Low-Temperature and High-Pressure Induced Swelling of a Hydrophobic Polymer-Chain in Aqueous Solution

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We report molecular dynamics simulations of a hydrophobic polymer-chain in aqueous solution between 260 K and 420 K at pressures of 1 bar, 3000 bar, and 4500 bar. The simulations reveal a hydrophobically collapsed state at low pressures and high temperatures. At 3000 bar and about 260 K and at 4500 bar and about 260 K, however, a transition to a swelled state is observed. The transition is driven by a smaller volume and a remarkably strong lower enthalpy of the swelled state, indicating a steep positive slope of the corresponding transition line. The swelling is stabilized almost completely by the energetically favorable state of water in the polymers hydrophobic first hydration shell at low temperatures. Although surprising, this finding is consistent with the observation of a positive heat capacity of hydrophobic solvation. Moreover, the slope and location of the observed swelling transition for the collapsed hydrophobic chain coincides remarkably well with the cold denaturation transition of proteins.

I. INTRODUCTION

Hydrophobic effects have been shown to be of relevance for a wide range of physicochemical and biophysical phenomena. In particular, they are seen as an important driving force with regard to the folding of proteins. Consequently, a wealth of studies of hydrophobic interactions using molecular simulation techniques have been undertaken over the past three decades. Simulation studies have revealed that the contact state of a pair of hydrophobic particles in aqueous solution is entropically stabilized at ambient conditions. In addition, the contribution of the solvation heat capacity has been recognized recently. In general, the dissolution of hydrophobic particles is accompanied with an increase of the associated heat capacity. As a consequence, the dissolution of a hydrophobic particle is found to be increasingly enthalpically stabilized with decreasing temperature.

Biopolymers such as proteins remain stable and functional only in a limited pressure and temperature range. Increasing temperature lead to structural changes differing from the native folded state. This is often accompanied with large fluctuations and aggregation phenomena. Hence, pressure effects on proteins are of interest in biotechnology and biology, as pressure is shifting the equilibrium of protein configurations without increasing thermal fluctuations. Proteins undergo unfolding upon addition of pressures above 2 kbars. High pressures are also able to dissociate protein complexes and the addition of co-solvents is found to have a significant influence on the size, location and shape of the stability region of proteins. At high pressures (> 2 kbars), the volume of proteins upon unfolding decreases. This seemed to be inconsistent with the assumption that protein unfolding is equivalent to the transfer of hydrophobic groups from the protein interior to the aqueous solvent, since the volume change upon transfer of hydrophobic groups to water is positive. Hummer et al. suggested a scenario in which pressure unfolding of proteins is modeled as the transfer of water into the protein hydrophobic core with increasing pressure. The transfer of water molecules into the protein interior is essential for the pressure unfolding process, leading to the dissociation of close hydrophobic contacts and subsequent swelling of the hydrophobic protein interior through insertions of water molecules. The characteristic features of water-mediated interactions between hydrophobic solutes in water are found to be pressure-dependent. In particular, with increasing pressure the solvent-separated configurations in the solute-solute potential of mean force is stabilized with respect to the contact configurations. In addition, the desolvation barrier increases monotonically with respect to both contact and solvent-separated configurations. The locations of the minima and the barrier move toward shorter separations, and pressure effects are considerably amplified for larger hydrophobic solutes.

Pressure also changes the entropy/enthalpy balance of the hydrophobic interactions. Ghosh et al. found that the contact minimum is dominated by entropy, whereas the solvent-separated minimum is stabilized by favorable enthalpy of association. Both the entropy and enthalpy at the contact minimum seem to change little with increasing pressure leading to the relative pressure insensitivity of the contact minimum configurations. In contrast, the solvent-separated configurations are increasingly stabilized at higher pressures by enthalpic contributions that prevail over the slightly unfavorable entropic contributions to the free energy.

In this contribution, we focus particularly on the scenario proposed by Hummer et al. of water penetrat-
ing into the protein interior at elevated pressures. We study, however, a very much simplified model system of protein: A fully hydrated polymer-chain, consisting of 20 interconnected hydrophobic particles. The polymer-chain approach has been recently advocated by Chandler and co-workers [38], suggesting that the collapse of a hydrophobic polymer chain is driven by a drying transition. In Ref. [37] the hydrophobic chain is modeled by repulsive polymer/water interactions only, whereas the solvent is represented by a coarse grained model. In the recent work of Ghosh et al. [39], molecular dynamics simulations of a polymer-chain in an explicit solvent reveal the effect of salt concentrations on the polymer configuration, increasingly favoring compact folded configurations of the polymer with increasing salt concentration. In the above mentioned studies a “stiff” polymer chain was employed, which exhibits a stretched equilibrium configuration. In Ref. [47] the hydrophobic chain is modeled by a hydrophobic polymer chain driven by a drying transition.

II. COMPUTATIONAL METHODS

A. MD Simulation details

We report molecular dynamics (MD) simulations of a purely hydrophobic polymer-chain dissolved in an aqueous solution. The polymer-chain consists of 20 hydrophobic polymer beads, represented by Lennard-Jones interaction sites with \( \sigma_{XX} = 3.975 \text{ Å} \), \( \epsilon_{XX} k_B T = 214.7 \text{ K} \). The water-polymer cross parameters were obtained using the conventional Lorentz-Berthelot mixing rules with \( \sigma_{ij} = (\sigma_{ii} + \sigma_{jj})/2 \) and \( \epsilon_{ij} = \sqrt{\epsilon_{ii} \epsilon_{jj}} \). The polymer sites are linked by rigid bonds of 4.2 Å length. This bond-length was determined to represent the bead-bead contact distance for non-linked particles in aqueous solution [27]. In addition, this value corresponds roughly to the distance between adjacent hydrophobic Valine sidechains in a polypeptide. All intramolecular non-bonded interactions were taken into account, except interactions between adjacent bonded sites. No additional bond-bending or torsional potentials were used. The water phase is represented by 1000 TIP5P water molecules [50]. The TIP5P model was chosen, since it represents the hydrophobic solvation behavior of water on the low-temperature side probably most realistically among the simple point charge models [28]. Moreover, the temperature dependent strength of the hydrophobic interaction was found to be quite critically linked to the temperature dependence of waters expansivity [29]. The simulations discussed here were carried over a broad temperature range at pressures of 1 bar, 3000 bar, and 4500 bar.

| \( T / \text{K} \) | \( \tau / \text{ns} \) | \( \langle V \rangle / \text{nm}^3 \) | \( \langle E \rangle / \text{kJ mol}^{-1} \) |
|-----------------|-----------------|-----------------|-----------------|
| 260 80          | 31.803 ± 0.006  | -38065 ± 6      |
| 280 54          | 31.710 ± 0.002  | -35036 ± 5      |
| 300 30          | 32.008 ± 0.003  | -32469 ± 6      |
| 320 30          | 32.563 ± 0.003  | -30128 ± 4      |
| 340 30          | 33.324 ± 0.003  | -27936 ± 5      |
| 380 27          | 35.450 ± 0.020  | -23799 ± 20     |
| 420 28          | 38.880 ± 0.050  | -19772 ± 30     |
| 260 100         | 27.868 ± 0.003  | -37432 ± 9      |
| 280 100         | 28.043 ± 0.002  | -35138 ± 3      |
| 300 50          | 28.305 ± 0.002  | -33067 ± 3      |
| 360 30          | 29.428 ± 0.002  | -27535 ± 2      |
| 400 30          | 30.384 ± 0.001  | -24252 ± 3      |
| 280 73          | 26.935 ± 0.002  | -35222 ± 3      |
| 300 73          | 27.212 ± 0.001  | -33220 ± 3      |
| 320 25          | 27.514 ± 0.002  | -31360 ± 3      |
| 360 25          | 28.206 ± 0.001  | -27918 ± 3      |

TABLE I: Simulation protocol for the performed MD-simulations. \( \tau \): Simulation time; \( \langle V \rangle \): Average box-volume; \( \langle E \rangle \): Average total energy.

Individual MD-simulations extend up to 100 ns, while the total simulation time adds up to about 0.76 μs. For completeness, a detailed simulation protocol is given in Table I.

The MD-simulations are carried out in the NPT ensemble using the Nosé-Hoover thermostat [51, 52] and the Rahman-Parrinello barostat [53, 54] with coupling times \( \tau_T = 1.5 \text{ ps} \) and \( \tau_p = 2.5 \text{ ps} \) (assuming an isothermal compressibility of \( \chi_T = 4.5 \times 10^{-5} \text{ bar}^{-1} \), respectively. The electrostatic interactions are treated in the “full potential” approach by the smooth particle mesh Ewald summation [55] with a real space cutoff of 0.9 nm and a mesh spacing of approximately 0.12 nm and 4th order interpolation. The Ewald convergence factor \( \alpha \) was set to 3.38 nm\(^{-1} \) (corresponding to a relative accuracy of the Ewald sum of \( 10^{-5} \)). A 2.0 fs timestep was used for all simulations. Solvent constraints were solved using the SETTLE procedure [56], while the SHAKE-algorithm was used for the polymer constraints [57]. For all simulations reported here the GROMACS 3.2 program [58, 59] was used. Statistical errors in the analysis were computed using the method of Flyvbjerg and Petersen [60]. For each system an initial equilibration run of about 1 ns length was performed using the Berendsen weak coupling scheme for pressure and temperature control (\( \tau_T = \tau_p = 0.5 \text{ ps} \) [61].
It should be mentioned that the completely stretched polymer extends to about 8 nm, which exceeds the used box length about 2.5 times. However, in practice, contacts between the polymer and its virtual image were not observed during the simulation runs discussed here.

### B. Energy Analysis

In order to assign potential energies to individual molecules and thus to be able to distinguish between contributions from the “hydration shell” and from the “bulk”, we determine energies by a reaction field method based on the minimum image cube. This “cubic” cut-off procedure has been originally proposed by Neumann. Roberts and Schnitker have shown that the obtained energy estimates are comparing very well with the Ewald-summation including tin-foil boundary conditions.

For convenience, we divide the potential energies in contributions assigned to the individual molecules with

$$ E = \sum_{i=1}^{M} E_i $$

$$ E_i = \left( \frac{1}{2} \sum_{j=1}^{M} E_{ij} \right) + E_{i,corr}, $$

where $E_i$ is the potential energy assigned to molecule $i$, $M$ is the total number of molecules. The molecule-molecule pair energy

$$ E_{ij} = \sum_{\alpha} \sum_{\beta} 4 \epsilon_{\alpha \beta} \frac{1}{r_{\alpha \beta}^{12}} - \frac{1}{r_{\alpha \beta}^{6}} + q_{i,\alpha} q_{j,\beta} \frac{1}{r_{\alpha \beta}} $$

is then obtained as sum over discrete interaction sites $\alpha$ and $\beta$, with $r_{\alpha \beta} = |\vec{r}_{j,\beta} - \vec{r}_{i,\alpha}|$ based on the molecule/molecule center of mass minimum image separation. We employ long range corrections $E_{i,corr} = E_{i,corr}^{el} + E_{i,corr}^{LJ}$ accounting for electrostatic, as well as Lennard-Jones interactions. The electrostatic correction

$$ E_{i,corr}^{el} = \frac{2\pi}{3} V \vec{D} \vec{d}_{i} $$

is a reaction field term, corresponding to the cubic cut-off, assuming an infinitely large dielectric constant. Here $\vec{d}_{i} = \sum_{\alpha} q_{i,\alpha} \vec{r}_{i,\alpha}$ is the dipole moment of molecule $i$, $\vec{D} = \sum_{i} \vec{d}_{i}$ is the total dipole moment of all molecules in the simulation cell and $V$ is the instantaneous volume of the simulation box. Finally, also the long range Lennard-Jones corrections for to the minimum image were taken into account, as outlined in Ref. [25].

### III. RESULTS

The structure of the dissolved hydrophobic polymer-chain is characterized by its radius of gyration $R_G^2 = 1/20 \sum_{i=1}^{20} (\vec{r}_i - \vec{c})^2$ with $\vec{c} = 1/20 \sum_{i=1}^{20} \vec{r}_i$. The typical conformation of this polymer at high temperatures and low pressures is a compact, collapsed state with a $R_G$ between 0.5 nm and 0.6 nm, as shown in Figure 3. A snapshot of a representative collapsed-chain configuration is shown in Figure 3. The conformational distribution with respect to $R_G$ is found to be narrow, with a half width at half maximum of $R_G$ of about 0.1 nm. Test-simulations at 300 K and ambient pressure conditions, starting with a swelled configuration of $R_G \approx 1$ nm, show a collapse on a timescale $<1$ ns. The polymer/water center of mass pair correlation functions (given in Figure 4) reveal that in the collapsed state water is completely excluded from the polymer interior.

The temperature dependence of the average radius of gyration at a pressure of 1 bar is characterized by a shallow minimum at about 300 K. With increasing temperature the distribution of $R_G$ more or less maintains its shape, but is becoming broader with its maximum shifting to larger values as shown in Figure 2. The high-

![FIG. 1: Representative configurations of the hydrophobic polymer in aqueous solution as obtained from simulations 3000 bar. Top: Collapsed configuration observed at 300 K. Bottom: Swelled configuration observed at 260 K.](image-url)
E(H₂O)/kJ mol⁻¹ & 260 K; 3000 bar & 280 K; 4500 bar & 260 K; 3000 bar & 280 K; 4500 bar \\ 
E(H₂O)/kJ mol⁻¹ & -43.657 ± 0.004 & -42.081 ± 0.003 & 16.02 ± 0.05 & 15.50 ± 0.02 \\ 

Hydration Shell: & 260 K; 3000 bar & 280 K; 4500 bar & 260 K; 3000 bar & 280 K; 4500 bar \\ 
N(H₂O) & 126.5 ± 2.1 & 184.2 ± 2.8 & 129.4 ± 1.3 & 173.0 ± 2.0 \\ 
V(Shell)/nm³ & 4.51 ± 0.09 & 5.96 ± 0.08 & 4.49 ± 0.03 & 5.58 ± 0.07 \\ 
Vₘ(H₂O)/cm³ mol⁻¹ & 21.47 ± 0.05 & 19.49 ± 0.05 & 20.97 ± 0.07 & 19.46 ± 0.07 \\ 
E(H₂O)/kJ mol⁻¹ & -43.63 ± 0.05 & -44.43 ± 0.03 & -41.85 ± 0.02 & -42.34 ± 0.03 \\ 
E(Polymer)/kJ mol⁻¹ & -149.7 ± 0.5 & -141.3 ± 0.6 & -141.2 ± 0.2 & -126.7 ± 0.5 \\ 

Here temperature behavior is more or less similar for all pressures discussed here. In addition, a slight penetration of water into the polymer interior is observed for 420K (see Figure 4).

At lower temperatures, however, a different behavior is starting to emerge. From the time evolution of R_G, shown in Figure 2 it is evident that at about 260 K and 1 bar the polymer increasingly starts to explore extended-chain configurations. These extended-chain states are occurring only rather infrequently and are short-lived with
a life-time of about 1 ns. We would like to emphasize that the 280 K/3000 bar and 300 K/4500 bar trajectories show a similar behavior. Again, the extended-chain configurations are rather short-lived with the chain quickly returning to the collapsed state. In a quantitative manner, an increase of the population of swelled states is found, which is due to the apparent higher frequency of large amplitude $R_G$-fluctuations. The most striking difference, however, is observed for 260 K at 3000 bar and 280 K at 4500 bar. Here the extended-chain configurations are dominating, although an equilibrium between collapsed and swelled states is still maintained. The radius of gyration is showing an apparently bimodal distribution at 260 K at 3000 bar and a broad distribution at 280 K at 4500 bar. The representation of both (swelled and compact) at each of these states, strongly suggests that the corresponding conformational transition temperatures are located in close proximity to the temperatures indicated. The temperature dependence of $R_G$ at different pressures, as given in Figure 3, suggests that the high-temperature collapsed-chain state is more compact at elevated pressures, which would correspond to a more compressed coiled state. At 280 K and 3000 bar and 260 K and 4500 bar, however, the situation has already changed, and the tendency to explore more extended configurations leads to an increase in $R_G$ which is quickly progressing upon cooling. The low temperature destabilization of the collapsed state is in line with the decreased stability of hydrophobic contacts observed for elevated pressures [18, 19, 45]. Figure 3 implies that the transition towards a swelled state at 3000 bar and 4500 bar occurs in a rather narrow temperature interval, suggesting a rather large enthalpy difference between collapsed and swelled state. In other words: it shows a large “cooperativity”. Thermodynamical consistency requires that the swelled low temperature state has to be energetically more stable than the compact state and that it has to occupy a smaller total volume. Figure 4 shows a superposition of the time-evolution of the potential energy and box-volume, as well as $R_G$ for 260 K at 3000 bar. In order to make the trends more clearly visible, the noise in the fluctuations have been reduced by Savitzky Golay filtering [65]. Both, the potential energy as well as the box-volume are clearly anti-correlated with respect to $R_G$. The swelled state of the polymer is enthalpically stabilized with energy difference of $\Delta E_u = E_{(swell.)} - E_{(coll.)}$ of $-182 \text{ kJ mol}^{-1}$ at 3000 bar and of $-82 \text{ kJ mol}^{-1}$ at 4500 bar. The swelled state leads also to smaller box volumes, with $\Delta V_u = V_{(swell.)} - V_{(coll.)}$ of $-20 \text{ ml mol}^{-1}$ at 3000 bar and of $-10 \text{ ml mol}^{-1}$. We would like to point out the observed enthalpy and volume changes are of similar magnitude as observed for some proteins [32] and peptides [66].

In the following paragraphs we would like to show that the energy and volume change can be attributed almost quantitatively to the first hydration shell of the polymer and have to be almost exclusively attributed to the solvent. Figure 4 depicts the polymer/water-oxygen site-site pair correlation functions for the collapsed and swelled states obtained at 260 K and 3000 bar. The first minimum, located at a distance of 0.52 nm, indicates the spatial dimension the first hydration shell. The average number of water neighbors around each polymer site changes about 40% from 11.5 to 16 upon swelling (at 3000 bar). At 4500 bar a qualitatively similar behavior is observed. In order to quantify the changes in the hydration shell, we define the volume occupied by the polymer and the first hydration shell as the volume with a distance of $R \leq 0.52 \text{ nm}$ with respect to any polymer-site. The properties obtained for the hydration shell of the collapsed and swelled polymer and the water bulk are summarized in Table III.

The polymer/water center of mass pair correlation function as well as the potential energy of water as a function distance to the polymer center for the collapsed and swelled states are given in Figure 7. From Figure 7 it is evident that for the region of the polymer/water interface ($r \approx 0.9 \text{ nm}$) water is $0.3 \text{ kJ mol}^{-1}$ more stable as in the bulk. However, due to the lack of water neighbors, a further penetration of individual waters into the

![FIG. 3: Average radius of gyration $R_G$ of the hydrated polymer as obtained for all temperatures at 1 bar, 3000 bar and 4500 bar. The lines are drawn to guide the eye.](image)

![FIG. 4: Polymer/water center of mass pair correlation functions for all simulated state points.](image)
The difference between collapsed and swelled states is about 0.26 kJ mol\(^{-1}\) at 4500 bar. The potential energy difference between collapsed and swelled state is about 4.4 kJ mol\(^{-1}\) at 4500 bar and ∆V\(_{m,u}\) ≈ −21 ml mol\(^{-1}\) for the 4500 bar-isobar. Origin of the negative volume change upon swelling is indicated in Figure 6. Here the free volume fraction accessible to a small hard sphere particle as a function of distance to the polymer center. Given are dependencies for the collapsed and swelled states at 260 K and 3000 bar. The volume change upon swelling is ∆V\(_{m,u}\) ≈ −21 ml mol\(^{-1}\) for the 4500 bar-isobar. Origin of the negative volume change upon swelling is indicated in Figure 6. Here the free volume fraction accessible to a small hard sphere particle as a function of distance to the polymer center. Given are dependencies for the collapsed and swelled states at 260 K and 3000 bar. The diameter of the particle with σ\(_{Y-OW}\) = 2.5 Å and σ\(_{Y-X}\) = 2.93 Å is scaling as the corresponding Lennard-Jones sigmas.

FIG. 5: Time evolution of the radius of gyration of the polymer (black), potential energy (red, top) and volume (red, bottom) as obtained from the simulation at 260 K and 3000 bar. Potential energies and box-volumes were directly taken from GROMACS simulation output. In order to reduce the noise, all data were smoothed using with the same Savitzky-Golay filter [65].

FIG. 6: Polymer-bead/water-oxygen site-site pair correlation function \(g_{X-OW}(r)\) and integrated number of nearest neighbors \(N_{n}(r)\) for the collapsed and swelled states at 260 K and 3000 bar.

collapsed hydrophobic coil is energetically unfavorable, as the steep increase in Figure 7a indicates. When drawing the balance over all water molecules in the hydration shell, energy gains and penalties almost cancel out completely and the average potential energy of a water molecule in the hydration shell of the collapsed polymer is nearly identical to the value observed for the water bulk at 3000 bar and slightly more unstable at 4500 bar (see Table III). For the case of the swelled polymer, the energy penalty is absent and the water in the hydration shell gains −0.78 kJ mol\(^{-1}\) potential energy per water molecule with respect to the bulk on average (−0.26 kJ mol\(^{-1}\) at 4500 bar). The potential energy difference between collapsed and swelled state is about

\[
\Delta E_u \approx [E(\text{shell,swell}) - E(\text{bulk})] N(\text{shell,swell}) - \left(E(\text{shell,coll}) - E(\text{bulk})\right) N(\text{shell,coll}) + E(\text{polymer,swell}) - E(\text{polymer,coll}) \approx -138 \text{ kJ mol}^{-1} (60 \text{ kJ mol}^{-1})
\]

which already accounts largely for the observed total energy difference of about −182 kJ mol\(^{-1}\) (−82 kJ mol\(^{-1}\)). The potential energy of the polymer changes just by 8.4 kJ mol\(^{-1}\) (14.5 kJ mol\(^{-1}\)), which is due to the fact that the loss of intramolecular interactions when going from the collapsed to the swelled state is almost completely compensated by polymer/solvent interactions (The values for 4500 bar are given in parentheses). Hence the the extended-chain configurations at low temperatures appear to be largely solvent-stabilized.

The volume change upon swelling is ∆V\(_{m,u}\) ≈ [V\(_{m}\)(shell,swell) − V\(_{m}\)(bulk)] N(Shell,swell) − [V\(_{m}\)(shell,coll) − V(bulk)] N(Shell,coll) = −57 ml mol\(^{-1}\) at 4500 bar and ∆V\(_{m,u}\) ≈ −21 ml mol\(^{-1}\) for the 4500 bar-isobar.
collapsed state, apparently an excess free volume in the polymer interior is available to a hard sphere particle which is absent in the swollen state. The hydration shell around the hydrophobic chain appears to be more tightly packed, so that the average free volume fraction is even lower than for the water bulk. Hence the increase in solvent accessible surface (increasing number of hydration shell waters) is overcompensated by the decrease in molar volume of water in the swollen hydration shell state. The hydration shell volume of the collapsed state includes, of course, the volume of the hydrophobic core, which is made accessible to the solvent upon unfolding of the chain.

Finally, we would like to compare the behavior of the hydrophobic polymer to the experimentally obtained stability diagrams of proteins. Interpolating the $R_G$-data from Figure 4 and assuming that the transition appears at $R_G \approx 0.7$ nm, we obtain $T_u \approx 268$ K and 282 K for 3000 bar and 4500 bar, respectively. Using the enthalpies $\Delta H_u \approx \Delta E_u + PV_u$ and volumes $\Delta V_u$ according the data from Table I, we obtain a slope of the coexistence line of $\partial P_{eq}/\partial T = \Delta H_u/T_u \Delta V_u$ of about 100 bar K$^{-1}$ for both pressures, seemingly consistent with the approximate transition temperatures. Although having more data would be desirable, we might conjecture that the slope does not seem to change much, but the enthalpy and volume differences tend to decrease with increasing pressure. Since the differences might disappear completely at higher pressures, the swelling transition of the hydrophobic polymer probably ends up in a “critical point” (The term “critical point” might not be fully appropriate here since swelling transition has a monotonous character due the finite size of the polymer). In Figure 9 the swelling transition line, as well as the stability diagrams for Staphylococcal Nuclease and ubiquitin are shown. We would like to point out that the location and slope of the swelling transition shows remarkable similarity to the given cold-denaturation lines. The hydrophobic polymer seemingly behaves as suggested by the water penetration scenario according Hummer et al. In the present case the energy stabilization of the swelled configuration is dominated the energy gain of the hydration water. In a real polypeptide this is not necessarily true. Backbone hydration and the equilibrium between intra- and intermolecular hydrogen bonds will certainly play an important role. How this delicate balance might influence the equilibrium between swelled and collapsed configuration should be further investigated. The high temperature side of the protein stability diagram might not be accessible by our simple model since it is largely related to internal secondary structural transformation to a “molten globule” state. In addition, we would like to mention that the observed cold swelling of the hydrophobic polymer has some similarity to the scenario proposed for the swelling of polymeric tropo-elastin upon cooling, as advocated by the work of D.W. Urry.

In the present simulation study the energy gain upon unfolding is apparently a consequence of the increasing water-water pair interactions in the hydration shell at low temperatures. The hydrogen bond network in the hydration shell starts disintegrating upon heating more strongly as in the bulk since no hydrogen bonds can be formed to the hydrophobic Lennard-Jones particles. The counterbalance between strengthened hydrogen bonds and enhanced disintegration of the hydrogen bond network is widely regarded as the mechanism leading to the positive solvation heat capacity associated with hydrophobic hydration. As a consequence, the observed transition temperature might be critically influenced by the change of the heat capacity of the solvent in the hydration shell compared to the bulk. This might also shed light on the mechanism how co-solvents affect the (cold) unfolding transition of proteins.

### IV. CONCLUSION

Molecular dynamics simulations of a hydrophobic polymer-chain in aqueous solution between 260 K and 420 K at pressures of 1 bar, 3000 bar, and 4500 bar reveal a hydrophobically collapsed state at low pressures and high temperatures. At about 268 K and 3000 bar and at 282 K and 4500 bar a transition to a swelled state is observed. The transition is driven by a smaller volume and a remarkably strong lower enthalpy of the swelled state. The volume effect is basically due to a smaller net-volume of the extended hydrated state compared to the collapsed state exhibiting hydrophobic cavity volumes and penetration of the internal volume by water. Moreover, the extended-chain structure is almost completely energetically stabilized by the lower potential energy of the water molecules in the hydration shell. Consequently, the increasingly stably water-water hydrogen bonds close to a
hydrophobic particle, leading the positive heat capacity of solvation, which is a signature for hydrophobic hydration [34], is the key to the observed behavior. The strong energy and volume differences indicates a steep positive slope of the corresponding transition line of about 100 bar K\(^{-1}\). The observed stability line for the collapsed hydrophobic chain shows strong similarity with the lower temperature side of the stability diagram of proteins in aqueous solution.

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