Further study of the effect of enzyme–enzyme interactions on steady-state enzyme kinetics
(linear enzyme chain/Ising problem/Bragg–Williams approximation/phase transition/dilute enzyme solution)

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ABSTRACT This paper continues an earlier one [Hill, T. L. (1977) Proc. Natl. Acad. Sci. USA 74, 3632–3636] and presents further introductory examples. Most attention is devoted to a closed linear chain of two-state enzyme molecules with nearest-neighbor interactions. The one-dimensional Ising theory can be used here. The Bragg–Williams (mean field) approximation is introduced to deal with a one-, two-, or three-dimensional lattice of enzyme molecules, at steady state, with an arbitrary kinetic diagram. The behavior of the flux in a phase transition is noted. Finally, a treatment is given for the first effect (second "virial" coefficient) of interactions on the flux in a dilute solution of two-state enzyme molecules.

This subject was introduced in a previous publication (1). The present paper is a continuation of the earlier one, using the same notation, in which we examine more complicated systems. We continue to use the non-unique rate constant convention in equation 4 of ref. 1. This is appropriate for present purposes, but is too restrictive for some biochemical applications.

Closed One-Dimensional Two-State Chain. As a theoretical prototype, we study a closed linear chain (ring) of M identical enzyme molecules, each of which has a two-state cycle with unperturbed (1) rate constants as shown in Fig. 1. There are interaction free energies \( w_{11}, w_{12}, w_{22} \) between nearest-neighbor pairs of molecules in the chain in states 11, 12 (or 21), 22, respectively. These interactions alter the unperturbed rate constants: the instantaneous rate constant for any transition of a given molecule will depend, in general, on the instantaneous states (1 or 2) of its two nearest neighbors. We shall make the symmetrical choice (1) for the "split" of inverse rates: \( f_0 = f_2 = 1/2 \). Explicitly, for the three kinds of nearest-neighbor pairs, the central molecule of a triplet of molecules is assumed to have rate constants as follows:

\[
\begin{align*}
111 & \rightarrow 121 \quad \alpha = \alpha_0 y_{12}/y_{11}, \quad \alpha' = \alpha_0 y_{11}/y_{12} \\
& \beta = \beta_0 y_{11}/y_{12}, \quad \beta' = \beta_0 y_{12}/y_{11} \\
211 & \rightarrow 221 \quad \alpha = \alpha_0 y_{22}/y_{11}, \quad \alpha' = \alpha_0 y_{11}/y_{22} \\
& \beta = \beta_0 y_{12}/y_{22}, \quad \beta' = \beta_0 y_{22}/y_{12} \\
212 & \rightarrow 222 \quad \alpha = \alpha_0 y_{22}/y_{12}, \quad \alpha' = \alpha_0 y_{12}/y_{22} \\
& \beta = \beta_0 y_{11}/y_{22}, \quad \beta' = \beta_0 y_{22}/y_{11},
\end{align*}
\]

where \( y_{ij} = e^{-w_{ij}/kT} \). As already explained (1), the "population" properties of this particular system at steady state will be those of a quasi-equilibrium system. Hence we can obtain these properties, as needed, from the well-known one-dimensional equilibrium Ising problem (2, 3), which can be solved exactly and easily by the matrix method (3–5).

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In the grand partition function of the ring of M molecules (regarding the system as "open" with respect to the number \( N_2 \) of molecules in state 2) can be written as the sum of a product of contributions \( L \) from each molecular pair in the ring:

\[
\Xi = \sum_{m=1}^{M} \prod_{m=1}^{M} \sum_{m=1}^{M} L(\sigma_m, \sigma_{m+1}),
\]

where \( \sigma_m \) is the state index for the \( m \)th molecule in the chain \((M + 1 \equiv 1)\). The sum is over the \( 2^M \) possible states of the whole chain \((\sigma_m = 1, 2; m = 1, \ldots, M)\). \( L \) is a \( 2 \times 2 \) matrix:

\[
L = \begin{pmatrix}
y_{11} & y_{12} x^{1/2} \\
y_{12} x^{1/2} & y_{22}
\end{pmatrix},
\]

where \( x = (\alpha_0 + \beta_0)/(\beta_0 + \alpha_0) \). In the notation of section 14-1 of ref. 2, \( x = q \lambda \). Eq. 6 gives proper weight to each kind of pair and, in \( \Xi \), to each of the \( 2^M \) system-states. If we perform the sum in Eq. 5 in the order \( \sigma_{M}, \sigma_{M-1}, \ldots, \sigma_2, \) we get

\[
\Xi = \sum_{\sigma_1} L^M(\sigma_1, \sigma_1) = L^M(1,1) + L^M(2,2) = \lambda^M + \lambda^M.
\]

where $L^M$ represents a matrix product and $\lambda_1$, $\lambda_2$ are the two eigenvalues of $L$.

$$\lambda_1, \lambda_2 = (y_{11} + y_{22}x \pm \sqrt{\cdot})/2 \quad [8]$$

with

$$\sqrt{\cdot} = [(y_{11} - y_{22}x)^2 + 4y_{12}^2x]/2. \quad [9]$$

We take $\lambda_1$ as the larger eigenvalue (+ sign).

If we define $\theta = N_2/M$, then because each of the $2^M$ states in $\Xi$ is weighted properly,

$$\theta = L^M(2,2)/\Xi. \quad [10]$$

If we sum over $\sigma_1, \ldots, \sigma_3$ in Eq. 5,

$$\Xi = \sum_s L(\sigma_1, \sigma_2) L^M(\sigma_2, \sigma_1). \quad [11]$$

Therefore, as in Eq. 10,

$$\bar{N}_{112}/M = L(\sigma_1, \sigma_2) L^M(\sigma_2, \sigma_1)/\Xi. \quad [12]$$

Similarly, for triplets,

$$\bar{N}_{112}/M = L(\sigma_1, \sigma_2) L(\sigma_2, \sigma_3) L^M(\sigma_3, \sigma_1)/\Xi. \quad [13]$$

To use Eqs. 10, 12, and 13, we need also (ref. 5, equation 2.80)

$$L'(\sigma, \sigma') = \lambda_1 \phi_1(\sigma) \phi_1(\sigma') + \lambda_2 \phi_2(\sigma) \phi_2(\sigma'), \quad [14]$$

where $\phi_1$ and $\phi_2$ are the eigenvectors belonging to $\lambda_1$ and $\lambda_2$, respectively. For example,

$$\phi_1(1) = (y_{11} - y_{22}x + \sqrt{\cdot})/A_1 \quad [15]$$

where the normalization constant is given by

$$A_1^{-2} = 2[(y_{11} - y_{22}x)^2 + 4y_{12}^2x + (y_{11} - y_{22}x)\sqrt{\cdot}]. \quad [16]$$

Eqs. 4 and 13 provide the flux $J$ for any finite value of $M \geq 3$. However, the algebra is quite involved except in simple special cases (e.g., equations 25 of ref. 1 with $x = 1$).

Fortunately, the case of most interest, $M \to \infty$, is easy to handle because the smaller eigenvalue $\lambda_2$ can be ignored. We find from Eqs. 10, 12, and 13,

$$\theta = 2y_{12}x/\sqrt{\cdot}(y_{11} - y_{22}x + \sqrt{\cdot}) \quad [17]$$

$$\bar{N}_{112}/M = y_{11}(y_{11} - y_{22}x + \sqrt{\cdot})/\sqrt{\cdot} \quad [18]$$

$$\bar{N}_{112}/M = 2y_{12}x/\sqrt{\cdot} \quad [19]$$

$$\bar{N}_{11112}/M = y_{22}x(y_{22}x - y_{11} + \sqrt{\cdot})/\sqrt{\cdot} \quad [20]$$

$$\bar{N}_{1111}/M = y_{11}(y_{11} - y_{22}x + \sqrt{\cdot})/\sqrt{\cdot} \quad [21]$$

$$\bar{N}_{1111}/M = 2y_{11}y_{12}x/\sqrt{\cdot} \quad [22]$$

$$\bar{N}_{111112}/M = y_{22}x(y_{22}x - y_{11} + \sqrt{\cdot})/\sqrt{\cdot} \quad [23]$$

$$\bar{N}_{11111}/M = 2y_{22}y_{12}x/\sqrt{\cdot} \quad [24]$$

where

$$\sqrt{\cdot} = [(y_{11} - y_{22}x)^2 + 4y_{12}^2x]/2. \quad [25]$$

Eq. 24 will be illustrated numerically below. When $y \to 0$ (22 pairs excluded),

$$J = \left(\frac{\alpha_0 \beta_0 - \alpha_0 \beta_0}{\beta_0 + \alpha_0}\right) \frac{[1 + (1 + 4x)^{-1/2}]}{(1 + 4x^{-1/2})1 + 2x + (1 + 4x)^{-1/2}}. \quad [26]$$

In this case, from Eq. 17, an alternative expression is

$$J = \left(\alpha_0 \beta_0 - \alpha_0 \beta_0\right)/(\alpha_0 + \beta_0), \quad [27]$$

which is the same as equation 26 of ref. 1 (for small $M$).

When $y \to \infty$ in Eq. 24 (strong attraction between state 2 neighbors),

$$J \to (\alpha_0 \beta_0 - \alpha_0 \beta_0)/(\alpha_0 + \beta_0) y \quad [28]$$

Almost all molecules are in state 2 ($\theta \to 1$); the flux is small.

**Excluded Pairs (y = 0).** There is an alternative way to obtain Eq. 26, which we digress to consider. This is based on the finite $M$ approach in ref. 1. $\Xi(M)$ is the grand partition function for a closed chain (Eq. 5); we denote the same function for an open chain by $\Xi_0(M)$ (we used subscripts $cl$ and $op$, respectively, in ref. 1). For finite $M$ the flux in Eq. 25 becomes (1)

$$J = [(\alpha_0 \beta_0 - \alpha_0 \beta_0)/(\beta_0 + \alpha_0)]\Xi_0(M - 3)/\Xi(M). \quad [29]$$
The $\Xi$ quotient here can be seen directly (1) to be equal to $\theta/x$. But we follow a more devious path. Using the relation (that can be observed in equations 25 of ref. 1; see also the Appendix)

$$\Xi_0(M) = \Xi(M) + x^2 \Xi_0(M - 4), \quad [29]$$

we have for the $\Xi$ quotient in Eq. 28:

$$\Xi \text{ quotient} = \frac{\Xi_0(M - 3)}{\Xi_0(M) - x^2 \Xi_0(M - 4)}. \quad [30]$$

In the limit $M \to \infty$, the subscript can be dropped because open and closed chains have the same properties. Thus,

$$\Xi \text{ quotient} \rightarrow \frac{[\Xi(M - 3)/\Xi(M)]}{1 - x^2 \Xi(M - 4)/\Xi(M)}. \quad [31]$$

Now, from Eqs. 8 and 17,

$$\frac{(\ln \Xi)/M}{\ln \lambda_1} = \ln[1 + (1 + 4x)^{1/2}/2]$$

$$= \ln[(1 - \theta)/(1 - 2\theta)]. \quad [32]$$

Also, we need the Taylor expansion

$$\Xi(M - m) = \Xi(M) + \frac{\partial \Xi}{\partial M}(-m) + \frac{1}{2!} \frac{\partial^2 \Xi}{\partial M^2}(-m)^2 + \cdots, \quad [33]$$

where $x$ and $T$ are constant in the derivatives. Because $\ln \Xi$ is an extensive property,

$$\frac{\partial^n \Xi}{\partial M^n} = \Xi \left( \frac{\partial \ln \Xi}{\partial M} \right)^n = \Xi \left( \frac{\ln \Xi}{M} \right)^n. \quad [34]$$

Thus, it follows from Eq. 33 that

$$\Xi(M - m)/\Xi(M) = \exp [-m(\ln \Xi)/M]$$

$$= [(1 - 2\theta)/(1 - \theta)]^m. \quad [35]$$

We substitute this result (for $m = 3$ and 4) into Eq. 31 and use

$$x = \theta/(1 - \theta)/(1 - 2\theta)^2 \quad [36]$$

from Eq. 32, to obtain, finally,

$$\Xi \text{ quotient} = (1 - 2\theta)^2/(1 - \theta) = \theta/x. \quad [37]$$

Some further properties of $\Xi$ and $\Xi_0$ are given in the Appendix.

Numerical Examples. The effect of the interaction free energy $w_{22}$ on the flux is illustrated in Fig. 2. Here the solid curve $M = \infty$ is a plot of $J/\rho_0$ from Eq. 24, as a function of $y = e^{-w_{22}/K}$ with $x$ held constant at $x = 1$ (i.e., there is no bias with respect to state 1 or 2). Either attraction or repulsion between state 2 molecules reduces the flux below $J_0$. When $y$ is large, $J/J_0 \to 2/y$ (Eq. 27). When $y \to \infty$, $J/J_0 \to 2\theta = 1 - 5^{-1/2} = 0.553$ (Eq. 28).

Also included in Fig. 2 is a solid curve labeled $\pm, M = \infty$. This is for the case $w_{11} = w_{22} = -w_{12}$ (compare Eq. 29) with $y = y_{22} = 1$ and $x = 1$. Here we find

$$J/J_0 = 4y^2/(1 + y^2)^2. \quad [38]$$

This curve has symmetry with respect to $y$ and $y^{-1}$. The dotted curve in Fig. 2 is the $x = 1$ case for a pair of molecules only ($M = 2$), with $w_{11} = w_{22} = 0$ and $w_{22} \neq 0$ as in Eq. 24 for $M = \infty$. From equation 19 of ref. 1,

$$J/J_0 = 2(1 + y)/(3 + y^2). \quad [39]$$

where here $y = y_{12}^2 = e^{-w_{22}/2K}$. The general definition of a comparable variable for any system of this type (22 interactions) is $y = e^{-w_{22}/2K}$, where $z$ is number of nearest neighbors (this is used in the next section). When $M = 2, z = 1$. Eq. 39 also gives $J/J_0 \to 2/y$ for $y \to \infty$, but $J/J_0 \to 2/3$ for $y \to 0$. There is surprisingly little difference between the $M = 2$ and $M = \infty$ curves.

As a final example, we consider Eq. 24 as a function of $x$, with $y$ constant. To attach an explicit physical significance to the $x$ variation, one can suppose that (Michaelis–Menten): $\beta_0$ is negligible; $\beta_0$ and $\alpha_0$ are held constant; and $\alpha_0 = (\beta_0 + \alpha_0) x$ is a pseudo-first-order rate constant that is proportional to the concentration of a ligand that is bound in the $\alpha_0$ (1 → 2) process. That is, $x$ is proportional to ligand concentration.

It is convenient to plot $J/\beta_0$ as a function of $x$. For the unperturbed system ($y = 1$), $J/J_0/\beta_0 = x/(1 + x) = \theta$ (Michaelis–Menten kinetics). For arbitrary $y$, $J(x)/\beta_0$ is given by Eq. 24 if we replace the $\alpha_0/\beta_0$ parentheses () in that equation by $y$. Fig. 3 shows $J(x)/\beta_0$ for $y = 0, y = 1$, and $y = 10$. The $y = 0$ and $y = 1$ curves are also plots of $\theta(x)$ (above, and Eq. 26). The $y = 1$ curve saturates at a value 1, the $y = 0$ curve at $1/2$ (Eq. 36). The $y = 10$ curve corresponds to fairly strong attraction. In the Bragg–Williams (BW) approximation (next section), there is a phase transition at this $y$ (the broken BW curve in Fig. 3 is taken from Fig. 4). As $y$ gets larger, the $J(x)/\beta_0$ curves become flatter and lower, $J/\beta_0 \to 1/y$ (Eq. 27), except for small $x$, where $J/\beta_0 \equiv x \equiv \theta$. Note the dominant qualitative fact that this flux ($1/y$) is very small compared to the maximum flux.
In the special case $f_1 + f_2 = 1$, in this two-state system (1), Eq. 43 takes the equilibrium form

$$\theta = \frac{Yx}{(1 + Yx)}$$

or

$$Yx = \theta/(1 - \theta).$$

As is well known (2), this equation can produce a phase transition. Also, Eq. 44 becomes

$$\frac{J}{J_0} = \frac{Yf_2(1 + x)/(1 + Yx)}{Y(f_1 + f_2)},$$

where $J_0$ is given by equation 10 of ref. 1. We take $f_2 = 1/2$, as usual (1).

We turn now to the special case $w_{11} = w_{12} = 0$, for which $Y = \beta_2$. We define $y = \beta_2^2$ (see the sentence following Eq. 39). Then $Y = \beta_2^2$ and

$$y^{2\theta}x = \theta/(1 - \theta).$$

When $w_{22}/kT$ is very small ($y \to 1$),

$$J/J_0 \to 1 + (yw_{22}/kT)Y_0(2\theta_0 - 1) + \cdots,$$

where $\theta_0 = x/(1 + x)$. The linear correction term here is zero in Fig. 2 because $\theta_0 = 1/2$ ($x = 1$).

Eq. 48 agrees with equation 20 of ref. 1 ($z = 1$) and with Eq. 24 ($z = 2$) in the limit $y \to 1$. It is well known (2) that BW is exact, in equilibrium systems, in the limit $y \to 1$. It appears that BW is also exact for any two-state, steady-state lattice system with $f_2 = 1/2$ (despite the additional kinetic instantaneous approach to steady-state assumption).

Numerical Examples. The broken curve in Fig. 2 is a plot of Eq. 47 ($J/J_0$ as a function of $y$) with $x = 1$. At large $y$, again $J/J_0 \to 2/y$. The slope of $J/y$ becomes infinite as $y \to 0$ (the BW approximation is especially unrealistic in this limit). Generally, the three top curves in Fig. 2 ($M = 2$, $M = \infty$, BW) are very similar.

We next consider $J$ as a function of $x$, with $y$ constant, as in Fig. 3. As in the preceding section, we take $\beta_0 = 0$ and $\alpha_0 = (\beta_0 + \alpha_0)x$. We have here

$$J(x)/\beta_0 = y^{\theta}x/(1 + y^{2\theta}x).$$

Fig. 4 shows $J(x)/\beta_0$ and $\theta(x)/\beta_0$ for a number of choices of $y$. The critical curve, with a cusp in the flux, is $y = e^2$. There is a phase transition for $y > e^2$, for example, for $y = 10$. At the phase transition, there is a discontinuity in $\theta(x)$ but only a discontinuity in the slope of $J(x)$ (the broken regions in the $y = 10$ curves are metastable or unstable). That is, the flux is the same in the two phases (as can be verified analytically). For large $y$, $J/J_0 \to 1/y$ (except at very small $x$, where $J/J_0 \equiv x \equiv \theta$. This is the same asymptotic behavior as in the one-dimensional chain (above).

This phase transition effect in $J$ is interesting physically but rather unlikely biologically. Note that whereas $\theta(x)$ allows the usual single hysteresis loop (ref. 2, p. 251), $J(x)/\beta_0$ would exhibit a corresponding “double loop” (like a bow tie).

In less symmetrical, non-quasi-equilibrium BW cases, the flux is not the same in the two phases (part four, to be published).

Dilute Two-State Enzyme in Solution. This problem will be considered more extensively elsewhere. Here we state a few results, without a detailed proof. We consider the simplest possible case: we take $f_2 = f_2 = 1/2$ so that the steady-state molecular distributions are as at equilibrium; we assume radially dependent distributions only; also, we find only the sec-
The explicit expression for $Z_2/V$ (2) is obtained from spontaneous distance below).

The interaction free energies are $w_i(r)$, with $i, j = 1, 2$. We use the grand partition function method employed in equations (15–78) and (15–79) of ref. 2 for the polarization of an imperfect gas in an electric field. For the second "virial" coefficient, we need consider only one molecule in the macroscopic volume $V$ (with flux $J_1$, denoted by $f_0$ above) and two molecules in $V$ (total flux $J_2$ for the two molecules, including interaction effects). From the above two equations (replacing the electric moment $M_0$ by $f_0$), we find

$$J/J_0 = 1 + \rho \int_0^\infty (1 - \theta_0)(y_{11}y_{12})^{1/2} + \theta_0(y_{22}y_{12})^{1/2}) dr. \quad [51]$$

The integral is the second "virial" coefficient for the flux.

**APPENDIX**

Inspection of Eqs. 18 and 19 shows the following "quasi-chemical" relations (2), mentioned in the text:

$$\overline{N}_{11}/\overline{N}_{22} = y_{11}/y_{12}$$

$$\overline{N}_{111}/\overline{N}_{211} = y_{11}/y_{11}$$

$$\overline{N}_{221}/\overline{N}_{121} = y_{22}/y_{12}$$

$$\overline{N}_{221}/\overline{N}_{121}/\overline{N}_{111}/\overline{N}_{111} = 1. \quad [55]$$

Alternative combinations are possible.

Relations involving $\Xi$ and $\Xi_0$ (Eqs. 28 and 29) are:

$$\phi = M^{-1} \ln \Xi_0 = \Xi_0(M - 3) = \Xi(M)$$

$$x \Xi(M - 2) + \Xi(M - 1) = \Xi(M)$$

$$x \Xi_0(M - 3) + \Xi_0(M - 1) = \Xi(M)$$

$$\Xi(M) = 1 + Mx + \frac{(M - 3)!}{2!} x^2 + \frac{(M - 4)(M - 5)!}{3!} x^3 + \cdots \quad [59]$$

$$\Xi_0(M) = 1 + Mx + \frac{(M - 1)(M - 2)!}{2!} x^2 + \frac{(M - 2)(M - 4)!}{3!} x^3 + \cdots \quad [60]$$

Eqs. 59 and 60 are polynomials that break off at the first zero term (1). Eq. 29 follows from Eqs. 57 and 58.