



Molecular interactions accounting for protein denaturation by urea



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ABSTRACT

Urea destabilizes proteins when added to aqueous solutions. To study its molecular mechanism we perform explicit all-atom molecular dynamics simulations of unrestrained poly-glycine, poly-alanine, and poly-leucine monomers as well as of extended poly-alanine and poly-leucine dimers. We show that poly-leucine monomers become less compact when urea is added to water whereas poly-glycine and poly-alanine monomers are only weakly affected by this co-solvent. Consistent with these results, we find that only the potential of mean force (PMF) of extended poly-leucine dimers changes significantly when urea is added to water. To rationalize these observations, we perform detail analysis of extended dimers. Urea is found to occupy positions between leucine's side chains that are not accessible to water. This allows extra van der Waals interactions between urea and side-chains to be formed which favor the monomeric state. In contrast, urea-solvent interactions are found to favor the dimeric state. The sum of these two energetic terms, i.e., urea-peptide and urea-solvent, provides the enthalpic driving force for urea denaturation. We show here that this enthalpy correlates with the potential of mean force of poly-leucine dimers. Moreover, the framework developed here is general and may be used to provide insights into effects of other small molecules on protein interactions.

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1. Introduction

One of the first scientific accounts of urea's effect on living systems dates back to 1902 when it was reported that dead frogs become translucent and fall into pieces in a saturated urea solution [1]. This molecule is now widely used as a denaturant in the lab whereby it perturbs native protein conformations to favor the unfolded state [2,3]. However, urea's atomic mechanism has been a question of debate since its effect was quantified in the 1960s [4]—although important insights have been obtained recently [5–9]. Urea may impede the formation of native structures either by forming strong bonds with the protein (direct mechanism) [7,10–18] or by modifying the structure of water accounting for different solvation properties (indirect mechanism) [19–23]. Moreover, it is still not clear whether urea affects mainly the backbone or side chains of the protein. Answers to these open questions can be obtained using simplified computational frameworks that can quantify interaction strengths in the solvent and protein at the atomic level.

In recent years, several studies have emerged showing that, in solution, urea can replace water without changing significantly its structural properties since both molecules are highly

polar [4,12,24–29]. This has been a strong argument against urea's indirect mechanism which assumes that the formation of water structures is disrupted by this molecule [19,30]. Urea's direct mechanism is, therefore, gaining increased acceptance in the scientific community. In particular, it is supported by explicit all-atom computer simulations [10,11] wherein urea is shown to form strong non-bonded interactions with the protein providing the driving force for unfolding. Some studies indicate that these protein-urea interactions emerge mainly from hydrogen bonding with the backbone [13,15,28,31,32] whereas others point to dispersion interactions with non-polar groups of the proteins [9,27,33–35]. Separating contributions from backbone and side-chain atoms is not an easy task even in computer simulations that have access to atomic detail [7,10,31].

Traditionally, model compounds have been used to estimate interaction strengths between different chemical groups in proteins [14,24,36–39]. In particular, free-energies to transfer non-polar amino acids from pure water to urea solutions were shown to be negative implying that urea solutions can accommodate non-polar amino acids better than pure water [36]. As a result hydrophobic interactions are weaker in urea solutions and this could account for protein denaturation. Small non-polar compounds (e.g., methane and alanine) as well as glycine were not found to be significantly affected by urea [36]. Computer simulations of model compounds point to the same conclusion [37,38] but also highlight strong

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hydrogen bonding of urea with small charged solutes [32]. Different conclusions can be drawn from studies of simple model compounds because they do not account simultaneously for contributions from backbone and side-chain atoms in the same proportion as in real proteins. Moreover, solvation of side chains are affected by the backbone as reported in a recent study [40,41]. These limitations of model compounds can be overcome by studying homopeptides [7,17,27,39]. Recently, Horinek and Netz computed free-energies τ to transfer homopeptides from pure water to aqueous solutions containing urea using all-atom molecular dynamics simulations and different force-fields [7]. Direct and indirect effects of urea were singled out by differentiating τ with respect to urea concentration. This provided important insights into effects of force-fields on urea's mechanism [7].

In this paper we study effects of urea on non-restrained poly-glycine, poly-alanine, and poly-leucine monomers as well as on the interaction of extended poly-alanine and poly-leucine dimers. For the extended dimers, we compute the free-energy required to bring two peptides that are initially non-interacting all the way to a distance ξ , i.e., we compute potentials of mean force (PMF). Our choice of peptides and approach allows us to probe the effect of urea on two types of interactions that are essential to account for protein folding, i.e., hydrophobic interactions between side chains of alanine and leucine residues and interactions between backbone atoms. Notice that, in addition to non-polar residues, urea has also been shown to bind favorably to polar and charged residues [11,24]. These types of residues are mainly located at the surface of proteins and, therefore, their energy of solvation is not expected to contribute significantly to protein unfolding.

Here, we show that poly-leucine monomers become less compact when urea is added to water whereas poly-glycine and poly-alanine monomers are only weakly affected by this co-solvent. Consistent with this result, changes in the PMF of extended poly-alanine and poly-leucine dimers when urea is added to water agree qualitatively with transfer free-energies of their respective side chains, i.e., only the PMF of poly-leucine dimers is destabilized (Fig. 2). Also, we show that urea molecules are more densely populated around poly-leucine's side chains than in the bulk and this difference increases when peptides dissociate (Fig. 3). This occurs because urea molecules can replace water in the first shell around peptides and they can occupy positions that are not accessible to water due to their planar distribution of partial charges [42,43] (Fig. 3). Thus, our results suggest that urea destabilize proteins through a direct mechanism whereby it binds favorably to side chains. Moreover, we observe a clear correlation between changes in non-bonded interactions (i.e., enthalpy) and changes in the PMF when urea is added to the system (Fig. 4). These changes when peptides are brought close together emerge from contributions of protein-urea interactions (mostly Lennard-Jones) that favor the dissociated state and solvent-solvent interactions (electrostatic and Lennard-Jones) that favor the folded state (Fig. 5).

2. Methodology

Microsecond-long molecular dynamics simulations are used to study unrestrained poly-glycine, poly-alanine and poly-leucine monomers in pure water and 3 M urea solution. Two independent simulations, starting from different stretched conformations of the monomer, are performed for each combination of solvent and peptide. Systems are equilibrated for 60 ns and they are analyzed along 940 ns trajectories. Simulations are performed in the NPT ensemble at 1 atm and 298 K.

Extended poly-alanine and poly-leucine dimers in this work are made of 10 residues which are immersed in pure water and aqueous urea solutions. Peptides are made “infinite” through the use of

periodic boundary conditions by attaching the carbonyl-group of residue 1 to the amine-group of residue 10 in the z-direction. This method to describe extended peptides was first used by Horinek and Netz [7]. Advantages of this method are that it eliminates effects from chain ends and all amino acids are equivalent. To maintain peptides stretched at an average length of ~ 3.5 nm, pressures of -38 atm and -100 atm are applied along the z-direction for poly-alanine and poly-leucine, respectively. A pressure of 1 atm is applied along x and y directions accounting for an average density of 990 kg/m^3 . Thus, the anisotropic pressure used in this work ensures that peptides are stretched and their solvation resembles experimental conditions [39,44].

To determine the free-energy landscape of extended dimers, we use an umbrella sampling protocol where the reaction coordinate is the x-y distance between centers-of-mass of peptides (ξ) in the range 0.4 nm to 2.0 nm. Notice that ξ does not correspond to backbone distance and peptides can rotate around the z-axis. We simulate different windows in which peptides are restrained by a spring that has an equilibrium constant of 5000 kJ/mol/nm^2 . The equilibrium distances of springs in neighboring windows differ in steps of 0.05 nm. Each window is simulated for 150 ns. Trajectories at the different windows are used to compute the “raw” PMF, i.e., PMF^{raw} , using the Weighted Histogram Analysis Method (WHAM) [45]. Since ξ evolves in a two-dimensional space (see Fig. S2 of supplemental material [46]), the PMF increases linearly with ξ . In this work we subtract this linear dependence on ξ and we use the average PMF, i.e., $\langle C \rangle$, computed in the ξ range 1.8–2.0 nm to define the reference PMF (i.e., its zero value):

$$\text{PMF}(\xi) = \text{PMF}^{\text{raw}}(\xi) + k_b T \log(\xi) - \langle C \rangle. \quad (1)$$

A detail account of the normalization procedure of the PMF is given in reference [47] and supplemental material of reference [48]. A discussion about the convergence of the computed PMF is given in the supplemental material [46].

Temperature (298 K) and pressure are controlled using the v-rescale thermostat [49] ($\tau_T = 1$ ps) and the Parrinello-Rahman barostat [50] ($\tau_P = 1$ ps), respectively. Simulations are carried out using GROMACS [51] and the AMBER99SB-ildn-force-field [52]. We use the TIP3P model for water. Urea, $(\text{NH}_2)_2\text{CO}$, is a highly polarized molecule with a dipole moment 6–8 Debye (D) in liquid state [53] (the value for water is 2.95 D [54]). Therefore, urea can easily form hydrogen bonds with water molecules [55]. For urea we used the AMBER-force-field [56] (see Table S1 of supplemental material [46]). Notice that other force-fields are also available to simulate urea [7,57,58]. We use the leap-frog algorithm with a time-step of 2 fs to integrate the equation of motion and the neighbor list is updated every 10 steps. Electrostatics is treated using the Smooth Particle Mesh Ewald method with a grid spacing of 0.13 nm and a 1.3 nm real-space cutoff [59].

Stretched fragments of proteins are of fundamental importance in biology [60] as they form the building blocks of β -sheets. After α -helices, β -sheets are the most frequent secondary structures of globular proteins serving as template for 20–28% of all the residues [61]. In our setup, the two peptides in the simulation box run in opposite directions, i.e., the displacement vector between carbonyl- and amine-groups point in the positive z-direction for one peptide and in the negative direction for the other. As a result, the type of β -sheet structures adopted by peptides in our simulations is called antiparallel. β -sheet structures formed by peptides that run in the same direction are called parallel. In globular proteins, the latter occur less frequently than antiparallel β -sheet structures and they will not be studied in this work. Both poly-alanine and poly-leucine have been found to form fibrils [62] which are stabilized by hydrogen bonds and side-chain interactions from facing and surrounding

residues. When a β -sheet is formed in our model, it is stabilized by hydrogen bonds and chain interactions from surrounding residues.

3. Results

In Fig. 1 we study effects of urea on the conformation of unrestrained poly-glycine, poly-alanine, and poly-leucine peptides by measuring the radius of gyration R_g of backbone atoms. Poly-glycine is a model of the protein backbone whereas poly-alanine and poly-leucine represent peptides with small and large non-polar side chains, respectively. Fig. 1a shows that conformations of poly-glycine are not significantly affected by urea: R_g distributions of poly-glycine in pure water (black line) and aqueous urea solution (red) are very similar to each other. In Fig. 1b we show that effects of urea on conformations of poly-alanine are more pronounced. Arrows in this figure highlight an increase in the population of extended conformations of this peptide, i.e., conformations that are characterized by large values of R_g , when urea is added to water. Thus, urea favors extended conformations of poly-alanine. Effects of urea on the distribution of R_g are even more significant for poly-leucine as shown in Fig. 1c. This points to the importance of side chains in accounting for urea's destabilization of compact protein conformations. In agreement with Fig. 1, a recent simulation of a fifteen residue poly-glycine peptide performed using the Kirkwood-Buff force field for urea reported that conformations of this peptide are not significantly affected by urea [9]. In this study, effects of urea on side chains of small proteins were found to be key in accounting for the unfolded state [9].

To shed light into urea denaturation mechanism, we study a simplified system, i.e., extended poly-alanine and poly-leucine dimers in aqueous urea solutions. These systems provide a framework to study effects of urea on atomic interactions responsible for the stability of native conformations, i.e., hydrophobic interactions and hydrogen bonds. Fig. 2 shows PMF of poly-alanine and poly-leucine dimers in pure water and in aqueous solutions containing urea. These PMF are characterized by a global minimum at $\xi \sim 0.5$ nm in which peptides assume anti-parallel β -sheet structures. For poly-leucine, PMF also exhibit a second minima at $\xi \sim 0.8$ nm. At this ξ value, we observe interdigitation of side chains with polar groups of the backbone pointing towards the solvent [44]. In addition to these minima, an energy barrier is observed between global and local minima for poly-leucine. Characteristic configurations at the barrier resemble the ones at $\xi \sim 0.8$ nm with side chains interdigitating more tightly.

In Fig. 2a, the addition of urea does not account for significant changes in the PMF of poly-alanine chains. In contrast, urea weakens the interactions between poly-leucine chains as depicted in Fig. 2b. Similar results for other concentrations of urea (2 M, 3.8 M, 5 M, and 7 M) are shown in the supplementary material [46]. A possible explanation for this difference in behavior is that urea affects mostly side

chain (not backbone) interactions [3,63] with side chains of leucine being more affected than side chains of alanine. This idea is supported by simulations of methane (CH_4) and neo-pentane (C_5H_{12}) dimers that are chemical compounds resembling side chains of alanine and leucine, respectively [37]. PMF describing the interaction of methane dimers do not change significantly in the presence of urea [37] whereas PMF of neo-pentane dimers become less negative with increasing urea concentration. If backbone interactions were the main energetic term affected by urea then PMF of both poly-leucine and poly-alanine in Fig. 2 would have changed by a similar amount. This is not the case suggesting that the backbone is not the main part of the protein affected by urea. This is also consistent with transfer free-energies of glycine from pure water to urea solutions [36]. Glycine is often used as model for the backbone of proteins and its transfer free-energy is small (smaller than for alanine) and positive implying that urea has little or no destabilizing effect on the interaction between glycine residues. Another case in point regarding the PMF in Fig. 2b is that the energy barrier separating global and local minima for poly-leucine increases significantly when urea is added to aqueous solution. A similar increase was observed in the PMF of non-polar model compounds [37]. This was related to enhanced cooperativity in folding/unfolding transitions of some proteins [64].

Additional support for the idea that urea weakens mainly side-chain interactions and not backbone interactions is provided by studying the preferential interaction Γ . This quantity compares the distribution of urea and water as a function of the distances r from the closest peptide [63,65]:

$$\Gamma(r) = \left\langle N_u(r) - \frac{N_u^{\text{bulk}}}{N_w^{\text{bulk}}} N_w(r) \right\rangle, \quad (2)$$

where $N_u(r)$ and $N_w(r)$ are the number of urea and water molecules, respectively, with minimal distance to peptide atoms between 0 and r . N_u^{bulk} and N_w^{bulk} are the number of urea and water molecules in the bulk. Distances r from the protein for which urea tends to accumulate will show positive Γ values whereas negative Γ values imply exclusion of urea from the protein. Fig. 3 provides a comparison of Γ when dimers are at their preferred interaction distance, i.e., at $\xi = 0.47$ nm (full line), and when they are non-interacting, i.e., at $\xi = 2.0$ nm (dashed line). Γ measured with respect to all atoms of poly-alanine or poly-leucine are shown in black. Poly-leucine has bigger side chains compared to poly-alanine and they interact favorably with urea. This accounts for the larger Γ values for poly-leucine compared to poly-alanine in Fig. 3. Moreover, Γ measured with respect to all atoms (in black) of poly-alanine or poly-leucine increases when chains dissociate. These changes in Γ due to dimer dissociation, i.e., $\Delta\Gamma(r) = \Gamma_{\text{monomer}}(r) - \Gamma_{\text{dimer}}(r)$ are shown in Fig. 3c–d for poly-alanine and poly-leucine, respectively. For attractive co-solvents, $\Delta\Gamma(r)$ is

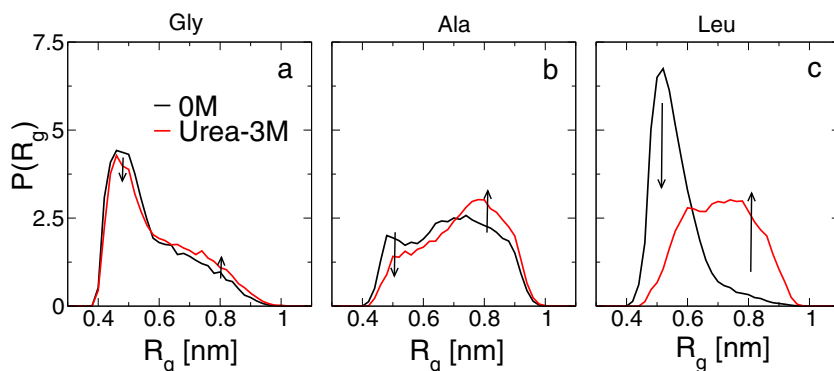


Fig. 1. Distribution of the radius of gyration R_g of backbone atoms for poly-glycine, poly-alanine, and poly-leucine in aqueous urea solutions at 298 K and 1 atm.

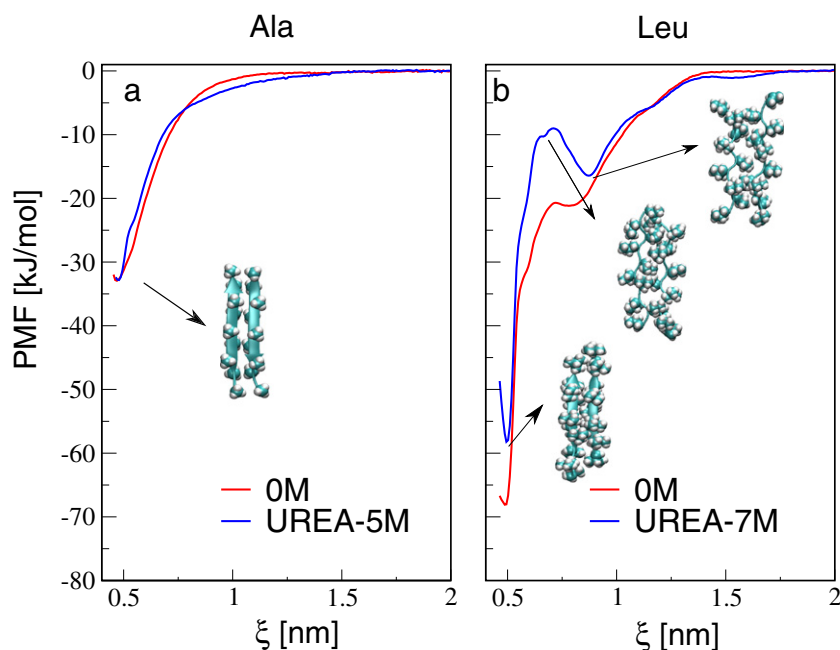


Fig. 2. Potential of mean force (PMF) of poly-alanine (a) and poly-leucine (b) dimers in pure water (red) and aqueous urea solutions (blue). PMF values computed at $\xi=2.0$ nm are used as our reference, i.e., zero value. Characteristic configurations of dimers are also shown.

proportional to the effect of the co-solvent on the free energy difference to unfold proteins [66]. In our simulations, $\Delta\Gamma(r)$ is significantly larger for poly-leucine compared to poly-alanine close to the peptide, i.e., for $r < 0.6$ nm.

To provide further insights, $\Gamma(r)$ is decomposed into contributions from backbone (Γ_{backbone} , in blue) and side chain ($\Gamma_{\text{sidechain}}$, in red) atoms. $\Gamma_{\text{backbone}}(r)$ is computed by counting the number of urea and water molecules (i.e., $N_u(r)$ and $N_w(r)$) that are closer to backbone than to side-chain atoms with a distance between 0 and r from the peptide. A similar definition is used for $\Gamma_{\text{sidechain}}$. These definitions ensure additivity of $\Gamma(r)$, i.e., $\Gamma(r) = \Gamma_{\text{backbone}}(r) + \Gamma_{\text{sidechain}}(r)$. $\Gamma_{\text{sidechain}}$ and Γ_{backbone} are shown in Fig. 3a–b. The corresponding change in these quantities, i.e., $\Delta\Gamma_{\text{backbone}}$ and $\Delta\Gamma_{\text{sidechain}}$, is shown in Fig. 3c–d. For poly-alanine, both backbone (blue) and side-chain atoms (red) contribute significantly to $\Delta\Gamma(r)$. In contrast, $\Delta\Gamma(r)$ for poly-leucine is mainly ascribed to side-chain atoms (red). Notice that $\Delta\Gamma_{\text{backbone}}$ is positive and its magnitude is similar for both poly-alanine and poly-leucine chains. Although $\Delta\Gamma_{\text{backbone}}$ accounts for a significant contribution of the overall $\Delta\Gamma$ in poly-alanine, dimers of this peptides are not destabilized significantly—see Fig. 2a.

The accumulation of polar urea molecules around the non-polar side chain can be explained by the planar distribution of partial charges in urea that allows this molecule to surround planar non-polar environments without breaking hydrogen bonds with the solvent. For example, urea was found to adopt preferentially parallel orientations close to protein surfaces and non-polar plates [42,43]. Planar non-polar environments are usually unfavorable to water that would have at least one non-saturated hydrogen bond because of its tetrahedral distribution of partial charges. Fig. 3e–f depicts spatial distribution functions of urea and water molecules around poly-leucine in aqueous urea and pure water solutions, respectively. To compute these functions the simulation box was divided in bins of size $0.02 \times 0.2 \times 0.2$ nm³. Spatial distribution functions are given as the ratio between the density of solvent molecules in the bin and the density of the solvent in an ideal fluid. In addition to replacing water molecules around the peptide, urea is also found between side chains

in the hydrogen bonded face of the β -sheet [67] (arrows in Fig. 3e) whereas these positions are left unoccupied in pure water solutions (Fig. 3f). Similarly, at other poly-leucine dimer distances (i.e., other ξ values), urea occupies spaces otherwise left empty by water.

In Fig. 4a we show changes in the PMF of poly-leucine when 7 M urea is added to water, i.e., $\Delta\text{PMF}(\xi) = \text{PMF}^{\text{urea}}(\xi) - \text{PMF}^{\text{water}}(\xi)$. This quantity is the result of both enthalpic and entropic terms. Since the volume of the system does not change significantly as a function of ξ (see Fig. S6 in SI [46]), the main term contributing to the enthalpy is the total energy of the system. In Fig. 4a, we show how this total energy changes when 7 M urea is added to water: $\Delta E_{\text{total}}(\xi) = E_{\text{total}}^{\text{urea}}(\xi) - E_{\text{total}}^{\text{water}}(\xi)$. The entropic term ($-\Delta S$) which is determined by subtracting ΔE_{total} from ΔPMF is also shown in Fig. 4a. Notice that ΔPMF , ΔE_{total} , and $-\Delta S$ are computed with respect to their values at $\xi = 2.0$ nm. Fig. 4a shows that an increase in $\Delta E_{\text{total}}(\xi)$ is accompanied by an increase in $\Delta\text{PMF}(\xi)$. For example, both ΔPMF and ΔE peak at $\xi = 0.7$ nm and their local minima are located at $\xi = 0.5$ nm. The entropic term opposes these changes and it is negative, i.e., favorable to dimerization, for small ξ values. This is consistent with recent studies in which the entropic energy of urea was shown to favor the folded state of some macromolecules [35,68,69]. Thus, when urea is added to water changes in ΔPMF of poly-leucine are caused by changes in total energy of the system.

In Fig. 4b we show changes in the non-bonded component of the total energy, i.e., $\Delta E_{\text{non-bonded}}$, when urea is added to water. This component is the sum of van der Waals and electrostatic energies in the system and it is shown with respect to its value computed at $\xi = 2.0$ nm. As for the total energy, changes in $\Delta E_{\text{non-bonded}}$ and ΔPMF occur at the same ξ values and in the same direction. Notice, however, that the magnitude of these changes differ by a factor of two as other energetic terms also contribute to ΔPMF , e.g., entropic energy in Fig. 4a. In Fig. 4c, we project $\Delta E_{\text{non-bonded}}$ into its peptide-peptide (ΔE_{pp}), peptide-solvent (ΔE_{ps}), and solvent-solvent (ΔE_{ss}) components. As peptides are brought close to each other, solvent molecules are released into the bulk breaking peptide-solvent

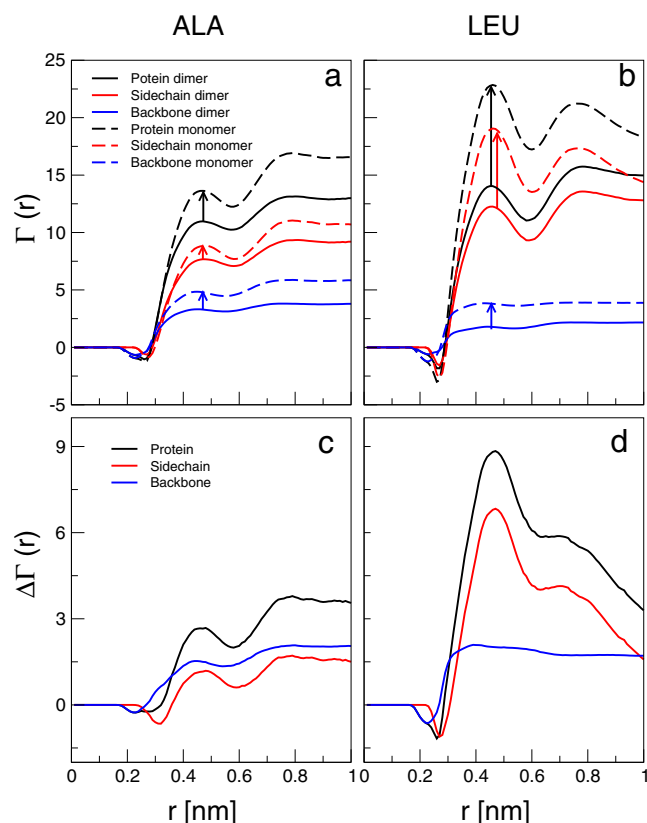


Fig. 3. (a–b) Preferential interaction $\Gamma(r)$ around dimers ($\xi = 0.47$ nm) and monomeric peptides ($\xi = 2.0$ nm). Decomposition of the preferential interaction (black) into backbone (blue) and side-chain (red) contributions. (c–d) Changes in the preferential interaction $\Delta\Gamma = \Gamma_{\text{monomer}} - \Gamma_{\text{dimer}}$ as peptides dissociate. Spatial distribution functions of urea (panel e) and water (panel f) around poly-leucine dimers in 7 M urea and pure water solutions, respectively. Isovalues for urea and water are 18 and 7.8.

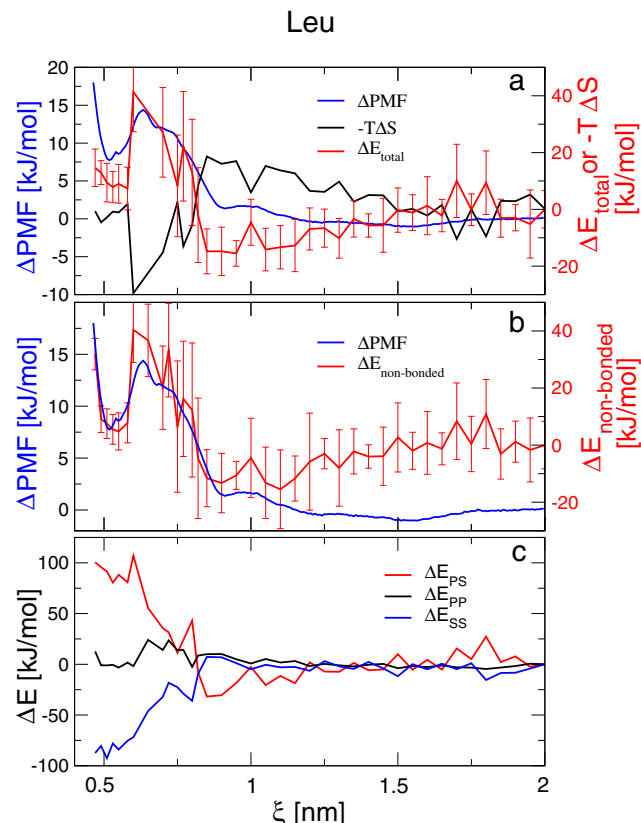


Fig. 4. (a) Changes in total energy (ΔE_{total}), entropic energy ($-T\Delta S$), and PMF (ΔPMF) when 7 M urea is added to water as a function of the distance ξ between polyleucine peptides. (b) Correlation between ΔPMF and changes in non-bonded energies when urea is added to water ($\Delta E_{\text{non-bonded}}$) as a function of ξ . Error bars are computed using block average of the different trajectories. (c) Projection of non-bonded energy into peptide-solvent (ΔE_{PS}), peptide-peptide (ΔE_{PP}), and solvent-solvent (ΔE_{SS}) components.

bonds. These bonds are stronger in urea solution than in pure water accounting for the positive ΔE_{PS} in Fig. 4c (red line). Congruently more solvent-solvent interactions are formed in this process. Solvent interactions involving urea molecules are stronger than in pure water accounting for the negative ΔE_{SS} in Fig. 4c (blue line). In contrast, peptide-peptide interactions are not strongly affected by urea (black line) except at intermediate distances where side chains interdigitate. Changes in peptide-urea interactions have been extensively studied in the literature and related to the destabilizing effect of this co-solvent [3,7,10,63]. However, as shown in Fig. 4c, unfavorable changes in the peptide-solvent interactions occur simultaneously with a favorable change in solvent-solvent interactions. It is the result of these two interactions that account for the destabilizing effect of urea.

Additional insights into how ΔE_{PS} and ΔE_{SS} are affected by urea can be obtained by studying their van der Waals and electrostatic components. To that purpose, we write $\Delta E_{\text{PS}} = E_{\text{PU}}^{\text{urea}} + E_{\text{PW}}^{\text{urea}} - E_{\text{PW}}^{\text{water}}$. Now, the term $E_{\text{PW}}^{\text{water}} - E_{\text{PW}}^{\text{urea}}$ can be interpreted as the energy of water molecules that have been replaced by urea. We refer to this term as $\Delta E_{\text{PW}}^{\text{replaced}}$. Lennard-Jones (red lines) and electrostatic (blue) contributions of both $\Delta E_{\text{PU}}^{\text{urea}}$ and $\Delta E_{\text{PW}}^{\text{replaced}}$ are shown in Fig. 5a–b. Differences in the magnitude of these quantities for poly-alanine (panel a) and poly-leucine (panel b) are due to differences in the size of the side chains of these peptides. When the distance ξ of both poly-alanine and poly-leucine dimers is small, electrostatic contributions of these two terms are comparable in magnitude and they cancel out when

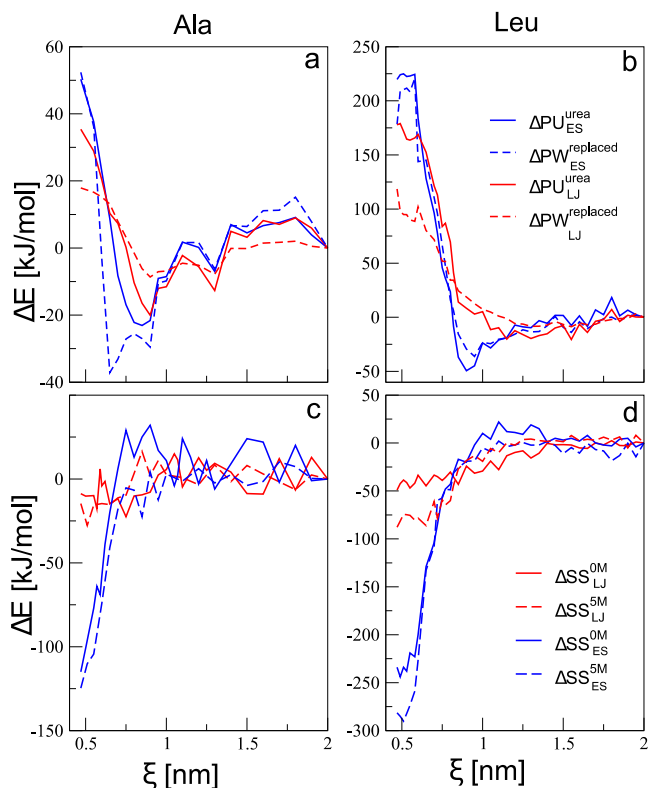


Fig. 5. Lennard-Jones and electrostatic energies of (a–b) peptide-solvent and (c–d) solvent-solvent interactions. Peptide-solvent is decomposed into replaced peptide water interactions $\Delta PW^{\text{replaced}}$ (see text) and peptide-urea ΔPU^{urea} energies. Solvent-solvent is shown for pure water and aqueous urea solution. Quantities computed at $\xi=2.0$ nm are used as our reference.

accounting for ΔE_{PS} . This implies that the electrostatic interaction between water and the peptide is very similar to the electrostatic interaction between urea and the peptide [24]. This is consistent with results from another simulation [70]. In contrast, Lennard-Jones energies of these two terms are very different when peptides are close to each other with the magnitude of $\Delta E_{\text{PU}}^{\text{urea}}$ dominating over $\Delta E_{\text{PW}}^{\text{replaced}}$. Thus, peptide-solvent Lennard-Jones energies favors non-interacting configurations of the dimer. This energetic analysis is consistent with the molecular picture in Fig. 2 where urea forms new Lennard-Jones bonds with non-polar side chains and it replaces some electrostatic bonds between peptide and water (e.g., hydrogen bonding).

In Fig. 5c–d, we decompose solvent-solvent interactions (i.e., ΔE_{SS}) into their Lennard-Jones and electrostatic components for simulations performed in pure water and urea solutions. As peptides are brought close to each other, solvent molecules are released into the bulk and this accounts for a favorable decrease in solvent-solvent energy. Fig. 5c–d shows that this reduction in energy is larger in urea solution and, therefore, the release of urea molecules into the bulk favors folding of the protein. This is consistent with experimental [4,25,26] and computational [24,27,71] studies in which urea was found to incorporate readily and tightly into water. In Fig. 5c–d both Lennard-Jones and electrostatic energies contribute to urea insertion into the solvent.

From Fig. 5 we can conclude that the increase in ΔE_{PS} (see Fig. 3b) when peptides are brought close together in urea solution compared to pure water emerges from loss of favorable Lennard-Jones interactions between peptide and urea. This occurs for both poly-alanine and poly-leucine dimers (see Fig. 5a–b) although the effect in poly-alanine is not significant enough to produce a change in its

PMF (see Fig. 2a). In contrast, reductions in ΔE_{SS} (see Fig. 3b) when peptides are brought close together in urea solution compared to pure water occurs because of changes in both Lennard-Jones and electrostatic energies—see Fig. 5c–d. This reduction is smaller for poly-alanine compared to poly-leucine due to the small size of its side chain.

4. Conclusions

In this work we study effects of urea on poly-peptides. We show that unrestrained poly-leucine peptides become less compact when urea is added to water whereas poly-glycine and poly-alanine peptides are only weakly affected by this co-solvent. To rationalize these results, we study interactions between stretched poly-alanine and poly-leucine dimers. In particular, we compute potential of mean force of these dimers in pure water and aqueous solutions containing urea. Urea is found to significantly destabilize poly-leucine dimers whereas it has little effect on the stability of poly-alanine dimers (Fig. 2). This is consistent with our simulations of unrestrained poly-peptides and it leads to the conclusion that urea has a main effect on interactions involving side chains since poly-alanine and poly-leucine are different only in their side chains. This is further confirmed by studying the distribution of urea molecules around peptides using the preferential interaction defined in Eq. 2. We found that the number of urea molecules close to side chains is larger than the expected number for the same volume in bulk solvent (Fig. 3a–b). Moreover, the difference between the number of urea molecules around peptides and the expected number increases when peptides dissociate (Fig. 3c–d). This is significantly larger for poly-leucine than for poly-alanine. Therefore, our results suggest that urea destabilizes proteins through a direct mechanism whereby it binds favorably to large non-polar side chains. An analysis of the energy of the system shows that urea forms mainly Lennard-Jones interactions with side chains while maintaining electrostatic bonds with the solvent (Fig. 5). This is possible due to the planar distribution of partial charges in urea that allows it to occupy positions between non-polar side chains which are not accessible to water (Fig. 3e–f).

In summary, we propose a simplified framework to study effects of urea on hydrophobic interactions and hydrogen bonds in proteins. This framework can be used to provide insights into effects of other cosolvents [72]. Results for urea agree with other studies in which this molecule is found to destabilize proteins through dispersion interactions with non-polar groups of the proteins [27,33–35,73]. The simplified nature of the framework used here allows us to provide a comprehensive description of the effects of urea on the interactions leading to the “association” of peptides. Despite the insights brought by the current study, limitations should also be noted. In particular, our framework does not account for the reduction in the conformational entropy of proteins associated with folding since peptides in our simulations are stretched. Effects of urea on the conformational entropy which is the main energetic term opposing dimerization still needs to be investigated. However, our simulations of unrestrained peptides provide evidence that these effects will not affect the main conclusion of this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.molliq.2016.10.022>.

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