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TWO ENZYME ACTIVE TRANSPORT IN VITRO WITH pH INDUCED ASYMMETRICAL FUNCTIONAL STRUCTURES I. THE MODEL AND ITS ANALYTICAL TREATMENT

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A class of systems is characterized by the asymmetrical distribution of a sink and a source reaction, the asymmetry of the global chemical equation (energy liberation) and by an asymmetrical one-wave space profile. These systems belong to the family of primary chemical cells and can deplete and enrich the media they separate. A "one way" transport-reaction chain is needed for specific "real" active transport. A two enzyme model of this class is described in which the spatial asymmetry is due to a (diffusive) pH gradient; this distribution of "potential" enzyme activities is called the "functional structure". Equal potential enzyme activities and absence of reactive back action on local pH are assumed in the "square model" version of the pump. Analytical expressions of the enzymatic diffusion reactions are derived for zero and first order kinetics, i.e. in function of substrate concentrations. Tables of equations are presented. The intrinsic properties of the pump are characterized by (dimensionless) transport reaction parameters, (membrane composition); the "potential" activity is controlled by the pH gradient; the "effective" pumping is also a function of the substrate concentrations on the boundaries.

1. Introduction

Our immediate aim is to introduce a diffusive active transport model capable of achieving the transport of molecules and ions due to asymmetrical spatial distribution of enzyme activities, under the influence of enzyme regulating pH gradients. It belongs to a family of models characterized by space oscillatory profiles.

Active transport can be defined in a very general way as the transport of a species through a barrier which can be carried out even against the electrochemical potential gradient of the species (pumping). Such a process has to be "fueled" by an other, — energy dissipating, — chemical process, taking place in the system (see Kedem [1]). This parallel process generates the driving force for active transport. A great number of mechanisms can lead to such a phenomenon depending on the nature of the driving forces and the translocation processes involved.

Experimental models using enzyme reactions have been listed in the review articles of Selegny [2] and Thomas and Caplan [3]; together they cover most of the papers published before their appearance. One can distinguish e.g. a class of asymmetrical one enzyme reaction systems which generate electric currents and which can transport charged species. These systems have been analysed in terms of linear irreversible thermodynamics by different research groups [4,5]. The systems to be discussed here belong to another class in which molecules and ions can be transported by a chain of coupled diffusion-chemical reaction processes.

1) Chemical reactions and diffusion processes can be coupled through the mass balance [6a] by common ions and molecules participating in them simultaneously [2]. Such systems can be properly evaluated by diffusion-reaction kinetics.

As already mentioned by Mitchell [7] couplings of compartmented reactions through selective membranes have been implicitly included in the original chemical cell imagined by Guggenheim [8] and the concept of chemical cells has been brought by Rosenberg [9] to the attention of biologists. The concept of primary

Table 1
List of symbols

a	Surface of the membrane (normal to direction X).
D	Mean effective diffusion coefficient of S and P in the membrane.
E	Total thickness of the membrane.
e	Thickness of active layers 1 and 3.
J_D	Diffusion flux of S.
J_{Si}	Diffusion-reaction flux of S in layer i.
j_{si}	Dimensionless diffusion-reaction flux of S in layer i: $j_S = J_S/(DK_m/e)$.
J_{SI1}	Entering diffusion-reaction flux of S.
K_m	Michaelis constant of enzymes E_1 and E_2.
n	Ratio of inactive/active layer thickness.
$P(X)$	Concentration of P (intermediate product).
pH'_1 or $_2$	Optimal pH of enzyme E_1 or E_2.
R_S	Stoichiometric yield of transport.
$S(X)$	Concentration of S (transported substrate).
$s(x)$	Dimensionless concentration of S: $s = S/K_m$.
S_0	Initial concentration of S at $t = 0$.
S_1, S_2	Concentration of S in compartment 1 or 2.
$S_i(X)$	Concentration of S in layer i at point X.
$V(E_i)$	Potential enzyme activity at local pH.
$V(C_i)$	Effective enzyme activity, a function of pH and local conc. S or P.
V_m	Maximum activity of enzymes E_1 and E_2.
V_M	Global rate of transfer of S.
v_M	Volume of the membrane: $v_m = aE$.
v_C	Volume of the cell compartments 1 and 2.
W	Concentration of W (substrate).
X	Distance from entering face along X.
x	Dimensionless distance: $x = X/e$.
Z	Concentration of Z (substrate or product).
αe (σ')	Dimensionless first order kinetic parameter: $\alpha e = \sigma^{1/2}$. (See ref. [30].)
Σ	Dimensionless thiele modules type enzyme diffusion-reaction parameter of membrane: $\Sigma = V_m E^2/K_m D$ (see ref. [25]).
σ	Dimensionless thiele modulus type enzyme diffusion-reaction parameter of an active layer: $\sigma = V_m e^2/K_m D$ (see ref. [29]). ($\Sigma/\sigma = (n+2)^2$).
ΔS	Concentration difference of S between compartments 2 and 1.
ΔpH	pH gradient between cells 2 and 1.
ΔpH_{II}	pH gradient in central inactive layer II.
θ	Time constant for first order kinetics.
χ	Time constant for zero order kinetics. $\chi = 2Dv_m/E^2 v_C$.

chemical cells in distinction with secondary (osmotic) cells [10] has been used by Mitchell [7] in the basic elaboration of his proton-motive chemio-osmotic theories. In such active transport systems the presence of charged groups is incidental. The theoretical treatment is simpler if our considerations are limited to molecules. In this case electrochemical potentials reduce to chemical potentials. At constant temperature and pressure, in absence of convective transport, concentrations i.o. activities can be utilised to unite chemical stoichiometries and diffusion processes in easily formulated classical equations. When necessary, the treatment can be extended to include all these neglected effects.

To further simplify we assume that all the reactions take place between well identified boundaries. We name the system closed between these boundaries "membrane", and assume that this membrane is big enough and that the number of events is sufficient to justify the use of macroscopic theories. These models are in essence one-dimensional diffusion-chemical reaction pumps.

2) A few remarks on the reaction chain and the spatial distribution of the components are now required.

2a) In the simplest situation a single species S is pumped through the membrane by entering it from one side (the donor compartment) and leaving on the other side (acceptor compartment).

The chemical chain in the membrane will be of the type:

$$\cdot S \rightarrow P_1 \cdots P_n \rightarrow S\,, \qquad (1)$$

where P_i are intermediate products.

In view of the first and second principles of thermodynamics, fully reversible reaction chains of the type $S \rightleftarrows P_{1,n}$ must be discarded as they alone cannot liberate usable chemical energy and are compatible only with "downhill" facilitated, or exchange transports. To liberate energy in the operating conditions at least one parallel reaction has to take place in which energy is dissipated (e.g. the transformation of A or A'). This leads to the coupling with an irreversible reaction [11] or a modification that makes the total chemical pathway irreversible. The name "inversible" [12] will be used for stoichiometric reversibility to avoid confusion with thermodynamic reversibility in the operating conditions.

The shortest possible reaction chain is that with one intermediate product $P^{\ddagger}$. Let the chemical difference $P - S = T$ be the carrier (positive if captured or nega-

‡ In a more general schemes longer chains may be needed: AT→ A'T and A→ A' stand for all irreversible processes inclusive of AT→ A+T.

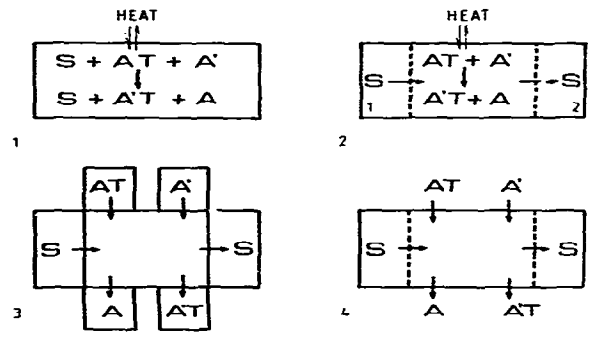

Fig. 1. Different (isotherm) arrangements and exchanges of the S + AT + A' → S + A + A'T system. 1. Closed homogeneous system; heat exchange; 2. Closed system but transfer of S from 1 to 2; 3. Open cross flows of AT → A and A' → A'T, transfer of S at T = Cte; 4. Same as 3. but out and in diffusion of all components.

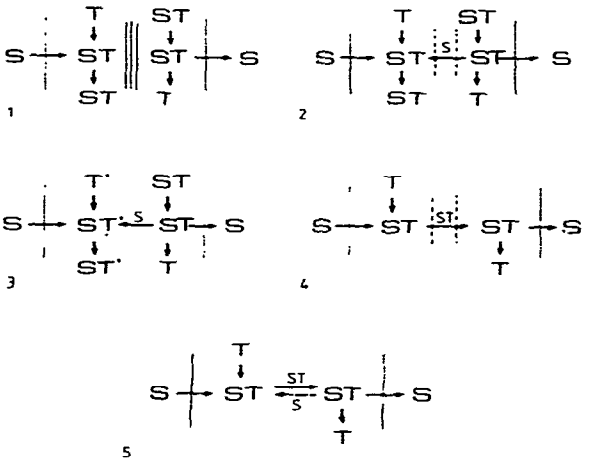

Fig. 2. Couplings of R_1 sink reaction (left) and R_2 source reaction (right) (correactants A or A' are not represented). Vertical: lines are films permeable to S; |i| "separators" permeable to i; arrows: inflows and outflows of carriers T and T'. Horizontally: diffusions and reactions of S and ST. 1. Impermeable separator, R_1 and R_2 are not coupled in the membrane: "apparent active transport", no oscillatory profile. 2. R_1 and R_2 coupled through S permeable separator; sink reaction is a dissipative S barrier, one wave S profile; (if T = OH^- and S = H^+: active transport of pH). 3. Coupling through S without separator; sink reaction is a dissipative S barrier; one wave S profile (if S = H^+ and T' = OH^-: active transport of pH); 4. Coupling through ST permeable but S impermeable separator "ideal" system. Interrupted S profile, space oscillatory ST profile. 5. R_1 and R_2 coupled through S and ST without separator or with an imperfect one. Active transport of S, typical one wave S and ST profiles.

tive if released by S), then a typical "inversible reaction couple" including both A and A' is

$$S + AT \rightarrow ST + A\,, \tag{2a}$$

$$ST + A' \rightarrow S + A'T\,, \tag{2b}$$

and the global chemical energy and carrier are furnished by the irreversible reaction

$$AT + A' \rightarrow A'T + A\,, \tag{3a}$$

coupled with the reversible

$$S + T \rightleftharpoons ST\,, \tag{3b}$$

reaction. As a result of inversibility, the two reaction steps of the couple can use different enzymes or catalysts if they are necessary and the chain reversal needs energy.

2b) At this stage one can represent the interactions of the reactants, AT, S, A' as a thermodynamic system or "black box" and consider the different situations schematically represented in figure 1. These situations will be evaluated in detail in another publication [13].

The spatial distributions need some attention. They must satisfy the conditions that allow the coupling of reactions and diffusion in the system. Local conditions are ruled by the Curie principle. Local anisotropies can lead to coupling even in symmetrical systems [14,6a]. In isotropic systems macroscopic asymmetry can play the same role as local anisotropy (see a fundamental study of DeSimone and Caplan [15] confirmed by recent demonstrations of Ripoll and Selegny [13]). This is reasonable as in a completely homogeneous and symmetrical medium scalar chemical reactions and statistically scalar diffusion (brownian diffusion) cannot generate undirectional flows. — Practically, we know that proper asymmetrical distribution of reactive sites and diffusive properties, compositions or conditions are needed for vectorial net flows to be produced as in chemical cells. A major part of the question is how they can be generated and characterized?

2c) In the systems under examination space oscillatory concentration profiles [16] (or in a more general context of electrochemical potentials [2,12]) provide

a tool for establishment of active transport.

Well known dissipative structures with local symmetry breaking but overall symmetry have been reported by Prigogine et al. [17] with diffusion–multireaction systems. We have also shown [16] that as a stationary solution of coupled diffusion–two enzyme systems with appropriate kinetic laws space oscillatory substrate concentration profiles are obtained. *Overall asymmetry* of this profile i.e. whole (or approximately whole) number of stationary space waves corresponds to negative fluxes, that is active transport of the substrate.

The simplest profile is composed of *one space wave only*. Its clear significance [2] is the association of a geometrically distinct substrate sink (R_1) and of a substrate source (R_2) (absolute or relative to each other). For stoichiometric reasons if R_1 and R_2 are chemical reactions then R_1 is a product source and R_2 a product sink. In figure 2 five possible arrangements of sink and source reaction couples and their transport links are represented. To form a chain R_1 and R_2 are connected with selective "passive" barriers (in agreement with the general Guggenheim concept of arranging physical or chemical compartments in the chemical cell [8]). Selectivities can be due to charge, solubility, pore size, carrier facilitated transport or similar conducting mechanisms. They can give rise to "uniport", "symport" or "antiport" as defined by Mitchell [7].

The external membrane boundaries are limited by S permeable and P (i.e. ST) impermeable "films". All five membrane systems are able to deplete one of the media they separate and enrich the other but the transport effect depends on the "separator" between the reaction sites.

The first three systems cannot transfer S, but they produce "apparent" active transport. In system 1 the "separator" is impermeable, and the sink and the source are not coupled inside the membrane. In system 2 the separator is permeable to S but not to ST; due to this compartmentization T can be the same in both reactions.

In system 3 without a separator the geometrical differentiation is more evident with distinct T and T'. As mentioned in the figure legend cases 2 and 3 can correspond for example to "active transport of pH".

System 4 is the ideal transport system. Its three passive barriers are permeable to S, ST and S respectively. The S profile is discontinuous, while the ST profile forms an incomplete wave. But perfect separators selectively impermeable to molecules are often not available in the laboratory and this fact was cited as the main reason [9] for the lack of development of chemical cells as compared to electrochemical ones.

When the separator is absent or imperfect, system 5 is obtained. Here sink and source are coupled by transport and reaction of S and ST and both will produce space wave profiles.

With systems 4 and 5 we have *"one way" diffusion-reaction chains* and "real" active transport. In the "perfect" system 4 the stoichiometric yield of transformation of the chemical reactions into transport (osmotic effect) is 100%. Each transport or reaction step controls the following one and in the steady state the transport and chemical rates J_j are equal:

$$J_{S\,in} = J_{R1} = J_{ST} = J_{R2} = J_{S\,out} \cdot \qquad (4)$$

In the leaking, "imperfect" system 5 a part of S ($J_{S\,back}$) is refed from R_2 to R_1 by passive back-diffusion and is being retransformed to ST.

$$J_{S\,in} = J_{R1} - J_{S\,back} = J_{R2} - J_{S\,back} = J_{S\,out} \cdot \qquad (5)$$

Further examination shows that R_1 and R_2 and convenient connecting barriers can also be united in systems transporting T or ST instead of S, but P as defined above can continue to represent the intermediate product in such systems. Moreover dissociation products of ST for example can also be exchanged through permeable barriers. Simultaneously coupled S, T etc.... pumps (including heat) using the same R_1 and R_2 thus appear possible.

2d) Enzymes deserve a special mention in relation to these systems. Many slow reactions will not take place at a significant rate when and where enzymes are absent even when the reactants are mixed together. Selectively lowering the activation energy of a chemical pathway enzymes orient the reactions and constitute selective chemical links in the system as selective separators do physically. Consequently they can simultaneously produce asymmetrical geometrical compartmentalization, reaction orientation and kinetic (energetic) effects and considerably enlarge the field of interest of chemico-motive chemical cells.

Heterogeneous enzyme kinetics, regulations, environmental and feed back effects have been intensively studied during the last decade with immobilized enzymes. They make many various situations and many

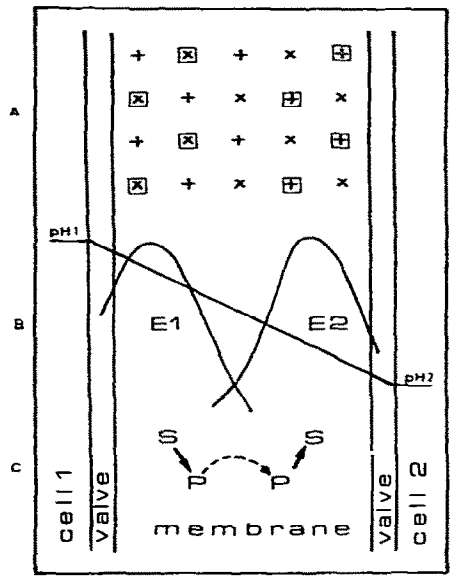

Fig. 3. Schematic diagram of an active transport membrane with asymmetric functional structure: A) Homogeneously distributed enzymes E1 (×) and E2 (+) and activity distribution with a pH gradient: active (□ □) and inactive (× +). B) Activity profile of enzymes E1 and E2 as a function of pH. C) Scheme of reactions. (→ reaction; – – → diffusion). The orientation of the pH gradient determines the direction of pumping: an inversion of the gradient inverses the pumping flow.

different sink and source reactions possible. Effectors of enzyme activity, their exchange or production provides the system with means of control and regulation. Hydrogen (or OH^-) ions are universal regulators as all enzyme activities are pH dependent.

Investigations of "leaking" systems have made much progress during the last decade.

The first experimental pump [18] used hexokinase and phosphatase enzymes to achieve the active transport of glucose. The system already included the necessary coupled reactions and ATP as a source of energy and of carrier. The membrane asymmetry obtained with two separate enzyme layers has been qualified of "permanent structure" [2].

This model has been analysed analytically in the stationary state, the effectiveness of the space oscillatory profile was known, and the possible biological implications were discussed [19–22]. Its evolutions have been treated numerically [23,24].

Other enzymatic [2,25–27] or fully synthetic [12, 28] models have followed.

It can be said that all these systems are characterized by the *triple asymmetry* of the chemical equation, of the one wave space profile and of the distribution of the sink and source reactions.

The highest degree of freedom and the greatest number of variables are found in the "dissipative" version of the "functional structure" model [2,25].

Our recent investigations have been focused on two points: first, on the communication of asymmetry from the "membrane" to the bounding media and back, and secondly on the progressive exploration of single, molecular or ion, pumps and coupled pumps and on the regulating mechanisms included in the model.

2. The functional structure model induced by diffusion

This model is generated by establishing a pH gradient through a homogeneous mixture of two enzymes. As a result an asymmetrical distribution of "potential" enzyme activities is achieved. The obtained system will be named "functional structure" (fig. 3).

When the pH gradient is due to diffusion of acid (base) enforced by proper boundary conditions the functional organization diminishes as soon as this diffusion process stops; it is clear that this system belongs to the family of "functional structures" coupled to dissipative phenomena [6b] and may be treated as such.

For the sake of simplicity in the following a one dimensional membrane-like model will be discussed. However, other configurations (e.g. cylindrical or spherical) can be materialized as well.

When such a pH gradient is applied three layers of different enzyme activities are induced in the membrane. The relative thicknesses (e and ne) of these layers depend on the nature of the pH gradient (fig. 3). The total thickness is $E = (n+2)e$.

Layer 1: The internal pH is equal to or not much different from the pH at which the activity of the first enzyme is optimal; enzyme E1 is activated and the transformation of S entering from the "donor" compartment 1 takes place. P accumulates and tends to diffuse; but as on the left it meets an impermeable valve film, the diffusion is oriented towards layers 2 and 3.

Layer 2: the local pH does not activate any of the

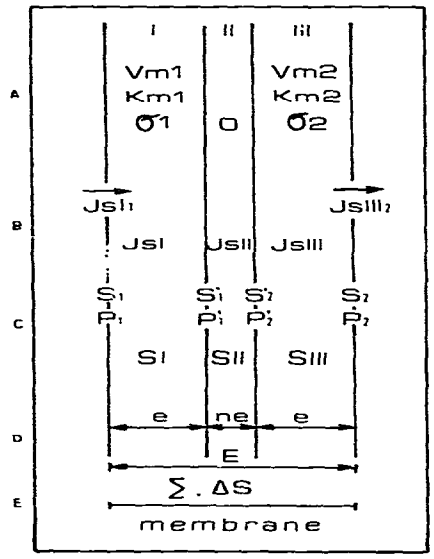

Fig. 4. Schematic diagram of active transport membrane parameters: A) Activity parameters of the layers; B) Substrate fluxes inside the layers and at membrane limits; C) Substrate and product concentrations; D) Thickness of layers and membrane; E) Global parameters of membrane.

enzymes, and S and P are only submitted to passive diffusion.

Layer 3: the internal pH is close to the optimal pH of the second enzyme, E2 is activated and P is retransformed into S. S diffusing to the left will be refed into layer 1, and to the right it will reach the acceptor compartment.

Thus the net effect will be the transport of S from the donor to the acceptor compartment, controlled by the diffusion process which is used to establish the pH gradient.

It must be stressed, that the enzymes are not necessarily fixed to the membrane matrix, but in fact they may freely diffuse. The only restrictions are that the pH gradient is maintained, the response of the enzymes of pH changes is immediate and their stationary amount constant. Nevertheless an enzyme and its substrates should not meet at an activating pH outside the membrane.

For the demonstration of the system a glucose pump was developed. The experiments will be reported in the next paper.

The analytical kinetic treatment of the system is presented here.

3. Theory

In th mathematical description of the system, the following assumptions have been made (see also fig. 4):

1. The pH profile through the membrane was assumed to be linear.

2. It was assumed that the activity of enzyme E1 (E2) in the first (third) layer is constant and in the other layers vanishingly small. Furthermore the activities (V_m) and affinities (K_m) of both enzymes were taken to be equal. In the following discussion this distribution will be referred to as the "Square model".

3. It was assumed that the enzymatic reactions do not influence the pH gradient and that the concentration of all the components participating in the reactions, except S and P, remain unchanged.

4. Michaelis-type enzymes were considered.

5. We also assumed that the diffusion coefficients of S and P are equal and constant all over the system.

6. P is limited to the membrane.

The concentration profiles and fluxes in the membrane are calculated from time-independent models. These results were incorporated when time-dependent global transport equations were derived. This is allowable if the relaxation-time of the separate events in the membrane is much shorter than the duration of the whole process.

The mass balance for any component Z is:

$$\left(\frac{\partial Z}{\partial t}\right)_{\text{total}} = \left(\frac{\partial Z}{\partial t}\right)_{\text{reaction}} + \left(\frac{\partial Z}{\partial t}\right)_{\text{diffusion}}, \qquad (6)$$

$(\partial Z/\partial t)_{\text{reaction}}$ for S and P in the first and third layer are as follows:

$$(\partial Z/\partial t)_R = \pm V_m W/(K_m + W), \qquad (7)$$

W is the reacting species, it is identical to Z or different of it, depending on S, P and layer considered; in detail: first layer:

$$(\partial P/\partial t)_R = -(\partial S/\partial t)_R = V_m S/(K_m + S), \qquad (8a)$$

third layer:

$$-(\partial P/\partial t)_R = (\partial S/\partial t)_R = V_m P/(K_m + P). \qquad (8b)$$

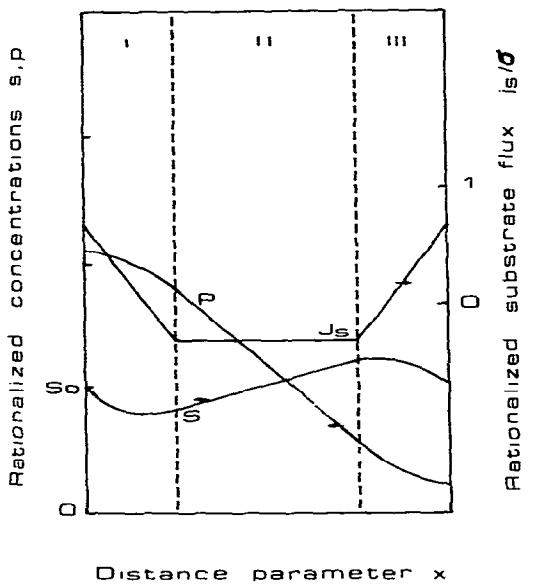

Fig. 5. Steady state concentration profiles and fluxes with $\Delta S = 0$, for zero order reactions. Substrate concentration profile (S); product concentration profile (P); substrate flux profile (J_S).

The diffusion fluxes are obtained from Fick's first law:

$$\vec{J_z} = -D_z \overrightarrow{\mathrm{Grad}}(Z) . \tag{9}$$

Under steady state conditions in the membrane $(\partial Z/\partial t)_T = 0$ and thus:

$$D\,\mathrm{d}^2Z/\mathrm{d}X^2 \pm V_m W/(K_m + W) = 0 . \tag{10}$$

In the following two further simplifying assumptions will be made:

$$(\partial Z/\partial t)_R = \pm(V_m/K_m)Z \quad \text{(first order reaction)} , \tag{11}$$

$$(\partial Z/\partial t)_R = \pm V_m \quad \text{(zero order reaction)} . \tag{12}$$

The solutions of the above equations are based on ref. [29] (zero order) and [30] (first order) and on computations of ref. [2].

The activating pH gradient must also be taken into account; this is done through the relative layer thickness n: its pH dependence will be approximated by eq. (13), implicitly assuming a linear pH profile $X = k(\mathrm{pH} - \mathrm{pH_0})$ in the membrane (k = constant; $\mathrm{pH_0}$ = pH at $X = 0$):

$$n = 2\Delta\mathrm{pH}_n/(\Delta\mathrm{pH} - \Delta\mathrm{pH}_n) , \tag{13}$$

where ΔpH is the gradient through the membrane and $\Delta\mathrm{pH}_n$ the difference between the active domains of the two enzymes.

3.1. Solutions with zero order reactions

3.1.1. Concentration profiles and fluxes (see fig. 5).

The situation in each layer is expressed separately:

layer 1 $(0 \leqslant X \leqslant E/(n+2))$:

$$D\,\mathrm{d}^2S/\mathrm{d}X^2 - V_m = 0 , \tag{14}$$

integration between the limits of the layer and boundary concentrations S_1 and S_1' gives:

$$S_I(X) = \frac{V_m}{2D}X^2 + \left(\frac{S_1' - S_1}{e} - \frac{V_m e}{2D}\right)X + S_1 , \tag{15}$$

layer 2 $(E/(n+2) \leqslant X \leqslant E(n+1)/(n+2))$:

$$D\,\mathrm{d}^2S/\mathrm{d}X^2 = 0 , \tag{16}$$

$$S_{II}(X) = \frac{S_2' - S_2}{ne}(X - e) + S_1' , \tag{17}$$

layer 3 $(E(n+1)/(n+2) \leqslant X \leqslant E)$:

$$D\,\mathrm{d}^2S/\mathrm{d}X^2 + V_m = 0 , \tag{18}$$

$$S_{III}(X) = -\frac{V_m}{2D}(X - (1+n)e)^2 + \left(\frac{S_2 - S_2'}{e} + \frac{V_m e}{2D}\right)(X - (1+n)e) + S_2' . \tag{19}$$

Concentrations S_1' and S_2' at the interfaces are obtained from the diffusion flux equation (Fick's first law eq. (9)) at $X = e$ and $X = (n+1)e$ respectively:

$$S_1' = \frac{1}{n+2}\left(S_2 + (n+1)S_1 - \frac{nV_m e^2}{2D}\right) , \tag{20a}$$

$$S_2' = \frac{1}{n+2}\left(S_1 + (n+1)S_2 + \frac{nV_m e^2}{2D}\right) . \tag{20b}$$

Substitutions in eqs. (15), (17) and (19) lead to the equations of concentration profiles and of local fluxes; for example in layer 1 the substrate concentration is

Table 2
Two enzyme active transport in a membrane with *zero order* reactions. Equations of (membrane type) unidirectional diffusion-reactions in the quasi stationary state with Michaelis type enzymes. Fundamental cases: no regulatory effects: zero order reaction: $s > 10$ in layer 1 and $p > 10$ in layer 3.

a) Concentrations, profiles, gradients, left: real forms; right: dimensionless forms.

Concentration profiles		
Substrate, layer 1	$S_I(X) = \frac{V_m}{2D}X^2 + \left(\frac{S_1' - S_1}{e} - \frac{V_m e}{2D}\right)X + S_1$	$s_I(x) = \frac{\sigma}{2}(x^2 - x) + (s_1' - s_1)x + s_1$
Substrate, layer 2	$S_{II}(X) = \frac{S_2' - S_1'}{ne}X + S_1' - \frac{S_2' - S_1'}{n}$	$s_{II}(x) = \frac{s_2' - s_1'}{n}(x-1) + s_1'$
Substrate, layer 3	$S_{III}(X) = -\frac{V_m}{2D}X^2 + \left(\frac{S_2 - S_2'}{e} + \frac{V_m e}{2D}(2n+3)\right)X + S_2 + (S_2' - S_2)(n+2) - \frac{V_m e^2}{2D}(n^2+3n+2)$	$s_{III}(x) = \frac{\sigma}{2}(-x^2 + (2n+3)x - n^2 - 3n - 2) + (s_2' - s_2)(n+2-x) + s_2$
Product, layer 1	$P_I(X) = -\frac{V_m}{2D}X^2 + \left(\frac{P_1' - P_1}{e} + \frac{V_m e}{2D}\right)X + P_1$	$p_I(x) = \frac{\sigma}{2}(-x^2 + x) + (p_1' - p_1)x + p_1$
Product, layer 2	$P_{II}(X) = \frac{P_2' - P_1'}{ne}X + P_1' - \frac{P_2' - P_1'}{n}$	$p_{II}(x) = \frac{p_2' - p_1'}{n}(x-1) + p_1'$
Product, layer 3	$P_{III}(X) = \frac{V_m}{2D}X^2 + \left(\frac{P_2 - P_2'}{e} - \frac{V_m e}{2D}(2n+3)\right)X + P_2 + (P_2' - P_2)(n+2) + \frac{V_m e^2}{2D}(n^2+3n+2)$	$p_{III}(x) = \frac{\sigma}{2}(x^2 - (2n+3)x + n^2 + 3n + 2) + (p_2' - p_2)(n+2-x) + p_2$
Interfacial concentrations		
Substrate, layers 1/2	$S_1' = \frac{1}{n+2}\left(S_2 + (n+1)S_1 - \frac{nV_m e^2}{2D}\right)$	$s_1' = \frac{1}{n+2}\left(s_2 + (n+1)s_2 - \frac{n\sigma}{2}\right)$
Substrate, layers 2/3	$S_2' = \frac{1}{n+2}\left(S_1 + (n+1)S_2 + \frac{nV_m e^2}{2D}\right)$	$s_1' = \frac{1}{n+2}\left(s_1 + (n+1)s_2 + \frac{n\sigma}{2}\right)$
Product, layers 1/2	$P_1' = \frac{1}{n+2}\left(P_2 + (n+1)P_1 + \frac{nV_m e^2}{2D}\right)$	$p_1' = \frac{1}{n+2}\left(p_2 + (n+1)p_1 + \frac{n\sigma}{2}\right)$
Product, layers 2/3	$P_2' = \frac{1}{n+2}\left(P_1 + (n+1)P_2 - \frac{nV_m e^2}{2D}\right)$	$p_2' = \frac{1}{n+2}\left(p_1 + (n+1)p_2 - \frac{n\sigma}{2}\right)$
Product, cells 1/2	$P_1 - P_2 = (n+1)\frac{V_m e^2}{D}$	$p_1 - p_2 = (n+1)\sigma$
Maximum load of the pump	$\Delta S_{max} = \frac{n+1}{(n+2)^2}\frac{V_m E^2}{D}$	$\Delta s_{max} = (n+1)\sigma = \frac{n+1}{(n+2)^2}\Sigma$
Kinetics (with $\chi = 2Dv_m/E^2 v_c$)		
Substrate concentration difference	$\Delta S = \frac{n+1}{(n+2)^2}\frac{V_m E^2}{D}(1 - \exp(-\chi t))$	$\Delta s = (n+1)\sigma(1 - \exp(-\chi t))$
Substrate concentration in cell 1	$S_1(t) = S_0 - \frac{n+1}{(n+2)^2}\frac{V_m E^2}{2D}(1 - \exp(-\chi t))$	$s_1(t) = s_0 - \frac{n+1}{2}\sigma(1 - \exp(-\chi t))$
Substrate concentration in cell 2	$S_2(t) = S_0 + \frac{n+1}{(n+2)^2}\frac{V_m E^2}{2D}(1 - \exp(-\chi t))$	$s_2(t) = s_0 + \frac{n+1}{2}\sigma(1 - \exp(-\chi t))$

Table 2
(Continued)

b) Fluxes, yield, extraction, left: real forms; right: dimensionless forms.

Diffusion-reaction fluxes		
Substrate, layer 1	$J_{SI}(X) = -V_m X - D\left(\frac{S_1' - S_1}{e} - \frac{V_m e}{2D}\right)$	$j_{SI}(x) = \sigma\left(-x + \frac{1}{2} + s_1 - s_1'\right)$
Substrate, layer 2	$J_{SII}(X) = -D\frac{S_2' - S_1'}{ne}$	$j_{SII}(x) = -\frac{s_2' - s_1'}{n}$
Substrate, layer 3	$J_{SIII}(X) = +V_m X - D\left(\frac{S_2 - S_2'}{e} + \frac{V_m e}{2D}(2n+3)\right)$	$j_{SIII}(x) = \sigma\left(x - \frac{2n+3}{2}\right) + s_2' - s_2$
Product, layer 1	$J_{PI}(X) = +V_m X - D\left(\frac{P_1' - P_1}{e} + \frac{V_m e}{2D}\right)$	$j_{PI}(x) = \sigma\left(x + \frac{1}{2}\right) + p_1 - p_1'$
Product, layer 2	$j_{PII}(X) = \frac{P_2' - P_1'}{ne}$	$J_{PII}(x) = -D\frac{p_2' - p_1'}{n}$
Product, layer 3	$J_{PIII}(X) = -V_m X - D\left(\frac{P_2 - P_2'}{e} - \frac{V_m e}{2D}(2n+2)\right)$	$j_{PIII}(x) = \sigma\left(-x + \frac{2n+3}{2}\right) + p_2' - p_2$
Substrate entering flux	$J_{SI1} = -D\frac{S_1' - S_1}{e} + \frac{V_m e}{2}$	$j_{SI1} = \frac{\sigma}{2} + s_1 - s_1'$
Substrate diffusion flux	$J_D = -D\frac{S_2 - S_1}{(n+2)e}$	$j_D = \frac{s_2 - s_1}{n+2}$
Global rate of transfer	$V_M = \frac{1+n}{(2+n)^2}\frac{v_m}{v_c}V_m$	$\frac{V_M}{V_m} = \frac{n+1}{(n+2)^2}\frac{v_m}{v_c}$
Stoichiometric yield	$R_S = \frac{1+n}{2+n}\frac{1-\exp(-\chi t)}{\chi t}$	$R_S = \frac{n+1}{n+2}\frac{1-\exp(-\chi t)}{\chi t}$
Extracting power	$S_2/S_1 = \frac{2DS_0 + (n+1)V_m e}{2DS_0 - (n+1)V_m e}$	$s_2/s_1 = \frac{2s_0 + (n+1)\sigma}{2s_0 - (n+1)\sigma}$

$$S_I(X) = \frac{V_m}{2D}X^2 + \left(\frac{S_2 - S_1}{(n+2)e} - \frac{n+1}{n+2}\frac{V_m e}{D}\right)X + S_1 \quad (21)$$

and the local substrate flux is:

$$J_{SI}(X) = -V_m X - \frac{D}{(n+2)e}(S_2 - S_1) + \frac{n+1}{n+2}V_m e. \quad (22)$$

The detailed flux and concentration equations are given in table 2. By the combination of these equations one can calculate the profiles, layer by layer, in the whole thickness of the membrane. Fig. 5 shows an example where one may particularly remark the "substrate space wave".

3.1.2. Global kinetics of active transport

The evolution of the donor compartment concentration S_1 and the flux J_{SI1} are linked together by the relation

$$dS_1/dt = -(a/v_{c1})J_{SI1}. \quad (23)$$

In the symmetric system ($v_{c_1} = v_{c_2} = v_c$) where $\Delta S = 0$ at $t = 0$, we have:

$$S_1 = S_0 - \tfrac{1}{2}\Delta S, \quad d(\Delta S)/dt = -2\,dS_1/dt. \quad (24)$$

The differential equation:

$$-\frac{d(\Delta S)}{dt} = \frac{2v_m D}{v_{c1}E^2}\left(\Delta S - \frac{1+n}{(2+n)^2}\frac{V_m E^2}{D}\right), \quad (25)$$

integrates to:

Table 3

Two enzyme active transport in a membrane with *first order* reactions. Equations of (membrane type) unidirectional diffusion-reactions in the quasi stationary state with Michaelis type enzymes. Fundamental cases: no regulatory effects; first order reaction: $s < 1/10$ in layer 1 and $p < 1/10$ in layer 3.

a) Concentrations, profiles, gradients; left: real forms; right: dimensionless forms.

Concentration profiles

Substrate, layer 1	$S_1(X) = \dfrac{S_1' \sinh(\alpha X) + S_1 \sinh(\alpha e - \alpha X)}{\sinh(\alpha e)}$	$s_1(x) = \dfrac{s_1' \sinh(\sigma' x) + s_1 \sinh(\sigma' - \sigma' x)}{\sinh\sigma'}$
Substrate, layer 2	$S_{II}(X) = \dfrac{S_2' - S_1'}{ne} X + S_1' - \dfrac{S_2' - S_1'}{n}$	$s_{II}(x) = \dfrac{s_2' - s_1'}{n}(x - 1) + s_1'$
Substrate, layer 3	$S_{III}(X) = -P_{III}(X) + \dfrac{P_2 + S_2 - P_2' - S_2'}{e} X + S_2' + P_2' - (1+n)(S_2 + P_2 - S_2' - P_2')$	$s_{III}(x) = -p_{III}(x) + (p_2 + s_2 - p_2' - s_2')(x - n - 1) + s_2' + p_2'$
Product, layer 1	$P_1(X) = -S_1(X) + \dfrac{P_1' + S_1' - P_1 - S_1}{e} X + P_1 + S_1$	$p_1(x) = -s_1(x) + (p_1' + s_1' - p_1 - s_1)x + p_1 + s_1$
Product, layer 2	$P_{II}(X) = \dfrac{P_2' - P_1'}{ne} X + P_1' - \dfrac{P_2' - P_1'}{n}$	$p_{II}(x) = \dfrac{p_2' - p_1'}{n}(x - 1) + p_1'$
Product, layer 3	$P_{III}(X) = \dfrac{P_2 \sinh(\alpha X - \alpha(n+1)e) + P_2' \sinh(\alpha(n+2)e - \alpha X)}{\sinh(\alpha e)}$	$p_{III}(x) = \dfrac{p_2 \sinh(\sigma'(x - 1 - n)) + p_2 \sinh(\sigma'(n+2-x))}{\sinh\sigma'}$

Interfacial concentrations

Substrate, layers 1/2	$S_1' = \dfrac{(S_2 \sinh(\alpha e) + \alpha e S_1(\cosh(\alpha e) + n))(\cosh(\alpha e) + 1) - S_1 \sinh(\alpha e)(\cosh(\alpha e) - 1)}{2\sinh(\alpha e)\cosh(\alpha e) + \alpha e(n\cosh(\alpha e) + 1)(\cosh(\alpha e) + 1)}$	$s_1' = \dfrac{s_2 \sinh\sigma' + \sigma' s_1(\cosh\sigma' + n)(\cosh\sigma' + 1) - s_1 \sinh\sigma'(\cosh\sigma' - 1)}{2\sinh\sigma'\cosh\sigma' + \sigma'(n\cosh\sigma' + 1)(\cosh\sigma' + 1)}$
Substrate, lyaers 3/2	$S_2' = \dfrac{S_1'(n\alpha e\cosh(\alpha e) + \sinh(\alpha e)) - n\alpha e S_1}{\sinh(\alpha e)}$	$s_2' = \dfrac{s_1'(n\sigma'\cosh\sigma' + \sinh\sigma') - n\sigma' s_1}{\sinh\sigma'}$
Product, layers 2/3	$P_2' = \dfrac{(S_2 - S_2')\sinh(\alpha e)\cosh(\alpha e) - \alpha e S_1' \cosh(\alpha e) + \alpha e S_1 \cosh^2(\alpha e)}{\sinh(\alpha e)(\cosh(\alpha e) - 1)}$	$p_2' = \dfrac{(s_2 - s_2')\sinh\sigma'\cosh\sigma' - \sigma' s_1' \cosh\sigma' + \sigma' s_1 \cosh^2\sigma'}{\sinh\sigma'(\cosh\sigma' - 1)}$
Product, layer 3/cell 2	$P_2 = \dfrac{P_2'}{\cosh(\alpha e)}$	$p_2 = \dfrac{p_2'}{\cosh\sigma'}$
Product, layers 1/2	$P_1' = S_2'(1+n) - nS_2 - S_1' + P_2'\left(n + 1 - \dfrac{n}{\cosh(\alpha e)}\right)$	$p_1' = s_2'(n+1) - ns_2 - s_1' + p_2'\left(n + 1 - \dfrac{n}{\cosh\sigma'}\right)$
Product, cell 1/layer 1	$P_1 = P_1' + S_1' - S_1 + \dfrac{\alpha e(S_1\cosh(\alpha e) - S_1')}{\sinh(\alpha e)}$	$p_1 = p_1' + s_1' - s_1 + \dfrac{\sigma'(s_1\cosh\sigma' - s_1')}{\sinh\sigma'}$
Maximum load of the pump	$\Delta S_{max} = 2S_0 \dfrac{A-2}{A}$ $\quad A = 2\cosh(\alpha e) + n\alpha e \sinh(\alpha e)$	$\Delta s_{max} = 2s_0 \dfrac{A-2}{A}$ $\quad A = 2\cosh\sigma' + n\sigma'\sinh\sigma'$

Table 3
(Continued)

Kinetics		
Substrate concentration difference	$\Delta S = 2S_0\frac{A-2}{A}(1-\exp(-\theta t))$ $B = \frac{2\sinh(\alpha e)\cosh(\alpha e)}{\cosh(\alpha e)+1} + \alpha e(n\cosh(\alpha e)+1)$	$\Delta s = 2s_0\frac{A-2}{A}(1-\exp(-\theta t))$ $B = \frac{2\sinh\sigma'\cosh\sigma'}{\cosh\sigma'+1} + \sigma'(n\cosh\sigma'+1)$
Substrate concentration in cell 1	$S_1(t) = S_0\frac{2}{A}(1-\exp(-\theta t))$ $\theta = \frac{v_m D\alpha A}{v_c EB}$	$s_1(t) = s_0\frac{2}{A}(1-\exp(-\theta t))$ $\theta = \frac{n+2}{2}\frac{A}{B}\chi_{\sigma'}$
Substrate concentration in cell 2	$S_2(t) = S_0\frac{2A-2}{A}(1-\exp(-\theta t))$	$s_2(t) = s_0\frac{2A-2}{A}(1-\exp(-\theta t))$
b) Flux, yield, extraction		
Diffusion-reaction fluxes		
Substrate, layer 1	$J_{S_I}(X) = -D\alpha\frac{S_1'\cosh(\alpha X) - S_1\cosh(\alpha X-\alpha e)}{\sinh(\alpha e)}$	$j_{S_I}(x) = -\sigma'\frac{s_1'\cosh\sigma' x - s_1\cosh(\sigma' x-\sigma')}{\sinh\sigma'}$
Substrate, layer 2	$J_{S_{II}}(X) = -D\frac{S_2'-S_1'}{ne}$	$j_{S_{II}}(x) = -\frac{s_2'\ \ s_1'}{n}$
Substrate, layer 3	$J_{S_{III}}(X) = -J_{P_{III}}(X) - D\frac{S_2+P_2-P_2'-S_2'}{e}$	$j_{S_{III}}(x) = -j_{P_{III}}(x) + p_2' + s_2'\quad p_2 - s_2$
Product, layer 1	$J_{P_I}(X) = -J_{S_I}(X) - D\frac{P_1'+S_1'\quad P_1-S_1}{e}$	$j_{P_I}(x) = -j_{S_I}(x) + p_1 + s_1 - p_1' - s_1'$
Product, layer 2	$J_{P_{II}}(X) = -D\frac{P_2'-P_1'}{ne}$	$j_{P_{II}}(x) = -\frac{p_2' - p_1'}{n}$
Product, layer 3	$J_{P_{III}}(X) = -D\alpha\frac{P_2\cosh(\alpha X-\alpha(n+1)e) - P_2'\cosh(\alpha X-\alpha(n+2)e)}{\sinh(\alpha e)}$	$j_{P_{III}}(x) = -\sigma'\frac{p_2\cosh(\sigma'(n+1-x)) - p_2'\cosh(\sigma'(n+2-x))}{\sinh\sigma'}$
Substrate entering flux	$J_{S_{I1}} = -D\alpha\frac{S_1'-S_1\cosh(\alpha e)}{\sinh(\alpha e)}$	$j_{S_{I1}} = -\sigma'\frac{s_1' - s_1\cosh\sigma'}{\sinh\sigma'}$
Substrate diffusion flux	$J_D = -D\frac{S_2-S_1}{(n+2)e}$	$j_D = -\frac{s_2-s_1}{n+2}$
Global rate of transfer	$V_M = \frac{v_m D\alpha}{v_c E}\frac{2S_0 - AS_1}{B}$	$\frac{V_M}{V_m} = \frac{1}{n+2}\frac{v_m}{v_c}\frac{1}{\sigma'}\frac{2s_0 - As_1}{B}$
Stoichiometric yield	$R_S = \frac{A-2}{A}\frac{v_c}{v_m}\frac{K_m}{V_m}\frac{1-\exp(-\theta t)}{t}$	$R_S = \frac{A-2}{A}\frac{2}{\Sigma}\frac{1-\exp(-\theta t)}{\chi t}$
Extracting power	$S_2/S_1 = A-1$	$s_2/s_1 = A-1$

$$\Delta S = \frac{1+n}{(2+n)^2}\frac{V_m E^2}{D}[1 \quad \exp(-\chi t)] \tag{26}$$

where

$\chi = 2Dv_m/E^2 v_c$.

and the maximum rate of global transfer can be expressed as a function of the elementary parameters of the membrane and of cell volume:

$$V_M = \frac{(1+n)v_m}{(2+n)^2 v_c} V_m . \tag{27}$$

3.1.3. Load and yields

The net flux through the cell becomes zero (static head) when the concentration difference between the acceptor and donor cells becomes so large that the passive back-diffusion becomes equal and opposite to the active transport flux. The maximum obtainable concentration difference, or the maximum load of the pump is:

$$\Delta S_{max} = \frac{1+n}{(2+n)^2}\frac{V_m E^2}{D} . \tag{28}$$

The stoichiometric yield is expressed as

$$R_S = \frac{\text{number of moles of transported substrate}}{\text{number of moles reacted}}$$

and from eqs. (12) and (23) we have:

$$R_S = \frac{1+n}{2+n}\frac{1-\exp(-\chi t)}{\chi t} . \tag{29}$$

The expressions obtained for P are similar and are given in table 2.

3.2. Reactions of first order kinetics

3.2.1. Concentration profiles and fluxes

Differential equation (10) becomes in layer 1

$$d^2S/dX^2 - (V_m/K_m)S = 0 . \tag{30}$$

using $\alpha = (V_m/K_m D)^{1/2}$ the concentration profiles and flux equations can be expressed for layer 1:

$$S_1(X) = \frac{S_1' \sinh(\alpha X) + S_1 \sinh(\alpha e - \alpha X)}{\sinh(\alpha e)} . \tag{31}$$

$$P_1(X) = -S_1(X) + (P_1' + S_1' - P_1 - S_1)X/e + P_1 + S_1 . \tag{32}$$

$$J_{S_1}(X) = -D\alpha \frac{S_1' \cosh(\alpha X) - S_1 \cosh(\alpha e - \alpha X)}{\sinh(\alpha e)} . \tag{33}$$

The analogous equations for P and S in layers 2 and 3 are given in table 3. The interface concentrations S_1', S_2', P_2', P_1', P_1 and P_2 are expressed as functions of S_1 and S_2.

3.2.2. Global kinetics of active transport and maximum load

A calculation similar to the one for zero order kinetics gives the evolution of the concentration gradient as a function of time:

$$\Delta S = 2S_0 \frac{A-2}{A}(1 - \exp(-\theta t)) . \tag{34}$$

constants A, B and θ are functions of n, α and e, they are defined in table 3.

The maximum load of the pump is then:

$$\Delta S_{max} = 2S_0(A-2)/A . \tag{35}$$

The equation of the maximum rate of global transfer is more complex than in the former case:

$$V_M = \frac{v_m D\alpha}{v_c E}\frac{2S_0 - AS_1}{B} = \theta(2S_0/A - S_1) . \tag{36}$$

To make the results of the calculations more accessible, we have recalculated the equations in dimensionless form using the dimensionless groups:

$$s = S/K_m , \quad x = X/e \quad t' = tD/E^2 , \quad j = Je/K_m D .$$

$$\chi t = \frac{2Dv_m}{E^2 v_c} t , \quad \sigma = \frac{V_m e^2}{K_m D} . \tag{37}$$

These equations can be found in the right hand columns of tables 2 and 3.

In the equations the concentration profiles in the membrane, the interface concentrations, the global concentration gradient, diffusion and diffusion-reaction fluxes inside and at the membrane-boundaries are expressed.

The concentration of S in the donor and acceptor cells (S_1 and S_2) may also be kept constant. In this case a stationary state with non-zero net flux is obtained. Similar situation occurs, when the volumes of the donor and acceptor cells are very large. In this case the system is best characterized by the net flux through the membrane.

If S_1 and S_2 are not kept constant the most significant system parameter is the maximum load. The time-dependent equations refer to the initial state $\Delta S = 0$

when $t = 0$. But similar equations for other starting conditions can be developed as well.

4. Discussion and conclusion

The simple theory described predicts pH dependent pumps. The exact nature of the two reactions (oxydative, hydrolytic, complexing etc ...) has no influence on its principles and T could be an electron.

The intrinsic transport-reaction properties of the membrane are linked to the composition and dimensions of the "membrane" and are well characterized by the dimensionless σ and Σ parameters of layers and membrane.

The model contains structures of two different types belonging to the thermodynamic domain and nevertheless both are coupled to dissipative phenomena.

The "functional" structure may exist even in the absence of substrate in the form of "potential enzyme activities" ($V(E_i)$) [31]; it is induced by a convenient pH gradient in the membrane. The PH gradient required is a function of the distance and values of the active pH domains of sink (E_1) and source (E_2) enzymes, as shown by the expression of n (eq. (13)). For a given orientation of sink ($V(E_1)$) and source ($V(E_2)$) sequences even *opposite pH gradients may be needed with enzymes of different pH dependences* (iso-enzymes, enzyme complexes). (See e.g. the acid and alkaline phosphatases.)

The space waves of S and P result from enzyme diffusion reaction activity. Their orientation is such that the pumping always goes from S sink downhill to S source uphill. The substrate concentrations on the boundaries control the order of reaction that is the "effective" enzyme activity and the corresponding space-wave and pumping output.

Three comments can come now, the first of them for thermodynamic and the others for biophysical reasons.

Let us assume that S is a molecule and let the valve-films be impermeable to ions (e.g. to H^+). Then the pH values and compositions in the bordering compartments can be assumed identical and the pH gradient may be limited to the "membrane" domain only. We see in this case that the functionally asymmetrical membrane pump will work in a way that *tends to destroy the symmetry of its boundaries.* This is exactly what active transport should do.

The second comment is that membrane potentials have no direct influence on the distribution of neutral molecules such as sugars. But pH changes can control the output of pumps of the type described here [‡], and establish an indirect coupling between molecular transport and pH gradients.

The third remark is that with some arrangements of the pump S could also be an ion that is not pH active by itself (e.g. K^+). Then "external" pH changes would produce uptake or release of the transported species through "enzyme regulation" in a more specific manner than by "phase specific" [7] electric potential differences through barriers. The two mechanisms could also contribute together.

With all the necessary precautions it can be recalled that e.g. deviations of pH from normal in extracellular liquids produce such transfers of molecules and ions from and to intracellular spaces [32].

The model also contains the "allotopic" concept of the possible displacement of inactive enzymes up to the spot where activity is needed and the activating environment is met.

Before any detailed discussion the examination of the characteristic properties of the pump and experimental verifications are needed. They will be reported in the next papers. Comparisons and combinations of permanent and functional effects will come later.

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[‡] Just as the position of the gas pedal controles that of the engine.

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