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Oscillations and multiple steady states in active membrane transport models

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Abstract. The dynamic behavior of some non-linear extensions of the six-state alternating access model for active membrane transport is investigated. We use stoichiometric network analysis to study the stability of steady states. The bifurcation analysis has been done through standard numerical methods. For the usual six-state model we have proved that there is only one steady state, which is globally asymptotically stable. When we added an autocatalytic step we found self-oscillations. For the competition between a monomer cycle and a dimer cycle, with steps of dimer formation, we have also found self-oscillations. We have also studied models involving the formation of a complex with other molecules. The addition of two steps for formation of a complex of the monomer with another molecule does not alter either the number or the stability of steady states of the basic six-state model. The model which combines the formation of a complex with an autocatalytic step shows both self-oscillations and multiple steady states. The results lead us to conclude that oscillations could be produced by active membrane transport systems if the transport cycle contains a sufficiently large number of steps (six in the present case) and is coupled to at least one autocatalytic reaction. Oscillations are also predicted when the monomer cycle is coupled to a dimer cycle. In fact, the autocatalytic reaction can be seen as a simplification of the model involving competition between monomer and dimer cycles, which seems to be a more realistic description of biological systems. A self-regulation mechanism of the pumps, related to the multiple stationary states, is expected only for a combined effect of autocatalysis and formation of complexes with other molecules. Within the six-state model this model also leads to oscillation.

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1. Introduction

Oscillations, spatial patterns and self-regulatory mechanisms seem to be an essential feature of biochemical cell signaling systems. Some examples are the calcium oscillations induced by hormonal or neurotransmitter signals observed for a large variety of cells (Petersen and Wakui 1990; Mayer et al. 1992), and those related to rhythmic motility in *Physarum* plasmodium (Yhosimoto and Kamiya 1982; Ueda et al. 1986). Although active transport is recognized to be responsible for the regulation of the concentration levels of ions in cells and in cell compartments, their role in these complex events have been largely unexplored. In fact, active transport is possibly one of the most important paths to self-regulation and self-control in living cells.

Active transport is performed by pump proteins coupled to a source of metabolic energy, usually ATP hydrolysis. The pumps have specific binding sites for the transported solute and for the molecule involved in the energy supply. The transport is accomplished by a conformational change of the pump in transferring the solute across the membrane. This process can be modeled by specific kinetic steps for the binding and conformational changes, similar to those which occur in standard chemical kinetics. For ATP driven systems there is a model which explains the essential features of the pumping mechanism (Tanford 1983; Läuger 1984). It is known as the alternating access model and it is used both for theoretical and experimental studies (Läuger 1984; Adamo et al. 1990).

Although active transport is known to be involved in many complex mechanisms of cells which require spatio-temporal organization, the models usually presented in the literature are based on simple steps of sequential kinetics involving only one pump unit. It is well known from non-equilibrium theory that spatio-temporal orga-

nization of non-equilibrium processes requires highly non-linear kinetics (Nicolis and Prigogine 1977). This requirement rules out the usual models of active transport for reproducing such complex behavior.

In the models describing complex biochemical behavior, such as calcium oscillations induced by hormones and neurotransmitters (Goldbeter et al. 1990), active transport is considered as a global process and not as a sequence of elementary steps. The fluctuating variables are the concentration of the transported ions in the cell compartments, and the non-linearity is due to the coupling between different transport mechanisms. In this case oscillations come from the competition between different sub-systems and cannot be attributed to active transport alone. However, some recent experimental results (Tepikin et al. 1992) showed that calcium oscillations in the cytosol were accompanied by a discrete pulse of calcium extrusion into the extracellular medium. The authors concluded that extrusion by the membrane Ca^{2+} pumps plays an important, possibly even dominant role, at least in the case of transient oscillations. Although the coupling with others transport mechanisms was not definitely excluded, it is possible that, at least in this case, the pumping mechanism could be the origin of oscillations. More experimental data are needed to confirm this assumption.

In order to investigate, from the theoretical point of view, the possibility of reproducing complex behavior induced by the pumping mechanism, we shall propose in this paper some extensions of the usual six-state alternating access model. In a previous paper (Weissmüller and Bisch 1993) it was shown that the introduction of one autocatalytic step in the four step model, combined with the formation of a regulatory complex, leads to hysteresis. This allows the pump to alternate between two quite different dynamic regimes, providing the pumps with a self-regulation mechanism. Another source of non-trivial dynamic behavior could be the dynamic formation of dimers, which would compete with the monomer cycle. In this paper we use a standard six-state alternating model as the basis of a systematic study of the effects of autocatalysis and of the formation of complexes with other molecules. Further, we investigate the effects caused by the introduction of a competing dimer transportation cycle including two kinetic steps for dimer formation.

The above dynamic steps are not usual in active transport models and must be discussed, starting from knowledge about the functioning of ATPase pumps. The role of dimers as the pumping unit has been suggested for many active transport systems, such as the Na/K- (Repke 1986) and Ca- (Andersen 1989; Kosk-Kosicka and Bzdega 1988, 1990) ATPase pumps. The dynamic formation of dimers from monomers during the transport cycle has never been considered in kinetic schemes proposed in the literature. From the experimental point of view, however, it has not been proved that dimers are still stable during the transport cycle. The dynamic transformation of monomers into dimers and dimers into monomers, in principle cannot be excluded from the kinetic scheme, although their importance depends on the kinetic constants of both processes.

The formation of complexes with other molecules resulting in activated monomers has been shown to be involved in some pumping mechanisms, for example calmodulin binding to the Ca-ATPase of erythrocyte membranes (Kosk-Kosicka and Bzdega 1988, 1990). In this case the formation of a complex regulates the pump function. Further, we have shown that when such a mechanism is combined with autocatalytic steps it could lead to a self-regulation of the pumping function (Weissmüller and Bisch 1993).

The autocatalytic conformational changes of the pump molecules means that the collision of the reacting molecules leads to the formation of a metastable dimer which decays instantaneously into its products. This type of kinetics could be possible in membranes with high pump density, like the Ca-ATPase in the sarcoplasmic reticulum, or for sufficiently large diffusion coefficients, where the collision rate will be larger than the pump transport rate (Weissmüller and Bisch 1993). Further, as will be shown later, the autocatalytic reaction could be seen as a simplification of the model which involves the kinetic conversion of monomers into dimers. From this point of view, this simple reaction could be used as the start point to investigate the dynamics of more complex models describing more realistic situations.

The models of active transport systems can be seen as chemical reaction networks. Their dynamics are given by a set of differential equations. In the present paper we intend to study the dynamic of some active transport models which could lead to complex behavior, such as oscillations and multiple steady states. For this purpose we have applied some of the methods which have been proposed for the analysis of complex reaction networks. The zero deficiency theorem (Horn and Jackson 1972; Horn 1972; Feinberg 1972, 1987) and the deficiency one theorem (Feinberg 1987) give important results about very complex networks. Some properties of the differential equations associated with chemical networks have been shown by Willamowski (1978). Among other things, he proved that standard chemical networks have an odd number of hyperbolic steady states. This result will be used in our analysis. However, our main tool will be Clarke's stoichiometric network analysis, which deals mainly with the stability of steady-states (Clarke 1980, 1981). We shall apply stoichiometric network analysis to identify the elements responsible for oscillatory or multiple steady-state behavior. All these mathematical tools are summarized in the appendix. The bifurcation analysis will be carried out through standard numerical methods (Kubíček and Marek 1983; Seydel 1988).

2. The models

A) Model 1

We start with the simplest model containing the essential features of active transport systems. These steps will be present in all models treated here. It is the six step standard alternating access model (Läuger 1984).

The model is shown in Fig. 1 where the carrier states are represented by A, B, C, D, E and F. The reaction

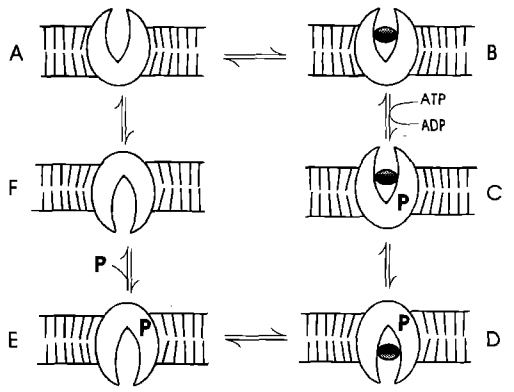

Fig. 1. Six-state alternating access model scheme for ATP-driven pumps. During the cycle A→B→C→D→E→F→A one ligand molecule is translocated from the external to the internal medium

network for the model is the following:

$$A + X' \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \qquad \text{I}$$

$$B + ATP \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C + ADP \qquad \text{II}$$

$$C \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} D \qquad \text{III}$$

$$D \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E + X'' \qquad \text{IV}$$

$$E \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} F + P_i \qquad \text{V}$$

$$F \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} A \qquad \text{VI}$$

where X′ is the ligand in the external medium and X″ is the ligand in the internal medium.

The main assumptions of the model are:

i) The binding sites are accessible alternatively only from the external or only from the cytoplasmic side of the membrane.
ii) Phosphorylation-dephosphorylation reactions and conformational transitions are treated as elementary steps.
iii) Each pump transports only one ion per cycle.
iv) We shall not assume the usual simplification of equilibrium between the states with empty or occupied ion binding sites (Läuger 1984).
v) The ligand concentration and the concentrations of ATP, ADP and P_i (adenosine tri- and di-phosphate and inorganic phosphate, respectively) are considered as externally controlled parameters.

With minor changes the model could be adapted to be applied to cotransport systems, by introducing a second ligand in steps I and IV. Hypothesis iii) would then not be necessary. To adapt the model to the transport of more than one ion per cycle we should consider that binding sites will always be either completely empty or completely full. We may assume that the pumps will be phosphorylated either by ATP only or by direct reaction with an inorganic phosphate. In fact, due to assumption v) the concentrations of ATP, ADP, P_i and X, or even of a second transported substance, are taken as fixed parameters during the reaction. The rate constants could thus be replaced in each case by the appropriate effective rate constants depending on the externally controlled concentrations, e.g. $k_1^{\text{eff}} = k_1 \cdot X'$. In this case one should consider that in this model each of the six steps is really a set of complex reactions containing other elementary steps.

This model has been applied in different theoretical studies, e.g., thermodynamic aspects and the dependence of the pump on membrane voltage of proton (Läuger 1984) and Na/K pumps (Lemieux and Koosey 1991). Similar models have also been applied to the analysis of experimental results of Ca-ATPase (Adamo et al. 1990).

B) Model 2

We start with model 1 and add one more reversible reaction:

$$A + F \underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}} 2A \qquad \text{VII}$$

This extra autocatalytic reaction is introduced to simulate dynamic cooperatively in active membrane transport. It is known that membranes of specialized cells of multicellular organisms and subcellular compartments of all eukaryotic cells have a restricted number of different proteins and usually a high concentration of each type, facilitating the interactions between them (Andersen 1989; Betts and Srivastava 1991). Some effects of this autocatalytic pathway in active transport have already been discussed (Weissmüller and Bisch 1993).

As will be shown later, this model provides a basis for the analysis of the further models. Indeed, it will be demonstrated that oscillatory behavior appears when some reaction paths involving autocatalysis or equivalent reactions are dominant in the reaction scheme. It should also be emphasized that the choice of a single autocatalytic step is made in order to simplify the model. As will be made clear in the following analysis, the addition of other autocatalytic steps would make the model still more nonlinear and all complex phenomena would be more likely to appear. The choice of states A and F in the autocatalytic step is also arbitrary; however, it is not relevant at the present stage of our analysis. In this paper we intend to investigate systematically the general features of the nonlinear steps leading to complex behaviour of the pumps. A detailed analysis and comparison with experimental data will be made in the future.

C) Model 3

In this model we assume the existence of two different cycles. The first one is identical to model 1. The second one is a cycle in which the pump units are dimers instead of monomers. In addition we introduce two steps of reversible associations of monomers into dimers. These additional steps lead to a coupling between monomer and dimer cycles. Although it is well known that dimers and, more generally, oligomers could be the functional units for active transport, the effect of transformation of dimers into monomers or vice-versa, during the transport cycle was not considered in the literature. Most of the work in

the literature tries to identify the transport units, and looks for them as stables units even when they are dimers, oligomers or molecular complexes, disregarding the fact that they could in principle be decomposed into monomers during the transport cycle. However, there is some recent experimental data on the Ca-ATPase of erythrocyte membranes (Kosk-Kosicka and Bzdega 1988, 1990) which shows the possibility of competition between monomer and dimer transport cycles. From the dynamic point of view, this new possibility furnishes an alternative reaction pathway which, as will be seen later, could lead to complex behaviour of the pumps.

We consider then an additional cycle with eight steps. The dimer states will be denoted by AA, AB, BB, CC, DD, DE, EE and FF. The reactions are:

$$AA + X' \underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}} AB \qquad \text{VII}$$

$$AB + X' \underset{k_{-8}}{\overset{k_8}{\rightleftharpoons}} BB \qquad \text{VIII}$$

$$BB + 2\,ATP \underset{k_{-9}}{\overset{k_9}{\rightleftharpoons}} CC + 2\,ADP \qquad \text{IX}$$

$$CC \underset{k_{-10}}{\overset{k_{10}}{\rightleftharpoons}} DD \qquad \text{X}$$

$$DD \underset{k_{-11}}{\overset{k_{11}}{\rightleftharpoons}} DE + X'' \qquad \text{XI}$$

$$DE \underset{k_{-12}}{\overset{k_{12}}{\rightleftharpoons}} EE + X'' \qquad \text{XII}$$

$$EE \underset{k_{-13}}{\overset{k_{13}}{\rightleftharpoons}} FF + 2\,P_i \qquad \text{XIII}$$

$$FF \underset{k_{-14}}{\overset{k_{14}}{\rightleftharpoons}} AA \qquad \text{XIV}$$

We also introduce two further reactions to accomplish the formation of dimer complexes as a dynamic process:

$$E + E \underset{k_{-15}}{\overset{k_{15}}{\rightleftharpoons}} EE \qquad \text{XV}$$

$$D + E \underset{k_{-16}}{\overset{k_{16}}{\rightleftharpoons}} DE \qquad \text{XVI}$$

We note that the dimer cycle contains the species DE and EE. This feature is essential to include the chemical pathway involving the dynamic formation of dimers from different monomer states. As will be shown later, this pathway is the basis of self-oscillations, like the autocatalytic reaction in the previous example. Once more we note the rather arbitrary choice of the monomers E and D involved in the dimer formation. In fact, other possible paths to dimer formation could be considered without loss of the complex behavior. We emphasize that the original motivation for model 3 is the idea, which comes from experimental results, that dimers could also be pumping units. The dynamic formation of dimers from monomers during the transportation cycle is a natural assumption coming from the chemical kinetics and a consequence of the fact that these dimers have not been definitively proved to be stable during the transport cycle.

D) Model 4

Next, we consider the formation of molecular complexes with other molecules, which should lead to activated monomers. An example is given by the binding of

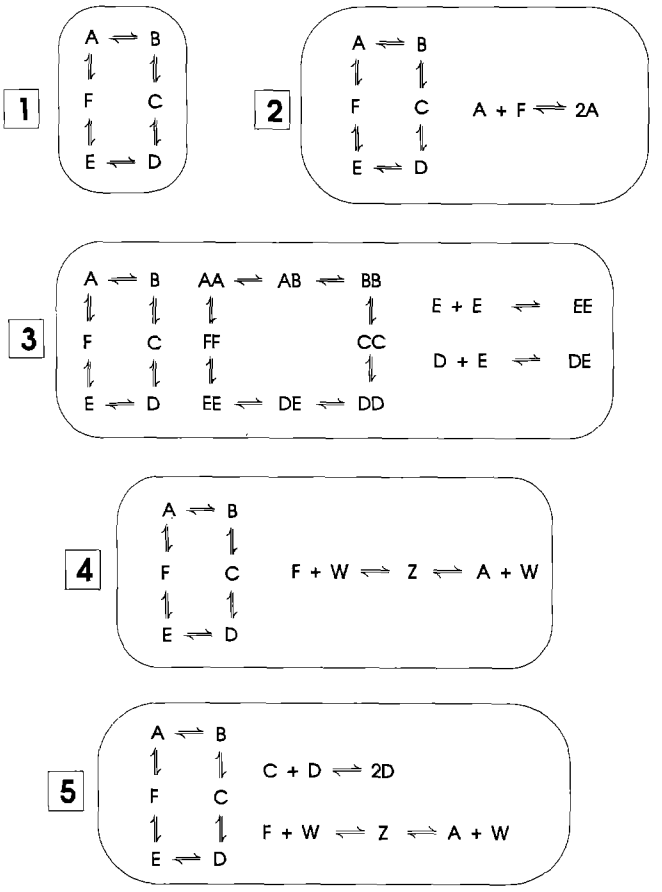

Fig. 2. Chemical networks representing the five models studied in the present paper

calmodulin to the erythrocyte Ca-ATPase (Kosk-Kosicka and Bzdega 1988, 1990). The presence of calmodulin leads to important changes in the ion flux and it could be regarded as an activator of the transport system. From the kinetic point of view, the formation of an intermediate complex generates a new chemical pathway, whose dynamic behaviour could be investigated in the present context.

We start with model 1 and add two reactions, representing the additional molecule by W and the complex pump-molecule by Z:

$$A + W \underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}} Z \qquad \text{VII}$$

$$F + W \underset{k_{-8}}{\overset{k_8}{\rightleftharpoons}} Z \qquad \text{VIII}$$

E) Model 5

In this model we put together the assumptions of models 2 and 4, i.e., one autocatalytic step and two reversible reactions involving the formation of a complex with another molecule.

$$C + D \underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}} 2D \qquad \text{VII}$$

$$A + W \underset{k_{-8}}{\overset{k_8}{\rightleftharpoons}} Z \qquad \text{VIII}$$

$$F + W \underset{k_{-9}}{\overset{k_9}{\rightleftharpoons}} Z \qquad \text{IX}$$

A similar model, involving only four steps in the transport cycle, has been proposed recently (Weissmüller and Bisch 1993). It was shown that even in this simple version the system exhibits steady states. We expect that model 5 could also lead to such complex behavior. As will be shown the autocatalytic step may be seen as a synthesis of the most important features of the dimer model. So, extensions of model 5 with monomer and dimer cycles and the formation of activated monomers would, at least, show the same kind of behavior. Of course, other activated monomer combinations are possible.

A concise representation of the five models appears in Fig. 2.

As discussed before the rate constants appearing in the above schemes can be replaced by effective constants which include the concentration of the externally controlled substrate. We deal with these effective constants in the next section. In fact the results will be valid for a larger class of systems which could be described by effective constants reproducing the same kinetic equations as the schemes presented above. Another advantage of this procedure is that the relations between the true kinetic constants imposed by the equilibrium conditions, for instance detailed balance, are not necessarily fulfilled by the effective constants, since these effective constants include the non-equilibrium concentrations of the substrates.

3. Results

A) Model 1

It is well known that model 1 presents one steady state, which is asymptotically stable. By applying the deficiency zero theorem (see appendix) we can prove in an elegant way that there is only one steady state, which is globally asymptotically stable. Only models with additional reactions may show complex behavior such as oscillations and multiple steady states.

The other models do not satisfy the conditions of the deficiency zero theorem (Feinberg 1987). Recently Feinberg (1988) proposed the so-called deficiency one algorithm, which gives the necessary and sufficient conditions for the existence of multiple steady states for some types of reaction networks. Unfortunately this algorithm does not apply to networks with cycles, which is the class to which our models belong.

B) Model 2

The system of differential equations for model 2 is

$$\begin{aligned}
\dot{A} &= -k_1 \cdot A + k_{-1} \cdot B + k_6 \cdot F - k_{-6} \cdot A + k_7 \cdot A \cdot F \\
&\quad - k_{-8} \cdot A^2 \\
\dot{B} &= k_1 \cdot A - k_{-1} \cdot B - k_2 \cdot B + k_{-2} \cdot C \\
\dot{C} &= k_2 \cdot B - k_{-2} \cdot C - k_3 \cdot C + k_{-3} \cdot D \\
\dot{D} &= k_3 \cdot C - k_{-3} \cdot D - k_4 \cdot D + k_{-4} \cdot E \\
\dot{E} &= k_4 \cdot D - k_{-4} \cdot E - k_5 \cdot E + k_{-5} \cdot F \\
\dot{F} &= k_5 \cdot E - k_{-5} \cdot F - k_6 \cdot F + k_{-6} \cdot A - k_7 \cdot A \cdot F \\
&\quad + k_{-8} \cdot A^2 \\
T &= A + B + C + D + E + F
\end{aligned}$$

This system has in principle two equations of second order. However, since the total number of pumps is conserved, one equation can be eliminated. If this eliminated equation is of second order, the system will have only one equation of second order and the others are of first order. We take advantage of this simplification in the analysis.

The maximum number of steady states for a system of equations may be obtained using Bézout's theorem (Abhyankar 1990). A useful version of this theorem states that given a system of m algebraic equations with order $g_1, \ldots, g_m$, the maximum number of solutions for the system is $\prod_{i=1}^{m} g_i$.

Applying Bézout's theorem to the system of equations for model 2 we see that this model has at most two steady states. As Willamowski has shown (Willamowski 1978) weakly reversible chemical systems with mass action kinetics of maximum order two always have an odd number of hyperbolic steady states. Thus, model 2 has (for almost every set of rate constants) only one steady state.

Now we shall apply the stoichiometric network analysis to study the stability of the stedy state of model 2 (see appendix). The extreme current matrix **E** is

$$\begin{pmatrix}
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 \\
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 1
\end{pmatrix}$$

These currents may be represented by current diagrams. In these diagrams the number of barbs and feathers represent the stoichiometry of produced and consumed species respectively. The current diagrams for model 2 are shown in Fig. 3.

The rate constants are parametrized by

$$\begin{aligned}
k_1 &= a \cdot (c_1 + c_{10} + c_{12}) & k_{-1} &= b \cdot (c_1 + c_{11} + c_{13}) \\
k_2 &= b \cdot (c_2 + c_{10} + c_{12}) & k_{-2} &= c \cdot (c_2 + c_{11} + c_{13}) \\
k_3 &= c \cdot (c_3 + c_{10} + c_{12}) & k_{-3} &= d \cdot (c_3 + c_{11} + c_{13}) \\
k_4 &= d \cdot (c_4 + c_{10} + c_{12}) & k_{-4} &= e \cdot (c_4 + c_{11} + c_{13}) \\
k_5 &= e \cdot (c_5 + c_{10} + c_{12}) & k_{-5} &= f \cdot (c_5 + c_{11} + c_{13}) \\
k_6 &= f \cdot (c_9 + c_6 + c_{10}) & k_{-6} &= a \cdot (c_5 + c_8 + c_{11}) \\
k_7 &= a \cdot f \cdot (c_7 + c_8 + c_{12}) & k_{-7} &= a^2 \cdot (c_7 + c_9 + c_{13})
\end{aligned}$$

where $\mathbf{j} = (c_1, \ldots, c_{13})$ and $\mathbf{h} = (a, b, c, d, e, f)$ are the stoichiometric network analysis parameters.

The currents 1 to 7, 10 and 11 are deficiency zero networks; they are mixing asymptotically stable. Using Routh-Hurwitz's criteria it could be shown that currents 8, 9 and 13 are asymptotically stable. On the other hand, current 12 will be unstable for an appropriate choice of parameters (Clarke 1980). As the network has only one steady state, an unstable steady state will probably come from a Hopf bifurcation (Willamowski 1978).

The bifurcation analysis was performed using well established numerical methods (Kubíček and Marek 1093; Seydel 1988). We have shown that, at least for some values of the parameters, the system exhibits a Hopf bifurcation, going from one steady state to oscillations through a well defined boundary in parameter space. Table 1 shows the parameters used for model 2. Figure 4 presents the results of the numerical analysis and shows the locus of a two parameter Hopf bifurcation in model 2.

From this analysis it can be deduced that if other autocatalytic combinations have been incorporated in the model the extreme currents of this new model will be the same as in Fig. 3 plus the news ones, including the new autocatalytic paths. This means that this new model will show the same complexity as model 2 and other unstable extreme currents do to new autocatalytic paths.

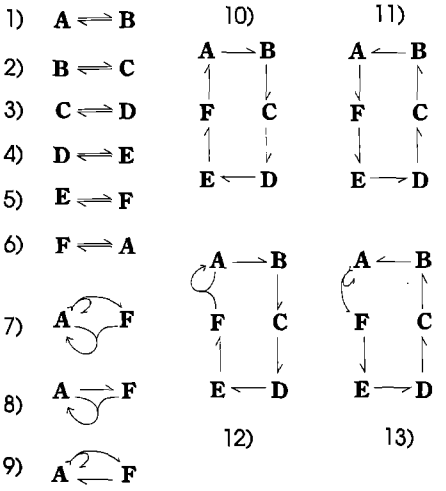

Fig. 3. Representation of the 13 extreme currents of model 2

C) Model 3

Model 3 has many currents which are equivalent to those of model 2, and most of them are also stable. Some typical current diagrams for model 3 are shown in Fig. 5.

We note that current 21 in model 3 is equivalent to an extension of current 12 in model 2, with D and E playing the part of F and A respectively. As the latter is a current which can produce instability, by the theorems of topologically similar networks (Clarke 1975, see Appendix) one can infer that current 21 in model 3 can also produce instability.

At this point it can be seen that the simplifying assumption of only two paths of dimer formation could be replaced by a more realistic assumption where all paths are considered. In this case there would appear other extreme currents similar to current 21, which would also be unstable. So, with this simplifying assumption model 3 has the basic elements responsible for complex behavior.

From Bézout's theorem and Willamowski's results the maximum number of steady states for model 3 is 7. The instability in this case could come either from a multiple steady-state bifurcation or a Hopf bifurcation. We have found a Hopf bifurcation associated with the instability produced by current 21.

Table 2 shows the parameters used for model 3. Figure 6 shows a two-parameter Hopf bifurcation diagram of model 3 for different values of k_1.

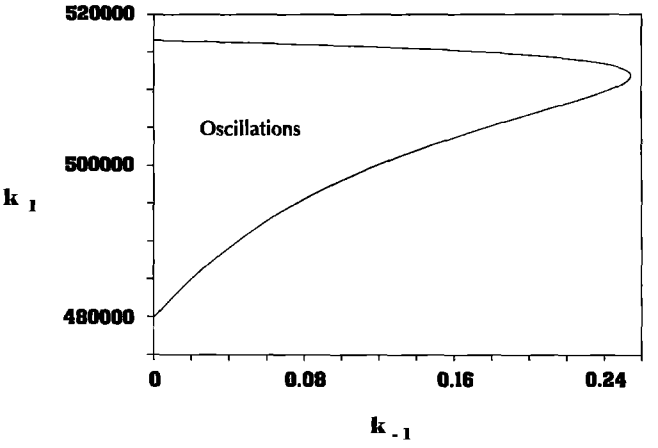

Fig. 4. Locus of Hopf bifurcation points for model 2 in the (k_{-1}, k_1) plane. The remaining rate constants are given in Table 1

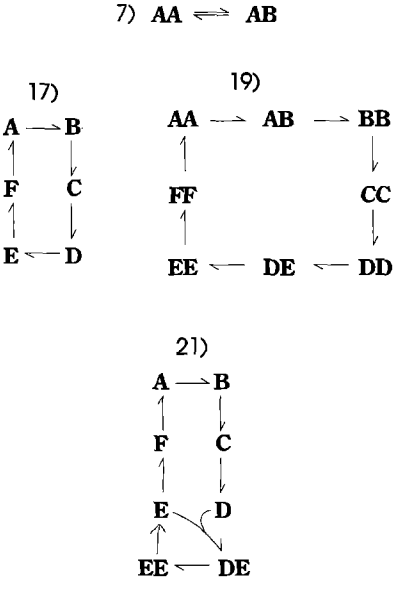

Fig. 5. Representation of some of the extreme currents found in model 3 (see text)

Table 1. Parameters for model 2. The k_i are the rate constants and T is the total pump concentration

Parameter	Value	Parameter	Value
k_2	1000	k_{-2}	0.003
k_3	1000	k_{-3}	0.003
k_4	1000	k_{-4}	0.003
k_5	1000	k_{-5}	0.00003
k_6	0.00003	k_{-6}	1.5
k_7	5000	k_{-7}	750
T	104		

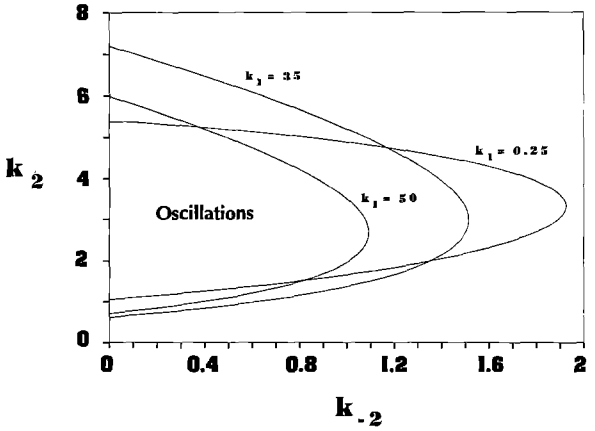

Fig. 6. Locus of Hopf bifurcation points for model 3 in the (k_{-2}, k_2) plane for different values of rate constant k_1 (given in the figure). The remaining rate constants are given in Table 2

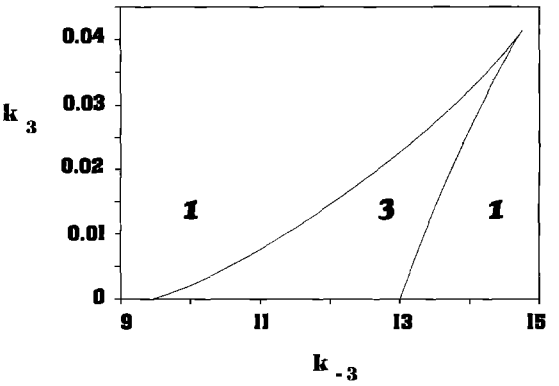

Fig. 8. Locus of the bifurcation that gives rise to multiple steady-states for model 5 in the (k_{-3}, k_3) plane. The numbers inside the figure represent the multiplicity of steady-states

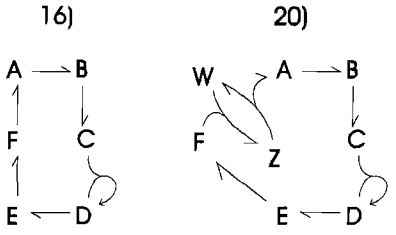

Fig. 7. Representation of two important model 5 extreme currents (see text)

D) Model 4

To study model 4 we shall add a reversible reaction $\square \rightleftharpoons A$, where $\square$ represents a pump reservoir, and apply stoichiometric network analysis to calculate the linearization matrix **M** for the network. In this case we can apply Daoyi's result (Daoyi 1985) to show that matrix **M** will have only eigenvalues with a negative real part.

If the rate constants of reaction $\square \rightleftharpoons A$ vanish, we can show that the linearization matrices of the original and the extended models can be made as close to each other as desired. As a consequence, the eigenvalues of the two models can also be made as close as desired (Palis and Melo 1982). As eigenvalues of extended models always have negative real parts, the eigenvalues for the linearization matrix of model 4 cannot have positive real parts. Therefore model 4 will have only one asymptotically stable hyperbolic steady state.

E) Model 5

The most important currents in model 5 are shown in Fig. 7.

Current 16 is equivalent to current 12 in model 2; therefore, model 5 should also exhibit Hopf bifurcations and limit cycles. We can show that current 20 can also be unstable and have multiple steady states when current 20 has a significant "weight".

Table 3 shows the parameters used for model 5. Figure 8 shows a two parameter bifurcation diagram for multiple steady states of model 5.

Table 2. Parameters for model 3. The k_i are the rate constants and T is equal to the total monomer concentration plus twice the total dimer concentration

Parameter	Value	Parameter	Value
		k_{-1}	0.00007
k_3	3	k_{-3}	0.000007
k_4	0.000007	k_{-4}	0.018
k_5	881	k_{-5}	0.00006
k_6	3	k_{-6}	0.00085
k_7	0.001	k_{-7}	0.0001
k_8	0.001	k_{-8}	0.0001
k_9	0.001	k_{-9}	0.0001
k_{10}	0.001	k_{-10}	0.0001
k_{11}	0.001	k_{-11}	0.0001
k_{12}	546	k_{-12}	0.0003
k_{13}	0.0014	k_{-13}	0.0001
k_{14}	0.001	k_{-14}	0.0001
k_{15}	0.003	k_{-15}	709
k_{16}	5.8	k_{-16}	0.0001
T	200		

Table 3. Parameters for model 5. The k_i are the rate constants, T is the total pump concentration plus complex Z concentration, and P is equal to complex Z plus molecule W concentrations

Parameter	Value	Parameter	Value
k_1	40	k_{-1}	4
k_2	8.3	k_{-2}	0.065
k_4	18.8	k_{-4}	6.16
k_5	10.6	k_{-5}	12
k_6	0.00002	k_{-6}	12
k_7	0.3	k_{-7}	0.00007
k_8	0.00004	k_{-8}	3.8
k_9	30	k_{-9}	0.000006
T	68	P	2.5

4. Discussion

The stoichiometric network analysis used in this paper enable steady-state studies of large and complex chemical networks, which would be rather difficult to do analyti-

cally without this method. It also gives the possibility of identifying the basic elements for instability through extreme currents and the theorem on the stability of topologically similar networks. Through this analysis it has also been possible to identify the minimum necessary steps leading to oscillations. The identification of unstable extreme currents makes it possible to simplify the models. Of course more realistic models may be analyzed by the same techniques, but at the price of increasing the number of variables and rate constants.

After the identification of unstable regions and after the appropriate choice of rate constants (which could be done in comparison with experimental results), numerical analysis seems to be the only way to carry out bifurcation analysis on such large systems. On the other hand, numerical analysis cannot supply a complete understanding of the system. Indeed the possibility of multiple steady states in model 3 and of chaos in models 2, 3 and 5 cannot be discarded.

The results show that, as expected, the usual six-state model (model 1) has only one stable steady state. Even when additional steps involving the formation of activated monomers are included (model 4), the system still has the same behavior. This means that although the formation of an intermediate complex could be relevant in some situations, as underlined by experiments of Kosk-Kosicka and Bzdega (1988, 1990), such a process alone is not able to produce oscillations or multiple stationary states.

The addition of one autocatalytic step to the six-state model (model 2), in contrast to results from the four-state model investigated previously (Weissmüller and Bisch 1993), presents a bifurcation to an oscillatory regime as shown in Fig. 4. This underlines the important role of autocatalysis in producing complex behavior when combined with sufficiently complex reaction schemes.

Even more interesting was the discovery of oscillations produced by coupling the monomer cycle to a dimer cycle (model 3). In fact, by comparing the extreme current 21 of model 3 with current 12 of model 2, both leading to oscillations, we note that the first is a generalization of the second by adding two more steps involving dimers. When the kinetics involving the dimers are sufficiently fast, such that we should consider the dimer states as metastable states instantaneously producing the product, as with autocatalytic reactions, then the current 21 of model 3 reduced to current 12 of model 2. This means that we could simulate oscillations by considering the formation of dimers or in a more simplified version, when the lifetime of dimers is very short, by an autocatalytic step. The simplifying assumption of only two dimer formation paths is not essential. Indeed, considering additional dimer formation steps makes the system become even more easily unstable.

As discussed by Kosk-Kosicka and Bzdega (1988) a remarkable increase of activity of Ca-ATPase is observed when the pump concentration is increased in a detergent-lipid preparation, which could be due to the association of enzyme molecules. These experimental findings show firstly that the association of pump molecules could be important in the dynamic behavior of the pumping mechanism, and secondly that, from the present theoretical results, we should expect even more complex behavior for this system independently of whether the association results in stable oligomers or in metastable complexes. In fact we have demonstrated that oscillations are expected for an active transport cycle when it involves a dynamic oligomerization of the pump units, which could be described by an autocatalytic step or by the coupling to an oligomer cycle (here represented by dimers).

Multiple stationary states have been found when autocatalytic steps are combined with the formation of activated monomers (model 5), as with the four state model. The relationship between multiple steady states and biological control has already been discussed (Weissmüller and Bisch 1993). However, model 5 could also lead to oscillations which would ensure that this model would exhibit the most complex behavior of all models presented here. We note that in model 5, the autocatalytic step could be replaced by coupling with a dimer cycle as in model 3, leading to even more complex kinetics able to produce both oscillations and multiple stationary states. In fact, the theorem on the stability of topologically similar networks enables us to argue that additional paths in the present models would not avoid instability. This means that complex behavior will still be present if we add more steps to the model in order to accomplish a more detailed biochemical model.

Self-oscillations are described in the literature (Nicolis and Prigogine 1977) and their relevance in biological systems is well known, but, as far as we know, a theoretical model with self-oscillations related to elementary steps of active transport in membranes has never appeared. However, there are some recent experiments for which the present results could be relevant. It has been suggested that oscillations of Ca^{2+} in *Physarum* plasmodium could be linked to active transport in mitochondria (Ueda et al. 1986). Further, it has been argued by Tepikin et al. (1992) that in calcium oscillations induced by hormonal or neurotransmitter signals Ca^{2+} pumps of the membrane play an important role. From the work of Tepikin et al. (1992) it seems that an autonomous mechanism of oscillation for the active transport systems is a possible way of explaining the experimental results, at least in the case of transient oscillations. In both cases the models presented here could be adapted or extended to account for the specificity of each system, although a detailed discussion depends on the physical accessibility of a proper rate constants set.

The theoretical approach used here leads us to the following conclusions. Firstly, to have oscillations induced by an active transport cycle, this cycle must have a large number of reaction steps and involve at least one autocatalytic step. A four state model does not lead to oscillations, as discussed before (Weissmüller and Bisch 1993). Further, the autocatalytic step could be seen as a simplification of coupling the monomer cycle to steps involving dimers or more complex oligomers. Secondly, owing to the large number of pump states needed to produce complex behavior, it seems that the only alternative way to treat this problem is the systematicc inspection of the reaction networks through the methods employed here. In fact we have made a first general analysis of the models, establishing the necessary conditions to have

complex behavior. A detailed comparison with experimental data and eventual adaptation to specific situations is still an open problem. Finally, although there is in the literature an alternative explanation fo Ca^{2+} oscillations – the main example cited here – it seems very attractive to think that the pumping mechanism could also be a source of oscillations. This idea can be checked in experiments involving only Ca^{2+} pumps, such as those performed by Kosk-Kosicka and Bzdega (1988). Indeed, our main conclusion is that if both monomer and dimer cycles occur, as seems to be the case in some systems, it would be possible to observe oscillatory behavior for appropriate experimental conditions.

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Appendix: Theoretical framework

In this appendix we present some results taken from the literature about the analysis of reaction networks.

The objects that appear before and after each reaction are called *complexes* (Horn and Jackson 1972) and we reserve the symbol y to denote the number of distinct complexes in a reaction network.

As usual, by *reversible* network we mean one in which each reaction is accompanied by its reverse. Some important results may be proven for networks satisfying the less restrictive conditions of weak reversibility. A network is *weakly reversible* if, whenever there is a directed arrow pathway pointing from one complex to another, there is also a directed arrow pathway pointing from the second complex back to the first (Horn and Jackson 1972).

A reaction network may be displayed diagrammatically in a standard form, in which each complex is written just once and arrows are drawn indicating all reactions between the complexes. The connected pieces of such a diagram are called the *linkage classes* of the network (Horn 1972). The symbol l will indicate the number of linkage classes of the network.

For a reaction network has r one-way chemical reactions $R_1, \ldots, R_r$, involving n species $x_1, \ldots, x_n$, one defines a $n \times r$ matrix $\mathbf{N}$ called the *stoichiometric matrix*, whose elements N_{ij} are equal to the stoichiometric coefficient of x_i on the right-hand side of reaction R_j minus that on the left (Clarke 1980).

If $\boldsymbol{x} = (x_1, \ldots, x_n)^T$ (symbol T denotes a transposition operation) is a vector of concentration, $\dot{\boldsymbol{x}} = \mathrm{d}\boldsymbol{x}/\mathrm{d}t$ and $\boldsymbol{v} = (v_1, \ldots, v_r)^T$ is a vector of one-way reaction rates, then the kinetic equations take the form (Clarke 1980)

$$\dot{\boldsymbol{x}} = \mathbf{N} \cdot \boldsymbol{v}. \tag{1}$$

Assuming standard mass action kinetics, the expression for the vector $\boldsymbol{v}$ will be

$$\boldsymbol{v} = (\operatorname{diag} \boldsymbol{k}) \cdot \boldsymbol{x}^{\mathbf{Y}}, \tag{2}$$

where $\boldsymbol{k}$ is a vector of the rate constants of the r reactions and $\operatorname{diag} \boldsymbol{k}$ is an $r \times r$ diagonal matrix with elements of $\boldsymbol{k}$ along its diagonal. By $\boldsymbol{x}^{\mathbf{Y}}$ we mean an r-component vector with its jth element defined as

$$(\boldsymbol{x}^{\mathbf{Y}})_j = \prod_{i=1}^{n} x_i^{Y_{ij}}, \tag{3}$$

where the symbol $\mathbf{Y}$ denotes the $n \times r$ *kinetic matrix*, whole element Y_{ij} is the order of x_i in reaction R_j. The components of $\boldsymbol{v}$ are always nonnegative and belong to the reaction space $\mathbb{R}^r$.

If the stoichiometric matrix $\mathbf{N}$ is of rank s, it is possible to choose s-linearly-independent columns of $\mathbf{N}$, which will span a subspace of the species space $\mathbb{R}^n$ called the *stoichiometric subspace*, denoted by S (Feinberg 1987). As a subspace of $\mathbb{R}^n$, S will contain the origin. The "parallel" of S containing $\boldsymbol{c}$, denoted by $\boldsymbol{c} + \mathrm{S}$, is obtained by adding the vector $\boldsymbol{c} \in \mathbb{R}^n$ to all vectors of S. For the $\boldsymbol{c}$ the intersection of $\boldsymbol{c} + \mathrm{S}$ with $\overline{\mathbb{R}}^n_+$, the nonnegative orthant of $\mathbb{R}^n$, is called a *stoichiometric compatibility class* (Feinberg 1987). It has been shown that, given the initial conditions of the system, the dynamics of a network are restricted to a unique stoichiometric compatibility class (Willamowski 1978).

The deficiency of a network denoted by δ is given by the formula

$$\delta = y - l - \mathrm{s}. \tag{4}$$

The steady states of the system are the first important element in the study of dynamic systems. Given a system $\dot{\boldsymbol{x}} = \mathbf{f}(\boldsymbol{x})$, the steady states are the points for which $\mathbf{f}(\boldsymbol{x}) = \mathbf{0}$. The linear equation associated with small perturbations around the steady state $\boldsymbol{x}^0$ is given by

$$\dot{\xi} = Df(\boldsymbol{x}^0) \cdot \xi \tag{5}$$

where $\xi = \boldsymbol{x} - \boldsymbol{x}^0$ and $Df(\boldsymbol{x}^0)$ is the Jacobian matrix of $\mathbf{f}$ at $\boldsymbol{x}^0$.

A steady state is *hyperbolic* if the eigenvalues of the Jacobian of $\mathbf{f}$ at the steady state have no zero real part. A theorem by Hartmann and Grobman (Guckenheimer and Holmes 1983) states that in the neighborhood of a hyperbolic point a nonlinear system is equivalent to the linear system given by the Jacobian at that point. It has been shown that systems having only hyperbolic steady states will not lose this property under "small perturbations" (Guckenheimer and Holmes 1983). On the other hand, the non-hyperbolic points will necessarily appear in the study of bifurcation points.

A hyperbolic steady state is asymptotically stable if all eigenvalues of the linearization matrix have negative real parts; otherwise, it will be unstable.

The structure of the differential equations associated with chemical systems has enabled Horn, Feinberg and Jackson (Horn and Jackson 1972; Horn 1972; Feinberg 1972, 1987) to prove the so-called deficiency zero theorem, from which we shall need the following simplified version:

Deficiency zero theorem: *If a reaction network of deficiency zero with mass action kinetics is weakly reversible, regardless, of the positive values of the rate constants, the associated system of differential equations will have only one steady state within each stoichiometric compatibility class. That steady state is asymptotically stable.*

In spite of its power, the deficiency zero theorem does not apply to a large number of chemical systems.

Willamowski (1978) showed that in the most common case of weakly reversible second order mass action kinetic systems, the systems will have at least one unstable steady state every time they have multiple steady states. As oscillations are assumed to be associated with Hopf bifurcations, which also implies unstable steady states, we may concentrate on the study of the stability of hyperbolic steady states.

Clarke (1980, 1981) has developed a method, which he calls *stoichiometric analysis*, that allows the study of the stability of steady states in complex reaction networks.

Let $\boldsymbol{x}^0$ be a steady state of the network and let $\boldsymbol{v}^0 = \boldsymbol{v}^0(\boldsymbol{x}^0, \boldsymbol{k})$. From Eq. (1) $\boldsymbol{v}^0$ will satisfy

$$\mathbf{N} \cdot \boldsymbol{v}^0 = \mathbf{0}. \tag{6}$$

Then all solutions $\boldsymbol{v}^0$ must lie in a $(r-s)$-dimensional subspace $\mathrm{P} \subset \mathbb{R}^r$, which is the right nullspace of $\mathbf{N}$. Since all components of $\boldsymbol{v}^0$ are nonnegative, $\boldsymbol{v}^0$ must be in the intersection of P and $\mathbb{R}^r_+$. This intersection is a convex polyhedral cone called the *current cone*. Let there be f cone vectors supporting the frame of the cone with directions given by $\boldsymbol{e}^1, \ldots, \boldsymbol{e}^f$, called extreme currents. Then every $\boldsymbol{v}^0$ in the current cone can be expressed as a nonnegative linear combination of these $\boldsymbol{e}^i$'s

$$\boldsymbol{v}^0 = \sum_{i=1}^{f} j_i \, \boldsymbol{e}^i \tag{7}$$

or in a matrix form

$$\boldsymbol{v}^0 = \mathbf{E} \cdot \mathbf{j} \tag{8}$$

where the $r \times f$ *current matrix* $\mathbf{E}$ has the vector $\boldsymbol{e}^i$ as its ith column and $\mathbf{j} \in \mathbb{R}^f_+$ is a vector whose components give the "weight" of each extreme current in the steady state.

It is worth noting that each extreme current is itself a chemical network, and the chemical diagram of each extreme current gives a good picture of their physical meaning.

Clarke has shown (Clarke 1980) that a zero deficiency extreme current is mixing asymptotically stable. Every combination of mixing asymptotically stable extreme currents have only one asymptotically stable steady state, and every combination of mixing stable extreme currents have only one stable steady state.

For each extreme current there is a purely numerical matrix, given by stoichiometric coefficients, current directions and the order of reactions

$$\mathbf{S}^{(i)} = -\mathbf{N} \cdot (\operatorname{diag} \boldsymbol{e}^i) \cdot \mathbf{Y}^T \tag{9}$$

and we can also define a symmetrical matrix

$$\mathbf{S}^{(i)}_{\mathrm{sym}} = (1/2)(\mathbf{S}^{(i)T} + \mathbf{S}^{(i)}). \tag{10}$$

Clarke has shown (Clarke 1980) that if all eigenvalues of $\mathbf{S}^{(i)}_{\mathrm{sym}}$ have nonnegative real part, then the ith current is mixing stable.

A second parameter is $\mathbf{h} \in \mathbb{R}^n_+$ defined by

$$\mathbf{h} = \mathbf{1}/\boldsymbol{x} = (1/x^0_1, \ldots, 1/x^0_n). \tag{11}$$

The linearization matrix can be written with the new parameters as

$$\mathbf{M} = \mathbf{N} \cdot (\operatorname{diag} \mathbf{E} \cdot \mathbf{j}) \cdot \mathbf{Y}^T \cdot (\operatorname{diag} \mathbf{h}). \tag{12}$$

To see whether a (numerical or algebraic) matrix has only eigenvalues with a negative real part Routh-Hurwitz's necessary and sufficient criterion is applied to the characteristic polynomial associated to the linearization matrix (Clarke 1980). In some cases it is possible to apply a result from Daoyi (1985) which gives a sufficient conditions for a matrix to have only eigenvalues with negative real part. This result is especially useful when the off-diagonal elemens of matrix $\mathbf{M}$ have a definite sign.

Clarke has also demonstrated (Clarke 1975) a theorem on the stability of topologically similar networks. Suppose that a network has a reaction $\mathrm{A} + \mathrm{B} \rightharpoonup \mathrm{C} + \mathrm{D}$. If another network has the same reactions, but with $\mathrm{A} + \mathrm{B} \rightharpoonup \mathrm{E} \rightharpoonup \mathrm{C} + \mathrm{D}$, then the latter is called an extension of the former. Clarke showed that if a network is unstable then its extensions will also be unstable.

The great advantage of this new method is that it is now possible to study the stability of all steady state without calculating them, by means of the new independent parameters $\mathbf{h}$ and $\mathbf{j}$.

References

Abhyankar SS (1990) Algebraic geometry for scientists and engineers. AMS, Providence, Rhode-Island

Adamo HP, Rega FA, Garrahan PJ (1990) Pre-steady-state phosphorylation of the humen red cell Ca^{2+}-ATPase. J Biol Chem 265:3789–3792

Andersen JP (1989) Monomer-oligomer equilibrium of sarcoplasmic reticulum Ca-ATPase and the role of subunit interaction in the Ca^{2+} pump mechanism. Biochim Biophys Acta 988:47–72

Betts GF, Srivastava DK (1991) The rationalization of high enzyme concentration in metabolic pathways such as glycolysis. J Theor Biol 151:155–167

Clarke BL (1975) Stability of topologically similar networks. J Chem Phys 62:3726–3738

Clarke BL (1980) Stability of complex reaction networks. In: Prigogine I, Rice SA (eds) Advances in chemical physics, Vol 43. Wiley, New York, pp 1–213

Clarke BL (1981) Complete set of steady state for the general stoichiometric dynamical systems. J Chem Phys 75:4970–4979

Daoyi X (1985) Simple criteria for stability of interval matrices. Int J Control 41:289–295

Feinberg M (1972) Complex balancing in general kinetic systems. Arch Rational Mech Anal 49:187–194

Feinberg M (1987) Chemical reaction network structure and the stability of complex isothermal reactors – I. Chem Eng Sci 42: 2229–2268

Feinberg M (1988) Chemical reaction network structure and the stability of complex isothermal reactors – II. Chem Eng Sci 43: 1–25

Goldbeter A, Dupont G, Berridge MJ (1990) Minimal model for signal-induced Ca^{2+} oscillations and their frequency encoding through protein phosphorylation. Proc Nacl Acad Sci, USA 87:1461–1465

Guckenheimer J, Holmes P (1983) Nonlinear oscillations, dynamical systems and bifurcation of vector fields. Springer, New York Berlin Heidelberg

Horn FJ (1972) Necessary and sufficient conditions for complex balancing in chemical kinetics. Arch Rational Mech Anal 49:172–186

Horn FJ, Jackson R (1972) General mass action kinetics. Arch Rational Mech Anal 47:81–116

Kosk-Kosicka D, Bzdega T (1988) Activation of the erythrocyte Ca^{2+}ATPase by either self-association or interaction with calmodulin. J Biol Chem 263:18184–18189

Kosk-Kosicka D, Bzdega T (1988) Activation of the erythrocyte Ca^{2+}ATPase by either self-association or interaction with calmodulin. J Biol Chem 263:18184–18189

Kosk-Kosicka D, Bzdega T (1990) Effects of calmodulin on erythrocyte Ca^{2+}-ATPase activation and oligomerization. Biochemistry 29:3772–3776

Kubíček M, Marek M (1983) Computational methods in bifurcation theory and dissipative structures. Springer, New York Berlin Heidelberg

Läuger P (1984) Thermodynamic and kinetic properties of electrogenic ion pumps. Biochim Biophys Acta 779:307–341

Lemieux DR, Koosey JM (1991) Simulation of the voltage dependence of the Na, Ka pump applied to cardiac cells. J Theor Biol 150:73–91

Mayer EA, Konder A, Sun XP, Wilkes J, Scott D, Sachs G (1992) Spatial and temporal patterns of intracellular calcium in colonic smooth muscle. J Membrane Biol 125:107–118

Nicolis G, Prigogine I (1977) Self-organization in non-equilibrium systems. Wiley, New York

Palis J, Melo W (1982) Geometric theory of dynamical systems: an introduction. Springer, New York Berlin Heidelberg

Petersen OH, Wakui MI (1990) Oscillating intracellular Ca^{2+} signals evoked by activation of receptors linked to inusitol lipid hydrolysis: mechanism of generation. J Membrane Biol 118:93–105

Repke KRH (1986) A model for allosteric regulation of Na^+/K^+ transporting ATPase. Biochim Biophys Acta 864:195–212

Seydel R (1988) From equilibrium to chaos. Practical bifurcation and stability analysis. Elsevier, New York

Tanford C (1983) Mechanism of free energy coupling in active transport. Annu Rev Biochem 52:379–409

Tepikin AT, Voronin SG, Gallacher DV, Petersen OH (1992) Pulsatile Ca^{2+} extrusion from single pancreatic acinar cells during receptor-activated cytosolic Ca^{2+} spiking. J Biol Chem 267: 14073–14076

Ueda T, Matsumoto K, Akitaya T, Kobatake Y (1986) Spatial and temporal organization of intracellular adenine nucleotides in relation to rhythmic motility in *Physarum* plasmodium. Exp Cell Res 162:486–494

Weissmüller G, Bisch PM (1993) Autocatalytic cooperativity and self-regulation of ATPase pumps in membrane active transport. Eur Biophys J 22:63–70

Willamowski KD (1978) Contributions to the theory of mass action kinetics – III. Z Natür 33a:989–992

Yoshimoto Y, Kamiya N (1982) Ca^{2+} oscillations in the homogenate of *Physarum* plasmodium. Protoplasma 110:63–65