Protein Dynamics and Solvent Effects

Proteins are the workhorses of living systems, supporting life by performing most cellular functions. Many textbooks render proteins with rigid and naked structures, real proteins are, however, dynamic molecules residing in a liquid environment. A central goal in protein physics is to understand how the dynamics bridge the structures and functions. Another topic of fundamental importance is the effects of environmental factors on the structure-dynamics-function relationship of proteins.

The protein dynamics have been best studied on the proteins whose structures and functions are well known, e.g. myoglobin (Mb). Mb is the O_2-shuttling protein in animal muscles including ours. Very interestingly the structure of Mb does not possess a static channel for O_2. In order to function Mb must undergo large-scale structural fluctuations to form a dynamic path so that O_2 or other gas ligands like CO can move in or out. Thus the gating of such a path involves not only energy (enthalpic) barriers but also a conformation (entropic) limit, i.e. the Mb protein needs an entropy reserve for its structural fluctuations. For a rough generalization this may be one of the reasons why proteins are usually large. A key question then arises: What determines the enthalpy and entropy so as to control the Mb dynamics? A recent work[1-2] in our group has found that the clue lies in the dynamical connection of Mb to its solvent environment: Large-scale Mb motions follow the fluctuations in the bulk solvent but are slower. Such a behavior of Mb is called slaving. The entry and exit of ligands are prototypes of slaved Mb motions. Take the exit of CO as an example: When measured in a fixed solvent at different temperatures (T), the exit rate (k_{exit}) varies with T as does the primary (α) dielectric relaxation rate (k_α) of the solvent, attenuated only by a number $n(T)$:

$$k_{exit}(T) = \frac{k_\alpha(T)}{n(T)}, \qquad (1)$$

where $n(T)$ depends on T very weakly. Eq. (1) implies that the exit of CO proceeds in $n(T)$ steps with each step having a rate of k_α. In other words, for CO to exit, Mb needs to fluctuate approximately $n(T)$ times and each fluctuation occurs only when the solvent moves. Thus the activation enthalpy for the exit of CO is dominated by the solvent while the activation entropy is provided by the Mb protein and its hydration shell. These concepts from Mb comprise the slaving model, which has also been found to apply to many other proteins.

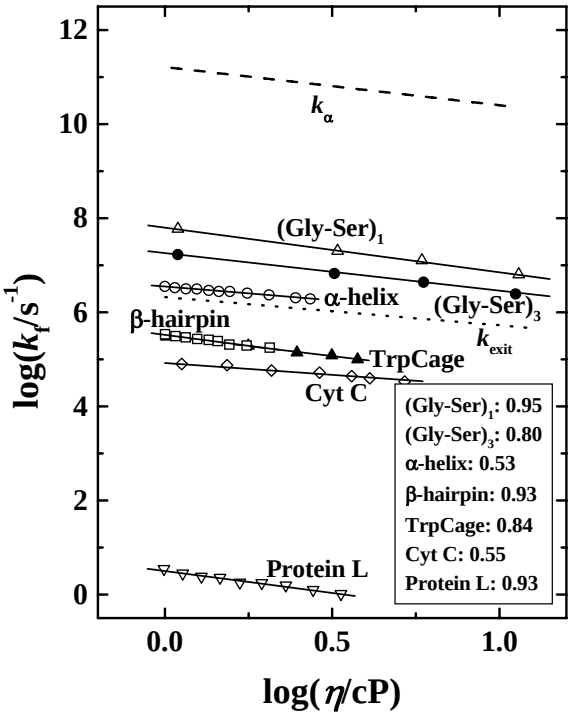

Fig.1[3] *Viscosity dependence of folding rates* (k_f) *at 293K for several polypeptides and proteins:* $(Gly\text{-}Ser)_1$ ( ), $(Gly\text{-}Ser)_3$ (●), *α-helix* (○), *β-hairpin* (□), *Tryptophan cage* (*TrpCage,* ▲), *cytochrome C* (*Cyt C,* ◊) *and protein L* ( ). *The solid lines show the fits to Eq.* (*2*) *with the values of* κ *given in the text box. For comparison the rates for the α-fluctuation* (k_α) *in glycerol/H_2O mixtures and for the exit of CO from Mb* (k_{exit}) *are also plotted as the dashed and dotted lines, respectively.*

The slaving model provides a tool to quantify the effects of solvents on the conformational dynamics of native proteins. More recently we have also applied this model to protein folding[3]. The motivation is straightforward: Protein folding, similar to the entry into, or exit from, Mb for ligands, also involves large-scale motions of polypeptides in solvents, then is it also slaved to the solvent dynamics? The key to this question is to directly compare the T dependence of the folding rate (k_f) to that of the fluctuation rates for the same solvent in which the folding is measured. Unfortunately such data are rarely available, if not lacking. This is, in great part, due to the fact that protein folding can only be examined in a very limited temperature range, so conventionally it is measured by varying solvents and k_f is usually reported as a function

of solvent viscosity (η) at a fixed temperature (Fig. 1). From such data detailed knowledge about the solvent types or concentrations is difficult to recover. Thus we can only resort to an indirect comparison in terms of η between k_f and the rates of solvent fluctuations. A first look at Fig. 1 shows that k_f varies among proteins across several orders of magnitude, but is much lower than k_α of solvents, here glycerol/H_2O mixtures. An analysis of the data furthermore indicates that k_f follow a power-law scaling with η:

$$k_f(\eta) \propto (\frac{\eta}{\eta_0})^{-\kappa} \quad . \qquad (2)$$

In Eq. (2) η_0=1cP, the viscosity of H_2O at 293K which is often used as a viscosity reference. The fits to Eq. (2) yield different values of exponent (κ) among the proteins: Some are close to 1 yet the others lower, about 0.5. The viscosity-dependence of k_α is, on the other hand, known to be well described by the Maxwell relation:

$$k_\alpha(\eta) = \frac{G(\eta)}{\eta}, \qquad (3)$$

where G is the high-frequency shear modulus. Eq. (3) means k_α scales inversely with η, differing from the fractional scaling of k_f in Eq. (2). Such a difference gives one an impression that protein folding is nonslaved to solvent motions because k_f k_α seems to break down. This impression is, however, not true because, for the indirect comparison in terms of η, one must not neglect the above-noted experimental fact: The solvents are varied; for each of the proteins in Fig. 1 it is actually measured in different solvents for the data points. On one hand, the data follow Eq. (2) fairly well, implying that the solvent change mainly affects the protein dynamically and η is the primary variable of this change, on the other hand, the proteins respond to the solvent change with a fractional κ, which suggests the effects of solvents upon the proteins should be in an indirect manner, otherwise one would expect $k_f(\eta)$ $1/\eta$ for a direct response to the dynamical nature of solvents. In other words, η directly reflects the solvent motions (k_α) but not the solvent-protein interactions and hence the protein motions (k_f). The data in Fig. 1 implies that, via the solvent-protein interactions, the change in solvent motions is conveyed to the protein but partially dampened, resulting in the observed fractional scaling of k_f with η. In the same sense, different proteins interact differently with the solvents and respond disparately to the solvent change, as manifested by their different fractional exponents. Moreover, because different solvents, in either types or concentrations, give rise to different compositional and configurational structures at the solvent-protein interface, the solvent-protein interactions are associated with a principal change in entropy. This entropy change means the number of protein folding motions depends on solvents or η. Thus, in such a case with varying solvents, the relation between k_f and k_α is of a form:

$$k_f(\eta) = \frac{k_\alpha(\eta)}{n(\eta)}. \qquad (4)$$

Eq. (4) represents a solvent-varying version of slaving as compared with the isosolvent relationship in Eq. (1). One can see, from Eq.s (2) through (4), $n(\eta)$ $G(\eta)/\eta^{1-\kappa}$, indicative of the complex effects upon the protein as solvents are varied.

Additional support to the above analysis has also been gained from the data of k_{exit} measured in different solvents. As plotted in Fig. 1, at 293K in different glycerol/H_2O mixtures, $k_{exit}(\eta)$ $\eta^{-0.55}$, also showing a fractional η dependence which seems to break down the slaving model because $k_\alpha(\eta)$ $1/\eta$. On the other hand, however, from the isosolvent data we already know that the exit of CO from Mb is slaved to the bulk solvent motions. Essentially this paradox is also caused by the complex effects in association with the solvent change, as in the measurements of protein folding. Thus one must be aware of the experimental details in understanding the solvent effects on the protein dynamics.

References

[1] P. W. Fenimore, H. Frauenfelder, B. H. McMahon and F. G. Parak, Proc. Natl. Acad. Sci. USA **99**(25), 16047(2002).

[2] P. W. Fenimore, H. Frauenfelder, B. H. McMahon and R. D. Young, Proc. Natl. Acad. Sci. USA **101**(40), 14408(2004).

[3] H. Frauenfelder, P. W. Fenimore, G. Chen and B. H. McMahon, Proc. Natl. Acad. Sci. USA **103**(42), 15469(2006).

Contact Information: Guo Chen ─ Theoretical Biology and Biophysics (T-10) and Center for Nonlinear Studies, MS K710, Los Alamos National Laboratory, Los Alamos, NM 87545. Phone: (505) 667-6870; e-mail: chenguo@lanl.gov.