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OSCILLATIONS AND MULTIPLE STEADY STATES IN
MEMBRANE ACTIVE TRANSPORT MODELS

by

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ABSTRACT

We have investigated the dynamic behaviour of some extensions of the alternating access model for membrane active transport. We have used the 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 along with dynamical dimer formation we have also found self-oscillations. We have also studied models involving complexes formation with other molecules. The addition of two steps of complex formation does not alter the number and the stability of the basic six-state model. The model with addition of two steps of complex formation along with a autocatalytic step shows both self-oscillations and multiple steady states.

Key-words: Membrane active transport; Dissipative structures; Stoichiometric network analysis; Oscillations and multiple steady states.

1 Introduction

The active transport in membranes is an essential feature of living cells. It is responsible for energy storage and for the regulation of ions concentration levels. Active transport is possibly the most important path to self-regulation and self-control in living cells.

For ATP driven systems there is a model showing the essential features of general systems ¹. It is known as the alternating access model and it is used both for theoretical and experimental studies ^{1,2}.

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 dynamics of some active transport models. We will propose some models showing complex behaviour such as oscillations and multiple steady states. This kind of behaviour can be linked to selfregulation and control of the cells.

We have used a standard six-state alternating model as a basis to study some effects of autocatalysis and of complexes formations. We will also study some of the effects caused by the introduction of a competing dimer transportation cycle along with dynamical dimer formations.

We have applied some of the methods which have been proposed for the analysis of complex reaction networks. The zero deficiency theorem ³⁻⁶ and the deficiency one theorem ⁶ give strong results about very complex networks.

Some properties of the differential equations associated with usual chemical networks have been shown by Willamowski ⁷. Among other things, he proved that usual chemical networks have an odd number of hyperbolic steady states.

Our main tool will be Clarke's stoichiometric network analysis, which deals mainly with steady-states stability ^{8,9}. We will apply stoichiometric network analysis to identify the elements responsible for oscillatory or multiple steady-state behaviour. The bifurcation analysis will be carried out through standard numerical methods ^{10,11}.

2 Theoretical framework

In this section we will present some results taken from literature as well as establish some essential notation.

The objects that appear before and after each reaction are called *complexes* ³ and we will reserve the symbol γ to denote the number of distinct complexes in a reaction network.

As usual, by *reversible network* is meant one in which each reaction is accompanied by its reverse. Some important results may be proven for networks satisfying the less restrictive condition 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 pointing from the second complex back to the first ³.

A reaction network may be displayed in a standard form diagram, in which each complex is written just once and then arrows are drawn indicating all reactions among the complexes. The connected pieces of such a diagram are called the *linkage classes* of the network ⁴. The symbol l will indicate the number of linkage classes of the network.

For a reaction network that has r one-way chemical reactions R_1, \dots, R_r , involving n species x_1, \dots, x_n , one defines a $n \times r$ matrix \mathbf{Q} called the *stoichiometric matrix*, whose elements Q_{ij} are equal to the stoichiometric coefficient of x_i on the right-hand side of reaction R_j minus that on the left ⁵.

If $\mathbf{x} = (x_1, \dots, x_n)^T$ (symbol T denotes a transposition operation) is a vector of concentration, $\dot{\mathbf{x}} = d\mathbf{x}/dt$ and $\mathbf{v} = (v_1, \dots, v_r)^T$ is a vector of one-way reaction rates, then the kinetic equations take the form ⁸

$$\dot{\mathbf{x}} = \mathbf{Q} \cdot \mathbf{v} \quad (1)$$

Assuming the usual mass action kinetics, then the expression for the vector \mathbf{v} will be

$$\mathbf{v} = (\text{diag } \mathbf{k}) \cdot \mathbf{x}^{\mathbf{Y}} \quad (2)$$

where \mathbf{k} is a vector of the rate constants of the r reactions and $\text{diag } \mathbf{k}$ is a $r \times r$ diagonal matrix with elements of \mathbf{k} along its diagonal. By $\mathbf{x}^{\mathbf{Y}}$ it is meant an r component vector with a j th element defined as

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

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

If the stoichiometric matrix \mathbf{Q} is of rank s , it is possible to choose s -linearly-independent columns of \mathbf{Q} , which will span a subspace of the species space \mathbb{R}^n called the *stoichiometric subspace* denoted by S ⁶. As a subspace of \mathbb{R}^n S will contain the origin. The "parallel" of S containing \mathbf{c} , denoted by $\mathbf{c} + S$, is obtained by adding the vector $\mathbf{c} \in \mathbb{R}^n$ to all vectors of S . For each \mathbf{c} the intersection of $\mathbf{c} + S$ with $\overline{\mathbb{R}}_+^n$, the nonnegative orthant of \mathbb{R}^n , is called a *stoichiometric compatibility class* ⁶. It has been shown that, given the initial conditions of the system, the dynamics of a network is restricted to a unique stoichiometric compatibility class ⁷.

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

$$\delta = y - l - s \quad (4)$$

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

$$\dot{\xi} = Df(\mathbf{x}^0) \cdot \xi \quad (5)$$

where $\xi = \mathbf{x} - \mathbf{x}^0$ and $Df(\mathbf{x}^0)$ is the Jacobian matrix of \mathbf{f} at \mathbf{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 Hartman and Grobman ¹² 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 by "small perturbations" ¹³. 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 to chemical systems has enabled Horn, Feinberg and Jackson ³⁻⁶ to prove the so-called deficiency zero theorem, from which we will 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 the rate constants take, 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 ⁷ showed that in the case of weakly reversible second order mass action kinetic systems, which are the usual ones, 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 ^{8,9} has developed a method, called by him *stoichiometric network analysis*, that allows the study of the stability of steady states in complex reaction networks.

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

$$\mathbf{Q} \cdot \mathbf{v}^0 = \mathbf{0} \quad (6)$$

Then all solutions \mathbf{v}^0 must lie in a $(r - s)$ -dimensional subspace $P \subset \mathbb{R}^r$, which

is the right nullspace of \mathbf{Q} . Since all components of \mathbf{v}^o are nonnegative, \mathbf{v}^o 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 $\mathbf{e}^1, \dots, \mathbf{e}^f$, called *extreme currents*. Then every \mathbf{v}^o in the current cone can be expressed as a nonnegative linear combination of these \mathbf{e}^i 's

$$\mathbf{v}^o = \sum_{i=1}^f j_i \mathbf{e}^i \quad (7)$$

or in a matrix form

$$\mathbf{v}^o = \mathbf{E} \cdot \mathbf{j} \quad (8)$$

where the $r \times f$ current matrix \mathbf{E} has the vector \mathbf{e}^i as its i th 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 ⁸ 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

$$\mathbf{S}^{(i)} = -\mathbf{Q} \cdot (\text{diag } \mathbf{e}^i) \cdot \mathbf{Y}^T \quad (9)$$

and we can also define a symmetrical matrix

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

Clarke has shown ⁸ that if all eigenvalues of $\mathbf{S}_{sym}^{(i)}$ have nonnegative real part, then the i th current is mixing stable.

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

$$\mathbf{h} = \mathbf{1}/\mathbf{x} = (1/x_1^o, \dots, 1/x_n^o) \quad (11)$$

The linearization matrix can be written with the new parameters as

$$M = Q \cdot (\text{diag } E, j) \cdot Y^T \cdot (\text{diag } h) \quad (12)$$

To see whether a (numerical or algebraic) matrix has only negative real part eigenvalues Routh-Hurwitz's necessary and sufficient criterion is applied to the characteristic polynomial associated to the linearization matrix ⁸. In some cases it is possible to apply a result from Daoyi ¹⁴. It gives a sufficient condition for a matrix to have only negative real part eigenvalues. This result is specially useful when the off-diagonal elements of matrix M have a definite sign.

Clarke has also demonstrated ¹⁵ a theorem on the stability of topologically similar networks. Suppose that a network has a reaction $A + B \rightarrow C + D$. If another network has the same reactions, but with $A + B \rightarrow E \rightarrow C + D$, 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.

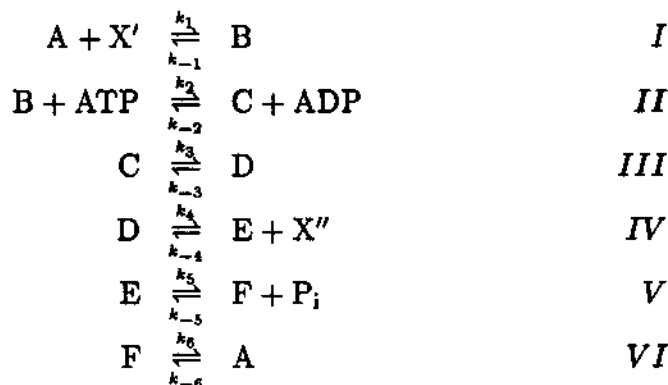
The great advantage of this new method is that it is possible now to study the stability of all steady states without calculating them, by means of the new independent parameters **h** and **j**.

3 The models

A) Model 1

We start with a simple model containing some of the essential features of active transport systems. These steps will be present in all next models. It is an usual alternating access model and it is composed by six states ¹.

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



where X' is the ligand in the external medium and X'' is the ligand in the internal medium.

The main assumptions for the model are :

- i) The binding sites are accessible only from one 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 will not assume the usual simplification of equilibrium between the empty and the full states ¹.
- 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. Hypothesis iii) would not be necessary. To adapt the model to the transport of more than one ion per cycle we should consider that the binding sites will always be either completely empty or completely full. We may assume that the pumps will be phosphorylated either only by ATP or by direct reaction with inorganic phosphate. In fact, the concentrations of ATP, ADP, P_i and X could be included in the effective rate constants.

It should be noted that in this model each one of the six elementary steps is in reality complex reactions containing other elementary steps.

This model has been applied in different theoretical studies, e.g., thermodynamical aspects and the dependence of the pump on membrane voltage of proton ¹ and Na, K pumps ¹⁶. Similar models have also been applied to the analysis of experimental results of Ca²⁺-ATPase ².

B) Model 2

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



This extra autocatalytic reaction is introduced to simulate dynamic cooperativity in membrane active 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, making easy the interaction among them ¹⁷⁻¹⁹. Some effects of this autocatalytic pathway in active transport have already been discussed in literature ¹⁹.

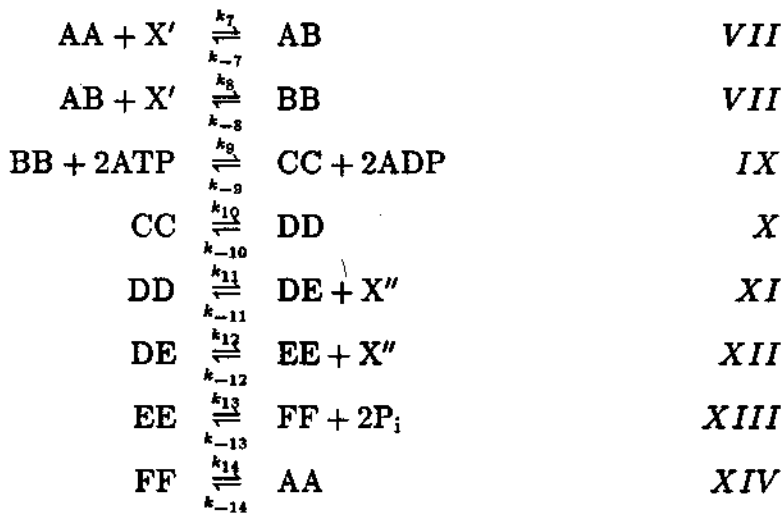
Furthermore, this model provides a mathematical basis for the analysis of the next models, which are more complex than the present one.

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 unities are dimers instead of monomers.

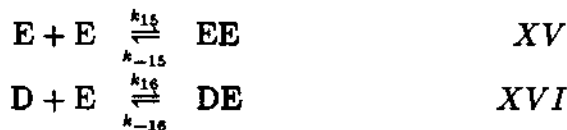
It is known that dimer and more generally oligomers may be the functional unities for active transport, and the possibility of competition between monomer and dimer transport cannot be discarded ²⁰.

We will consider 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 :



Again, as ATP, ADP and P_i concentrations are constants. They will be included in the effective rate constants.

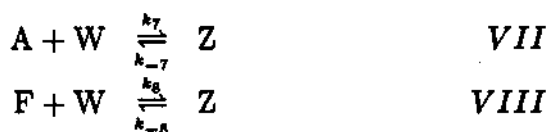
We also introduce two more reactions to accomplish the formation of dimer complexes, considering it as a dynamic process :



D) Model 4

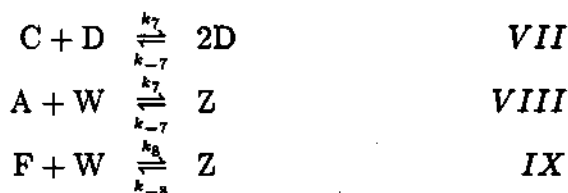
Next, we will consider the formation of protein complexes involving other molecules, e.g. calmodulin ²⁰. The phenomenon may be responsible for important changes in the ion flux ²¹.

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



E) Model 5

In this model we will join the assumptions of models 2 and 4, i.e., one autocatalytic and two complex formation reversible reactions.



A similar simpler model has been able to show multiple steady states ¹⁹.

A concise form of the five models appears in Figure 2.

4 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 we may 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 behaviour such as oscillations and multiple steady states.

The other models do not satisfy the conditions of deficiency zero theorem ⁶. Recently Feinberg ²² proposed the so-called deficiency one algorithm, which gives the necessary and sufficient conditions to 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 of our models.

B) Model 2

The system of differential equations for model 2 is

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

It has two equations with degree two and the other equations with degree one. As we have the conservation of the total number of pumps we can eliminate one linearly dependent equation. If we eliminate one equation with degree two, the system will have only one equation with degree two and the other with degree one.

The maximum number of steady states for a system of equations may be obtained from the Bezout's theorem ²³. A useful version of this theorem says that given a system of m algebraic equations with degrees g_1, \dots, g_m , the maximum number of solutions for the system is $\prod_{i=1}^m g_i$.

Applying Bezout's theorem to the system of equations for model 2 we will see that this model will show two steady states at most. As Willamowski has shown ⁷ weakly reversible chemical systems with mass action kinetics of order two at most have always an odd number of hyperbolic steady states. Thus, model 2 has (for almost every set of rate constants) only one steady state.

Now we will apply the stoichiometric network analysis to study the stability of the model 2 steady state. The extreme current matrix E is

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

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

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

where $\mathbf{j} = (c_1, \dots, c_{13})$ 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; therefore, they are mixing asymptotically stable. Using Routh-Hurwitz's criterion it may be shown that currents 8, 9 and 13 are asymptotically stable. We can show that current 12 will be unstable for a convenient choice of parameters. As the network has only one steady state, an unstable steady state will probably come from a Hopf bifurcation ⁷.

The bifurcation analysis can be done through established numerical methods ^{10,11}. Table 1 displays the parameters used for model 2. Fig. 4 presents the results of the numerical analysis and shows the locus of a two parameter Hopf bifurcation in model 2.

C) Model 3

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

As we can see, the 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 ¹⁵ one can infer that current 21 in model 3 can also produce instability.

From Bezout'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 displays the parameters used for model 3. Fig. 6 shows a two-parameter Hopf bifurcation diagrams of model 3 with different values of k_1 .

D) Model 4

To study model 4 we will add a reversible reaction $\square \rightleftharpoons A$, where \square represents a pump reservoir. Then we will apply stoichiometric network analysis to calculate the linearization matrix M for the network. In this case we can apply Daoyi result ¹⁴ to show that matrix M will have only negative real part eigenvalues.

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 as desired. The consequence is that the eigenvalues of the two models can also be made as close as desired¹³. As extended model eigenvalues always have negative real parts, the eigenvalues for model 4 linearization matrix cannot have positive real parts. So 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 will also possibly have Hopf bifurcations and limit cycles. We can show that current 20 is another current that can be unstable. We found multiple steady states when current 20 has a significant "weight".

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

5 Discussion

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

After the identification of unstable regions and after the choice of the initial rate constants set (which could be done in comparison with experimental results), the 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 theorem on the stability of topologically similar networks enables us to see that additional paths in the basic six-states alternating access model would not avoid instability. That means, complex behaviour will still be present if we add more steps to the model in order to accomplish a more detailed biochemical model.

A model for active transport which shows multiple steady states has already appeared and the relationship between multiple steady states and biological control has already been discussed in literature¹⁹. On the other hand, model 5 is more complete, including the main characteristics of active transport systems. The stability of the steady state of model 4 shows the important role of the autocatalytic reaction in chemical systems with complex behaviour. Nevertheless, it is not the only way to produce this kind of behaviour.

Self-oscillations are common in literature²⁴ and their relevance in biological systems is well known. Indeed, recently oscillations of Ca^{2+} in *Physarum plasmodium* have been found experimentally^{25,26} and it has been suggested that these oscillations could

be linked to active transport in mitochondria. Anyway, as far as we know, a theoretical model with self-oscillations related to active transport in membranes has never appeared.

We would like to stress the fact that in model 3 the assumptions of active transport through either a monomer or a dimer cycle and of the dynamical dimer formation seem to be in complete accordance with experimental knowledge. The usefulness of this path for the creation of biological rhythms depends, among other things, on the physical accessibility of a proper rate constants set.

Acknowledgments

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FIGURE CAPTIONS

Figure 1. Six-states alternating access model scheme for ATP-driven pumps. During the cycle $A \rightarrow B \rightarrow C \rightarrow D \rightarrow E \rightarrow F \rightarrow A$ one ligand molecule is translocated from the external to the internal medium.

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

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

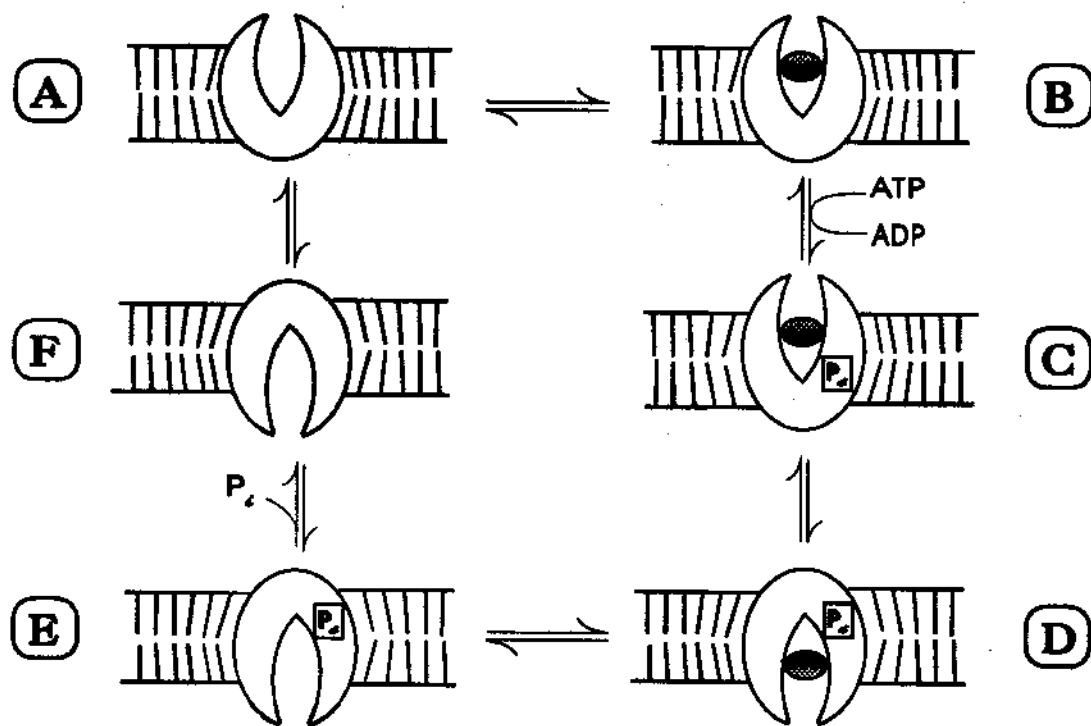
Figure 4. Locus of Hopf bifurcation points for model 2 in the (k_{-1}, k_1) plane. The remaining rate constants are given in Table 1.

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

Figure 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.

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

Figure 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. 1*

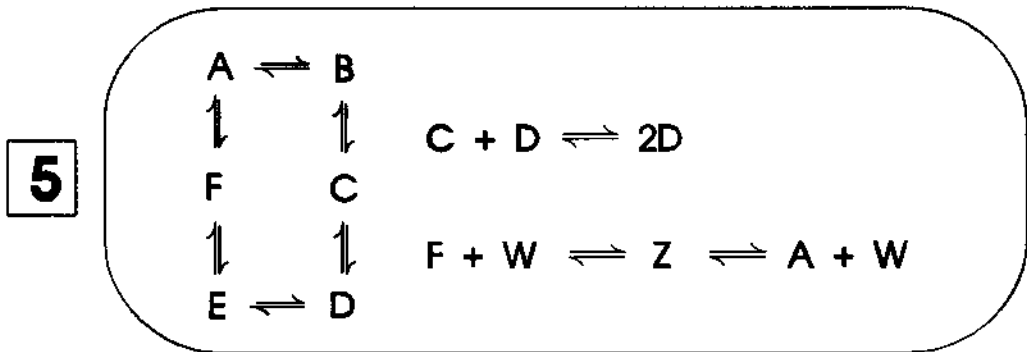
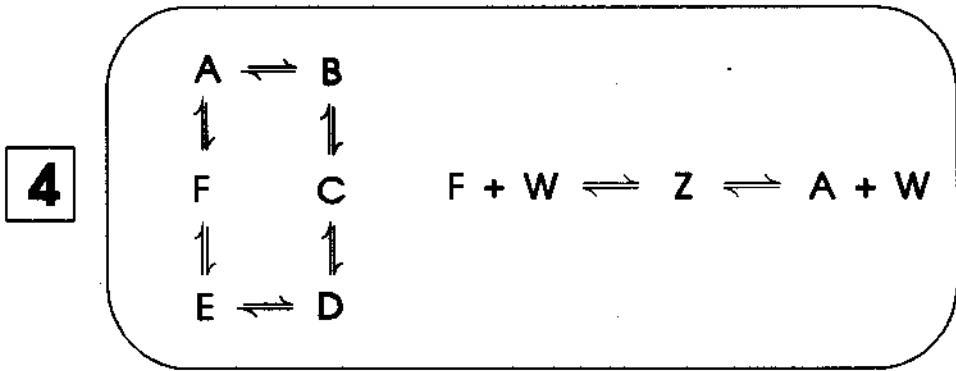
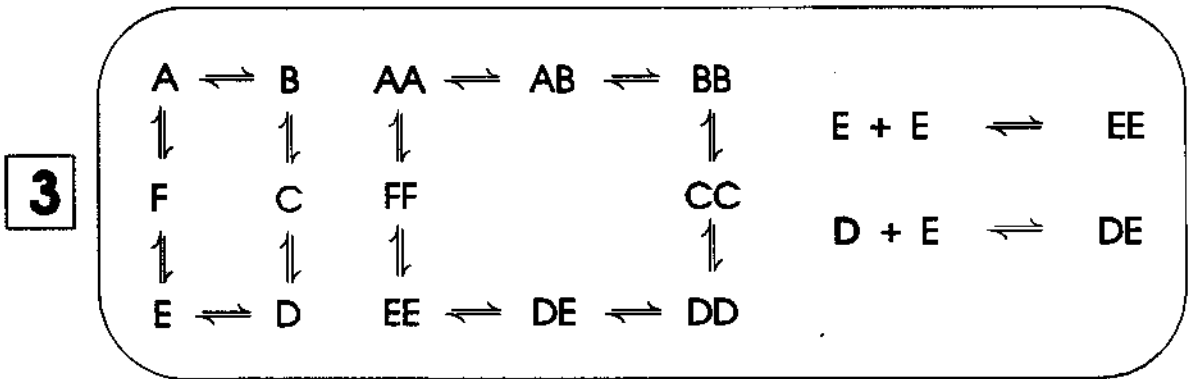
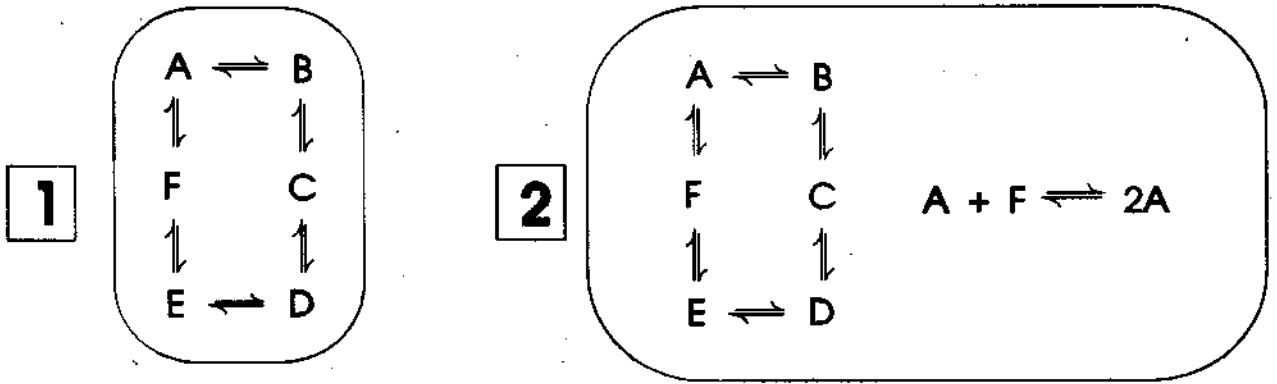
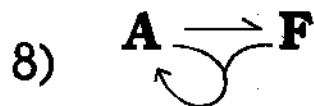
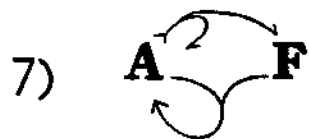
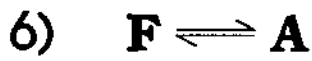
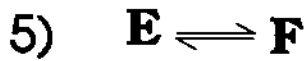
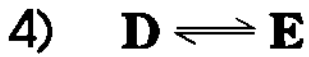
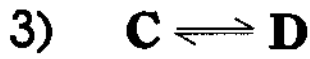
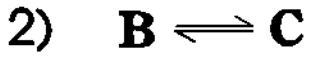
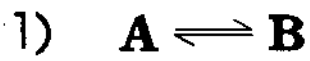
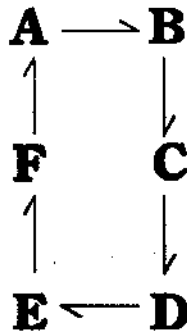


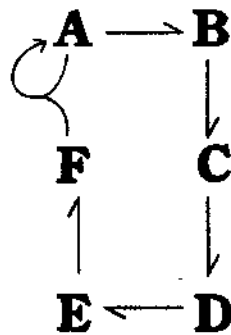
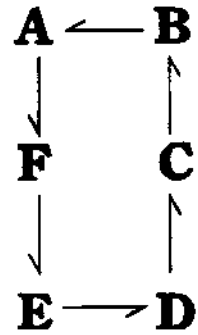
Fig. 2



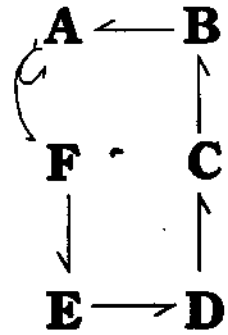
10)



11)

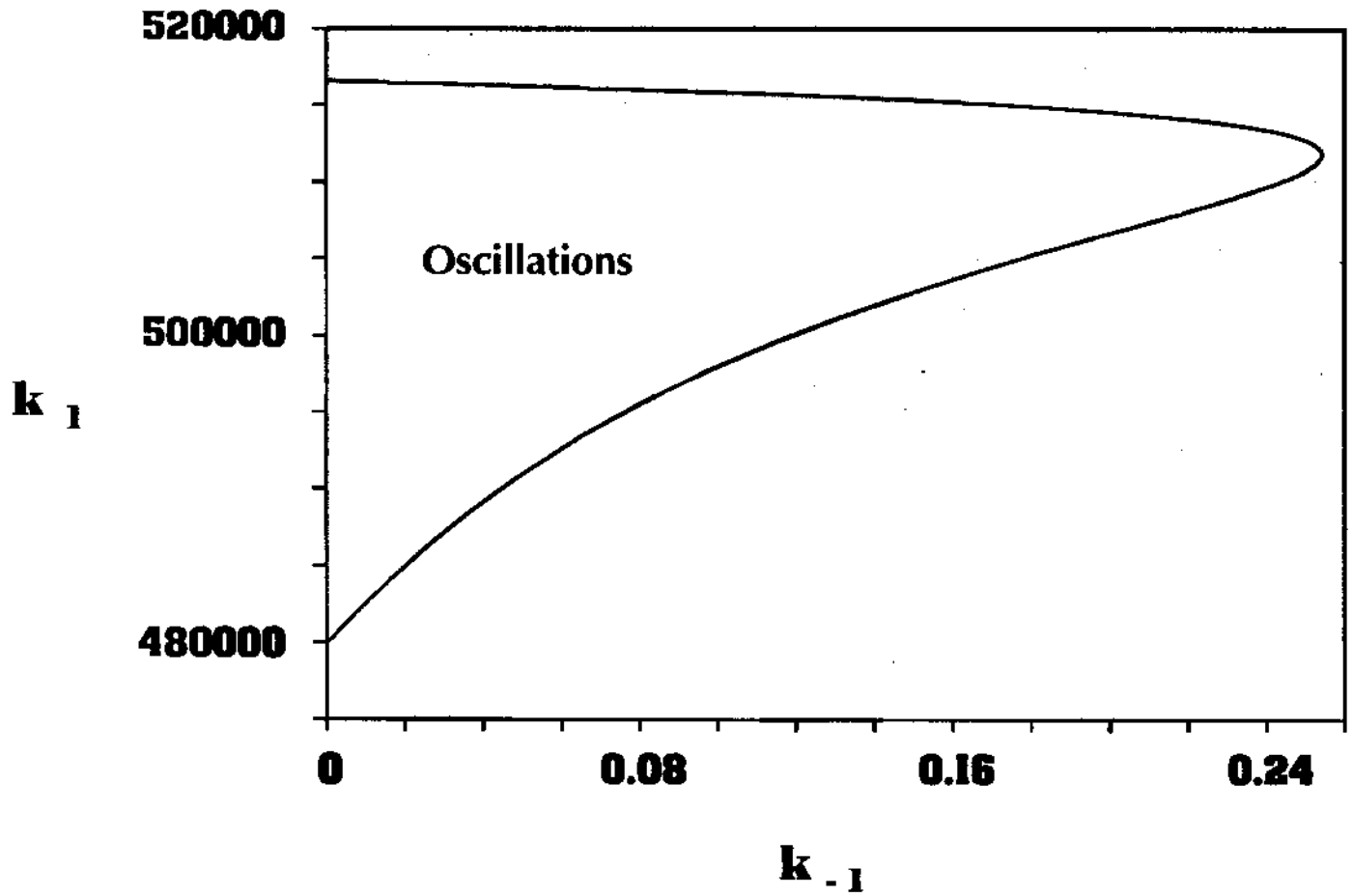


12)



13)

Fig. 3

*Fig. 4*

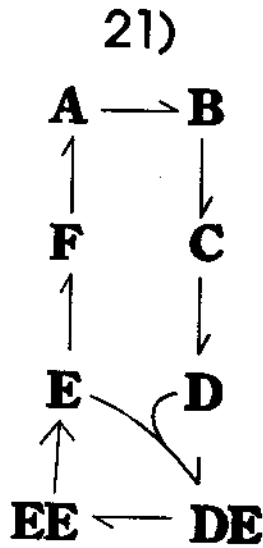
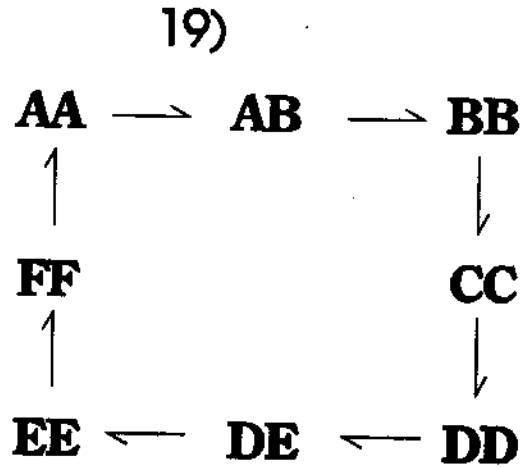
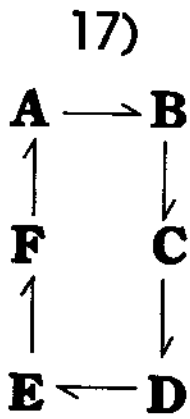
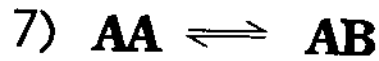


Fig. 5

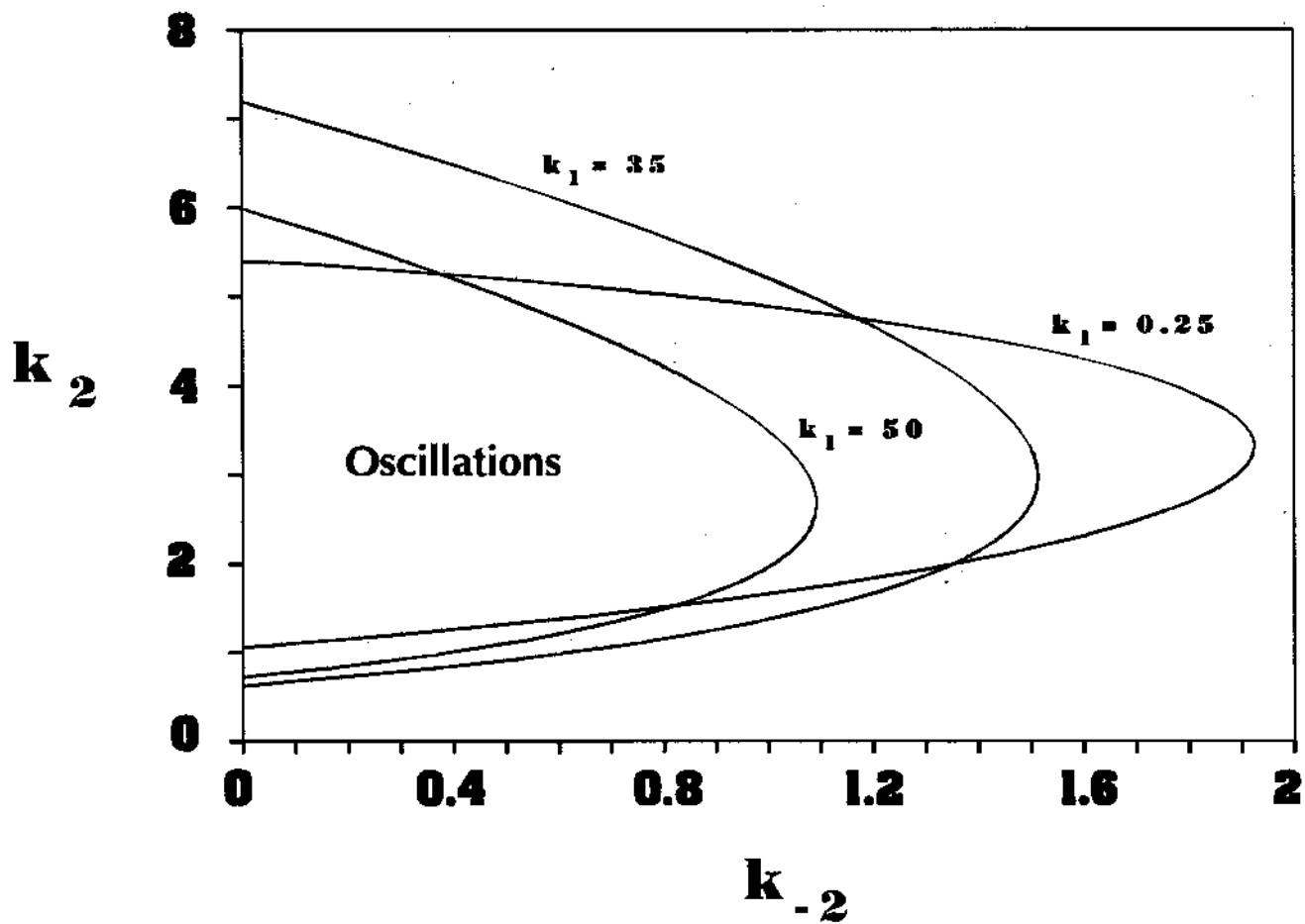
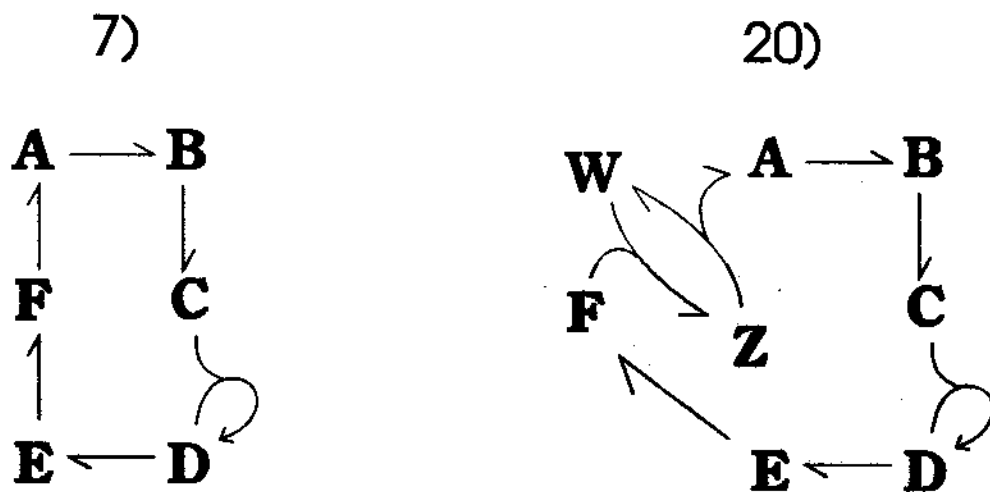
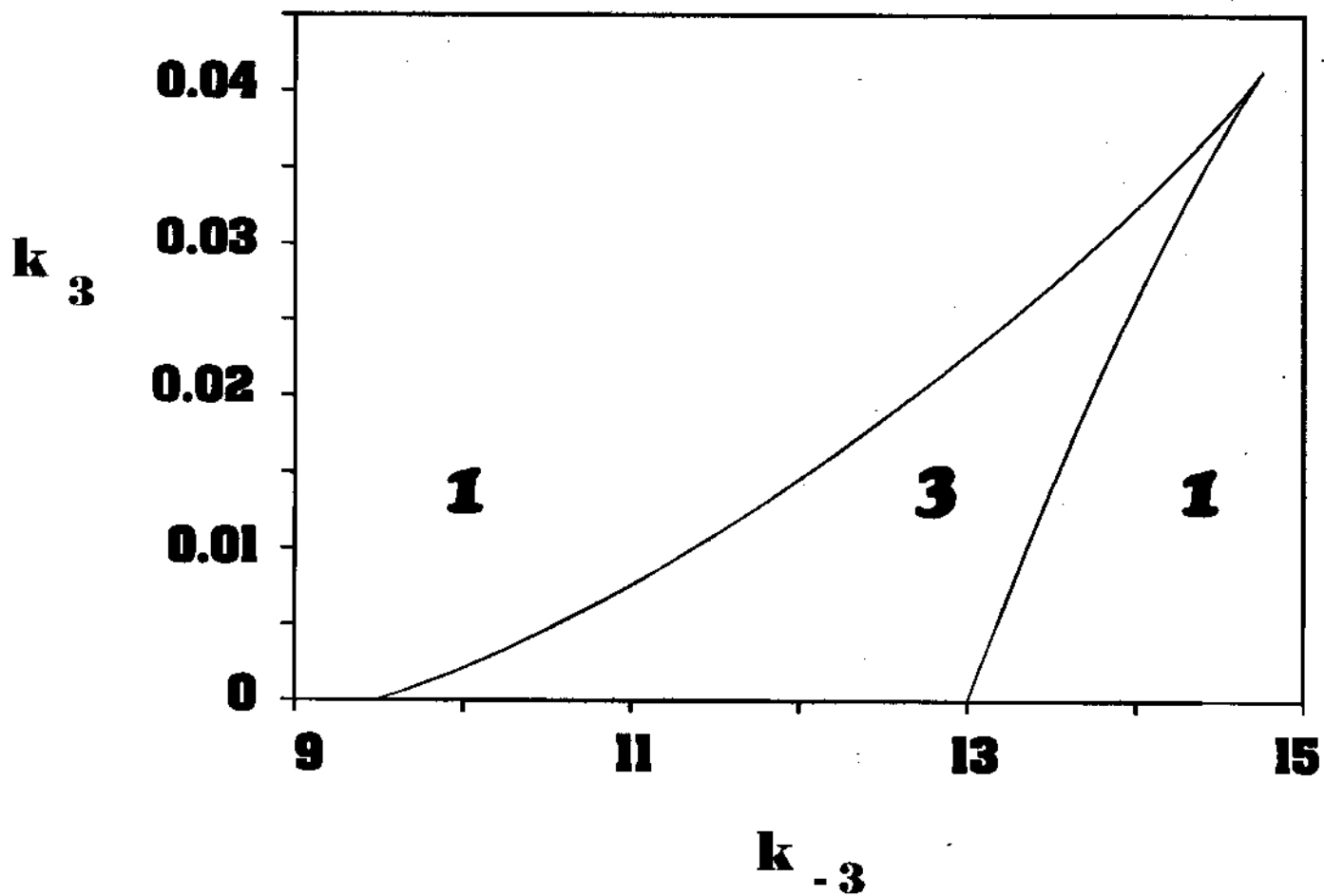


Fig. 6

*Fig. 7*

*Fig. 8*

Tables

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		

Table 1 : Parameters for model 2. The k_i 's are the rate constants and T is the total pump 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 2 : Parameters for model 3. The k_i 's are the rate constants and T is equal to the total monomer concentration plus twice the total dimer concentration.

<i>parameter</i>	<i>value</i>	<i>parameter</i>	<i>value</i>
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	30	k_{-8}	0.000006
k_9	3.8	k_{-9}	0.00004
T	68	P	2.5

Table 3 : Parameters for model 5. The k_i 's 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.

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