Simulations of Proteins with Inhomogeneous Degrees of Freedom: The Effect of Thermostats

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Received 13 December 2007; Revised 20 January 2008; Accepted 31 January 2008
DOI 10.1002/jcc.20951
Published online 25 March 2008 in Wiley InterScience (www.interscience.wiley.com).

Abstract: Proteins with a long flexible polymeric tail attached at their N- or C-terminus are studied using molecular dynamics (MD) simulations of a coarse-grained model for protein folding where the temperature is regulated by either the Berendsen or the Langevin thermostat. These thermostats show different abilities to regulate the temperature of these systems that include flexible and more rigid regions. In the simulations with the Berendsen thermostat, the flexible tail is significantly hotter than the protein, both in its folded and unfolded states. Upon weakening the strength of the Berendsen thermostat, the temperature gradient between the fast and the slow degrees of freedom is significantly decreased, yet linkage between the temperatures of the flexible tail and the protein remains. The Langevin thermostat is proven to regulate the temperature of these inhomogenous systems reliably, without discriminating between the slow and fast degrees of freedom. The Langevin thermostat is less sensitive than is the Berendsen thermostat to the strength of the coupling between the protein system and the thermal bath. Our study calls for special care in choosing the thermostat for MD simulations of systems with inhomogenous degrees of freedom. Using the Berendsen thermostat with strong coupling would result in mistaken thermodynamic descriptions of such systems.

 J Comput Chem 29: 1992–1998, 2008

Key words: molecular dynamic simulation; thermostat; Langevin dynamics; protein folding; Coarse-grained model

Introduction

Many proteins include unstructured polypeptide segments at their N- or C-terminus. The disordered regions at the protein termini are a specific category of a large family of proteins, termed intrinsically disordered proteins, that often adopt a specific structure only upon binding.[1] Bioinformatic studies have suggested that over 30% of eukaryotic proteins have at least one long (>50 residues) disordered region. The intrinsically unstructured proteins encompass a variety of biological functions including an improved ability to specifically recognize several target molecules in the cell.[2–4] It was previously demonstrated that the terminal can affect the biophysical properties of the proteins. In some cases, possession of a flexible tail was found in the laboratory to have no significant effects on protein characteristics and it could therefore be viewed, in these cases, as an inert conjugation to the protein. In other cases, the flexible region was found to exert a more significant effect. For instance, in the prion protein that is composed of two domains of similar length (a helical domain and a disordered domain at the N-terminal), it was found that truncating the length of the flexible tail gradually reduces the thermodynamic stability of the protein.[5] The stabilization origin upon truncation of the disordered domain is unclear. One might conjecture that removing the flexible region that includes many charged residues affects the balance between enthalpy and entropy in favor of the folded state. Alternatively, the tail might be viewed as an entropic tail that interacts with the foldable chain by affecting its entropy.

In this article, we focus on exploring the appropriate protocol for undertaking molecular dynamics (MD) simulations of proteins, where each protein bears a flexible tail. MD simulations are widely used to study the dynamic properties of various systems such as liquids, macromolecules, and large biomolecules. MD studies provide important physical understanding of molecular systems and the opportunity to link macroscopic observations to their microscopic origin. MD studies, which are currently routinely used for various biomolecular systems (such as proteins, nucleic acids, membranes, oligosaccharides, and their assemblies) enable the establishment of a linkage between MD and biological function.[6–8]

An MD simulation can be performed at constant energy, yielding the microcanonical ensemble (having a constant

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Contract/grant sponsors: the Kimmelman Center for Macromolecular Assemblies, the Clore Center for Biological Physics, the Center for Complexity Science, and Foundation Fernande et Jean Gaj

number (N) of particles, volume (V), and enthalpy (E), i.e., constant NVE). Because of the energy conservation, the Newtonian equations of motion (i.e., disregarding quantum effects) are likely to represent the dynamics of the system. Simulating NVE ensembles has some drawbacks, among them, the possibility of large energy drift and of common experimental conditions not corresponding to constant energy and volume conditions. MD simulations are therefore frequently performed by probing instead the more convenient canonical ensemble in which constant enthalpy is replaced by constant temperature (T) (i.e., constant NVT).[9] The temperature in MD simulations is translated to kinetic energy. The kinetic energy of each degree of freedom is equal to 0.5 k_BT, where k_B is the Boltzmann constant. Accordingly, the total kinetic (thermal) energy (i.e., $0.5\sum_{i=1}^{N} M_iV_i^2$) of a molecule of N atoms is given by (3N-6) $k_BT/2$, and the subtracted 6 degrees of freedom correspond to those of the translation and rotation of the center of mass.

Several thermostats are available to adapt MD simulations to the canonical ensemble by maintaining a fixed temperature.[10] These temperature regulation approaches include the Nosé-Hoover thermostats that generate the true canonical distributions of velocities, and rescaling velocity methods such as the Andersen and Berendsen thermostats. The rescaling approaches ensure that the average kinetic energy of the system corresponds to the expected value at the desired temperature and can be simply implemented in integration algorithms. In all approaches, the degree of perturbation of the real time evolution of the system can be adjusted by manipulating various parameters of the thermostats.

The Berendsen thermostat[11] employs a proportional scaling of the velocities at each time step with the scaling factor given by

$$\lambda = \left[1 + \frac{\Delta t}{\tau_B}\left(\frac{T_{ref}}{T(t)} - 1\right)\right]^{1/2}$$

where Δt is the integration time step, τ_B is the time constant of the Berendsen thermostat that describes the strength of the coupling of the system's temperature to that of the heat bath, T_{ref} is the desired temperature and $T(t)$ is the instantaneous temperature at each time step.[11] By varying the thermostat time constant, τ_B, one can, in effect, increase or decrease the degree of coupling to an external bath. A larger value for τ_B indicates weaker coupling, since more time is required to achieve a given T_{ref} after an instantaneous change from that temperature. At the limit, $\tau_B \to \infty$, the Berendsen thermostat is inactive and the simulations sample a microcanonical ensemble. At the other extreme, if the value of τ_B is too small, the temperature fluctuations become unrealistically small.

In the Langevin thermostat, at each time step all the particles experience a stochastic force (white noise) and have their velocities lowered since kinetic energy is dissipated to the bath. The loss of kinetic energy is proportional to the velocity of each particle and to the damping constant (also called the frictional coefficient) γ that determines the strength of the coupling to the bath. In contrast to this energy dissipation, the system may gain energy by occasional implicit kicks from the surroundings. Introducing friction and stochastic terms in the equations of motion yields the Langevin equation

$$m_i\dot{v}_i = F_i - \gamma m_i v_i + R_i(t)$$

where F_i is the systematic force acting on the particle and R_i is a Gaussian stochastic variable with zero mean and with variance

$$\langle R_i(t)R_j(t+\tau)\rangle = 2m_i\gamma k_B T\delta(\tau)$$

The average magnitude of the random (R_i) and the frictional ($-\gamma m_i v_i$) forces satisfies the fluctuation-dissipation theorem. The balance between these forces (as dictated by the variance of the random force) keeps the temperature constant. The magnitude of γ determines the relative strength of the frictional force with respect to the random forces. The larger the value of γ, the greater the influence of the surrounding fluctuating force.

Each thermostat has some limitations and the simulator has to pay attention to the choice of thermostat used. Although the performance of the thermostat is critical to properly regulate the temperature and to simulate the desired thermodynamic ensemble, to date, these aspects have not been systematically investigated. Previous studies have focused on inspecting the parameters of some thermostats and the nature of the associated trajectories[12–14] and pointed out the effects that the thermostat had on dynamic behavior. For example, a problem is encountered when simulating molecular systems involving distinct sets of degrees of freedom with different characteristic frequencies. In this case, the joint coupling of all degrees of freedom to a thermostat may lead to different effective temperatures for the distinct subsets of degrees of freedom, because of a too slow exchange of kinetic energy between them. A typical example is the so called "hot solvent–cold solute" problem in simulations of macromolecules. Because the solvent is significantly more sensitive than the solute to noise in the simulation, the coupling of the whole system to a single thermostat may cause the average solute temperature to be lower than the average solvent temperature. A possible solution to this problem is to separately couple the solute and solvent degrees of freedom to two different thermostats. Although the "hot solvent–cold solute" problem is a relatively known phenomenon, it has been vaguely described in the literature.[15] In that regard, the current manuscript can be treated as a systematic description of this phenomenon.

A second problem is encountered when using a simulation program that applies the thermostat directly to the atomic velocities without taking stochastic effects into account. In this case, the system's linear and angular momenta are not conserved and the thermostat may shift kinetic energy from high frequency to low-frequency degrees of freedom that lead to the "flying ice cube effect."[16]

In this article, we study the folding thermodynamics and kinetics of proteins with long flexible tails using a coarse-grained model based on the native topology of the studied protein. The dynamics of the systems at hand are simulated here using MD protocols with coupling to the Berendsen and the

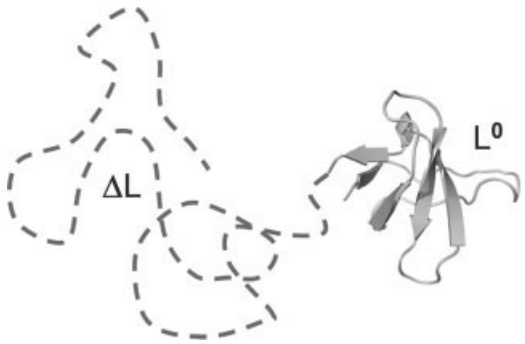

Figure 1. A line drawing of an SH3 domain (composed of $L^0 = 56$ residues) bearing a flexible tail of ΔL residues at its C-terminal.

Langevin thermostats. We show that the dynamic properties of the biomolecules highly depend on the nature of the thermostat.

Methods

Coarse grained MD simulation protocols were applied on a set of protein models having an attached flexible polymer tail to investigate the possible effect the choice of thermostat in those simulations might have. We studied the folding features of an SH3 domain (pdb 1srl) with a long polymeric tail (see Fig. 1) using two types of thermostats: the Berendsen and the Langevin thermostats. Protein dynamics is addressed using the native structure-based (Gō) model. This model relies on the idea, which was supported by several experimental and theoretical studies,[17–22] that protein sequences are evolutionarily selected to be minimally frustrated to reduce conflicts between non-native interactions and interactions that support the folded state. The native topology based model, in other words, assumes a smooth funneled energy landscape for folding. This model, although neglecting the potential contribution of non-native interactions,[23] has reproduced many of the experimental observations of the mechanisms of folding.[18,20,24,25]

In the coarse-grained model, each amino acid is represented by a single bead centered at its Cα atom. The Hamiltonian is a summation over bonded and nonbonded potential terms. In the native structure-based models, the terms get their lowest energy at the corresponding values of the native state. The bonded terms that maintain the local structure of the protein include a harmonic potential for bond distances (the distance between adjacent Cα beads is 3.8 Å) and bond angles between the pertinent Cα atoms of the model. The dihedral angles term treats angles of subsequent four Cα beads. The non-bonded terms that maintain the secondary and tertiary structure of a protein include two contributions. One term represents each native interaction using the Lennard-Jones potential and the other term is exclusively repulsive, and acts upon Cα atoms that do not form native interactions (termed non-native interactions). The non-native repulsive interactions can be viewed as excluded-volume terms that do not allow crossing of the polymeric chain. The distance between beads that form native contacts is defined by the crystal structure, and the repulsive distance for non-native contacts is 4 Å (i.e., the radius of each Cα bead is set to 2 Å). The details of the native topology based models can be found in previous studies.[25–27]

A flexible polypeptide tail is attached to each protein at its C- or N-terminus. The tail dynamics is dictated by the harmonic potential for the covalent bonds and the repulsive potential that exerts excluded volume effect between the tail's beads and between the tail and the protein. The polypeptide tail, accordingly, is defined by two terms while the protein is defined by five terms. The tail does not form any specific interactions with the structured protein besides the bond that links the tail to the protein. The tail, therefore, may have only an entropic effect on the folding of the protein domain. The values of the bond distance and repulsion distance of the tail beads are identical to those of the structured protein.

We have linked a tail of various lengths (10–150 residues) to the SH3 domain (β-sheet protein, 56 residues). In addition to investigating the effect of the tail's length on SH3 folding, we have studied the effect of tethering a flexible tail to the 434 repressor (α helical protein, pdb 1r69), the villin headpiece (α protein, pdb 1vii), protein L (α/β protein, pdb 2ptl), and the ribosomal L9 protein (α/β protein, pdb 1cqu) at either the N- or C-terminus.

To characterize the folding thermodynamics of the protein with the flexible tail, each system was simulated at various temperatures for long runs. The WHAM (weighted histogram method)[28] analysis was used to estimate some thermodynamic properties of the systems. These include the energetic barrier that governs the reaction as well as its thermodynamic stability measured by the folding temperature, T_F, where the free energy of the unfolded and folded states is identical (i.e., ΔG for folding is zero). The T_F is characterized by the peak of the heat capacity curve where $C_v = k_B T^2[\langle E\rangle^2 - \langle E^2\rangle]$. A larger T_F value correlates with greater thermal stability.

Results and Discussion

The effect of a flexible tail on the SH3 domain was explored using two different thermostats; the Berendsen and the Langevin thermostats. The polypeptide tails in these simulations have no stabilizing interactions with the protein and their dynamics is governed solely by an excluded volume term (i.e., a repulsion potential) that prevents crossing of the protein and tail chains. Figure 2 shows the folding temperatures of SH3 with a tail of length 10, 20, 40, 80, 100, or 150 residues using the two thermostats. Using the Berendsen thermostat, the T_F of the protein-tail systems increases significantly with the increasing tail length (Fig. 2, filled triangles). This increase levels off for a tail of about 100 residues. Using the Langevin thermostat, the T_F of identical protein-tail models was found to be independent of tail length.

The discrepancy between the thermodynamics of the SH3-tail systems when simulated with the Berendsen and Langevin thermostats may arise from an artifact in the Berendsen thermostat in estimating the redistribution of kinetic energy. To examine the origin of the apparent difference in stability, as described by the two thermostats, we plotted the temperature of the tail and

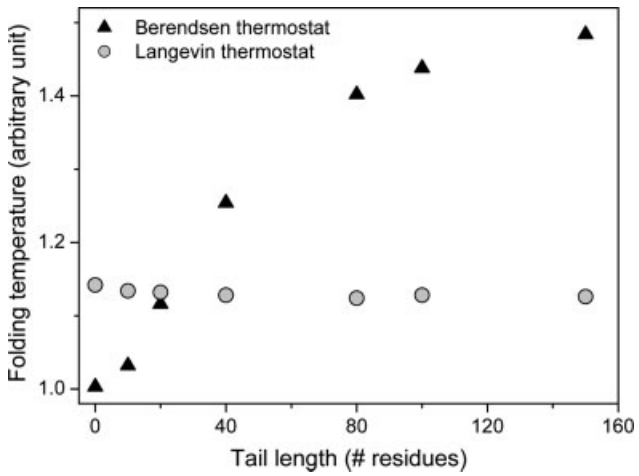

Figure 2. The folding temperature, T_F, of an SH3 domain with a flexible polymeric tail of various lengths (10–150 beads) attached to its C-terminal as calculated through molecular dynamic simulations using the Berendsen and Langevin thermostats. The T_F is estimated from the peak of the specific heat curve. The integration time in all the simulations is 0.005, the time constant (τ_B) of the Berendsen thermostat is 0.2 ps, and the friction constant (γ) of the Langevin thermostat is 0.05 s^{-1}. The error of the T_f is about 2%.

protein beads in each trajectory, based on the total kinetic energy of the corresponding beads (see Fig. 3). The temperatures of the tail and the protein are very similar along each of the Langevin thermostat simulation trajectories. These temperatures are evidently not affected by the tail's length (the relatively large noise in the temperature of the SH3 with a short tail of 10 residues is because it was calculated based on the average kinetic energy of only a very few beads). The temperatures of the tail and the protein when simulated with the Berendsen thermostat are very different. In these trajectories, the temperature of the tail is significantly larger than that of the protein by up to 50%. The polarization between the temperature of the tail and protein becomes more severe as tail length increases. The increase in the T_F of SH3, as shown in Figure 2 for the Berendsen thermostat (filled triangles), is therefore not because of the greater stability of the protein but because the flexible polymeric tails in these simulations absorb the thermal energy and become much hotter than the foldable protein. One may note that the temperature of the flexible tail simulated with the Berendsen thermostat depends on the state (i.e., folded/unfolded) of the SH3 protein. That is, the tail is hotter when the SH3 is folded (has smaller fluctuations) than when the SH3 is unfolded. The temperature gradient formed in the simulation with the Berendsen thermostat is similar to that associated with the so-called "hot solvent–cold solute" artifact. A similar effect was observed in simulations of mixtures of water and heavier molecules, such as octane, where the water gained more thermal energy.[29]

To examine the effects of thermostat strength on the temperature of the tail and the foldable protein, the SH3 protein with a tail of 80 beads was studied using four different values of τ_B and γ for the Berendsen and Langevin thermostats, respectively (see Fig. 4). Temperature regulation by the Langevin thermostat with γ = 0.1–100 is very similar and shows no temperature gradient between the temperatures of the tail and the protein. In addition, these simulations indicate that folding transitions are less frequent for larger γ (stronger coupling to the bath), which indicates a disadvantage in using a too high frictional coefficient due to it slowing down the system kinetics, which in return reduces the sampling efficiency. Simulating the SH3-tail80 (80 beads) system using the Berendsen thermostat with larger values of τ_B (i.e., weaker coupling to the bath) significantly influences the temperature gradient between the tail and the protein. For τ_B = 10 ps the difference between the temperatures of the tail and the protein is small, however, a linkage is still observed between their temperatures. Fluctuations that increase the temperature of the tail are mirrored by a decrease in the temperature of the SH3 protein. This coupling between the degrees of freedom of the flexible and rigid parts of the protein exist also for τ_B = 100 ps (data not shown).

To test whether the performance of the Berendsen thermostat depends on the protein topology, we used the Berendsen thermostat to study the thermodynamics of four additional proteins that have different secondary structure content and topological complexity, namely: protein L, the ribosomal protein L9, the 434 repressor, and the villin headpiece. A flexible polymeric tail that is composed of 10–150 residues was attached to one (or both) terminus of these proteins. Figure 5 shows the shift of the specific heat capacity (Cv) plot when a flexible tail is attached to the C-terminal of protein L. In addition to the higher T_F of the tailed protein L variants, their kinetics severely slows down as reflected by the increased free energy barrier for folding. On the basis of these results, one may mistakenly conclude that attaching a long flexible entropic tail to a protein results in larger thermodynamic and kinetic stability.

Figure 6 summarizes the change in T_F and in the free energy barrier for folding ($\Delta F^{\#}$) relative to the corresponding values for the wild-type protein, (T_F^0 and $\Delta F^{\#0}$, respectively) when simulated with the Berendsen thermostat. For all the four proteins studied, irrespective of whether the tail was tethered to the C- or N-terminal, a strong correlation is seen between tail length and the folding temperature of the proteins when simulated using the Berendsen thermostat. In all cases, the maximal increase in T_F relative to the wild-type proteins is about 20–30%. This effect is achieved in most of the cases with a tail that is about 2–3 times longer than the protein. For the villin headpiece, the maximal increase in T_F is achieved for tails that are 6 times longer than the protein—an effect that can originate from the fact that it has a very low folding barrier in our simulation model. The folding barrier for all the proteins with a long tail is significantly larger than that of the wild-type proteins. The dependency of the folding barrier on tail length, however, shows less uniform behavior than the behavior of the T_F. For some systems, the barrier is smaller when short tails are used, yet, for all the cases, long tails slow down folding kinetics (the barrier is about 80% higher than that of the wild-type protein) when simulated using the Berendsen thermostat.

Conclusions

Flexible terminals of naturally occurring proteins often carry out an important functional role since they participate in specific molecular recognitions. In our study, we explored the effects

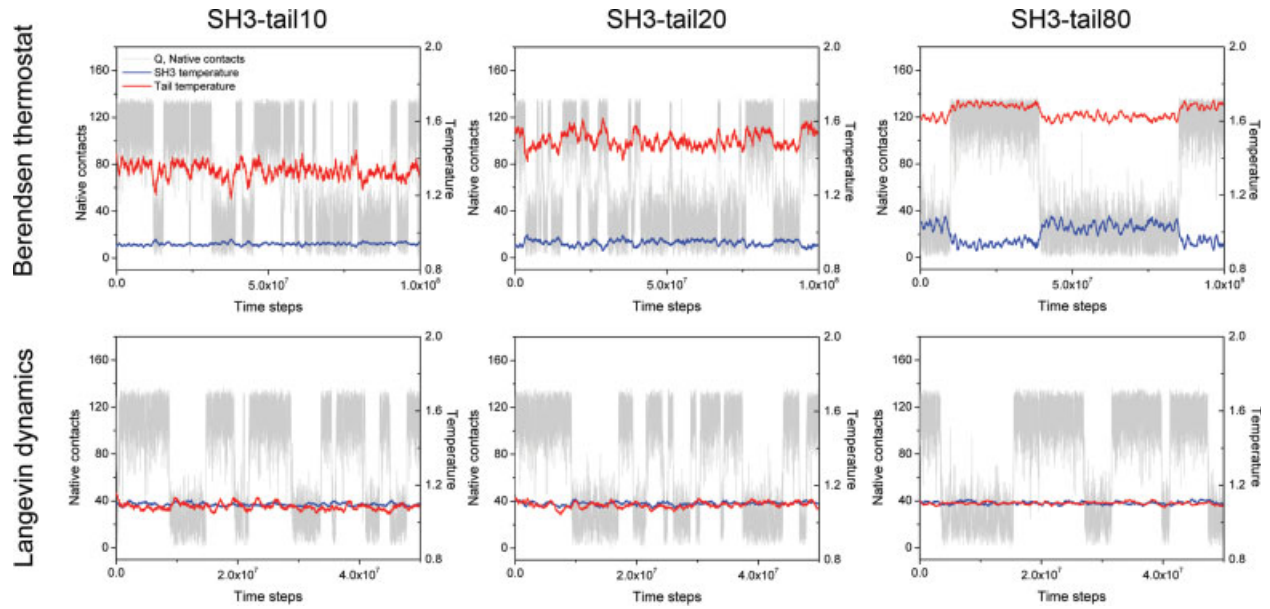

Figure 3. Time evolution of the temperatures of the flexible polymeric tail and the SH3 domain simulated with the Berendsen thermostat (top panels) and the Langevin thermostat (bottom panels). For each thermostat, three trajectories that correspond to three SH3-tail systems having different tail lengths (10, 20, and 80 beads) are shown. In addition to the temperature of the tail (red line) and the SH3 domain (blue line), the time evolution of the number of native contacts is reported for each trajectory (grey line). The native contacts exhibit two-state behavior that corresponds to the unfolded (low value) and folded (high value) states of the SH3 domain. The parameters of these simulations are identical to those shown in Figure 2. The depicted trajectories were simulated at the folding temperatures.

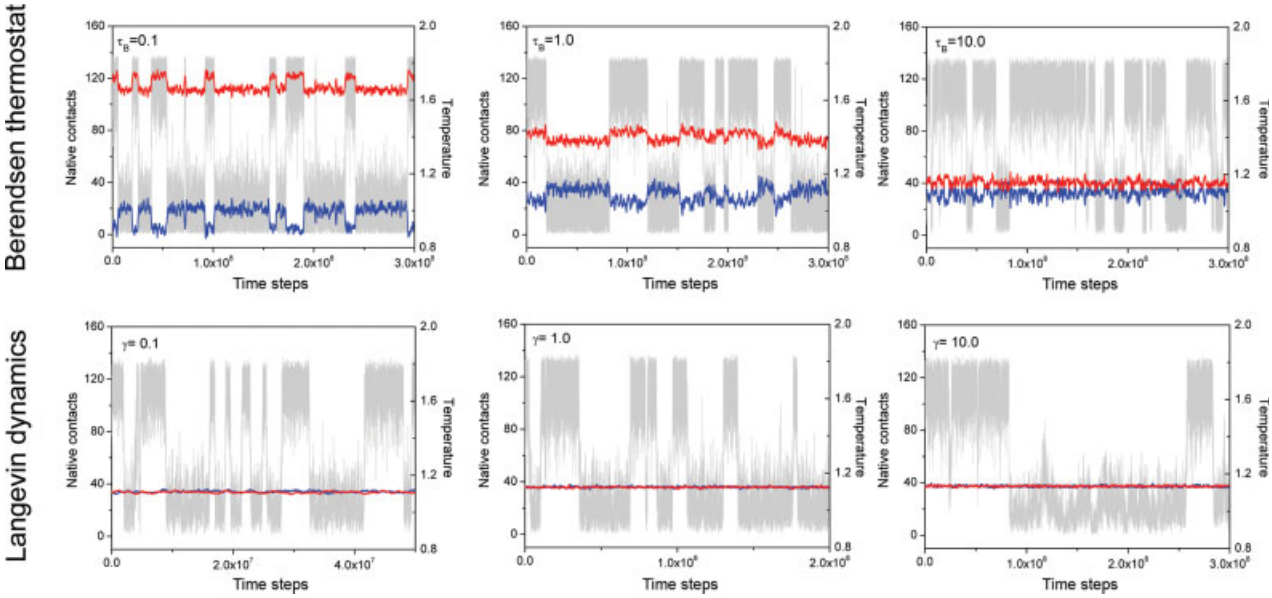

Figure 4. The effect of the strength with which the temperature of the protein is coupled to that of the thermal bath on the temperature gradient between the flexible tail and the foldable protein. The SH3 protein with a tail of 80 beads (the SH3-tail80 system) was simulated using three values for the constants of thermostat time (τ_B) and friction (γ): τ_B = 0.1, 1, and 10 ps for the Berendsen thermostat, and γ = 0.1, 1, and 10 for the Langevin thermostat. The other simulation settings are identical to those shown in Figure 2. The color scheme is identical to that of Figure 3.

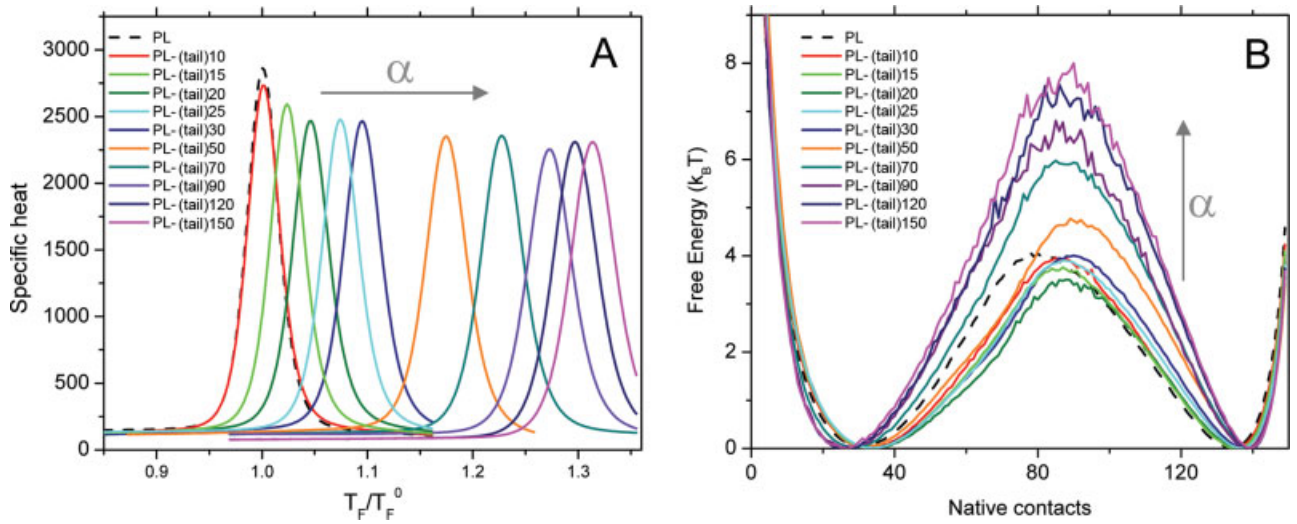

Figure 5. The thermodynamics of protein L (PL) with a flexible tail attached at the C-terminal as obtained from molecular dynamic simulations using the Berendsen thermostat with $\tau_B = 0.2$ ps. The plot of specific heat capacity (Cv) against simulated folding temperature (T_F) as a proportion of the native protein's folding temperature (T_F^0) and the free energy profiles for folding (B) show a strong correlation with tail length. α indicates the length of the tail, ΔL, in the units of length of the wild-type protein, L^0 (i.e., $\alpha = \Delta L/L^0$).

that tethering a flexible entropic polypeptide chain to either the C- or N-terminal of several proteins had on their thermodynamic properties. More specifically, we asked whether a long tail could affect protein stability and folding rates. Some experimental evidence supports this notion by demonstrating that the removal of the flexible tails causes a significant destabilization of the proteins to which these tails are attached. In this scenario, one may envision that the tethered flexible polymer acts as an inter-

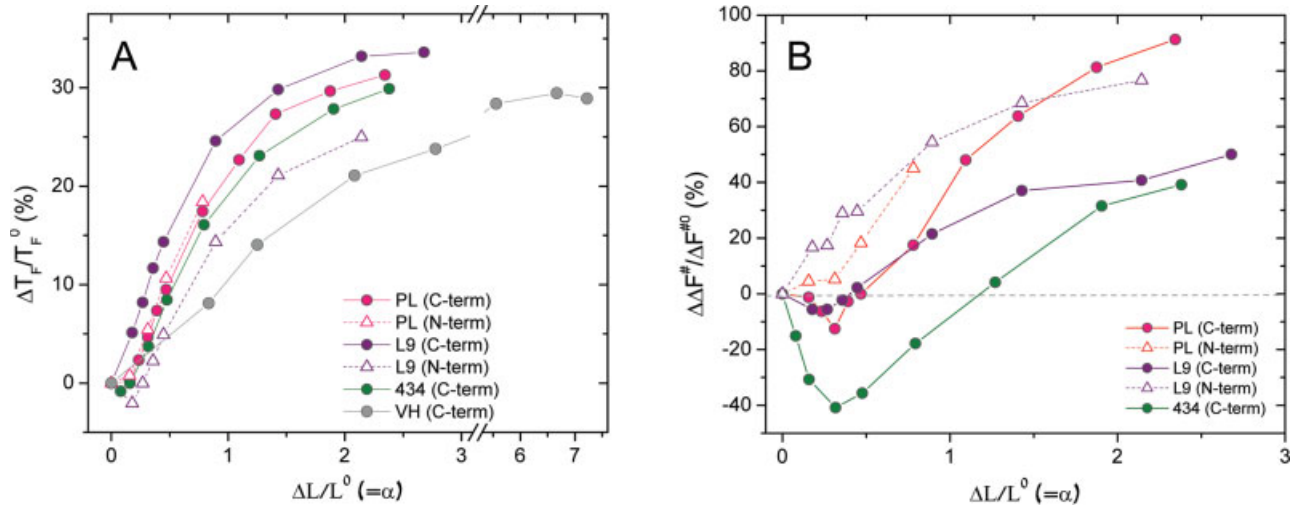

Figure 6. The dependency of the folding temperature (A) and the folding barrier (B), as obtained from molecular dynamic simulations when temperature is regulated using the Berendsen thermostat ($\tau_B = 0.2$ ps), on the length of the flexible tails that are attached to four proteins. The simulated folding temperatures (T_F) and free energy barriers ($\Delta F^{\#}$) are shown as a function of the tail length (L) as measured in the units of length of the wild-type protein, α, which has T_F^0, $\Delta F^{\#0}$, and L^0. When α equals 1, the tail has the same length as that of the wild-type protein. In these plots, PL, L9, 434, and VH stand for protein L, the ribosomal protein L9, 434 repressor, and the villin headpiece, respectively.

nal crowding agent that can shift the balance between enthalpy and entropy and so dictates protein stability, similarly to the effect of molecular crowding molecules and confinement.[30,31]

The effect of tails of various lengths on the thermodynamics of five protein systems was explored using a coarse-grained model for protein folding and two types of thermostats; the Berendsen and Langevin thermostats. The thermodynamic characteristics of folding for the tailed proteins studied differ significantly according to the thermostat used. MD using the Berendsen thermostat results in much higher protein stability and much slower kinetics than are actually the case. For tails that are 2–3 times longer than the protein, the T_F is 20–30% higher and the folding barrier in some cases is ~80% larger than the wild type values. These effects are not present in simulations that use the Langevin thermostat and the protein thermodynamics in such simulations appears to be unaffected by a tail of any length.

Our study calls for special care in choosing the thermostat for MD simulations of systems with inhomogeneous degrees of freedom. MD with the Berendsen thermostat yields temperature gradients between the fast to the slow degrees of freedom. The flexible polymeric tail is significantly hotter than the foldable protein for MD with strong coupling to the thermal bath. For weaker coupling between the system and the bath (larger τ_B) the gradient can vanish, but nevertheless, a correlation is still seen between the fluctuations of the temperatures of the tail and the protein. We demonstrate that the Langevin thermostat reliably regulates the temperature of the inhomogenous system without discriminating between the slow and fast degrees of freedom. The Langevin thermostat is less sensitive to its strength-modulating parameters than the Berendsen thermostat.

Acknowledgments

Y.L. is the incumbent of the Lilian and George Lyttle Career Development Chair. One of the authors (G.Z.) wishes to acknowledge, with gratitude, the support of the Clore Foundation.

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