A minimum-reaction-flux solution to master-equation models of protein folding

Huan-Xiang Zhou[a)]
Department of Physics and Institute of Molecular Biophysics and School of Computational Science, Florida State University, Tallahassee, Florida 32306, USA

(Received 25 January 2008; accepted 24 April 2008; published online 20 May 2008)

Master equations are widely used for modeling protein folding. Here an approximate solution to such master equations is presented. The approach used may be viewed as a discrete variational transition-state theory. The folding rate constant k_f is approximated by the outgoing reaction flux J, when the unfolded set of macrostates assumes an equilibrium distribution. Correspondingly the unfolding rate constant k_u is calculated as $Jp_u/(1-p_u)$, where p_u is the equilibrium fraction of the unfolded state. The dividing surface between the unfolded and folded states is chosen to minimize the reaction flux J. This minimum-reaction-flux surface plays the role of the transition-state ensemble and identifies rate-limiting steps. Test against exact results of master-equation models of Zwanzig [Proc. Natl. Acad. Sci. USA **92**, 9801 (1995)] and Muñoz *et al.* [Proc. Natl. Acad. Sci. USA **95**, 5872 (1998)] shows that the minimum-reaction-flux solution works well. Macrostates separated by the minimum-reaction-flux surface show a gap in p_{fold} values. The approach presented here significantly simplifies the solution of master-equation models and, at the same time, directly yields insight into folding mechanisms. © *2008 American Institute of Physics*.
[DOI: 10.1063/1.2929824]

I. INTRODUCTION

Master equations have been used to develop both conceptual and quantitative models for protein folding.[1–15] In such models, the conformational space of a protein is reduced to a discrete collection of macrostates, and transitions between the macrostates are described as rate processes. With the increase in the number of macrostates, the folding problem can be modeled more and more realistically. However, concomitantly the solution of the master equations becomes more cumbersome, and moreover, mechanistic insight becomes more difficult to be gained from such a solution. Here we present an approximate solution, based on a variational transition-state theory approach. This approach significantly simplifies the solution of master-equation models and, at the same time, directly yields insight into folding mechanisms.

The approach presented here can trace its origin to a 2005 review paper.[15] It was recognized that an approximate solution, proposed by Zwanzig[2] based on a local thermodynamic equilibrium assumption for his master-equation model can be obtained from a transition-state theory. Zwanzig's model is one dimensional, i.e., the macrostates are specified by a single discrete variable, which is the number of ordered residues in a protein. There is a natural choice for the dividing surface between the unfolded and folded states. The idea of applying transition-state theory to master-equation models seems promising. However, for higher-dimensional models it was not clear how to determine the dividing surface.

In a transition-state theory for calculating the folding rate constant k_f,[15] the unfolded set of macrostates is assumed to take an equilibrium distribution (and the folded state unoccupied). One then obtains

$$k_f = J, \tag{1}$$

where J is the outgoing reaction flux. Because the transition-state theory overestimates the folding rate constant, we thus determine the dividing surface between the unfolded and folded states by minimizing the reaction flux J. This way of obtaining k_f is similar in spirit to a variational transition-state theory, which was originally developed for the (continuous) phase space of a three-body system.[16] Our approach is specifically applied to problems with discrete macrostates, modeled by master equations, and will be referred to as the minimum-reaction-flux (MRF) approach.

In Sec. II we outline the MRF approach. Section III presents applications to master-equation models of Zwanzig[2] and of Muñoz *et al.*[5] Implications and extensions of the present approach are discussed in Sec. IV.

II. MRF APPROACH

We consider a model for protein folding in which the protein conformational space is reduced to discrete macrostates specified by more than one index. For concreteness, we consider the case of two indices, i and j. For example, in the model of Muñoz *et al.*,[5] all but one macrostate are specified by a single stretch of contiguous ordered peptide bonds, with i representing the start position and j representing the length of the ordered stretch. Let the transition rate from macrostate (i,j) to macrostate (i',j') be $\omega(i,j\to i',j')$. These rates are always assumed to satisfy the detailed balance condition:

a)Electronic mail: zhou@sb.fsu.edu. Tel.: (850) 645-1336. FAX: (850) 644-7244.

$$P_{eq}(i,j)\omega(i,j \to i',j') = P_{eq}(i',j')\omega(i',j' \to i,j), \tag{2}$$

where $P_{eq}(i,j)$ is the equilibrium probability of macrostate (i,j), which is normalized, i.e.,

$$\sum_{i,j} P_{eq}(i,j) = 1. \tag{3}$$

The detailed balance condition guarantees that, once all the macrostates take their equilibrium probabilities, they will stay in that distribution. If the macrostates start from a different distribution, the time dependence of the probabilities $P(i,j,t)$ is governed by

$$\frac{dP(i,j,t)}{dt} = \sum_{(i',j')\neq(i,j)} P(i',j',t)\omega(i',j' \to i,j) - P(i,j,t)\sum_{i',j'} \omega(i,j \to i',j'). \tag{4}$$

In matrix-vector, this equation can be written as

$$\frac{d\mathbf{P}(t)}{dt} = -\omega \cdot \mathbf{P}(t). \tag{5}$$

No matter what the initial conditions are, $P(i,j,t)$ will relax to the equilibrium value, $P_{eq}(i,j)$, at long times. Note that $P(i,j,t)$ is normalized at all times. The exact solution of Eq. (4) can be obtained by numerical integration,[2,5] by diagonalizing the transition matrix[4,6,11] or by kinetic simulation.[8]

Experimental observables such as fluorescence intensity can be obtained as a weighted average of the probabilities of the macrostates,

$$y(t) = \sum_{i,j} \theta(i,j)P(i,j,t). \tag{6}$$

Let the unfolded and folded sets of macrostates be denoted by Γ_u and Γ_f, respectively. Then the fractional populations $u(t)$ and $f(t)$ of the two states can be calculated as

$$u(t) = \sum_{(i,j)\in\Gamma_u} P(i,j,t), \tag{7a}$$

$$f(t) = \sum_{(i,j)\in\Gamma_f} P(i,j,t) = 1 - u(t), \tag{7b}$$

which can be viewed as special cases of Eq. (6) [the second identity of Eq. (7b) is derived from the normalization condition of $P(i,j,t)$]. The equilibrium probabilities, p_u and $p_f = 1-p_u$, are obtained when $P(i,j,t)$ takes the equilibrium value, $P_{eq}(i,j)$,

$$p_u = \sum_{(i,j)\in\Gamma_u} P_{eq}(i,j), \tag{8a}$$

$$p_f = \sum_{(i,j)\in\Gamma_f} P_{eq}(i,j) = 1 - p_u. \tag{8b}$$

Master-equation models typically produce a single-exponential relaxation for $y(t)$,

$$y(t) = y(\infty) + [y(0) - y(\infty)]e^{-k_R t} \tag{9}$$

after a brief transient period. A single-exponential relaxation occurs when the smallest nonzero eigenvalue λ_1 of the transition matrix ω is much smaller than other nonzero eigenvalues. The relaxation rate constant k_R is then simply λ_1. Single-exponential relaxation is an indication that equilibration among the macrostates follows a two-state rate process

$$U \underset{k_u}{\overset{k_f}{\rightleftarrows}} F. \tag{10}$$

In such a process, $u(t)$ and $f(t)$ are governed by

$$\frac{du(t)}{dt} = -k_f u(t) + k_u f(t), \tag{11a}$$

$$\frac{df(t)}{dt} = k_f u(t) - k_u f(t). \tag{11b}$$

The folding and unfolding rate constants k_f and k_u can be obtained from the following relations:

$$k_f + k_u = k_R, \tag{12a}$$

$$k_f/k_u = p_u/p_f = p_u/(1-p_u). \tag{12b}$$

Let us consider a special initial condition, with the unfolded set of macrostates occupied according to an equilibrium distribution and the folded state unoccupied. That is,

$$P(i,j,0) = P_{eq}(i,j)/p_u \quad \text{if } (i,j) \in \Gamma_u \tag{13a}$$

$$= 0 \quad \text{if } (i,j) \in \Gamma_f. \tag{13b}$$

Correspondingly $u(0)=1$ and $f(0)=0$. Summing Eq. (4) over the unfolded state set of macrostates and evaluating at $t=0$, we find

$$\left.\frac{du(t)}{dt}\right|_{t=0} = -\sum_{(i,j)\in\Gamma_u} P(i,j,0) \sum_{(i',j')\in\Gamma_f} \omega(i,j \to i',j') = -p_u^{-1} \sum_{(i,j)\in\Gamma_u} P_{eq}(i,j) \sum_{(i',j')\in\Gamma_f} \omega(i,j \to i',j') \equiv -J, \tag{14}$$

where J represents the total reaction flux, at $t=0$, from the unfolded set of macrostates to the folded set of macrostates (see Fig. 1). If we assume that the rate equation, Eq. (11a), is valid at $t=0$, then

$$\left.\frac{du(t)}{dt}\right|_{t=0} = -k_f u(0) + k_u f(0) = -k_f. \tag{15}$$

Comparison of Eqs. (14) and (15) leads to Eq. (1). Correspondingly the unfolding rate constant k_u is given by $Jp_u/(1-p_u)$ [see Eq. (12b)].

Predicting the folding rate constant by the reaction flux calculated under the special initial condition given by Eq. (13) may be viewed as a general form of transition-state theory. This prediction actually overestimates the folding rate constant (see Appendix). The "derivation" of Eq. (1) for the folding rate constant presented above hinges on the use of the rate equation, Eq. (11), at $t=0$. This use is not rigorous because single-exponential relaxation takes place only after a transient period and thus does not strictly apply at $t=0$. In approximating the folding rate constant by the reaction flux

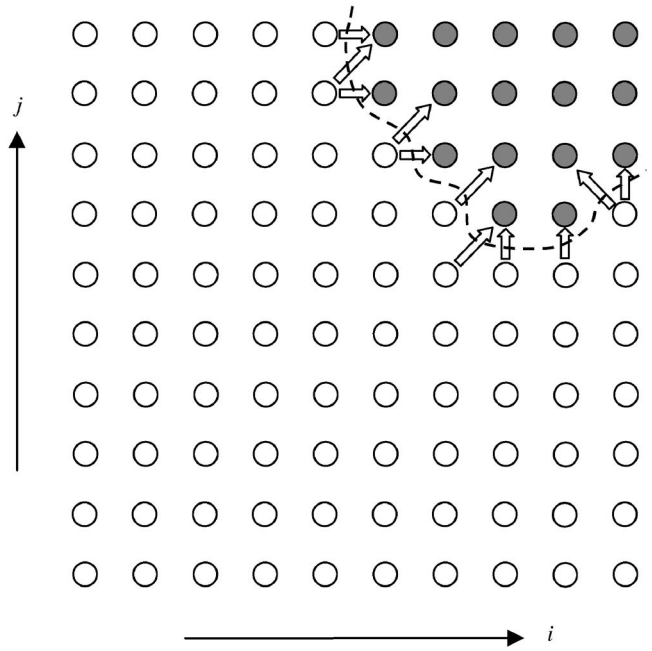

FIG. 1. Illustration of the MRF approach. Circles, as specified by indices i and j, represent macrostates. Open and filled circles are located in the unfolded and folded states, respectively. The dividing surface, as shown by the dotted curve, minimizes the outgoing reaction flux J. This flux consists of individual transitions, as indicated by block arrows, from macrostates in the unfolded state to those in the folded state.

J, we still have the freedom of choosing the dividing surface between the unfolded and folded states. To make this approximation as accurate as possible, it is then necessary to choose the dividing surface which would minimize the reaction flux (in the spirit of variational transition-state theory). A procedure to locate the MRF surface is presented in the next section.

III. APPLICATIONS

Here we first give the procedure for locating the dividing surface leading to the minimum reaction flux and then present its applications to two well-known master-equation models of protein folding.

A. Location of MRF surface

Our simple procedure consists of the following steps:

(1) Start with a subset of manifestly unfolded macrostates; calculate the outgoing flux J_0 for this subset and identify all macrostates not in the initial subset but are "connected" to it. A connection from one macrostate, (i,j), to another, (i',j'), exists if the transition rate $\omega(i,j\rightarrow i',j')$ is nonzero.

(2) For each macrostate connected to the initial subset, calculate the change in the reaction flux if this macrostate is included in the unfolded subset. If there are more than one such connected macrostate, the one leading to the largest decrease in J_0 is included. The expanded subset is then taken as the new initial subset and the process is repeated until J_0 can be no longer decreased.

(3) The last subset is taken as the unfolded set and the last J_0 taken as the minimized reaction flux.

The applications below will further illustrate this procedure.

B. Model of Zwanzig

In this model,[2] each of N residues in a protein can be either ordered or disordered. There are thus a total of 2^N microstates. A macrostate is specified by the total number i of ordered residues, regardless where these residues occur within the protein sequence.[17] There are a total of $N+1$ macrostates. The multiplicity Ω_i, i.e., the number of microstates corresponding to a single macrostate with i ordered residues, is $C_N^i=N!/i!(N-i)!$. Each additional ordered residue brings changes of ΔH in enthalpy and of ΔS in entropy. In addition, when the last residue becomes ordered, the free energy of the protein is lowered by ε. The free energy of macrostate i (with i ordered residues) is

$$\Delta G(i) = i(\Delta H - T\Delta S) - \delta_{iN}\varepsilon, \tag{16}$$

where T is the absolute temperature and δ_{iN} is a Kronecker delta. The partition function is

$$Q = \sum_{i=0}^{N} C_N^i e^{-\Delta G(i)/RT} = [1 + e^{-(\Delta H - T\Delta S)/RT}]^N + (e^{\varepsilon/RT} - 1)e^{-N(\Delta H - T\Delta S)/RT}, \tag{17}$$

where R is the gas constant. The equilibrium probabilities of the macrostates are

$$P_{\mathrm{eq}}(i) = Q^{-1}C_N^i e^{-i(\Delta H - T\Delta S)/RT}, \quad 0 \leqslant i \leqslant N-1 \tag{18a}$$

$$= Q^{-1}e^{\varepsilon/RT}e^{-N(\Delta H - T\Delta S)/RT}, \quad i = N. \tag{18b}$$

To assign the transition rates, the rate constant for the disorder-to-order transition of a single residue is assumed to be k_0. For each of the microstates comprising macrostate i, any of the $(N-i)$ disordered residues can undergo a disorder-to-order transition to reach macrostate $i+1$. The transition from macrostate i to macrostate $i+1$ thus has rate

$$\omega(i \rightarrow i+1) = (N-i)k_0, \quad 0 \leqslant i \leqslant N-1. \tag{19}$$

The rate for the reverse transition can be obtained by detailed balance [see Eq. (2)]. The result is

$$\omega(i+1 \rightarrow i) = (i+1)k_0 e^{(\Delta H - T\Delta S)/RT}, \quad 0 \leqslant i \leqslant N-2 \tag{20a}$$

$$= Nk_0 e^{-\varepsilon/RT}e^{(\Delta H - T\Delta S)/RT}, \quad i = N-1. \tag{20b}$$

Rates for all other transitions are zero.

To obtain the MRF solution, we take the subset of macrostates from 0 to i as the initial guess for the unfolded state. From this subset, there is only one outgoing transition, i.e., the one from macrostate i to macrostate $i+1$. The outgoing reaction flux [see Eq. (14)] is

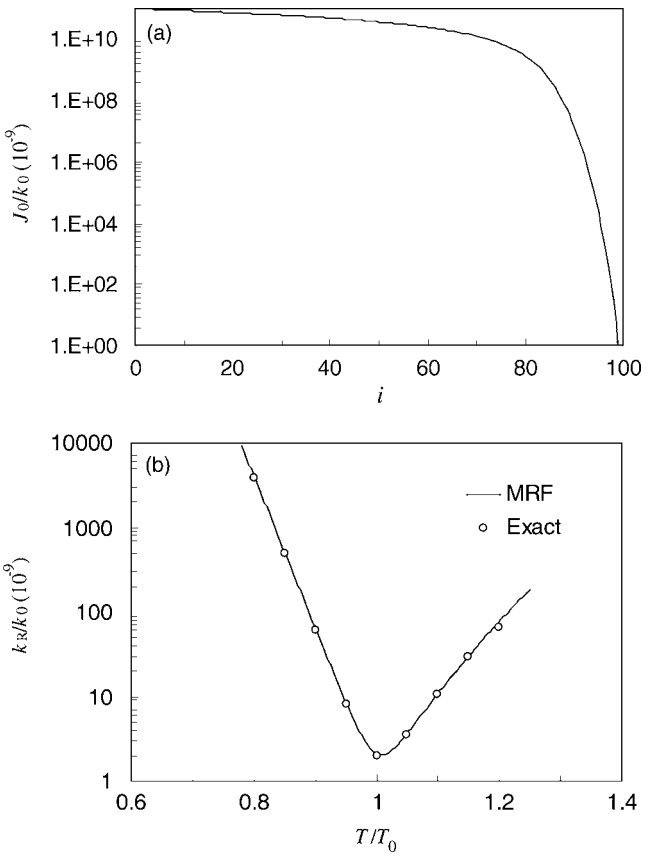

FIG. 2. (a) Dependence of the outgoing reaction flux on the putative upper bound of the unfolded set of macrostates in the model of Zwanzig. Results at $T/T_0=1$ are shown, but the same qualitative dependence on i is also found for other temperatures. (b) Comparison of the MRF prediction for k_R against the exact result. The exact result is obtained from numerical integration of the master equation, with the initial condition $P(i,t=0)=\delta_{i0}$. All numerical integration is done by the LSODE program (Ref. 24).

$$J_0(i)=\frac{P_{\rm eq}(i)\omega(i\to i+1)}{\Sigma_{i'=0}^{i}P_{\rm eq}(i')}. \tag{21}$$

In Fig. 2(a) we show the values of J_0 as the upper bound i of the putative set of unfolded macrostates expands from 0 to $N-1$. Model parameters are those selected by Zwanzig ($N=100$, $\Delta H/RT_0=-2$, $\Delta S/R=-\ln 2$, and $\varepsilon/RT_0=24$; T_0 is a reference temperature). It can be seen that J_0 decreases monotonically with increasing i. The minimum of the reaction flux occurs at $i=N-1$, when the unfolded set includes all but the last macrostate. That macrostate, with all the N residues ordered, then constitutes the folded state. The values of J_0 span 11 orders of magnitude, and the decrease becomes very sharp near the minimum. The disparity in J_0 between $i=N-1$ and nearly all preceding values of i is an indication that equilibration among macrostates with $i<N-1$ is fast and that the transition from macrostate $N-1$ to macrostate N is the rate-limiting step for folding.

The minimum J_0 gives the predicted folding rate constant

$$k_f=J_0(i=N-1)=\frac{Nk_0e^{(\Delta H-T\Delta S)/RT}}{(1+e^{(\Delta H-T\Delta S)/RT})^N-1}. \tag{22a}$$

Correspondingly the unfolding rate constant is

$$k_{\rm u}=\frac{[1-P_{\rm eq}(N)]k_f}{P_{\rm eq}(N)}=Nk_0e^{(-\varepsilon+\Delta H-T\Delta S)/RT}. \tag{22b}$$

These results are identical to those obtained by Zwanzig[2] by "guessing" that the unfolded state is comprised of macrostates 0 to $N-1$ and assuming that equilibration within the unfolded state is fast. As noted previously,[15] Eq. (22a) and (22b) is what is predicted by transition-state theory if the unfolded state is as specified by Zwanzig. What we show here is that such a specification is indeed optimal, in the sense that the predicted folding rate constant is the most accurate among all possible specifications for the unfolded state.

In Fig. 2(b) the predicted relaxation rate constant, $k_R=k_f+k_u$, by Eq. (22a) and (22b) is compared against the exact result obtained by numerically integrating the master equation and then fitting the time dependence of the fractional population of macrostate N to a single exponential.[18] We find that $P(N,t)$ fits well to a single exponential in the temperature range $0.8<T/T_0<1.2$ but poorly outside this range. In line with what is reported by Zwanzig, the agreement between the exact result and the prediction by Eq. (22a) and (22b) is very good.

C. Model of Muñoz *et al.*

Muñoz *et al.*[5] developed a model for β-hairpin formation and used it to analyze their experimental data[19] on a peptide consisting of residues 41-56 of streptococcal protein G B1 (Fig. 3). Instead of residue, peptide bond is used as the basic unit of the peptide. Each of the 15 peptide bonds can be either ordered or disordered, resulting in a total of $2^{15}=32\,768$ microstates. Each microstate with a single stretch of ordered peptide bonds constitutes a unique macrostate, which is specified by two indices: i, the starting position of the stretch, and j, the length of the stretch. The microstate without any ordered peptide bonds and all microstates with more than one stretch of ordered peptide bonds are lumped into a single macrostate. This macrostate will be assigned indices $(i,j)=(0,0)$. There are 15 macrostates with $j=1,14$ macrostates with $j=2,\ldots$, and 1 macrostate with $j=15$. Along with the (0, 0) macrostate, there are a total of 121 macrostates. Below we denote the total number of peptide bonds in the peptide as n and use n and 15 interchangeably.

Each ordered peptide bond brings a change of ΔS in entropy. Additional changes in free energy only apply to macrostates with a single stretch of ordered peptide bonds (i.e., $j>0$). First, hydrogen bonds are formed between peptide bonds i and $16-i$ if they are ordered (which means that the intervening peptide bonds are also ordered due to the single-stretch restriction). Each hydrogen bond brings a change of ΔH in enthalpy. Second, three hydrophobic interactions, between residues W43 and F52, between Y45 and F52, and between W43 and V54, can occur. The first interaction occurs when peptide bonds 3 and 11 are both ordered; for the second and third hydrophobic interactions, the pairs of peptide bonds involved are 5 with 11 and 3 with 13, respectively. Each hydrophobic interaction changes the free energy by $\Delta G_{h\phi}$. If the total numbers of hydrogen bonds and

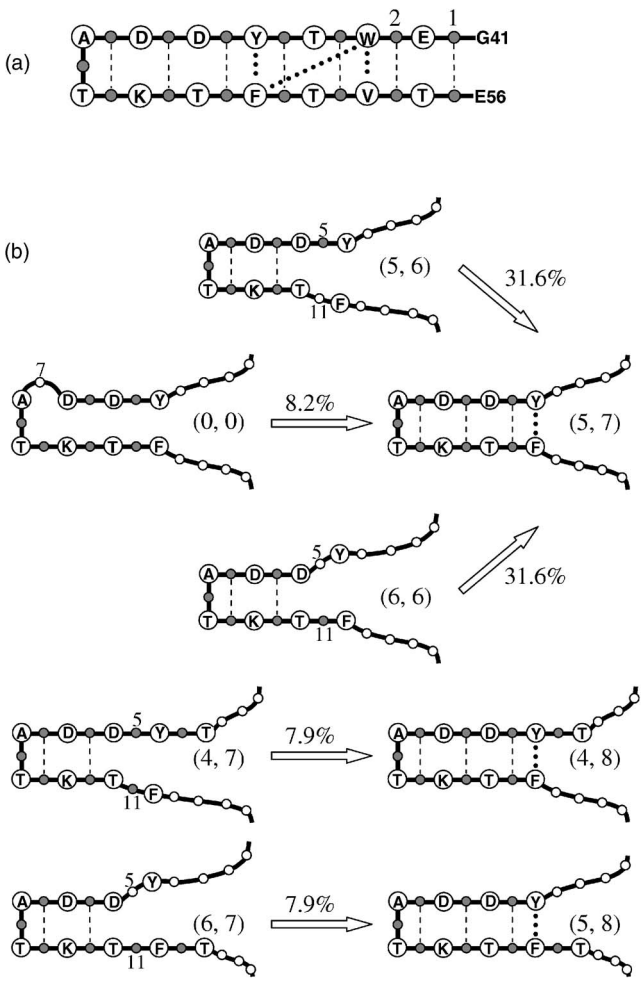

FIG. 3. (a) The model of Muñoz *et al.* Filled small circles represent peptide bonds; position numbers of the first two peptide bonds are labeled. Thin dashed lines represent hydrogen bonds, and thick dotted lines represent hydrophobic interactions. (b) The five transitions making the most contributions to the minimum reaction flux. Filled and open small circles represent ordered and disordered peptide bonds, respectively. Macrostates are identified by a pair of numbers in parentheses, with the first number indicating the start position of the stretch of ordered peptide bonds and the second number indicating the length. Position numbers for some peptide bonds are shown. Percent contributions of the transitions to the reaction flux are shown next to block arrows.

hydrophobic interactions in macrostate (i,j) (with $j>0$) are $n_{hb}(i,j)$ and $n_{h\phi}(i,j)$, respectively, then the free energy of this macrostate is

$$\Delta G(i,j) = n_{\mathrm{hb}}(i,j)\Delta H + n_{h\phi}(i,j)\Delta G_{h\phi} - jT\Delta S. \tag{23}$$

The partition function is

$$Q = (1+e^{\Delta S/R})^n - \sum_{j=1}^{n}\sum_{i=1}^{n-j+1} e^{j\Delta S/R} + \sum_{j=1}^{n}\sum_{i=1}^{n-j+1} e^{-\Delta G(i,j)/RT}, \tag{24}$$

where the first line is the statistical weight, denoted as $w(0,0)$ for future reference, of the (0, 0) macrostate, and the exponential factor inside the double summation in the second line is the statistical weight of macrostate (i,j) (with $j>0$). The ratios of these statistical weights and Q give the equilibrium probabilities of the macrostates.

Transitions between macrostates with $j>0$ are assumed to occur through extension or contraction at the ends of the single stretch of ordered peptide bonds. The rates are

$$\omega(i,j \to i,j+1) = \omega(i,j \to i-1,j+1) = k_0 e^{\Delta S/R}, \tag{25a}$$

$$\omega(i,j \to i,j-1) = k_0 e^{\Delta S/R} e^{-[\Delta G(i,j-1)-\Delta G(i,j)]/RT}, \tag{25b}$$

$$\omega(i,j \to i+1,j-1) = k_0 e^{\Delta S/R} e^{-[\Delta G(i+1,j-1)-\Delta G(i,j)]/RT}, \tag{25c}$$

which by design satisfy the detailed balance condition. Transitions can also occur between the (0, 0) macrostate and macrostates with $j>0$. These rates are

$$\omega(0,0 \to i,j) = k_0 e^{j\Delta S/R}[|j-2| + \delta(i,j)e^{\Delta S/R}]/w(0,0), \tag{26a}$$

$$\omega(i,j \to 0,0) = k_0 e^{j\Delta S/R}[|j-2| + \delta(i,j)e^{\Delta S/R}]e^{\Delta G(i,j)/RT}, \tag{26b}$$

where

$$\delta(i,j) = 0 \quad \text{if } i=1 \text{ and } j=n \tag{27a}$$

$$= n-j-1 \quad \text{if } i=1 \text{ and } i+j-1<n \text{ or } i>1 \text{ and } i+j-1=n \tag{27b}$$

$$= n-j-2 \quad \text{otherwise}. \tag{27c}$$

Applying the procedure presented in subsection III A, the dividing surface between the unfolded and folded states which minimizes the reaction flux is found. All the folded macrostates thus identified, 25 altogether, have at least three hydrogen bonds, between peptide bonds 5 and 11, 6 and 10, and 7 and 9, which are accompanied by at least the Y45-F52 hydrophobic interaction. There are 36 transitions going from the unfolded set of macrostates to the folded set of macrostates, with the contributions to J differing by five orders of magnitude. Five of these transitions contribute 87.2% of the total flux. These five transitions and their percent contributions to J are shown in Fig. 3(b). The two top contributors, each at 31.6% of J, start from the (5, 6) and (6, 6) macrostates, respectively, with peptide bonds 11 and 5 disordered, thus precluding hydrogen bonding between them. Both transitions lead to the (5, 7) macrostate, in which the hydrogen bond between peptide bonds 5 and 11 and simultaneously the Y45-F52 hydrophobic interaction are formed. The third contributor, at 8.2% of J, is the transition to the (5, 7) macrostate from the (0, 0) macrostate. The next two contributors, each at 7.9% of J, start from the (4, 7) and (6, 7) macrostates, and end at the (4, 8) and (5, 8) macrostates, respectively.

The finding on the transitions from the unfolded macrostates to the folded macrostates indicates that the rate-limiting step for the β-hairpin formation is the formation of the hydrogen bond between peptide bonds 5 and 11. Muñoz *et al.* came to the same conclusion based on considering a free-energy profile calculated as a function of the number of ordered residues (essentially the potential of mean force

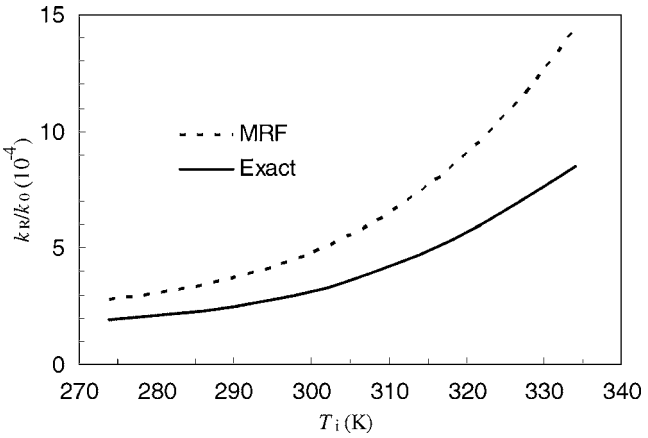

FIG. 4. Comparison of the MRF prediction for k_R and the exact result for the model of Muñoz *et al.* The exact result is obtained by numerical integration of the master equation, with initial condition given by the equilibrium distribution at a particular initial temperature (T_i). At $t=0$, the temperature is switched to T_i+15 K (modeling a 15 degree temperature jump). Thereafter the fractional population of the nine macrostates with all the three hydrophobic interactions formed is fitted to a single exponential, yielding the exact result for k_R.

along j). In addition to energetics, our approach also takes dynamics into consideration, since the reaction flux also depends on the transition rates between macrostates. In the model of Muñoz *et al.*, all disorder-to-order transitions have the same rate [see Eq. (25a)]. When transition rates are macrostate dependent, the landscape of the flux can be different from the energy landscape (see Sec. IV for further discussion).

In Fig. 4 we compare the relaxation rate constant obtained from the MRF approach with the exact result obtained by numerical integration. The MRF prediction overestimates k_R by ~50%. Given its simplicity, the MRF approach seems to perform quite well.

IV. DISCUSSION

We have presented an approach for calculating the folding and unfolding rate constants in master-equation models, based on minimizing the reaction flux. Test against exact results shows that the MRF solution works well. This solution suggests that the folding and unfolding rate constants are determined by a small number of transitions. This finding is important in itself. In designing master-equation models, one has to assign free energies to macrostates and rates to transitions between them. The above finding suggests that attention should be focused on the few parameters that critically affect the folding and unfolding rates. Below we further discuss several related issues.

A. Accuracy of the MRF solution

As a transition-state theory, our approach provides upper bounds on the folding and unfolding rate constants. The use of the MRF dividing surface makes the upper bounds as tight as possible.

In comparing the results on the two models tested, the MRF solution works much better for the model of Zwanzig than for the model of Muñoz *et al.* In the former model, the folded state consists of a single macrostate, which is connected to a single unfolded macrostate. The folded macrostate has a significantly lower free energy (by $\sim\varepsilon$) than the unfolded macrostate. Therefore once the protein makes the forward transition between the two macrostates, it is very unlikely to go backward. In contrast, the folded state in the model of Muñoz *et al.* consists of 25 macrostates, with modest decreases in free energy relative to neighboring macrostates across the dividing surface. There are thus appreciable chances of recrossing the dividing surface. This situation is typically found in some of the more realistic master-equation models,[7,13] and hence the relatively larger error found in the model of Muñoz *et al.* is perhaps more representative of the performance of our approach.

Accuracy on the calculated folding and unfolding rate constants aside, we emphasize that the MRF surface directly yields insight into folding mechanisms.

B. MRF surface and transition-state ensemble

In our approach, the dividing surface between the unfolded and folded states which minimizes the reaction flux plays the role of the transition-state ensemble. This specification of the transition-state ensemble is more intuitive than previous proposals such as the eigenvector corresponding to the smallest nonzero eigenvalue of the transition matrix[9–11] or the stochastic separatrix.[20] In the latter approach, $p_{\rm fold}$, the probability, starting from an arbitrary macrostate, of first reaching manifestly folded macrostates (as opposed to first reaching manifestly unfolded macrostates) is calculated from the eigenvectors of the symmetrized transition matrix. When this approach is applied to the models of Zwanzig and of Muñoz *et al.*, we find that, depending on the starting macrostates, $p_{\rm fold}$ values are close to either 0 or 1, with a large gap between the two subsets of values. Contrary to conventional wisdom, we choose to define the transition-state ensemble as a collection of transitions rather than a collection of macrostates, which is supposed to be identified by $p_{\rm fold}$ values close to 0.5. The gap in $p_{\rm fold}$ values presents strong argument in favor of our way of defining the transition-state ensemble, as it allows for a clear-cut division of the macrostates into folded and unfolded subsets; the transitions between the two subsets naturally constitute the transition-state ensemble. Moreover, for both the models of Zwanzig and Muñoz *et al.*, we find that the two subsets of $p_{\rm fold}$ values are precisely separated by the MRF surface!

Conceptually, the MRF surface is just what is represented by the transition state in classical transition-state theory.[16,21] Among the forward transitions contributing to the minimized reaction flux, we can further identify the one(s) making the largest contribution as the "saddle point." However, the MRF approach differs from classical transition-state theory in one important respect: the dividing surface minimizing the reaction flux is determined not only by the free energies of the macrostates but also by the transition rates. In principle, dynamic information incorporated by the transition rates can lead to a landscape significantly different from free energy landscape. This situation is similar to diffusional models of protein folding in which the diffusion coefficient

is conformation dependent. A conformation-dependent diffusion coefficient can significantly shift the kinetic transition state and barrier height of protein folding.[22]

C. Search for the MRF surface

The procedure outlined in Subsection III A has worked well for the two models studied here. It is possible that refinement has to be introduced for other models. For example, during the iterative process, we can test for both adding and removing macrostates.

A procedure that more exhaustively searches for the optimal dividing surface can also be contemplated. Each macrostate can belong to either the unfolded state or the folded state. For a model with M macrostates, there are a total of 2^M possible choices for the dividing surface. This search problem can be mapped to the problem of finding the protonation states of titratable groups in a protein (each titratable group can be either protonated or deprotonated). Methods[23] developed for protein titration can thus be adapted for searching for the optimal dividing surface.

D. Beyond two-state folding

The MRF approach has been applied to master-equation models exhibiting two-state folding behavior. For an on-pathway intermediate, the folding scheme becomes

$$U \rightleftarrows I \rightleftarrows F. \tag{28}$$

In this case, our approach can still be used. The reaction flux will have two local minima, one corresponding to the transition between U and I while the other corresponding to the transition between I and F. For models with more intermediates and more complicated connections among U, F, and the intermediates, the approach presented here will need modifications.

In conclusion, the MRF approach significantly simplifies the solution of master-equation models and, at the same time, directly yields insight into folding mechanisms.

ACKNOWLEDGMENTS

This work was supported in part by NIH Grant No. GM058187.

APPENDIX: PROOF OF $J \geqslant K_F$

Here we show that Eq. (1) is an overestimate of the folding rate constant. Let the nonzero eigenvalues, λ_l, of the transition matrix ω be ordered from small to large. The fractional occupation of the unfolded state can be written as

$$u(t) = p_u + \sum_l A_l e^{-\lambda_l t}, \tag{A1}$$

where A_l are constants determined by the initial condition. Applying the initial condition given by Eq. (13), one has

$$u(0) = p_u + \sum_l A_l = 1, \tag{A2a}$$

hence

$$\sum_l A_l = 1 - p_u. \tag{A2b}$$

. Taking the time derivatives of both sides of Eq. (A1) and evaluating at $t=0$, we find

$$J \equiv -\left.\frac{du(t)}{dt}\right|_{t=0} = \sum_l A_l \lambda_l \leqslant \sum_l A_l \lambda_1 = (1-p_u)k_R = k_f, \tag{A3}$$

which is the desired result. In the second last step we equated k_R with λ_1, and the last identity is a consequence of Eq. (12).

[1] R. Zwanzig, A. Szabo, and B. Bagchi, Proc. Natl. Acad. Sci. U.S.A. **89**, 20 (1992).
[2] R. Zwanzig, Proc. Natl. Acad. Sci. U.S.A. **92**, 9801 (1995).
[3] R. Doyle, K. Simons, H. Qian, and D. Baker, Proteins **29**, 282 (1997).
[4] M. Cieplak, M. Henkel, J. Karbowski, and J. R. J. R. Banavar, Phys. Rev. Lett. **80**, 3650 (1998).
[5] V. Muñoz, E. R. Henry, J. Hofrichter, and W. A. Eaton, Proc. Natl. Acad. Sci. U.S.A. **95**, 5872 (1998).
[6] P. A. Thompson, V. Muñoz, G. S. Jas, E. R. Henry, W. A. Eaton, and J. Hofrichter, J. Phys. Chem. B **104**, 378 (2000).
[7] E. Alm, A. V. Morozov, T. Kortemme, and D. Baker, J. Mol. Biol. **322**, 463 (2002).
[8] D. Makarov, C. A. Keller, K. W. Plaxco, and H. Metiu, Proc. Natl. Acad. Sci. U.S.A. **99**, 3535 (2002).
[9] S. B. Ozkan, K. A. Dill, and I. Bahar, Protein Sci. **11**, 1958 (2002).
[10] S. B. Ozkan, K. A. Dill, and I. Bahar, Biopolymers **68**, 35 (2003).
[11] J. Schonbrun and K. A. Dill, Proc. Natl. Acad. Sci. U.S.A. **100**, 12678 (2003).
[12] D. A. Evans and D. J. Wales, J. Chem. Phys. **121**, 1080 (2004).
[13] T. R. Weikl, M. Palassini, and K. A. Dill, Protein Sci. **13**, 822 (2004).
[14] C. Merlo, K. A. Dill, and T. R. Weikl, Proc. Natl. Acad. Sci. U.S.A. **102**, 10171 (2005).
[15] H.-X. Zhou, Phys. Biol. **2**, R1 (2005).
[16] J. C. Keck, J. Chem. Phys. **32**, 1035 (1960).
[17] The notations used here are somewhat different from the original paper in order to maximize connections between this model and the model of Muñoz *et al.* However, there is no substantive change from the original paper.
[18] Zwanzig solved the master equation by numerical integration for a single temperature. He reported results for the relaxation time (the inverse of k_R), obtained from the time integral of the fractional population of macrostate N. He found an analytical solution for this integral.
[19] V. Muñoz, P. A. Thompson, J. Hofrichter, and W. A. Eaton, Nature (London) **390**, 196 (1997).
[20] A. Berezhkovskii and A. Szabo, J. Chem. Phys. **121**, 9186 (2004).
[21] H. Eyring, J. Chem. Phys. **3**, 107 (1935).
[22] J. Chahine, R. J. Oliveira, V. B. Leite, and J. Wang, Proc. Natl. Acad. Sci. U.S.A. **104**, 14646 (2007).
[23] M. K. Gilson, Proteins **15**, 266 (1993).
[24] http://computation.llnl.gov/casc/odepack/download/lsode_agree.html.