Conformational change path between closed and open forms of C2 domain of coagulation factor V on a two-dimensional free-energy surface

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We test a hypothesis that the closed form of the C2 domain of coagulation factor V is more stable than the open form in an aqueous environment using a two-dimensional free-energy calculation with a simple dielectric solvent model. Our result shows that while the free-energy difference between two forms is small, favoring the closed form, a two-dimensional free-energy surface (FES) reveals that a transition state (1.53 kcal/mol) exists between the two conformations. By mapping the one-dimensional order parameter ΔQ onto the two-dimensional FES, we search the conformational change path with the highest Boltzmann weighting factor between the closed and open form of the factor V C2 domain. The predicted transition path from the closed to open form is not that of simple side chain movements, but instead concerted movements of several loops. We also present a one-dimensional free-energy profile using a collective order parameter, which in a coarse manner locates the energy barriers found on the two-dimensional FES.

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I. INTRODUCTION

Factor V (FV) plays an important role in blood coagulation [1]. The FV circulates in an aqueous environment as a glycoprotein of a single chain (A1-A2-B-A3-C1-C2) [2]. It is converted into an active form of FV (FVa) through the proteolytic cleavages of Arg709, Arg1018, and Arg1545 by thrombin, which results in the loss of the B domain and leaves two unconnected chains (A1-A2:A3-C1-C2). Active FVa, bound to a platelet membrane, facilitates the binding of the activated factor X (FXa) and prothrombin to form the prothrombinase complex, which activates the blood clotting cascade through the generation of α-thrombin [3–5]. The C2 domain has attracted attention since it is found not only in FV, but also in FVIII [6], a protein homologous to FV, and in lactadherin [7,8]. The C2 domain (159 residues) effectively holds FVa on the surface of activated platelets and accelerates the cascade reactions of blood clotting [9].

Two distinct x-ray crystal structures are found for the FV C2 domain: closed and open forms [10]. These are compared in a later figure with the results of this study. Both forms have three adjacent spikelike loops at the lower part: loop 1 (Ser21–Trp31), loop 2 (Asn39–Asn45), and loop 3 (Gly75-Tyr84). The backbone root mean square deviation (RMSD) of the closed (PDB code: 1czv) to the open form (PDB code: 1czt) is 0.7 Å. One small difference, however, occurs near the loop 1 region (Ser21-Trp31) [10], where Trp26 and Trp27 at the apex of loop 1 are solvent exposed, presumably for immersion into the hydrophobic chains of the membrane in the open form [10]. On the other hand, the orientation of the two residues in the closed form provides components of an intramolecular hydrophobic core [10]. The orientations of Leu79, Trp26, and Trp27 provide a hydrophobic entrance when viewed from the membrane direction. The entrance is smaller for the closed form due to a tight hydrophobic packing [10]. These distinctive conformations of the FV C2 subdomain lead to the hypothesis that the closed form is stable in an aqueous environment and the open form on/in a lipid membrane [10]. This hypothesis has been previously evaluated by using molecular-dynamics (MD) simulations based on energetics [11]. In this article, we investigate general conformational stability of the two conformations of the FV C2 domain in simple dielectric solvents by free-energy calculation. Based on the free-energy landscape, we are also able to estimate the conformational change path from the closed to the open form.

II. MODEL

A. Order parameter

To construct a free-energy landscape, we first must define a reaction coordinate or order parameter, which describes the conformational change from theclosed to open form of the C2 domain of FV. Since the conformation of a protein is multidimensional in conformational space, an appropriate conformational change coordinate should also be multidimensional if it is to describe all conformational motions in detail. However, a free-energy calculation is normally performed in one or two dimensions. The projection of the information of the free-energy landscape of the multidimensional conformation space onto a lower dimension leads to the inevitable loss of some information. Thus, it is a nontrivial task to choose a proper coordinate or order parameter in a lower dimension with minimal loss of information from the higher dimension while calculating the free energy [12]. We choose the Q value, the similarity index between two conformations, as an order parameter for free-energy calculation. It is widely used in the free-energy studies of protein folding [13–16]. The Q value is defined as

$$Q_A = \frac{1}{N}\sum_{ij} \exp\left[-\frac{(r_{ij}-r_{ij}^A)^2}{2\sigma^2}\right], \tag{1}$$

where r_{ij} is the distance between the ith and jth atom in the conformation of interest, r_{ij}^A is the corresponding distance in the conformation A for which the Q_A value is defined, and the normalization factor N is equal to the number of pairs of atoms whose positions define the conformation. σ [17] in Eq.

(1) controls a resolution of the order parameter and is set to 2 Å. The similarity index Q_A changes from 1 (for the conformation A) to 0 (for a conformation with no resemblance to A). Generally, only C_α carbons are chosen in the calculation of the Q_A value. However, since the main conformational difference between closed and open forms of the FV C2 domain may lie in the disposition of side chains, e.g., Trp26 and Trp27 rather than backbone chains, we extend the range of atoms to also include all C_β, C_γ, C_δ, C_ε, C_Z, and C_H atoms [18]. Such an extension is for tracking the detailed movement of side chains at a high resolution. A total of 684 atoms are considered in the calculation of the Q_A value; this definition is essential in tracking the conformational change which involves small movements or rotations of side chains. This definition for Q_A also leads to a RMSD of the closed to open form approximately 1.6 Å considering 684 atoms. A total of 233 586 components in the Q_A pair sum were calculated. We performed a two-dimensional (2D) free-energy calculation using two order parameters Q_{closed} and Q_{open}; this involves significant computation. For a one-dimensional (1D) free-energy calculation we used ΔQ $(=Q_{\text{closed}}-Q_{\text{open}})$ as an order parameter.

B. Biasing potential and free-energy calculation

The conformational change from the closed to open form on a two-dimensional free-energy surface is guided by a biasing potential using the weighted histogram analysis method (WHAM) [19–23],

$$V(Q_{\text{closed}},Q_{\text{open}}) = \frac{1}{2}k_{\text{closed}}(Q_{\text{closed}} - Q_{\text{closed}}^{\text{min}})^2 + \frac{1}{2}k_{\text{open}}(Q_{\text{open}} - Q_{\text{open}}^{\text{min}})^2, \quad (2)$$

where k_{closed} and k_{open} are spring constants and $Q_{\text{open}}^{\text{min}}$ and $Q_{\text{closed}}^{\text{min}}$ are the locations at which biasing potentials are applied. The spring constants k_{closed} and k_{open} are in the range from 33.6 to 125.5 kcal/mol. A total of 213 windows are used for each different $Q_{\text{closed}}^{\text{min}}$ and $Q_{\text{open}}^{\text{min}}$ ranging from 0.7 to 1. MD simulation was performed for 0.54 ns for each window. The total sampling corresponds to 115.0 ns (0.54 ns/window × 213 windows) for dielectric constant ϵ =80.0 and 117.7 ns (0.54 ns/window × 218 windows) for dielectric constant ϵ=4.0. On a 100 ns timescale, significant side chain motions and loop dynamics can be observed [24]. For the one-dimensional free-energy calculation, $V(\Delta Q)$ $=\frac{1}{2}k(\Delta Q-\Delta Q^{\text{min}})^2$ is used as the biasing potential. The spring constants k are in the range from 14.0 kcal/mol to 365.0 kcal/mol. The timescale of the one-dimensional free-energy calculation corresponds to 16.8 ns (56 windows × 0.3 ns/window) for dielectric constant ϵ=80.0 and 19.2 ns (64 windows × 0.3 ns/window) for dielectric constant ϵ =4.0. All MD simulations for each window were performed using a large-scale atomic/molecular massively parallel simulator (LAMMPS) at the atomistic level [25] with the CHARMM27 protein-lipid force field [26]. The open and closed forms of the FV C2 domain were minimized using the steepest descent gradient method and equilibrated for 70 ps using target MD to keep the innate x-ray crystallographic structures less than 0.24 Å of the backbone RMSD for generation of the initial structures. NVT simulations were performed in a simple dielectric solvent model with different dielectric constants of 4.0 and 80.0 at a room temperature 300 K. The Coulombic and Lennard-Jones interactions were computed with a 9.0/14.0 Å twin-range cutoff, the same conditions employed by Mollica *et al.* [11] in their prior MD study of this system. As a simple dielectric solvent model, we adopted the linear distance dependent dielectric model, $\epsilon(r)=\epsilon r$, implemented in the LAMMPS [27,28]. The biasing potential is implemented using the chain rule, $F_\mu=-\frac{\partial V(Q)}{\partial \mu}$ $=-\frac{\partial V(Q)}{\partial Q}\frac{\partial Q}{\partial \mu}$. $V(Q)$ is the biasing potential and μ corresponds to x, y, or z components [18].

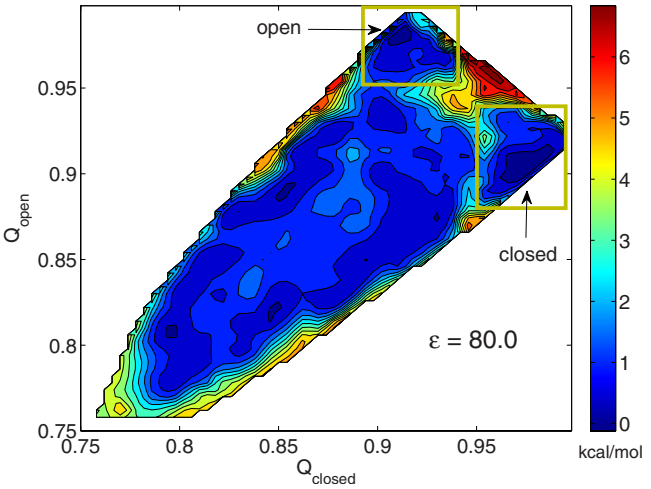

FIG. 1. (Color online) The two-dimensional free-energy surface (FES) of the FV C2 domain for dielectric constant ϵ=80.0. Yellow boxes denote the basins of closed and open forms.

III. RESULTS

A. Basin of open and closed forms

The two yellow boxes in Fig. 1 show the basins of open and closed forms of the FV C2 domain in the 2D map of the computed free energy. Generally, a basin is defined as the set of inherent structures [29–32], which is obtained by the minimization of the potential energy surface (PES). The conformations within a basin show a strong similarity to each other. From the viewpoint of statistical mechanics, the open or closed forms are not single points and thus should be treated as an ensemble of the corresponding basin. The advantage of a two-dimensional FES is that one can easily visualize the approximate size of a basin. Once the basins are determined from the two-dimensional FES, the free-energy difference can be obtained by numerical integration for each basin using the partition functions [33],

$$F_{\text{open}} = -\frac{1}{\beta}\ln\int_{\Gamma_{\text{open}}} e^{-\beta F(Q_{\text{closed}},Q_{\text{open}})}dQ_{\text{closed}}dQ_{\text{open}},$$

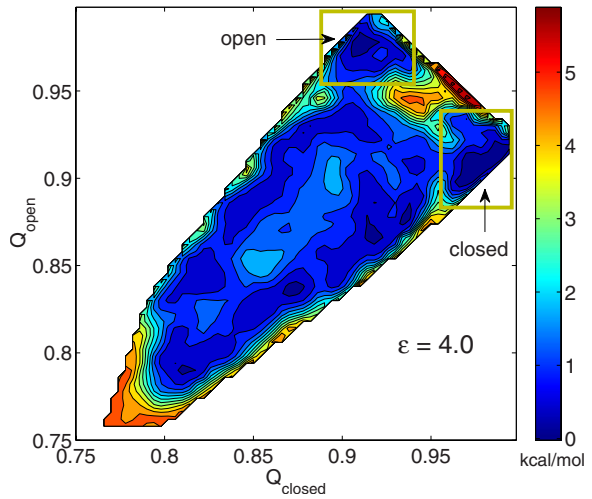

FIG. 2. (Color online) The two-dimensional free-energy surface (FES) of the FV C2 domain for dielectric constant ϵ=4.0. The shape of two basins are well conserved irrespective of the dielectric constant. However, the location of the highest energy barrier on two-dimensional FES is changed according to the different dielectric constant.

$$F_{\text{closed}} = -\frac{1}{\beta}\ln\int_{\Gamma_{\text{closed}}} e^{-\beta F(Q_{\text{closed}},Q_{\text{open}})} dQ_{\text{closed}} dQ_{\text{open}}, \quad (3)$$

where β is $\frac{1}{kT}$ and k and T denote the Boltzmann constant and temperature, respectively. Γ_{closed} and Γ_{open} correspond to the conformational space of the basins of the closed and open forms, which are denoted as boxes in Fig. 1. Figures 1 and 2 show the two-dimensional FES of the FV C2 domain for dielectric constant ϵ=80.0 and dielectric constant ϵ=4.0, respectively. Despite the two extremes of the dielectric constant, the two two-dimensional FESs show a similar landscape around the two basins. This implies that the hydrophobic effect is dominant over the hydrophilic effect in this case. However, the energy landscape beyond the basins on the two-dimensional FES shows somewhat different features depending on the dielectric constant. Especially, two-dimensional FESs manifest a shift of energy barriers in the middle regime of the conformational space (Q_{closed}, Q_{open}: 0.86–0.9, 0.86–0.92).

B. Free-energy difference between closed and open forms: Testing a hypothesis

The border line of a basin on the two-dimensional FES does not play any significant role due to the exponential decay of the Boltzmann weighting factor. The main contributions to free energy arise from structures with lower free energy within the basin. The free-energy difference between closed and open forms for dielectric constant ϵ=80.0 is approximately $\Delta F(F_{\text{closed}}-F_{\text{open}}) \sim -0.28$ kcal/mol according to Eq. (3) when the sizes of the basins are represented in Table I. In the case of dielectric constant ϵ=4.0, ΔF corresponds to −0.32 kcal/mol. Since the ΔF is similar for both dielectric constants, the implication is that specific lipid-protein interactions are responsible for driving the system to the open state in the environment of the lipid/membrane. The "hypothesis" that the closed form is more stable in an aqueous environment has been supported by Mollica *et al.* [11] even for the relatively short MD simulations (~1.5 ns in explicit solvent) based only on the energetics. However, there is a limitation in the criterion for the stability of the two forms based on the energetics only, with the exclusion of entropy. The magnitude for ΔF that we find also supports the hypothesis that the closed form is more stable in an aqueous environment. The free-energy difference, however, is small. On the other hand, considering the x-ray crystallographic structures of the open and closed forms, the main structural difference lies in loop 1 as shown in Fig. 4, especially the orientation of the Trp26 and Trp27 side chains. Thus, the small free-energy difference between the two conformations is perhaps not surprising. A more interesting feature of the conformational change of the FV C2 domain is that there exists a free-energy barrier corresponding to the approximate middle of the conformational space defined by Q_{closed} and Q_{open}. Such a free-energy barrier will discourage the direct conversion between the open and closed forms in an aqueous environment.

TABLE I. The size of the basins for closed and open forms in terms of Q_{closed} and Q_{open} for dielectric constant ϵ=80.0.

	Basin of closed form	Basin of open form
Q_{closed}	0.95–1	0.88–0.94
Q_{open}	0.88–0.94	0.95–1

C. Conformational transition path

For switching from the closed to open form, the transition path must overcome the free-energy barrier even though the two conformations have a small free-energy difference. The transition path for the switching from the closed to open form can be built on the two-dimensional FES by connecting the local free-energy minima [34]. In this article, the local free-energy minima are searched along ΔQ, $Q_{\text{closed}}-Q_{\text{open}}$, mapped onto the two-dimensional FES. This transition path with a dependence on the choice of order parameter may be the plausible path among the multiple possible paths for the conformational change. In Fig. 3, a predicted transition path is displayed for dielectric constant ϵ=80.0. The yellow line corresponds to the transition path determined from the highest Boltzmann weighting factor (local free-energy minimum). Transition state (TS) with 1.53 kcal/mol denotes the highest energy point located only along the transition path, respectively. One can clearly visualize the detour of the transition path between the two conformations on the two-dimensional FES. Such a detour of the transition path can be also seen for dielectric constant ϵ=4.0, for which the TS barrier is 1.44 kcal/mol (not shown).

Despite the significant computational demands, a two-dimensional FES shows clearly the conformational space of the FV C2 domain. By constructing the local free-energy minimum points, one of the possible multiple conformational transition paths is obtained. Figure 4 shows the superim-

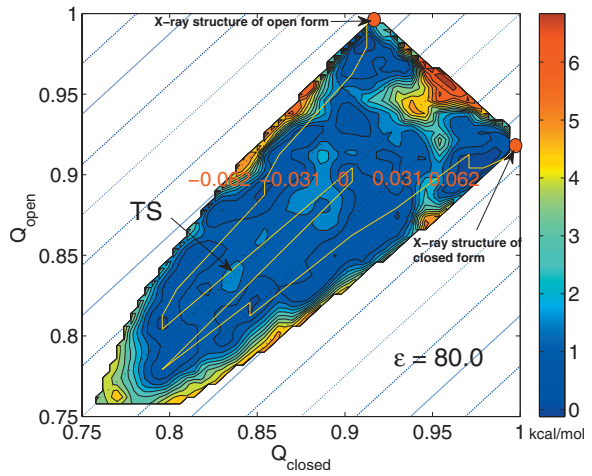

FIG. 3. (Color) The conformation transition path between the closed and open forms on two-dimensional FES for dielectric constant ϵ=80.0. The transition path with thin solid line is determined from the highest Boltzmann weighting factor (lowest free-energy minimum). Transition state (TS) denotes the highest energy point along the predicted transition path. The x-ray crystallographic structures for the closed and the open form are denoted as circles in two-dimensional FES. The dotted diagonal lines correspond to the contours of ΔQ when it is mapped onto two-dimensional FES.

posed images of conformations taken from the dominant path from the closed to open form for dielectric constant ϵ =80.0. One of the striking results from our free-energy calculation is that there is no direct simple conversion path from the closed to open form. As Fig. 4 shows, the conversion from the closed to open form is rather indirect, accompanied by the concerted motions of the flexible loops adjacent to loop 1 (Ser21–Trp31), loop 2 (Asn39–Asn45), and loop 3 (Gly75–Tyr84) as well as (Gly115–His122) without any significant backbone change in the folded core that has secondary structure. Indeed, when the conformational change from the closed to open form is triggered by the binding of the closed form on lipid, C6PS (1,2-dicaproyl-*sn*-glycero-3-phospho-L-serine), any significant backbone change is not detected [35]. Rather, the conversion seems to be processed by the conformational change of three spikelike loops that are very flexible parts in the FV C2 domain [11,36]. The conversion from the closed to open form is not only adjustments of simple side chain movement of Trp26 and Trp27. It is apparently a concerted movement of flexible loops including loop 1, loop 2, loop 3, and other flexible parts. Such a concerted movement comes mainly from the interconnected geometry [10] of these three spikes.

D. One-dimensional free-energy landscape

A one-dimensional free-energy calculation has an advantage over a two-dimensional free-energy calculation in saving computational time if the order parameter can be properly chosen. The definition of a one-dimensional collective order parameter, ΔD_{RMSD}, for two different conformations for DNA [37] and for the allosteric adenylate kinase [38] has proven to be useful in predicting the free-energy landscape.

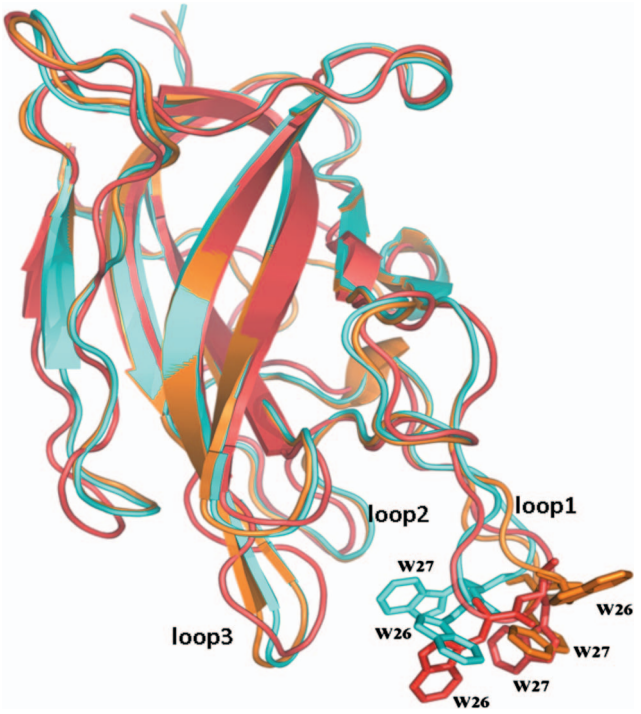

FIG. 4. (Color) The three superimposed images, the closed form (cyan), the open form (orange), and the TS conformation (red), taken from the conformational transition path from the closed to open form for dielectric constant ϵ=80.0. The RMSD for C_α, C_β, C_γ, C_δ, C_ε, C_Z, and C_H atoms of the TS form to the closed form is 2.12 Å; to the open form 2.18 Å.

However, despite the advantage of simplicity and rapid convergence in the free-energy calculation, the utility of a one-dimensional free-energy calculation using a collective order parameter remains problematic. A two-dimensional FES enables us to verify how effectively a one-dimensional free-energy calculation reflects the free-energy landscape. It is, however, meaningful to employ a one-dimensional free-energy calculation with a one-dimensional collective order parameter ΔQ to find a plausible transition path on the two-dimensional FES.

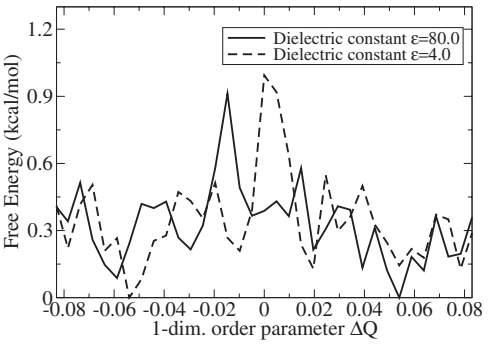

FIG. 5. One-dimensional free-energy landscape using a one-dimensional collective coordinate ΔQ for dielectric constant ϵ =80.0 and dielectric constant ϵ=4.0. The free-energy barrier in dielectric constant ϵ=80.0 corresponds to ~0.91 kcal/mol around −0.015; for the dielectric constant ϵ=4.0, the free-energy barrier is located in the vicinity of ΔQ=0 with 0.99 kcal/mol.

Figure 5 shows a one-dimensional free-energy landscape using the collective order parameter ΔQ for dielectric constant $\epsilon=4.0$ and dielectric constant $\epsilon=80.0$, respectively. The dielectric constant ϵ changes the location of the energy barrier as shown in Fig. 5. The one-dimensional free-energy calculation gives the energy barrier of ~0.91 kcal/mol at $\Delta Q=-0.015$ for dielectric constant $\epsilon=80.0$ and ~0.99 kcal/mol at $\Delta Q=0$ for dielectric constant $\epsilon=4.0$. This shift of the energy barrier in the one-dimensional free-energy landscape according to the change in dielectric medium is also shown in the two-dimensional FES from Figs. 1 and 2. On the other hand, the TS on the two-dimensional FES corresponds to 1.53 kcal/mol at $\Delta Q=-0.004$ (Q_{closed}, Q_{open}: 0.834, 0.838) for dielectric constant $\epsilon=80.0$ in Fig. 1. For dielectric constant $\epsilon=4.0$, the TS is 1.44 kcal/mol at (Q_{closed}, Q_{open}: 0.822, 0.826). Thus, there is some difference between the TS on the two-dimensional FES and the energy barrier found for the one-dimensional free-energy landscape. However, strictly speaking, the one-dimensional free-energy landscape corresponds to the ensemble average of the free energy on the two-dimensional FES as $e^{-\beta F(\Delta Q')} \sim \int e^{-\beta F(Q_{\text{closed}},Q_{\text{open}})} \delta[\Delta Q' - (Q_{\text{closed}} - Q_{\text{open}})] dQ_{\text{closed}} dQ_{\text{open}}$.

It has been shown that the one-dimensional free energy reduces to the ensemble average of the two-dimensional FES in the case of Trp-cage [33]. For a fair comparison of the reconstructed 1D and the pure 1D free energy, the same level of sampling should be effected in 1D and 2D, using a new, yet to be discovered, localized collective order parameter unlike ΔQ. As shown in Figs. 1, 2, and 5, ΔQ extracts the information on two dimensions and displays it in one dimension to some extent, especially the middle regime of the transition from the closed to open form. However, ΔQ shows some limitations in reproducing the two-dimensional FES information near the open- and closed-form basins. This limitation is mainly due to the peculiar geometry of basins relevant to the open and closed forms. The basins of the closed and open forms fall in a perpendicular direction to ΔQ. Thus, in defining the basins with the one-dimensional free-energy landscape, contributions outside the basins cannot be excluded. In other words, the basin is not well *localized* in terms of ΔQ. The geometry of the basin is quite relevant to the choice of the effective order parameter in a lower dimension [33]. A detailed comparison of the pure and reconstructed one-dimensional free energy may be found in Ref. [40].

IV. CONCLUSION

The two-dimensional FES shows that the closed form is more stable by 0.28 kcal/mol in dielectric solvent with $\epsilon=80.0$, which partially supports the "hypothesis" that the closed form is more stable in an aqueous environment. However, the magnitude $\Delta F=(F_{\text{closed}}-F_{\text{open}})$ is small. The probability ratio of the closed to the open form in dielectric constant $\epsilon=80.0$ corresponds to $P_{\text{closed}}/P_{\text{open}}=\exp[-\Delta F/kT] \sim 1.6$. To provide a precision error estimate for the free-energy calculations, we adapted the Monte Carlo bootstrap analysis method [23] to our 2D WHAM calculations ($\epsilon=80.0$). We found an average error of 2.7% in our free-energy calculation. In the Monte Carlo bootstrap analysis [23,39], we produce a "trial data" set by randomly drawing the samples with the replacement from the original data set and compute the standard deviation of the average of the trial data set. This corresponds to the estimation of the statistical precision of the average value using "real data." The empirical force field and solvent model used in these calculations will surely contribute to overall errors inaccuracy of the method.

We calculated both the two-dimensional FES and the one-dimensional free-energy landscape of the FV C2 domain the in simple dielectric solvent model. We found that the one-dimensional free-energy landscape is reasonably consistent with the free-energy profile along the predicted transition path on the two-dimensional FES in the middle regime of conformational change between closed and open forms. The energy barrier of TS on the predicted transition path along ΔQ on the two-dimensional FES is 1.53 kcal/mol for dielectric constant $\epsilon=80.0$ (1.44 kcal/mol for dielectric constant $\epsilon=4.0$). We infer that only 1.53 kcal/mol or 2.6 kT could drive the conversion process from the closed to the open form.

Despite the nature of our simple implicit solvent model as compared, for instance, to the Generalized Born (GB) model [41], the shape of two basins is well conserved irrespective of the dielectric constants. From that, we conjecture that the free-energy difference between the closed and open form will not be greatly changed even in an explicit solvent model. However, for a more complete test of the hypothesis, the free-energy calculation of the FV C2 domain bound to a membrane should be performed.

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