Anomalous properties of a simple small-volume membrane transport system at steady state

(master equation/discrete N /mean concentration/chemical potential/thermodynamic force)

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ABSTRACT We investigate the transport of a single ligand L , between two macroscopic baths with different concentrations of L , by a simple membrane system. The center of this system is a very small volume V that might contain $N = 0, 1, 2, \dots$ molecules of L . Access to V from either bath is by means of a single binding site for L . The steady-state properties of this simple system are studied exactly by a discrete- N master-equation approach. It is found that the mean concentration of L in V , $\bar{c} = \bar{N}/V$, is not a significant quantity either kinetically or thermodynamically. Further, the chemical potential of L in V is not defined and hence the overall thermodynamic force on L between the two baths cannot be subdivided at V . In fact, because V is small, the transport system must be treated as an indivisible system with interdependent parts. In the limit when V and $\bar{N}$ are very large, V becomes in effect a third macroscopic bath and two well-defined transport subsystems emerge (between V and either bath).

The recent paper of Westerhoff and Chen (1), which has to do primarily with novel small-volume effects in the transport of protons across the mitochondrial membrane, suggested to us that it would be interesting to examine as simple a small-volume transport system as possible to see what anomalies persist in such a case. This is the purpose of the present paper.

The Model

The hypothetical model that we study is shown in Fig. 1. A single ligand L can be transported between two macroscopic baths, 1 and 2, where L has concentrations c_1 and c_2 ($c_1 \geq c_2$, for concreteness) by means of the small membrane system shown in the figure. Part of this system is a very small volume V that serves as an intermediate reservoir of L molecules (of number $N = 0, 1, 2, \dots$). Access to V from either bath, for L , is via a single binding site, designated ℓ (left) and r (right). Each site can exist in two states, empty (designated 0) or occupied (designated 1). The two binding sites plus the reservoir V thus comprise the transport system for L . We shall deal with mean steady-state properties of a large ensemble of such systems (or, alternatively and equivalently, with long-time averages at steady state for a single system).

The first-order rate constants adopted for binding to and detachment from site ℓ are shown in Fig. 2. The corresponding rate constants for site r have subscript 2. The primed rate constants are related to V transitions in both cases. The rate constants are interconnected by the thermodynamic relations

$$\alpha_1/\beta_1 = \alpha'_1/\beta'_1 = K_1, \quad \alpha_2/\beta_2 = \alpha'_2/\beta'_2 = K_2, \quad [1]$$

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FIG. 1. Model of transport system for ligand L from bath 1 to bath 2 via binding sites ℓ and r and the central small volume V , which may contain $N = 0, 1, 2, \dots$ molecules of L .

where K_1 and K_2 are the binding constants of L on sites ℓ and r , respectively.

If V is very large, the relative fluctuations of N about the mean $\bar{N}$ (at steady state) will be very small and V can be treated as a third macroscopic bath. Let $\bar{c} = \bar{N}/V$ be the steady-state concentration of L in V ; $\bar{c}$ is determined below. The two-state (0, 1; empty, occupied) kinetic diagram for site ℓ for this case is shown in Fig. 3. There is a similar diagram for site r . Let $P_{0\ell}$ be the probability that the left site is empty, etc. Then, at steady state (2),

$$P_{0\ell} = \frac{\beta_1 + \beta'_1}{\alpha_1 c_1 + \alpha'_1 \bar{c} + \beta_1 + \beta'_1}, \quad [2]$$

$$P_{0r} = \frac{\beta_2 + \beta'_2}{\alpha_2 c_2 + \alpha'_2 \bar{c} + \beta_2 + \beta'_2}.$$

The two sites are independent of each other except that they share $\bar{c}$. The four fluxes of L , shown in Fig. 3b, must be equal at steady state:

$$J_A = \alpha_1 c_1 P_{0\ell} - \beta_1 (1 - P_{0\ell}) = J_B = \beta'_1 (1 - P_{0\ell}) - \alpha'_1 \bar{c} P_{0\ell}$$

$$= J_C = \alpha'_2 \bar{c} P_{0r} - \beta'_2 (1 - P_{0r}) = J_D$$

$$= \beta_2 (1 - P_{0r}) - \alpha_2 c_2 P_{0r}. \quad [3]$$

FIG. 2. Rate constant notation for transitions involving site ℓ . There are similar rate constants for site r (with subscript 2).

FIG. 3. (a) Two-state cycle for site ℓ when V is very large (0, empty site; 1, occupied site). (b) Four fluxes of L that must be equal at steady state.

If we substitute Eqs. 2 into Eqs. 3 we find (for the four equal fluxes)

$$J = \frac{\alpha_1 \beta'_1 (c_1 - \bar{c})}{\alpha_1 c_1 + \alpha'_1 \bar{c} + \beta_1 + \beta'_1} = \frac{\alpha_2 \beta'_2 (\bar{c} - c_2)}{\alpha_2 c_2 + \alpha'_2 \bar{c} + \beta_2 + \beta'_2}. \quad [4]$$

The two right-hand expressions here furnish a quadratic equation that determines $\bar{c}$ as a function of c_1 , c_2 , and the eight rate constants (3). Then J follows from this $\bar{c}$.

When V is very large, there is no ambiguity about the chemical potential μ of L in V at concentration $\bar{c}$. The thermodynamic forces driving L are

$$X_1 = \mu(c_1) - \mu(\bar{c}), \quad X_2 = \mu(\bar{c}) - \mu(c_2) \quad [5]$$

$$X = X_1 + X_2 = \mu(c_1) - \mu(c_2), \quad [6]$$

where X_1 is the force between bath 1 and V , X_2 is the force between V and bath 2, and X is the overall force between bath 1 and bath 2. The relations to the rate constants in Fig. 3a are (2)

$$e^{X_1/kT} = \alpha_1 c_1 \beta'_1 / \alpha'_1 \bar{c} \beta_1 = c_1 / \bar{c} \quad [7]$$

$$e^{X_2/kT} = \alpha'_2 \bar{c} \beta_2 / \alpha_2 c_2 \beta'_2 = \bar{c} / c_2 \quad [8]$$

$$e^{X/kT} = c_1 / c_2. \quad [9]$$

FIG. 4. Kinetic diagram for the transport system. There is a square of four binding substates, N_{ij} , for each value of N . N increases indefinitely. Each line represents a forward and backward transition. Only a few rate constants (from Fig. 2) are included; the others are available in Eqs. 10–16.

We return now to the small V case of primary interest, in which the important N values may be very small and must be treated discretely. Each binding site may be empty or occupied; thus there are four binding substates for each value of $N = 0, 1, 2, \dots$ (the number of L molecules in V). The kinetic diagram for this system is shown in Fig. 4, with a sample of rate constants (from Fig. 2). The states are labeled N_{ij} , where $i = 0, 1$ and $j = 0, 1$ specify the states of sites ℓ and r , respectively. The mean probability of state N_{ij} at time t is P_{Nij} ; these probabilities are normalized to unity. That is, P_{Nij} is the fraction of systems in an infinitely large ensemble that are in state N_{ij} . The linear differential equations that govern the P_{Nij} are

$$\frac{dP_{000}}{dt} = -(\alpha_1 c_1 + \alpha_2 c_2) P_{000} + \beta_1 P_{010} + \beta_2 P_{001} \quad [10]$$

$$\begin{aligned} \frac{dP_{010}}{dt} = & -(\alpha_2 c_2 + \beta_1 + \beta'_1) P_{010} + \beta_2 P_{011} \\ & + \frac{\alpha'_1}{V} P_{100} + \alpha_1 c_1 P_{000} \end{aligned} \quad [11]$$

$$\begin{aligned} \frac{dP_{001}}{dt} = & -(\alpha_1 c_1 + \beta_2 + \beta'_2) P_{001} + \beta_1 P_{011} \\ & + \frac{\alpha'_2}{V} P_{100} + \alpha_2 c_2 P_{000} \end{aligned} \quad [12]$$

$$\begin{aligned} \frac{dP_{N11}}{dt} = & -(\beta_1 + \beta'_1 + \beta_2 + \beta'_2) P_{N11} \\ & + \alpha'_2 \frac{(N+1)}{V} P_{N+1,10} + \alpha'_1 \frac{(N+1)}{V} P_{N+1,01} \\ & + \alpha_2 c_2 P_{N10} + \alpha_1 c_1 P_{N01} \quad (N \geq 0) \end{aligned} \quad [13]$$

$$\begin{aligned} \frac{dP_{N00}}{dt} = & -\left(\alpha'_1 \frac{N}{V} + \alpha'_2 \frac{N}{V} + \alpha_1 c_1 + \alpha_2 c_2\right) P_{N00} \\ & + \beta'_1 P_{N-1,10} + \beta'_2 P_{N-1,01} \\ & + \beta_1 P_{N10} + \beta_2 P_{N01} \quad (N \geq 1) \end{aligned} \quad [14]$$

$$\begin{aligned} \frac{dP_{N10}}{dt} = & -\left(\alpha_2 c_2 + \alpha'_2 \frac{N}{V} + \beta_1 + \beta'_1\right) P_{N10} \\ & + \beta_2 P_{N11} + \alpha'_1 \frac{(N+1)}{V} P_{N+1,00} \\ & + \beta'_2 P_{N-1,11} + \alpha_1 c_1 P_{N00} \quad (N \geq 1) \end{aligned} \quad [15]$$

$$\begin{aligned} \frac{dP_{N01}}{dt} = & -\left(\alpha_1 c_1 + \alpha'_1 \frac{N}{V} + \beta_2 + \beta'_2\right) P_{N01} \\ & + \beta_1 P_{N11} + \alpha'_2 \frac{(N+1)}{V} P_{N+1,00} \\ & + \beta'_1 P_{N-1,11} + \alpha_2 c_2 P_{N00} \quad (N \geq 1). \end{aligned} \quad [16]$$

The omitted rate constants in Fig. 4 can be filled in from these equations, if desired. At steady state, the left-hand sides of Eqs. 10–16 are all replaced by zero. The resulting linear algebraic equations in the P_{Nij} can then be solved easily by computer provided that the P_{Nij} become negligible by $N = 40$ or so (160 linear equations). The parameters of the problem, in numerical calculations, are c_1 , c_2 , V , and the eight rate constants (with the two constraints in Eqs. 1).

The steady state is an equilibrium if $c_1 = c_2 = c$. Equilibrium statistical mechanics then applies to V ; the chemical potential of L in V is determined by and equal to μ in the two baths. We can treat V formally as an open one-component ideal gas. Thus the canonical and grand partition functions are (4)

$$Q_N = q^N/N!, \Xi = \sum_N Q_N \lambda^N = e^q = e^{\bar{c}V}, \quad [17]$$

where q is proportional to V and $\lambda = e^{\mu/kT}$. The probability P_N of N molecules in V is

$$P_N = \frac{Q_N \lambda^N}{\Xi} = \frac{(q\lambda)^N}{N! e^{q\lambda}} = \frac{(\bar{c}V)^N}{N! e^{\bar{c}V}}. \quad [18]$$

Note that this is a Poisson distribution. We can therefore anticipate, and easily verify by substitution, that the solution to the steady-state Eqs. 10–16 in this case is

$$P_{Nij} = \frac{(cV)^N p_{ij}}{N! e^{\bar{c}V}} \quad (N = 0, 1, 2, \dots; i, j = 0, 1) \quad [19]$$

$$\begin{aligned} p_{00} &= 1/\xi, & p_{10} &= K_1 c/\xi, & p_{01} &= K_2 c/\xi, \\ p_{11} &= K_1 K_2 c^2/\xi, & \xi &= (1 + K_1 c)(1 + K_2 c), \end{aligned} \quad [20]$$

where $\bar{c} = c$ and ξ is the grand partition function for the two binding sites (4). In this equilibrium case, no matter how small V and $\bar{N}$ are, the chemical potential μ in V is well-defined by the μ in the two macroscopic baths with which V is in equilibrium (via the binding sites). Just as in the two baths, μ in V has the conventional form

$$\mu = \mu^\circ + kT \ln \bar{c}. \quad [21]$$

Thus μ and $\bar{c} = \bar{N}/V$ have true thermodynamic significance; also $\bar{c}$ and $\bar{N}$ are operational quantities because, in principle, they can be measured using an ensemble of these small systems in a membrane.

The Steady-State Small System

Consider an arbitrary steady state in which V is very small, $c_1 > c_2$, and the eight rate constants are specified (subject to Eqs. 1). Eqs. 10–16 can be solved numerically to give the P_{Nij} . In terms of these P_{Nij} , the mean value of N in V is

$$\bar{N} = \sum_{N,i,j} NP_{Nij}, \quad [22]$$

where the sum is over $N \geq 0$ and $i, j = 0, 1$. The probabilities that site ℓ is empty or that site r is empty are, respectively,

$$P_{0\ell} = \sum_{N,j} P_{N0j}, \quad P_{0r} = \sum_{N,i} P_{Ni0}. \quad [23]$$

The four steady-state fluxes in L (Fig. 3b), which must be equal at steady state, are then

$$\begin{aligned} J_A &= \alpha_1 c_1 P_{0\ell} - \beta_1 (1 - P_{0\ell}) = J_B \\ &= \beta'_1 (1 - P_{0\ell}) - \frac{\alpha'_1}{V} \sum_{N,j} NP_{N0j} \\ &= J_C = \frac{\alpha'_2}{V} \sum_{N,i} NP_{Ni0} - \beta'_2 (1 - P_{0r}) = J_D \\ &= \beta_2 (1 - P_{0r}) - \alpha_2 c_2 P_{0r}. \end{aligned} \quad [24]$$

If we rewrite these equations in the usual way (2), in terms of the two states of each binding site (as in Fig. 3a and Eqs. 3), they become

$$\begin{aligned} J_A &= \alpha_1 c_1 P_{0\ell} - \beta_1 (1 - P_{0\ell}) = J_B \\ &= \beta'_1 (1 - P_{0\ell}) - \alpha'_1 c_{0\ell} P_{0\ell} \end{aligned} \quad [25]$$

$$\begin{aligned} &= J_C = \alpha'_2 c_{0r} P_{0r} - \beta'_2 (1 - P_{0r}) = J_D \\ &= \beta_2 (1 - P_{0r}) - \alpha_2 c_2 P_{0r}, \end{aligned} \quad [26]$$

where

$$c_{0\ell} \equiv \bar{N}_{0\ell}/V, \quad c_{0r} \equiv \bar{N}_{0r}/V \quad [27]$$

$$\bar{N}_{0\ell} \equiv \sum_{N,j} NP_{N0j}/P_{0\ell}, \quad \bar{N}_{0r} \equiv \sum_{N,i} NP_{Ni0}/P_{0r}. \quad [28]$$

Note that $\bar{N}$ is the mean value of N over all states Nij but that $\bar{N}_{0\ell}$ and $\bar{N}_{0r}$ are mean values of N only over subsets of Nij (all states in which site ℓ is empty or all states in which site r is empty, respectively).

Note that $\bar{c} = \bar{N}/V$ does not occur in the basic flux relations, Eqs. 24, 25, and 26; rather, two other concentrations appear, $c_{0\ell}$ and c_{0r} . Of course, when V and $\bar{N}$ are very large, $\bar{c}$, $c_{0\ell}$, and c_{0r} would become equal to each other because in this case the perturbation of the N distribution in V by the states of the two binding sites would be negligible. When V is small, $\bar{N}$ and $\bar{c}$ are still operational variables (as described above) at steady state, but they are no longer significant variables kinetically. The reverse is true of $\bar{N}_{0\ell}$, $c_{0\ell}$, $\bar{N}_{0r}$, and c_{0r} : they are significant but not operational. What we are beginning to see here is that it is useful and legitimate to regard V as a separate system if V is large or at equilibrium but not if it is small and in a nonequilibrium steady state. In this case, V and the two binding sites have to be treated as an inseparable package.

Incidentally, if V is replaced in the membrane by a row of binding sites, to provide an alternative transport mechanism for L , analogous behavior is encountered: mean occupancies of neighboring sites *cannot* be used to write correct flux expressions (ref. 2, p. 190), as one might expect.

Let $\bar{N}_{1\ell}$ be the mean value of N when site ℓ is occupied and similarly for $\bar{N}_{1r}$. Then clearly

$$\begin{aligned} \bar{N} &= \bar{N}_{0\ell} P_{0\ell} + \bar{N}_{1\ell} (1 - P_{0\ell}) \\ &= \bar{N}_{0r} P_{0r} + \bar{N}_{1r} (1 - P_{0r}). \end{aligned} \quad [29]$$

Thus $\bar{N}_{1\ell}$ and $\bar{N}_{1r}$ are not additional independent properties. From Eqs. 25 and 26,

$$\begin{aligned} P_{0\ell} &= \frac{\beta_1 + \beta'_1}{\alpha_1 c_1 + \alpha'_1 c_{0\ell} + \beta_1 + \beta'_1}, \\ P_{0r} &= \frac{\beta_2 + \beta'_2}{\alpha_2 c_2 + \alpha'_2 c_{0r} + \beta_2 + \beta'_2}. \end{aligned} \quad [30]$$

These are generalizations of Eqs. 2 for small V . If $P_{0\ell}$ and P_{0r} are now eliminated from Eqs. 25 and 26,

$$\begin{aligned} J &= \frac{\alpha_1 \beta'_1 (c_1 - c_{0\ell})}{\alpha_1 c_1 + \alpha'_1 c_{0\ell} + \beta_1 + \beta'_1} \\ &= \frac{\alpha_2 \beta'_2 (c_{0r} - c_2)}{\alpha_2 c_2 + \alpha'_2 c_{0r} + \beta_2 + \beta'_2}. \end{aligned} \quad [31]$$

This generalizes Eq. 4 to small V . The two right-hand expressions provide a relation between $c_{0\ell}$ and c_{0r} ; these two quantities are not independent of each other. However, the complete set P_{Nij} is needed to calculate $c_{0\ell}$ and c_{0r} .

As a quite arbitrary numerical example, we use

$$\begin{aligned} \alpha_1 &= 0.5, & \beta_1 &= 1.0, & \alpha'_1 &= 2.0, & \beta'_1 &= 4.0 \\ \alpha_2 &= 4.0, & \beta_2 &= 2.0, & \alpha'_2 &= 1.0, & \beta'_2 &= 0.5 \\ c_1 &= 40.0, & c_2 &= 2.0, & V &= 0.5. \end{aligned} \quad [32]$$

In this case, $4 \times 31 = 124$ states were included in the computer calculation. If we define P_N as the sum of P_{Nij} over the four substates ij , then we find in this example $P_0 = 7.99 \times 10^{-4}$, $P_6 = 0.1496$ (maximum), and $P_{28} = 1.58 \times 10^{-9}$. Several properties of interest are included in Table 1. $\bar{N}$, $\bar{N}_{0\ell}$, and $\bar{N}_{0r}$ are significantly different. The fact that $\sigma^2 \neq \bar{N}$ (σ^2 is the variance in N) shows that P_N does not have a Poisson distribution, as at equilibrium. This effect is larger if the individual NP_N/P_{N-1} are examined; these quantities would all be equal to $\bar{N}$ in a Poisson distribution. For example, $NP_N/P_{N-1} = 7.45, 6.99$, and 7.10 for $N = 1, 10$, and 28 , respectively. Also, the quotient $P_{N00}P_{N11}/P_{N10}P_{N01}$ varies somewhat with N (it is equal to 1 at equilibrium): e.g., 0.985 at $N = 0$, 1.033 at $N = 5$, and 1.009 at $N = 28$.

These results show that this small steady-state system does not exhibit a local equilibrium within V or the binding sites. Because of this, the chemical potential of L in V , when V is small and at steady state, is not a defined thermodynamic quantity. At equilibrium, the two macroscopic baths determine μ in V (and on the two sites); when $c_1 \neq c_2$, a μ in V does not exist. One could, of course, define a μ formally by Eq. 21, because $\bar{c}$ is an operational quantity, but this μ has no particular interest or significance. Thus, on both kinetic (see above) and thermodynamic grounds, $\bar{N}$ is an inconsequential quantity when V is small.

Thermodynamic Forces

The transport system we are considering is operating under the well-defined force

$$X = \mu(c_1) - \mu(c_2) = kT \ln(c_1/c_2) \quad [33]$$

between baths 1 and 2. When V is small, this force cannot be subdivided in any thermodynamically significant way, as in Eqs. 5 and 6 (large V). Rather, the transport system must be treated as a single indivisible system. In particular, as just explained, $\mu(\bar{c})$ in V should not be used for such a subdivision of X .

It is of some interest to define subforces *kinetically*, using the quotient of products of rate constants (2) in the two-state binding cycles (see Eqs. 7, 8, 25, and 26):

$$e^{X_{\ell}/kT} \equiv \frac{\alpha_1 c_1 \beta'_1}{\alpha'_1 c_{0\ell} \beta_1} = \frac{c_1}{c_{0\ell}}, \quad [34]$$

$$e^{X_r/kT} \equiv \frac{\alpha'_2 c_{0r} \beta_2}{\alpha_2 c_2 \beta'_2} = \frac{c_{0r}}{c_2}. \quad [35]$$

These are nonthermodynamic and nonoperational subforces. Furthermore, because in general $c_{0\ell} \neq c_{0r}$, the sum of the two subforces is not equal to X in Eq. 33.

Non-Uniqueness of $J(\bar{c})$

Here we pursue the above numerical example further to illustrate in a different way (1) that V , when small, cannot be treated as an ordinary bath, at $\bar{c}$, in a transport system.

The right-hand transport subsystem (V , site r , bath 2) in Fig. 1 has parameters V , α_2 , β_2 , α'_2 , β'_2 , and c_2 (aside from the N distribution in V). If we keep all of these parameters constant at their values in Eq. 32 but alter the left-hand transport subsystem in Eq. 32 by varying α_1 and β'_1 (holding $\alpha_1 \beta'_1$ con-

FIG. 5. Flux J in the right-hand subsystem, as a function of $\bar{c}$ and c_{0r} , when the left-hand subsystem is varied. Solid curve, $J(\bar{c})$ when α_1 and β'_1 are varied holding $\alpha_1 \beta'_1$ constant; points, $J(\bar{c})$ when β_1 and α'_1 are varied holding $\beta_1 \alpha'_1$ constant; broken curve, unique curve for $J(c_{0r})$ (any variation in the left subsystem).

stant), then J and $\bar{c}$ will both change as a consequence. From the point of view of the right-hand subsystem, considered as an independent system, all of its parameters are being held constant except for a variation in $\bar{c}$ in the bath V , which produces a change in J . The solid curve in Fig. 5 shows $J(\bar{c})$ calculated in this way. J approaches zero as $\alpha_1 \rightarrow 0$, $\beta'_1 \rightarrow \infty$, $\bar{c} \rightarrow c_2$, because, in this limit, V comes to equilibrium with bath 2 (the left-hand transport subsystem becomes inactive).

If we now repeat the calculation but this time vary only β_1 and α'_1 (in Eq. 32), holding $\beta_1 \alpha'_1$ constant, again we will obtain $J(\bar{c})$ for the right-hand subsystem. In this case the left-hand subsystem becomes inactive as $\beta_1 \rightarrow 0$, $\alpha'_1 \rightarrow \infty$, $\bar{c} \rightarrow c_2$ because site ℓ becomes saturated. The points in Fig. 5 result from this second method of varying $\bar{c}$ for the right-hand system. Because the points (except the reference point at the top) do not fall on the solid curve, we conclude that $J(\bar{c})$ is not a unique function for the right-hand system: this function depends on how the left-hand subsystem is altered to produce variations in $\bar{c}$. The conclusion (again) is that, when V is small, one cannot treat either of the two transport subsystems (left or right) as an independent system; they are inseparable. Also, we confirm that $\bar{c}$ by itself is not an adequate state variable for V (the complete distribution in N_{ij} is involved).

Incidentally, the lower expression in Eq. 31 gives J as a *unique* function of c_{0r} , if α_2 , β_2 , α'_2 , β'_2 , and c_2 are held constant as above, regardless of how the left-hand subsystem is altered to vary c_{0r} . This function is the broken line in Fig. 5. This property of $J(c_{0r})$ is of no great consequence, however, because c_{0r} is a nonoperational quantity.

Table 1. Steady-state properties in an example

$\bar{N}$	7.0113	$P_{0\ell}$	0.09290
$\bar{N}_{0\ell}$	7.2060	P_{0r}	0.10492
$\bar{N}_{0r}$	6.6638	J	0.95080
σ^2	6.9595	$\bar{c}$	14.0226

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