Effect of the ordered water on protein folding: An off-lattice Gō-like model study

Guanghong Zuo,[1,2] Jun Hu,[2,3] and Haiping Fang[1,2,4,*]

[1]*T-Life Research Center and Department of Physics, Fudan University, Shanghai 200433, China*

[2]*Shanghai Institute of Applied Physics, Chinese Academy of Sciences, P.O. Box 800-204, Shanghai 201800, China*

[3]*Bio-X Life Science Research Center, College of Life Science and Biotechnology, Shanghai JiaoTong University, Shanghai 200030, China*

[4]*Theoretical Physics Center for Science Facilities (TPCSF), CAS, 19(B) Yuquan Road, Beijing 100049, China*

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Recent experiments and numerical simulations have shown that the water molecules confined on the surfaces of some substrates, including the surfaces of cellular components in tissues and cells, form icelike ordered structures. If a protein folds in an environment with those icelike ordered water molecules, its behavior may be different from that in bulk water. Here, the effect of this ordered water environment on protein folding is studied by using an off-lattice Gō-like model. It is found that the ordered water environment significantly improves the native state stability and greatly speeds up the folding rate of the proteins.

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I. INTRODUCTION

Water has been called the matrix of life [1]. It plays an important role in the structures and functions of biomolecules [2–4]. Protein folding, which is one of the most important and only partly solved problems in structural biology [5–10], is also believed to be greatly impacted by water inside or on the surface of the protein [11–17].

Inside the cell, proteins and other biomolecules together with water are usually in crowded environments [18,19]. In recent years, it became well recognized that water confined in nanoscale dimensions usually displays phenomena different from its bulk counterpart [3,20–26]. In 1995, Hu *et al.* [20] observed an icelike structure of water on mica surfaces at room temperature. Later, this two-dimensional ordered structure of water has been found at the surfaces of many other substances [21]. A very recent study showed that the icelike structure of water reached the third layer from the surface [23]. The ordering structures have also been observed in the confined water on the surfaces of biological membranes and the biomolecules. Cheng *et al.* [26] obtained direct experimental evidence for the ordering of water on a membrane surface and found that the amount of ordered water depended on the surface polar groups. Pal and Zewail [3] showed that some water molecules on the surfaces of DNA and protein molecules were ordered.

In the past few years, it became well recognized that both the spatial confinement and crowded environment significantly improve the stability and speed of the folding of the proteins due to the conformational entropy loss of the denatured states induced by the spatial confinement [27–33]. However, the effect of the ordered water, which may form on the walls of the confined spaces or the surfaces of other biomolecules, has rarely been discussed. Very recently, by using infrared spectroscopy, Sen *et al.*, [34] showed that the behavior of the folding of the protein *cytochrome c* next to a solid surface is different from the behavior of its folding in bulk water. This difference may result from the structure of the water next to the solid surface. In our previous work, we have presented a primary investigation on the folding of a single type of protein, chymotrypsin inhibitor II (CI2) (protein datebase (PDB) code 1COA), in a confined space with the existence of the ordered water [35]. It has been found that at the physiological temperature, both the folding rate and the stability of the protein have tendencies to be promoted, since the ordered water has a low mobility compared with bulk water [36,37], which weakens the random fluctuations.

In this paper, we present a systematic approach toward the effect of the ordered water on protein folding by numerical simulations. Six proteins with different structural classes and sizes have been studied in order to demonstrate the universality. The simulation results show that the existence of the ordered water significantly improves the native state stability and greatly speeds the folding rates of the proteins. While it is thought that both the spatial confinement and ordered water enhance the native state stability of the proteins, our study shows that the effect of the ordered water is much more significant than that of the spatial confinement.

II. MODEL AND METHOD

A. Classical Gō-like model

The Gō-like potential [38] has been widely used to describe the interactions between residues in both lattice [39,40] and off-lattice [31,41–44] models of protein folding. In these models, residues are represented by beads centered in their C_α atoms; all beads are connected into a polymer chain by virtual bonds. The interactions include terms related to the virtual bonds, angles, dihedral angles, and nonbonded pairs of the beads. In this work, the terms and parameters of the Gō-like interactions are taken from Ref. [41] by Clementi *et al.* except for the dihedral term. For a certain conformation of a protein, the total Gō-like potential energy is thus given by the expression

*Corresponding author; fanghaiping@sinap.ac.cn

$$V_{\mathrm{Go}} = V_{\mathrm{bonded}} + V_{\mathrm{bond\text{-}angle}} + V_{\mathrm{dihedral}} + V_{\mathrm{nonbonded}}$$
$$= \sum_{\mathrm{bonds}}^{N-1} K_r(r-r_0)^2 + \sum_{\mathrm{bond\text{-}angles}}^{N-2} K_\theta(\theta-\theta_0)^2 + \sum_{\mathrm{dihedral}}^{N-3} \{K_\phi^{(1)}[1-\cos(\phi-\phi_0)] + K_\phi^{(3)}[1-\cos 3(\phi-\phi_0)]\}$$
$$+ \sum_{i<j-3}^{\mathrm{native}} \epsilon\left[5\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - 6\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{10}\right] + \sum_{i<j-3}^{\mathrm{non\text{-}native}} \epsilon\left(\frac{2\sigma_0}{r_{ij}}\right)^{12}. \quad (1)$$

Here, N is the total number of residues. r, θ, and ϕ are the bond length, bond angle, and dihedral angle, respectively. r_0, θ_0, and ϕ_0 correspond to the values in the native conformation. r_{ij} is the spatial distance between two C_α atoms which are separated by at least four residues along the chain. For every native contact, a 10–12 Lennard-Jones (LJ) potential is used, and σ_{ij} is the C_α-C_α distance between residues i and j in the native conformation. Here the native contact is defined as the contact between two residues i and j in the native structure if the distance between any two heavy atoms which belong to different residues is less than 5 Å. For every nonnative contact, the parameter $2\sigma_0$ represents the excluded volume repulsion between these residues. In our simulations, σ_0=2 Å is the radius of the hard cores of residues. The interaction parameters are taken as $K_r=100\epsilon$, $K_\theta=20\epsilon$, $K_\phi^{(1)}=\epsilon$, and $K_\phi^{(3)}=0.5\epsilon$, respectively. During the simulation, a native contact between two residues i and j is considered to be formed if the distance between their C_α atoms is smaller than $\kappa\sigma_{ij}$. Here $\kappa=1.2$ is used.

B. Confinement and ordered water effect

A schematic of our simulation model is shown in Fig. 1. A protein is confined in the space between two L-apart parallel hard plates, and the ordered water is assumed to form on the inside walls of these two plates. There is a repulsion interaction between a residue and any of the inside walls of the plates when their distance is less than σ_0. This is realized by a Lennard-Jones form potential [30,31],

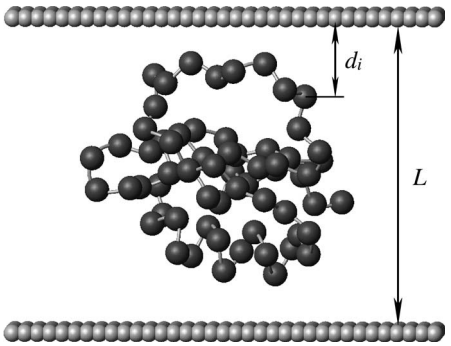

FIG. 1. Schematic for protein folding confined in the space between two parallel plates. In this model, the connected black beads, which are confined by two gray-bead plates, represent the residues of the protein. The water molecules are implicit and not shown here. L denotes the distance of the two parallel plates and d_i denotes the distance of the ith residue to its nearer plate.

$$V_{\mathrm{rep}} = \sum_i 50\epsilon\left[\left(\frac{\sigma_0}{d_i}\right)^4 - 2\left(\frac{\sigma_0}{d_i}\right)^2 + 1\right]\Theta(\sigma_0 - d_i), \quad (2)$$

where d_i is the distance between the ith residue and its nearer plate, and $\Theta(x)=1$ when $x>0$ and 0 otherwise.

To realize the effect of the ordered water, we introduce a factor $\rho_i(d_i)$ in the Langevin equation [see Eq. (5) below] to characterize the reduction in the random force near the plates, which results from the low mobility of the ordered water near the plates [36,37]. Since the ordered water only exists in the region very close to the plates and the degree of the ordering of the water decreases quickly with respect to the distance from the solid plates [23], the factor ρ_i decreases very quickly as d_i increases. Moreover, $\rho_i(d_i)=1$ at a position very far from both plates and $\rho_i(d_i)$ is a very small value at the boundary of any plate. In this paper, we take the function

$$\rho_i(d_i) = 1 - e^{-d_i/d_0}, \quad (3)$$

which meets those conditions, as an example to illustrate the effect of the ordered water. Here we use d_0=4.34 Å, so that ρ_i=0.9 for d=10 Å, which is about the thickness of three layers of water molecules, considering the observation that the icelike hydrogen-bond network reached the third layer from the surface [23]. We note that another choice of the value d_0 slightly different from 4.34 Å and even the other choice of the function ρ_i do not change the conclusion obtained in this paper, provided that the low mobility of the water molecules close to the plates is taken into account.

C. Langevin dynamics

Langevin dynamics is applied to simulate the folding process while the initial conformation of a protein is set as a random coil. The Langevin equation of motion in our simulation is

$$m\dot{\mathbf{v}}(t) = \mathbf{F}(t) - \gamma\mathbf{v}(t) + \mathbf{\Gamma}(t), \quad (4)$$

where $\mathbf{v}$, $\dot{\mathbf{v}}$, and m are the velocity, acceleration, and mass of a bead, respectively. $\mathbf{F}=-\nabla(V_{\mathrm{G}\sigma}+V_{\mathrm{rep}})$, here $V_{\mathrm{G}\sigma}$ is the Gō-like potential shown in Eq. (1) and V_{rep} is the potential between a protein residue and its closer plate which is displayed in Eq. (2). γ is the friction constant. Here we use $\gamma=0.5\tau^{-1}$ with τ being the time scale defined below [45]. $\mathbf{\Gamma}$ is the random force, which is produced from the Gaussian distribution with a standard variance at the simulation temperature times the effect factor of the ordered water,

$$\langle \mathbf{\Gamma}_i(t)\mathbf{\Gamma}_i(t')\rangle = 6\rho_i^2 \gamma k_B T \delta(t-t'). \quad (5)$$

k_B is the Boltzmann constant, T is the temperature, t is the time, and $\delta(t-t')$ is the Dirac delta function. ρ_i is the parameter to realize the effect of the ordered water and i is the index of the residue shown in Eq. (3). Every bead is subjected to a random force at each integration time step. The components of the random force are independently generated by setting $\Gamma_{ij}=\rho_i\Phi\sqrt{2m\gamma k_B T/\delta t}$, where j denotes the three components of random force in the x, y, and z directions, Φ is a random value taken from a standard Gaussian distribution (zero mean and unit variance), and δt is the integration time step. At each simulation, the initial velocities of the beads are assigned to be zero.

The leap-frog algorithm [46] is used to integrate Eq. (4). The time scale of the model is always controlled by a quantity $\tau=\sqrt{m\lambda^2/\epsilon_0}$ with the length scale $\lambda=3.8$ Å and the energy scale $\epsilon_0=1$. The time step of the simulation is set as $\delta t=0.005\tau$. Simulation time in this study is presented in units of δt. The length is measured in units of λ. The energy parameter ϵ and temperature T are given in units of ϵ_0 and ϵ_0/k_B, respectively. The length is measured in units of λ. To simplify this notation, other units are chosen as $m=1$ and $k_B=1$ in the simulation, as used by Veitshans *et al.* [47].

D. Weighted histogram analysis method

The weighted histogram analysis method (WHAM) is used to calculate relevant thermodynamic quantities based on the statistical physics [10,41,48]. WHAM yields an optimal estimate of the density of state of the system. In the canonical ensemble at temperature T, the probability distribution, P, of potential energy E is given by

$$P(E) = (1/Z_c)w(E)e^{-E/k_B T}, \quad (6)$$

and the probability distribution of other reaction coordinate, such as the radius of gyrate (R_g), follows

$$P(R_g) = (1/Z_c)\sum_E w(R_g,E)e^{-E/k_B T}, \quad (7)$$

where w is the density of state of the system and is obtained by solving two self-consistent equations [10]. Z_c is the canonical partition function. The free energy $F(R_g)$ is estimated by the logarithm of probability $P(R_g)$ [41].

III. RESULTS

A. Folding trajectory and free-energy landscape

The hydrophobic and other water-mediated effective interactions are sensitive to temperature, specially for the desolvation barriers in the enthalpy of protein folding. However, for the models without elementary desolvation barriers, it was found that the folding and unfolding are essentially controlled only by the ratio ϵ/T [43]. Recently, Kaya and Chan [13] suggested that the parameter $\Psi=-\epsilon/k_B T$ can be taken as a rudimentary model for changes in denaturant concentration under a constant T and variance ϵ. Here, we use Ψ to describe the folding behavior and called it denaturing intensity.

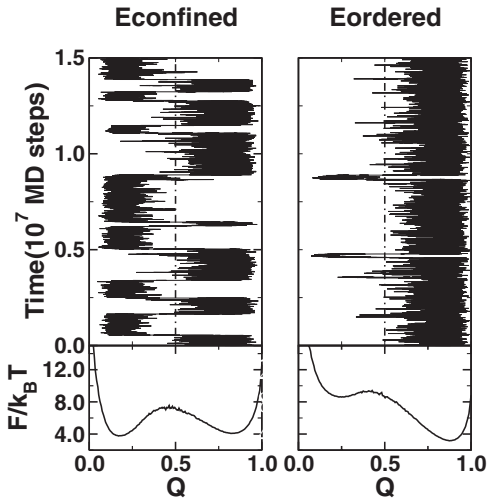

FIG. 2. Folding time course and distribution of the nativeness measure Q both for protein 1COA folding in the environment with and without the ordered water (Econfined and Eordered) at Ψ_m^0. Here, Ψ_m^0 is the denaturing intensity of the folding transition midpoint for the protein in bulk water, and the distance of the two plates is $L=76.0$ Å for both systems. (top) Time series $Q(t)$ plotted for representative trajectories. (bottom) Free-energy profiles $F(Q)$ as a function of Q. The existence of ordered water makes the protein fold in a "downhill-like" manner (bottom right).

Figure 2 shows the folding trajectories and free-energy landscapes for protein 1COA under the condition $\Psi=\Psi_m^0 \approx -0.95$ in the confined space between two parallel plates ($L=76.0$ Å) with and without the ordered water, respectively. Here, Ψ_m^0 is the denaturing intensity of the folding transition midpoint, where the folded probability is $P_{\text{folded}}=0.5$ for the protein folding in bulk water. The folding trajectories of proteins are characterized by Q, which is the measure of the similarity to the native structure of the protein [41]. Protein 1COA is a well-known two-state folder and usually regarded as a model protein in the simulation studies of protein folding [13,44]. It is found that when the protein is in the environment without ordered water (this environment is denoted by "Econfined" hereafter), as shown in the top-left panel of Fig. 2, the folding is hopping between the native state ($Q\approx 0.8$) and the denatured state ($Q\approx 0.2$). The probabilities for the protein staying at the native state and the denatured state are close. In the free energy for the protein folding in Econfined (bottom-left panel of Fig. 2), there are two basins, which correspond to the native state and the denatured state, respectively. A barrier, which corresponds to the transition state (TS), exits between these two basins. Moreover the height of these two basins is almost equal. According to the free-energy landscape theory, the free energy of a state denotes the stability of the state in equilibrium, and for a two-state folder, the TS barrier is the major obstruct for the folding/unfolding. It indicates a very close probability for the folding from the denatured state to the native state and the unfolding from the native state to denature. The native state is as stable as the denatured state in Econfined at this condition.

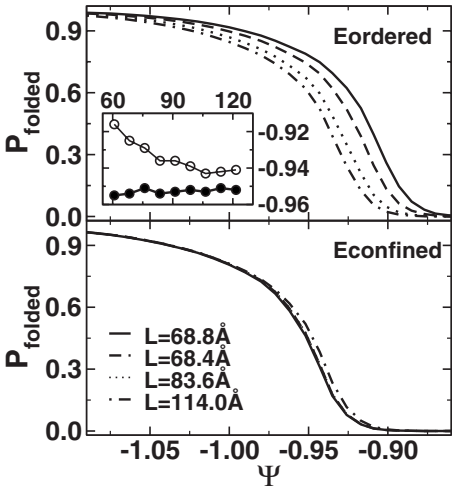

FIG. 3. Folded probability as a function of denaturing intensity for protein 1COA folding in the environment with and without the ordered water (top: Eordered; bottom: Econfined) for various L. The denaturing intensity is denaturing by $\Psi=-\epsilon/k_BT$. The inset figures show the denaturing intensity of the folding transition midpoint (Ψ_m) of the protein as a function of L in Eordered (hollow circles) and Econfined (solid circles), respectively. Here the folding transition midpoint means $P_{\text{folded}}=0.5$.

For the protein in the environment with ordered water (this environment is denoted by "Eordered"), the behavior of the folding trajectory and free-energy landscape, however, are quite different from those in Econfined (see the right panel of Fig. 2). In the folding trajectory, though there are still two states for the folding of the protein, the time duration of the protein in the native state is much longer than that in the denatured state. Most of the time, the protein is in the native state. Sometimes, it jumps to the denatured state from the native state and returns to the native state very quickly. In the free-energy landscape, the basis for the denatured state is much higher than that of the native state. The folding from the denatured state to the native state only requires one to overcome a very small barrier, which is much lower than that of unfolding. The folding from the denatured state to the native state, therefore, is much easier than the unfolding from native state to the denatured state, and the protein spends more time in the native state. This is, the native state of the protein is much more stable than the denatured state in these conditions.

B. Stability of native state

Figure 3 shows the folding transition for protein 1COA in four confined spaces. The sharp sigmoidal transitions of these curves imply that the protein folds in a two-state manner under every condition. As shown in the bottom panel of Fig. 3, the folding transition curves are close for different confined spaces in Econfined. All the denaturing intensity for the folding transition midpoint (Ψ_m) are very close to that of the bulk water ($\Psi_m^0 \approx -0.95$), as shown in the inset of the figure. This suggests that the effect on the folding transition by the confinement is small. However, for protein 1COA in Eordered, as shown in the upper panel, the folding transition midpoint is considerably high. With the decreasing of L, the folding transition midpoint moves to the right. Ψ_m increases from -0.94 for $L=100$ Å to about -0.91 for $L=60.8$ Å (shown in the inset of Fig. 3). Since Ψ implies the denaturant concentration, a bigger value of Ψ for the folding transition midpoint implies that a higher denaturant concentration is required to denature the protein. Therefore, the ordered water greatly promotes the stability of protein 1COA; the smaller the confined space, the more significant the improvement.

Similar behavior can also be observed in the other five proteins studied in this paper (shown in Table I). We have computed the relative variance of the denaturing intensity of the folding transition midpoint of the proteins in Eordered and Econfined, denoted by $\delta\Psi_m=(\Psi_m^i-\Psi_m^c)/|\Psi_m^c|$, for different L. The results are shown in Fig. 4. It is found that the relationship between $\delta\Psi_m$ and L can be described by the exponential law $\delta\Psi_m=\mu e^{\zeta L}$. The parameters μ and ζ for different proteins are listed in Table I. It should be noted that the exponential parameters ζ for all six proteins are close to a value of $\bar{\zeta}=-0.024$, suggesting a possibility of universal behavior of the effect of the ordered water for different proteins.

C. Folding rate

The kinetics of protein folding is described by the folding rate (k_f) in this study. Here, the folding rate k_f is taken to be

TABLE I. Parameters in the exponential fittings on the dependence of the relative variation in the folding transition environment ($\delta\Psi_m$) together with the folding rate ratio (k_f^i/k_f^c) on the distance of two plates (L) for different proteins in the environment of ordered water on the plate boundaries. Here, R_g is the radius of gyrate of the native conformation of a protein, which indicates the spatial size of the protein.

Proteins					$\delta\Psi_m=\mu e^{\zeta L}$		$k_f^i/k_f^c=\eta e^{\xi L}$	
Protein name	PDB code	Structure class	Chain length	R_g (Å)	μ	ζ	η	ξ
Barstar	1BTA	α	89	11.1	0.14	−0.024	11.5	−0.018
ACBP	2ABD	α	86	11.8	0.13	−0.025	6.3	−0.015
CspB	1CSP	β	67	10.1	0.14	−0.023	9.1	−0.017
α-spectrin SH3	1SHG	β	57	9.3	0.16	−0.024	8.8	−0.014
Ubiqution	1UBQ	α/β	76	11.0	0.13	−0.023	9.1	−0.015
CI2	1COA	α/β	64	10.3	0.17	−0.025	10.3	−0.018

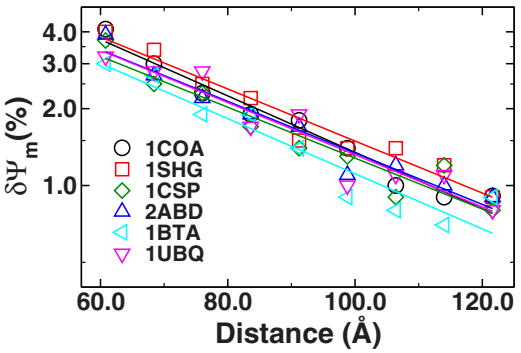

FIG. 4. (Color online) Relative variation in the folding transition midpoint caused by the ordered water $[\delta\Psi_m=(\Psi_m^i-\Psi_m^c)/|\Psi_m^c|]$ at various L for six proteins. For every protein, the dependence of $\delta\Psi_m$ on L can be fitted by a negative exponential law. Each fitting line is labeled as the same color of the data.

$(\mathrm{MFPT})^{-1}$ (mean first passage time), and MFPT implies the average time required to fold a protein from a random coil conformation to its native state. Figure 5 shows k_f of the protein 1COA folding in Eordered and Econfined at six different denaturing conditions for four different confined spaces. To obtain a reasonable value of k_f, 100 runs have been processed to obtain each point in this figure. As shown in Fig. 5, k_f of protein 1COA increases as the distance between two plates (L) decreases in the range of the denaturing intensity we studied. In the same confined space (L) and condition (Ψ), k_f of protein 1COA in Eordered is faster than that in Econfined. Moreover, the dependence of the folding rate on L is more significant in Eordered than that in Econfined.

We have also computed the ratios between the folding rates in Eordered and Econfined (k_f^i/k_f^c) for all the six proteins. The results are shown in Fig. 6. Similar to the relationship between $\delta\Psi_m$ and L in Sec. III B, this ratio with respect to L can also be fit by a negative exponential law $k_f^i/k_f^c = \eta e^{\xi L}$ for every protein. Table I lists the fitting parameters. The fitting exponents (ξ) for all the six proteins are very close to a value of $\bar{\xi}=-0.016$.

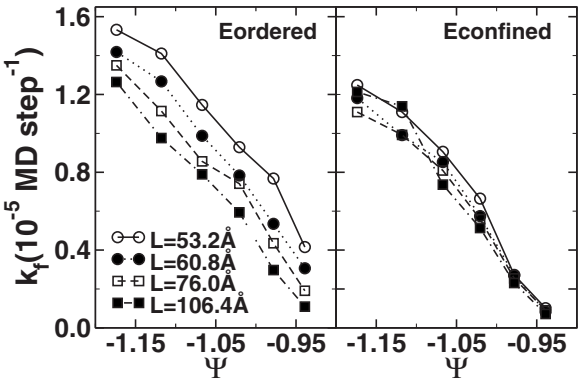

FIG. 5. Folding rate as a function of the degree of denaturing intensity for protein 1COA folding in the environment with and without the ordered water.

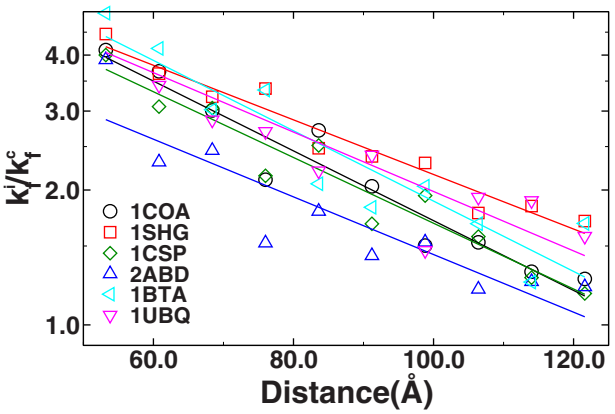

FIG. 6. (Color online) Ratio between the folding rates of proteins in the environment with and without ordered water (k_f^i/k_f^c) as a function of L at Ψ_m^0. The variation in k_f^i/k_f^c with respect to L for every protein can be described by a negative exponential law. The fitting lines are labeled as the same colors of the data. Ψ_m^0 is the denatured degree of the folding transition midpoint for a protein in bulk water.

IV. CONCLUSION

The effect of the low mobility of the ordered water on protein folding has been studied by using an off-lattice Gō-like model. Our results show that the effect is remarkable. It significantly improves the native state stability and greatly speeds up the folding rates of the proteins. Moreover, if we assume that the low mobility of the ordered water can be characterized by an exponential function shown in Eq. (3), negative exponential laws are found in both the stability and folding rate with respect to the size of the confined space for all the proteins we considered. We note that the quantitative behavior of the low mobility of the ordered water is not still clear yet; the exponential function we used may not correctly characterize the low mobility of the ordered water. Consequently, further studies are required on both the behavior of the ordered water and its impact on the protein folding.

Furthermore, it is thought that both the spatial confinement and ordered water enhance the native state stability of proteins but their mechanisms are different. The main contribution by the ordered water is the reduction in random noises, which somewhat likes to decrease the effective temperature. In contrast, for the spatial confinement, the reduction in the conformation entropy of the unfolded state is the main responsibility. However, in this paper, we have only applied the confinement to the protein in one dimension by two parallel plates so that the effect due to the confinement is weak. The remarkable effect of the ordered water only on those two parallel plates on protein folding demonstrates the importance of this study.

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