and nucleus is included. This network is sketched in Figure 4 below, where Raf = $X_1$, MEK = $X_2$, ERK = $X_3$. A second is that the fact is included that ERK and MEK which are not fully phosphorylated can bind to each other (→←→←→← in Figure 4). This protects ERK from phosphorylation by MEK and thus represents a kind of negative feedback. Note that the tendency of this type of feedback to encourage bistability was already pointed out in [28]. A third way is that a negative feedback due the repression of SOS by ERK is included (→ in Figure 4). This is important since SOS influences the rate of phosphorylation of Raf. This last effect is modelled in a simple phenomenological manner. It was discovered that certain aspects of the experimental data do not fit the mathematical model with an isolated cascade. In particular this concerns the facts that the oscillations are found for a large range of total amounts of ERK and that the period of the oscillations is found to depend only weakly on the total amount of EGF, the substance being used to stimulate the cascade. It is assumed that the phosphorylation of Raf is proportional to the amount of EGF. Incorporating the negative feedback loop via SOS in the model allows the experimental results to be reproduced.

Yet another type of negative feedback which may lead to oscillations arises via the competition of a substrate of ERK with a phosphatase being reduced by increasing degradation of the activated substrate [29]. In this paper the authors present both mass action and reduced models and find periodic solutions in simulations.

6  Alternative phosphorylation mechanisms

In this paper we have concentrated on distributive phosphorylation. If this is partly replaced by processive (but still sequential) phosphorylation then this often results in simpler dynamics. For instance it was proved in [9] that when phosphorylation, dephosphorylation or both are replaced in the dual futile cycle by their processive versions, then there is a unique steady state for fixed values of the total amounts. This follows from the deficiency one algorithm of chemical reaction network theory. The result was generalized to the analogue of the multiple futile cycle with strictly processive and sequential phosphorylation in [10]. In addition it
was proved that all other solutions converge to the steady state. Note that other types of (partially) processive phosphorylation may also be considered [19].

One of the most important roles of oscillations in biology is that they can act as clocks, for instance those defining circadian rhythms. Many of these clocks are dependent on translation but a clock has been found in cyanobacteria which is not. It uses only phosphorylation states of the proteins KaiA, KaiB and KaiC. This has been demonstrated by reconstructing the clock \textit{in vitro} [31]. In KaiC the phosphorylation is cyclic rather than sequential. In other words the first of two sites to be phosphorylated is also the first to be dephosphorylated. This motivated the study of oscillations in dual phosphorylation models more general than the usual dual futile cycle [24]. In that paper the case of unordered phosphorylation was considered, i.e. that where the two (de-)phosphorylations may take place in any order. Simulations indicate that this is sufficient to produce sustained oscillations.

While the MAPK cascade is a type of phosphorylation system of central importance in eukaryotic signalling pathways, signalling pathways in prokaryotes more often use a different type of phosphorylation system known as a two-component system [40]. These are uncommon in eukaryotes and unknown in mammals. The central mechanism is as follows. The two components are proteins generically called HK and RR. The protein HK is a histidine kinase which, under appropriate conditions, phosphorylates itself on a histidine. RR, the response regulator, catalyses the transfer of the phosphate group from the histidine of HK to an aspartate in RR. In this way the RR is activated. HK can also dephosphorylate RR.

What is the advantage of the process of phosphorylation of RR taking place in two steps rather than directly? It has been suggested that the motivation is that the two-step process leads to absolute concentration robustness [38]. This means that the output signal is independent of the total concentrations of the enzymes, so that the system achieves independence from the stochastic variation in protein levels between different cells.

Bistability has been observed in two-component systems. The conditions for bistability in these systems have been discussed in [23]. This dynamical property has also been studied in [2]. The case treated there is that of a split kinase. This means that instead of one kinase HK phosphorylating itself one kinase binds to a second which then phosphorylates the first. These authors investigated bistability in different models using both dynamical simulations and the Chemical Reaction Network Toolbox. The latter is a computer programme which provides positive or negative results on bistability on the basis of chemical reaction network theory.

Some signaling pathways include phosphorelays in which the phosphate group is transferred from one species to the next. They also have a cascade structure, in many cases with four layers. In some layers, a phosphate group can easily be lost by hydrolysis. Furthermore some species can be bifunctional, i.e. able to give as well as take a phosphate group to/from the species on the next layer. As for the MAPK cascade, such phosphorelays can be embedded in transcriptional feedback loops. See for example [26] where mathematical results have been obtained on the form
of response functions and bistability in certain cases. There are many possible topologies for these systems and questions arise which are similar to those which could be posed for the MAPK cascade: how complicated should the architecture of the network be in order to achieve a ‘good switch’ property of the input-output relation? We saw that bistability could give an answer. Furthermore the methods explained in the previous sections could give insight on the dynamics beyond the steady states (heteroclinic structure, oscillations).

7 Summary and outlook

The MAPK cascade is a system of chemical reactions occurring as a part of many signal transduction networks. This cascade on its own has the potential to give rise to complicated dynamical behaviour such as multi-stability, sustained oscillations and even chaos. The positive and negative feedback loops in which the cascade is embedded in real biological systems present even more possibilities for generating this type of phenomena. One way of trying to obtain deeper insights into the conditions leading to different types of dynamical behaviour is to carry out mathematically rigorous investigations of systems of ordinary differential equations modelling the cascade. In this context it is natural to start by studying small building blocks in detail and build up from there. In particular we can pass from single layers of the cascade to the full cascade without feedback and then the full cascade with feedback.

In the first four sections of this paper the results on the MAPK cascade or parts of it which have been proved rigorously are summarized. In the case of a single phosphorylation loop it could be shown that the dynamics are simple: there is only one steady state and it is globally stable. For a series of several phosphorylation loops it has been known for some time that multiple stationary solutions are possible and information is available on their number. More recently it could be proved that for a system with two loops there are parameter values which give bistability, thus confirming previous conclusions based on numerical simulations and heuristic considerations. It could also be proved that for the MAPK cascade (or even for the first two layers of it) there exist periodic solutions. An introduction is given to some of the mathematical techniques used to obtain these results, geometric singular perturbation theory and bifurcation theory.

Cases are pointed out where further progress would be desirable. It could not yet be proved that the oscillations in the MAPK cascade are stable although simulations indicate that this is the case. In fact if they were not stable then it would be hard to find them by simulations. There are also no analytical results available on the presence of chaos. Obtaining results of this kind would require analysing bifurcations much more complicated than those treated up to now. It would also be valuable to obtain more results which in addition to showing that certain types of behaviour can occur in a given system also give useful information on the range of parameters for which it occurs.

The fifth section contains some remarks of the influence of feedback
loops on the dynamics of the cascade. This should be a fruitful field of application for the techniques already developed for the cascade on its own. Interestingly it seems based on simulations that the range of parameters for which bistability or sustained oscillations occur is increased by the presence of feedback loops and this may be important for the question of whether these features are present for biologically reasonable parameters and if so, whether the ranges of these parameters are large enough to allow the oscillations to be observed experimentally.

The sixth section discusses some generalizations to other types of phosphorylation systems. The focus in this paper is on distributive and sequential phosphorylation and this mirrors a more general tendency in the theoretical literature on this subject. Replacing distributive by processive phosphorylation in some reactions appears to lead to a simplification of the dynamics. On the other hand distributive unordered phosphorylation can lead to oscillations not present in the corresponding sequential case. Here again there is a lot of potential for further analytical investigations. Remarks are also made on the relations to signal transduction networks based on two-component systems.

Phosphorylation systems involved in signal transduction give rise to many challenges for mathematical analysis which have only started to be addressed. It is to be hoped that further progress in this direction will be rewarded by a deeper understanding of the mechanisms of the biological processes involved.

References

[1] Altan-Bonnet, G. and Germain, R.N. 2005 Modelling T cell antigen discrimination based on feedback control of digital ERK responses. PloS Biol. 3 (11): e356

[2] Amin, M., Porter, S. L. and Soyer, O. S. 2013 Split histidine kinases enable ultrasensitivity and bistability in two-component signalling networks. PloS Comp. Biol. 9 (3): e1002949.

[3] Angeli, D., De Leenheer, P. and Sontag, E. D. 2007 A Petri net approach to the study of persistence in chemical reaction networks. Math. Biosci. 210:598–618.

[4] Angeli, D., Ferrell, J. E. Jr. and Sontag, E. D. 2004 Detection of multistability, bifurcation and hysteresis in a large class of biological positive-feedback systems. Proc. Natl. Acad. Sci. USA 101, 1822–1827.

[5] Angeli, D., Hirsch, M. and Sontag, E. 2009 Attractors in coherent systems of differential equations. J. Diff. Eq. 246, 3058–3076.

[6] Angeli, D. and Sontag, E. D. 2006 Translation-invariant monotone systems and a global convergence result for enzymatic futile cycles. Nonlin. Anal. RWA 9, 128–140.

[7] Conley, C. 1978 Isolated invariant sets and the Morse index. CBMS Regional Conference Series in Mathematics 38.
[8] Conradi, C. and Mincheva, M. 2014 Catalytic constants enable the emergence of bistability in dual phosphorylation. Royal Society Interface 11, 20140158.

[9] Conradi, C., Saez-Rodriguez, J., Gilles, E.-D. and Raisch, J. 2005 Using chemical reaction network theory to discard a kinetic mechanism hypothesis. IEEE Syst. Biol. 152, 243–248.

[10] Conradi, C. and Shiu, A. 2015 A global convergence result for progressive multisite phosphorylation systems. Bull. Math. Biol. 77, 126–155.

[11] Errami, H., Eiswirth, M., Grigoriev, D., Seiler, W. M. and Weber, A. 2015 Detection of Hopf bifurcations in chemical reaction networks using convex coordinates. J. Comp. Phys. 291, 279–302.

[12] Feinberg, M. 1980 Lectures on chemical reactions networks. Available at https://crnt.osu.edu/lectures-chemical-reaction-networks

[13] Fenichel, N. 1979 Geometric perturbation theory for ordinary differential equations. J. Diff. Eq. 31, 53–98.

[14] François, P., Voisinne, G., Siggia, E. D., Altan-Bonnet, G. and Vergassola, M. 2013 Phenotypic model for early T cell activation displaying sensitivity, specificity and antagonism. Proc. Natl. Acad. Sci. USA. 110 E888-E897.

[15] Gedeon, T. 2010 Oscillations in monotone systems with a negative feedback. SIAM J. Dyn. Sys. 9, 84–112.

[16] Gedeon, T. and Sontag, E. D. 2007 Oscillations in multi-stable monotone systems with slowly varying feedback. J. Diff. Eq. 239, 273–295.

[17] Goldbeter, A. and Koshland, D. E., Jr. 1981 An amplified sensitivity arising from covalent modification in biological systems. Proc. Natl. Acad. Sci. USA 78, 6840–6844.

[18] Guckeheimer, J. and Holmes, P. 1983 Nonlinear oscillations, dynamical systems, and bifurcations of vector fields. Springer.

[19] Gunawardena, J. 2007 Distributivity and processivity in multisite phosphorylation can be distinguished through steady-state invariants. Biophys. J. 93, 3828–3834.

[20] Hell, J. and Rendall, A. D. 2015 A proof of bistability for the dual futile cycle. Nonlin. Anal. RWA. 24, 175–189.

[21] Hell, J. and Rendall, A. D. 2015 Sustained oscillations in the MAPK cascade. Preprint.

[22] Huang, C.-Y. F. and Ferrell, J. E., Jr. 1996 Ultrasensitivity in the mitogen-activated protein kinase cascade. Proc. Natl. Acad. Sci. USA 93, 10078–10083.

[23] Igoshin, O. A., Alves, R. and Savageau, M. A. 2008 Hysteretic and graded responses in bacterial two-component signal transduction. Mol. Microbiol. 68, 1196–1215.

[24] Jolley, C. C., Ode, K. L. and Ueda, H. R. 2012 A design principle for a posttranslational biochemical oscillator. Cell Reports 2, 938–950.
22
[41] Vanderbauwhede, A. 1989 Centre Manifolds, Normal Forms and Elementary Bifurcations. Dynamics Reported 2, 89–169

[42] Ventura, A. C., Sepulchre, J.-A. Merajver, S. D. 2008 A hidden feedback in signalling cascades is revealed. PLoS Comp. Biol. 4(3):e1000041.

[43] Ventura, A. C. and Sepulchre, J.-A. 2013 Intrinsic feedbacks in MAPK signalling cascades lead to bistability and oscillations. Acta Biotheor. 61, 59–78.

[44] Wang, L. and Sontag, E. D. 2008 On the number of steady states in a multiple futile cycle. J. Math. Biol. 57, 29–52.

[45] Wang, L. and Sontag, E. D. 2008 Singularly perturbed monotone systems and an application to double phosphorylation cycles. J. Nonlin. Sci. 18, 527–550.

[46] Widmann, C., Gibson, G., Jarpe, M. B. and Johnson, G. L. 1999 Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. Physiol. Rev. 79, 143–180.

[47] Zumsande, M. and Gross, T. 2010 Bifurcations and chaos in the MAPK signalling cascade. J. Theor. Biol. 265, 481–491.
Figure 4: Two-compartment model with a full MAPK cascade in the cytosol, a lower half of the MAPK cascade in the nucleus, transport of $X_3PP$ from the cytosol to the nucleus and transport of $X_3$ from the nucleus to the cytosol.