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Interacting enzyme systems at steady state: Location of the phase transition in approximations of the mean field type

(van der Waals loop/Maxwell's theorem/Ising system/enzyme lattice)

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ABSTRACT **We consider a phase transition "loop," obtained from a mean field type of approximate treatment of a closed *steady-state* Ising system. Where is the cut (stable path) across the loop located? The general procedure, in answering this question, is to pass to an open version of the same system and use the cut that appears automatically in this case (no loop is possible in an open system). This is equivalent to finding the point at which the two phases have equal total probability in the open system. It is shown here that this procedure, when applied to a system of *two-state* enzyme molecules, is formally equivalent to well-known thermodynamic methods (Maxwell's theorem, etc.). These can be applied directly to the closed system without considering the open system explicitly. However, for enzyme molecules with more than two states, the "thermodynamic" method generally fails and one must fall back on the open system procedure mentioned above. Practical implementation of this procedure is not easy.**

In earlier papers of this series (1, 2), the Bragg–Williams (BW) or "mean field" approximation was introduced as one method of treatment of the steady-state kinetics of a lattice of enzyme molecules with nearest-neighbor interactions (i.e., a steady-state Ising system). With strong enough attractive interactions, phase transitions appear in the form of van der Waals "loops." The question arises (just as at equilibrium): if the stable phase transition path is followed, cutting across the loop, where is the cut located? The answer is well known at equilibrium. We discuss the steady-state situation here.

Our comments are limited to systems of the type mentioned above, treated by any approximation that produces a loop. Keizer (3) has recently discussed the same question but for a different class of systems.

Review for a one-component equilibrium system

Certain features of this case, which have already been discussed at some length (4), provide the general approach to the steady-state problem as well. Hence a very brief summary is called for. Consider a lattice gas of M sites, N of which are occupied by molecules. There are attractive interactions between the molecules, leading to a phase transition. If an approximation of the BW or quasichemical (5) type is used on the closed system (independent variables N, M, T), a loop is obtained, for example, in a plot of $\theta \equiv N/M$ against $\lambda = e^{\mu/kT}$ (5). However, if the open version of the same system, to the same approximation, is treated (fluctuations in N are allowed; independent variables μ, M, T), the loop in θ plotted against λ is now missing and a vertical (or almost vertical, if M is large but finite) line takes its place (4). The "open" vertical line cuts the "closed" loop at just that λ prescribed by Maxwell's equal area theorem (applied to a plot of θ against $\ln \lambda$).

The probability that the open system contains N molecules is proportional to $R(N) \equiv Q(N, M, T)\lambda^N$, where Q is the canonical partition function. Let $P(N)$ be $R(N)$ normalized. At an "ordinary" point, $P(N)$ has a single peak centered at $N = \theta(\lambda)M$ for a given λ. However, there are two peaks in $P(N)$ at or near a phase transition, at $N = \theta_1 M$ and $\theta_2 M$, one peak corresponding to each phase (with θ_1 or θ_2). At the center of the phase transition (with λ located by the vertical line mentioned above), $\theta = (\theta_1 + \theta_2)/2$ and the two peaks in $P(N)$ necessarily have the same area or total weight (4).

Thus, although it is not at all necessary at equilibrium, the cut across the "closed" loop *could* be located on the λ axis by passing to the open system and noting where the vertical step in the plot of θ against λ occurs. Alternatively, and completely equivalently, one could note where the two peaks in $P(N)$ have the same area or weight. Exactly the same procedure (based on passing from the closed to the open system) can be followed at steady state. This procedure provides a general method, at least in principle, for steady-state systems. This is noteworthy because, as we shall see, thermodynamic-like methods cannot be applied to an arbitrary closed steady-state system—in fact, such methods can be applied, in general, only to a steady-state system comprised of *two-state* molecules.

Steady-state system of two-state molecules

We show in this section that the treatment of any steady-state system of two-state enzyme molecules, using an approximation of the BW type, can be arranged to parallel the equilibrium treatment of the same kind of system. Hence we find a Maxwell theorem, etc.

We have a lattice system of M interacting enzyme molecules, where M is very large. Each molecule has an unperturbed (i.e., no interactions) kinetic diagram (6) as shown in Fig. 1*a*. Each molecule may be in state 1 or state 2; the system is therefore "open" with respect to the number of molecules N in state 2. In the general interaction problem (7), the open system has 2^M separate states that must be considered. The kinetic diagram for the entire system is therefore *extremely* complicated. However, in the BW approximation, all of the $M!/N!(M-N)!$ states with exactly N molecules in state 2 are equivalent and hence can be grouped together. The kinetic diagram for the complete system therefore simplifies tremendously: the diagram is *linear*, as shown in Fig. 1*b*, with system states characterized by the value of the single variable N. The circumstance that, in the BW approximation, the kinetic diagram for the system is linear is the key point: the equilibrium-like treatment below is practically an automatic consequence of this feature.

In Fig. 1*b*, α_{N-1} is the rate constant for the α transition 1 $\rightarrow$ 2 of any one of the $M - N + 1$ molecules in state 1 (when N

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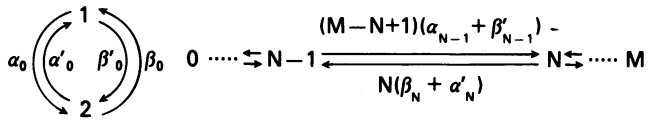
Abbreviation: BW, Bragg–Williams.



a b

FIG. 1. (*a*) First-order rate constants and diagram for two-state enzyme molecule in the absence of interactions. (*b*) Kinetic diagram for a system of M two-state molecules, according to the BW (mean field) approximation. N is the number of molecules in state 2.

− 1 molecules are in state 2). The value of α_{N-1} is α_o multiplied by a factor that takes nearest neighbors into account, according to the BW approximation (1, 2). Similar remarks apply to the other three rate constants in Fig. 1*b*.

If there are no interactions, $\alpha_{N-1} = \alpha_o$, etc., in Fig. 1*b*. In this case. Fig. 1*b* does not represent an approximation. If we define R_N as proportional to the steady-state probability of system-state N, with $R_0 \equiv 1$, then the R_N can be found *seriatim* (because of the linear diagram):

$$R_0 = 1, R_1 = Mx R_0, R_2 = (M-1)\,x\,R_1/2, \text{ etc.,} \quad [1]$$

where $x = (\alpha_o + \beta'_o)/(\beta_o + \alpha'_o)$. Thus we find (see also Eq. 6.53 of ref. 6 and Eq. 7.43 of ref. 8)

$$R_N = M!x^N/N!(M-N)! \quad (0 \le N \le M) \quad [2]$$

In effect, because of the linear diagram, there is "detailed balance" between successive states of the diagram. *At equilibrium*, in lattice gas notation (5), $x = q\lambda$, where q ($\equiv q_2$) is the partition function of a single molecule in state 2 (with $q_1 \equiv 1$). That is (5),

$$R_N = Q_N\lambda^N, Q_N = M!q^N/N!(M-N)!, \quad [3]$$

where Q_N is the canonical partition function.

With interactions included, as is well known (5), R_N can still be written as $Q_N\lambda^N$ at equilibrium, either in an exact or in an approximate treatment. However, at steady state, with interactions, R_N cannot, *in an exact treatment*, be put in the form $\Gamma_N x^N$, as in Eq. **2**. This follows because there is no detailed balance, real or simulated, in the exact system diagram at an *arbitrary* steady state, even with two-state enzyme molecules (1, 7).

In the BW approximation, however, with interactions included, the linear diagram in Fig. 1*b* does allow steady-state R_N of the form $\Gamma_N x^N$. To arrange this, we make the same change of independent kinetic variables as in earlier papers (2, 7): instead of β_o, α'_o, α_o, β'_o (Fig. 1*a*), we use $\beta_o \equiv 1$, α'_o, x, F, where

$$x = (\alpha_o + \beta'_o)/(1 + \alpha'_o) \quad [4]$$

$$F = e^{X/kT} = \alpha_o/\alpha'_o\beta'_o \quad [5]$$

$$\alpha_o = \alpha'_o(1 + \alpha'_o)\,x\,F/(1 + \alpha'_oF) \quad [6]$$

$$\beta'_o = (1 + \alpha'_o)x/(1 + \alpha'_oF). \quad [7]$$

Here X is the thermodynamic force, and α_o and β'_o are both proportional to x. Consequently, when we write R_1/R_0, R_2/R_1, etc., essentially as in Eq. **1**, a factor x appears at each stage (each α_{N-1} and β'_{N-1} have factors α_o and β'_o, respectively, which in turn have factors x). Thus, by virtue of a simulated "detailed balance", we again obtain $R_N = \Gamma_N x^N$, but here Γ_N includes not only $M!/N!(M-N)!$, as in Eq. **2**, but also a factor involving the intermolecular interactions of the model.

The final ingredient required, before proceeding to the main argument, is the observation that, when M is very large, the steady-state system we are considering here will have extensive and intensive properties, just as an equilibrium system does. Thus, for example, if we double the size of the system (M), holding all kinetic and interaction parameters constant (these are intensive variables), then all other extensive variables will also double; for example, the total enzyme flux, $\ln \Gamma_N$ (see Eq. **2**, for example), N, etc.

The method to be outlined below is critically dependent on the definitions of (*a*) an intensive variable x that appears at each stage R_1/R_0, R_2/R_1, etc., and of (*b*) an extensive variable $\ln \Gamma_N$ (N large) such that the unnormalized probability ($R_0 \equiv 1$) R_N has the form $\Gamma_N x^N$.

The normalized probability $P(N)$ (we discontinue the use of *subscript* N in the remainder of this section) that the open BW system has N molecules in state 2 is

$$P(N; x, M) = R(N; x, M)/S(x, M) \quad [8]$$

$$R(N; x, M) = \Gamma(N, M)x^N, S(x, M) = \sum_N R(N; x, M). \quad [9]$$

Here, S is analogous to a grand partition function and Γ to a canonical partition function (we are omitting the temperature T from the notation). It follows from Eqs. **8** and **9** that

$$\overline{N} = x(\partial \ln S/\partial x)_M \quad [10]$$

$$\sigma^2/M \equiv (\overline{N^2} - \overline{N}^2)/M = x\partial\theta/\partial x, \quad [11]$$

where $\theta = \overline{N}/M$. The variance σ^2 is of order M; the peak in $P(N)$, for an "ordinary" point, occurring at, say, $N = N^*$ will be extremely sharp if M is very large. Therefore, we can replace $\ln S$ by the logarithm of the maximum term in S (5).

To do this, we use

$$\ln R = \ln \Gamma + N \ln x \quad [12]$$

$$\partial \ln R/\partial N = 0 = (\partial \ln \Gamma/\partial N)_M + \ln x. \quad [13]$$

The value of N that satisfies Eq. **13** is $N = N^*$. In the equations below it is to be understood that N represents N^*. In effect, we thereby treat the open system as virtually closed, because of the small fluctuations in N (5). In Eq. **12**, we replace $\ln R$ by $\ln S$ and use Eq. **13** to eliminate $\ln x$:

$$\ln S = \ln \Gamma - N(\partial \ln \Gamma/\partial N)_M. \quad [14]$$

We also have

$$d\ln \Gamma = (\partial \ln \Gamma/\partial N)_M\, dN + (\partial \ln \Gamma/\partial M)_N dM \quad [15]$$

$$\ln \Gamma = N(\partial \ln \Gamma/\partial N)_M + M(\partial \ln \Gamma/\partial M)_N, \quad [16]$$

where Eq. **16** follows (Euler's theorem) because $\ln \Gamma$, N, and M are extensive properties while the two derivatives are intensive properties of the system. On comparing Eqs. **14** and **16**, we have

$$\ln S = M\Phi, \Phi \equiv (\partial \ln \Gamma/\partial M)_N. \quad [17]$$

Here Φ is the analogue of an equilibrium pressure (in dimensionless units).

Equations **14** and **15** can be rewritten as

$$\ln S = M\Phi = \ln \Gamma + N \ln x \quad [18]$$

$$d\ln \Gamma = -\ln x\, dN + \Phi dM. \quad [19]$$

Thus we find

$$d\ln S = d(M\Phi) = \Phi dM + N d\ln x \quad [20]$$

and also

$$d\Phi = \theta\, d\ln x, \Phi(x) = \int_0^x \theta)x')d\ln x', \quad [21]$$

where $\theta = N/M$. Eq. 21 shows how to calculate Φ if $\theta(x)$ is known [e.g., from the BW treatment of the closed system (2)].

The above equations all have well-known thermodynamic analogues but it should be noted particularly that thermodynamics *per se* has not been used in the derivation.

We turn now to the situation at a phase transition. In the open steady-state system, just as at equilibrium (4), $P(N)$ will have two sharp peaks (phases) centered at $N_1 = \theta_1(x)M$ and $N_2 = \theta_2(x)M$. Suppose the two peaks have equal areas (total probabilities) at $x = x_t$. Then, at this x,

$$\theta = \bar{N}/M = (\theta_1 + \theta_2)/2. \quad [22]$$

This is where the vertical step in $\theta(x)$ occurs. For x slightly different from x_t, one or the other peak will be very small (4) (a metastable state, possibly followed in a hysteresis loop). For an x at or near x_t, let $R_1(x)$ and $\sigma_1^2(x)$ be the largest $R(N)$ and the variance of the first peak, respectively, and let $R_2(x)$ and $\sigma_2^2(x)$ have the same meaning for the second peak. The condition that the two peaks have equal areas (to locate x_t) is equivalent to, for Gaussian peaks,

$$R_1(x_t)\sigma_1(x_t) = R_2(x_t)\sigma_2(x_t) \quad [23]$$

or, to terms of macroscopic order of magnitude,

$$\ln S_1(x_t) = \ln S_2(x_t) \text{ or } \ln R_1(x_t) = \ln R_2(x_t). \quad [24]$$

Here, S_1 represents the sum in Eq. 9 over the first peak, etc., and the second of Eqs. 24 follows because $\ln R_i$ is of order M while $\ln \sigma_i$ is of order $\ln M$ (this is, of course, also the justification for the maximum term procedure in Eqs. 12–14).

Finally, we note that the equal peak area condition, $\ln S_1 = \ln S_2$, is the same as the condition $\Phi_1 = \Phi_2$, in view of Eq. 17. Thus, if we have derived $\theta(x)$ for a closed BW system, we can find $\Phi(x)$ from Eq. 21 and then locate the phase transition at $x = x_t$ by the condition $\Phi_1 = \Phi_2$. Although we have used the mathematically more complicated open system to deduce this procedure, we need not actually consider the open system in the *application* of the method. It suffices to work with the simpler closed system exclusively.

Maxwell's equal area theorem holds here if applied to a plot of θ against $\ln x$. This follows from Eq. 21:

$$\Phi_2 - \Phi_1 = 0 = \int_1^2 \theta(x')d\ln x', \quad [25]$$

where the integral is over the loop from θ_1,x_t to θ_2,x_t. Maxwell's theorem is obviously equivalent to $\Phi_1 = \Phi_2$.

The above results exactly parallel those for an equilibrium system. Unfortunately, the formal resemblance is limited to two-state enzyme molecules.

Three-state enzyme molecules

As a simple example of a more complicated system, suppose we have M three-state enzyme molecules (1, 9) in a lattice. The diagram for a single unperturbed molecule is shown in Fig. 2*a*. In the absence of interactions, the steady-state probabilities p_i for each of three states can easily be expressed in terms of the six rate constants α_{ij} belonging to Fig. 2*a* (6). If we then define $x_2 = p_2/p_1$ and $x_3 = p_3/p_1$, the generalization of Eq. 2, with the same physical significance, is (ref. 6, Eq. 6.53)

$$R_{N_2N_3} = M!{x_2}^{N_2}{x_3}^{N_3}/N_2!N_3!(M - N_2 - N_3)! \quad [26]$$

However, it is easy to see, from simple examples (M small), that for the BW approximation of the system *with interactions*, it is *not* possible in general to put $R_{N_2N_3}$ in the form

$$R_{N_2N_3} = \Gamma_{N_2N_3}{x_2}^{N_2}{x_3}^{N_3}, \quad [27]$$

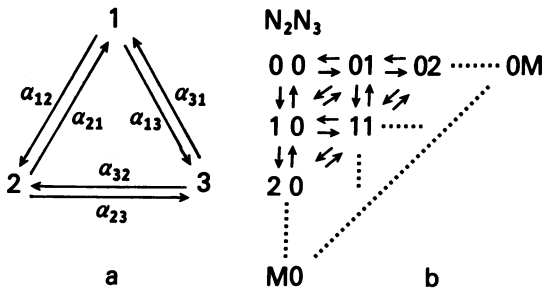


FIG. 2. (*a*) First-order rate constants and diagram for three-state enzyme molecule in the absence of interactions. (*b*) Schematic kinetic diagram for a system of M three-state molecules, according to the BW approximation. N_2 is the number of molecules in state 2, etc.

with $\Gamma_{N_2N_3}$ independent of x_2 and x_3. This is a consequence of the absence of any sort of "detailed balance" in this case.

At equilibrium, for a two-component system (*not* based on Fig. 2*a*), the right-hand side of Eq. 27 is $Q_{N_2N_3}{\lambda_2}^{N_2}{\lambda_3}^{N_3}$, in conventional notation (5), whether there are interactions or not and whether or not any approximation is used in handling the interactions. Because the three-state steady-state BW system does not follow this same pattern, equilibrium-like methods of locating a phase transition are inapplicable in this case (unlike the two-state steady-state situation above). This will generally be true for arbitrary steady-state BW systems.

How, then, is the phase transition located, for example, in the three-state BW case above? The only potentially exact method we are aware of is to fall back on the basic property that, at a transition point (x_{2t}, x_{3t}) in the *open* system, the total probabilities of the two peaks in $P_{N_2N_3}$ ("volumes" under the peaks, in this case) must be equal. One is therefore obliged to use the open BW system explicitly.

We mention two possible approaches to the implementation of the above "equal peak volume" requirement, though we have not actually used either one as yet.

At the outset, Fig. 1*b* must be extended to two dimensions, as shown *schematically* in Fig. 2*b*. This is the system diagram for the three-state model in Fig. 2*a* when treated according to the BW approximation. There are 3^M system states altogether, but they can be collected, in this approximation, according to N_2, N_3 values. Because the limit of very large M is of primary interest, one approach would be to use the differential equation in the continuous function $P(N_2,N_3)$, derived from the difference equation in $P_{N_2N_3}$. One would need, for arbitrary x_2, x_3, the solution of the differential equation in the neighborhood of the two Gaussian peaks and in the region *between the peaks* (to establish their relative sizes). Incidentally, it is easy to locate the *position* (N_2,N_3) of the peaks and of the minimum between them [from the closed system treatment (1, 9)], for given x_2,x_3. The problem is to find the relative peak sizes.

A second method would be to use an extremely long Monte Carlo walk (10) on the diagram in Fig. 2*b* for M large but finite. This walk would generate $P_{N_2N_3}$ values (10). An alternative and much more efficient procedure might be to start each of a very large number of walks at the minimum (which is generally extremely flat and low) between the two peaks and then record the fraction of walkers "captured" by each of the two peaks ("capture" meaning first crossing of a perimeter around the base of a peak).

An explicit two-state example

For the BW model already introduced in Fig. 1 and Eqs. 4–7, the explicit expression for $x(\theta)$, in a closed system, is (2)

$$x = \frac{(1 + \alpha_o' F)\theta\,(1 + \alpha_o' Y^{f-1})}{(1 + \alpha_o')(1 - \theta)Y\,(1 + \alpha_o' F Y^{f-1})}, \quad [28]$$

where

$$f = f_\alpha + f_\beta,\ Y = ry^{2\theta}$$
$$r = (y_{12}/y_{11})^z,\ y = (y_{11}y_{22}/y_{12}^2)^{z/2}$$
$$y_{ij} = e^{-w_{ij}/kT}\ (i,j = 1,2).$$

Here f_α and f_β are constant kinetic parameters (2, 9), z is the number of nearest neighbors of a molecule in the lattice, and w_{ij} is the interaction free energy between nearest neighbors in states i and j. From Eqs. **21** and **28**, after integrating by parts, we find for Φ, expressed as a function of θ,

$$\Phi = -\ln(1 - \theta) - \theta^2 \ln y + \theta \ln\left[\frac{\alpha_o' + r^{1-f}\eta(\theta)}{\alpha_o' F + r^{1-f}\eta(\theta)}\right] + [H(\theta)/2(1 - f)\ln y], \quad [29]$$

where

$$H(\theta) = \int_1^{\eta(\theta)} \ln\left(\frac{\alpha_o' F + r^{1-f}\eta'}{\alpha_o' + r^{1-f}\eta'}\right)\frac{d\eta'}{\eta'} \quad [30]$$
$$\eta(\theta) = y^{2(1-f)\theta}.$$

Usually the integral H must be evaluated numerically. Using Φ from Eq. **29**, and Eq. **18**,

$$\ln S = M\Phi, \quad (1/M)\ln \Gamma = \Phi - \theta \ln x. \quad [31]$$

A number of examples of phase transitions (in the form of loops), based on Eq. **28**, have been presented elsewhere (2). In several of these cases, as a check, we have located x_t by: (*a*) use of Eq. **29** and $\Phi_1 = \Phi_2$; (*b*) numerical integration of Eq. **25** (Maxwell's theorem); and (*c*) for the open system, explicit step-by-step calculation of all the R_N and P_N (normalized) based on Fig. 1*b*, for M = 1000, 5000, or 10,000. Equality of the two Gaussian peaks in P_N was used to find x_t. The three methods agree, as expected. For example, in one case (y = 50 in figure 12 of ref. 2), x_t = 1.6902 from $\Phi_1 = \Phi_2$ ($M = \infty$), x_t = 1.6897 for M = 10,000 in method *c*, and x_t = 1.6855 for M = 1000 in method *c*. Other properties were cross-checked (open compared to closed), as well, such as θ_1, θ_2, Eq. **11** (for each peak), and Eq. **23**.

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1. Hill, T. L. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4111–4115.
2. Hill, T. L. (1978) *J. Chem. Phys.*, in press.
3. Keizer, J. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3023–3026.
4. Hill, T. L. (1956) *Statistical Mechanics* (McGraw-Hill, New York), Appendix 9, pp. 413–423.
5. Hill, T. L. (1960) *Statistical Thermodynamics* (Addison-Wesley, Reading, MA).
6. Hill, T. L. (1977) *Free Energy Transduction in Biology* (Academic, New York).
7. Hill, T. L. (1978) *J. Chem. Phys.*, in press.
8. Hill, T. L. (1968) *Thermodynamics for Chemists and Biologists* (Addison-Wesley, Reading, MA).
9. Hill, T. L. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3632–3636.
10. Hill, T. L. & Chen, Y. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1291–1295.