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# Theoretical study of the effect of enzyme–enzyme interactions on steady-state enzyme kinetics

(enzyme lattice/enzyme solution/Ising problem/phase transition/diffusion and reaction)

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ABSTRACT **Equilibrium statistical mechanics is much concerned with problems involving intermolecular interactions, either in lattices or in pure fluids or solutions. The possibility of enzyme–enzyme interactions suggests that the same problems might be studied profitably at steady state as well as at equilibrium. In the systems we consider, each of the identical enzyme molecules of the system undergoes steady-state stochastic cycling among states $i = 1, \ldots, n$. But the molecules do not cycle independently. Two neighboring molecules, in states $i$ and $j$, interact with a free energy $w_{ij}$ (a function of the distance $r$ in the solution case). The instantaneous transition probabilities between states for a given molecule will depend on the instantaneous interactions between the molecule in question and its neighbors. The primary question of interest is how the enzyme flux is influenced by the interactions. The general problem is outlined here and some simple special cases are treated. The discussion will be continued in a following paper [Hill, T. L. (1977) *Proc. Natl. Acad. Sci. USA* 74, in press].**

Problems that involve interactions between neighboring molecules, in a lattice or in a gas or solution, are among the most interesting in equilibrium statistical mechanics. That these same problems can be studied at steady state rather than at equilibrium is suggested by interacting enzyme systems. However, new theoretical difficulties abound in the steady-state systems. Monte Carlo computer methods will usually be required. In this paper and its sequel [Hill, T. L. (1977) *Proc. Natl. Acad. Sci. USA* **74,** in press], we outline the general problem and then illustrate the subject with assorted simple special cases. We use enzyme terminology throughout, but the problem is not really limited in this way.

These two papers are an extension of recent discussions (1–3) of interacting enzymes considered as free energy transducing systems. Here we emphasize the interactions themselves. In much earlier work (4–12), certain more or less related special cases have been investigated.

This topic is put forward here primarily as an interesting problem in statistical physics. In this respect, large systems present a more challenging theoretical problem, but finite (oligomeric) systems are more important biologically. The conventional analysis of the latter problem is basically an equilibrium (12–15), or approach to equilibrium (14), treatment. In contrast, we refer here, for large or small systems, to an explicit *kinetic* study of *steady states*.

**Lattice Problem.** Let us now outline the problem for a lattice of enzyme molecules (in one, two, or three dimensions). Our primary interest is in the steady state, but the model would obviously allow treatment of transients as well. The lattice contains $M$ identical enzyme molecules, each of which can exist in $n$ discrete molecular states with various possible transitions between pairs of states. In the simplest cases, the $n$ states comprise a single kinetic cycle, but in general the kinetic diagram might contain several cycles (2). There is a complete set $\alpha^0_{ij}$ of first-order, or pseudo-first-order, rate constants for the possible transitions $i \to j$ of each unperturbed enzyme molecule (i.e., in the absence of interactions). In general, the $\alpha^0_{ij}$ set is chosen to correspond to a steady state at time $t = \infty$, rather than to equilibrium (2).

There is an interaction free energy $w_{ij}$ between any two nearest-neighbor molecules of the lattice, in states $i$ and $j$, as in the equilibrium Ising problem (15). The $w_{ij}$, incidentally, will have no effect on the thermodynamic force or forces driving the steady-state enzymatic cycling of each enzyme, because these forces are determined solely by the fixed concentrations of the ligand molecules that bind to or are released from the enzyme molecule, in some of its states, during its cycling (2). But the $w_{ij}$ *will* affect the basic free energy levels (1, 2) and the steady-state flux or fluxes, per enzyme molecule, because the rate constants $\alpha^0_{ij}$ for the transitions of a particular enzyme molecule of the lattice are altered instantaneously to new values that depend on the instantaneous states of all the nearest neighbors of the particular molecule. The rate constant alterations have, of course, to be consistent with the $w_{ij}$. This still allows much latitude in the construction of a kinetic model of interaction effects, but we shall adopt a definite and rather natural convention in this regard in the following section.

We have to imagine, then, that each of the $M$ enzyme molecules of the lattice is undergoing its own stochastic behavior, i.e., making occasional instantaneous transitions within its kinetic cycle or diagram (2). However, this stochastic behavior is not that of an independent enzyme but rather that of an enzyme whose transition probabilities at any time $t$ depend in some prescribed way (see the next section) on the states of all of its nearest neighbors at $t$. This is a steady-state interactive or cooperative (positive or negative) system, a 2-fold generalization of the equilibrium Ising problem (the usual Ising problem is limited to $n = 2$).

The steady-state properties of immediate interest are the probabilities $p_i$ of each enzyme state ($\Sigma_i p_i = 1$) of the kinetic diagram and the net mean flux or fluxes per enzyme molecule (there is only one operational flux for a single-cycle enzyme). These properties will be the same for all enzymes in the lattice if $M \to \infty$ or if $M$ is finite but the lattice has periodic boundary conditions (e.g., a one-dimensional ring of $M$ molecules). Otherwise there will be end effects. In addition to the above, there are of course a great many other topics of obvious interest such as probabilities for groups of molecules in various states, fluctuations, noise, correlation functions, phase transitions, critical behavior, cycle completion stochastics (16), etc. In general, one can ask: (*a*) what influence do the nearest-neighbor

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interactions within the lattice have on the steady-state properties of the unperturbed enzyme? and, conversely, (*b*) how do steady-state conditions alter the equilibrium properties of cooperative Ising systems?

**Fluid Problem.** The above is a sketch of the problem for a lattice of enzyme molecules. The counterpart is a system of identical enzyme molecules moving in a solvent and at a sufficient concentration $c$ so that inter-enzyme interactions are significant. The interaction free energy $w_{ij}(r)$ now depends on the distance $r$ between enzyme pairs (and on their mutual orientation, in a more general treatment). To be interesting, in the present context, we need $w_{ij}(r)$ to depend significantly on $ij$ as well as on $r$. The problem can be considered, again, in one, two, or three dimensions. The three-dimensional case is obviously realistic, and in fact a particular equilibrium case (multiple binding of a ligand—e.g., protons—to interacting protein molecules) has been studied in some detail (15, 17, 18). The two-dimensional case is also realistic because many protein or enzyme molecules (or complexes) in membranes are believed to be able to diffuse in the plane of the fluid-like phospholipid matrix. But whether there are experimental enzyme-interaction effects of significant magnitude in real two- or three-dimensional fluids is, of course, another question.

In the fluid problem, a given enzyme molecule has, instantaneously, neighboring molecules in various states and at varying distances $r$. All of these neighbors, within range, contribute to the total free energy of interaction of the given molecule with its instantaneous environment. Correspondingly, the unperturbed rate constants $\alpha^0_{ij}$ (applicable when $c \to 0$) of the given molecule are altered instantaneously in such a way as to be consistent with the total interaction free energy (see below).

There are two extreme cases (and a much more difficult intermediate case): (*a*) diffusion of enzyme molecules is fast relative to the time scale of transitions of the kinetic diagram; and (*b*) transitions are fast compared to diffusion. Case *a* would appear to apply to enzyme molecules in aqueous solution and possibly also to enzymes in membranes, though the intermediate situation is probably also of importance in the membrane case.

**Lattice–Fluid Problem.** Finally, we mention a generalized lattice problem that serves as a bridge between the above lattice and fluid (diffusion) problems. In fact, this model represents a lattice approximation to the fluid problem. In a lattice of $M$ sites, $N$ are occupied by enzyme molecules and $M - N$ sites are empty. A given enzyme molecule (on a site) may now have both nearest-neighbor enzyme molecules and nearest-neighbor empty sites (with no interaction). Otherwise, transitions $ij$ of an enzyme at a site, and the influence of nearest-neighbor interactions on these transitions, are handled as in the original lattice problem. A new feature here, however, is the additional possibility that a molecule in state $i$ at a given site can jump to a nearest-neighbor site, provided that this site is empty. The molecule is still in state $i$ after the diffusional transition. Such a transition competes, stochastically, with "biochemical" transitions of the type $i \to j$ at the original site. The unperturbed rate constant for a jump between neighboring sites in either direction is $\kappa_0$, which one might take, for simplicity, to be the same for all states $i$ (because enzyme states usually differ from each other only at the local level, e.g., by a relatively small conformation change or by binding a small ligand). That is, the "diffusion coefficient" is taken to be independent of state $i$. "Unperturbed" refers here to jumps in which both the initial and final sites in the jump have only empty nearest-neighbor sites.

The jumping enzyme molecule, in state $i$, has a set of instantaneous nearest neighbors in the initial site and another set in the final site. The corresponding interaction free energies will alter the two unperturbed rate constants $\kappa_0$, as described at the end of the next section.

**Relation between Rate Constants and Interactions.** We present in this section the convention (not unique) to be used in assigning what we have called, above, "altered" rate constants. Consider the inverse transitions $i \to j$ and $j \to i$ for a particular "central" enzyme molecule in the presence of an arbitrary instantaneous nearest-neighbor environment that we designate by $e$. In the solution problem, $e$ includes neighbors at all values of $r$ within range of the central molecule. Let $w_{ie}$ and $w_{je}$ be the interaction free energies between the central molecule (in states $i$ and $j$) and the enzyme molecules comprising $e$. For example, for a square lattice, if $e$ happens to include two nearest-neighbor molecules in state $k$ and two in state $m$, then

$$w_{ie} = 2w_{ik} + 2w_{im}, \qquad w_{je} = 2w_{jk} + 2w_{jm}. \qquad [1]$$

That is, pair-wise additivity of interaction free energies is assumed (these are potentials of mean force; see p. 349 of ref. 15).

If $\alpha_{ij}$ and $\alpha_{ji}$ are the altered rate constants in the presence of $e$, then detailed balance in a hypothetical equilibrium between states $i$ and $j$ requires that

$$\alpha_{ij}/\alpha_{ji} = (\alpha^0_{ij}/\alpha^0_{ji})e^{(w_{ie}-w_{je})/kT}. \qquad [2]$$

Note that, because $ij$ is arbitrary, the product of instantaneous relations such as Eq. 2 around any cycle in the diagram will give, because of cancellation of the $w$s,

$$\Pi_+/\Pi_- = \Pi^0_+/\Pi^0_- = e^{X/kT}, \qquad [3]$$

where $X$ is the thermodynamic force (2) operating in the cycle in the positive ($i \to j$) direction ($X$ is determined by ligand concentrations only), $\Pi_+$ is the product of $\alpha$s around the cycle in the $i \to j$ direction, etc. Eq. 3 confirms that interactions do not alter the force $X$.

The explicit assumption we make about individual rate constants is that

$$\begin{aligned} \alpha_{ij} &= \alpha^0_{ij}e^{f_{ij}(w_{ie}-w_{je})/kT} \\ \alpha_{ji} &= \alpha^0_{ji}e^{(1-f_{ij})(w_{je}-w_{ie})/kT}, \end{aligned} \qquad [4]$$

where $f_{ij}$ is a constant fraction that depends on the pair $ij$ (in general) but not on $e$. In the language of Eyring's rate theory, this is equivalent to assuming that the interaction free energy between the $ij$ transition state ("activated complex") of the central molecule and $e$ is an average of $w_{ie}$ and $w_{je}$,

$$(1 - f_{ij})w_{ie} + f_{ij}w_{je}, \qquad [5]$$

with the *same* fractional weights $1 - f_{ij}$ and $f_{ij}$ used for *all* contributors to $e$ (e.g., the enzymes in states $k$ and $m$ in Eq. 1). This seems plausible, at least. In fact, for simplicity, the choice we shall usually make is the *symmetrical* one: $f_{ij} = 1/2$ for *all* transitions $ij$.

Finally, we consider the altered rate constants for site-to-site jumps in the hybrid lattice–fluid model. We consider the jump for an enzyme molecule in biochemical state $i$ from an initial site with instantaneous environment $e$ to a final (nearest-neighbor) site with environment $e'$. Let $\kappa_+$ be the forward rate constant and $\kappa_-$ the reverse. Then, as in Eq. 2,

$$\kappa_+/\kappa_- = e^{(w_{ie}-w_{ie'})/kT}. \qquad [6]$$

It seems particularly natural, for this purely physical process,

to use the symmetrical choice for the individual rate constants:

$$\kappa_+ = \kappa_0 e^{(w_{1e}-w_{1e}')/2kT}, \qquad \kappa_- = \kappa_0 e^{(w_{1e}'-w_{1e})/2kT}. \quad [7]$$

**Rate Constant Relations in Two-State Systems.** Most single-cycle enzyme systems have at least three states in the cycle. However, some rate constants may be much larger than others so that a more extensive cycle may reduce, effectively, to only two states (2). Fig. 1*A* shows a two-state cycle, including the special unperturbed rate constant notation to be used for this simple case. The dominant cycling direction is, say, counterclockwise ($\alpha_0\beta_0 > \alpha'_0\beta'_0$). The well-known Michaelis–Menten case is $\beta'_0 = 0$ with $\alpha_0$ pseudo-first-order.

Fundamental rate constant relations for the unperturbed enzyme in Fig. 1 *A* and *B* are (2)

$$\alpha_0/\alpha'_0 = e^{\Delta/kT}, \qquad \beta_0/\beta'_0 = e^{(X-\Delta)/kT}$$
$$\alpha_0\beta_0/\alpha'_0\beta'_0 = e^{X/kT}, \quad [8]$$

where $\Delta$ and $X - \Delta$ are "basic free energy" (2) drops in the transitions [1 → 2 (left in Fig. 1*A*) and 2 → 1 (right), respectively], and $X$ is the thermodynamic force, or total free energy drop for one counterclockwise cycle (Fig. 1*B*).

The steady-state probabilities of the two states of the unperturbed enzyme are (2)

$$p_1 = (\beta_0 + \alpha'_0)/(\alpha_0 + \beta'_0 + \beta_0 + \alpha'_0)$$
$$p_2 = (\alpha_0 + \beta'_0)/(\alpha_0 + \beta'_0 + \beta_0 + \alpha'_0) \quad [9]$$

and the corresponding counterclockwise net flux per enzyme molecule is

$$J = (\alpha_0\beta_0 - \alpha'_0\beta'_0)/(\alpha_0 + \beta'_0 + \beta_0 + \alpha'_0). \quad [10]$$

In the presence of an instantaneous environment $e$ (i.e., one or more enzyme molecules in states 1 and/or 2), the altered rate constants are, according to Eq. 4,

$$\alpha = \alpha_0 e^{f_\alpha(w_{1e}-w_{2e})/kT}$$
$$\alpha' = \alpha'_0 e^{(1-f_\alpha)(w_{2e}-w_{1e})/kT}$$
$$\beta = \beta_0 e^{f_\beta(w_{2e}-w_{1e})/kT}$$
$$\beta' = \beta'_0 e^{(1-f_\beta)(w_{1e}-w_{2e})/kT}. \quad [11]$$

Although we are considering a system at steady-state, *because there are only two states in the cycle*, Eqs. 9 for the unperturbed system have the same form they would have at *equilibrium* for the hypothetical system indicated in Fig. 1*C*, in which there is a *single* inverse pair of transitions. That is, there is a simulated combined "detailed balance" at the steady state:

$$(\alpha_0 + \beta'_0)p_1 = (\beta_0 + \alpha'_0)p_2. \quad [12]$$

This quasi-equilibrium behavior has been pointed out before (19).

In the presence of an instantaneous interacting environment $e$, the combined rate constants corresponding to $\alpha_0 + \beta'_0$ and $\beta_0 + \alpha'_0$, above, are now $\alpha + \beta'$ and $\beta + \alpha'$, as given by Eqs. 11. If we form the quotient $(\alpha + \beta')/(\beta + \alpha')$, we obtain the simple result

$$\frac{\alpha + \beta'}{\beta + \alpha'} = \frac{(\alpha_0 + \beta'_0)}{(\beta_0 + \alpha'_0)} e^{(w_{1e}-w_{2e})/kT} \quad [13]$$

*if and only if* the condition $f_\alpha + f_\beta = 1$ is satisfied. The significance of Eq. 13 is that it is precisely the altered "detailed balance" relation for the simulated "equilibrium" system of Fig. 1*C* in the presence of $e$ (just as in Eq. 2 for any single transition pair). It therefore follows from Eq. 13 that the quasi-equilibrium nature of the unperturbed two-state system will persist

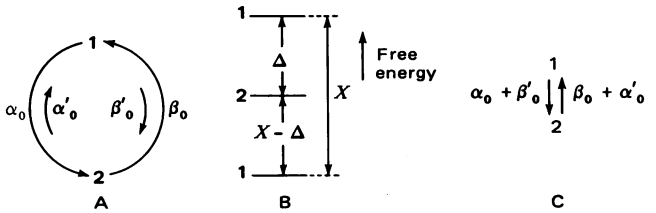


FIG. 1. (*A*) Two-state cycle with unperturbed rate constant notation. The counterclockwise direction is dominant. (*B*) Basic free energy changes for the unperturbed enzyme. See *text* (Eq. 8). (*C*) Effective or simulated "detailed balance" at steady state (see *text*).

even in the presence of an arbitrary interactive environment, in the special case $f_\alpha + f_\beta = 1$. Mathematically, in this case, the steady state cannot be distinguished from an equilibrium. When $f_\alpha + f_\beta = 1$, then, steady-state *population* properties such as state probabilities, probabilities of nearest-neighbor pairs and triplets of different types, correlation functions, spatial distribution functions, etc., will be the same as for the corresponding equilibrium system with interactions.

The applicability of equilibrium population properties to steady-state, two-state systems is, of course, extremely helpful mathematically. We shall illustrate this, in the following paper, for a one-dimensional lattice of two-state enzymes. In this case we can take over results from the exactly soluble one-dimensional equilibrium Ising problem. As another example, the distinction between the two extreme diffusion cases *a* and *b*, mentioned under *Fluid Problem*, disappears at steady state for a two-state enzyme with $f_\alpha + f_\beta = 1$.

**Example: Two Enzyme Molecules with Two States.** This is the simplest example possible to illustrate interaction effects on enzyme flux (or turnover). It can easily be worked out completely, though this is not done here. The same problem for two *different* enzyme molecules has been discussed elsewhere (1, 2). To simplify the algebra, we deal primarily with the special case of a one-way cycle ($\alpha'_0 = \beta'_0 = 0$ in Fig. 1*A*). Each of the two identical molecules ($M = 2$) has states 1 and 2 ($n = 2$) and the pair of molecules (at a fixed distance apart, as in a lattice) then has states 11, 12, 21, 22. The interaction free energies are $w_{11}$, $w_{12}$, etc., and we introduce the notation $y_{11} \equiv e^{-w_{11}/kT}$, $y_{12} \equiv e^{-w_{12}/kT}$, etc. Using Eqs. 11, the rate constants for the transitions in the one-way cycle are

$$11 \to 12 \text{ or } 11 \to 21: \alpha_0(y_{12}/y_{11})^{f_\alpha}$$
$$12 \to 22 \text{ or } 21 \to 22: \alpha_0(y_{22}/y_{12})^{f_\alpha}$$
$$12 \to 11 \text{ or } 21 \to 11: \beta_0(y_{11}/y_{12})^{f_\beta}$$
$$22 \to 12 \text{ or } 22 \to 21: \beta_0(y_{12}/y_{22})^{f_\beta}. \quad [14]$$

Thus, in the top line here, the transition is of type 1 → 2 (left in Fig. 1*A*) and the environment $e$ consists of one molecule in state 1. For two-way cycles, the rate constants involving $\alpha'_0$ and $\beta'_0$ are similar. Using the fact that the transition fluxes (2) into and out of each pair-state (11, 12, etc.) must be equal at steady state, we find

$$p_{12}/p_{11} = (\alpha_0/\beta_0)(y_{12}/y_{11})^{f_\alpha+f_\beta}$$
$$p_{22}/p_{12} = (\alpha_0/\beta_0)(y_{22}/y_{12})^{f_\alpha+f_\beta} \quad [15]$$

Together with $\Sigma p_{ij} = 1$ and $p_{12} = p_{21}$, Eqs. 15 suffice to determine the four $p_{ij}$. Incidentally, if we had included back reactions ($\alpha'_0$ and $\beta'_0$), we would have found, instead of Eq. 15a,

$$\frac{p_{12}}{p_{11}} = \frac{\alpha_0(y_{12}/y_{11})^{f_\alpha} + \beta'_0(y_{12}/y_{11})^{1-f_\beta}}{\beta_0(y_{11}/y_{12})^{f_\beta} + \alpha'_0(y_{11}/y_{12})^{1-f_\alpha}}. \quad [16]$$

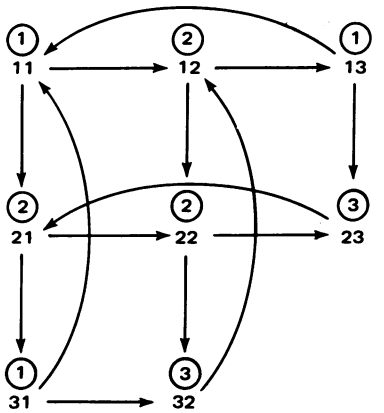


FIG. 2. Kinetic diagram for a pair of three-state enzyme molecules ($M = 2, n = 3$) with one-way transitions (arrows). All rate constants are $\alpha_0$. State 33 is excluded because of strong repulsion between molecules in this state. There are no other interactions. The numbers in circles indicate relative steady-state probabilities of states.

Eqs. **15** and **16** illustrate an important point made in the previous section: the steady-state probability ratios in these equations correspond to those of a quasi-equilibrium system if and only if $f_\alpha + f_\beta = 1$. For example, from Eq. **16** when $f_\alpha + f_\beta = 1$,

$$p_{12}/p_{11} = [(\alpha_0 + \beta'_0)/(\beta_0 + \alpha'_0)](y_{12}/y_{11}), \qquad [17]$$

which simulates a detailed balance relation (compare Eq. **13**). But there is no need to employ this simplification here.

The flux per molecule, around the one-way cycle in Fig. 1*A*, is the property of primary interest. It can be calculated in several ways from Eqs. **14** and **15**. For example,

$$J = 11 \to 12 + 21 \to 22 = 12 \to 11 + 22 \to 21$$
$$= \alpha_0(y_{12}/y_{11})^{f_\alpha}p_{11} + \alpha_0(y_{22}/y_{12})^{f_\alpha}p_{21}$$
$$= \frac{(\alpha_0/\beta_0)(\alpha_0 y_{22}^{f_\alpha}y_{12}^{f_\beta} + \beta_0 y_{11}^{f_\beta}y_{12}^{f_\alpha})}{y_{11}^{f_\alpha+f_\beta} + 2(\alpha_0/\beta_0)y_{12}^{f_\alpha+f_\beta} + (\alpha_0/\beta_0)^2 y_{22}^{f_\alpha+f_\beta}}. \qquad [18]$$

This is to be compared with the unperturbed flux $\alpha_0\beta_0/(\alpha_0 + \beta_0)$ (Eq. **10**), which also follows from Eq. **18** on putting $y_{11} = y_{12} = y_{22} = 1$ (no interactions).

In the special case $w_{11} = w_{12} = 0$ and $f_\alpha = f_\beta = 1/2$, Eq. **18** becomes

$$J = \frac{(\alpha_0/\beta_0)(\alpha_0 y_{22}^{1/2} + \beta_0)}{1 + 2(\alpha_0/\beta_0) + (\alpha_0/\beta_0)^2 y_{22}}. \qquad [19]$$

That is, in this case the two molecules interact ($w_{22}$) only when both are in state 2. Eq. **19** will be illustrated numerically in the sequel. In the limit $w_{22} \to \infty$ (strong repulsion), $J \to \alpha_0\beta_0/(2\alpha_0 + \beta_0)$ (state 22 is not allowed). In the limit $w_{22} \to -\infty$, $J \to \beta_0/y_{22}^{1/2} \to 0$. That is, $p_{22} \cong 1$ (see Eqs. **14**). When $w_{22}/kT$ is very small,

$$J \to \frac{\alpha_0\beta_0}{\alpha_0 + \beta_0}\left\{1 + \frac{w_{22}}{2kT}\cdot\frac{\alpha_0(\alpha_0 - \beta_0)}{(\alpha_0 + \beta_0)^2} + \cdots\right\}. \qquad [20]$$

If $\alpha_0 = \beta_0$ in Eq. **19**,

$$J = \alpha_0(1 + y_{22}^{1/2})/(3 + y_{22}). \qquad [21]$$

By the method of Fig. 2 (2, 3), it is easy to derive the result corresponding to Eq. **21** for a pair of molecules, each with a one-way *three*-state cycle ($n = 3$). That is, we take (the states are 1, 2, 3) $\alpha_{12} = \alpha_{23} = \alpha_{31} = \alpha_0$, all $w_{ij} = 0$ except $w_{33}$, and $f_{23} = f_{31} = 1/2$. We find, per molecule,

$$J = \alpha_0(4 + 5y_{33}^{1/2})/3(5 + 3y_{33}^{1/2} + y_{33}). \qquad [22]$$

The unperturbed flux ($y_{33} = 1$) is $\alpha_0/3$.

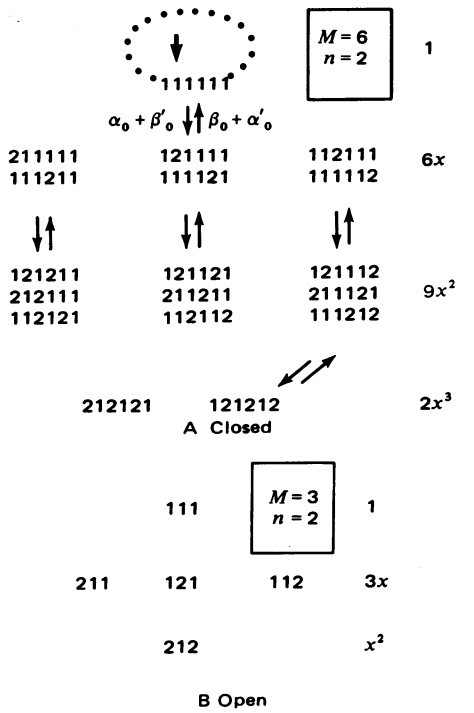


FIG. 3. (*A*) Enumeration of all possible states of a *closed* linear chain of six two-state molecules, with nearest-neighbor 22 pairs excluded owing to strong 22 repulsion. The only transitions indicated (pairs of arrows) are for the *second* molecule in the chain (as an example). See *text* for further details. (*B*) The five possible states of an *open* chain of three two-state molecules, with 22 neighbors excluded. These five states correspond to the five transitions shown in *A* (note the states of the last three molecules in the chain of six).

**Example: Two Enzyme Molecules with Excluded Pair-States.** Fig. 2 shows the kinetic diagram for a pair of three-state molecules ($M = 2, n = 3$). There is a one-way cycle and all three rate constants in the cycle are $\alpha_0$ (as in Eq. **22**). State 33, however, is excluded because of strong repulsion, $w_{33}/kT \to \infty$. There are no other interactions. This is the case $y_{33} = 0$ in Eq. **22** (see also Eq. **21** with $y_{22} = 0$). All arrows in Fig. 2 are associated with a rate constant $\alpha_0$. The numbers in circles give the relative steady-state populations of the pair-states (2, 3). For example, $p_{12} = 2/15$. This algebraic solution can be checked by inspection (the net transition flux is zero at each pair-state). Note that simulated detailed balance is absent because $n > 2$. The flux per molecule can be seen, in various ways, to be $4\alpha_0/15$. The following is a summary of fluxes per molecule for such cases, with $n = 2, 3, 4$:

$$n = 2: J/\alpha_0 = 1/2[0], 1/3[1]$$
$$n = 3: J/\alpha_0 = 1/3[0], 4/15[1], 1/5[2]$$
$$n = 4: J/\alpha_0 = 1/4[0], 9/41[1], 8/45[2], 1/7[3]. \qquad [23]$$

The number in brackets indicates the number of enzyme states (out of $n$) involved in the strong repulsive interaction. For example, for $n = 4$, [1] means that the pair-state 44 is excluded, [2] means 33, 34, 43, 44 are excluded, etc. The [0] fluxes are the unperturbed values. For a given $n$, the flux of course decreases with an increasing number of exclusions.

**Example: Closed One-Dimensional Two-State Chain with 22 Pairs Excluded.** Consider a linear chain of $M$ molecules of the type shown in Fig. 1A. The chain (or ring) is closed, as indicated by the dotted line at the top of Fig. 3A (where $M = 6$). All molecules have equivalent properties in a closed (but not in an open) chain. The only interaction is $w_{22}/kT \to \infty$. Hence, all nearest-neighbor 22 pairs are excluded from the chain, but otherwise there are no interaction effects.

The possible system-states for $M = 6$, as an example, are enumerated in Fig. 3A. The only transitions shown explicitly in Fig. 3A are those (as an example) for the *second* molecule in the chain (see the arrow at the top of the figure). These are combined transitions, as in Fig. 1C. It is obvious that "detailed balance" will obtain at steady state, producing quasi-equilibrium system-state probabilities. The relative probabilities of *individual* states in Fig. 3A are (reading downward) 1, $x$, $x^2$, $x^3$, where $x \equiv (\alpha_0 + \beta'_0)/(\beta_0 + \alpha'_0)$.

Note that, because 22 pairs are excluded, the second molecule in the chain can undergo the transition $1 \to 2$ only when the first three molecules in the chain are in the state 111. This can occur in five ways, because the remaining three molecules can exist in the five states shown in Fig. 3B. These five states are all of those possible for an *open* linear chain with $M = 3$ (i.e., the state 212 in Fig. 3B is not excluded because this group of three molecules has a state 1 molecule on either side of it in the complete $M = 6$ chain).

The probability of the top state in Fig. 3A is $1/\Xi_{cl}$, of state 121111 is $x/\Xi_{cl}$, etc., where $\Xi_{cl}$ ($cl \equiv$ closed) is the sum $1 + 6x + 9x^2 + 2x^3$ (Fig. 3A). This sum has the form and significance of an equilibrium grand partition function (15). The flux per molecule (see Fig. 1A) is then, from the five transitions,

$$J = [(\alpha_0 - \alpha'_0 x) + 3(\alpha_0 x - \alpha'_0 x^2) + (\alpha_0 x^2 - \alpha'_0 x^3)]/\Xi_{cl}. \quad [24]$$

Proceeding in this way, we find for $M = 1$ to 7,

$$\begin{aligned} J &= [(\alpha_0\beta_0 - \alpha'_0\beta'_0)/(\beta_0 + \alpha'_0)] \times \text{p.q.} \\ M &= 1\text{: } 1/(1 + x) = \text{p.q. for } M = 1 \\ M &= 2\text{: } 1/(1 + 2x) = \text{p.q. for } M = 2, \text{ etc.} \\ M &= 3\text{: } 1/(1 + 3x) \\ M &= 4\text{: } (1 + x)/(1 + 4x + 2x^2) \\ M &= 5\text{: } (1 + 2x)/(1 + 5x + 5x^2) \\ M &= 6\text{: } (1 + 3x + x^2)/(1 + 6x + 9x^2 + 2x^3) \\ M &= 7\text{: } (1 + 4x + 3x^2)/(1 + 7x + 14x^2 + 7x^3). \quad [25] \end{aligned}$$

The polynomial quotients (p.q.) can be written (see above) $\Xi_{op}(M - 3)/\Xi_{cl}(M)$, where $op \equiv$ open.

Let $\theta(= p_2)$ be the probability that any molecule of the chain is in state 2. We can then easily calculate $\theta$ from $\Xi_{cl}(M)$. We find that the p.q. in Eq. **25** is just $\theta/x$. Thus, for any $M$,

$$J = (\alpha_0\beta_0 - \alpha'_0\beta'_0)\theta/(\alpha_0 + \beta'_0). \quad [26]$$

This result can be verified or understood as follows. The two separate rates for $2 \to 1$ are $\beta_0\theta$ and $\alpha'_0\theta$ (see Fig. 1A), because there is no interactive restraint on these processes. Then we have

$$J = \alpha_0 P - \alpha'_0\theta = \beta_0\theta - \beta'_0 P, \quad [27]$$

where $P$ is the probability that any one of the $M$ triplets in the chain is of the type 111. From Eq. **27** we first find that $P = \theta/x$ and then deduce for $J$ the same result as in Eq. **26**. Note, finally, that $P$ = p.q.

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