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Properties of Membrane Stationary States

I. The Microcanonical Membranes

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Summary. The flux of permeant species through a membrane is examined using discrete state stochastic models for the transport process within the membrane. While a membrane flux is maintained due to a concentration gradient between bathing solutions, the distribution of species within the membrane evolves to a time invariant configuration which can differ significantly from the equilibrium configuration. Some special properties of these stationary states are examined using linear, microcanonical models for the membrane transport process. Analysis of these models reveals properties which are masked by the phenomenological analysis of irreversible thermodynamics. For example, the models can be used to study the nature of multi-state relaxation within the membrane by observation of the time dependence of the net membrane flux when the membrane is perturbed from its stationary state distribution. Under some conditions, multi-state models will produce relaxation similar to that observed for single-state processes. The symmetry within the membrane is a critical factor for monitoring relaxation processes within the membrane. Because of the stationary nature of the membrane configuration, statistical thermodynamic variables can be defined for the membrane configuration. The total system is not in equilibrium since the baths must still be described by dissipation functions. In the stationary state, the configurational entropy of the membrane is lowered relative to equilibrium and is shown to depend quadratically on the time independent parameter (j/p) where j is the membrane flux and p is a characteristic transition probability for intra-membrane transitions. The basic membrane system serves as a quantitative example of the entropy reduction possible in a stationary state system. An allosteric transition mediated by the stationary state configuration is examined as a means of utilizing this negentropy production.

Although the transport of ions and molecules across biological membranes is the subject of extensive experimental study, the theoretical frameworks available for analysis of these observations are still extremely limited. The phenomenological equations of irreversible thermodynamics are used most frequently since they can be related directly to the observed experimental fluxes and forces. Alternatively, thermodynamic-kinetic equations such as the Nernst-Planck equation or discrete state models such as the

Parlin-Eyring model (1954) provide an effective approach to the description of membrane transport processes although they require a detailed model of the membrane. Analytical solutions require approximations such as the constant field approximation (Goldman, 1943). For membrane models which more accurately reflect specific situations, numerical integration methods must be used.

Although both continuum and discrete state models require detailed knowledge of membrane structure, the discrete state models do possess a number of advantages over the continuum models. In a bilayer membrane with its molecular dimensions (7.5 nm), a continuum model provides a less accurate picture than a discrete site model where motion across the membrane proceeds in a series of jumps between "holes" generated by the thermal motions of the membrane model. Transitions between the hydrophobic phase and the polar boundaries of the bilayer can be described by discrete transition probabilities eliminating some of the problems of a multi-boundary analysis.

Experiments on excitable membranes indicate that potassium and sodium ions do not diffuse through the membrane but permeate through specific independent channels. The mechanisms for the passage of sodium and potassium ions are quite different. Potassium ion in excitable membranes exhibits the anomalous "long pore" effect suggesting an interaction between the inward and outward fluxes (Hodgkin & Keynes, 1955). Hille (1971) has noted that sodium ion passes through the sodium channel with at least one water of hydration and suggests a hydrogen bonding mechanism for the transport process. Although these developments can be incorporated into the phenomenological framework of irreversible thermodynamics, the detailed picture of membrane transport permits a more basic starting point. By suggesting a molecular model for the transport process, membrane fluxes may be calculated for comparison with both experimentally observed phenomena and the predictions of irreversible thermodynamics. A primary purpose of this work will be the illustration of events within the membrane itself which may be masked by the phenomenological approach of irreversible thermodynamics.

Utilization of a discrete state stochastic approach to membrane transport permits the definition of parameters which do not arise naturally in irreversible thermodynamics. Using stochastic membrane models for the membrane, the distribution of permeant within the membrane will approach a time invariant configuration although a membrane flux is maintained. This configuration can differ significantly from the equilibrium configuration and is determined by the membrane flux. Since under steady-state conditions the

entropy production associated with the diffusion flux is continuously absorbed by the external bulk solutions, the membrane state and entropy remain constant in time. Because of the time invariance of the membrane configuration, a statistical entropy can be determined for the stationary membrane state for comparison with the equilibrium entropy. The entropy of the stationary state is lowered relative to the equilibrium state due to the ordering effect of the flux. This serves as a specific quantitative example of the entropy reducing properties of a flow system which has been discussed generally by Morowitz (1968).

Membrane Models

A stochastic formalism for discrete state processes in the membrane functions as a common basis for a variety of models which are based on the existence of discrete states within the membrane. Because of the generality of the approach, carrier models, discrete site binding models and "hole" diffusion models differ only in the definition of states for the system. For example, Parlin and Eyring (1954) developed membrane transport as a series of discrete site transitions using transition state theory. Both Hladky and Harris (1967) and Macey and Oliver (1967) have suggested models for the "long pore" effect observed in the potassium channels of excitable membrane (Hodgkin & Keynes, 1955). Hill and Kedem (1966) have considered a variety of transport models using stochastic processes and graph theory. Although their approach is excellent for the determination of stationary configurations for complex systems, it is difficult to extend to relaxation phenomena. Vol'kenshtein (1969) uses transform techniques to develop the time dependence in the framework of graph theory.

For channel models of membrane transport, two models representing opposing ends of the spectrum are often used. In the "long pore" channels, a set of binding sites are accessible to the ions. When the channel is opened, ions bind to all the sites creating an electroneutral configuration. Transport then proceeds by a knock-on mechanism in which all the ions are simultaneously displaced by one site producing a net transport through the membrane. Only a single membrane state is possible and this state is not altered by a change in the net membrane flux. Temporal variation is observed only when the channel is initially opened (Macey & Oliver, 1967).

In the sodium channel, the independence principle suggests the presence of a single ion in a channel at a given time. In this case a variety of states are possible. The hydrated sodium ion generates a new state for each site to which it binds. If there are N such sites, the hydrated sodium bound to the

first site will be state 1, etc. If the hydrogen-binding energy is relatively small, the transport will be similar to a free diffusion through the channel.

Intermediate cases can also be incorporated in the stochastic framework as well. For example, if more than one ion can enter the channel at a given instant, this situation can be included as an additional set of stochastic states. The method is also easily extended to competitive systems such as the calcium-sodium competition model recently proposed by Heckmann, Lindemann, and Schakenberg (1972). Carrier systems are included in a similar manner by defining the carrier permeant complex as a new membrane state. The inclusion of carriers will introduce a bimolecular condition into the stochastic transition probabilities but this analysis is also amenable to stochastic analysis (Staff, 1967). For this study, bimolecular systems will be assumed pseudo-first order to facilitate a linear analysis. The implications of higher order membrane processes will be explored in a future paper. Some of the major modes of membrane transport are illustrated in Fig. 1 including "hole" diffusion which is the discrete state analogue of the diffusion process.

To illustrate some of the basic properties of stationary state membrane configurations, the transitions between states are assumed linear

$$p_{ij} f_j \tag{1}$$

where p_{ij} is the transition probability per second from state j to state i and f_j is the concentration in the j-th state. The concentration f_j will be the average concentration in the j-th state determined by examination of an ensemble of channels or regions in the membrane. The normalized concentration gives the probability that a given channel is in a specific state. The normalized distribution will be used here. The choice of linear transition probabilities permits the use of matrix analysis.

In this first paper, analysis will be restricted to microcanonical systems where all states have identical energies. This choice restricts the absorption or emission of energy from the membrane during the flow process. The transition probabilities may differ by having different activation energies but this will affect only the rate of transition rather than the system energy. The microcanonical membrane system is illustrated in Fig. 2. One consequence of the equal energy provision is the equality of forward and reverse transition probabilities for a given transition between two states. In the microcanonical system, permeant redistribution within the membrane can be considered in the absence of energy redistribution. This restriction is eliminated in the second paper.

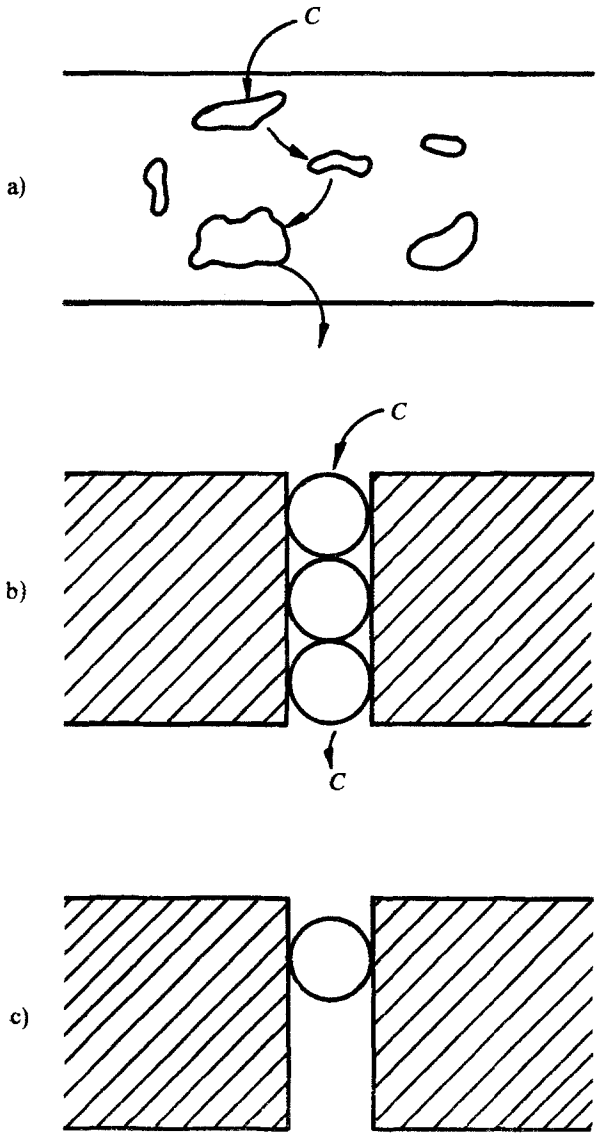

Fig. 1. Discrete membrane permeation models. (a) Diffusion via thermally generated "holes" in the membrane structure. (b) Single file diffusion through potassium channels in excitable membrane; an entering ion displaces an ion into the opposite bath. (c) Individual ion diffusion through membrane channels. Binding sites may be present within the channel

From the transition probabilities, the total rate of change for the state i can be determined as

$$df_i/dt = \sum_j p_{ij} f_j - f_i \sum p_{ji} \quad i=1, \ldots, N \tag{2}$$

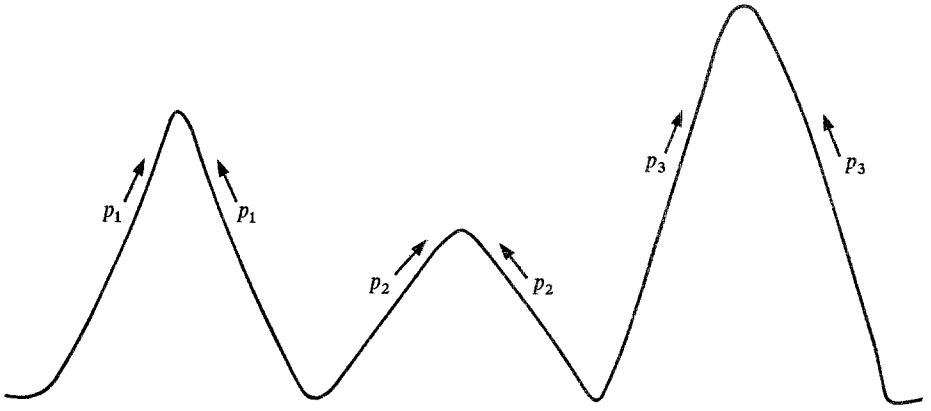

Fig. 2. Activation energy diagram for microcanonical transition probabilities in a four-state nearest model

where the first term determines the net gain of state i while the second term determines its net loss. The choice of linear transition probabilities permits the reduction of the N separate rate equations for the states i into the matrix "Master" equation of the system,

$$d|f\rangle/dt = -\hat{P}|f(t)\rangle; \quad |f(0)\rangle = |f^0\rangle \tag{3}$$

where $|f\rangle_i = f_i$, $[\hat{P}]_{ij} = -p_{ij}$ and $[\hat{P}]_{ii} = +\sum p_{ji}$. Since there are no transitions to states outside the system, the columns of the matrix sum to zero. This is the formal condition of mass conservation. Under such conditions, the matrix $\hat{P}$ is singular and at least one zero eigenvalue exists. Such a system will be defined as a conservative system since, in the stationary state, the total concentration of species within the membrane will not be altered. The membrane flux will alter only the distribution of these species. Non-conservative systems where the total number of permeant species in the membrane depends on the coupling between the membrane and the bathing solution will also be considered in detail since their properties may differ markedly from the conservative systems.

For a system with a state distribution vector $|f^0\rangle$ at the initial time $t=0$, the Master equation can be solved formally as

$$|f(t)\rangle = \exp(-Pt)|f^0\rangle \tag{4}$$

which can be spectrally decomposed to the more tractable form

$$f(t) = \hat{Z}_0|f^0\rangle + \sum \exp(-\lambda_i t)\hat{Z}_i|f^0\rangle \tag{5}$$

where λ_i is the i-th eigenvalue and $Z_i = |\psi_i\rangle\langle\psi_i|$ is the projection operator for the i-th eigenvalue. If sufficient time is allowed, the components of the

nonzero eigenvalues will decay to zero leaving only the equilibrium, $\lambda_0 = 0$ component,

$$|f^{eq}\rangle = \hat{Z}_0 |f^0\rangle = |\psi_0\rangle \langle \psi_0 | f^0\rangle \tag{6}$$

which is time invariant. Since mass must be conserved throughout the relaxation process, the total mass of the system must reside entirely in the equilibrium component. If the summation vector $\langle u| = (1, 1, \ldots, 1)$ is applied to the eigenvalue projections, we find

$$\langle u \hat{Z}_i f^0\rangle = \delta_{0i}. \tag{7}$$

Although the nonzero eigenvalues can perturb the distribution they can add no net mass to the conservative system (Prater & Wei, 1962). When the system is perturbed from equilibrium, the relaxation back to equilibrium is described by the exponential decay of the eigenvalues. A pure exponential decay will be observed only for a single-state relaxation or under conditions where the lowest nonzero eigenvalue is well separated from the other eigenvalues. The larger eigenvalue terms then decay rapidly relative to the lowest eigenvalue and an exponential decay is observed on the slower time scale.

The Stationary State

If the membrane is placed between two baths of different concentrations of permeant species, then the baths can influence the membrane configuration in two distinct ways. If the transition probabilities are altered by the concentration or electrochemical gradient between the two baths, the conservative system will evolve to a new equilibrium distribution consistent with the new transition probabilities. Such a situation could arise even if there were no flow of material through the membrane.

To create a nonequilibrium configuration within the membrane, permeant molecules must flow through the membrane. Molecules or ions will be assumed to populate the state i at some constant rate j_i. Although these inputs will normally populate states located near the membrane boundaries, the actual states populated from the baths will depend on the specific model chosen. The rates will remain constant if the baths are large enough so that the bath concentrations are unaltered by the passage of permeant. The choice of large concentration maintains the total system in a nonequilibrium state since the net rate approaches zero as the system comes to equilibrium. The flux between the membrane and bulk phase can be related to the bath concentration c_i through the relation (Mazur & deGroot, 1962)

$$j_i = k_i c_b \tag{8}$$

where c_b is the bath concentration. This concentration can be held effectively constant on the time scale of interest through the use of large volumes of bathing solution so that the input of permeant remains constant. However, the output from membrane to the baths may be either independent or dependent on the concentration of permeant species in the membrane. Since these two possibilities generate two distinct types of stationary state behavior, they will be examined individually.

Conservative Systems

A conservative system is a system where the net flux through the membrane remains constant independent of the state of the membrane and the input and output fluxes are equivalent. For example, the "long pore" model requires an entering ion to displace an ion into the bath at the opposite end of the channel to preserve electroneutrality. In this case, since only a single state is possible, the rate of flow will not alter the membrane configuration. If the membrane concentration is high relative to one of the baths, the rate of efflux will obey Eq. (8). Thus, when $c(\text{Bath 1}) > c(\text{membrane}) > c(\text{Bath 2})$ steady inputs and outputs can be generated. The rates of influx and efflux j_i for each state i can be expressed as a vector $|j>$ and the transport equation becomes

$$d|f\rangle/dt = -\hat{P}|f\rangle + |j\rangle; \quad |f(0)\rangle = |f^0\rangle. \tag{9}$$

Because the conservative $\hat{P}$ matrix is singular, this equation cannot be solved formally. Integration of the spectrally decomposed matrix produces the solution

$$|f(t)\rangle = Z_0(|f^0\rangle + |j\rangle t) + \sum \exp(-\lambda_i t)\hat{Z}_i|f^0\rangle + |j\rangle)/\lambda_i + \sum Z_i|j\rangle/\lambda_i \tag{10}$$

for the general case. To prevent the linear increase of the equilibrium population distribution with time, the flux $|j>$ must have no projection on the equilibrium eigenvector. The flux components j_i must be chosen so the inputs exactly balance the outputs, i.e., $\sum j_i = 0$. This conservative flux redistributes the populations of the states but does not augment the total population. In the limit of long times, the system evolves to a stationary state distribution

$$|f^{ss}\rangle = \hat{Z}_0|f^0\rangle + \sum \hat{Z}_i|j\rangle/\lambda_i. \tag{11}$$

Since the equilibrium configuration appears in the first term, the remaining terms represent the difference between the equilibrium configuration and the stationary state.

Nonconservative Systems

In the analysis of the conservative system, the invariance of the total population of permeant species within the membrane was established. An alternative situation arises when the rate of efflux from the membrane is dependent on the population of the membrane states. By assuming a constant influx vector and a population dependent efflux vector, the rate vector for the nonconservative stationary state becomes

$$|j'\rangle = |j\rangle - \hat{K}\,|f(t)\rangle \tag{12}$$

where K is a diagonal matrix of the form $[K]_{ii} = k_i$ where k_i is the first-order rate constant for the transition from membrane state i to one of the bulk solutions when such a transition is consistent with the specific membrane geometry for the model. For example, in an N-site channel model, k_1 and k_N will be nonzero. The transport equation becomes

$$d|f\rangle/dt = -(\hat{P}+\hat{K})\,|f(t)\rangle + |j\rangle = -\hat{R}\,|f\rangle + |j\rangle. \tag{13}$$

Because of the matrix $\hat{K}$, the matrix R is nonstochastic and no zero eigenvalue exists. The general solution is

$$|f(t)\rangle = \exp(-Rt)\,|f^0\rangle + \{(1-\exp(-Rt)\}\,R^{-1}\,|j\rangle \tag{14}$$

with spectral decomposition

$$|f(t)\rangle = \sum \exp-(\lambda_i t)\,\hat{Z}_i\,|f^0\rangle + \sum Z_i\,|j\rangle/\lambda_i - \sum \exp(-\lambda_i t)(\hat{Z}_i\,|j\rangle)/\lambda_i. \tag{15}$$

After a relaxation period, the distribution evolves to the stationary state

$$|f^{ss}\rangle = \sum Z_i\,|j\rangle/\lambda_i = R^{-1}\,|j\rangle. \tag{16}$$

In the absence of fluxes j from the bulk solutions, all permeant molecules are lost irreversibly from the membrane. The configuration must be maintained by arbitrary input fluxes from either or both bathing solutions. If both flux inputs are equal (equal bath concentrations) the membrane comes to dynamic equilibrium with the baths.

Properties

Because the properties of the stationary state systems depend on the nature of the transition probabilities which constitute the $\hat{P}$ matrix, the characteristics of these transition probabilities will be explored in detail. Because the microcanonical transition probability matrix is symmetric, the eigenvalues which determine both the relaxation times and the stationary state distribution will all be real. For relaxation to a stable stationary state

they must also be greater than or equal to zero. For these stochastic systems this is easily verified using Gersgorin's theorem; the system eigenvalues all lie within circles of radius $\sum |P_{ij}|$ centered at $[P]_{ii}$. Since $[P]_{ii} = \sum |P_{ij}|$ for the conservative case,

$$0 \leqq \lambda_i \leqq +2 \sum_j |P_{ij}|. \tag{17}$$

The addition of the matrix K in the nonconservative case retains the same radius but shifts the eigenvalue spectrum along the positive axis so that the eigenvalues lie entirely in the positive region with no zero eigenvalue. The matrices will relax monotonically to a stable stationary state.

Since the membrane will relax to the stationary state and will remain time invariant in that state, a statistical entropy can be defined. Since the transition probabilities are determined by averaging over a large number of channels, the configuration is the average configuration over these channels. The statistical entropy is

$$S = -k \sum f_i \ln f_i. \tag{18}$$

The entropy will be the characteristic variable for the microcanonical system considered here. Since configuration redistribution has no effect on the total energy of the membrane, the free energy for the system can be defined as

$$G = kT \sum f_i \ln f_i. \tag{19}$$

The entropy of Eq. (18) is an entropy per channel. In the conservative case, the flux through the membrane does not alter the total number of particles in the channel and the normalizing factor remains constant. For the nonconservative case, the total number of permeant species within the channel varies with the flux and the system must be normalized for each stationary state. The normalized stationary state is

$$f^{ss} = (\sum \hat{Z}_i |j\rangle / \lambda_i) / \sum \langle u \hat{Z}_i j \rangle. \tag{20}$$

Since the total number of species varies during the relaxation to the stationary state, this normalization is valid only at the stationary state.

Although the stationary configurations permit thermodynamic analysis, such analysis is dependent on the determination of the transition probabilities for the system. Although the stationary configurations are a function of the transition probabilities through the relaxation constants λ_i, a time dependent analysis of the change in membrane flux with time provides a more accessible experimental bridge. The system can be perturbed from its stationary configuration and its relaxation to the same or a new stationary state can be monitored by observing the resultant variation in membrane flux. Such analysis is possible only for the nonconservative systems. If the

conservative flux is suddenly changed to a new value, an internal redistribution of permeant species occurs which is not observable as a change in flux since the flux is fixed. In the nonconservative system, the net flux through the membrane will exhibit a temporal variation since the flux depends on the instantaneous internal membrane configuration. The net flux through the membrane can be determined by observing the net change in flux at an interface. The net flux from bath 1 into the membrane is

$$j_{\text{in}}^{\text{net}} = j_1 - k_1 f_1(t) \tag{21a}$$

while the net efflux is

$$j_{\text{out}}^{\text{net}} = j_N - k_N f_N(t). \tag{21b}$$

For the nonconservative systems, these fluxes will be equivalent in the stationary state.

If the flux vector $|j\rangle$ is suddenly jumped to a new flux vector $|j'\rangle$, the relaxation of the membrane to its new stationary state is

$$\begin{aligned} |f(t)\rangle &= R^{-1}|j'\rangle + \exp(-Rt)R^{-1}|(j+j')\rangle \\ &= \sum\{Z_i|j'\rangle/\lambda_i\} + \sum_i\{\exp(-\lambda_i t)Z_i|(j+j')\rangle/\lambda_i\}. \end{aligned} \tag{22}$$

From Eq. (21a, b) the net flux is then

$$\begin{aligned} &\langle(|j'\rangle - KR^{-1}|j'\rangle) + K\exp(-Rt)R^{-1}|(j+j')\rangle \\ &\quad = \langle j'\rangle - \sum\{KZ_i|j'\rangle/\lambda_i\} + \sum\{\exp(-\lambda_i t)KZ_i|(j+j')\rangle/\lambda_i\}. \end{aligned} \tag{23}$$

The first and N-th components will be the only nonzero components corresponding to influx and efflux, respectively. The fluxes will relax as a sum of exponentials as expected for such a linear system.

Eq. (22) assumes an ideal relaxation condition where the flux changes instantaneously to its new value and variation in the net flux is due entirely to redistribution of the configurations. If this change to the final input flux takes place on a time scale comparable with the relaxation times, the time dependent variation in the flux must be included in the integration of the transport equations.

In practice, a rapid concentration change in the bathing solutions to facilitate a relaxation is difficult to accomplish on a time scale short relative to the relaxation time scale. A more rapid flux change is possible when the permeant species are ions flowing along a potential gradient. A double pulse experiment can be used to change the membrane potential rapidly and the relaxation to the new stationary configuration can be observed as a variation in membrane current. The relaxation will occur even if the membrane parameters do not change during the relaxation. When both the

transition probabilities and the currents change with potential on a comparable time scale, the analysis becomes more complex but the basic stochastic framework can be retained.

Nearest Neighbor Models

Conservative Systems

Because of the restricted geometry expected for membrane channels, nearest neighbor transitions, where a permeant may move only to adjacent sites within the channel, may provide an accurate description of membrane transport. Nearest neighbor models have been developed by Parlin and Eyring (1954), Hladky and Harris (1967) and Macey and Oliver (1967). A nearest neighbor transition probability matrix has the Jacobi or tridiagonal form. Because of the restriction of microcanonical transition probabilities, the forward and reverse transition probabilities between a pair of sites are identical although the magnitudes of transition probabilities between different pairs of sites may differ. The nearest neighbor models are shown in Figs. 2 and 3. The matrix for the transition probabilities will be symmetric of the form

$$[P]_{ii}=b_i; \quad [P]_{i,i+1}=[P]_{i,i+1}=-a_i. \tag{24}$$

For a conservative system

$$b_i=[P]_{ii}=\sum_{i\neq j} P_{ji}. \tag{25}$$

To illustrate the properties of nearest neighbor models most effectively, an identical site model is used. The N sites will then have identical transition probabilities. If a channel model is assumed with no more than one permeant species in a channel at a given instant, then the transition probabilities are averaged transition probabilities based on an ensemble average of a large number of identical channels in the membrane. The final distribution will be an average distribution for all these channels. The relaxation eigenvalues are (Hildebrand, 1968)

$$\lambda_r=4p\sin^2(r\pi/2N) \quad r=0, \ldots, N-1. \tag{26}$$

Despite the equality of the transition probabilities, a spectrum of eigenvalues is produced. As the number of channel sites increases, the lowest nonzero eigenvalue approaches the equilibrium zero eigenvalue as illustrated in Fig. 4.

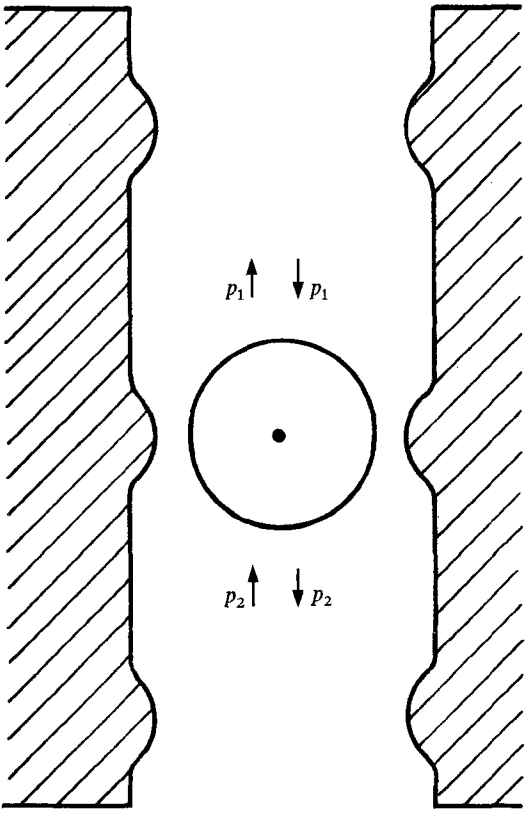

Fig. 3. A three-site membrane channel for membrane transport in a nearest neighbor model with microcanonical transition probabilities

The normalized equilibrium eigenvector is

$$\lambda_0 = 1/N \tag{27}$$

and all states are occupied with equal probability. If a conservative flux $|j\rangle$ is applied to the terminal states, the k-th state will evolve to a stationary population

$$\begin{aligned} |f^{ss}\rangle_k &= |f^{eq}\rangle_k + (j/p)\sum A_r (2\tan\phi \sin\phi)^{-1} \cos(2k-1)\phi \\ A_r &= \sum \cos^2(2k-1) \end{aligned} \tag{28}$$

$$|f^{ss}\rangle_k = +|f^{eq}\rangle_k + |f\rangle_k ; |f\rangle_k = -|f\rangle_{N-k} = (j/p)(N-2i+1)/2 \tag{29}$$

where $\phi = r\pi/2N$ and the summation is restricted to odd n since the symmetric (even) projection operators are orthogonal to the (odd) flux vector $|j\rangle$.

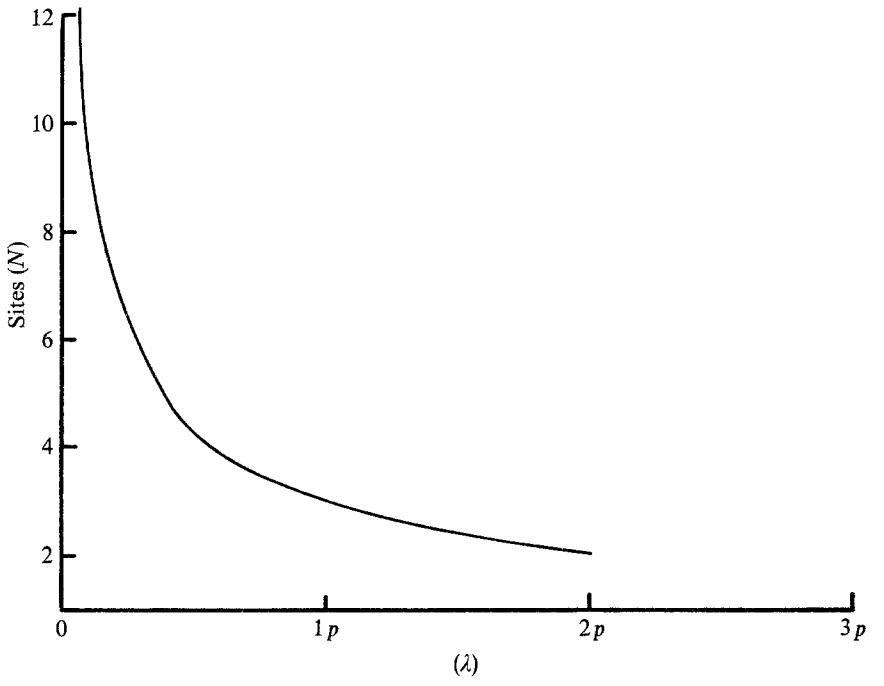

Fig. 4. Relationship between smallest nonzero eigenvalue λ and the number of membrane binding sites N

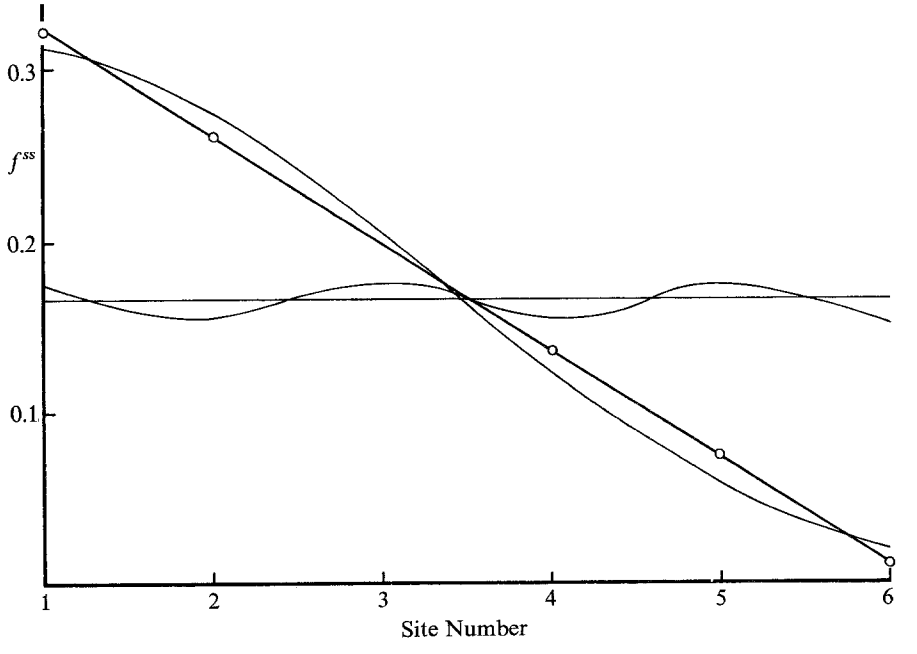

Fig. 5. The eigenvalue components of the stationary state distribution of a nearest neighbor model. The components produce the linear distribution

Fig. 5 illustrates the final stationary state distribution and contributions from individual eigenvalue projections for a six-site model. Although the relative contributions from the higher eigenvalues decrease rapidly the summation of all contributions produces a linear membrane configuration as stated in Eq. (29). By comparison, a linear concentration distribution is postulated in the Henderson solution of the Nernst-Planck equation to predict the membrane potential. The linear distribution arises naturally from first-order transition probabilities and steady fluxes.

The Eyring-Parlin model is more complex because arbitrary transition probabilities are allowed. The model is canonical since the states need not be at the same energy. In the Eyring-Parlin model, the nearest neighbor transition probabilities have the form

$$[P]_{ii} = -(k_i + k_i'); \quad [P]_{i+1,i} = k_i; \quad [P]_{i-1,i} = k_i \tag{30}$$

and the membrane flux is conservative with $j_1 = j$ and $j_N = -j$ for the terminal states. To derive the Eyring-Parlin form, the general stochastic equation with conservative flux is solved under stationary state conditions

$$d|f\rangle/dt = 0 = -P|f\rangle + |j\rangle \tag{31}$$

$$|j\rangle = P|f\rangle \tag{32}$$

which produces the equations

$$\begin{aligned} j &= k_1 f_1 - k_2' f_2 \\ j &= k_2 f_2 - k_3' f_3 \\ &\vdots \\ j &= k_{N-1} f_{N-1} - k_N f_N \end{aligned} \tag{33}$$

which are identical to the starting equations of Eyring and Parlin. The stochastic formalism can determine both the stationary state behavior and the relaxation properties of the system to the stationary state. However, the conservative system relaxation will not be manifested in an external flux variation since the external flux is fixed for these models. Relaxation must be observed with an internal probe.

While nearest neighbor channel models with equal transition probabilities provide excellent models for examining the properties of membrane flow systems, they introduce a high degree of symmetry into the membrane structure which may not appear in an actual membrane. The symmetry leads to cancellation of the symmetric projection operators in the membrane distribution function. These modes will be lost for any system where the

transition probabilities are symmetric about the membrane center since such conditions will lead directly to sets of symmetric and antisymmetric projection operators. This observation is important for lipid bilayers which would be expected to have symmetry about a central point. In a canonical system where an external field can alter the transition probabilities, this symmetry can be destroyed by the application of such a field. If the transition probability matrix possesses no intrinsic symmetry, the stationary distribution will be a summation of all the eigenvalue projection operators of the system although the symmetric contribution will be small in a conservative system.

The Nonconservative Nearest Neighbor Model

For the nonconservative microcanonical nearest neighbor model, the transition probability from a terminal membrane site to the bulk solution is finite. Because of the transition across a phase boundary, the transition probability can be markedly different from the internal transition probabilities. To facilitate comparison between these models and the conservative models with equal transition probabilities, the terminal transition probability is assumed to depend only on its membrane state and will thus be identical to the internal transition probabilities.

The transition probabilities are

$$[P]_{ii}=2p; \quad [P]_{i-1,i}=[P]_{i+1,i}=-p \quad i=1, \ldots, N \tag{34}$$

with eigenvalues (Hildebrand, 1968)

$$\lambda_r=4p\sin^2\{r\pi/2(N+1)\} \tag{35}$$

and eigenvector components

$$\psi_{rk}=(2/N+1)\sin\{4k\pi/(N+1)\} \tag{36}$$

leading to the stationary state distribution

$$\begin{aligned}|f^{ss}\rangle_k=&\sum_r(2/N+1)\{\sin r\pi/(N+1)\}(j_1+(-1)^{r+1}j_N)\\&\cdot(p\lambda_r)^{-1}\sin\{rk\pi/(N+1)\}\\=&[(N-k+1)/(N+1)]\,(j_1/p)+[k/(N+1)]\,(j_N/p)\end{aligned} \tag{37}$$

where j_1 and j_N are uncorrelated fluxes from the bulk solutions. Despite flux imbalances between j_1 and j_N, the system will attain the stationary value

$$j^{\text{net}} = (j_1 + j_N)/N + 1. \tag{38}$$

The net membrane flux through the membrane is the *sum* of the input fluxes reduced by the factor $N+1$. The net flux for a multi-state model is not the flux difference between the input fluxes. Instead both fluxes contribute to give a stationary distribution which regulates the net flux observed as a net influx or net efflux at an interface.

Because both input fluxes j_1 and j_N are uncorrelated, the input vector $|j\rangle$ is completely arbitrary with contributions from both the symmetric and antisymmetric projection operators. However, the symmetric projection operators will give terms of the form

$$(j_1 + j_N) \exp -\lambda_i t \, |c_i\rangle \tag{39a}$$

while the asymmetric projection operators will give expressions involving the difference in input fluxes

$$(j_1 - j_N) e^{-\lambda_j t} \, |c_j^{\text{odd}}\rangle. \tag{39b}$$

For this reason the observed relaxation of the flux to its stationary configuration will be dominated by the symmetric projection operators when the fluxes into the membrane are large and of comparable magnitude so that $j_1 + j_N$ is very large relative to $j_1 - j_N$. Under such circumstances, the observed decay of the net flux to its stationary state value will appear exponential. The apparent exponential nature of the decay is enhanced since the smallest eigenvalue, which dominates the relaxation process, is associated with a symmetric projection operator. The decay of multi-state models with two, three and six states is shown in Fig. 6.

Although the net flux relaxation for two- or three-level models will not be pure exponential, the difference between input and output fluxes can differ from zero and the relaxation of this difference will be a pure exponential decay. The situation arises because of the cancellation of the symmetric modes when the difference between influx and efflux is formed. For ionic systems, the difference between influx and efflux during relaxation creates a charge imbalance within the membrane during relaxation. This phenomena will be explored in a later paper.

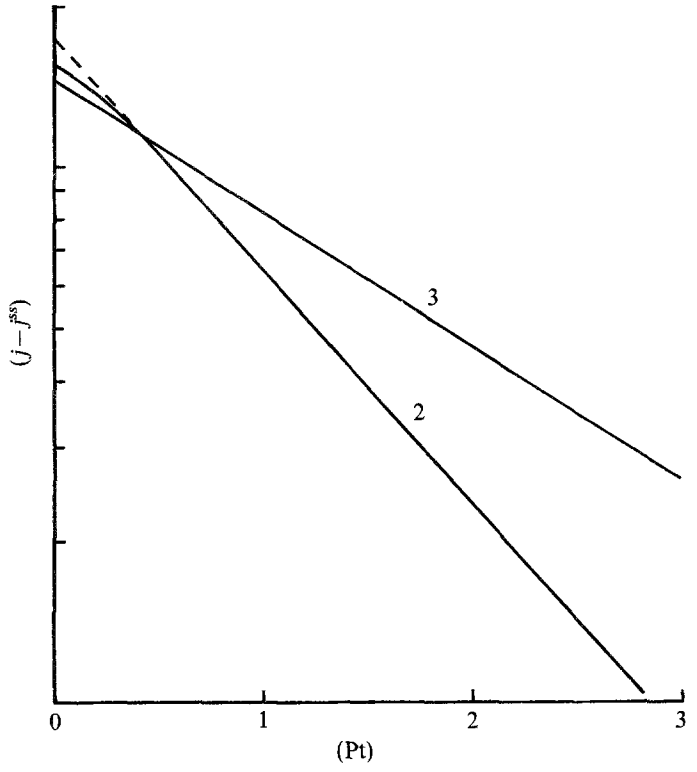

Fig. 6. Relaxation of the logarithm of net flux $(j-j^{ss})$ for two- and three-site membrane models illustrating the first-order relaxation properties of symmetric two- and three-level models

Thermodynamic Analysis

Although the stationary state distribution of permeant species led directly to information on the net membrane flux, the time invariance of this state permits the definition of statistical thermodynamic variables as well. The entropy and free energies arise naturally in the microcanonical system and provide a measure of organization and work potential within the membrane. The parameter characteristic of both the stationary state distribution and the thermodynamic variables is the time independent parameter (j/p).

For the conservative system, the entropy is determined from complementary pairs of distribution states

$$|f^{eq}\rangle_k \pm d_k(j/p) \tag{40}$$

where

$$d_k=\sum_r^M A_r(2\tan\phi\sin\phi)^{-1}=(j/p)(N-2k+1)/2$$
$$M=k/2,\ M \text{ even};\qquad M=(k-1)/2,\ M \text{ odd}. \tag{41}$$

This separation will hold whenever the membrane has a center of symmetry; in other cases, all terms must be summed individually. The statistical entropy is

$$S=-k\sum_k\{|f^{eq}\rangle_k+d_k(j/p)\}\ln\{|f^{eq}\rangle_k+d_k(j/p)\}$$
$$+\{|f^{eq}\rangle-d_k(j/p)\}\ln\{f^{eq}-d_k(j/p)\}. \tag{42}$$

When the membrane flux is small, the entropy may be expanded about equilibrium to give

$$S=S^{eq}-\sum_k^M(j/p)^2 d_k^2 N^2. \tag{43}$$

The entropy of the membrane is reduced quadratically with a linear increase in the membrane flux. The time independent ratio (j/p) can be contrasted with the irreversible thermodynamic dissipation function for the system

$$T\phi=L(T)J^2 \tag{44}$$

where L is the linear coupling coefficient for the phenomenological forces as a function of the fluxes. Although the baths create entropy, the membrane entropy remains constant. The entropy in the stationary state is independent of the flux direction and always leads to a reduction in the equilibrium entropy which is consistent with the equilibrium state as the configuration of maximum entropy.

The nonconservative system has no stable equilibrium state in the absence of a membrane flux. The nonconservative microcanonical system has the normalized stationary state distribution

$$|f\rangle_k=\{2/N(N+1)\}\{(N+1-k)j_1+kj_N)/(j_1+j_2)\} \tag{45}$$

which separates into symmetric and antisymmetric distributions of the form

$$|f^{ss}\rangle_k=(1/pN)(j_1+j_N)+\{(j_1-j_N)/pN(N+1)\}(N-2k+1). \tag{46}$$

Since the nonconservative system will normally involve two positive inputs from the bathing solutions, the symmetric term involving the sum of input fluxes is dominant and provides a point of expansion. The statistical entropy is

$$S=-k[\{(j_1+j_N)/p\}\ln\{(j_1+j_N)/pN\}]$$
$$+(j_1-j_N)^2(N-1)/\{(j_1+j_N)(6p)(N+1)\}. \tag{47}$$

Both the symmetric and antisymmetric terms in the stationary state expression contribute to the total entropy.

Because only microcanonical systems are examined in this paper, the energy and enthalpy are independent of the stationary state configuration. The internal energy of the equilibrium state can be defined as zero. For the microcanonical system, the free energy is

$$G = -TS \tag{48}$$

so that the stationary state entropy is a direct measure of the system's ability to do work at a temperature T.

The presence of a stationary configuration permits a mode of coupling between the flux and allosteric transitions involving membrane constituents which is illustrated with a simple model. Assume at least one of the membrane sites is amenable to an allosteric transition which is mediated by the concentration of permeant at that site. The flow of permeant is assumed fast relative to changes in membrane configuration so that the residence time of the permeant ion at the sites produces a steady concentration on the allosteric time scale. For convenience a single membrane site undergoes an allosteric transition since the generalization to multiple sites is readily apparent. The situation is illustrated in Fig. 7. The average concentration

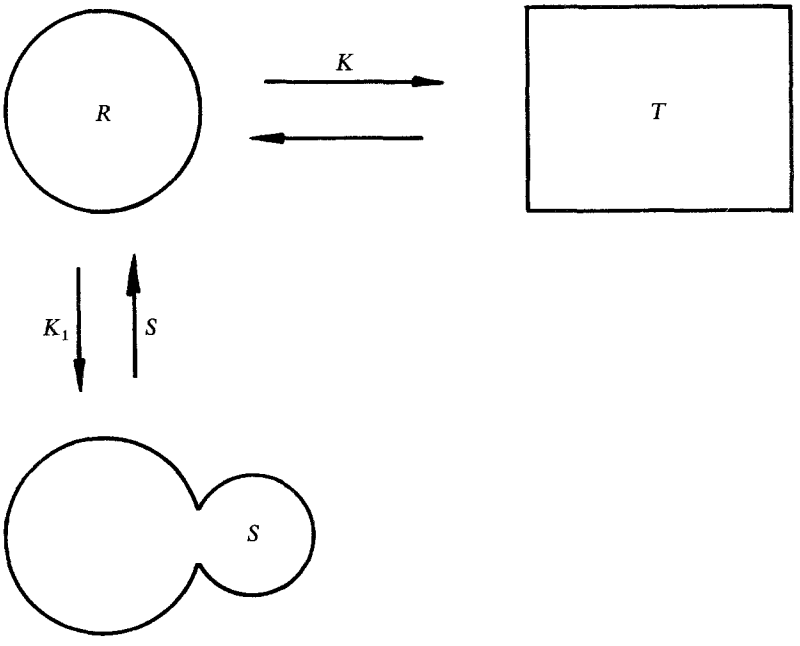

Fig. 7. A simple allosteric model for a membrane channel site where the site is capable of two distinct allosteric sites R and T and substrate S binds only to R

at site k is $S=Mf_k$ where M is the total number of channels. If binding is permitted only to the allosteric state R, the fraction of channels with the site in the R configuration is

$$\langle R\rangle=(1+K_1 S)/(1+K_1 S+K) \tag{49}$$

where K_1 is the association constant for the permeant and K is the allosteric transition equilibrium constant. Blumenthal, Changeux, and Lefever (1970) have utilized an allosteric transition model to describe nerve excitation phenomena by including a cooperative interaction between allosteric units. Graph theory is used to establish the stationary concentrations. The stochastic method developed here emphasizes that the energy necessary to facilitate such transformations is stored in the stationary state configuration.

Discussion

The choice of a discrete state model for the description of membrane transport was dictated by a variety of reasons. The examination of specific models provided a basis for comparison with the phenomenological approach of irreversible thermodynamics and showed that certain relaxation modes could dominate the relaxation of the membrane influx or efflux.

The discrete membrane models possess some computational advantage over continuum equations such as the Nernst-Planck equation for membranes less than 100 nm thick. For such molecular dimensions, transport through the membrane is accurately described as a series of jumps between "holes" in the membrane structure or passage through channels where interaction between the channel walls and the permeant species is possible. These intramembrane transitions can be extended to include transitions to dipole layers, unstirred boundary layers, etc., by introducing additional transition probabilities and including these regions as new membrane states.

The development of nonconservative transport models for the membrane rather than conservative models such as the Eyring-Parlin model provides a basis for observation of relaxation of the membrane when perturbed from its stationary distribution. The time dependence of the net flux through the membrane is determined by the relaxation times generated by the transition probability matrix. In the models considered here, the transition probabilities were assumed constant over the linearized range of stationary state behavior. This hypothesis can be tested by observing the relaxation period for a variety of stationary states. The situation is more complex when

changes in the bathing solution potentials can affect the transition probabilities as well. This will be discussed in the second paper.

To determine the nature of relaxation within the membrane, internal probes must be used. Allosteric transitions which are mediated by the stationary state configuration provide one possibility. An alternative possibility is the addition of spectroscopic probes to the membrane system. For example, aequorin can be used as a sensitive monitor of calcium ion changes during a relaxation process if it is present near one of the membrane states of interest.

Because the membrane configuration remains stationary for a constant dissipation rate in the baths, statistical thermodynamic variables could be defined to provide a common basis of comparison between a variety of membrane systems. Morowitz (1968) has emphasized the necessity of stationary state conditions for effective biological organization and the stationary state membranes provide a quantitative example of such behavior. The steady flux creates a potential which can be utilized to do work within the membrane when this flux is changed. Because of this potential which is characteristic of the stationary state, the actual potentials necessary to facilitate electron transfer or chemical processes within the membrane may differ considerably from equilibrium predictions. The use of equilibrium free energies to describe the intra-membrane process must be corrected for this stationary state potential if the system under consideration is a flow system.

Both the stationary state behavior and the thermodynamic properties are dependent on the ratio of membrane flux to the characteristic transition probability for the system (j/p). This time independent ratio is a measure of the ordering effect of the flux relative to the randomizing effect of the transitions within the membrane. If the net flux through the membrane is examined, the factor (j/p) in the stationary state expression will be multiplied by p to reproduce the flux which is the observable of irreversible thermodynamics. Although expressions for p do not arise naturally in the phenomenological approach of irreversible thermodynamics, the magnitudes of the transition probabilities can be determined experimentally from relaxation studies of the net flux through the membrane under perturbations from the stationary state. The experimental studies can be used to establish the range of the linear stochastic models and the nature of the stationary configuration.

The statistical thermodynamic variables defined for the stationary state are analogous to those defined for the equilibrium state. Since the Boltzmann entropy expression is valid for any time independent system, it

provides the common bridge between the stationary and equilibrium states. Equilibrium thermodynamic variables are characterized by their state property and it is interesting to determine whether a similar property can be defined in the stationary state systems. If a transition to a new stationary state is induced by a change in the membrane flux, a relaxation process will ensue and the system will evolve through a series of states which depend on the nature of the perturbation applied. To provide consistency with equilibrium thermodynamics, the stationary state entropy must be defined in terms of "reversible" transitions; the only path permitted for the calculation of stationary state entropy changes will be the path with minimal relaxation states. The transition between stationary states proceeds through a series of stationary states. This eliminates time dependence from the calculation of the stationary state properties. The approach is limited to the linear range postulated in the stochastic equations since the presence of phase transitions or dissipative instabilities will induce discontinuities in the equations and complicate the analysis.

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