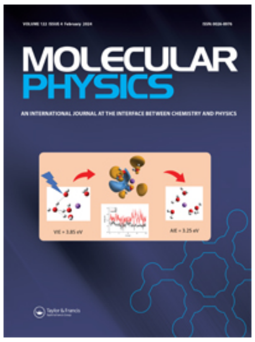

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Molecular Physics
An International Journal at the Interface Between Chemistry and Physics

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/tmph20

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To cite this article: Ali Eltareb, Janel Rivera-Cancel, Gustavo E. Lopez & Nicolas Giovambattista (05 Mar 2024): Backbone hydration of α-helical peptides: hydrogen-bonding and surface hydrophobicity/hydrophilicity, Molecular Physics, DOI: 10.1080/00268976.2024.2323637

To link to this article: https://doi.org/10.1080/00268976.2024.2323637

MOLECULAR PHYSICS e2323637
https://doi.org/10.1080/00268976.2024.2323637

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RULL-ABASCAL SPECIAL ISSUE FOR STATISTICAL PHYSICS IN SPAIN (BY INVITATION ONLY)

Backbone hydration of α-helical peptides: hydrogen-bonding and surface hydrophobicity/hydrophilicity

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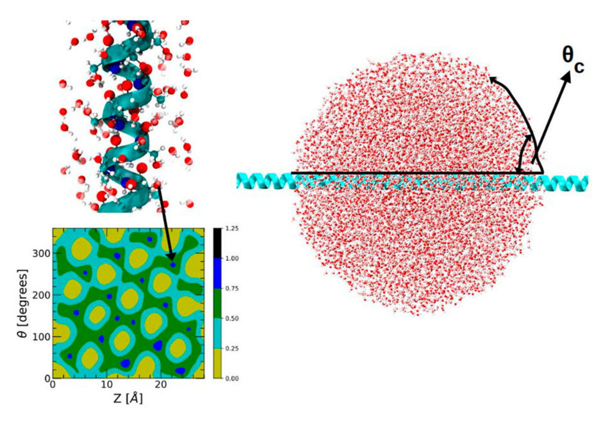
ABSTRACT

The stability of proteins and small peptides depends on the way they interact with the surrounding water molecules. For small peptides, such as α-helical polyalanine (polyALA), water molecules can weaken the intramolecular hydrogen-bonds (HB) formed between the peptide backbone O and NH groups which are responsible for the α-helix structure. Here, we perform molecular dynamics simulations to study the hydration of polyALA, polyserine (polySER) and other homopolymer peptide α-helices at different temperatures and pressures. We find that water molecules form HB with most polyALA carbonyl O atoms, despite ALA hydrophobic CH_3 side chain. Similar water-peptide backbone HB are found in other (hydrophobic and hydrophilic) homopolymer α-helices with large side chains, including polyvaline, polyleucine and polyphenylalanine. A novel hydration mechanism is observed in polySER: the backbone peptide rarely forms HB with water and, instead, the carbonyl O atoms tend to form HB with polySER side chain OH groups. We also quantify the hydrophobicity/hydrophilicity of polyALA and polySER by calculating the contact angle θ_c of a water droplet pierced by a long polyALA/polySER α-helix. Unexpectedly, even when polyALA α-helix is supposed to be hydrophobic ($\theta_c > 90^0$), we find that $\theta_c \approx 79^0$. For polySER, $\theta_c \approx 70^0$, consistent with α-helical polySER being hydrophilic.



ARTICLE HISTORY
Received 12 December 2023
Accepted 20 February 2024

KEYWORDS
Water; hydrophobicity; hydrogen-bond; α-helix; peptide hydration

1. Introduction

The role of water in the structure, stability and function of biomolecules and self-assembled structures in aqueous solutions is fundamental. For example, the way water hydrates a given protein can affect how the protein interacts with cell membranes, other proteins/biomolecules, and drugs (e.g. Refs. [1–3]). Hydration can also affect the stability of proteins and secondary structure elements, such as α-helices and β-sheets [4–7]. Not surprisingly, understanding the hydration of biomolecules, including proteins, has implications in a wide range of engineering and scientific applications.

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/00268976.2024.2323637.

To shed light into the protein stability, function, and protein-protein interactions, early studies have focussed on the interactions between water and protein secondary structure elements, particularly, α-helices. Alanine-based α-helices have been used to study water-mediated interactions [6] and the α-helix/β-sheet relative stability of small peptides [8]. The role of water on the thermal stability of alanine-based α-helices has been studied in detail by Garcia and collaborators [9,10]. α-helices are the classical secondary structure elements of proteins [11]. The α-helical structure of a peptide is maintained by hydrogen-bonds (HB) formed among the different amino acids. Specifically, in an α-helix, the carbonyl O atom of residue i forms a HB with the amino N atom of residue $i+4$; in addition, the amino N atom of the same residue i forms a HB with the carbonyl O atom of residue i—4. This sequence of intramolecular HB between the (acceptor) O and (donor) N atoms of the peptide backbone is the fingerprint of the α-helical structure. By doing so, the amino acid side chains project out from the α-helix, covering the peptide surface and remaining exposed to water [11] (see, e.g., Figure 1). For example, if the α-helix is composed solely of (hydrophobic) alanine residues then the corresponding CH_3 side chains will be exposed to the solvent which, presumably, will make the α-helix hydrophobic [Figure 1(a)]. Early studies have shown that alanine-based α-helices can experience thermal destabilisation due to the interaction of water molecules with the peptide backbone. Specifically, the hydration of the peptide backbone O and NH groups can weaken the O-HN intrapeptide HB [9,10,12] which, in turn, can lead to the α-helix unfolding. Indeed, it has been shown that the shielding of the α-helix backbone from water by large residue side chains can favour the thermal stabilisation of alanine-based α-helices [9].

In this work, we perform molecular dynamics (MD) simulations of homopolymer α-helical peptides, including polyalanine (polyALA) and polyserine (polySER), and study the peptide hydration (backbone-water interactions) at different pressures ($P = 0.1 - 700\,\text{MPa}$) and temperatures ($T = 220 - 300\,\text{K}$). In the case of polyALA, and in agreement with previous studies, we observe partial unfolding of the α-helix at $T = 300\,\text{K}$ while, at low temperatures, polyALA α-helix is stable. At all temperatures studied, most polyALA carbonyl O atoms form one HB with water molecules, consistent with Refs. [9]. Hence, the CH_3 side chains of polyALA are unable to shield the peptide backbone from water. Interestingly, similar backbone-water HBs are found in other homopolymer α-helical peptides composed of hydrophobic and hydrophilic residues. Even in the case of homopolymer α-helical peptides with large side chains, such as of polymethionine, we find that the side chains are not able to fully shield the α-helix backbone from water indicating that 'side chains backbone shielding' may be limited in stabilising α-helical structures. Interestingly, the hydration picture described above, where water easily forms HB with the α-helix backbone, does not always hold. In the case of polySER, we find that water molecules rarely make HB with the α-helix backbone and, instead, the backbone carbonyl O atoms tend to form HB with the OH side chains of polySER. Overall, our results show that the α-helix backbone (specifically, carbonyl O atoms) participate actively in the hydration of the α-helix and that they compete with the peptide side chains in determining the α-helix hydration. Since the α-helix hydration and surface hydrophobicity/hydrophilicity are intimately related, we also look into the hydrophobicity/hydrophilicity of polyALA and polySER by simulating a long α-helix piercing a water nanodroplet. As expected, the water contact angle for polySER is $\theta_c \approx 70^0$, consistent with polySER α-helix being hydrophilic (i.e. $\theta_c < 90^0$). However, in the case of polyALA, we find that $\theta_c \approx 79^0$ which is inconsistent with polyALA being hydrophobic ($\theta_c > 90^0$). While θ_c may not be the only measurement of hydrophobicity, and may not be the best parameter to quantify the hydrophobicity/hydrophilicity at the molecular level, our results suggest that it is not impossible that the HB formation between the α-helix carbonyl O atoms and water may affect the hydrophobicity/hydrophilicity of the corresponding peptide [e.g. by lowering the hydrophobicity expected for an α-helix composed of hydrophobic residues (e.g. polyALA), or the hydrophilicity of a α-helix composed of hydrophilic peptide (polySER)].

This work is organised as follows. The computer simulation details are presented in Section 2. The results are included in Section 3 where we discuss the hydration and hydrophilicity/hydrophobicity of polyALA and polySER. Also included in Section 3 are results obtained for other single-residue α-helical peptides. A summary and discussion is included in Section 4.

2. Computer simulation details

We perform two kinds of MD simulations: (i) an α-helix immersed in a water bath, and (ii) a long α-helix in contact with a water droplet.

(i) *An α-helix in a water bath.* We perform molecular dynamics (MD) simulations of short peptides composed of 20 identical residues at different pressures and temperatures. Most of our discussions are focussed on polyALA and polySER (Figure 1) immersed in a bath of water; see Sections 3.1 and 3.2. In these cases, the peptides are caped by an NH_2 and acetyl group at the C- and N-terminus, respectively. MD simulations for polyALA are performed at $P = 0.1,\ 400,\ 700$ MPa and $T = 220,\ 240,\ 300$ K; MD simulations for polySER are performed at $P = 0.1$ MPa and $T = 220,\ 240,\ 300$ K.

A second set of MD simulation is performed for homopolymer α-helical peptides (immersed in water) composed of either valine (VAL), isoleucine (ILE), leucine (LEU), methionine (MET), phenylalanine (PHE), cysteine (CYS), tyrosine (TYR), tryptophan (TRP), glycine (GLY), or ALA; see Section 3.3. In these cases, the peptides are caped with NH_2 and COOH groups at the N- and C-terminus, respectively, and the simulations are performed at $P = 0.1$ MPa and $T = 240,\ 300$ K.

In all cases, the peptide is initially in an α-helical configuration and has a length of approximately 3–3.5 nm. The system box is cubic with a side length of at least 5.9 nm and is filled with $N \approx 7000$ water molecules; periodic boundary conditions apply along the x-, y- and z-direction. All peptides are modelled using the CHARMM36 force field [13] and water molecules are represented using the TIP4P/2005 model [14]. All MD simulations run for 100 ns using a simulation time step of 2 fs. Simulations are performed using the GROMACS software package [15]. A cutoff $r_c = 1.1$ nm is used to calculate the Lennard-Jones interactions. Electrostatic interactions are treated using the Particle-Mesh Ewald (PME) method with the same cutoff $r_c = 1.1$ nm. The temperature and pressure are controlled using a Nose–Hoover thermostat and a Berendsen barostat.

(ii) *An α-helix wet by a water droplet.* In order to quantify the hydrophobicity/hydrophilicity of polyALA and polySER, we perform MD simulations of a water droplet composed of $N \approx 10{,}200$ water molecules and pierced by a long, straight α-helix composed of 1000 ALA or SER residues. In these simulations, the C_α atoms of the peptide remain immobile throughout the 30-ns long MD run (while all other atoms of the peptide are free to move); see Section 3.1.4.

3. Results

The results are organised as follows. In Section 3.1, we discuss the hydration of a polyALA α-helix at $T = 220,\ 240,\ 300$ K and $P = 0.1,\ 400,\ 700$ MPa, for which the peptide side chains (CH_3) are hydrophobic. In Section 3.2, we study polySER at $T = 220,\ 240,\ 300$ K and $P = 0.1$ MPa for which the peptide side chains (CH_2–OH) are hydrophilic. Homopolymer α-helical peptides composed of amino acids other than ALA and SER are the focus of Section 3.3 ($P = 0.1$ MPa and $T = 240,\ 300$ K).

3.1. PolyALA

3.1.1. Structure

Experimental data on polyALA is limited due to its low solubility in water [10,16–21]. Early experiments of small ALA-based peptides indicate a significant helical composition at room temperature and a broad thermal helix-coil transition at about $T_{H-C} = 320 - 340$ K [18,22,23]. Computer simulations of polyALA at normal pressure show that the coil-helix transition can vary with the force field considered, with $T_{H-C} \approx 275 - 350$ K [4,9]. Our MD simulations are consistent with these results and show that polyALA remains in an α-helical configuration over a wide range of pressures ($P = 0.1 - 700$ MPa) at $T = 240$ K but exhibits partial unfolding at $T = 300$ K. To show this, we calculate the number of hydrogen bonds (HB) formed between the O and N-H atoms of polyALA backbone. After all, the structure of an α-helix peptide is maintained by (i) HB formed by the carbonyl O of residue i and the amino group N of residue $i + 4$, and (ii) HB formed by the amino group N of the same residue i and the carbonyl O of residue i–4 [11]. Figure 2 shows the average number of HB that the O atom of polyALA residue i ($i = 1, 2, 3 \cdots 20$) forms with any of the N-H groups of the polyALA backbone, $n_{\mathrm{O-N}}(\mathrm{i})$ [red line] at $P = 0.1,\ 400,\ 700$ MPa and $T = 240$ K. As expected, at all pressures considered, $n_{\mathrm{O-N}}(\mathrm{i}) \approx 1$ for $1 \leq i \leq 16$ and $n_{\mathrm{O-N}}(\mathrm{i}) \approx 0$ for $i \geq 17$. This is because there are only 20 residues in polyALA and hence, only for the O atoms of residues $1 \leq i \leq 16$ there exist a residue $i + 4 \leq 20$ with an available N-H group to hydrogen-bond to. Also included in Figure 2 is the average number of HB that the N-H group of polyALA residue i forms with the polyALA carbonyl O atom, $n_{\mathrm{N-O}}(\mathrm{i})$ [black line]. As expected, $n_{\mathrm{N-O}}(\mathrm{i}) \approx 1$ for $5 \leq i \leq 20$ and $n_{\mathrm{O-N}}(\mathrm{i}) \approx 0$ for $i \leq 4$; only for the N-H group of residues $5 \leq i \leq 20$ there exist a residue $i - 4 \geq 0$ with an available O atom to hydrogen-bond to. The values of $n_{\mathrm{O-N}}(\mathrm{i})$ and $n_{\mathrm{N-O}}(\mathrm{i})$ are fully consistent with polyALA maintaining its α-helical structure at all pressures considered ($T = 240$ K). We confirmed that polyALA remains in an α-helical configuration at $T \leq 240$ K by calculating the end-to-end distance of the corresponding peptide (in general, our conclusions regarding the α-helical structure of the peptides studied are consistent with the corresponding end-to-end distance calculated from the MD simulations and visual inspection of the trajectories).

In order to study the hydration of polyALA, we will limit our study to residues $5 \leq i \leq 16$. This is because residues $i \leq 4$ are missing a HB with the O atom of

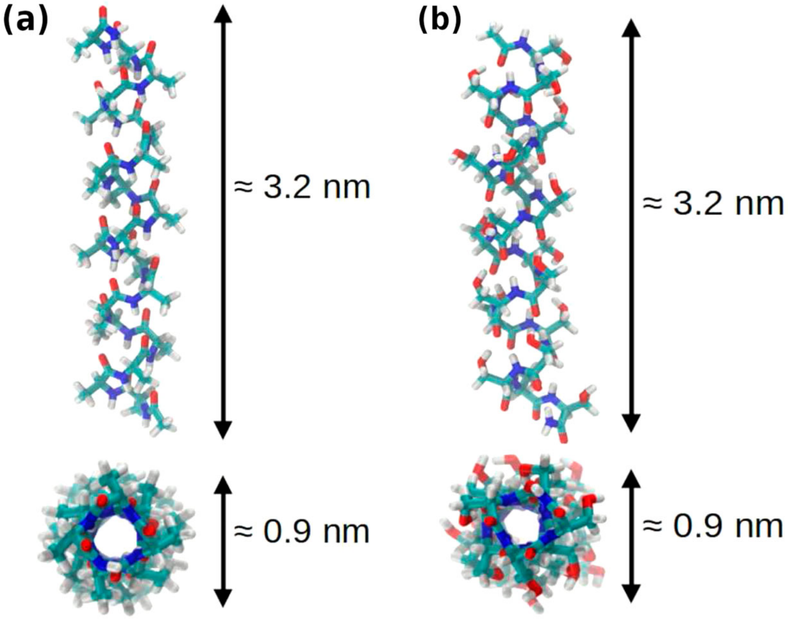

Figure 1. Snapshot of the 20-residue (a) polyALA and (b) polySER α-helical peptides used in our MD simulations. In (a) and (b), polyALA and polySER are capped by an NH_2 and acetyl group at the C- and N-terminus, respectively.

residue (i−4); and residues $i \geq 17$ are missing a HB with the N-H group of residue ($i + 4$). Accordingly, residues $i \leq 4$ and $i \geq 17$ are prompt to form HB with water (see Figure 2). Table 1 shows the fractional helicity f_α of polyALA, defined as the average fraction of residues $5 \leq i \leq 16$ whose dihedral angles correspond to an α-helical structure. The fact that $f_\alpha = 100\%$ at all pressures considered ($T = 240$ K) confirms, again, that polyALA middle residues maintain an α-helical structure. Also included in Table 1 is the average number of HB [24] that the O and N-H atoms of residues $5 \leq i \leq 16$ form with any other residue ($1 \leq j \leq 20$), n_{int} (the HB formed with the $COCH_3$ and NH_2 caps are not considered in the calculations). We find that $n_{int} > 15.0$ which is consistent with the expected value, for a perfect α-helix, $n_{int} = 16$. Table 1 also shows (a) the number of HB that the O atoms of residues $5 \leq i \leq 16$ form with the N-H groups ($1 \leq j \leq 20$), $n_{\mathrm{O-N}}$; and (b) the number of HB that the N-H atoms of residues $5 \leq i \leq 16$ form with the O atoms ($1 \leq j \leq 20$), $n_{\mathrm{N-O}}$ (HB formed with the $COCH_3$ and NH_2 caps are ignored). The fact that $n_{\mathrm{O-N}}$, $n_{\mathrm{N-O}} > 11.1$, again, is fully consistent with residues $5 \leq i \leq 16$ of polyALA being in an α-helix structure (for a perfect α-helix, one should have $n_{\mathrm{O-N}} = n_{\mathrm{N-O}} = 12$).

Table 1. Average properties of polyALA at $T = 240$ K and $P = 0.1,\ 400,\ 700$ MPa.

P [MPa]	f_α [%]	n_{int}	$n_{\mathrm{O-N}}$	$n_{\mathrm{N-O}}$
0.1	100(0.0)	15.03(0.96)	11.15(0.86)	11.62(0.52)
400	100(0.0)	15.22(0.87)	11.31(0.81)	11.66(0.53)
700	100(0.0)	15.24(0.91)	11.32(0.95)	11.69(0.56)

Statistics are based on residues numbered 5–16 (see text). f_α is the fraction of residues $5 \leq i \leq 16$ that are in an α-helical configuration. n_{int} is the number of internal hydrogen-bonds that residues $5 \leq i \leq 16$ form with other atoms of polyALA residues $1 \leq j \leq 20$ (the acetyl and NH_2 caps are not included). $n_{\mathrm{O-N}}$ is the average number of HB that the carbonyl O atoms of residues $5 \leq i \leq 16$ form with an N-H group of polyALA residues $1 \leq j \leq 20$ (the NH_2 cap is not included). Similarly, $n_{\mathrm{N-O}}$ is the average number of HB that the backbone N-H groups of residues $5 \leq i \leq 16$ form with an O atom of polyALA residues $1 \leq j \leq 20$ (the acetyl cap is not included). Numbers in parenthesis are the standard deviations.

3.1.2. Hydrogen-bonds with water

To characterise the hydration of polyALA, we show in Figure 2(a–c) the average number of HB that the O atom of residue i forms with the water molecules, $n_{\mathrm{O-H_2O}}(\mathrm{i})$ [green lines]. Also included is the number of HB that the N atom of residue i forms with the water molecules, $n_{\mathrm{N-H_2O}}(\mathrm{i})$ [blue lines]. Interestingly, for residues $5 \leq i \leq 16$, $n_{\mathrm{O-H_2O}}(\mathrm{i}) = 0.75 - 1$ depending on the pressure, while $n_{\mathrm{N-H_2O}}(\mathrm{i}) = 0$ in all cases. Hence, at all pressures studied, the CH_3 side chains of the ALA residues are not able to shield the carbonyl O atoms from water. Indeed, our results are consistent with Ref. [10] where it is shown that the carbonyl O atoms of polyALA are surrounded, in average, by approximately one water molecule (within an OO distance ≤ 3.6 Å). Our results are also consistent with density functional theory and MD calculations of an isolated polyALA α-helix hydrated by a few water molecules [25,26].

Included in Table 2 are the total average number of water-polyALA (external) HB, n_{ext}, as well as the average number of HB that the O and N-H atoms

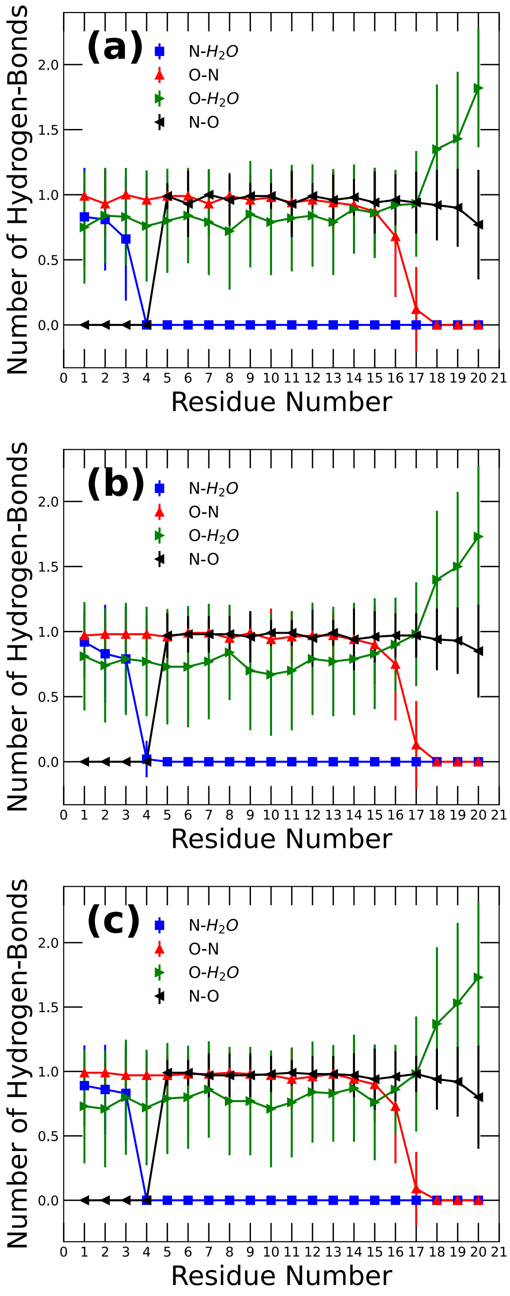

Figure 2. Average number of HB [24] formed by the O and N-H atoms of polyALA α-helix backbone at $T = 240$ K and (a) $P = 0.1$ MPa, (b) $P = 400$ MPa and (c) $P = 700$ MPa. The O atom of residue i, for $1 \leq i \leq 16$, forms an average of 1 HB with the N-H group of residue $i + 4$ (red line) and 0.75–1 HB with the water molecules (green line). Similarly, the N-H group of residue i, for $5 \leq i \leq 20$, forms an average of 1 HB with the O atom of residue i—4 (black line) and no HB with water (blue line). This pattern does not hold for the end residues, $i \leq 4$ and $i \geq 17$, these residues are particularly prompt to form HB with water (see text).

Table 2. Average number of HB formed by polyALA with water molecules at $T = 240$ K and $P = 0.1$, 400, 700 MPa.

P [MPa]	n_{ext}	n_{O-H_2O}	n_{N-H_2O}
0.1	9.95(1.07)	9.91(1.06)	0.04(0.20)
400	9.26(1.24)	9.22(1.26)	0.04(0.19)
700	9.67(1.43)	9.62(1.43)	0.05(0.22)

n_{ext} is the total average number of HB that polyALA residues numbered 5–16 form with water molecules. n_{O-H_2O} and n_{N-H_2O} are, respectively, the average number of HB that the O and N-H atoms of polyALA residues numbered 5–16 form with water molecules. Numbers in parenthesis are the standard deviations.

of polyALA residues $5 \leq i \leq 16$ form with the water molecules, n_{O-H_2O} and n_{N-H_2O}, respectively. Consistent with Figure 2, we find that $n_{O-H_2O} \approx 10.0$ at $P = 0.1$ MPa which is close to the value of 12 corresponding to one HB per O atom; note also that $n_{N-H_2O} \approx 0$. Increasing pressure reduces the number of O-water HB to $n_{O-H_2O} = 9.26 - 9.67$ implying that pressure does not affect the HB-network around polyALA. Note that, as expected, $n_{ext} \approx n_{O-H_2O} + n_{N-H_2O}$ meaning that only the N-H and O atoms of polyALA can form HB with water.

We conclude this section with a brief discussion of the HB formed by the residues located at the polyALA ends. From our previous discussion, it follows that the O atoms of residues $i \geq 17$ should be able to form HB with water. After all, these O atoms do not have an N-H group to HB to. Accordingly, Figure 2 shows that $n_{O-H_2O}(\mathrm{i}) > 1$ for $i \geq 17$ [green lines]. Similarly, the N-H group of residues $i \leq 4$ should be able to form HB with water since these N-H groups do not have a O atom to HB to. Accordingly, Figure 2 shows that $n_{N-H_2O}(\mathrm{i}) \approx 1$ for $i \leq 4$ [blue lines]. Overall, Figure 2 indicates that in a polyALA α-helix, the carbonyl O atoms (of any residue) try to form approximately a total of 2 HB (with an N-H group and a water molecule, or with two water molecules). Similarly, the N-H groups try to form approximately a total of 1 HB (with an O atom or a water molecule).

The results discussed above are obtained at $T = 240$ K. Similar results hold for $T = 220$ and 300 K ($P = 0.1$ MPa) indicating that the findings reported so far for polyALA are rather general. For example, as shown in Table 3, water molecules do form HB with the O atoms of polyALA residues $5 \leq i \leq 16$ at $T = 220$ and 300 K. Not surprisingly, the number of HB among the O and N-H atoms decreases with increasing temperatures as the peptide fluctuations increase and the α-helical structure of polyALA weakens. Indeed, at $T = 300$ K, we find $f_\alpha = 87\%$ indicating that polyALA starts to unfold and becomes unstable in an α-helical configuration. Previous studies of the thermal stability of polyALA show that the α-helix-coil transition occurs over a wide temperature range, about 275–350 K, depending on the force-field employed. Evidently, the partial unfolding of polyALA

Table 3. Average properties of polyALA at $T = 220,\ 240,\ 300$ K ($P = 0.1$ MPa); values for $T = 240$ K are taken from Tables 1 and 2.

T [K]	f_α [%]	n_{int}	$n_{\mathrm{O-N}}$	$n_{\mathrm{N-O}}$	n_{ext}	$n_{\mathrm{O-H_2O}}$	$n_{\mathrm{N-H_2O}}$
220	100(0.0)	15.37(0.82)	11.45(0.62)	11.72(0.49)	9.75(1.02)	9.73(0.99)	0.02(0.14)
240	100(0.0)	15.03(0.96)	11.15(0.86)	11.62(0.52)	9.95(1.07)	9.91(1.06)	0.04(0.20)
300	87(12.1)	11.47(1.49)	7.85(1.51)	10.62(1.33)	11.13(1.92)	10.90(1.70)	0.23(0.52)

All quantities are defined in Tables 1 and 2. Statistics are based on residues numbered $5 \le i \le 16$ (the acetyl and NH_2 caps are not excluded). Numbers in parenthesis are the standard deviations.

Table 4. Average properties of polySER at $T = 220, 240,\ 300$ K and $P = 0.1$ MPa.

T [K]	f_α [%]	n_{int}	$n_{\mathrm{O-N}}$	$n_{\mathrm{N-O}}$	$n_{\mathrm{O-OG}}$	$n_{\mathrm{N-OG}}$
220	100.0(0.0)	24.84(1.07)	11.82(0.48)	11.90(0.30)	7.50(1.00)	0.00(0.00)
240	100(0.0)	24.27(1.35)	11.73(0.51)	11.85(0.50)	6.79(1.17)	0.00(0.00)
300	86.5(8.4)	14.24(2.27)	6.86(1.83)	8.30(1.70)	4.04(1.35)	0.46(0.54)

Statistics are based on residues numbered 5–16 (see text). f_α, n_{int}, $n_{\mathrm{O-N}}$ and $n_{\mathrm{N-O}}$ are defined in Table 1. $n_{\mathrm{O-OG}}$ and $n_{\mathrm{N-OG}}$ are the average number of HB that the O and N-H atoms of polySER residues $5 \le i \le 16$ form with the oxygen atom (OG) of any side chain (residue $1 \le j \le 20$); the acetyl and NH_2 caps are not included in the calculations. Numbers in parenthesis are the standard deviations.

Table 5. Average number of HB formed by polySER with water molecules at $T = 220, 240,\ 300$ K and $P = 0.1$ MPa.

T [K]	n_{ext}	$n_{\mathrm{O-H_2O}}$	$n_{\mathrm{N-H_2O}}$	$n_{\mathrm{OG-H_2O}}$
220	20.50(1.77)	0.86(0.82)	0.00(0.00)	19.64(1.69)
240	20.82(2.10)	0.91(0.86)	0.03(0.17)	19.88(1.92)
300	27.74(3.05)	5.52(1.90)	1.50(0.92)	20.72(2.58)

n_{ext}, $n_{\mathrm{O-H_2O}}$ and $n_{\mathrm{N-H_2O}}$ are defined in Table 2. $n_{\mathrm{OG-H_2O}}$ and $n_{\mathrm{OG-H_2O}}$ are the average number of HB that the oxygen atoms of polySER side chains (OG) form with water molecules. Only residues $5 \le i \le 16$ are considered in the calculations. Numbers in parenthesis are the standard deviations.

Table 6. Average number of HB formed by polySER side chains OG atoms at $T = 220, 240,\ 300$ K and $P = 0.1$ MPa.

T [K]	$n_{\mathrm{OG-O}}$	$n_{\mathrm{OG-N}}$	$n_{\mathrm{OG-H_2O}}$
220	6.68(0.85)	0.00(0.00)	19.64(1.69)
240	6.18(1.11)	0.00(0.00)	19.88(1.92)
300	4.48(1.49)	0.12(0.32)	20.72(2.58)

Only OG atoms of residues $5 \le i \le 16$ are considered in the calculations. $n_{\mathrm{OG-O}}$, $n_{\mathrm{OG-N}}$ and $n_{\mathrm{OG-H_2O}}$ are, respectively, the average number of HB that the OG atoms (residues $5 \le i \le 16$) form with polySER backbone O and N-H atoms (residues $1 \le j \le 20$), and water. Numbers in parenthesis are the standard deviations.

exposes the carbonyl O atoms to water and $n_{\mathrm{O-H_2O}}$ increases upon heating.

3.1.3. Hydration

To characterise the spatial distribution of the water molecules surrounding polyALA, we generate water density contour (WDC) maps. The WDC maps show the distribution of water molecules within the cylindrical hydration layer of polyALA α-helix and are calculated as follows. For each snapshot extracted from our MD simulations, we define an axis for the α-helix. Specifically, for each snapshot, we split polyALA into two ('upper' and 'lower') halves. We then identify the coordinates of the centre of mass of the alpha-carbon atoms C_α belonging to the upper half of polyALA ($R_{CM,u}$); similarly, we also calculate the coordinates of the centre of mass of the C_α atoms belonging to the lower half of polyALA ($R_{CM,l}$). The axis of polyALA is the line that passes through points $R_{CM,u}$ and $R_{CM,l}$. Using the polyALA axis as a reference, we define cylindrical coordinates (z, θ, r) to locate any water O atom within the hydration layers of polyALA. The WDC maps at a given point in the (z, θ)-plane are proportional to the corresponding local water density within the cylindrical shell extending over a distance $4 \le r \le 6$ Å from the polyALA axis.

Figure 3(a) shows the WDC map of α-helical polyALA at $T = 240$ K and $P = 0.1$ MPa. The regularly located light green domains are dry. These domains coincide with the location of the CH_3 side chains of the ALA residues; see brown spots in Figure 3(b). The green and blue domains represent the regions of polyALA that are hydrated. Consistent with our previous discussions, water hydrates preferentially the regions close to polyALA O atoms. Indeed, as shown in Figure 3(b), the blue domains of the WDC maps are located in-between the N-H groups (orange spots) and O atoms (red spots) of polyALA backbone. The elongated dark green regions expanding between the O atoms of polyALA are characterised by intermediate water densities. As shown in Figure 3(c,d), the effect of increasing pressure is to increase the overall density of water in the first hydration layer of polyALA but it does not change the water density next to polyALA O atoms, and it does not induce wetting of polyALA next to the CH_3 side chains. Not surprisingly, the WDC maps are sensitive to the thickness of the hydration layer considered. As shown in the supplementary material (SM), the picture resulting from Figure 3(a–d) is consistent with the WDC maps obtained by using hydration layers of different thickness.

3.1.4. On the hydrophobicity/hydrophilicity of polyALA

The formation of backbone-water HB in α-helical polyALA peptide may also affect the peptide overall hydrophobicity. To address this issue, we perform

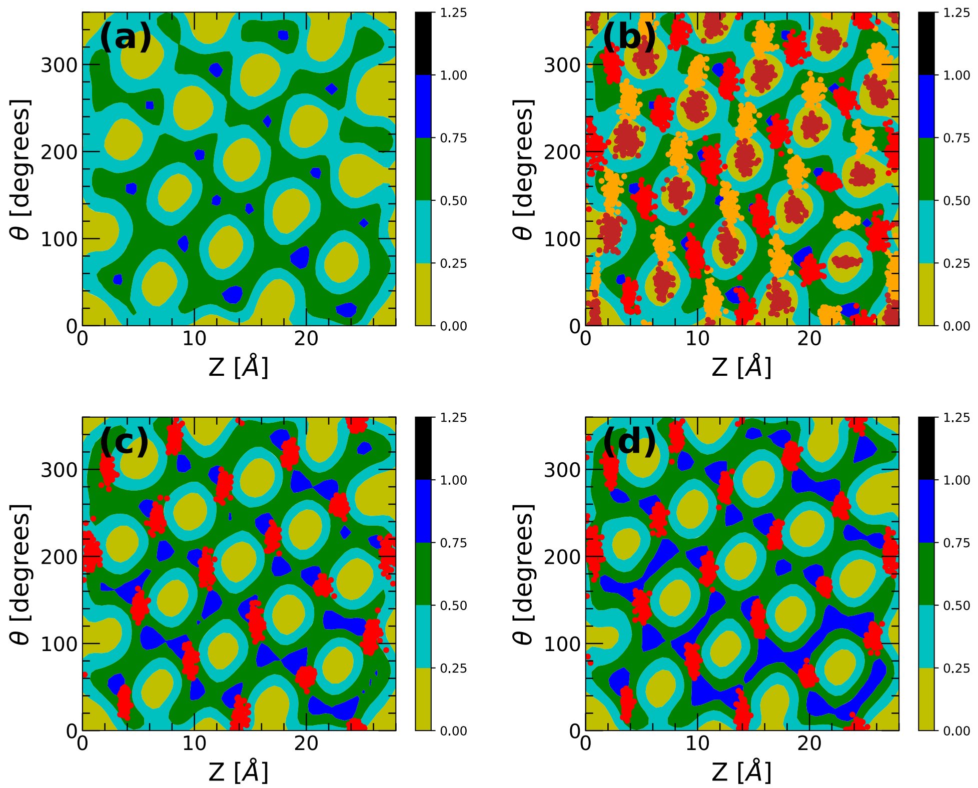

Figure 3. Water density contour maps of a polyALA α-helix at $T = 240$ K and (a,b) $P = 0.1$ MPa, (c) $P = 400$ MPa and (d) $P = 700$ MPa. Z and θ are cylindrical coordinates where z is measured along the α-helix axis and θ is the corresponding azimuth angle (the α-helix extends from $z = 0$ to $z \approx 30$ Å). Only molecules within a distance $4 \le r \le 6$ Å from the α-helix axis are considered in the calculations. Light green regions are dry and correspond to the location of polyALA CH_3 side chains [brown spots in (b)]. Dark green and blue indicate regions of polyALA surface that are hydrated. Water molecules tend to locate preferentially next to the O atoms [red spots in (b–d)]. Orange spots in (b) correspond to the location of polyALA N atoms. The density colour scale is in arbitrary units.

the following additional MD simulations. We consider an initial configuration composed of a water droplet ($N = 10{,}239$ water molecules) that is pierced by a long polyALA α-helix (composed of 1000 residues). As shown in Figure 4(a,b), in the starting configuration of our MD simulations, the polyALA α-helix passes through the centre of the water droplet. The simulation is then run for 30 ns while keeping the C_α atoms of polyALA at fixed positions (all other atoms are able to move). The final configuration of the system is shown in Figure 4(c,d) where one can observe that the droplet remains wetting the polyALA α-helix. While the droplet oscillates with time, it remains rather centred at the polyALA α-helix Figure 4(d). From the MD simulations, we also calculate the contact angle that the water droplet forms with the polyALA α-helix (see SM for details on how the contact angle is calculated). The calculated average contact angle of water with the polyALA α-helix is $\theta_c = 79.2°$. For comparison, we also run a similar MD simulations where the partial charges of polyALA are turned off; this makes 'polyALA' fully hydrophobic. The final configurations of the system after a 30 ns-run are shown in Figure 4(e,f). In this case, the water droplet remains tangent to the 'polyALA' α-helix as the system tries to minimise the water-polyALA interface.

The finding that the water contact angle of polyALA α-helix is $<90^0$ is counter-intuitive since, in principle, this implies that the polyALA α-helix is hydrophilic. The polyALA CH_3 side chains exposed to water strongly suggest that polyALA is hydrophobic. Indeed, in Ref. [6], computer simulations were used to calculate the potential of mean force (PMF) between two polyALA α-helices. Although the α-helices had a fix relative orientation and the motion of the backbone C_α atoms were constrained, it was found that the polyALA-polyALA PMF was comparable to the PMF between hydrophobic nanoscale solutes. In the stable state of the system, the two polyALA alpha-helices were in contact with one another, without water molecules trapped in between – the PMF in Ref. [6] is qualitatively very similar to the PMF between nanoscale graphene plates characterised by a water contact angle of $\theta_c = 94^0$ [27]. Our results and Ref. [6] suggest that a single polyALA α-helix is weakly hydrophilic, but when two *of such* α-helices get close together, the flexible CH_3 side chains of both peptides work jointly in expelling the water molecules from the confined space. We also note that computer simulations of water nanodroplets on artificial planar peptide networks composed of ALA indicate a contact angle of $\theta_c \approx 120^0$, also consistent with polyALA being hydrophobic [28]. However, the exposure of the carbonyl O atoms (and side chains) to water is different in the planar peptide networks than in the corresponding α-helical configurations. We conclude that, although our result for polyALA based on the θ_c of a water nanodroplet is counter-intuitive, it is not unthinkable that the backbone-water HB formation may reduce the overall hydrophobicity of α-helical polyALA.

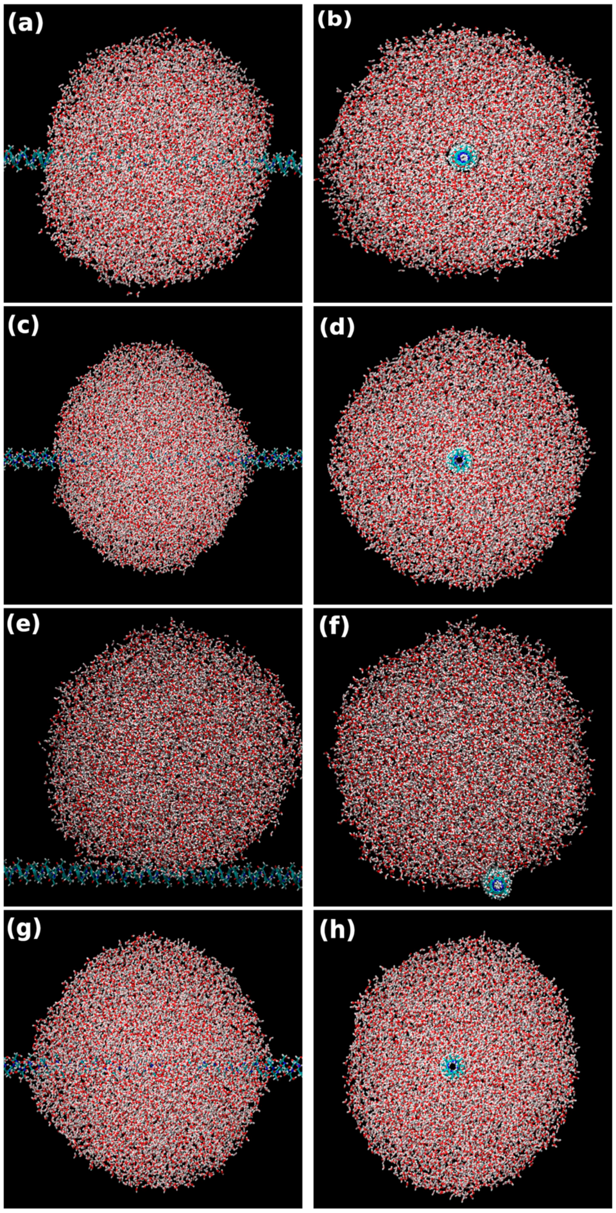

Figure 4. Snapshots taken during MD simulations of a water nanodroplet wetting a long polyALA α-helix; the C_α atoms of polyALA are kept at fixed position during the MD simulations. (a,b) Starting configuration of the system along the view (a) perpendicular and (b) parallel to the polyALA α-helix. PolyALA pierces the water droplet through its centre. (c,d) Snapshots of the droplet-polyALA system at $t = 30$ ns. Although the droplet oscillates with time during the MD simulations, it remains centred at the polyALA α-helix [the average contact angle of water with the polyALA α-helix is $\theta_c = 79.2°$]. (e,f) Snapshots of the droplet-polyALA system when all the partial charges of polyALA are turned off; $t = 30$ ns. In this case, the water droplet remains tangent to the polyALA α-helix as the system tries to minimise the water-polyALA interface. (g,h) Snapshots of a droplet-polySER system at $t = 30$ ns. As for polyALA, the droplet oscillates with time during the MD simulations but it remains centred at the polySER α-helix [the average contact angle of water with the polySER α-helix is $\theta_c = 70.0°$].

3.2. PolySER

The hydration analysis of polyALA suggests that the carbonyl O atoms of α-helices play an important role in defining the surface chemistry of the corresponding peptide. One may wonder if these O atoms can also interact with amino acid side chains, particularly, with side chains that are polar and able to form HB. To address this question, we study the hydration of polySER at $T = 220, 240, 300$ K and $P = 0.1$ MPa. PolySER side chain, CH_2–OH, can clearly form HB with water via its OH group. As we show next, the side chains OH groups form HB with the carbonyl O atoms of polySER.

We first confirm that polySER is able to maintain an α-helical structure during our MD simulation. Figure 5(a,b) show, respectively, $n_{\mathrm{O-N}}(\mathrm{i})$ [red line] and $n_{\mathrm{N-O}}(\mathrm{i})$ [black line] at $T = 240$ K and $P = 0.1$ MPa. A comparison of Figures 5(a,b) and 2(a) [red and black lines] shows practically the same behaviour of $n_{\mathrm{O-N}}(\mathrm{i})$ and $n_{\mathrm{N-O}}(\mathrm{i})$ for polySER and polyALA. Specifically, (a) $n_{\mathrm{O-N}}(\mathrm{i}) \approx 1$ for residues $1 \le i \le 16$ while $n_{\mathrm{O-N}}(\mathrm{i}) \approx 0$ for $i \ge 17$, and (b) $n_{\mathrm{N-O}}(\mathrm{i}) \approx 1$ for residues $5 \le i \le 20$ while $n_{\mathrm{N-O}}(\mathrm{i}) \approx 0$ for $i \le 4$. As explained in Section 3.1.1 for the case of polyALA, these results indicate that polySER maintains an α-helical structure at the conditions studied. Next, following the analysis of polyALA hydration, we focus on polySER residues $5 \le i \le 16$. As expected, we find that these residues are in an α-helical structure. Specifically, we find that at low temperature ($T = 220, 240$ K) these residues are in an α-helical configuration with $f_\alpha = 100\%$ Table 4. At higher temperature ($T = 300$ K), polySER starts to show partial unfolding, as for the case of polyALA, with $f_\alpha = 86.5\%$. Consistent with these observations, we find that $n_{\mathrm{O-N}}, n_{\mathrm{N-O}} = 11.82, 11.73, 6.86$ at $T = 220, 240, 300$ K (again, $n_{\mathrm{O-N}}, n_{\mathrm{N-O}} = 12$ for a perfect α-helix). Indeed, a comparison of Tables 3 and 4 shows that the values of $n_{\mathrm{O-N}}$ and $n_{\mathrm{N-O}}$ are very close, if not identical, for polyALA

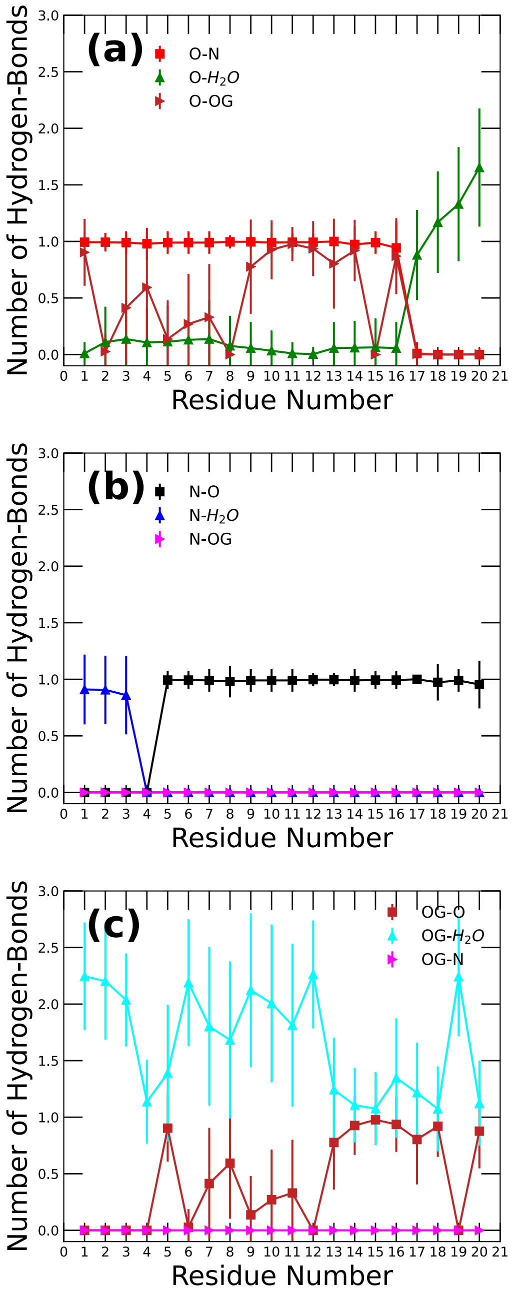

Figure 5. (a,b) Average number of HB formed by the carbonyl O and amino N atoms of polySER α-helix at $T = 240$ K and $P = 0.1$ MPa; same colours as in Figure 2 are used. The carbonyl O atom of residue i, for $1 \leq i \leq 16$, form an average of 1 HB with the N-H groups (of residues $1 \leq j \leq 20$) (red line), and ≈ 0 HB with water (green line). Similarly, the amino N atom of residue i, for $5 \leq i \leq 20$, form an average of 1 HB with the carbonyl O atoms ($1 \leq j \leq 20$) (black line), and 0 HB with water (blue line). The carbonyl O atom can form up to 1 HB with the side chains oxygen atoms (OG) (brown line); amino N atoms do not form HB with the side chains OG atoms (magenta line). (c) Average number of HB formed by the side chain OG oxygen of residue i, $1 \leq i \leq 20$ ($T = 240$ K, $P = 0.1$ MPa). The OG atoms form a total average of ≈ 2 HB, either ≈ 2 HB with water, or ≈ 1 HB with a water molecule plus ≈ 1 HB with a carbonyl O atom (no HB forms between the OG and N atoms).

and polySER ($T = 220,\ 240,\ 300$ K, $P = 0.1$ MPa). The only difference between Tables 3 and 4 is that $n_{int} = 11.47 - 15.37$ for polyALA while $n_{int} = 14.24 - 24.84$ for polySER. The larger value of n_{int} for polySER is due to the side chains oxygen atoms, OG, which can form HB with the carbonyl O atoms (contributing to n_{int}). As shown in Table 4, the number of HB formed by the carbonyl O atoms (residues $5 \leq i \leq 16$) with any OG atom (residues $1 \leq j \leq 20$) is $n_{\mathrm{O-OG}} = 7.50,\ 6.79,\ 4.04$ at $T = 220,\ 240,\ 300$ K. Interestingly, a close inspection of polySER shows that the O atom of residue i forms a HB with the OG atom of residue $i+4$ (as for the HB formed between O and N-H atoms). We note that the N-H groups of polySER do not form HB with the side chains, $n_{\mathrm{N-OG}} = 0.00$. Next, we focus on the hydration of polySER. The presence of HB between O and OG atoms makes the hydration of polySER complex. To simplify the discussion, we exclude the end residues of polySER in the following analysis and focus on residues $5 \leq i \leq 16$. Below are the main points.

(i) As shown in Figure 5(b), the N-H groups of polySER do not form HB with water; they do not form HB with the OG atoms either. As for the case of polyALA, the N-H groups are rather dormant and the N-H group of residue i only forms 1 HB with the O atom of residue i–4 ($5 \leq i \leq 20$). As discussed previously, the N-H groups of the end residues $i<4$ do form HB with water because they lack an O atom to HB to; see Figure 5(b).

(ii) The behaviour of the O atoms in polySER is very different to that observed in polyALA. While the O atoms of residues $5 \leq i \leq 16$ form one HB with the N-H group of residue $i+4$, as found in polyALA, they form practically no HB with water; see Figure 5(a) and Table 5. The lack of O-water HB ($5 \leq i \leq 16$) is partially compensated by the formation of O-OG hydrogen-bonds. For example, the O atoms of residues $i = 16$ and $9 \leq i \leq 14$ form, in average, one HB with side chain OG atoms while the O atoms of residues $i = 5, 8, 15$ do not. It follows that the hydration of polySER is highly heterogeneous with O atoms forming a total of either 1 or 2 HB. We note that the O atoms at the end of polySER, residues $i \geq 17$, form $\approx 1 - 2$ HB with water molecules, as found for polyALA. However, this is because such O atoms are exposed to water since they do not have an N-H group to hydrogen-bond to.

(iii) The OG atoms form, in average, a total of approximately 2 HB. As shown in Figure 5(c), the OG atoms either form (A) two HB with water, or (B) one HB with water and one HB with a carbonyl O

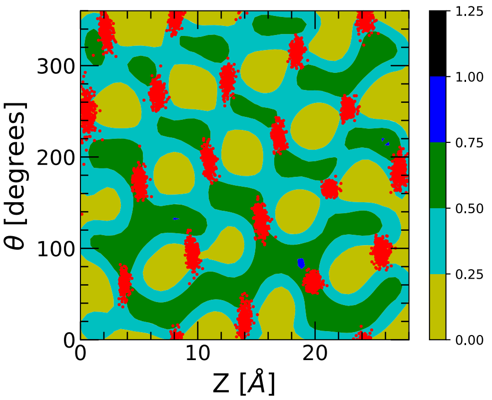

Figure 6. Water density contour map of polySER α-helix hydration layer at $T = 240$ K and $P = 0.1$ MPa (see caption of Figure 3 for details). Density colour scale is in arbitrary units.

atom. Interestingly, at least for the 20-residue polySER α-helix considered, the OG-O HB seem to be grouped in small sets of residues. For example, among residues $5 \leq i \leq 16$, residues $i = 6$-12 form (A) while residues 5, 13-16 form (B). The number of HB that an OG atom of polySER residues $5 \leq i \leq 16$ form with water, O and N-H atoms are included in Table 6. In particular, we note that OG atoms form, in average, a total of ≈ 25 HB with water molecules and O atoms; again, this corresponds to ≈ 2 HB per OG atom.

For comparison with polyALA, we include in Figure 6 the WDC map of polySER at $T = 240$ K ($P = 0.1$ MPa). The WDC maps for polyALA and polySER are, not surprisingly, different. The dry (light green) regions of the WDC map in Figure 6 correspond to the location of polySER side chains; the blue and dark green regions, indicating the presence of water molecules, are non-uniform throughout polySER surface. We note that even when the OG atoms form HB with the carbonyl O atoms of polySER, the overall surface of polySER remains hydrophilic. As shown in Figure 4(g,h), a water droplet easily wets a long polySER peptide. Indeed, the water contact angle for polySER is $\theta_c \approx 70.0 \pm 1^\circ$ Figure 4(g), smaller than the corresponding value for polyALA, $\theta_c \approx 79.2 \pm 1^\circ$ Figure 4(c). The value of θ_c of polySER is $<90^0$ and hence, it consistent with polySER α-helix being hydrophilic (although weakly hydrophilic).

3.3. *α-Helices composed of residues other than alanine and serine*

One may wonder if the active role of the polyALA and polySER carbonyl O atoms is common to other α-helices. For example, homopolymer α-helical peptides with large side chains are expected to shield the backbone from water, which is a mechanism that could provide enhanced thermal stability to α-helices [9,10,12]. To show that polyALA and polySER α-helices are not isolated cases, we perform MD simulations of 20-residue homopolymer α-helical peptides composed of VAL, ILE, LEU, MET, PHE, CYS, TYR, TRP and GLY. MD simulations are performed for 100 ns at $T = 240,\ 300$ K and $P = 0.1$ MPa. Here, we focus on the case $T = 240$ K; results at $T = 300$ K are included in the SM.

The average properties of the α-helices studied at $T = 240$ K are included in Table 7 (the corresponding tables for $T = 300$ K are included in the SM). Table 7 is divided into three groups; peptides numbered 1–6 have hydrophobic side chains while peptides numbered 7–9 have polar side chains that can form HB; polyGLY is listed separately since it is a special residue (with only a H atoms as the side chain). It follows from Table S1 of the SM that most peptides unfold, at least partially, at $T = 300$ K ($f_\alpha < 100\%$). Instead, Table 7 indicates that most α-helices maintain their structure at $T = 240$ K during the 100 ns simulation time ($f_\alpha = 100\%$).

The two main points from Table 7 are the following. (a) In all cases, the carbonyl O atoms of the peptide make HB with water with $n_{\mathrm{O-H_2O}} > 5.7$. This may not be surprising for peptides that are totally or partially unfolded (e.g. polyGLY) since, in these cases, the peptide has O atoms that are exposed to water [29]. However, even in cases where the α-helices are partially or mostly folded, ($f_\alpha \approx 100\%$ and $n_{\mathrm{O-N}} \approx n_{\mathrm{N-O}} > 9$), there are HB formed between water and the carbonyl O atoms of the α-helices; specifically, $n_{\mathrm{O-H_2O}} \approx 5.7 - 10.2$ (the maximum number of O-water hydrogen bonds is $n_{\mathrm{O-H_2O}} = 12$). These observations apply to α-helices composed of residues with both hydrophobic and hydrophilic side chains. In particular, even for the case of α-helices with large side chains, we find that water can form HB with the carbonyl O atoms. For example, $n_{\mathrm{O-H_2O}} = 6 - 7$ for the case of polyTRP and polyPHE (over a maximum of 12). Evidently, the large side chains interfere with one another and can only partially shield the backbone from water.

(b) None of the polar side chains of polyCYS, polyTYR and polyTRP form HB with the carbonyl O atoms (or any other atom of the peptide backbone), i.e. $n_{\mathrm{sc-O}} \approx 0$. While this may seem to be at odds with the results found for polySER, it is possible that the differences are due to the different size of the side chains of polyCYS, polyTYR and polyTRP. It is unclear whether other peptides composed of small polar/charged amino acids, such as threonine and asparagine, could form HB with the corresponding α-helix backbone, as found for polySER. We note that, not surprisingly, there are HB formed between water and the side chains of polyTYR and polyTRP (but not for the case of polyCYS – polyCYS is sometimes

Table 7. Average properties of different homopolymer α-helical peptides studied at $T = 240$ K and $P = 0.1$ MPa. All peptides are composed of 20 amino acids and are terminated with NH_2 and COOH in the N- and C-terminus, respectively. Properties are defined in Tables 1 and 2; number in parenthesis are the standard deviations. α-helices (1)–(6) are composed of residues with hydrophobic side chains; the size of the residue side chain increases from (1) polyALA to (6) polyPHE. α-helices (7)–(9) are composed of residues with a polar side chains; the size of the residue side chain increases from (7) polyCYS to (9) polyTRP. PolyGLY is listed separately as it is usually considered to be a special residue. Results obtained at $T = 300$ K are included in the SM.

T [K]	f_α [%]	n_{int}	$n_{\mathrm{O-N}}$	$n_{\mathrm{N-O}}$	n_{ext}	$n_{\mathrm{O-H_2O}}$	$n_{\mathrm{N-H_2O}}$	$n_{\mathrm{sc-H_2O}}$	$n_{\mathrm{sc-O}}$
(1) polyALA	100	14.62(1.01)	10.82(0.84)	11.54(0.64)	10.24(1.11)	10.18(1.10)	0.06(0.24)	0.0	0.0
(2) polyVAL	85.25	9.40(1.94)	7.22(1.55)	7.48(1.79)	7.80(1.55)	7.22(1.55)	0.12(0.32)	0.0	0.0
(3) polyILE	78.20	7.68(1.61)	6.62(1.35)	6.06(1.32)	9.24(1.26)	7.86(0.87)	1.38(0.87)	0.0	0.0
(4) polyLEU	100	14.40(0.91)	10.70(0.85)	11.42(0.69)	7.88(0.84)	7.86(0.82)	0.02(0.14)	0.0	0.0
(5) polyMET	100	12.40(1.41)	9.66(1.50)	10.14(1.02)	7.96(1.04)	7.96(1.04)	0.0	0.0	0.0
(6) polyPHE	97.38	10.84(1.75)	7.68(1.30)	7.64(1.65)	7.90(1.64)	7.36(1.34)	0.54(0.73)	0.0	0.0
(7) polyCYS	100	14.78(0.93)	11.22(0.76)	11.40(0.66)	5.74(1.43)	5.70(1.42)	0.04(0.20)	0.0	0.0
(8) polyTYR	100	12.40(1.23)	9.22(1.01)	9.14(1.04)	31.73(2.50)	6.41(1.33)	0.23(0.42)	25.10(2.04)	0.0
(9) polyTRP	100	12.46(1.31)	9.12(1.14)	9.44(0.98)	21.06(2.50)	6.04(1.26)	0.04(0.20)	14.98(2.18)	0.0
(10) polyGLY	0.0	4.50(1.33)	4.50(1.27)	1.74(0.82)	19.62(1.81)	11.72(1.55)	7.90(1.10)	0.0	0.0

All peptides are composed of 20 amino acids and are terminated with NH_2 and COOH in the N- and C-terminus, respectively. Properties are defined in Tables 1 and 2; number in parenthesis are the standard deviations.

classified as a 'special' amino acid, as for the case of polyGLY).

4. Summary and discussion

In this work, we explored the hydration of short, homopolymer α-helical peptides at different T and P, with particular emphasis on polyALA and polySER. Our results indicate that, the carbonyl O atoms of α-helical peptides are not dormant but play a relevant role in the hydration process. Specifically, in addition to forming one HB with an N-H group of the peptide backbone, the carbonyl O atoms can also form HB with water molecules or, as in the case of polySER, with the residues side chain. The number of carbonyl O-water HB depends on the nature of the residues side chain. For example, in the case of polyALA, we find that the carbonyl O atoms form an average of 0.75–1 HB with water molecules (plus 1 HB with a backbone N-H group). In the case of polySER, the carbonyl O atoms rarely form HB with water molecules and, instead, they form 1 HB with the serine side chain OH groups.

Our MD simulations of homopolymer α-helical peptides composed of hydrophobic and hydrophilic residues, other than polyALA and polySER (see Table 7), indicate that the formation of carbonyl O-water molecules in such peptides is rather general, but that they do not form HB with the residues side chain (in contrast to polySER). Interestingly, even in the case of homopolymer α-helices with large side chains (e.g. polyMET and polyTRP), we find HB formed between the peptide backbone and water molecules. Evidently, the high density of large side chains along the α-helix suppresses the 'backbone shielding effect', where the side chains protect the peptide backbone from water – backbone shielding can provide thermal stability to the α-helix [9].

In principle, the formation of HB among the carbonyl O and water molecules could affect the overall hydrophobicity/hydrophilicity of the corresponding α-helix. To explore this possibility, we also calculated the wetting of a water droplet pierced by a polyALA and polySER long α-helix. Our simulations show that the contact angle of a water droplet in contact with straight polyALA and polySER ' fibres' are $\theta_c \approx 79^0$ and $\theta_c \approx 70^0$, respectively. Surfaces are usually classified as hydrophobic if $\theta_c > 90^0$ and hydrophilic, otherwise. Hence, our results indicate that polySER is hydrophilic, as one would expect. However, they also indicate that polyALA is not hydrophobic, which is counter intuitive. Contact angles are the standard macroscopic property of water to define the hydrophobicity/hydrophilicity of flat surfaces. Such a definition has been used extensively in computational studies of nanodroplets to characterise the hydrophobicity/hydrophilicity of flat surfaces, including surfaces composed of peptide networks [28,30]. Yet, it may be possible that, somehow, θ_c is not a proper quantity to characterise the hydrophobicity/hydrophilicity of an α-helix peptide. With the available information, however, our results for θ_c should not be overlooked given the large number of HB formed between the polyALA carbonyl O and water molecules (10 of the 12 carbonyl groups of polyALA form, in average, one HB with water molecules).

We also note that the θ_c of polySER is not that small as one would expect given the OH groups in serine side chains. For example, computer simulations find that the contact angle of a water nanodroplet on an artificial, planar peptide network composed of serine residues is $\theta_c \approx 21^0$ [28]. The apparent low hydrophilicity of polySER can be explained by the formation of HB between the carbonyl O atoms and side chains OH. This phenomenon not only shields the backbone carbonyl O from water, but it also constrains the side chain OH to form only one HB with water; OH groups that are fully exposed to the solvent are expected to form an average of 2–3 HB with the water molecules. It follows that such a reduction in the number of HB that the side chain and backbone O atoms can form with water could decrease the hydrophilicity of polySER. We note that there are computer simulations showing that it is possible for polar surfaces composed of hydrogen-bonding sites to be hydrophobic [31]. Indeed, the formation of HB is not sufficient for a surface to be hydrophilic; the relative distribution of the hydrogen bonding sites on the surface and the compatibility with water's tetrahedral hydrogen bond-network, the surrounding chemical groups, among other surface properties, all play a relevant role to define the hydrophobicity/hydrophilicity of a surface [32–35].

Our findings may play an important role in understanding the thermal stability of small peptides [9] as well as self-assembly processes involving proteins and small peptides in aqueous environments [36]. These processes are driven by water-mediated interactions which depend on the hydrophobicity/hydrophilicity of the protein secondary structure elements and/or interacting peptides. Similarly, the results presented here can also be important in understanding the interactions of biomolecules and drugs (e.g. docking) with protein surfaces containing α-helices [37].

The results presented in this work should be particularly important to understand (or to spur further investigation of) the behaviour and mechanical response of peptides and proteins subjected to stretching forces (applied tension), as studied in stretching-induced protein unfolding experiments and in steered MD simulations [38–44]. For example, our finding that the backbone O atoms of α-helical peptides can form HB with water strongly suggests that the mechanical response of α-helical peptides during the stretching process can be affected if, somehow, water molecules are impeded to form HB with the peptide backbone. Indeed, experimental and computational studies probing the mechanical response of α-helices subjected to stretching forces show that the response of the corresponding peptide can depend on the pH, side chains and amino acid sequence [39–41]. We also note that computational studies performed on single stable α-helices, using implicit water models, find that the intrahelical HB breaking in small peptides occurs individually, and is not a significant barrier to the α-helix unfolding [43]. These implicit solvent studies also find that peptide deformation mechanism is highly cooperative, and that the α-helix unfolding starts from the termini [42,43]. Our results point to the need of revisiting such studies and the mechanical response of α-helices and related structures, such as coiled coils, using computer simulations with explicit water/solvent models [42,43].

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the SCORE Program of the National Institutes of Health under Award No. 1SC3GM139673 and the NSF CREST Center for Interface Design and Engineered Assembly of Low Dimensional systems (IDEALS) (NSF Grant No. HRD-1547380). This work used computational resources at San Diego Supercomputer Center (SDSC) through allocation CHE230026 from the Advanced Cyberinfrastructure Coordination Ecosystem: Services & Support (ACCESS) program, which is supported by National Science Foundation grants #2138259, #2138286, #2138307, #2137603, and #2138296 [45].

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Supplementary Material for 'Backbone Hydration of α-Helical Peptides: Hydrogen-Bonding and Surface Hydrophobicity/Hydrophilicity'

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In this supplementary material (SM), we (i) include additional water density contour (WDC) maps, (ii) properties that further characterize the hydration of the different α-helix peptides studied in the main manuscript [cylindrical distribution functions (CDF)], and (iii) the details of the calculation of the contact angle for the polyALA/polySER α-helix pierced by a water nanodroplet. Also included are (iv) the average properties of the studied homopolymer α-helical peptides at $T = 300$ K.

1 Water Density Contour Maps

The WDC maps shown in Figs. 2 and 5 of the main manuscript were calculated using cylindrical shells expanding over a distance $4 \leq r \leq 6$ Å from the polyALA/polySER α-helix axis. Here, we focus on polyALA and compare the WDC maps obtained by using cylindrical shells expanding over a distance $4 \leq r \leq 5$ Å, $4 \leq r \leq 6$ Å, and $4 \leq r \leq 7$ Å from polyALA α-helix axis; see Fig. S1.

Fig. S1(a) shows that for thin cylindrical hydration shells there are no water molecules close to polyALA except in the close proximity to the O atoms of polyALA backbone (dark green/cyan spots in the WDC map). As the cylindrical shell thickness is increased, Figs. S1(b) and S1(c), the WDC maps show an increase of water molecules covering the surface of polyALA. As mentioned in the main manuscript, the dry (light green) regions of the WDC map in Fig. S1(b)(c) correspond to the CH_3 side chains of the alanine residues while the water high-density regions of the WDC maps (blue and black spots) coincide with the location of polyALA O atoms. Fig. S1(c) shows that increasing the thickness of the cylindrical shell, does not change qualitatively the pattern observed in the WDC maps discussed. Specifically, even for a hydration shell expanding over $4 \leq r \leq 7$ Å from the α-helix axis, one can still identify the location of the alanine CH_3 side chains (cyan and dark green spots) as well as the α-helix backbone O atoms (black spots).

2 Cylindrical Distribution Functions

To characterize the thickness of polyALA first hydration layer, we include in Fig. S2 the cylindrical distribution function (CDF) of water introduced in Ref.[1] Briefly, the CDF quantifies the distribution of water molecules as function of the distance r from polyALA axis. The CDF is analogous of the radial distribution function used to characterize the structure of liquids. For example, the CDF of a uniform distribution of water molecules about polyALA would be equal to 1 at all distances from polyALA. Fig. S2 shows the total CDF of water at $T = 240$ K and $P = 0.1,\ 400,\ 700$ MPa (black lines). The first peak of the total CDF corresponds to the first hydration layer of polyALA. Depending on pressure, the first hydration shell of polyALA expands from $r = 3.5 - 4.0$ Å to $r = 6.0 - 7.5$ Å.

Also included in Fig. S2 are the CDF of water molecules with $n = 3,\ 4, \ldots 8$ nearest-neighbor oxygens [two water molecules are nearest-neighbors if their O-O distance is less than $r_c = 3.4$ Å]. Solid (dashed) lines are obtained when polyALA O atoms are (are not) taken into account when calculating the number of nearest-neighbor oxygens of a given water molecule. While at $P = 0.1$ MPa most water molecules have $n = 4$ nearest-neighbor oxygens, at higher pressures most water molecules have $n \geq 5$ nearest-neighbor oxygens. In the first hydration layer of polyALA, water molecules have $n = 3 - 5$ nearest-neighbors depending on the pressure. The number of molecules with $n = 3$ nearest-neighbor oxygens (in contact with polyALA) depends considerably on whether one considers the polyALA backbone O atoms as possible nearest-neighbors of a given water molecules (see solid and dashed green lines).

3 Contact Angle of a water droplet in contact with a polyALA/polySER α-helix

To calculate the contact angle of the water nanodroplet in contact with a polyALA or polySER α-helix, we first obtain the water density profile as a function of (r, z), $\rho(r, z)$; z and r are the cylindrical coordinates defined in Sec. 3.2 of the main manuscript where the z-axis coincides with the α-helix axis. Fig. S3(a) shows an example of the $\rho(r, z)$ calculated for the case of polyALA. To calculate $\rho(r, z)$, we divided the (r, z)-plane into a grid with cell dimensions $\delta r = 0.25$ nm and $\delta z = 0.50$ nm. $\rho(r, z)$ is the density of water in the cell located at (r, z), averaged over the last 4 ns of the MD run (400 snapshots). Fig. S3(b) and (c) are scatter plots where each point correspond to the location of a water molecule in (r, z)-plane.

From the water density profile $\rho(r, z)$, one can calculate the droplet profile and hence, the water contact angle. The droplet profile $r_{drop}(z)$ is obtained in two different ways, (i) by dividing $\rho(r, z)$ in stripes parallel to the α-helix axis [Fig. S3(b)], and (ii) by dividing $\rho(r, z)$ in stripes perpendicular to the α-helix axis [Fig. S3(c)]. The procedure is similar in both cases, as we explain next.

(i) For the *i*-th stripe perpendicular to the z-axis [Fig. S3(c)], centered at $z = z_i$, we consider the corresponding density profile of water as a function of r, i.e., $\rho(r, z = z_i)$; e. g., see Fig. S2(d). The radius r_i of the water droplet at $z = z_i$ is given by the value of r at which $\rho(r, z = z_i) = 0.20$ g/cm^3. The set of points (r_i, z_i) obtained define the droplet profile, $r^i_{drop}(z)$.

(ii) For the *i*-th stripe parallel to the r-axis [Fig. S3(c)], centered at $r = r_i$, we consider the corresponding density profile of water as a function of z, i.e., $\rho(r = r_i, z)$; e.g., see Fig. S2(e). The boundaries of the water droplet at $r = r_i$, defined as $z_{high,i}$ and $z_{low,i}$, are given by the values of z at which $\rho(r = r_i, z) = 0.20$ g/cm^3. The set of points $(r_i, z_{high,i})$ and $(r_i, z_{low,i})$ obtained define the droplet profile, $r^{ii}_{drop}(z)$.

The water drop profiles $r^i_{drop}(z)$ and $r^{drop}_{ii}(z)$ are then averaged, giving the final droplet profile $r_{drop}(z)$. Fig. S3(f) shows $r_{drop}(z)$ for the case of polyALA together with the corresponding $\rho(r, z)$. The contact angle of the water droplet is obtained by following the construction shown in Fig. S3(g). Specifically, we fit $r_{drop}(z)$ for $z > 3.2$ nm using a polynomial of the form $f(z) = Az^2 + Bz + C$. The contact angle is then defined as $\vartheta_c = \frac{dr_{drop}}{dz}\Big|_{r=0.5nm} = \pi/2 + tan^{-1}(df/dz|_{f=0.5})$. We obtain $\vartheta_c = 79.2 \pm 1^o$ for the case of polyALA and $\vartheta_c = 70.0 \pm 1^o$ for the case of polySER-helix (Fig. S4).

4 Temperature-Effects on the Hydration of single-residue α-Helices

In Table 7 of the main manuscript there are a few relevant average properties of different homopolymer α-helical peptides (polyX with X being VAL, ILE, LEU, MET, PHE, CYS, TYR, TPR, GLY, ALA) at $T = 240$ K and $P = 0.1$ MPa. The same properties of the peptides studied at $T = 300$ K are included in Table S1.

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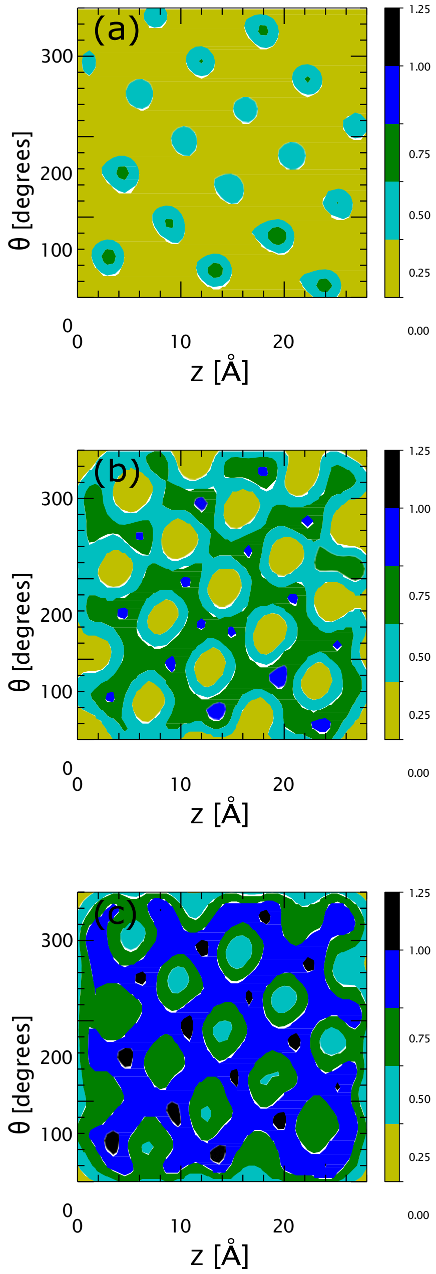

Figure S1: Water density contour maps of polyALA α-helix at $T = 240$ K and $P = 0.1$ MPa. Density contour maps are calculated in cylindrical shells expanding over a distance (a) $4 \leq r \leq 5$ Å, (b) $4 \leq r \leq 6$ Å, and (c) $4 \leq r \leq 7$ Å from the α-helix axis. The periodic spots with highest densities [see, e.g., (a)] correspond to the location of the O atoms of polyALA backbone.

Table S1: Same as Table 7 of the main manuscript for the case T = 300 K. Average properties of different single-residue α-helices studied at T = 300 K and P = 0.1 MPa. All peptides are composed of 20 amino acids and are terminated with NH_2 and COOH at the N- and C-terminus, respectively. Properties are defined in Table 7 of the main manuscript; number in parenthesis are the standard deviations. Alpha helices (1)-(6) are composed of residues with hydrophobic side changes; the size of the residue side chain increases from (1) polyALA to (6) polyPHE. Alpha helices (7)-(9) are composed of residues with a polar side changes; the size of the residue side chain increases from (7) polyCYS to (9) polyTRP. PolyGLY is listed separately as it is usually considered to be a special residue.

T [K]	f_α [%]	n_{int}	n_{O-N}	n_{N-O}	n_{ext}	n_{O-H_2O}	n_{N-H_2O}	n_{sc-H_2O}	n_{sc-O}
(1) polyALA	96.80	9.78(1.87)	8.76(1.61)	7.98(1.58)	12.26(1.76)	10.32(1.40)	1.94(0.95)	0.0	0.0
(2) polyVAL	58.30	7.86(1.88)	5.98(1.61)	6.40(1.68)	8.64(1.83)	8.20(1.65)	0.44(0.57)	0.0	0.0
(3) polyILE	81.40	8.12(1.88)	6.44(1.67)	5.52(1.50)	9.02(1.46)	7.26(1.21)	1.76(0.81)	0.0	0.0
(4) polyLEU	98.72	11.10(1.53)	9.12(1.32)	8.72(1.11)	7.24(1.68)	9.12(1.32)	0.86(0.60)	0.0	0.0
(5) polyMET	30.15	11.10(1.65)	5.56(1.31)	5.06(1.32)	10.72(2.13)	8.76(1.84)	1.96(0.85)	0.0	0.0
(6) polyPHE	64.10	10.62(1.75)	7.48(1.40)	7.82(1.40)	7.60(1.80)	6.62(1.44)	0.98(0.81)	0.0	0.0
(7) polyCYS	94.80	11.06(1.02)	9.06(0.81)	9.44(1.00)	6.94(1.40)	6.32(1.33)	0.62(0.63)	0.0	0.0
(8) polyTYR	87.85	11.18(1.66)	8.34(1.38)	8.30(1.30)	27.88(2.95)	5.50(1.32)	0.28(0.49)	22.10(2.19)	0.0
(9) polyTRP	100	13.70(1.45)	10.0(1.04)	10.08(1.25)	15.02(2.82)	3.86(1.17)	0.06(0.24)	11.10(2.24)	0.0
(10) polyGLY	0.0	0.70(1.09)	0.46(0.98)	0.44(0.73)	26.20(2.84)	17.48(2.36)	8.72(1.40)	0.0	0.0

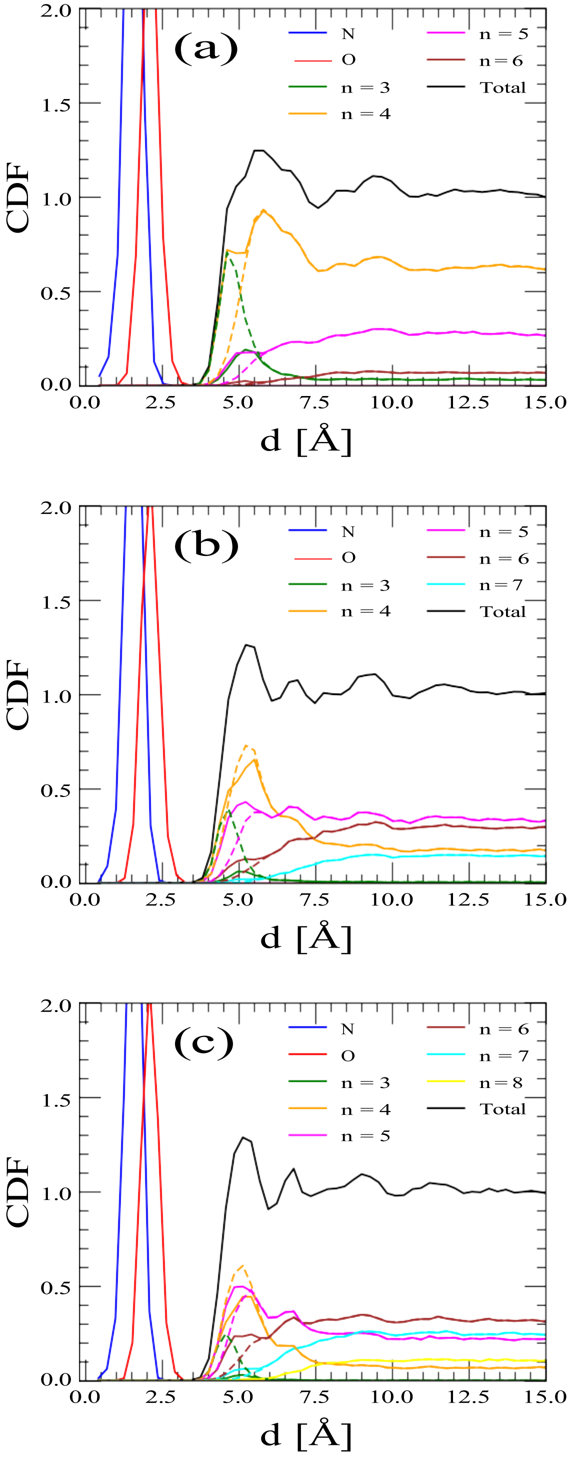

Figure S2: Cylindrical distribution functions of water surrounding the polyALA α-helix at T = 240 K and (a) P = 0.1 MPa, (b) P = 400 MPa, and (c) P = 700 MPa. CDFs are calculated considering all water molecules in the system (total CDF), and for water molecules with $n = 3 - 8$ nearest-neighbor oxygens. Solid (dashed) lines are the CDFs obtained when the O atoms of polyALA backbone are included (excluded) in the calculation of water molecules nearest-neighbor oxygens. For comparison we also include the CDF for polyALA O and N atoms.

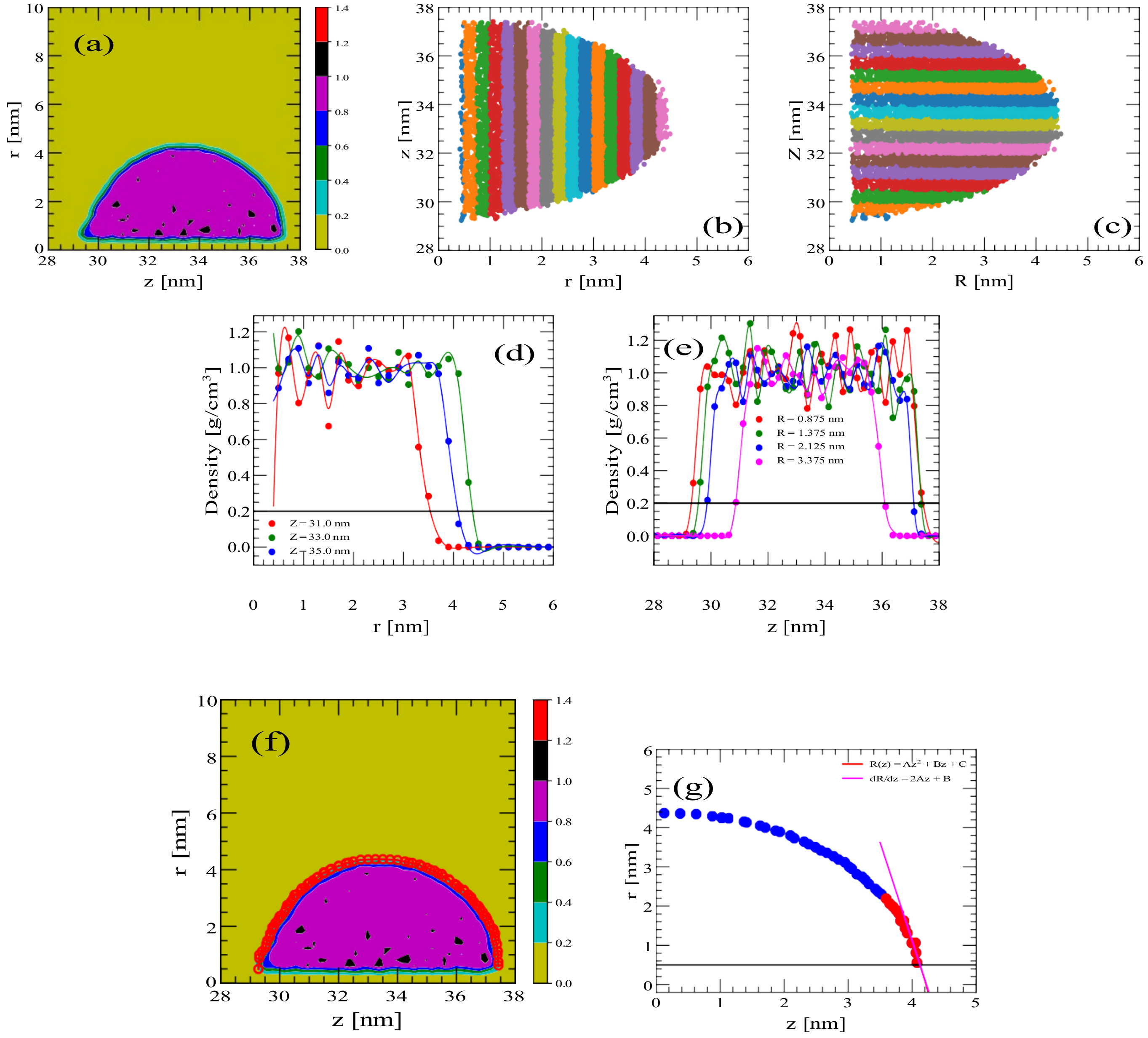

Figure S3: (a)Water density $\rho(r, z)$ map of polyALA α-helix at T = 300 K and P = 0.1 MPa averaged over the last 4 ns of the MD simulation. (b) Scatter plot for one snapshot showing the distribution of water droplet molecules around polyALA. Colors correspond to the stripes parallel to the z-axis used to calculate the droplet profile (see text; δr = 0.25 nm). (c) Same as (b) with the colors corresponding to the stripes perpendicular to the z-axis used to calculate the droplet profile (see text; δz = 0.5 nm). (d) Selected density profiles along stripes indicated in (c). (e) Selected density profiles along stripes indicated in (b). (f) Water droplet profile (red circles) along with the corresponding water density map, $\rho(r, z)$. (g) Construction used to calculate the contact angle ϑ_c of the water droplet with polyALA (see text); ϑ_c = 79.2 ° for the case of polyALA.

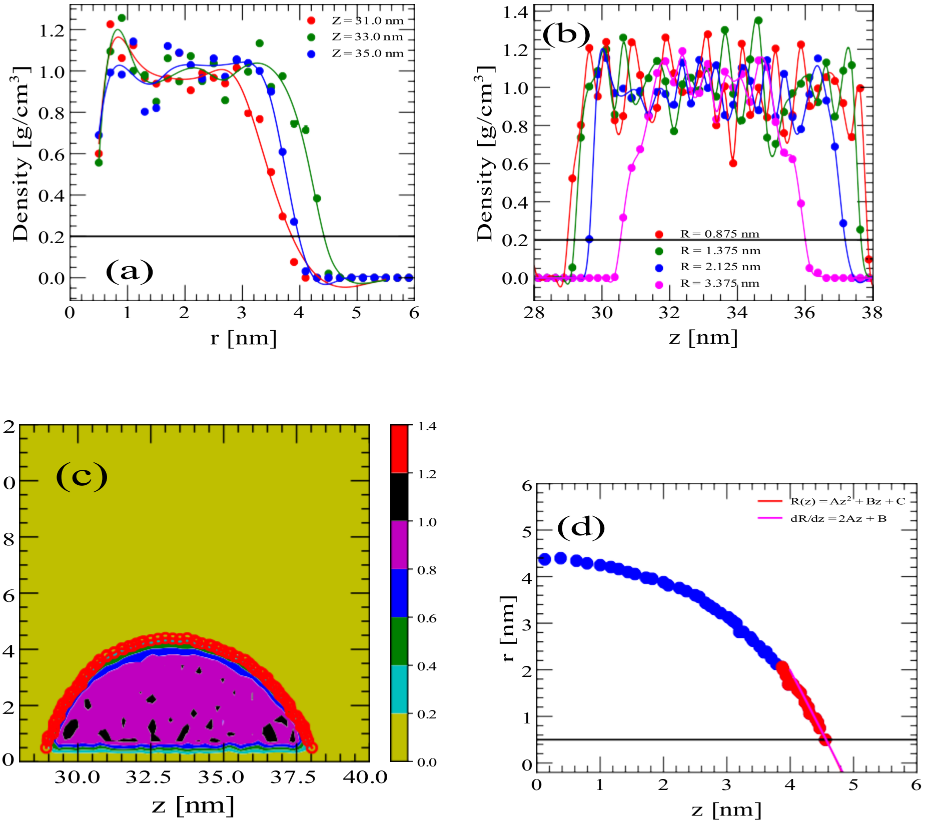

Figure S4: Same as Fig. S3(d)-(g) for the case of polySER. ϑ_c = 70.0 ∘ for polySER.