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Free energy transduction in membrane transport systems induced by externally imposed fluctuations in ligand concentrations

Yi-der Chen

Section on Theoretical Molecular Biology, Laboratory of Molecular Biology, National Institute of Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD (U S A)

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The effect of externally induced fluctuations in ligand concentration on the transport of ligand across a membrane is studied theoretically. It is found that, when only one of the two baths is externally perturbed to fluctuate the concentration, a net transport of ligand from the unperturbed to the perturbed side of the membrane is always observed, even though the average concentrations of ligand of the two baths are equal. As a result, apparent up-hill transport against a gradient can be realized in this case. In the case of both baths being perturbed independently, apparent up-hill transport is also possible. However, the direction of net transport of ligand will depend on the amplitudes and the frequencies of the two fluctuations as well as the kinetic mechanism of the transport system. From these results, it is concluded that externally imposed fluctuations in ligand concentration act to reduce the chemical potential of the ligand so that the effective activity is always less than the mean concentration of the ligand. The conclusion is very general in that it applies to any transport model that involves one ligand per turnover. The same principle can be used to analyze the effect of fluctuations in equilibrium ligand binding and cyclic enzyme reactions in solution.

1. Introduction

The study of effect of random fluctuations in external (environmental) parameters on the stability properties or dissipative structures of nonlinear dynamic systems have attracted considerable attention in recent years [1–9] Using a nonlinear positive feedback enzyme model, Hahn et al [1] were the first to demonstrate that fluctuations in the injection rate of substrate (an external parameter to the system) could induce quasi-periodic concentration oscillations in the system, which would not appear without the fluctuation On the other hand, Boiteux et al [2] found that the periodic behavior of the well-known glycolytic oscillator could be retained in the presence of a fluctuating substrate injection rate extending partly over the non-oscillating domain of the deterministic equations Recently, Horsthemke, Lefever and their colleagues [3–9] have studied the direct effect of fluctuations in rate constants on some chemical and biological nonlinear systems and found that fluctuations could stabilize macroscopic states and induce phase transitions at branching points which would not show up in the usual stability diagrams based on the deterministic equations

More recently, we have been investigating another aspect of the external noise-induced phe-

Correspondence Y Chen, Laboratory of Molecular Biology, Room 310, Building 2, National Institute of Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, U S A

nomenon the effect of fluctuations in rate constants on the flux of cycle completions in cyclic reacting systems at steady-state [10,11] Specifically, we are interested in the induction of directional flow or free energy transduction in enzyme-mediated cyclic reactions by applying a fluctuating external parameter to the system which alters the rate constant(s) of the reactions in the system For example, as discussed in Refs 10 and 11, a ligand carrier inside a membrane undergoing a four-state cyclic reaction can be made to transport ligand across the membrane against the chemical potential of the ligand (up-hill transport) by applying a fluctuating potential across the membrane, provided that some of the rate constants of the elementary reactions of the system are voltage-dependent and satisfy some asymmetry requirements (see Ref 11) In other words, the energy of the fluctuating membrane potential applied externally to a cyclic kinetic system can be harnessed or transduced to perform biochemical work These studies were motivated by the experimental and theoretical findings that periodic oscillations (a special kind of fluctuation) in membrane potential could induce up-hill transport of ligand across membranes [12–15]

In principle, any external parameter that alters the value of one or more rate constants of a cyclic kinetic system is capable of producing fluctuation-induced free energy transduction So far, our model studies on fluctuation-induced up-hill transport have been limited to only one external parameter the electrical potential In this paper, we will extend our studies to another external parameter the concentration of ligand That is, we are interested in the possible change in the transport flux of a carrier-mediated membrane system for ligand L when the concentration of L is externally perturbed to fluctuate

The reasons for this study are 2-fold First, different external parameters have different modes of interaction with the system For example, the electrical membrane potential affects those reactions that involve states (or species) with charges or dipole moments, while the concentration of ligand affects the binding reactions of the system Since fluctuation-induced free energy transduction depends critically on the kinetic mechanisms of the system (see Ref 11), it is expected that fluctuations in different external parameters should exhibit different transduction characteristics Thus, it is interesting to investigate whether concentration fluctuation can induce up-hill transport in cyclic kinetic systems and, if it can, does the system require any asymmetry condition in the kinetic rate constants, as in the potential-induced case studied in Refs 10 and 11? Second, oscillations in concentrations of metabolites at (nonequilibrium) steady-state are very common in biological systems (the oscillation of glycolytic intermediates in cells is a typical example [16]) Large fluctuations in metabolite concentrations are also expected to be present in biological systems It is thus important to examine the possible effect of these oscillations and fluctuations in biological functions This paper is the first of a series of studies on this subject We will consider dichotomous noise (i.e, the ligand concentration is randomly fluctuating between two discrete values) in this paper We will report elsewhere the study on multi-level fluctuations and regular oscillations

In Section 2, we describe briefly the model and the biochemical diagram of the system to be studied In Section 3, we show that up-hill transport can indeed be achieved for a class of models by externally perturbing the concentration of ligand in one bath and that the direction of transport is always from the non-fluctuating to the fluctuating bath Furthermore, the occurrence and the direction of up-hill transport does not depend on the existence of asymmetry in the kinetic mechanism The case that both ligand concentrations in the two baths are perturbed independently is discussed in Section 4 Up-hill transport is also shown to be possible in this case, but the direction of active transport depends on the frequencies and the amplitudes of the two fluctuations and the values of rate constants of the model Finally, the implication of this fluctuation induced free-energy transduction in biological functions and possible experimental procedures to study this phenomenon are discussed in the last section

2. The Model

For simplicity, the main part of this paper will be discussed in terms of a simplified two-state model shown in Fig 1 But, as will be shown in

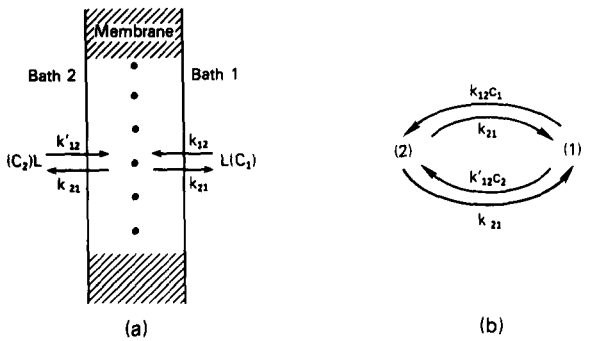

Fig 1 (a) A membrane transport system for ligand L (b) The kinetic diagram for the transport system A binding site (or carrier molecule) is in state 1 when it is empty and in state 2 when bound with a ligand

the Appendix, the general conclusions obtained for this model are in fact valid for models with an arbitrary number of states

As shown in Fig 1a, the system consists of a membrane placed between two large bathing solutions that contain an uncharged ligand L Inside the membrane are a number of independent binding sites that only one molecule of ligand L can bind from the bathing solutions on either sides of the membrane The kinetic diagram for this transport system in the absence of external perturbation is shown in Fig 1b, in which an empty site is denoted by 1 and an occupied site by 2 c_1 and c_2 are the mean concentrations of ligand L in baths 1 and 2, respectively

At equilibrium ($c_1 = c_2$), the detailed balance condition must be obeyed Thus, the rate constants k_{12}, etc, in the diagram must satisfy the condition

$$k_{11}k'_{21} = k'_{12}k_{21} \tag{1}$$

In the absence of external perturbation in both c_1 and c_2, the mean flux of transport of ligand L from bath 1 to bath 2 can be expressed as (see Ref 17)

$$J = \frac{\Pi}{\sum_0}(c_1 - c_2) \tag{2}$$

where

$$\Pi = k_{12}k'_{21} = k'_{12}k_{21} \tag{3}$$

$$\sum_0 = k_{12}c_1 + k_{21} + k'_{12}c_2 + k'_{21} \tag{4}$$

Thus, at $c_1 = c_2$, $J = 0$ and there is no net ligand transport across the membrane One must note that the model in Fig. 1 is equivalent to a four-state carrier model when the rate constants of the translocation steps are very large compared to the others

In the next two sections, we will examine the situation that c_2 (or c_1) alone (Section 3) or both c_1 and c_2 (Section 4) are perturbed externally to fluctuate randomly We will show that in the presence of external fluctuations in c_1 and/or c_2, the transport flux can be non-zero even though the mean values of c_1 and c_2 are equal

3. Fluctuations in one bath

In this section we consider the case that only one of the ligand concentrations (c_2 in Fig 1) is subjected to external perturbations In the next section, the case that the ligand concentrations of both bars are fluctuating will be considered We will show that external fluctuation-induced apparent up-hill transport can be realized in both cases But before going into that, we would like to discuss briefly the type of fluctuation (or noise) considered here. In general, due to the stochastic nature of the transport reactions, the two concentrations of ligand in both baths in Fig 1 will fluctuate around their mean values even in the absence of external perturbation This kind of fluctuation is usually referred to as the 'internal' fluctuation of the system Internal fluctuations in ligand concentrations diminish when the size of the bath is increased (or the number of carrier molecules of the transport system is decreased) In contrast, 'external' fluctuations are generated by some external sources (see Discussion for possible experimental set-ups) and are assumed to be independent of the size of the bath In the present study, internal fluctuations are neglected, because the baths are assumed to be very large

As in Ref 11, we will consider a special kind of fluctuation a random telegraph (or dichotomous) noise That is, the concentration of ligand L in bath 2 is assumed to be randomly switched be-

tween a high and a low value with a mean of c_2

$$c_2 + \Delta \underset{\gamma}{\overset{\gamma}{\rightleftarrows}} c_2 - \Delta \tag{5}$$

where γ is the transition rate constant of the switching Then, as discussed in Ref 11, at steady-state the biochemical diagram describing the kinetic system in Fig 1 can be expanded into the one shown in Fig 2, in which symbols 1 and 2, as in Fig 1b, represent an empty and an occupied site and the superscripts + and − signs indicate whether the concentration of ligand in bath 2 is above or below the mean value

$$c_2^+ = c_2 + \Delta$$

$$c_2^- = c_2 - \Delta$$

As shown in Fig 3, there are a total of six cycles for the kinetic diagram in Fig 2 Four of them can contribute to the transport of ligand across the membrane (cycles 1–4 in Fig 3) The fluxes of them can be expressed as (see Hill [17] for details)

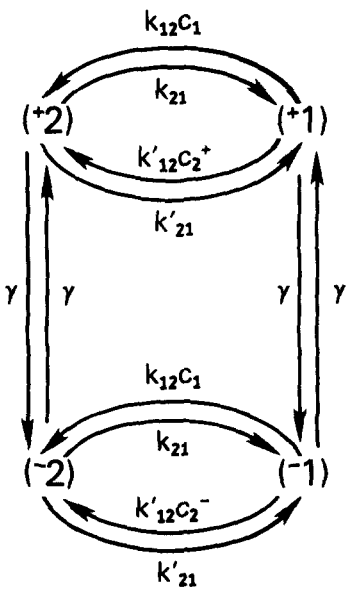

Fig 2 The 'expanded' kinetic diagram for the transport system in Fig 1 in the presence of a two-level (dichotomous) noise in c_2 The rate constants k_{12}, etc , are the same as those in Fig 1 γ is the transition rate constant of the dichotomous noise

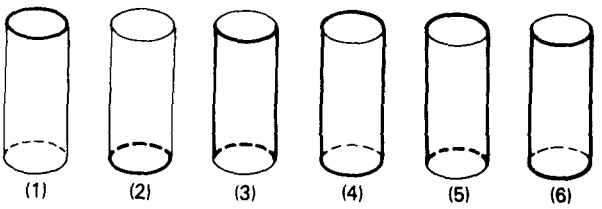

Fig 3 Cycles of the kinetic diagram in Fig 2

$$J_1 = \frac{\Pi}{\Sigma}(c_1 - c_2 - \Delta)\left(\gamma^2 + \gamma \sum_0 - \gamma\, k'_{12}\, \Delta\right) \tag{6}$$

$$J_2 = \frac{\Pi}{\Sigma}(c_1 - c_2 + \Delta)\left(\gamma^2 + \gamma \sum_0 + \gamma\, k'_{12}\, \Delta\right) \tag{7}$$

$$J_3 = \frac{\Pi}{\Sigma}\gamma^2(c_1 - c_2 - \Delta) \tag{8}$$

$$J_4 = \frac{\Pi}{\Sigma}\gamma^2(c_1 - c_2 + \Delta) \tag{9}$$

where Π and Σ_0 are given in Eqns 3 and 4 and

$$\Sigma = 4\gamma^2 \sum_0 + 2\gamma\left(\left(\sum_0\right)^2 - (k'_{12}\, \Delta)^2\right) \tag{10}$$

The sign of the J's in Eqns 6–9 is defined as positive when the ligand is transported from bath 1 to bath 2

One must note that the Σ in Eqns 6–10 is the sum of the product of rate constants of all directional diagrams [17], and is therefore a positive quantity Since Δ cannot be greater than c_2 (otherwise $c_2 - \Delta$ will become negative), $\gamma\ \Sigma$ is always larger than $\gamma\ k'_{12}\ \Delta$ (see Eqn 4) As a result, the quantity inside the second parentheses of Eqn 6 must be positive Thus, J_1 and J_3 become negative and J_2 and J_4 positive at $c_1 = c_2$ This means that a system at equilibrium initially ($c_2 = c_1$, $\Delta = 0$) can be induced to generate cycling cycles with non-zero fluxes by fluctuating the concentration of ligand in one bath Summing up Eqns 6–9, we obtain the net ligand transport flux at steady-state from bath 1 to bath 2 at arbitrary c_2 and Δ values

$$J = \left(\frac{2\Pi\gamma}{\Sigma}\right)\left(2\gamma(c_1 - c_2) + \sum_0 (c_1 - c_2) + k'_{12}\, \Delta^2\right) \tag{11}$$

At $c_2 = c_1$, Eqn 11 becomes

$$J(c_1 = c_2) = \left(\frac{2\Pi\gamma}{\sum}\right) k'_{12}\,\Delta^2 \tag{12}$$

Two surprising conclusions can be drawn from Eqn 12 First, the value of J in Eqn 12 is always positive This implies a net transport of ligand from bath 1 to bath 2 even though the averaged concentration of ligand in bath 2 is equal to that in bath 1 ($c_2 = c_1$) In other words, the fluctuation in ligand concentration applied externally to bath 2 acts to reduce the effective activity (or concentration if the solution is dilute) of ligand in bath 2 so that ligand is always transported from bath 1 to bath 2 (but not from bath 2 to bath 1) at $c_2 = c_1$ Consequently, up-hill transport of ligand from bath 1 to bath 2 can be realized (i e , the J in Eqn 11) is positive even at $c_1 < c_2$) Second, since the asymmetry is never used in deriving Eqn 12, the active transport induced by ligand concentration fluctuations does not require asymmetry or any other conditions in the kinetic mechanism As discussed in Ref 11, asymmetry is always required in potential-fluctuation induced active transport systems

As shown in the Appendix, the same conclusions can be obtained for general models containing more than two states in their kinetic diagrams, provided that the model involves only one ligand per turnover We will show elsewhere that the same results can be obtained also for systems with more complicated noise than the dichotomous one and systems with regular oscillations Thus, it seems the above two conclusions are rather general

In order to quantify the reduction in effective concentration of ligand due to the presence of externally imposed fluctuations for this two-state model, let us define the effective concentration of ligand in bath 2 as

$$(c_2)_{\text{eff}} = c_2 - \delta \tag{13}$$

That is, the net transport flux becomes zero when $(c_2)_{\text{eff}} = c_1$ Thus, by replacing the c_1 with $(c_2)_{\text{eff}}$ and setting $J = 0$ in Eqn 11, we get

$$(c_2)_{\text{eff}} = c_2 - \frac{k'_{12}\Delta^2}{2\gamma + \sum_0} \tag{14}$$

Thus, from Eqn 13 and 14, we obtain

$$\delta = \frac{k'_{12}\Delta^2}{2\gamma + \sum_0} \tag{15}$$

It is obvious from Eqn 15 that δ is always positive, independent of the values of the rate constants This implies that in the presence of external fluctuations the effective ligand concentration always becomes smaller than its mean value and that apparent up-hill transport can be realized only from bath 1 to bath 2 (one-way active transport) In fact, apparent up-hill transport of ligand from bath 1 to bath 2 will occur as long as the mean concentration of ligand in bath 2 satisfies the conditions

$$c_1 < c_2 < c_1 + \delta \tag{16}$$

As can be seen from Eqn 15, δ becomes larger when Δ increases and γ decreases In other words, in the presence of concentration fluctuations the effective activity of the ligand becomes smaller when the amplitude of the fluctuation is increased and the frequency is reduced

As will be shown in the next section, the δ derived in Eqn 15 is very useful in predicting the direction of ligand flow when both c_1 and c_2 are fluctuating independently

4. Fluctuation in both baths

In this section, we examine the case that the ligand concentrations on both sides of the membrane are perturbed to fluctuate independently (i e , the fluctuation of c_1 is not correlated to that of c_2) Let Δ_1 and γ_1 be the amplitude and the frequency of fluctuations of ligand concentration in bath 1 and Δ_2 and γ_2 be those in bath 2 (see Eqn 5) Then, the expanded biochemical diagram of the two-state transport system in the presence of independent external fluctuations in c_1 and c_2 can be shown as in Fig 4 The + or − signs on the right and the left sides of each state indicate, respectively, c_1 and c_2 having high or low values For example, $^-2^+$ indicates that the site is in state 2 (ligand bound state) with c_1 at high value ($c_1 + \Delta_1$) while c_2 is at the low value ($c_2 - \Delta_2$)

There are a total of 134 cycles for the diagram

in Fig 4 64 of them can contribute to the net transport of ligand between the two baths Since it would be rather difficult to analyze or calculate the ligand transport flux based on these cycle diagrams, the analysis will be based on numerical model calculations That is, each rate constant in Fig. 4 is assigned a value and the steady-state probabilities of the states are then calculated by the standard matrix inversion method The flux is then calculated from these state probabilities We will examine whether ligand transport is possible when c_1 is equal to c_2 And, if transport exists, how it is affected by the frequency and the amplitude of the fluctuations

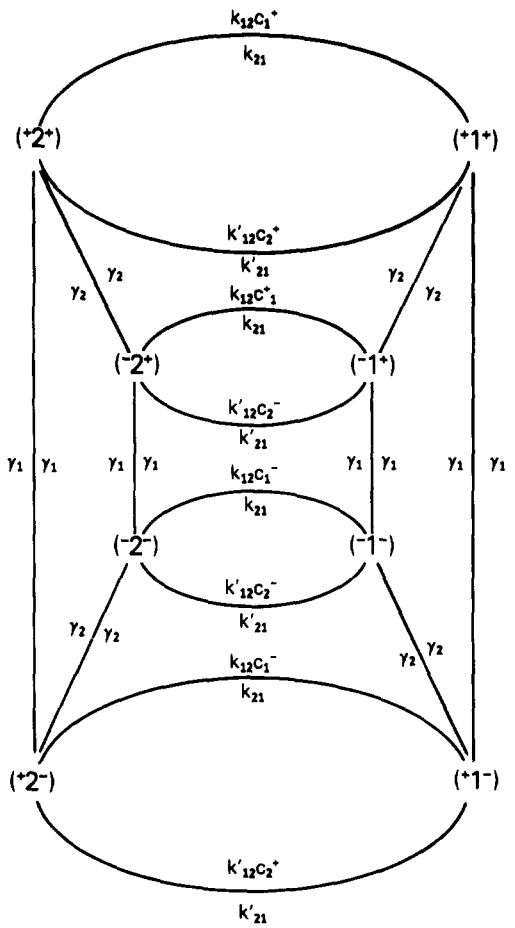

Fig 4 The expanded kinetic diagram for the transport system in Fig 1 in the presence of dichotomous fluctuations in both c_1 and c_2 The + and − signs on the right and the left side of each state indicate whether c_1 and c_2 are in the high or low value γ_1 and γ_2 are, respectively, the transition rate constants of fluctuations in c_1 and c_2 The rate constants k_{12}, etc , are the same as those in Fig 1

In Fig. 5, the calculated J (the net transport flux of ligand from bath 1 to bath 2) at constant Δ_1 and Δ_2 is plotted as a function of γ_2 at different γ_1 values for two kinetic models The calculated J at constant γ_1 and γ_2 is plotted as a function of Δ_2 and Δ_1 in Fig 6 As one can see from Figs 5 and 6, net transport of ligand between the two baths can occur at $c_1 = c_2$ even when both ligand concentrations on the two sides of the membrane are fluctuating Consequently, up-hill transport can be realized in this case One must note that up-hill transport can take place from bath 1 to bath 2 when the J in Figs 5 and 6 is positive and from bath 2 to bath 1 when J is negative

As shown in Fig 5, when the values of Δ_1 and Δ_2 are fixed (constant amplitude), decreasing the frequency in bath 2 will favor the transport of ligand from bath 1 to bath 2 On the other hand, when the values of γ_1 and γ_2 are fixed, ligand transport from bath 1 to bath 2 is favored at high

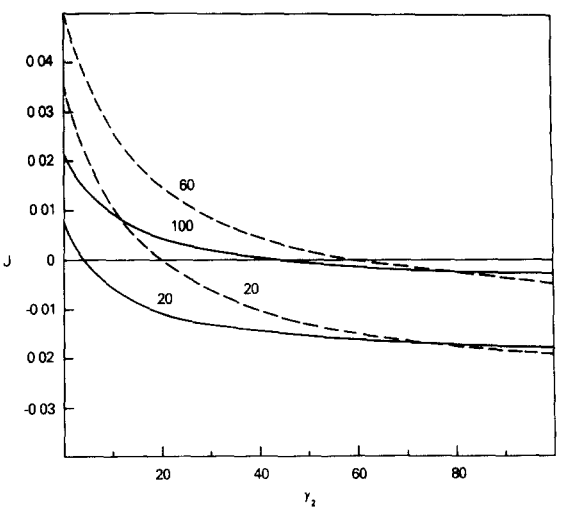

Fig 5 The net flux of transport of ligand L in the presence of fluctuations in both baths, calculated at constant Δ_1 and Δ_2 The calculated J (positive when L is flowing from bath 1 to bath 2) is plotted as a function of γ_2 at given values of γ_1 for two kinetic models Parameters used in the calculations are $c_1 = c_2 = 1\,5$, $\Delta_1 = \Delta_2 = 0\,5$ The value of γ_1 is indicated for each curve The rate constants for model A (dashed curves) are $k_{12} = 8$, $k_{21} = 4$, $k'_{12} = 8$, $k'_{21} = 4$ and for model B (solid curves) are $k_{12} = 8$, $k_{12} = 4$, $k'_{21} = 4$, and $k'_{21} = 2$

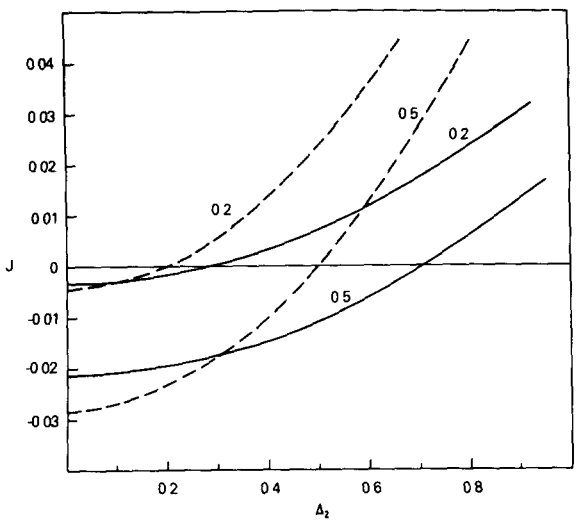

Fig 6 The net flux of transport of ligand L in the presence of fluctuations in both baths, calculated at constant γ_1 and γ_2 $c_1 = c_2 = 1\,5$, $\gamma_1 = \gamma_2 = 20$ The values of Δ_1 are indicated for each curve The rate constants for the two models are the same as those in the legend of Fig 5

Δ_2 value, as shown in Fig 6 In other words, in order to generate a net transport of ligand from bath 1 to bath 2 at $c_1 = c_2$, one would have to increase the amplitude and decrease the frequency of fluctuations in bath 2, while keeping the amplitude low and frequency high in bath 1 And, to favor the transport from bath 2 to bath 1, the condition has to be reversed

As we did for Figs 5 and 6, the exact way to determine the sign and magnitude of the transport flux J for any given model is to solve the kinetic equations of the system However, for the simple two-state model in Fig 1, the sign of J and the transition point at which $J = 0$ can be estimated approximately using the activity reduction factor δ, defined in Eqn 15, as discussed below

In the presence of fluctuations in both baths, the activity reduction factor can be written down separately for the two baths as

$$\delta_1 = \frac{k'_{12}\Delta_1^2}{2\gamma_1 + \sum_0} \tag{17}$$

$$\delta_2 = \frac{k_{12}\Delta_2^2}{2\gamma_2 + \sum_0} \tag{18}$$

where Σ_0 is given in Eqn 4 Thus, when δ_1 is less than δ_2, the effective activity of ligand in bath 1 is greater than that in bath 2 As a result, ligand will flow from bath 1 to bath 2 Similarly, ligand will flow from bath 2 to bath 1 if δ_1 is larger than δ_2 The transition point ($J = 0$) occurs at

$$\delta_1 = \delta_2 \tag{19}$$

For illustration, let us consider the transition points in Fig 5 ($\Delta_1 = \Delta_2$ case) Since k_{12} is equal to k'_{12} in model A, it is easy to see that the value of γ_1 in Eqn 17 must equal to γ_2 in Eqn 18 in order to have Eqn 19 This is indeed the case, as shown in Fig. 5 When applied to model B ($k_{12} = 8$, $k'_{12} = 4$), the transition points were estimated to occur at $\gamma_2 = 44$ for the $\gamma_1 = 100$ case and at $\gamma_2 = 4$ for the $\gamma_1 = 20$ case The exact values (from kinetic equations) were found to be 44 029 and 4 124, respectively

5. Discussion

The main results of this study are (1) In nonlinear membrane transport systems, the effective activity of ligand in the two baths is no longer described by its mean concentration, if the ligand concentration is externally induced to fluctuate In fact, the effective activity of ligand in this case is always reduced by a factor determined by the frequency and amplitude of the external fluctuations and the kinetic rate constants of the model under consideration As a result, apparent up-hill transport of ligands can be realized in these systems That is, ligand can be transported from the non-fluctuating side of the membrane to the fluctuating side, although the averaged mean concentration of ligand of the latter is greater than that of the former (2) In contrast to the external potential fluctuation induced case studied in Refs 10 and 11, the existence of apparent up-hill transport in the present case does not depend on whether the system possesses asymmetrical rate constants or not In other words, up-hill transport can be observed in concentration-fluctuation-induced systems with no asymmetry at all (3) When both ligand concentrations of the two baths are perturbed independently, up-hill transport is also possible However, the direction of transport will

depend on the amplitudes and the frequencies of the two fluctuations and the kinetic mechanism (rate constants) of the system

Although we have considered only symmetric dichotomous fluctuations in this paper, the same results have been found also for systems with complicated fluctuations or regular oscillations Thus, the above three conclusions are in fact very general, in that they are applicable to models with an arbitrary number of states in their kinetic diagrams and to fluctuations of an arbitrary form The only limitation is that the number of ligand transported by each transport unit per each turnover cannot be greater than one (see Appendix)

We would like to point out that although only ligand transport systems are discussed in this paper, the same principle can be applied to equilibrium binding or cyclic enzymatic reacting systems in solution (unpublished data) For example, in the ligand-receptor binding process

$$R + L \rightleftarrows RL$$

if the concentration of L is externally induced to fluctuate, the effective concentration of L is reduced and the observed concentration of ligand-bound receptors ([RL]) will be smaller than that obtained without the fluctuation As a result, the equilibrium binding constant measured in this case will be smaller than the actual value Similarly, in the enzymatic reaction of S (substrate) to P (product) via the enzyme E

$$E + S \rightleftarrows ES$$

$$ES \rightleftarrows EP$$

$$EP \rightleftarrows E + P$$

if P is externally perturbed to fluctuate, a net reaction of S to P can be observed at steady-state, even though the mean concentration of P is in equilibrium with S

We would like to emphasize that the fluctuation discussed here must be 'external' to the system under consideration That is, it must come from an external source, not from the system itself There are direct and indirect ways to generate these external concentration fluctuations In the direct method, fluctuations of ligand (substrate or product) concentrations are generated directly by changing the volume of the system through some physical means In the indirect method, an independent concentration fluctuation-generating system is coupled to the transport (or enzymatic) system Thus, if the fluctuation-generating system is large compared to the transport system, then the fluctuation of ligand concentration can be considered as 'external' to the transport system A possible experimental set-up to produce oscillations (a special kind of fluctuation) would be to place a 'large' oscillating glycolytic system in one of the baths of a 'small' transport system (or an enzymatic reacting system) for an intermediate substance Since the transport system is small, fluctuation is generated by the transport reaction steps would not interfere with the oscillations generated by the glycolytic system Thus, oscillations in intermediate concentrations will be stationary and can be considered as 'external' to the transport system

It is important to note that the external fluctuation-induced phenomena discussed here would not have happened if the system had been linear That is, the reason that directional flow (or up-hill transport) can be induced by external fluctuations in ligand concentration for the model in Fig 1 (or any carrier model) is due to the fact that the permeability coefficient of the system is not constant, but inversely proportional to the ligand concentrations (see Eqns 2 and 4) Consequently, positive and negative perturbations in ligand concentrations have different effects on the flux

Finally, the effect of nonequilibrium concentration fluctuations on ligand transport fluxes in small membrane systems have been discussed before [18,19] As in this study, the activity of the fluctuating ligand was found to be different from the mean concentration of the ligand In other words, in a small system where fluctuations are large and non-Poissonian the averaged mean number or ligands in the system is not a proper thermodynamic parameter to describe the dynamic properties of the system However, as discussed in Ref 18, the apparent up-hill transport seems to occur in the direction of from the fluctuating side to the non-fluctuating side, in contrast to that observed in this study This apparent contradiction to the present finding stems

from the facts that fluctuations in those small systems are not induced by an external source but are generated by the system itself and that the number of ligand involved in each turnover is more than one in the input and the output pumps (see Ref 18)

Appendix

In Section 2, we have demonstrated that active transport can be realized in ligand-transport systems when the concentration of ligand is perturbed externally to fluctuate The result was obtained based on the kinetic study of a simple two-state model with symmetric dichotomous (two-level) fluctuations In this appendix, we would like to show that the conclusions obtained in this paper are indeed very general, in that they are applicable to models with an arbitrary number of states

In general, the cycles (e g , see Fig 3) of an expanded kinetic diagram (Fig 2) that can contribute to the ligand transport in the presence of a symmetric two-level noise can be grouped into pairs of mirror-images with equivalent flux expressions For example, cycle 1 in Fig 3 is the mirror image of cycle 2 when they are placed end-to-end Similarly, cycle 3 is the mirror image of cycle 4 Furthermore, J_1 in Eqn 6 is identical to J_2 in Eqn 7, except that c_2 is in the high value $(c_2 + \Delta)$ in J_1 and in the low value $(c_2 - \Delta)$ in J_2 In other words, the flux expression of a diagram can be obtained from that of its mirror image by replacing all the c_2^+ with c_2^- and all the c_2^- with c_2^+ This symmetry property holds for models with an arbitrary number of states as long as the fluctuation is dichotomous and symmetric

The flux of a cycle involved in the transport of ligand L in an arbitrary diagram can be expressed as (see Hill [17])

$$J = \frac{\Pi \sum'(c_1 - c_2)}{\Sigma} \tag{A1}$$

where Σ is the sum of products of rate constants of all directional diagrams, Σ' is the sum of products of rate constants of all feed-in terms, and Π is the product of all rate constants of the cycle in either direction (because of detailed balance condition at equilibrium, the product of rate constants in one direction has to equal to that of the other direction, see Eqn 1) By definition, Π is a constant and both Σ and Σ' are functions of c_1 and c_2 In the presence of fluctuations in c_2, the expanded diagram contains c_2^+ and c_2^- (see Fig 3) If each carrier can bind with only one ligand, just one c_2^+ and one c_2^- will appear in the expanded diagram Then, if the c_2^+ term appears in the cycle part, it will not appear in the feed-in part (see Fig 3) Similarly, c_2^- will not appear in the feed-in part if it appears in the cycle part In other words, if the c_2 in Eqn A1 is c_2^+, Σ' can be either a constant or a function of c_2^- On the other hand, if c_2 in Eqn A1 is c_2^-, Σ' can be either a constant or a function of c_2^+ As a result, the fluxes for any two cycles that are mirror images of each other can be expressed generally as

$$J_a = \frac{\Pi}{\Sigma}(c_1 - c_2 + \Delta)(\Sigma_0 + \Sigma'_1 \Delta), \tag{A2}$$

$$J_b = \frac{\Pi}{\Sigma}(c_1 - c_2 - \Delta)(\Sigma'_0 - \Sigma_0 \Delta) \tag{A3}$$

When the feed-in terms do not contain c_2, Σ' is equal to zero (seen Eqns 8 and 9) The sum of these two fluxes at $c_1 = c_2$ is then equal to

$$J = J_a + J_b = \frac{\Pi \sum_1 \Delta}{\Sigma} \geq 0 \tag{A4}$$

Eqn A4 shows that the sum of fluxes of any two cycles that are mirror images of each other can never be less than zero As a result, the sum of all mirror-image pairs must be a positive quantity also, implying that ligand is always flowing from bath 1 to bath 2 even though c_1 is equal to c_2

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