Effects of oscillations and energy-driven fluctuations on the dynamics of enzyme catalysis and free-energy transduction

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It has been shown that many enzymes should be capable of utilizing free energy supplied by external time-dependent perturbations to drive the chemical or transport reactions they catalyze away from equilibrium. This property is analyzed in terms of thermodynamic and kinetic theory. An explicit demonstration using irreversible thermodynamics, through second order, is given for the case of a simple model enzyme in the presence of a periodic external perturbation. Three reasons for an enzyme to drive a reaction in a nonstationary environment may be identified: the average values of the forces, the root mean square of the external time-dependent perturbation, and the frequency-dependent correlation between the applied perturbation and the enzyme response. The extent to which the output reaction responds to any of these is governed by the kinetic constants of the enzyme. Even if the catalyzed reaction (e.g., the transport of an uncharged substance across a membrane) is in and of itself thermodynamically independent of the periodic perturbation (e.g., an ac electric field), the enzyme is competent to mediate free-energy exchange between the two. This originates from the fact that at high frequencies, the enzyme response lags behind the applied perturbation. It is sufficient that the enzyme interact with the applied field, and that the catalytic rate constants display the kinetic asymmetry typical of many, and particularly transport, enzymes. These results highlight the role of enzymes as free-energy converters in addition to that of biological catalysts.

INTRODUCTION

Random fluctuation and periodic oscillation of physical and chemical parameters are inherent to the environment of many proteins, particularly those embedded within the membranes of living cells.[1-5] We will consider, from a theoretical standpoint, how such fluctuations may influence the catalytic properties of enzymes.

Current theories used to model the effects of environmental nonstationarity on dynamic systems typically make a number of assumptions. Most of these are not necessarily reasonable for systems that are away from equilibrium, whereas biological processes of interest tend to be displaced from equilibrium by quite a few RT units,[6] where R is the gas constant and T is the Kelvin temperature. Fluctuations resulting from exergonic (dissipative) processes may be arbitrarily large and do not necessarily scale with the inverse of the square root of the volume of the system. Additionally, there is no guarantee that the correlation time of the fluctuation is much different than the relaxation time of the chemical system, and the response of the system may be intimately cross correlated with the fluctuating parameter.

One parameter particularly relavent for membrane enzymes is the membrane potential $\Delta\psi$, which is typically between 50 and 250 mV.[5,7] The electric field strength effective for processes occurring within the membrane is of the order 10^7 V/m. The effect of a *static* $\Delta\psi$ on enzyme kinetics and thermodynamics has been well elaborated.[8] However, despite many experimental observations of dramatic oscillations and fluctuations of $\Delta\psi$ ($\pm$ 40 mV) (Refs. 9 and 10), a similar, general theory for the effects of a dynamic field on enzyme catalysis has not been given.

It has been recently demonstrated theoretically that oscillating[5,11] or stochastic fields[12,13] can cause an enzyme to drive a reaction away from equilibrium. The main requirements were that some enzyme conformational transitions be influenced by the field and that the fluctuations in the field be driven by a free-energy-dissipating process. It has also been demonstrated that enzymes should be able to drive reactions against their *average* free-energy differences in response to concentration fluctuations in small systems.[14] As a result of these theoretical considerations, enzymes should be held capable of transducing free energy from external fluctuations in their environment. This finding is particularly timely since endergonic reactions have been observed in the apparent abscence of a sufficient stationary free-energy source in a number of biological systems.[5,15,16]

Other workers have focused on how oscillations may

influence dissipation and efficiency in free-energy transduction[17,18] and on how sensitivity and stability of signalling in biological systems may be improved by autonomous oscillation of a chemical messenger.[19,20] Horsthemke and Lefevre have treated the subject of noise-induced transitions in a wide variety of systems,[21] including those of biological interest.[22]

Enzyme catalysis is coupled to the environment by interactions between the catalytic transitions and thermodynamic parameters such as temperature, pressure, and local electric field strength. Much previous work has stressed only the influence of the environment on the free energy of the output (catalyzed) reaction. In the present paper, the effects of environmental parameters on the conformational transitions intrinsic to the protein are also emphasized. It is this possibility of such an interaction which is one important factor distinguishing enzymes from small-molecule catalysts. The treatment given applies, in principle, to any catalyst even in a homogeneous solution. However, the nonlinear terms giving rise to free-energy transduction would typically be negligibly small. It is the conformational flexibility of proteins and the positioning of certain enzymes within the bilayer where, e.g., local electric-field-strength fluctuations may be 10^7 V/m, that imply the possibly significant expression of this fundamental nonlinearity.

We present a detailed analysis of the interaction between a simple enzyme system and externally driven fluctuations. There are three major points we wish to emphasize. First, dynamically varying parameters such as concentrations of substrate, temperature, pressure, and electric field strength may influence enzyme catalysis in ways significantly different from what would be predicted solely on the basis of their time-averaged values. Second, external, or free-energy driven fluctuations, are qualitatively different from those which might arise in a system at equilibrium and do, in fact, "contain" free energy. The third theme is that many enzymes are capable of transducing this inflowing energy into a form usable in metabolism.

To develop these ideas, we first will describe a simple two-state enzyme. Then we will consider the effect of changing environmental parameters such as pressure, temperature, substrate concentrations, and electric field strength on the thermodynamic and kinetic properties of this enzyme. Using irreversible thermodynamic and kinetic theory, the influence of environmental fluctuations on the dynamics of a (pseudo-) first-order process are investigated. Finally, analytical results calculated for the catalytic flux, free-energy dissipation, and thermodynamic efficiency of free-energy transduction of our model enzyme in the presence of various types of environmental fluctuations are given and discussed.

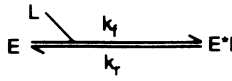
To clarify the major issue in which we are interested, let us imagine an elementary interconversion between two chemicals (we will designate this as the output reaction) which does not involve any net movement of electric charge, or change in dipole moment between the two reactants. Thus an external electric field is not coupled to this process, and the imposition of a field of arbitrary direction and magnitude will change neither the sign nor magnitude of the ΔG of reaction. If, however, an enzyme which catalyzes the interconversion between substrate and product has conformational transitions within its catalytic cycle which do involve intramolecular charge transfer or change in dipole moment, the enzyme can absorb energy from an alternating electric field. It has been shown computationally that under certain circumstances part of this energy may be converted to do work on the electrically silent output reaction by driving it away from equilibrium. In this paper we illustrate in the context of an analytically solvable model the fundamental thermodynamic and kinetic principles involved in this free-energy transduction.

The concepts presented may prove useful in understanding how a biological organism makes use of energy-driven fluctuations for the purpose of signal and free-energy transduction, and how "noise" may be a source of order rather than disorder.

MODELS AND CALCULATIONS

First-order reaction

In terms of mathematical description, the simplest possible reversible biochemical reaction is a (pseudo-) first-order transition involving a protein conformational change:



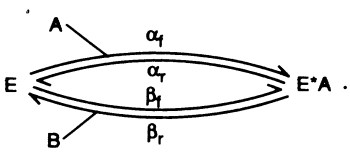
(1)

where the rate coefficients for the forward (k_f) and reverse (k_r) processes depend on the instantaneous, local, thermodynamic parameters such as p, T, and ϵ (electric field strength). We have included in the description the binding of a single molecule of ligand L in the transition. To allow for pseudo-first-order behavior the concentration of L is taken to be externally controlled, along with the other, thermodynamic parameters. The differential equation describing the evolution of the system in terms of the normalized concentration (state probability) of E, represented by $\tilde{E}$, is

$$\frac{d\tilde{E}}{dt} = -(k_f L + k_r)\tilde{E} + k_r \ . \tag{2}$$

We have taken advantage of the fact that $\tilde{E} + \tilde{E}^* \tilde{L} = 1$.

Such a protein conformational transition may be used to describe a simplified catalytic cycle of an enzyme (e.g., see Appendix D) summarized by



(3)

Here, both the top and bottom transition pathways, with rate constants denoted by α's and β's, respectively, may be taken as short-hand notation representing a series of elementary transitions, where the concentrations of the intermediates not explicitly shown are assumed to be

small and stationary. The overall cyclic pathway describes the catalytic interconversion between substrate A and product B (i.e., the "output reaction"). The kinetic equation for $\tilde{E}$ is

$$\frac{d\tilde{E}}{dt}=-(\alpha_f\tilde{A}+\alpha_r+\beta_f\tilde{B}+\beta_r)\tilde{E}+(\alpha_r+\beta_r)\ . \tag{4}$$

For simplicity, α_f and β_f represent pseudo-first-order rate coefficients into which reference *equilibrium* activities (concentrations) of A and B have been subsumed and $\tilde{A}$ and $\tilde{B}$ have been normalized to these reference values (i.e., $\alpha_f=k_{\alpha f}A_{\text{eq,ref}}$, where $k_{\alpha f}$ is the "true" second-order rate coefficient, and $\tilde{A}$ multiplied by $[A_{\text{eq,ref}}]$ is the actual instantaneous concentration of A). Detailed balance requires that $\alpha_f/\alpha_r=\beta_f/\beta_r$, but the reference equilibrium state may always be chosen such that $\alpha_f=1$, simplifying subsequent calculations.

Since $\tilde{A}$, $\tilde{B}$, and $\tilde{E}$ may be dependent variables, Eq. (4) is fundamentally a nonlinear differential equation, the analytical solution of which is quite complicated, although numerical integration can be carried out.[5,23] However, $\tilde{A}$ and $\tilde{B}$ are often either controlled externally, or changes in their activities due to the reaction shown in Eq. (3) are negligible: The number density of enzyme molecules is often much smaller than that of substrates and products.[24,25] In this case, $\tilde{A}$ and $\tilde{B}$ may be treated as parameters rather than dependent variables, reaction (3) becomes a pseudo-first-order process, and Eq. (4) is a linear differential equation in the single variable $\tilde{E}$. α_f, α_r, β_f, β_r, $\tilde{A}$, and $\tilde{B}$ may, because of external environmental fluctuations, depend on time.

The instantaneous rate of binding of $\tilde{A}$ and $\tilde{B}$ to the enzyme, J_α and J_β, respectively, may be written

$$\begin{aligned}J_\alpha&=(\alpha_f\tilde{A}+\alpha_r)\tilde{E}-\alpha_r\ ,\\ J_\beta&=(\beta_f\tilde{B}+\beta_r)\tilde{E}-\beta_r\end{aligned} \tag{5}$$

and by conservation of mass,

$$J_\alpha+J_\beta-\frac{d\tilde{E}}{dt}=0\ . \tag{6}$$

We define the flux $J_{AB}=(J_\alpha-J_\beta)/2$, which reduces to the usual definition for the net flux of $A\rightarrow B$ over any time period in which the average $\langle d\tilde{E}/dt\rangle=0$. We will show that parametric fluctuations may lead to the reaction $A\rightarrow B$ being driven away from its equilibrium, and we will perform a kinetic and thermodynamic analysis of this free-energy transduction.

We can also view this in terms of an external fluctuating or oscillating parameter causing a breakdown in detailed balance for the enzyme. The derivations could be done for the three-state triangle reaction discussed by Onsager,[26] except in this case, there would be two equations necessary to describe the time evolution of the chemical concentrations relevant for the reaction, and an analytic solution to the system of differential equations would be more complicated. At true equilibrium for the system of Eq. (3), the transitions from left to right along the top (α) path would be identically counterbalanced by transitions from right to left along the α path, and the same would be true for the bottom (β) path. If such a cyclic system is brought from equilibrium to a steady state by the input of a static energy source (e.g., for an enzyme this could be done by externally enforcing a constant nonequilibrium distribution of product and substrate), the system will begin to cycle through its states in either a clockwise or counterclockwise direction,[24] depending on the external force. This would imply the breakdown of detailed balance. In a three-or-more-state kinetic system, where each state may have a different property with respect to some physical property (such as fluorescence), this breakdown could be revealed by observing that fluctuations in that property would be asymmetric with respect to time reversal.[27–29] In Eq. (3), having A in excess of its equilibrium concentration will lead to clockwise, and excess B to counterclockwise, cycling. We will demonstrate that the application of external time-dependent perturbations, either random (noisy) or regularly oscillating will also impart a tendency for the system to undergo cyclic flux through its states and that the direction of this cycling is determined solely by the kinetic coefficients of the system. Furthermore, if two forces, static or dynamic, are acting on the system to cause cycling, one in the clockwise and the other in the counterclockwise direction, free energy can be transduced from the larger to the smaller force, irrespective of any *a priori* coupling between the two forces. An enzyme, through its cyclic catalytic process, can serve as the sole intermediary for this free-energy transduction.

We will now provide a thermodynamic analysis of the effect of an external perturbation on the free energies of reaction for each transition in Eq. (3). This will allow us to obtain a thermodynamically consistent, though not necessarily unique, set of rate coefficients which explicitly depend on the applied perturbation. Using these, we can evaluate the fluxes of Eq. (4) and thereby (5).

Equilibrium thermodynamics of the interaction between an enzyme and its environment

The equilibrium constant of a chemical reaction depends on the thermodynamic parameters of its environment such as p, T, and ϵ, and this is no different for the conversion of an enzyme from one state to another. Thus changes in such a parameter, designated in general as F, from a reference state will influence the values of the effective rate constants as well as the basic free-energy change of the transition. The magnitude of this effect will depend on the value of the conjugate parameter (volume V, entropy S, or molar polarization M, respectively), designated x, associated with the transition. As derived in Appendix A, for a finite perturbation in an $\tilde{F}$, $\tilde{A}$, or $\tilde{B}$, from the reference state (denoted by δF, $\delta\tilde{A}$, and $\delta\tilde{B}$, respectively) the instantaneous basic free energies of reaction may be written

$$\begin{aligned}\Delta G^0_\alpha&=\Delta G^0_{\alpha,0}+\delta\Delta G^0_\alpha\ ,\\ \Delta G^0_\beta&=\Delta G^0_{\beta,0}+\delta\Delta G^0_\beta\ ,\\ \delta\Delta G^0_\alpha&=(\Delta x_E+x_A)\delta F+\delta\ln(\tilde{A})\ ,\\ \delta\Delta G^0_\beta&=(\Delta x_E+x_B)\delta F+\delta\ln(\tilde{B})\ ,\end{aligned} \tag{7}$$

where the subscript α (β) refers to the top (bottom) transition in Eq. (3), and subscript 0 refers to the value evaluated at the reference state. With this formulation, we have formally "split up" the influence of external environmental parameters between changes of structure inherent in a transition from one enzyme state to another ($\Delta x_E \delta F$) from those due to the catalytic transformation of substrate to product ($x_A \delta F + \delta \ln A$) and ($x_B \delta F + \delta \ln B$). The free energy of the output reaction $A \rightarrow B$ may also depend on the environmental parameter F,

$$\begin{aligned} \Delta G_{AB} &= \Delta G_{AB,0} + \delta \Delta G_{AB} , \\ \delta \Delta G_{AB} &= \delta \mu_A - \delta \mu_B = (\Delta_{AB} x) \delta F + RT \delta \ln(A/B) , \end{aligned} \tag{8}$$

where ($\Delta_{AB} x$) refers to a molar volume, polarizability, or entropy difference between A and B, depending on which F is considered to vary. We may introduce the parameters

$$\begin{aligned} \phi_E &= \exp[\Delta x_E \delta F/(RT)] , \\ \phi_A &= \exp[x_A \delta F/(RT)] , \\ \phi_B &= \exp[x_B \delta F/(RT)] , \\ \phi_{AB} &= \exp[\Delta_{AB} x \delta F/(RT)] . \end{aligned} \tag{9}$$

The apportionment of the influence of the environment on the various rate coefficients is subject to thermodynamic constraint:

$$\phi_E \phi_A \alpha_{f0}/\alpha_{r0} = \phi_E \phi_B \beta_{f0}/\beta_{r0} . \tag{10}$$

Within this constraint, the details of the catalytic transitions give the appropriate distribution among the various transitions, as denoted below by apportionment factors l, m, n, and p. For the enzyme of Eq. (3), a general set of rate coefficients is given by

$$\begin{aligned} \alpha_f &= \alpha_{f0} \phi_E^m \phi_A^l , \\ \alpha_r &= \alpha_{r0} \phi_E^{m-1} \phi_A^{l-1} , \\ \beta_f &= \beta_{f0} \phi_E^n \phi_B^p , \\ \beta_r &= \beta_{r0} \phi_E^{n-1} \phi_B^{p-1} . \end{aligned} \tag{11}$$

Using the rate coefficients given in Eqs. (11), analytic solutions of the kinetic equations of the system of Eq. (3) for the case of a periodic square wave, or random stochastic dichotomous perturbations of F, $\tilde{A}$, or $\tilde{B}$ about their reference values, may be obtained as described in Appendixes B (square-wave perturbation) and C (random dichotomous perturbation). Here, let us look at what may be expected from an enzyme system exposed to environmental fluctuations from the point of view of nonequilibrium thermodynamics and compare this with a kinetic derivation for a simple specific case.

Nonequilibrium thermodynamics (NET) for the interaction between a nonstationary environment and an enzyme

In this subsection we shall discuss the driving forces that arise when a parameter is oscillating or fluctuating. These forces may cause fluxes which would not be expected on the basis of only the stationary (or time-average) forces.

The rate of entropy production within a system, $d_i S/dt$, multiplied by the temperature, is equal to the rate of free-energy dissipation and is known as the dissipation function. The dissipation function is typically written, in general, as[6,30]

$$T \frac{d_i S}{dt} = \Phi = \sum_k J_k X_k , \tag{12}$$

where the sum is over all conjugates of generalized flows and forces. Around equilibrium, any flow J_i may be related to the forces X (or vice versa) by a Taylor-series expansion

$$\begin{aligned} J_i = \sum_k \frac{\partial J_i}{\partial X_k} \delta X_k + \tfrac{1}{2} \sum_k \frac{\partial^2 J_i}{\partial X_k^2} \delta X_k^2 \\ + \tfrac{1}{2} \sum_k \sum_l \frac{\partial^2 J_i}{\partial X_k \partial X_l} \delta X_k \delta X_l + O(3) , \end{aligned} \tag{13}$$

where $O(3)$ represents third- and higher-order terms. In principle, depending on the operational definition of the flows and forces, the flows may in addition depend on factors other than the forces. For example, if both $\tilde{A}$ and $\tilde{B}$ of the enzyme reaction Eq. (3) are doubled, the magnitude (but not the sign) of the catalytic flux perhaps would change, even though the ΔG_{AB} of the output reaction would be the same. In the examples we will discuss, all effects on the fluxes will be contained within the forces as we define them.

The first term on the right-hand side of Eq. (13) represents the first-order contributions to the flux J_i and sufficiently close to the reference state all higher-order terms vanish since all $\delta X \rightarrow 0$. In this case, we may write

$$J_i = J_{i0} + \sum_k L_{ik} \delta X_k , \tag{14}$$

where $L_{ik} = \partial J_i/\partial X_k$. This familiar equation, in which each flux is expressed as a linear combination of all the forces, forms the basis for the theory of *linear* irreversible thermodynamics,[31] and when the reference state is equilibrium, for a simple formulation of the Onsager reciprocity relations.[26,32]

One question is, how close is sufficiently close? A purely thermodynamic treatment is unable to provide a definitive answer because few constraints can be placed on thermodynamic grounds on the magnitudes of $\partial^2 J_i/\partial X_k^2$, $\partial^2 J_i/(\partial X_k \partial X_l)$, etc. It is typical to attempt to establish a domain in which the linear equation (14) is sufficiently accurate by experimentally obtaining a flow-force curve plotting J_i versus an X_k while keeping the other external forces constant at their reference values. These experiments are usually performed under steady-state conditions such that the enzyme-state probabilities may be considered constant during the period in which a J_i is measured at a particular value of X_k. In any range of X_k where a linear relationship is found to hold, the terms $(\partial^n J_i/\partial X_k^n)\delta X_k^n$ are shown to be negligible. On the basis of enzyme kinetics, various authors[6,33,34] have prov-

en theoretically that for many enzyme systems this range is quite large. Of course, even in this linear range for X_k, under conditions where another force, X_l, may not be constant (or during any time before the enzyme-state probabilities have attained their steady-state values), significant contributions from the cross-force terms in Eq. (13) may still be expected.

Indeed, when an external force is applied to a system so as to displace it from a stable steady state, the system will respond to counteract the applied force and to return to a (new) stable state according to a principle reminiscent of the Le Chatlier–van t'Hoff principle. The distance of the system from the unperturbed steady state takes on the role of a restoring force, which is correlated with the applied (time-dependent) perturbation. Particularly in situations where the external force is dynamically fluctuating, this may give rise to non-negligible terms of the form $[\partial^2 J_i/(\partial X_k \partial X_l)]\delta X_k \delta X_l$, where X_k is the perturbing, and X_l the restoring, force.

To better illustrate these concepts for the case of an enzyme-catalyzed conversion of A to B [Eq. (3)], we write the dissipation function

$$\begin{aligned}\Phi &= J_\alpha \Delta G_\alpha + J_\beta \Delta G_\beta \\ &= J_\alpha(\Delta G_{\alpha 0} + \delta \Delta G_\alpha) + J_\beta(\Delta G_{\beta 0} + \delta \Delta G_\beta) \ . \end{aligned} \tag{15}$$

$\Delta G_{\alpha 0}$ and $\Delta G_{\beta 0}$ are the gross (concentration-dependent) free-energy differences between the two states under some reference conditions for the α and β paths, respectively, and $\delta \Delta G_\alpha$ and $\delta \Delta G_\beta$ represent contributions of perturbations from these conditions to the free-energy differences. If we consider the deviations to arise because of external perturbation through F, or from direct externally enforced concentration fluctuation, $\delta \ln \tilde{A}$ or $\delta \ln \tilde{B}$, we may write

$$\begin{aligned}\delta \Delta G_\alpha &= (\Delta x_E + x_A)\delta F + RT \delta \ln[\tilde{E}/(1-\tilde{E})] + \delta \ln(\tilde{A}) \ , \\ \delta \Delta G_\beta &= (\Delta x_E + x_B)\delta F + RT \delta \ln[\tilde{E}/(1-\tilde{E})] + \delta \ln(\tilde{B}) \ . \end{aligned} \tag{16}$$

In order to highlight the influence of dynamic perturbation on the catalytic flux, we define the following composite fluxes:

$$J_1 = (J_\alpha - J_\beta)/2, \quad J_2 = (J_\alpha + J_\beta)/2 \ , \tag{17}$$

and forces

$$\begin{aligned} X_1 &= (\mu_{A0} - \mu_{B0}) \ , \\ \delta X_1 &= (x_A - x_B)\delta F + \delta \ln(\tilde{A}/\tilde{B}) \ , \\ X_2 &= (\mu_{A0} + \mu_{B0} + 2\mu^0_E - 2\mu^0_{E^*A}) \ , \\ \delta X_2 &= (x_A + x_B + 2\Delta x_E)\delta F + \delta \ln(\tilde{A}\tilde{B}) \ , \\ X_3 &= 2RT \ln[\tilde{E}_0/(1-\tilde{E}_0)] \ , \\ \delta X_3 &= 2RT\{\ln[(\tilde{E}_0 + \delta\tilde{E})/(1-\tilde{E}_0 - \delta\tilde{E})] \\ &\qquad - \ln[\tilde{E}_0/(1-\tilde{E}_0)]\} \ . \end{aligned} \tag{18}$$

J_1 is the flux of the enzyme around its catalytic cycle, and at steady state in the absence of time-dependent perturbations, becomes the net catalytic flux (i.e., J_{AB}). J_2 is one-half the net rate of conversion of enzyme from $E \rightarrow E^* A$. Of the forces, X_1 represents the cyclic driving force in the context of steady-state (nonfluctuating) enzyme kinetics, X_2 the force driving the enzyme to the left- or right-hand state, and X_3 is the internal "restoring" force, counteracting the applied perturbations and driving the return of the system to its original condition if the external perturbation would be removed. $\tilde{E}_0$ refers to the enzyme-state probability when $\delta X_1 = \delta X_2 = \delta X_3 = 0$.

The dissipation function (15) may be transformed to

$$\Phi = J_1(X_1 + \delta X_1) + J_2[(X_2 + X_3) + (\delta X_2 + \delta X_3)] \ . \tag{19}$$

Often, $X_2 + X_3$ is treated as a single force. However, this treatment is valid only near equilibrium. Even then, if the perturbation is dynamic, the phase relationship between δX_2 (or δX_1) and δX_3 may depend on frequency. In the following we shall concern ourselves with terms only through second order, dropping $O(3)$.

We are interested in how a periodically fluctuating perturbation influences the enzymic catalysis of the reaction $A = B$. External perturbations can be applied such that $\delta F(t) = \langle \delta F \rangle + \delta_F$, with $\langle \delta_F \rangle = 0$. The time-dependent external forces are

$$\delta X_1 = \langle \delta X_1 \rangle + \delta_1, \quad \delta X_2 = \langle \delta X_2 \rangle + \delta_2 \ . \tag{20}$$

The resulting internal force will, after a sufficient number of periods, reach a stationary oscillation such that $\langle J_2 \rangle = 0$, and

$$\delta X_3 = \langle \delta X_3 \rangle + \delta_3 \tag{21}$$

with $\langle \delta_3 \rangle = \langle \delta_2 \rangle = \langle \delta_1 \rangle = 0$. The angular brackets denote the operation of averaging over time, and in terms of single cyclic integrals can be written $\langle X \rangle = \oint X\,dt / (\oint dt) = f \oint X\,dt$, where f is the period of oscillation. Subsequently, we will denote $\oint X\,dt = \oint X$, where the variable under which integration is carried out is implicitly time, and the integration is carried out over a single period of the oscillation. The time-averaged catalytic flux may be obtained

$$\langle J_i \rangle = \sum_j L_{ij} \langle \delta X_j \rangle + \sum_j \sum_k L_{ijk} \left[\langle \delta X_j \rangle \langle \delta X_k \rangle + f \oint \delta_j \delta_k \right] \ , \tag{22}$$

where $i = 1,2$, $j,k = 1,2,3$. For symmetric fluctuations such that $\langle \delta X_1 \rangle = \langle \delta X_2 \rangle = 0$, the stationarity condition $\langle J_2 \rangle = 0$ results in a quadratic equation that expresses $\langle \delta X_3 \rangle$ into second-order terms of the applied forces. Consequently, for sufficiently small symmetric perturbations, $\langle \delta X_3 \rangle$ can be taken as approximately zero. That is, in a first-order approximation, zero average fluctuations do not cause the average $\langle \tilde{E} \rangle$ to deviate from its nonfluctuating steady-state value.

At larger perturbation amplitudes the second-order terms in Eq. (22) become relavent. Then, we can identify several types of terms contributing to the net catalytic flux at larger perturbation amplitudes. First, there are linear ($\langle \delta X_1 \rangle$, $\langle \delta X_2 \rangle$) and nonlinear ($\langle \delta X_1 \rangle^2$, $\langle \delta X_2 \rangle^2$, $\langle \delta X_1 \rangle \langle \delta X_2 \rangle$) direct contributions from the average

values of the external forces. These are the classical driving forces which would also pertain for static perturbations from the reference state. All of these are identically zero for symmetric perturbation. Specific for the case of dynamic perturbation, there are three types of driving forces. First, there are rectification terms (involving $f\oint\delta_1^2dt$, $f\oint\delta_2^2dt$, $f\oint\delta_1\delta_2dt$). These reflect the nonlinear flow-force relationship and indicate that net flux can be induced even if the average driving force is zero. All of these terms are frequency independent (since integration of a cosine or sine yields a $1/f$ factor to cancel with the premultiplier) and are operative for ensembles of systems with a distribution of constant δ_1 and δ_2 at steady state as well as for a single system subject to dynamic perturbation of arbitrary period.

There are also frequency-dependent terms. These include the cross correlation between the system response and the imposed perturbation ($f\oint\delta_1\delta_3dt$ and $f\oint\delta_2\delta_3dt$), and the "rectification" term for internal response ($f\oint\delta_3^2dt$). The former are frequency dependent because the "phase lag" between δ_1 and $-\delta_3$ goes from 0° in the low-frequency limit to 90° in the high-frequency limit, and the latter is frequency dependent because the amplitude of δ_3 approaches zero in the high-frequency limit. Since we have assumed the δ's to be Fourier analyzable, they may be expressed as sums of sines and cosines, with a frequency-dependent phase lag between the perturbing (δ_1 and δ_2) and responding (δ_3) function. Finally, there are those terms containing the average value of the induced internal restoring force, $\langle\delta X_3\rangle$ and $\langle\delta X_3\rangle^2$, which are frequency dependent through their dependence on all the other frequency-dependent terms.

Let us look now at a few special cases which will also be investigated analytically using the kinetic equations derived in the Appendices. If an external perturbation interacts with the enzyme but does not influence the ΔG_{AB} of the output reaction, δ_1 and $\langle\delta X_1\rangle=0$. This is equivalent to taking $\phi_E\neq1$ and $\phi_{AB}=1$. Furthermore, if the perturbation is symmetric, $\langle\delta X_2\rangle=0$. If $\tilde{A}=\tilde{B}=1$,

$$\langle J_1\rangle=L_{12}\langle\delta X_3\rangle+L_{133}\langle\delta X_3\rangle^2+f\left[L_{122}\oint\delta_2(\delta_2+\delta_3)+L_{133}\oint\delta_3(\delta_2+\delta_3)\right]. \tag{23}$$

From the stationarity condition (i.e., $\langle J_2\rangle=0$) we can obtain the stable solution for $\langle\delta X_3\rangle$ to be

$$2L_{233}\langle\delta X_3\rangle=-L_{22}+\left[L_{22}^2-4fL_{233}\left[L_{222}\oint\delta_2(\delta_2+\delta_3)+L_{223}\oint\delta_3(\delta_2+\delta_3)\right]\right]. \tag{24}$$

In the low-frequency limit, $\delta_3=-\delta_2$, so $\oint\delta_2^2=\oint\delta_3^2=-\oint\delta_3\delta_2$. By use of the relationship between the L's, we then find that both $\langle J_1\rangle$ and $\langle\delta X_3\rangle$ are zero. Similarly, imposition of a static δX_2, which does not influence the free energy of the output reaction, does not cause flux when $\mu_A=\mu_B$. In the high-frequency limit, the phase difference between the forcing and response functions approaches 90°; hence $f\oint\delta_2\delta_3dt$ goes to zero and neither $\langle\delta X_3\rangle$ nor $\langle J_1\rangle$ are, in general, zero. This is an interesting result since at no time is there a nonzero ΔG_{AB} ($=X_1$) of the output reaction. The sign of the induced flux is governed by the L constants and is independent of the amplitude and frequency of the external force. Thus, we can surmise that even if we applied a small constant $\langle\delta X_1\rangle$ (e.g., by setting $\delta\ln A<\delta\ln B$) that a situation could arise with time-dependent δX_2 where there would be net flux from $A\rightarrow B$ even if $\mu_B>\mu_A$. This would represent free-energy transduction from a force not coupled to the ΔG_{AB} to do work on the output reaction. Such a coupling would be mediated solely by the interaction between the enzyme and its environment. In the results section, quantitative calculations for a specific kinetic model will be given.

If $\delta_2=\langle\delta X_2\rangle=0$ and $\delta_1\neq0$ but with $\langle\delta X_1\rangle=0$ (i.e., $\phi_{AB}\neq1$, $\phi_E=1$), as would be the case if $\delta\ln\tilde{A}$ and $\delta\ln\tilde{B}$ were caused to oscillate 180° out of phase with one another, thereby keeping $\ln(\tilde{A}\,\tilde{B})$ constant and zero but causing $\ln(\tilde{A}/\tilde{B})$ to oscillate symmetrically if $\tilde{A}=\tilde{B}=1$, the flux is

$$\langle J_1\rangle=L_{12}\langle\delta X_3\rangle+L_{111}f\oint\delta_1^2+L_{123}\left[\langle\delta X_3\rangle^2+f\oint\delta_3^2\right]+L_{113}f\oint\delta_1\delta_3. \tag{25}$$

As before, $\langle\delta X_3\rangle$ may be obtained by solving the quadratic equation derived from Eq. (24) under stationary oscillation. No general statements concerning the low- and high-frequency limits may be made here except that neither need be zero.

The L coefficients, which govern the extent to which any of the driving forces, including those which arise from dynamic perturbation, lead to net flux, are parameters depending on the kinetic mechanism of the enzyme and on its catalytic constants. In our discussions, we have implicitly assumed that they need not take on trivial values such as zero. This may be directly shown by evaluating these coefficients, and by doing so we could also explicitly calculate the fluxes under a variety of conditions. However, it is usually much simpler to calculate the fluxes directly from a kinetic formulation. Thus, an explicit kinetic treatment seems in order.

Kinetic picture

We consider the model enzyme of Eq. (3). In order to allow for clear illumination of the underlying physical principles, we perform the derivations in this section for only one set of kinetic parameters, using $\tilde{A}=1$,

$\alpha_{f0}=\alpha_{r0}=\beta_{f0}=\beta_{r0}=1$, $\phi_A=1$, $m=1$, $n=p=0$ [see Eq. (11)]. The average catalytic flux $\langle J_{AB}\rangle=\langle J_\alpha-J_\beta\rangle/2$ is a measure of the effect of the fluctuating parameter F on catalysis, and $\langle J_{AB}\rangle>0$ when $\langle\mu_B\rangle>\langle\mu_A\rangle$ will be indicative of free-energy transduction. We shall strive to emphasize the effect of first- and second-order terms as discussed in the preceding subsection.

The instantaneous state probability $\tilde{E}$ can be written in terms of its reference steady-state value and some distance Δ from that value,

$$\tilde{E}=\tilde{E}_0+\Delta,\quad \tilde{E}_0=2/(3+\tilde{B})\ . \tag{26}$$

Substituting $x'=[\Delta x_E\delta F/(RT)]$ and $y'=[x_B\delta F/(RT)]$, and dropping third- and higher-order terms (including, e.g., $x'^2\Delta$), we may derive

$$\frac{d\tilde{E}}{dt}=-(1+x'+x'^2/2+\tilde{B})\tilde{E}_0 +(2-y'+y'^2/2-x'+x'y'+x'^2/2)(1-\tilde{E}_0) +(3+\tilde{B}-y')\Delta\ ,$$

$$J_\alpha=(1+x'+x'^2/2)\tilde{E}_0-(1-\tilde{E}_0)+(2+x')\Delta\ , \tag{27}$$

$$J_\beta=\tilde{B}\,\tilde{E}_0-(1-y'+y'^2/2-x'+x'y'+x'^2/2)(1-\tilde{E}_0) +(\tilde{B}+1-y'-x')\Delta\ .$$

Under stationary oscillation conditions,

$$\langle J_{AB}\rangle=f\left[\oint J_\alpha-\oint J_\beta\right]\Big/2\ ,$$

and

$$\langle J_{AB}\rangle=(1-\tilde{B})\tilde{E}_0/2+f\tilde{E}_0\left[\oint x'^2\right]\Big/2+f(1-\tilde{E}_0)\left[\oint y'^2+2\oint x'y'+\oint x'^2\right]\Big/4+f\oint\Delta(x'+y'/2)\ . \tag{28}$$

This shows that net flux from A to B, even with $\tilde{B}=1$ (or slightly greater than 1) may result due to second-order terms $(x')^2$, and $(y')^2$ and cross terms $x'\Delta$ and $y'\Delta$.

The time-averaged entropy production may be written using Eq. (15),

$$\langle\Phi\rangle/(RT)=f\oint J_\beta y'+f\oint J_\beta\ln(\tilde{B}) +\oint(J_\alpha+J_\beta)x'+\Delta G_{AB}\langle J_{AB}\rangle\ . \tag{29}$$

From the second law of thermodynamics, $\langle\Phi\rangle\geq 0$. However, one or more of the individual terms of the right-hand side of Eq. (29) may be negative. In particular, if $\oint\delta F=0$, $\Delta G_{AB}\langle J_{AB}\rangle<0$ would be indicative of the reaction A,B being driven in a direction opposite to what would be predicted on the basis of the average difference in the electrochemical potentials of A and B, i.e., free-energy transduction from the fluctuating force to do "work" on the output reaction.

Interestingly, with the special set of parameters we have chosen, and in the case that $y'=0$, by redefining Δ in terms of the distance from the steady state (SS) to which the system would relax if the instantaneous value of F were maintained for a long time, i.e.,

$$\tilde{E}=\tilde{E}_{SS}+\Delta'=(1+\phi_E)/(2+\phi_E+\phi_E^{-1})+\Delta'\ . \tag{30}$$

$d\tilde{E}/dt$ can be written as a strictly linear equation[35] (i.e., with neither square nor cross-terms) through second order,

$$d\tilde{E}/dt=(3+\tilde{B})\Delta'\ . \tag{31}$$

Thus the response of $\tilde{E}$ to a periodic perturbation in this case is appropriately modeled by a linear-response theory so long as the perturbation strength, $x'<1$. Nevertheless, J_α and J_β are not given by linear equations, even to this second-order level of approximation, nor is the cyclic integral $\oint(J_\alpha-J_\beta)$ necessarily equal to zero (not even if $\tilde{B}=1$). The effect of a dynamic perturbation on the enzyme catalytic flux is not interpretable by a linear response theory except at much smaller perturbation strengths, and even then, the enzyme can act as an integrator of an external fluctuating signal. This shows that a linear dielectric response of an enzyme is not necessarily inconsistent with its ability to transduce free energy from a periodic electric field. The above, however, is a special case, and in general we must expect $d\tilde{E}/dt$ to be given by a nonlinear equation also.[36]

Efficiency of free-energy transduction

Of fundamental importance in our considerations are the free-energy dissipation in, and efficiency of free-energy conversion, which have been touched on briefly in Eq. (29). First, there must be the notion of input (free-energy-supplying) and output (energy-absorbing) reactions. There is a certain arbitrariness in these definitions, which rely on the observer's interpretation as to the function of the system. Any two reactions can be written down such that the one with the larger affinity may appear to drive the one with the smaller affinity away from its equilibrium. However, as Koenig *et al.*[37] have pointed out, a simple linear transform (or as these authors put it, a stroke of the pen) allows a set of reactions to be written down such that each reaction appears to proceed in its thermodynamically spontaneous direction independent of the other. The choice of what belongs to the input and what belongs to the output comes from the observer's concept of the function of the system, or from the mechanism by which the free-energy transduction occurs.

Even when an overall cyclic enzyme mechanism is written such that the net result of turnover is that one reaction goes up its chemical-potential gradient at the expense of another, of larger thermodynamic affinity, going down its gradient, the situation is not unambiguous. If the entropy production is written in terms of a sum of flows through each elementary enzyme transition J_{ij} in the mechanism, each multiplied by the corresponding ΔG_{ij} for that transition,

$$T\left[\frac{dS}{dt}\right]=\sum_{i,j}J_{ij}\Delta G_{ij}\ , \tag{32}$$

each term on the RHS of Eq. (32) is individually positive.[38] Thus, there can be no question of one thermodynamically downhill elementary transition driving some other elementary transition uphill. The role of an enzyme in free-energy coupling may be viewed as providing a sequence of elementary steps (*mechanism*) which reconcile the fact that net flux through each possible transition always occurs in the thermodynamically spontaneous direction with the desired (and observed) overall result that a net reaction occurring in the thermodynamically downhill direction can "drive" another net reaction uphill.

Typically, a reasonable definition for input versus output processes can be arrived at by splitting the various contributions of the environment and the chemical potentials within the ΔG's and grouping them together. For the system of Eq. (3), with the parameter values used previously, the entropy production in terms of elementary steps is given by Eq. (15). The ΔG's can be written as

$$\Delta G_{\alpha}=\Delta G^{0}_{\alpha 0}+\Delta x_E\delta F+RT\ln[\tilde{E}/(1-\tilde{E})]\ , \tag{33}$$

$$\Delta G_{\beta}=\Delta G^{0}_{\beta 0}+(\Delta x_E+x_B)\delta F+RT[\ln(\tilde{B}+\delta\tilde{B})-\ln\tilde{B}]+RT\ln\tilde{B}+RT\ln[\tilde{E}/(1-\tilde{E})]\ .$$

The dissipation per cycle once steady-state oscillation has been reached may be written

$$\oint\Phi=\left[\Delta x_E\oint(J_{\alpha}+J_{\beta})\delta F+x_B\oint J_{\beta}\delta F+RT\oint\ln(1+\delta\tilde{B}/\tilde{B})J_{\beta}\right]-\left[[RT\ln(\tilde{B})+x_BF_0]\oint J_{\beta}\right]\ , \tag{34}$$

where $-[RT\ln(\tilde{B})+x_BF_0]=\Delta G_{AB,0}$. We may define the first term on the RHS in large parentheses as the input, since it contains all contributions from fluctuations about the average values, and the second term to be the output, as this characterizes flow of A to B times the force according to the average values of B and F. Other interpretations may also be possible.

Using these definitions, an efficiency may be defined:

$$\eta=\frac{\left[[RT\ln(\tilde{B})+x_BF_0]\oint J_{\beta}\right]}{\left[\Delta x_E\oint(J_{\alpha}+J_{\beta})\delta F+x_B\oint J_{\beta}\delta F+RT\oint\ln(1+\delta\tilde{B}/\tilde{B})\right]J_{\beta}}\ . \tag{35}$$

Quantitative analysis of the effects of periodic perturbations on enzyme catalysis

$\tilde{E}(t)$, and consequently $J_{\alpha}(t)$ and $J_{\beta}(t)$, can in *general* be obtained directly from Eq. (4), since the formal solution to this linear first-order differential equation is

$$\tilde{E}(t)=\tilde{E}(0)\exp\left[-\int P\,dt\right]+\exp\left[-\int P\,dt\right]\int Q\exp\left[\int P\,dt\right]dt\ , \tag{36}$$

where $P=(\alpha_f\tilde{A}+\alpha_r+\beta_f\tilde{B}+\beta_r)$ and $Q=(\alpha_r+\beta_r)$. For any defined functional form for $P(t)$ and $Q(t)$, this can be solved by direct numerical integration. Eigen has provided an analytic solution to this equation for the analysis of data obtained by stationary relaxation techniques,[39] in which P (i.e., the inverse relaxation time τ^{-1}) was assumed to be constant, and Q to be a harmonically oscillating forcing function. This is the same point of view as adopted in linear dielectric theory.[35] In this case it could be shown that the dynamic variable follows the forcing function with the identical frequency, but with an attenuated amplitude and phase lag depending on the ratio between the forcing frequency and the relaxation time of the chemical system. In the situation of interest in this paper, neither can P be taken as constant, nor Q be assumed to be a purely harmonic forcing function even if the perturbation may be written $F(t)=F_0+\delta F\sin(2\pi ft)$. This significantly complicates the mathematics, but numerical integration of Eq. (36) can still typically be effected. Even if the underlying reason for nonstationarity of P and Q would be a random stochastic (but externally defined and periodic) noise in the environment, $F(t)$ can be expressed as a Fourier series, which would allow computational solution of Eq. (36) to arbitrary accuracy. An analytic solution, even for a special case, is nevertheless greatly desirable, since it will facilitate understanding of the importance of the various parameters with respect to the qualitative behavior of the system. Thus, we will focus on two cases of environmental nonstationarity, one for regular periodic oscillation, and the other for stochastic fluctuation for which an analytic formulation for $\tilde{E}(t)$, as well as for fluxes and free-energy dissipation, may be obtained. In both cases, the key will be that only two possible fluctuation levels, denoted + and −, will be considered. Derivations of the relevant equations for these two cases are presented in Appendixes B and C.

RESULTS

In this section we report on the behavior of our model enzyme (3) under the influence of square-wave periodic and dichotomous stochastic fluctuations. The calculations were done using the equations derived in the Appendixes. Unless otherwise specified, the parameter values are the same as given in the preceding section on the kinetic picture, where here, the numerical value of ϕ_E (or ϕ_B depending on the circumstance) is equal to 256,

which corresponds to a value for $\Delta x \delta F/(RT)=5.5$.

Concentration fluctuations—$\tilde{B}(t)=\bar{B}\pm\delta\tilde{B}(t)$. Symmetric (e.g., Gaussian) fluctuations of concentration do not imply symmetric chemical potential fluctuations, and in fact $\langle \mu_B(\tilde{B})\rangle < \mu_B(\langle \tilde{B}\rangle)$. Thus, it may not be too surprising that, as seen in Fig. 1, symmetric fluctuation of $\tilde{B}$ such that $\tilde{B}(t)=\bar{B}\pm 0.5$ could lead to net flux converting A to B even when $\bar{B}=1.1$ (with $\bar{A}=1$). This does not require any nonlinearity (asymmetry) in the flow-force relationship, and arises simply because the *average* chemical-potential difference

$$\langle \mu_A(\tilde{A})-\mu_B(\tilde{B})\rangle = -[\ln(1.05+0.5)+\ln(1.05-0.5)]/2 \simeq 0.16$$

is positive even though

$$\mu_A(\langle \tilde{A}\rangle)-\mu_B(\langle \tilde{B}\rangle) = -\ln(1.05) \simeq -0.05$$

is negative. Thus, the *symmetric* fluctuation of one reactant always leads to a bias of the flux (that may or may not be counteracted by other forces), which disappears at high frequencies, in the direction from the constant to the fluctuating component. The "efficiency" of free-energy transduction due to this effect, as calculated according to Eq. (35), is very low, and we shall not further consider this effect here. We note also that if the fluctuations would be such that around an average $\langle \tilde{B}\rangle=1$, the average chemical potential of B would be equal to the chemical potential of the average concentration of B (as we might expect for equilibrium fluctuations), the average chemical-potential difference when $\bar{A}=1$, $\langle \mu_A-\mu_B\rangle$, would be zero despite the fluctuation. The nonstationarity of the chemical-potential difference (i.e., force on the output reaction) would then be symmetric, and entirely cognate with the environmental fluctuations when $\Delta x_E=0$, $\Delta x_{AB}\neq 0$, as discussed below.

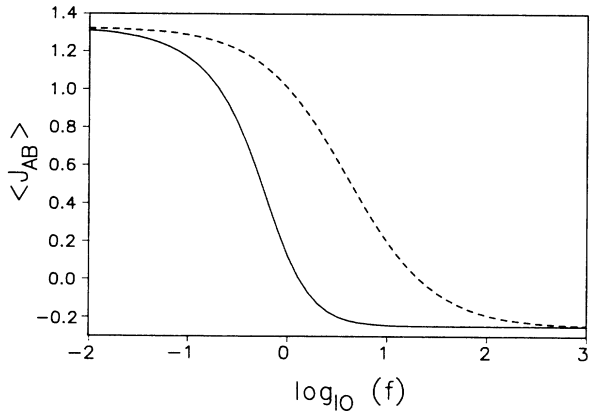


FIG. 1. Demonstration that symmetric concentration fluctuations can induce flux in a direction opposite to that predicted based on the average concentration. The average value of the parameter $\tilde{B}$ was 1.1, with fluctuations [random stochastic dichotomous (— — —) or regular periodic square wave (——)] superimposed on this value, where $\delta\tilde{B}=0.5$ (i.e., $\tilde{B}_+=1.6$, $\tilde{B}_-=0.6$) with $\delta_F=0$. All of the parameters in this and all other figures are as given in the section on the kinetic picture except where stated otherwise. The flux is calculated by use of Eqs. (B10), (C2)–(C8), and (C11). At low frequency, it is seen that flux is induced from A to B despite the fact that $\langle \tilde{B}\rangle > \langle \tilde{A}\rangle$.

Environmental fluctuations $F(t)=F_0\pm\delta F$ *with* $\phi_E=1$ *and* $\phi_B\neq 1$. Here we consider that an environmental fluctuation, such as in p, T, ϵ, μ_A, or μ_B, influences directly the output reaction through the term $x_B\delta F$, but not the intrinsic relative free energies of the enzyme conformational states ($\Delta x_E=0$). A steady-state (nonfluctuating) flow-force relationship for the model enzyme is shown in Fig. 2 ($\bar{B}=1$). This plot is quite asymmetric even in the range $-RT<x_B\delta F<RT$ and thus an ensemble average with one-half of the enzyme systems operating at steady state where $x_B\delta F=-RT$ and one-half where $x_B\delta F=+RT$ will show net flux even though the ensemble-average force $\langle x_B\delta F\rangle=0$. Accordingly, calculations based on Eqs. (14B) and (13C) for the low-frequency (LF) limit show nonzero net flux even when $\langle F\rangle=0$ and $A=B$. This behavior is analogous to that of a half-wave rectifier in electronics and requires asymmetry of the flow-force relation within the range of the fluctuation. In our NET analysis, we call this the rectification component. If we set the apportionment factor $p=\frac{1}{2}$ (keeping $\bar{B}=\bar{A}=1$), an antisymmetric flow-force relation about $\delta F=0$ is obtained over the entire range of forces, and the low-frequency (LF) limit net flux is abolished.

The bias to the flux due to the nonantisymmetric components of the flow-force relation [originating from the $f\oint\delta_1^2$ in Eq. (25)] is frequency *independent*. As the frequency increases, cross terms between the applied time-dependent force, which work opposite to terms $f\oint\delta_1^2$ and $f\oint\delta_3^2$, decrease, and the induced flux increases. This contribution to the fluctuation-induced bias of the output flux is strongly frequency dependent, being maximal as $f\to\infty$ as shown in Fig. 3. The net flux may be viewed as the sum of contributions arising from the steady-state chemical-potential difference $\langle \Delta\mu_A-\Delta\mu_B\rangle$, the frequency-independent rectification, and the frequency-dependent cross correlation between the perturbation and the restoring force. We note that setting $p=1$ (instead of 0) leads to a counterclockwise rather than clockwise bias

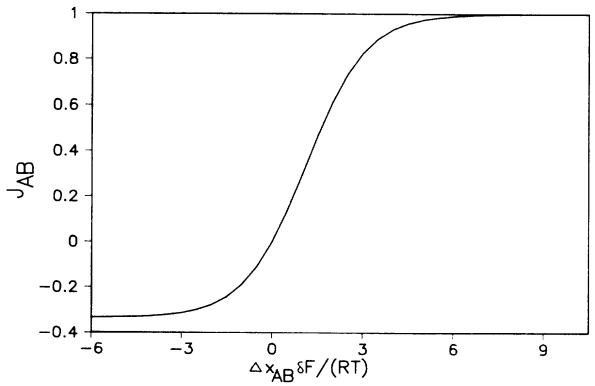


FIG. 2. Steady-state flux of the model enzyme when $\bar{A}=\bar{B}=1$ as a function of the force ΔG_{AB} (as modulated by $\Delta x_{AB}\delta F$) with $\Delta x_E=0$. Notice that this flow-force relation is quite asymmetric, even in the region $\pm 1.5RT$.

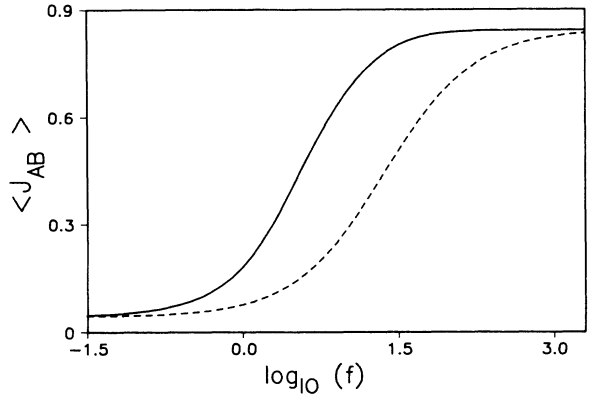


FIG. 3. Flux induced by symmetric fluctuation of the force (through δF_k) on the reaction, such that the average $\langle F \rangle$ was zero [(— — —) is for random, and (——) for regular periodic perturbation]. The flux is seen to be positive ($A \rightarrow B$) even though $\tilde{B} = 10$ and $\tilde{A} = 1$. At low frequency, this is solely due to a frequency-independent rectification effect (i.e., to the fundamental asymmetry of the flow-force relation). At high frequency an additional effect arises due to the cross correlation between the fluctuating δF and the nonstationary affinity of the enzyme transitions.

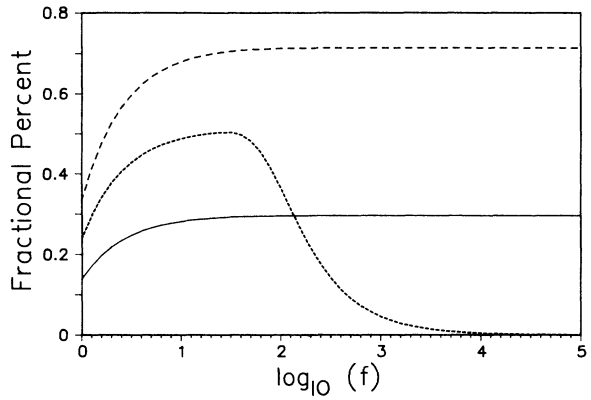


FIG. 5. Effects of dynamic perturbation of δF when $\Delta x_{AB} = 0$, but $\Delta x_E \neq 0$ with $\tilde{B} = 100$. The yield (- - -) (net $A \rightarrow B$ transitions per field cycle); flux ratio (— — —) (net $A \rightarrow B$ transitions per $E \rightarrow E^* A \rightarrow E$ cycle; this is essentially the fraction of fluctuation-induced enzyme transitions which actually go into doing work on the output reaction); and efficiency (——) (power out/power in, as explained in the text).

from both LF and HF contributions, and $p = \frac{1}{2}$ abolishes both.

Environmental fluctuations $F(t) = F_0 \pm \delta F$ *with* $x_B = 0$ *and* $\Delta x_E \neq 0$. Here, the fluctuating parameter interacts only with the intrinsic protein conformational equilibrium and not with ΔG_{AB}. Thus, F does not influence the "steady-state" driving force for the output reaction. Nevertheless, variation of the stationary value of F can significantly regulate the catalytic efficiency of the enzyme, even operating under steady-state conditions, as seen in Fig. 4. When F is dynamically varied, a bias of the output flux is induced which for our chosen set of parameters is in the clockwise direction. The frequency dependence of both stochastic and regular periodic perturbations (not shown) is similar to that for the previous case where $x_B \neq 0$, but here, when $\tilde{B} = 1$, the LF limiting flux is identically zero, and otherwise is of the same sign as that for the enzyme operating at steady state with constant $\delta F = 0$.

The high-frequency flux when $\tilde{B} = \tilde{A}$ arises when the cross term $f \oint \delta_2 \delta_3$ between the restoring force and the dynamic perturbation becomes small, failing to cancel $f \oint \delta_2^2$ and $f \oint \delta_3^2$. The yield ($A \rightarrow B$ transitions per cycle), flow ratio ($A \rightarrow B$ transitions per net $E \rightarrow E^* A \rightarrow E$ cycle), and efficiency for $\tilde{B} = 100$ are shown versus $\log_{10} f$ in Fig. 5. Figure 6 shows the input, output, and dissipated power as functions of frequency, and Figs. 7(a) and 7(b) show the flux ratio and efficiency versus the output free energy ΔG_{AB} and perturbation strength $\ln(\phi_E)$, re-

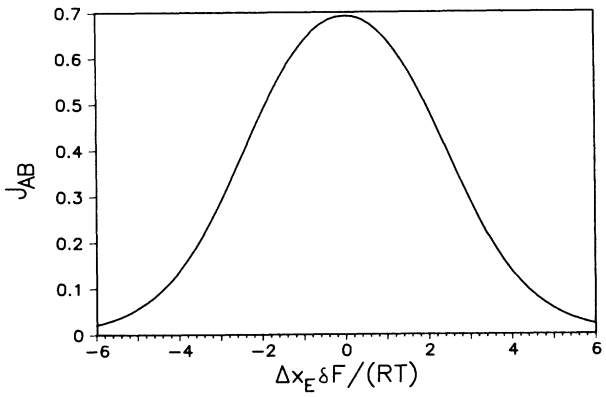


FIG. 4. Steady-state flux of the enzyme with $\tilde{A} = 1$ and $\tilde{B} = 0.1$ as a function of a stationary perturbation $\Delta x_E \delta F/(RT)$ with $\Delta x_{AB} = 0$. Since the term $\Delta x_E \delta F$ is not a part of the driving force of the output reaction, the flux when $\tilde{A} = \tilde{B} = 1$ was identically zero independent of the value of δF. That changing the static value of δF influences the rate of catalysis illustrates the thermodynamic regulation of enzyme activity.

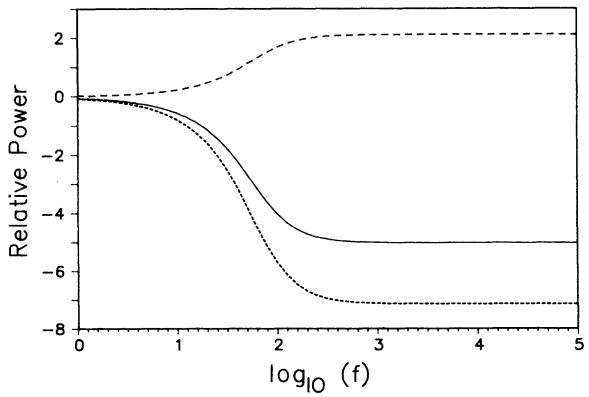


FIG. 6. Illustration of how the total dissipation (——) may be broken up into an input (- - -) and output (— — —) power ($\tilde{B} = 100$).

spectively. Of these, only the yield has a maximum value, falling off at high frequencies. As will be noted later, this is due to our selection of a very simple two-state model, and with more complicated models, the behavior is more complex. Note that the high-frequency bias is reversed by setting $m=0$, $n=1$, and abolished when $m=n$.

The phenomena discussed in this section emphasize the role of an enzyme as a free-energy converter, by which an energy source that is not thermodynamically coupled directly to the output reaction $A \rightleftharpoons B$ may, through differential interactions with two or more protein conformations, be used to drive this reaction away from equilibrium.

Comparison between fluctuations interacting with the output reaction versus those that interact with the intrinsic protein conformational equilibrium. Two parameters which might be indicative of the viability of fluctuation-induced free-energy transduction in biological systems are the thermodynamic efficiency Eq. (35) [(power in)/(power out)] and the static-head concentration of product (B) in excess of the equilibrium value which can be supported by various amplitude perturbations. In the high-frequency limit, using the equations derived in Appendix A, the static head for the case $\phi_E=1$, $\phi_{AB}\neq 1$ with all other parameters as before is

$$(\tilde{B}_{SH})_{HF}=(\phi_B+\phi_B^{-1})/2 \tag{37}$$

and for $\phi_{AB}=1$, $\phi_{enz}\neq 1$ is

$$(\tilde{B}_{SH})_{HF}=(\phi_E^2+\phi_E^{-2}+2)/4\ . \tag{38}$$

The low-frequency limit B_{SH} with $\phi_{AB}\neq 1$ and $\phi_{enz}=1$ is

$$(\tilde{B}_{SH})_{LF}=(\phi_B+\phi_B^{-1}+2)^{1/2}-1\ , \tag{39}$$

while for the case with $\phi_E\neq 1$ and $\phi_B=1$ $(\tilde{B}_{SH})_{LF}$ is identically unity. Notice that for equal magnitude ϕ's, in the high-frequency limit the static-head value supported by fluctuations which interact with the enzymes intrinsic conformational equilibrium is much larger than that supported by fluctuations which interact only with the output reaction. This is because for the case where the interaction is intrinsic to the enzyme conformational dynamics, the positive phase of the field serves to preferentially "push" the enzyme clockwise along the α path, and the negative stimulates clockwise transitions along the β path. If the interaction is only with the output reaction, then either only the α or β clockwise path is enhanced. If the interaction is split between A and B, then both paths will be stimulated, but each to a lesser extent than in the intrinsic case.

The efficiency in the high-frequency limit may be calculated, with $\phi_B\neq 1$, and $\phi_{enz}=1$, to be (see Appendix A)

$$(\eta)=-[\ln(\tilde{B})/\ln(\phi_B)]\times\{[2\tilde{B}-(\phi_B+\phi_B^{-1})]/[(\tilde{B}+1)(\phi_B+\phi_B^{-1})]\} \tag{40}$$

and for $\phi_{enz}\neq 1$, $\phi_B=1$,

$$(\eta)=-[\ln(\tilde{B})/\ln(\phi_E)]\times\left[\frac{4\tilde{B}-(\phi_E^2+\phi_E^{-2}+1)}{2\phi_E^2+2\phi_E^{-2}+2(\phi_E-\phi_E^{-1})(1+\tilde{B})}\right]\ . \tag{41}$$

For the latter case, efficiencies of 30% (see Fig. 7) may be obtained. However, for none of the conditions we have studied did the efficiency of the former case exceed 10% and most often was less than 5%.

In general it seems that in the high-frequency limit fluctuation-induced free-energy transduction is more effective when the perturbing parameter interacts with the intrinsic enzyme conformational equilibrium rather than when the only interaction is with the output reaction. This result may be very important in better understanding the conformational coupling mechanism for free-energy transduction in biological systems.[40]

Comparison between random stochastic and regular periodic perturbations. So far, we have not discussed much concerning the differences between random versus regular perturbations. This is because there really are not many. The high- and low-frequency-limit behavior is in fact identical for the two cases. The major difference lies in the frequency response, where it is seen (Figs. 1 and 3) that the response curve for random stimulation is broader and less sharp than the curve for regular periodic input. This is understandable since the stochastic perturbation

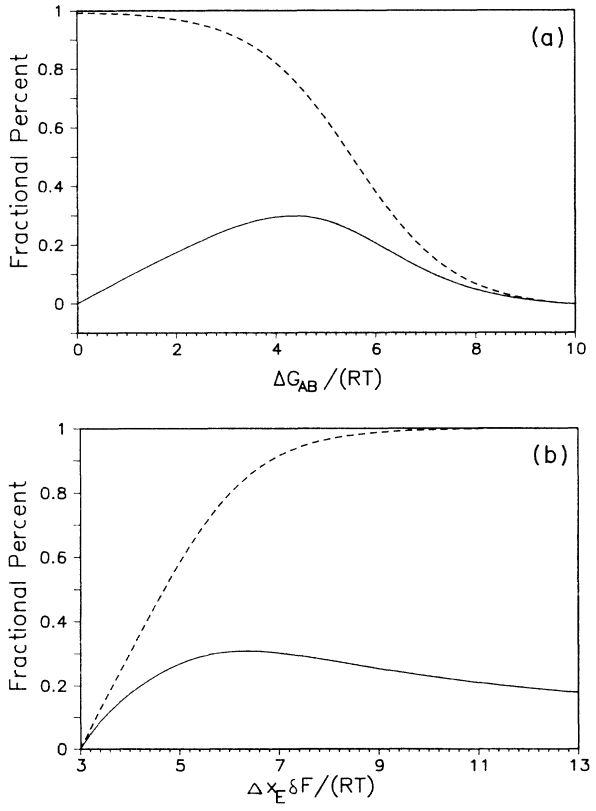


FIG. 7. Calculations of the flux ratio and efficiency of the enzyme subjected to high-frequency (HF-limit) fluctuations of δF with $\Delta x_{AB}=0$. (a) While ϕ_E was kept constant at 256, the output reaction free energy was varied by changing $\tilde{B}$. With respect to efficiency, the optimal value was approximately $4.6RT$, or $\tilde{B}=100$. (b) When $\tilde{B}$ was constant at 100 and ϕ_E was varied, the optimum was found to be $\ln(\phi_E)=6$ or $\phi_E=400$.

arising from a Lorentzian process (which is equivalent to what is known in some cases as random telegraph noise[10,41]) has a continuous distribution of Fourier components at frequencies both higher and lower than the inverse relaxation time of the noise-generating process, while the regular square-wave signal contains discrete components at the fundamental and *higher* harmonic frequencies.

The result that random perturbation can cause an enzyme to drive the reaction it catalyzes away from equilibrium may at first seem surprising since it is a common aphorism that random fluctuations occur even in systems at equilibrium. Let us consider more closely why an externally defined random signal (particularly a multiplicative noise term such as used in the nonlinear Langevin equation[42]) is not and cannot be descriptive of fluctuations at equilibrium, particularly for a noninertial system such as a chemical reaction, and why, even for a simple unimolecular process such as in Eq. (1), symmetric external fluctuations may cause a shift in the average probability distribution except when $k_f = k_r$ and $\delta = \frac{1}{2}$ (as we might imagine to be the case for the diffusion of a Brownian particle[35,36]). For the sake of concreteness, let us concentrate on the case of electric fluctuations.

We can imagine a protein in which a charge moves from one point to another via a Lorentzian process. An enzyme which is located close to this field-generating protein will thus sense a stochastically fluctuating electric field. If the output reaction and/or the conformational transitions of this enzyme are electrically sensitive (i.e., if Δx_E and/or x_A or x_B are not zero), the overall reaction, including the generator transitions, can be represented in terms of a single diagram[11,12] (see Appendix C). If we assume that the generator transitions are independent of the enzyme, the noise may be considered to be *autonomous*. In this case net flux of reactant to product away from equilibrium may be obtained, i.e., the free energy of the output reaction is increased. But, in order to sense an electric field, the enzyme must itself generate a field. In a local picture of a system at equilibrium, the field generated by the enzyme would of necessity exert an influence on the transitions of the generating protein, and when these

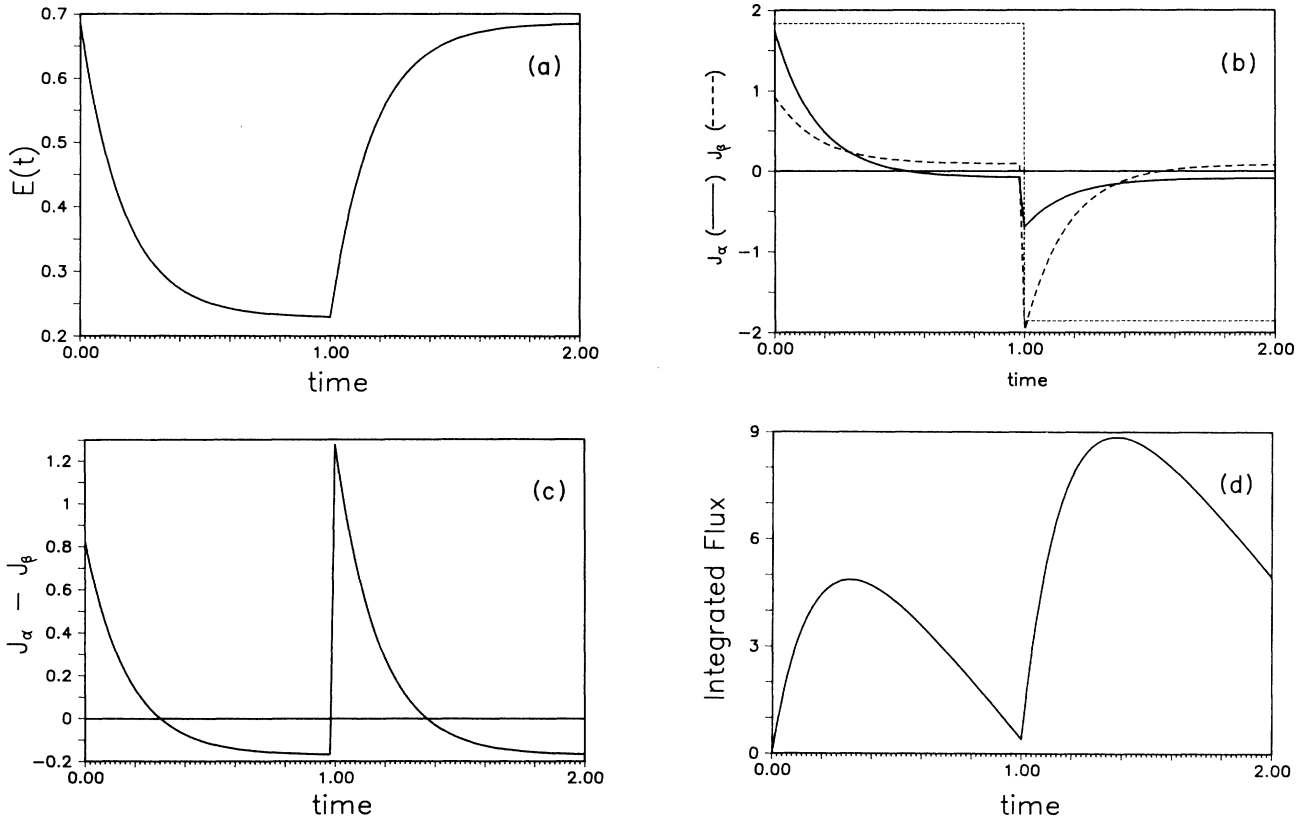


FIG. 8. Illustration of how an enzyme breaks the symmetry of an input fluctuation. The calculations were based on the enzyme having already reached a stationary fluctuating state such that $\oint dE = 0$. The parameters ($\phi_E = 30$, $\tilde{B} = 2$) which differed from those used in the other calculations were chosen so as to better illustrate the principles involved. (a) Probability of the E state [Eq. (3)] of the enzyme as a function of time is shown for one cycle of the oscillating δF. (b) The resulting instantaneous fluxes along the top (α) and bottom (β) transition pathways as a function of time. Notice that the two cross. If δF were allowed to remain at a constant value for a long time after a sudden perturbation, the integrals of J_α and J_β taken to infinity would be identically equal if $\tilde{A} = \tilde{B}$. (c) Here we show $J_\alpha - J_\beta$ as a function of time. (d) In the case of a dynamic perturbation, δF does not remain constant, and the integral $\int J_\alpha - J_\beta$ may be positiven even when $\tilde{B} > \tilde{A}$, and this represents free-energy transduction from the source of the fluctuations to do work on the output reaction mediated by the enzyme. In all plots time is dimensionless.

reciprocal interactions are explicitly included (a situation we have termed *endogenous* noise[12]), it has been shown that the ability of the electric fluctuations (which are still of course present) to do work is lost.

We have used these concepts to formulate a model for free-energy transduction by enzymes. In this picture, we visualize an energy-transducing unit to be composed of two *a priori* independent enzymes, each catalyzing some reaction toward equilibrium. As a specific example, we mention the F_0F_1 ATPase of the mitochondrial membrane[43] (where ATP is adenosine 5'-triphosphate). The F_0 is a proton translocator and F_1 an ATPase. During the course of their independent catalytic cycles each enzyme undergoes some conformational transitions which might involve, e.g., intramolecular charge-transport or dipole-moment changes. These result in local electric fluctuations, and as a result, when the two enzymes are (in a *gedanken* experiment) brought close together, they begin to interact, where the reciprocal influences of one on the other are specifically included in the model. Calculations reveal that flux of one reactant pair down its (electro-) chemical-potential gradient can drive flux of the other (with smaller affinity) up its gradient.[12,13,44]

DISCUSSION AND CONCLUSIONS

We have presented an analysis of the effects of free-energy-driven oscillations and fluctuations on the kinetics of catalysis by a simple enzyme. It was shown that the dynamic behavior cannot be understood in terms of the time average values of the environmental parameters alone. A steady-state force ΔG_{AB} imposes a directional bias on the cyclic catalytic flux of the system. It was demonstrated here that energy-driven fluctuations can also impose directional biases on this flux which may be opposite to that given by the steady-state force and strong enough to induce flux against the time-averaged force. This may be identified as free-energy transduction from the fluctuations to a time-averaged chemical-potential gradient.

A key factor determining whether an enzyme can use energy-driven fluctuation for doing useful output work is the kinetic asymmetry displayed by the system. Concentration fluctuations were seen to provide a bias in the direction of the nonfluctuating to the fluctuating component (in our case from B to A), even when $\tilde{A} = \langle \tilde{B} \rangle$. This did not require any *a priori* asymmetry of the enzyme system because symmetric *concentration* fluctuations give rise to asymmetric fluctuation of the output free energy $[\langle \Delta G(A) \rangle \neq \Delta G(\langle A \rangle)]$ and therefore a nonzero average driving force. Similar concepts have been discussed recently by Mou, Luo, and Nicolis,[45] who generalize the entropy production to include a term relating to non-Poissonian concentration fluctuations. As in the case discussed here, significant power output resulted only if the system were driven far enough from equilibrium. If the fluctuation of B were taken to be Poissonian, *this* bias due to asymmetric fluctuation of the output force disappeared.[45] However, as we see in the present paper, even fluctuations which lead to symmetric force fluctuations can impose bias given kinetic asymmetry typical for an enzyme.

Fluctuation of F (or chemical potential of substrate or product) influencing only the output reaction implies a symmetric fluctuation of the output free energy. In this case, symmetry breaking requires the flow-force relation for the enzyme to be nonantisymmetric about F_0. When this condition was met, significant flux from A to B could be induced, even when $\tilde{A}$ was 1 and $\tilde{B}$ was 10 as shown in Fig. 3.

Fluctuation of an F that influences the relative basic free energies of E and E^* but is not thermodynamically coupled to the reaction $A \rightleftharpoons B$, induces no fluctuation of the output free energy ΔG_{AB}. Here the required asymmetry to allow such a fluctuation to drive the reaction $A \rightarrow B$ even when $\mu_B > \mu_A$ is provided by having the greater F dependence in the forward process along the top (α) branch and in the reverse process along the bottom (β) branch of reaction equation (3). Although this may seem somewhat artificial, it turns out that increase of the "interaction energy" (which we have termed the conformational bias, or asymmetry parameter b in previous publications[11-13,44]) to be very large for a four-state enzyme with two transitions having symmetric dependence on F, and subsequent reduction of the diagram to two states, results in precisely this form, as shown in Appendix D.

In general, if a plot of steady-state flux J_{AB} versus some parameter of interest is nonantisymmetric (in the absence of fluctuation), a symmetric fluctuation of that parameter may lead to a time-averaged flux different from the flux calculated by steady-state theory for the time-averaged value of the parameter. This directional bias is frequency independent. In addition, there may be a kinetic contribution which is maximized at high frequencies. The way in which this situation works to allow flux in a preferred direction to be induced by symmetric fluctuations is shown in Figs. 8(a)-8(d) which demonstrates how the temporal symmetry of an input perturbation may be broken by the interaction with a very simple enzyme system such that a directed output is induced. In Fig. 8(a) the symmetric oscillation (under stationary oscillating conditions) of the enzyme-state probability is shown. Figure 8(b) demonstrates that, nevertheless, the instantaneous fluxes through the α and β branches may be different and that for the parameters chosen, initially following a positive perturbation $+\delta F$, $J_\alpha > J_\beta$ and following a negative perturbation $-J_\beta > -J_\alpha$. Figure 8(c) results if their difference $(J_\alpha - J_\beta)$ is plotted, and integration of this quantity yields the accumulated excess clockwise flux [Fig. 8(d)] from $A \rightarrow B$ even when $\mu_B > \mu_A$. This symmetry breaking results, for instance, if the affinity for substrate and product is different for different enzyme states. Under certain situations this output may be translated into an increase in the free energy of the system at the expense of the environment.

Throughout our discussions, we have emphasized the interaction between an environmental parameter and a protein conformational equilibrium, which makes an enzyme unique among catalysts. This interaction allows for the exchange of free energy between an enzyme and its surroundings and may be unrelated to any reaction catalyzed by the enzyme. If the environmental conditions

are caused to fluctuate rapidly by some energy-releasing process, the free-energy exchange becomes irreversible. The catalytic properties of an enzyme, coupled with its conformational interaction with the environment, may allow some of the input free energy to be utilized to drive an output-catalyzed reaction away from equilibrium, thereby storing free energy. For the case of electric field fluctuations, we have termed this to be free-energy transduction by electroconformational coupling,[4,12,13,15,46] stressing the interaction between the electric field strength and the protein conformational transitions.

Up to this point, we have been talking only about "fluxes" of A to B or vice versa, with the actual normalized concentrations $\tilde{A}$ and $\tilde{B}$ being kept fixed. This was necessary in order that the kinetic equation for the model be a *linear* differential equation, allowing for the analytic solutions provided here. Hopefully, this will not obscure the fact that, as shown previously,[5,23] direct numerical integration of the nonlinear differential equation demonstrates a buildup of $\tilde{B}$, at the expense of $\tilde{A}$, with a static-head condition where $\tilde{B} > \tilde{A}$ as the final result. Thus, the system would have a higher free-energy content than before the imposition of the (possibly random) external fluctuations. This free energy within the system, absorbed from the energy-driven environmental randomness, can be used to create order, introducing a new facet to the concept of order through fluctuation.[30,47,48]

Although we have framed our discussion in as general a context as possible, we have been primarily motivated by considerations of fluctuations (particularly electric field fluctuations) at and across biological membranes, and the effects on membrane-bound proteins (reviewed in Refs. 4, 7, and 15). Macroscopic oscillations of the membrane potential of ± 50 mV (equivalent to $\pm 100\,000$-V/cm field strength across the bilayer) have been observed experimentally,[9] and even larger stochastic fluctuations may well occur in the vicinity of ion channels in free-energy-transducing membranes.[10] The latter have ambient membrane potentials of up to 250 mV. Furthermore, large-amplitude oscillations of the membrane potential are relatively simple to attain experimentally due to the fact that an externally applied field is amplified across closed cell membranes. It has been experimentally demonstrated that external alternating electric fields can induce Na^+ and K^+ ATPase of erythrocyte membranes to drive Rb^+ (Ref. 49) and Na^+ (Refs. 50 and 46) up their respective electrochemical gradients, apparently in the abscence of significant ATP hydrolysis. Two important observations made experimentally but not demonstrated within the context of the simple model used in the present paper were the appearance of both frequency and amplitude windows. Simple extensions of the theory presented here allow for both of these to be qualitatively reproduced. The introduction of an induced dipole-moment term [i.e., inclusion of the functional dependence of Δx_E on F in Eq. (A7)] revealed a perturbation amplitude optimum.[5] Numerically solving the equations for a four-state model such as discussed in Appendix D, a frequency optimum with respect to the perturbation-induced flux (which may be thought of as a "quasiresonant" frequency) is observed.[7,46] This is related to the finite rate of association and dissociation of substrate and product. If the frequency is much greater than these rate coefficients, the electric field will simply stimulate back and forth movement along the top and bottom transitions (with large dissipation), but does not allow for significant binding and subsequent conversion of substrate to product.

Enzymes found in the bilayer represent an extremely important class of proteins, as it is these molecules which handle the tasks of communication between the cell and its environment, and, for a large part, of conversion of free energy from one form to another (e.g., from a redox potential difference through a proton electrochemical gradient to the synthesis of ATP[6,8,43]). The parameter values used in obtaining the figures displayed were chosen so as to be physically realistic within this context. For example, a ϕ_E value of 256 corresponds to a protein transition $E \rightleftharpoons E^*$ involving the transfer of one elementary charge across the membrane being subjected to a fluctuating membrane potential of ± 142 mV.

We have made no attempt to exhaustively characterize the properties of the model-enzyme system under the influence of fluctuations. Rather, we attempted to outline fundamental aspects of the behavior which may be important in understanding how proteins accomplish their roles as catalysts and free-energy converters. Along these lines, the basic concept of fluctuation-induced free-energy transduction may be used to model coupled systems, such as redox-driven proton pumps,[44] or ATPases which couple ion transport to ATP hydrolysis and synthesis.[5,23,51] As discussed in detail elsewhere, an energetically downhill reaction may serve to generate electric (or other) fluctuations which are sensed by an enzyme catalyzing a reaction which it is desired to drive thermodynamically uphill.[12,13,44]

Also, we have tried to present ideas which might be helpful in designing experiments, such as dielectric spectroscopy,[35,36] based on fluctuating perturbations to study the properties of membrane proteins. The equations presented in the Appendices can form the general basis for developing a qualitative picture of what to expect, and numerical techniques described elsewhere[5,11,12] can be used to model more realistic (and therefore more complex) systems of interest.

Although we have focused in this paper on the effects of macroscopic, temporal oscillations, the general ideas developed are also applicable to consideration of spatial inhomogeneity, and/or fluctuations arising in ensembles of small systems.[6,14,16] While classical chemical kinetics has been developed as a predominately mean-field theory, in which it is assumed that fluctuations occurring spontaneously are small and rapidly relaxing enough such that they may be averaged out, it is beginning to be realized that in small systems and systems which are not in equilibrium this may not be the case. Furthermore, the importance of such systems in biology is the current focus of much attention. We have demonstrated here, by explicit inclusion of environmental nonstationarity in the equations describing the dynamic behavior of a *very simple* enzyme system, that energy-driven fluctuations may

lead to behavior entirely different from what would be predicted solely on the basis of time- and space-averaged values of the parameters governing the system.

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APPENDIX A: THERMODYNAMICS OF THE INTERACTION BETWEEN AN ENZYME AND ITS ENVIRONMENT

In many (even most) cases, an enzyme operates away from equilibrium with respect to the reaction it catalyzes. Within the framework of steady-state enzyme kinetics, the activities of substrate and product are considered to be externally defined. Let us, therefore, first consider how the environmental parameters influence the equilibrium of a simple protein which has no catalytic role, such as depicted in Eq. (1), and then modify this description to encompass enzymes.

The macromolecule may be viewed as a small, open thermodynamic system immersed in a bath in which the intensive thermodynamic parameters are externally controlled. These include pressure p, temperature T, electric field strength ϵ, and chemical potential of any ligand such as, e.g., proton μ_{H^+}, that may have many binding sites on the protein. A conformational state in this picture consists of a set of molecular configurations which are *in internal equilibrium with one another.* For each state there is a set of extensive parameters which are conjugate to the intensive parameters. These are, respectively, volume V, entropy S, polarization M, and number of bound protons n_{H^+}.

We wish to determine how a small change in an external parameter will influence the state-concentration-independent (i.e., the basic[24]) free-energy difference (and thus the equilibrium constant) between two conformational states i and j. For clarity, we take these to denote the left and right states of Eq. (1), respectively. The differential of the appropriate generalized basic free energy (denoted by G^0) of i (per molecule) is[24,52,53] the differential of the state-probability-independent part of the chemical potential of state i plus the chemical potential of the stoichiometric ligand $\tilde{L}$ [see Eq. (1)] present in the bulk but not bound in state i,

$$\begin{aligned} dG_i^0 &= d\mu_i^0 + d\mu_L \\ &= (V_i + V_L)dp - (M_i + M_L)d\epsilon - (S_i + S_L)dT \\ &\quad + (n_{H^+ i})d\mu_{H^+} + RTd\ln(\tilde{L}) , \end{aligned} \tag{A1}$$

where

$$RTd\ln\tilde{L} + V_L dp - M_L d\epsilon - S_L dT = d\mu_L . \tag{A2}$$

We have implicitly taken the electrical contribution to the total energy of state i to be given by $M_i d\epsilon$ (change of the electric field strength at constant polarization).[54] This seems most consistent for development of a theory for the basic free energy of a molecular dipole. Other points of view developed for ions[55] and ion-pair[56] reactions lead to the same equation.

Note the difference in the treatment of a stoichiometrically binding ligand such as L, where $n_L = 0$ in state i and $n_L = 1$ in state j, and a freely associating ligand such as H^+, where $n_{H^+ i}$ represents the average number of protons bound in state i. In the former case, the free-energy difference is treated analogously to a chemical reaction such as $A + B = C$, where $\Delta G_{react} = (\mu_A + \mu_B - \mu_C)$. For proton binding to a protein, this would not be convenient since every substrate with a different number of bound protons would have to be explicitly considered separately. Instead, we treat $n_{H^+ i}$ analogously with V_i, S_i, M_i, etc., i.e., as an average value with a Boltzmann distribution being maintained at all times. We have assumed that ligand L does not bind protons.

An equation analogous to (A1) may be written for state j, and thus

$$\begin{aligned} d(\Delta_{ij}G^0) &= (\Delta_{ij}V + V_L)dp - \Delta_{ij}M + M_L)d\epsilon \\ &\quad - (\Delta_{ij}S + S_L)dT + (\Delta_{ij}n_{H^+})d\mu_{H^+} \\ &\quad + RTd\ln(\tilde{L}) . \end{aligned} \tag{A3}$$

For clarity, we note that V_j is the partial molar volume of E^*L, and hence contains the contribution of the bound ligand to the volume of the complex. In other words, $\Delta_{ij}V + V_L$ corresponds to the total volume change accompanying the binding. This is likewise for the other parameters. Equation (A3) may be generalized to

$$\begin{aligned} d(\Delta_{ij}G^0) &= \sum_k [\Delta_{ij}(x)_k + (x_L)_k]dF_k \\ &\quad + RTd\ln(\tilde{L}) . \end{aligned} \tag{A4}$$

The x_k's are extensive and F_k's intensive parameters, and $\Delta_{ij}(x)_k = (x_i)_k - (x_j)_k$. In this formulation, all of the intensive parameters are considered to be externally defined and consequently any cyclic process $i \rightarrow j \rightarrow i$ in a stationary environment occurs without change in the basic free energy of the macromolecule ($\oint dG^0 = 0$). Using the relation

$$\begin{aligned} \Delta_{ij}G^0 &= RT\ln(k_{ij}L/k_{ji}) \\ &= RT\ln(K_{ij}) \\ &= RT\ln(p_i/p_j) , \end{aligned} \tag{A5}$$

where k_{ij} (k_{ji}) is the rate coefficient for transition from state i to j (j to i) and $K_{ij} = (p_i/p_j)$ is the ratio of the probabilities of these two states to exist at equilibrium, we may write a generalized Van 't Hoff relation for the effect of changing one F_k (keeping all others constant) on K_{ij} ($= k_{ij}\tilde{L}/k_{ji}$)

$$\left[\frac{\partial \ln K_{ij}}{\partial F_k}\right]_{F \neq F_k} = [\Delta_{ij}(x)_k + (x_L)_k]/(RT) . \tag{A6}$$

In the remainder we will drop the subscript k, and it will be implicit that only one F will be varied from its reference value, with all others held constant.

In principle, all x_L, x_i, x_j, and hence $\Delta_{ij}x$ depend on the value of F. Thus, integration of Eq. (A6) for a finite change of F yields[56]

$$\ln K_{ij}=\ln(K_{ij})_0+\int[(\Delta_{ij}x+x_L)/(RT)]dF\ , \qquad \text{(A7)}$$

where $(K_{ij})_0$ is the equilibrium constant at some reference state (i.e., with $F=F_0$).

If the difference δF between F and its reference value is small enough for the dependence of $(\Delta_{ij}x+x_L)$ on F in this range to be negligible, Eq. (A7) becomes

$$\ln K_{ij}=\ln(K_{ij})_0+(\Delta_{ij}x+x_L)\delta F/(RT)\ . \qquad \text{(A8)}$$

Dropping the explicit reference to states i and j, and denoting $\Delta_{ij}x=\Delta x_E$, we may write

$$K=K_0\exp[(\Delta x_E+x_L)\delta F/(RT)]=K_0\phi_E\phi_L \qquad \text{(A9)}$$

with $\phi_E=\exp[\Delta x_E\delta F/(RT)]$ and $\phi_L=\exp[x_L\delta F/(RT)]$. Thermodynamically consistent rate coefficients are seen to be

$$k_f=k_{f0}\phi_E^m\phi_L^n,\quad k_r=k_{r0}\phi_E^{m-1}\phi_L^{n-1}\ . \qquad \text{(A10)}$$

m and n are apportionment constants which are a measure of the fraction of the overall δF relevant to the forward process and depend on the kinetic details of the transition. Although not thermodynamically necessary, it is usually taken that $0\le m,n\le 1$. Effects of changing $\tilde{L}$ on the equilibrium and rate coefficients may be handled analogously. Since $K=K_0\tilde{L}$, when the reference state is $\tilde{L}_0=1$, and with $F=F_0$, if we consider that the entire dependence on the concentration of $\tilde{L}$ is in the forward process, reduction to the typical kinetic formulation in which $\tilde{L}$ is a multiplier of k_f is possible. This is the way we will treat the effects of varying stoichiometric ligand (substrate and product) concentrations throughout this paper.

When the macromolecule E in question is an enzyme, we must take into consideration that there may be more than one transition pathway between any pair of states, as in Eq. (3). Typically F may have different effects on the two transition pathways. Thus, there may be an influence of F on the ΔG_{AB} due to the transformation of A to B as well as on the transition from one enzyme state to another. In this case, the formulation for the ratio between forward and reverse rate coefficients must be done separately for each pathway. For the top (α) path in reaction equation (3), which involves the binding and release of one molecule of substrate A, we may write

$$d\Delta G_\alpha^0=(\Delta x_E+x_A)dF+RTd\ln(A)\ . \qquad \text{(A11)}$$

The bottom (β) path involves the binding and release of one B from the bath, and

$$d\Delta G_\beta^0=(\Delta x_E+x_B)dF+RTd\ln(B)\ . \qquad \text{(A12)}$$

We may identify $RT\ln(\Delta G_\alpha^0)$ $[RT\ln(\Delta G_\beta^0)]$ as the pseudo-first-order equilibrium constant which would pertain for the α (β) path if the β (α) path were blocked. The free-energy difference impelling transitions between enzyme states is not equal for the α and β paths except when the overall system is in equilibrium.

In our derivations, we have used a Gibbs equation (A1) in analyzing the influence of changes in environmental parameters on the enzyme reaction. As we intend to use the results to describe systems possibly far from equilibrium, we must question its appropriateness and describe those implicit assumptions which will, if reasonable, be sufficient to warrant our approach. In the case of chemical reactions, it is necessary (and sufficient) that the reaction rate be sufficiently slow so as not to perturb the internal Boltzmann partitioning of energy among the various degrees of freedom other than that of the reaction itself.[30] Thus, we have assumed that the two conformational states described in the kinetic model (3) are individually in internal equilibrium. That is, the various configurations making up each conformational state are at all times distributed based on their individual basic free energies according to the Boltzmann equation. This is equivalent to assuming "quasiergodic" behavior for each individual conformation within the time scale of interest. Furthermore, the correct usage of the kinetic model for enzyme catalysis requires that all processes not in a steady state be explicitly denoted. For example, it is implicit that we consider molecular transport of A and B to and from the enzyme to be quite rapid relative to the transitions explicitly written down. If these assumptions of internal equilibrium are not met a more complicated diagram, displaying more complex behaviors, is sometimes[57] required for the description of the system (see also, e.g., Appendix D). For the purpose of revealing the basic concepts involved for the interaction between a nonstationary environment and an enzyme reaction, it is adequate to consider the two-state model given in Eq. (3). The results so obtained provide a lower limit to the complexity of behavior to be anticipated.

APPENDIX B: FIRST-ORDER PROCESS IN A PERIODIC SQUARE-WAVE FIELD

During any time period in which P and Q are constant, the analytic solution of the integral equation (36) is

$$\tilde{E}(t)=\tilde{E}(0)\exp(-Pt)+[1-\exp(-Pt)]Q/P\ , \qquad \text{(B1)}$$

where $\tilde{E}(0)$ is the value of $\tilde{E}$ at the beginning of the time period of interest. The effect of a periodic square-wave perturbation can be analyzed in terms of a series solution of Eq. (B1) (see Fig. 9). The value of $\tilde{E}$ at the nth field reversal ($\tilde{E}_n$) can be written in terms of its value at the $(n-1)$st reversal according to the recursion relations

$$\tilde{E}_n=\tilde{E}_{n-1}\exp[-P_n/(2f)]+\{1-\exp[-P_n/(2f)]\}Q_n/P_n\ , \qquad \text{(B2)}$$

where f is the frequency of the field and P_n and Q_n are the values of P and Q during the nth half-wave of the input. We define

$$P_\pm=(\tilde{A}_\pm\alpha_{f\pm}+\alpha_{r\pm}+\tilde{B}_\pm\beta_{f\pm}+\beta_{r\pm})\ ,$$
$$Q_\pm=(\alpha_{r\pm}+\beta_{r\pm})\ , \qquad \text{(B3)}$$

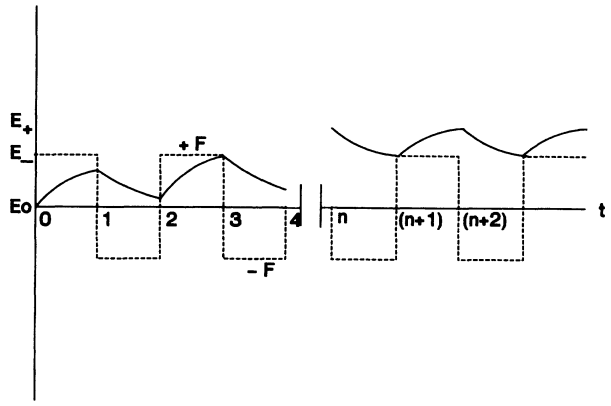


FIG. 9. Illustration of the parameters for the analytic series solution for the enzyme-state probability in the presence of a square-wave perturbation. The numerals on the time axis represent the n value. In the case shown, the relaxation time during the positive pulse was smaller than during the negative pulse, and consequently, the average value of $\langle E \rangle$ after many perturbation cycles was different than $\tilde{E}_0$. Under stationary oscillation, the value of $\tilde{E}$ oscillates symmetrically about $\langle E \rangle$, from $\tilde{E}_+$ to $\tilde{E}_-$.

where the + (−) subscripts refer to the values during the positive (negative) phase of the perturbation. Introducing the terms

$$g_\pm = \exp[-P_\pm/(2f)], \quad z_\pm = Q_\pm/P_\pm \tag{B4}$$

we may rewrite Eq. (B2) in terms of an arbitrary value of $\tilde{E} = \tilde{E}_0$, typically, the steady-state value of $\tilde{E}$ at the average environmental conditions in the absence of perturbations. For n = odd,

$$\begin{aligned}\tilde{E}_n = {} & \tilde{E}_0 g_+^{[(n+1)/2]} g_-^{[(n-1)/2]} \\ & + g_+[(1-g_+)g_- z_+ + (1-g_-)z_-] \\ & \times [1-(g_- g_+)^{(n-1)/2}]/(1-g_- g_+)\,,\end{aligned} \tag{B5}$$

and for n = even,

$$\begin{aligned}\tilde{E}_n = {} & \tilde{E}_0 (g_+ g_-)^{(n/2)} \\ & + [(1-g_-)z_- + (1-g_+)g_- z_+] \\ & \times [1-(g_+ g_-)^{n/2}]/(1-g_- g_+)\,.\end{aligned}$$

In the limit that $n \to \infty$, a stationary oscillation will be attained such that $\tilde{E}_n = \tilde{E}_{n+2}$. Then, the value of $\tilde{E}$ following a positive (negative) pulse, $\tilde{E}_+$ (E_-), is

$$\tilde{E}_\pm = [(1-g_\pm)z_\pm + (1-g_\mp)g_\pm z_\mp]/(1-g_+ g_-)\,. \tag{B6}$$

In the following consideration, we limit ourselves to the case that stationary oscillation has been attained. Then, using the values calculated from Eq. (B6), $\tilde{E}(t)$ at any time may be calculated. During a positive or negative phase of the cycle

$$\tilde{E}(t_\pm) = \tilde{E}_\mp \exp(-P_\pm t) + z_\pm[1-\exp(-P_\pm t)]\,. \tag{B7}$$

The instantaneous flux along each (α and β) branch of reaction equation (3) is given by Eq. (5). Thus, the integrated (total) number of net transitions along each branch during each half-cycle is

$$\begin{aligned}\int J_{\alpha\pm} = {} & [(\alpha_{f\pm}A_\pm + \alpha_{r\pm})/P_\pm](\tilde{E} - z_\pm)(1-g_\pm) \\ & + [z_\pm(\alpha_{f\pm}A_\pm + \alpha_{r\pm}) - \alpha_{r\pm}]/(2f)\,, \\ \int J_{\beta\pm} = {} & [(\beta_{f\pm}B_\pm + \beta_{r\pm})/P_\pm](\tilde{E} - z_\pm)(1-g_\pm) \\ & + [z_\pm(\beta_{f\pm}B_\pm + \beta_{r\pm}) - \beta_{r\pm}]/(2f)\,.\end{aligned} \tag{B8}$$

If we define

$$\begin{aligned}M_\pm &= (\alpha_{f\pm}\tilde{A}_\pm + \alpha_{r\pm} - \beta_{f\pm}\tilde{B}_\pm - \beta_{r\pm})\,, \\ N_\pm &= (\beta_{r\pm} - \alpha_{r\pm})\,,\end{aligned} \tag{B9}$$

the rate of conversion of $A \to B$, $\langle J_{AB} \rangle$, averaged over one cycle, is

$$\begin{aligned}\langle J_{AB} \rangle = {} & f[(M_+/P_+)(E_- - z_+)(1-g_+) \\ & + (M_-/P_-) + (E_+ - z_-)(1-g_-)]/2 \\ & + (z_+ M_+ + N_+ + z_- M_- + N_-)/4\,.\end{aligned} \tag{B10}$$

Limiting our discussion to symmetric perturbations such that $\tilde{A} = \tilde{A}_0 \pm \delta_A$, $\tilde{B} = \tilde{B}_0 \pm \delta_B$, and $F = F_0 \pm \delta_F$, the basic free energies for binding of A and B (driving force along the α and β branch from left to right, respectively) during the positive and negative phases of the perturbation are

$$\begin{aligned}\Delta G^0_{\alpha\pm} &= \pm(\Delta x_E + x_A)\delta_F + RT \ln(\tilde{A} \pm \delta_A) + \Delta G^0_{\alpha 0}\,, \\ \Delta G^0_{\beta\pm} &= \pm(\Delta x_E + xB)\delta_F + RT \ln(\tilde{B} \pm \delta B) + \Delta G^0_{\beta 0}\,.\end{aligned} \tag{B11}$$

The average rate of dissipation of free energy and thermodynamic efficiency may be calculated from Eqs. (34) and (35), respectively.

We may investigate also the low- and high-frequency limits of the flux. Evaluating Eq. (B6) as $f \to 0$,

$$(\tilde{E}_\pm)_{\rm LF} = z_\pm \tag{B12}$$

and as $f \to \infty$,

$$(\tilde{E}_\pm)_{\rm HF} = (P_+ z_+ + P_- z_-)/(P_+ + P_-)\,. \tag{B13}$$

Using these results, we may obtain

$$\begin{aligned}\langle J_{AB} \rangle_{\rm LF} = {} & \{[(Q_+ M_+/P_+) + N_+] \\ & + [(Q_- M_-/P_-) + N_-]\}/4\end{aligned} \tag{B14}$$

and

$$\begin{aligned}\langle J_{AB} \rangle_{\rm HF} = {} & \{2[(Q_+ + Q_-)(M_+ + M_-)/(P_+ + P_-)] \\ & + (N_+ + N_-)\}/4\,.\end{aligned} \tag{B15}$$

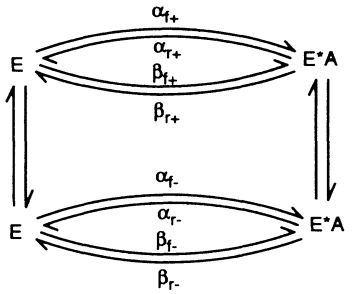
Similarly, the high- and low-frequency-limiting values for the dissipation, $\langle \Phi \rangle$, and efficiency, $\langle \eta \rangle$ [given as Eqs. (40) and (41) for the standard set of parameters], may be obtained. The limiting values for the static-head ratio for $\tilde{A}/\tilde{B}$ may be evaluated by setting the right-hand side of

Eqs. (A16) and (A17) to zero and solving for $\tilde{B}$, taking $\tilde{A}=1$ [see e.g., Eqs. (37)–(39)].

APPENDIX C: EFFECT OF STOCHASTIC DICHOTOMOUS NOISE ON A FIRST-ORDER PROCESS

Although regular periodic oscillations of parameters such as membrane potential and metabolite concentration do occur in biological systems, stochastic fluctuation is more ubiquitous.[10,58] The relation between such external "noise" and regular periodic perturbations can be recognized by writing arbitrary external fluctuations as a Fourier series of sine and cosine waves. While approximate solutions for the effects of external "noise" on an arbitrary chemical system can be obtained in the two extreme cases that the correlation time of the noise is either very large or very small compared to the relaxation time of the system on which it is to act,[21] so far a general solution has not been obtained. Here, let us consider the case of Markovian dichotomous noise influencing a first-order chemical process, a situation for which a general analytic formulation can be obtained. In this case, only two noise states are considered.

We have shown that such a case can be modelled by a single kinetic diagram,[12,13] where transitions from + to − noise states are explicitly included. The validity of this approach was later independently confirmed by Chen.[59] The diagram (3) becomes



(C1)

For convenience $\tilde{A}_+$, $\tilde{A}_-$, $\tilde{B}_+$, and $\tilde{B}_-$ are here considered to be subsumed into the coefficients α_{f+}, α_{f-}, β_{f+}, and β_{f-}, respectively. The lateral transitions represent the change from one set of external parameters to the other, $+\rightarrow-$. The overall kinetic diagram can be solved for the stationary-state probabilities, cyclic fluxes, thermodynamic driving forces, and free-energy dissipation. The rate coefficients for the lateral transitions can be assigned in two ways, as described previously.[11,12] For modeling noise which is not subject to feedback influence by the system, a case we have termed autonomous noise, and will treat here, these transition constants are independent of the states from which they originate, and may all be taken to be equal. When this is the case, the diagram does not in general conform to the principle of detailed balance when $\tilde{A}=\tilde{B}$, but requires an average concentration ratio different from unity between $\tilde{A}$ and $\tilde{B}$ to attain zero net flux. This implies the continual flow of energy from the environment into the system. The fluxes and forces for each of the six individual cycles shown in Fig. 10 are given below,

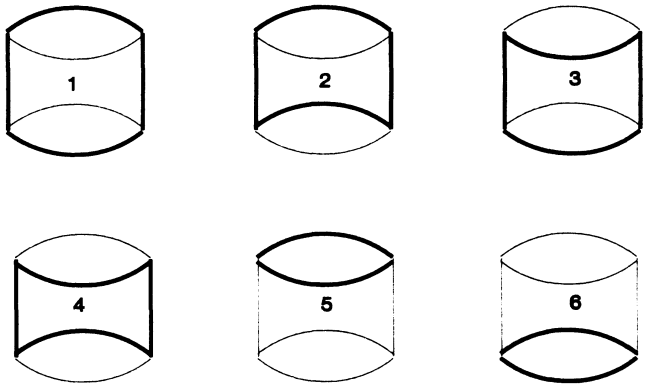


FIG. 10. The six possible cycles, delineated by boldface lines, resulting from Eq. (B1). Only cycles 1, 4, 5, and 6 contribute to the net flux, but all cycles contribute to the dissipation.

$$J_1=[f^2/(4\Sigma)](\alpha_{f+}\beta_{r-}-\beta_{f-}\alpha_{r+}) ,$$
$$X_1=RT\ln[(\alpha_{f+}\beta_{r-})/(\alpha_{r+}\beta_{f-})] , \quad \text{(C2)}$$

$$J_2=[f^2/(4\Sigma)](\alpha_{f+}\alpha_{r-}-\alpha_{r+}\alpha_{f-}) ,$$
$$X_2=RT\ln[(\alpha_{f+}\alpha_{r-})/(\alpha_{f-}\alpha_{r+})] , \quad \text{(C3)}$$

$$J_3=[f^2/(4\Sigma)](\beta_{f+}\beta_{r-}-\beta_{f-}\beta_{r+}) ,$$
$$X_3=RT\ln[(\beta_{f+}\beta_{r-})/(\beta_{f-}\beta_{r+})] , \quad \text{(C4)}$$

$$J_4=[f^2/(4\Sigma)](\beta_{f+}\alpha_{r-}-\alpha_{f-}\beta_{r+}) ,$$
$$X_4=RT\ln[(\beta_{f+}\alpha_{r-})/(\alpha_{f-}\beta_{r+})] , \quad \text{(C5)}$$

$$J_5=(1/\Sigma)[(f^2/4)+(fP_-/2)](\alpha_{f+}\beta_{r+}-\alpha_{r+}\beta_{f+}) ,$$
$$X_5=RT\ln[(\alpha_{f+}\beta_{r+})/(\alpha_{r+}\beta_{f+})] , \quad \text{(C6)}$$

$$J_6=(1/\Sigma)[(f^2/4)+(fP_+/2)](\alpha_{f-}\beta_{r-}-\alpha_{r-}\beta_{f-}) ,$$
$$X_6=RT\ln[(\alpha_{f-}\beta_{r-})/(\alpha_{r-}\beta_{f-})] , \quad \text{(C7)}$$

with

$$\Sigma=f^2(P_++P_-)/2+f(P_+P_-) . \quad \text{(C8)}$$

The individual state probabilities are

$$E_\pm=[f^2(\alpha_{r+}+\beta_{r+}+\alpha_{r-}+\beta_{r-}) + f(\alpha_{r\pm}+\beta_{r\pm})P_\pm]/\Sigma , \quad \text{(C9)}$$

$$E^*A_\pm=[f^2(\alpha_{f+}+\beta_{f+}+\alpha_{f-}+\beta_{f-}) + f(\alpha_{f\pm}+\beta_{f\pm})P_\pm]/\Sigma . \quad \text{(C10)}$$

The total net flux of conversion of A to B is

$$\langle J_{AB}\rangle=J_1-J_4+J_5+J_6 , \quad \text{(C11)}$$

and the rate of free energy dissipation is

$$\langle\Phi\rangle=\sum_{k=1}^{6}X_kJ_k . \quad \text{(C12)}$$

Once again it is instructive to consider the low- and high-frequency limits for $\langle J_{AB}\rangle$. As $f\rightarrow0$, the limit is

$$\langle J_{AB}\rangle_{f\to 0}=[(\alpha_{f+}\beta_{r+}-\alpha_{r+}\beta_{f+})P_- +(\alpha_{f-}\beta_{r-}-\alpha_{r-}\beta_{f-})P_+]/(2P_+P_-) \quad \text{(C13)}$$

and as $f\to\infty$

$$\langle J_{AB}\rangle_{f\to\infty}=[(\alpha_{f+}+\alpha_{f-})(\beta_{r+}+\beta_{r-})-(\alpha_{r+}+\alpha_{r-})\times(\beta_{f+}+\beta_{f-})]/[2(P_++P_-)] . \quad \text{(C14)}$$

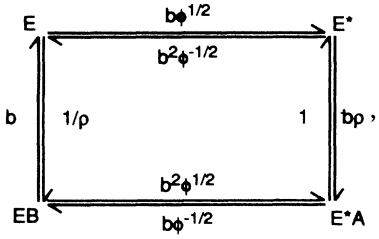
These limits may be shown to be identical to those obtained for regular square-wave perturbations as Eqs. (B14) and (B15).

The force acting on the various cycles may be decomposed as done in Eq. (35), in order to define an input and output. However, here, a natural (and equivalent) definition of efficiency may be given to be

$$\eta=\langle J_{AB}\rangle(\mu_A-\mu_B)/[\langle\Phi\rangle-\langle J_{AB}\rangle(\mu_A-\mu_B)] . \quad \text{(C15)}$$

APPENDIX D: REDUCTION OF A FOUR-STATE TO A TWO-STATE ENZYME DIAGRAM—STEADY-STATE APPROXIMATE

In previous publications[5,11,12] we have considered a four-state enzyme diagram and the effects of fluctuations and oscillations. This diagram, along with its rate coefficients may be written as

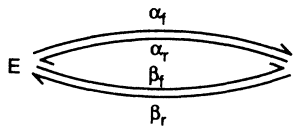


(D1)

where it was assumed $\Delta x_{AB}=0$ for the output reaction (and so we simply write $\phi_E=\phi$). It was found that the primary requirement for obtaining oscillation or fluctuation-induced excess clockwise flux was that b should be greater than unity. This produces a situation where, under stationary condition $\tilde{E}=\tilde{E}^*\tilde{A}\gg\tilde{E}^*=\tilde{E}\tilde{B}$ with $\delta F=0$ and $\rho=1$. $\rho=(\tilde{A}/\tilde{B})^{1/2}$ parametrizes the concentration (A and B) dependence of the transition rates, b is what we called the bias factor, and $b>1$ indicates that the substrate binds tighter to the enzyme than it does to the product. This differential binding energy has been referred to by Jencks,[60] particularly in the context of membrane transport, as the interaction energy. If we consider b to be large, the occupancy of states E^* and EB becomes very small, and these then may be treated as steady-state intermediates. Rigorously, if the following conditions are met,

$$(b^2\phi^{-1/2}+b\rho)>1, b\phi^{+1/2} , \quad (b^2\phi^{+1/2}+b)>1/\rho, b\phi^{-1/2} , \quad \text{(D2)}$$

over the entire range of ρ and ϕ of interest, diagram (D1) may be reduced[24] to



(D3)

where the effective rate constants are

$$\alpha_f=b^2\rho\phi^{1/2}/(b^2\phi^{-1/2}+b\rho) , \quad \alpha_r=b^2\phi^{-1/2}/(b^2\phi^{-1/2}+b\rho) , \quad \beta_f=b^2\phi^{1/2}\rho^{-1}/(b^2\phi^{1/2}+b) , \quad \beta_r=b^2\phi^{-1/2}/(b^2\phi^{+1/2}+b) . \quad \text{(D4)}$$

In the limit that $b\to\infty$, we find

$$\alpha_f=\phi\rho, \quad \alpha_r=1, \quad \beta_f=1/\rho, \quad \beta_r=\phi^{-1} , \quad \text{(D5)}$$

which gives us (as in the text) $m=1$ and $p=0$, i.e., all of the F dependence of the α branch in the forward rate coefficient α_f and that for the β branch in β_r.

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