

From Discrete Protein Kinetics to Continuous Brownian Dynamics: A New Perspective

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Conformational fluctuation is a fundamental characteristic of proteins in aqueous solution, which differentiates the macromolecules from small molecules. The intrinsic beauty and the remarkable details of the protein structures from crystallography often resulted in the view that proteins are static. However, it is the conformational flexibility along with the well-defined structures which give rise to the versatile, almost magic, functionalities of proteins and enzymes (Karplus and McCammon, 1983). Ever since the conception of allosteric enzyme (Koshland et al., 1966, Monod et al., 1966), the multiple state notion of proteins has been widely appreciated. Two particular notable examples are the models for **folding kinetics** of soluble globular proteins and the **gating kinetics** of membrane channel proteins. Both these models introduce discrete conformational states which are macroscopic and operationally defined by kinetic experiments (Tsong et al., 1972, Ehrenstein et. al., 1974). These models are usually expressed as



where U and N are unfolded and native states of a soluble protein, with k_1 and k_2 as folding and unfolding rate constants. Similarly, O and C are open and closed states of a membrane channel protein, with k_α and k_β as closing and opening rate constants. When such simple models can not explain specific experiments, usually more intermediate states are added (Bezannilla et al., 1994, Baldwin, 1995).

The discrete state description of proteins, however, neglects conformational fluctuations within each state. The energy landscape theory, treating the polypeptides as polymers, is an more realistic view of the protein dynamics (Frauenfelder et al., 1991, Wolynes et al., 1995, Zwanzig, 1995, Doyle et al., 1997). More importantly, recent experimental studies on several proteins indicate that it is also necessary to invoke continuous energy landscapes in order to provide comprehensive interpretations for the experiments (Sigg et al., 1999, Qian and Chan, 1999). There are now a host of laboratory observations which call attentions to interpretations based on continuous energy. Most notably are (*i*) rapid early conformational changes in relaxation kinetics and (*ii*) nonactivated transitions induced by strong external forces. From a conceptual standpoint, as we shall show, these two types of observations are intimately related to the hysteresis and “bond rupturing” phenomena recently

observed in the receptor-ligand dissociation under atomic force microscopy (Florin et al., 1994; Moy et al., 1994; Evans and Ritchie, 1997, Shapiro and Qian, 1997, 1998, Merkel et al., 1999).

In the continuous approach, the kinetics of a molecule is viewed as a Brownian motion on an energy surface. The theoretical basis of this approach has been extensively studied by Smoluchowski, Kramers, and others and is summarized in a review article on the fifty years after Kramers theory (Hänggi et al., 1990). It is interesting to see that the merging of discrete with continuous kinetic models has been a long process in chemistry, and it is now the turn for proteins. The following is a quotation from the article, which gives us a historical perspective.

... For unimolecular gas phase reactions, a description of the rate in terms of discrete energy exchange was more suitable than the continuous energy-exchange mechanism underlying energy diffusion in Kramers' model (1941). Work on chemical reactions in condensed phase, for which the Kramers theory is most appropriate, had to await the experimental progress achieved in the late seventies and eighties.

It is important to point out that every kinetic relaxation experiment has to involve two conditions for the protein under study. For protein folding they are usually the solvent condition or temperature, and for ion-channel gating it is membrane electrical potential. Recent experiments also apply external mechanical forces on proteins. At time zero, the protein under condition 1 is subjected to condition 2, which initiates the relaxation kinetics of the molecule. In terms of the energy function (potential of mean force), there are two different energy functions corresponding to the two conditions (Karplus and Shakhnovich, 1992, Qian and Chan, 1999). For the two-state kinetics shown in Eq. 1, the corresponding energy landscapes are shown in Fig. 1A. Each discrete state is associated with an energy well. The continuous model, however, also points out that the shape of an energy well, as well as their relative heights, change with the condition. Immediately after the initiation, not only the thermal equilibrium between the two wells are perturbed, the equilibrium within each of the wells too is perturbed. Therefore the response of a protein to the perturbation is to readjust its equilibrium distributions within each energy well, as well as to redistribute between the two wells. Since the latter process is thermally activated while the former process is energetically down-hill, the readjustment contributes a rapid early conformational change in any relaxation kinetics of proteins. One unique feature of this process, however, is that it is not thermally activated, hence non-exponential. Experimentally, from such non-exponential diffusion process one is expected to observe faster relaxation time with faster measurement temporal resolution, reminiscent of a fractal behavior.

Since the down-hill readjustment within each energy well is usually much faster than the two-state barrier crossing, and since the magnitude of the readjustment is usually small relative to that of the two-state transition, such early kinetic events are difficult to observe experimentally. However, recent experiments on gating of voltage-dependent membrane ion channel proteins have observed such fast kinetic phase (Stefani and Bezanilla, 1996), and a Brownian diffusion mechanism has been proposed for the early fast ($\sim \mu s$) components in the movement of gating-charge of the channel responding to a sudden change in membrane voltage (Sigg et al., 1999). In the kinetics of protein folding, such a fast energetically down-hill event is also observed (Sosnick et al., 1996,

Hagen et al., 1996, Qi et al., 1998). In addition, a large amount of experimental observation of various intermediate states in the early time of protein folding kinetics (known as molten globular states) can be interpreted by a readjustment step responding to a sudden change in denaturant concentration in solvent (Karplus and Shakhnovich, 1992, Qian and Chan, 1999).

A second situation under which discrete kinetics fail to provide a cogent interpretation is when the perturbation is so large that it completely eliminates the activation barrier, as shown in Fig 1B. Under such conditions, the traditional rate process with thermal activation loses its meaning all together and the relaxation is a very fast energetically down-hill diffusion. This phenomenon has not been observed in monomeric protein folding (unfolding) kinetics. However, such a mechanism lies behind streptavidin-biotin bond rupturing with atomic force microscopy (Shapiro and Qian, 1997, 1998), as well as successive unfolding of giant muscle protein titin by force (Qian and Shapiro, 1999). A similar behaviour also has been observed in the gating kinetics of K^+ channel with an extreme holding potential (Sigg et al., 1999).

The introduction of continuous energy landscape does not invalidate the discrete transition between two energy wells, rather it generalizes the discrete model with an increasing molecular details. It is well known that with a sufficient large activation barrier separating two energy wells, there is a rapid equilibrium within each well. Furthermore, the transition from one well to another is essentially exponential, i.e., Arrhenius (also known as discrete-state Markovian). This is the theoretical basis for the practice of discrete-state kinetics. One should recognize, however, that the “molecular structures” of the discrete states, which usually are defined experimentally through spectroscopy, change with the environmental condition for the protein. These changes also reflected in the baselines when fitting discrete multi-state models to equilibrium measurements (Qian, 1997, Qian and Chan, 1999).

Another important reason for introducing the continuous energy function to augment the discrete-state kinetics is the inability of relating energy to force in the latter framework. Force is the change of energy in response to a change in distance. As we can see the concept of distance is completely missing in the discrete-kinetics. In the continuous energy landscape, no matter how ill-defined the reaction coordinates are, they provides a conceptual framework. Therefore, the continuous energy landscape provides a bridge between the experimental studies on kinetic of proteins and more direct measurements of force and displacement on single protein molecules (Kellermayer et al., 1997, Reif et al., 1997, Tskhovrebova et al., 1997, Qian and Shapiro, 1999).

In summary, one important consequence of the energy landscape concept is that within each discrete kinetic state, there could be significant conformational readjustment due a changing condition (perturbation) for the protein, such as changes in temperature, solvent, or membrane potential. Therefore, following a sudden change in one of these conditions, a protein has two characteristic kinetic steps: an energetically down-hill readjustment into the new equilibrium position within the same discrete state, and then an thermally activated rate process which jumps from one discrete state into another with lower energy. When the perturbation is sufficiently larger, it is also possible that the activation barrier is completely eliminated. Then the kinetics becomes a down-hill diffusion, and relaxation kinetics is no longer exponential. The continuous energy perspective on protein kinetics provides a comprehensive theoretical framework for a host of experimental obser-

vations, ranging from protein folding, to membrane channel gating, to protein-ligand dissociation and protein unfolding under external force.

The conceptual thrust of the continuous energy landscape approach to proteins is that it provides a theoretical language for discussing a wide range of dynamical behavior of proteins. It has laid a foundation for developing a macromolecular mechanics at a mesoscopic level between the discrete models and the atomic-level molecular dynamics (Qian and Shapiro, 1999). It allows important concepts such as force and movement to be discussed on an equal footing as energy and thermodynamic states. With the recent significant progress in biophysical measurements of forces and movements in single protein molecules, models based on continuous Brownian dynamics will become an indispensable part of the protein science. In a similar spirit, Eisenberg and his colleagues have developed a diffusion theory for ion movement (not to be confused with protein movement in the gating kinetics) in open channels to augment traditional discrete-state models. For a review see Cooper et al. (1988) and Eisenberg (1996).

Here is an example to show how the continuous energy function serves as a unifying theoretical edifice in molecular biophysics. One interesting phenomenon observed from protein-ligand interaction under external force is hysteresis: the association process under a force and the dissociation process under a force are significantly different. This can be quantitatively interpreted as in Fig. 2. Compare this model with the well-known protein folding-unfolding kinetics scheme below, one immediately sees that the main feature of the two molecular processes are indeed identical.

	unfolded state		folded state
native condition	wet molten globule	\implies	native structure
	\uparrow		\downarrow
denaturing condition	random coil	\longleftarrow	dry molten globule

where we introduce the term “wet molten globule” referring the collapsed intermediate state commonly observed in the protein refolding kinetics (cf. Baldwin, 1993). It has been interpreted as the unfolded state under a native condition (Dill and Shortle, 1991, Qian and Chan, 1999). The dry molten globule (Kiefhaber et al., 1995), on the other hand, has been interpreted as the folded state under a denaturing condition (Qian and Chan, 1999). Both these two states are kinetic intermediates which appear in the transient folding and unfolding processes, respectively. The wet molten globule is a kinetically metastable state before the major activation barrier in the folding process, while the dry molten globule is again a kinetically metastable state before the major activation barrier but in the unfolding process. This difference is the same as the hysteresis.

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Figure Captions

Figure 1 Schematic diagram showing continuous energy functions for a two-state protein kinetics (Eq. 1) under two different conditions. (A) It is shown that the shape of each energy well, as well as the relative heights of the two wells, change with the condition. (B) Under extreme condition, the activation-energy barrier can completely disappear. In this case, the relaxation process is an energetically down-hill diffusion.

Figure 2 When applying a force to a protein-ligand pair, one can either pull the ligand apart from the protein, or load the protein with the ligand. The events in these two kinetics processes are schematically shown here. The particle in the diagram represents the ligand which experiences both intermolecular force from the protein (modeled as a 6-12 potential) and the force probe (modeled as a Hookean spring). The energy minimum on the left is the equilibrium position for the intermolecular energy; while the energy minimum on the right is the equilibrium position of the spring. For more discussion, see Qian and Shapiro, 1999.

Running Title: Discrete Kinetics and Continuous Dynamics

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