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Hydration of non-polar anti-parallel β -sheets

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In this work we focus on anti-parallel β -sheets to study hydration of side chains and polar groups of the backbone using all-atom molecular dynamics simulations. We show that: (i) water distribution around the backbone does not depend significantly on amino acid sequence, (ii) more water molecules are found around oxygen than nitrogen atoms of the backbone, and (iii) water molecules around nitrogen are highly localized in the planed formed by peptide backbones. To study hydration around side chains we note that anti-parallel β -sheets exhibit two types of cross-strand pairing: Hydrogen-Bond (HB) and Non-Hydrogen-Bond (NHB) pairing. We show that distributions of water around alanine, leucine, and valine side chains are very different at HB compared to NHB faces. For alanine pairs, the space between side chains has a higher concentration of water if residues are located in the NHB face of the β -sheet as opposed to the HB face. For leucine residues, the HB face is found to be dry while the space between side chains at the NHB face alternates between being occupied and non-occupied by water. Surprisingly, for valine residues the NHB face is dry, whereas the HB face is occupied by water. We postulate that these differences in water distribution are related to context dependent propensities observed for β -sheets. © 2014 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4871663>]

I. INTRODUCTION

β -sheets¹ are prominent structural templates that accommodate 20%–28% of all amino acids in globular proteins² and they are the building blocks of cross- β structures.^{3–6} Adding to their importance are experimental results suggesting that all amino acid sequences under appropriate denaturing conditions form cross- β structures.^{7,8} In recent years, tremendous progress has been gained in our understanding of the driving forces, stability, and mechanisms of β -sheet formation.^{9–13} However, this progress has not been translated yet into better predictive algorithms of β -sheet structures. One main reason is that propensity scales of β -sheet structures are strongly context dependent and the variables of this dependence are not clearly understood.¹⁴ This contrasts with propensity scales for α -helices which are transferable to different contexts.¹⁵ Understanding how propensity scales emerge from atomic interaction is important to design new β -sheet prone peptides and to understand diseases resulting from misfolding and aggregation.^{16,17} To shed light into the context dependence of β -sheets, we study here the distribution of water molecules around anti-parallel β -sheets made of model peptides.

A lot of effort has been devoted to study solvation of compounds that mimic chemical groups in proteins. For example, several studies have focused on methane and dimethylacetamide to unravel molecular mechanisms responsible for hydrophobic interactions and intra-backbone hydrogen bonding in proteins, respectively.^{18–22} Solvation of peptides has also received a lot of attention.^{23–28} In particular, computer simulations have found that compared to bulk water the first

solvation shell around peptides is more rigid, its translational and rotational diffusion coefficients are smaller, while its density and water-water hydrogen bond lifetime are larger.^{23,25} These results are in agreement with experiments reporting slower correlation times for hydration water compared to bulk water.

Water hydration and its properties depend on peptide conformation. Characterizing solvation for all peptide structures is a daunting and expensive task. However, it is reasonable to study solvation for key peptide structures, e.g., around α -helix, β -sheet, and unfolded conformations. In α -helices made from alanine residues, adjacently spaced C_β groups are not close enough to form van der Waals contact while the space left between these groups is also not enough to accommodate water molecules.³⁰ As a result, small cavities are found between C_β groups. When rationalized using a methane-dimer models,^{18,19} these side chain configurations can be mapped to desolvation barriers.^{31,32} Moreover, since desolvation barriers contribute unfavorably to the free energy^{20,29} and they become more prominent with increasing pressure,^{32–34} it was suggested that pressure could be used to destabilize α -helices. By contrast, water mediated interactions were shown to favor β -strands.^{31,32} This could rationalize the tendency of some amyloid peptides to assume β or coil like structures in water and α -helix conformations in membrane-like environments.^{35,36}

The energetic principles of packing preformed secondary structures are also related to hydration. For example, the presence of cavities in the packing of α -helices was related to desolvation barriers which account for enthalpic penalties in the system.³⁷ Furthermore, computer simulations showed that water mediated interactions can drive the assembly of

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parallel β -sheets made from amyloid β peptides ($A\beta_{16-22}$).³⁸ This result was latter corroborated by experiments.³⁹ In studies of peptide addition onto a preformed fibril, water expulsion was shown to occur late in the assembly process and cooperatively over a very short period of time—suggesting that dewetting is an essential process in stabilizing amyloid fibrils.⁴⁰ The energetics of β -sheet formation from preformed β -strands was studied recently.⁴¹ For model peptides made of residues containing large aliphatic side chains (valine and leucine), the main stabilizing energy was related to the transfer of water molecules from the vicinity of the strands to bulk water. By contrast, for peptides made of small side chains van der Waals interactions involving atoms of the protein were shown to be a main force accounting for the formation of β -sheets. Non-optimized packing of strands was also related to enthalpic penalties.⁴¹

Taken together, the above mentioned studies suggest that energies related to both desolvation and the transfer of shell water to bulk water can drive packing of secondary structures and determine preferences for a given peptide conformation over others. Here we focus on solvation around side chains and polar groups of the backbone in anti-parallel β -sheets. With respect to solvation around polar groups of the backbone we show that: (i) water distribution around the backbone does not depend significantly on amino acid sequence, (ii) more water molecules are found around oxygen than nitrogen atoms of the backbone, and (iii) water molecules around nitrogen are localized in the plane formed by peptide backbones. To study solvation around side chains, we note that anti-parallel β -sheets can exhibit two types of cross-strand pairing: Hydrogen-Bond (HB) and Non-Hydrogen-Bond (NHB) pairing.^{13,42} In the HB pairing two hydrogen bonds form between both residues of the pair, whereas carbonyl and amine groups in NHB pairing form hydrogen bonds with water—see Fig. 1. As a result, side chains are closer to each other in NHB pairings compared to HB.^{31,32,43} Figure 1 shows that all side chains forming HB pairs are on one side of the β -sheet, while side chains forming NHB pairs are on other side. We name the former side of a β -sheet HB face and the later NHB face.

We show here that distributions of water around alanine, leucine, and valine side chains are very different at HB compared to NHB faces. For alanine pairs, the space between side chains has a higher concentration of water if residues are located in the NHB face of the β -sheet as opposed to the HB face. For leucine residues, the HB face is found to

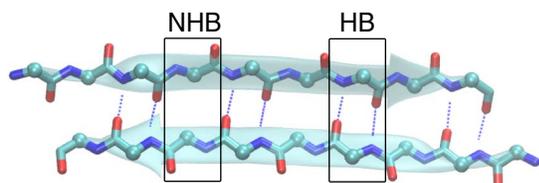


FIG. 1. Non-Hydrogen-Bond (NHB) and Hydrogen-Bond (HB) pairs of an anti-parallel β -sheet. C_{α} atoms are represented by spheres, oxygen, and nitrogen atoms are drawn in red and blue, respectively. Hydrogen bonds are represented by blue dotted lines. Notice that 14 and 10 backbone atoms are enclosed within the hydrogen bond network in NHB and HB pairs, respectively.¹³

be dry, while the space between side chains at the NHB face alternates between being occupied and non-occupied by water. Surprisingly the opposite trend is observed for valine: the NHB face is dry, whereas the HB face is occupied by water. We speculate that these differences in water distribution are related to context dependent propensities in β -sheets.

II. METHODS

In this work, we simulate two infinite homepeptides held apart from each other by a spring (spring constant of 5 000 kJ/mol/nm²) at a distance of $\xi = 0.5$ nm. Each of the homodimeric peptides, which are ten amino acids long, are made infinite through periodic boundaries in the z-direction: the carbonyl-group of residue 1 is attached to the amine-group of residue 10. The two peptides are placed in anti-parallel orientations resulting in β -sheet conformations. Three homodimeric peptide systems composed of alanine, valine, and leucine residues, respectively, were used. The use of infinite chains eliminates effects from chain ends and all amino acids become equivalent, resembling amino acids in the middle of a strand. A potential constraint of this setup is that it does not allow the formation of twists which have been shown to affect the stability of β -sheets through increased side chain interactions.^{44,45}

Peptides are immersed in a box of ~ 5500 water molecules (TIP4P) and a negative pressure is applied along z-direction (main axis of the peptides) to keep them stretched at an average distance of 3.5 nm. A positive pressure of 1 atm is applied along x and y directions to account for water density at ambient pressure. Simulations are carried out using GROMACS and CHARMM27 forcefield. Temperature and pressure were controlled using the velocity-rescale thermostat ($\tau_T = 1$ ps) and the Parrinello–Rahman barostat ($\tau_P = 1$ ps), respectively. Simulations were performed with a time-step of 2 fs and the neighbor list was updated every 10 steps. The first 10 ns of simulations were used to equilibrate the system, while statistics were gathered during the next 90 ns. Electrostatics were treated by the smooth particle mesh Ewald with a grid spacing of 0.13 nm and a 1.3 nm real-space cutoff.⁴⁶ To compute spatial distribution functions of water we use the `g_spatial` package provided with GROMACS in which we divided our simulation box in bins of size 0.01 nm and 0.02 nm for alanine and leucine/valine systems, respectively. Spatial distribution functions are given in units of the ratio of the density of water in the simulation and the density of water of an ideal fluid for each cell.

III. RESULTS

A. Solvation of polar groups of the backbone

In Fig. 2(a) we show the Radial Distribution Function (RDF) of O_w (oxygen atom of water) around solvent exposed N_b (backbone nitrogen), H_N (hydrogen atom covalently bonded to N_b), and O_b (carbonyl oxygen atom) for peptides made of alanine residues. The first solvation shell around O_b is well defined exhibiting a minimum at 3.5 Å. In this first shell, O_b is surrounded by an average of 2.32 water

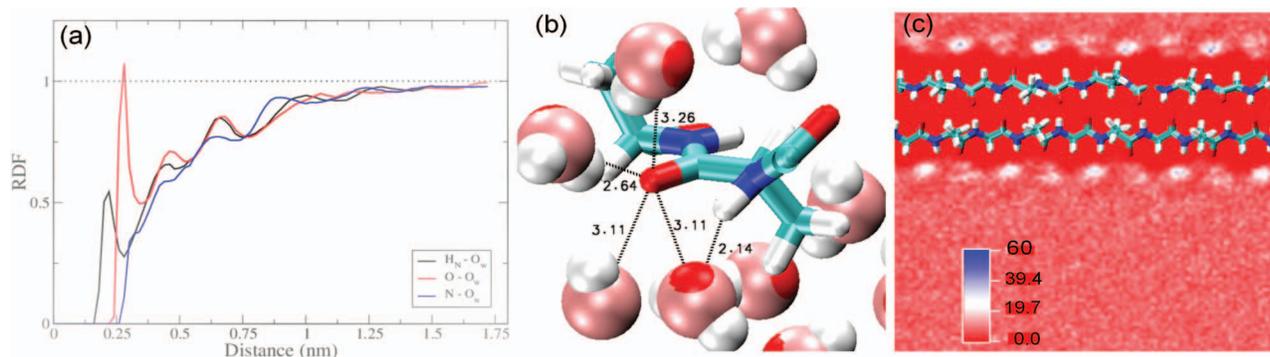


FIG. 2. (a) Radial distribution function of water around nitrogen (blue line), hydrogen (black), and oxygen (red) atoms of the backbone which are exposed to the solvent for dimers made of alanine residue. (b) Representative configuration illustrating the distribution of water molecules around backbone nitrogen (blue) and oxygen (red). Distances of water to backbone atoms are represented by dotted lines and they are given in Angstrom. (c) Spatial distribution function of water in the plane formed by the two peptides.

molecules with which it can form hydrogen bonds. This large number of neighbors is possible because O_b is hydrogen-acceptor, implying that when it binds to water molecules, O_w has freedom to orient itself. As a result, several water molecules can surround the backbone while maintaining their partial negative O_w at a fair distance from each other (see Fig. 2(b)).

To understand the distribution of water around amine groups of the backbone, we show in Fig. 2(a) the RDF of O_w around nitrogen and hydrogen atoms. Water molecules are found at all distances around N_b without forming a distinct first shell. By contrast, the first hydration shell around H_N accommodates 1.66 water molecules on average and its minimum occurs at a distance of 2.88 Å. Nitrogen is hydrogen-donor implying that it forms directional hydrogen bonds with O_w . As a result, O_w has reduced freedom to orient itself around H_N impairing the ability of the amine group (NH atoms) to have several partially charged O_w atoms as neighbors. In addition O_w found at close proximity of H_N (~2.7 Å) are also within hydrogen bond range to backbone oxygen—see Fig. 2(b). Hence water molecules close to amino groups are expected to be more localized in space. In Fig. 2(c), we

show the spatial distribution function of water in the plane formed by the two peptides. This distribution confirms an important concentration of water facing nitrogen atoms in this plane. This concentration is more pronounced than the one facing oxygen atoms because water around backbone oxygen is not localized in a plane but surrounds it.

To measure how side chain size affects the distribution of water around polar groups of the backbone we compare in Fig. 3 RDFs for the three dimeric peptides studied in this work. These functions show a similar distribution of water at the first shell around the backbone: positions and intensities are the same for all three dimers at the first peak of the RDF. However, the first minimum in the RDF is more pronounced for leucine and valine compared to alanine. This is particularly noticeable for the RDF around N_b : for alanine dimers this minimum is barely noticeable while for leucine and valine it is well defined. This can be rationalized by the extent with which water penetrates the space between side chains. In β -sheets formed by alanine residues, water can penetrate in between side chains—see molecule *a* in Fig. 4. These “penetrated” water molecules occupy distances close to the first

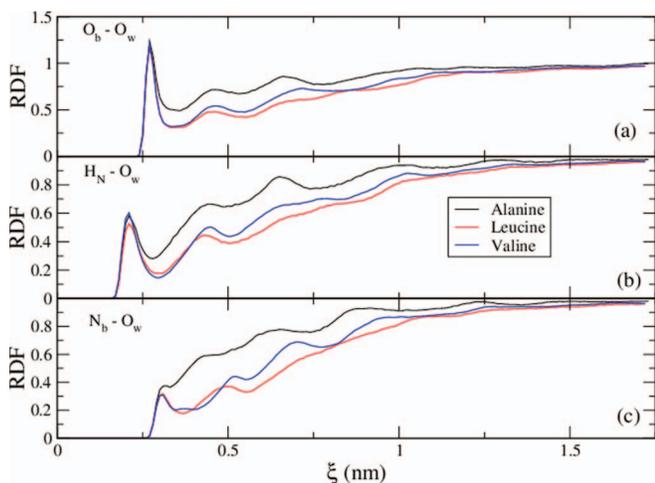


FIG. 3. Radial distribution function of O_w around carbonyl oxygen atoms (a), hydrogen atom covalently bonded to nitrogen (b), and nitrogen atom (c) for alanine (black), leucine (red), and valine (blue).

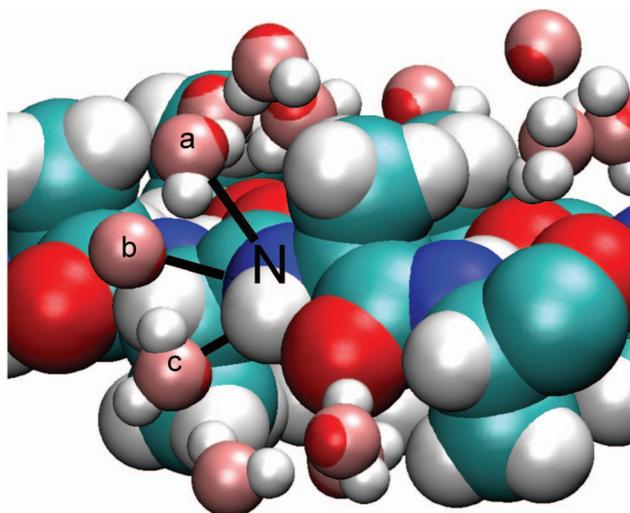


FIG. 4. Characteristic structure showing water distribution around exposed nitrogen atom. O_w atoms with index a, b, and c in the figure are at a distance of 3.55 Å, 4.15 Å, and 3.27 Å from N_b .

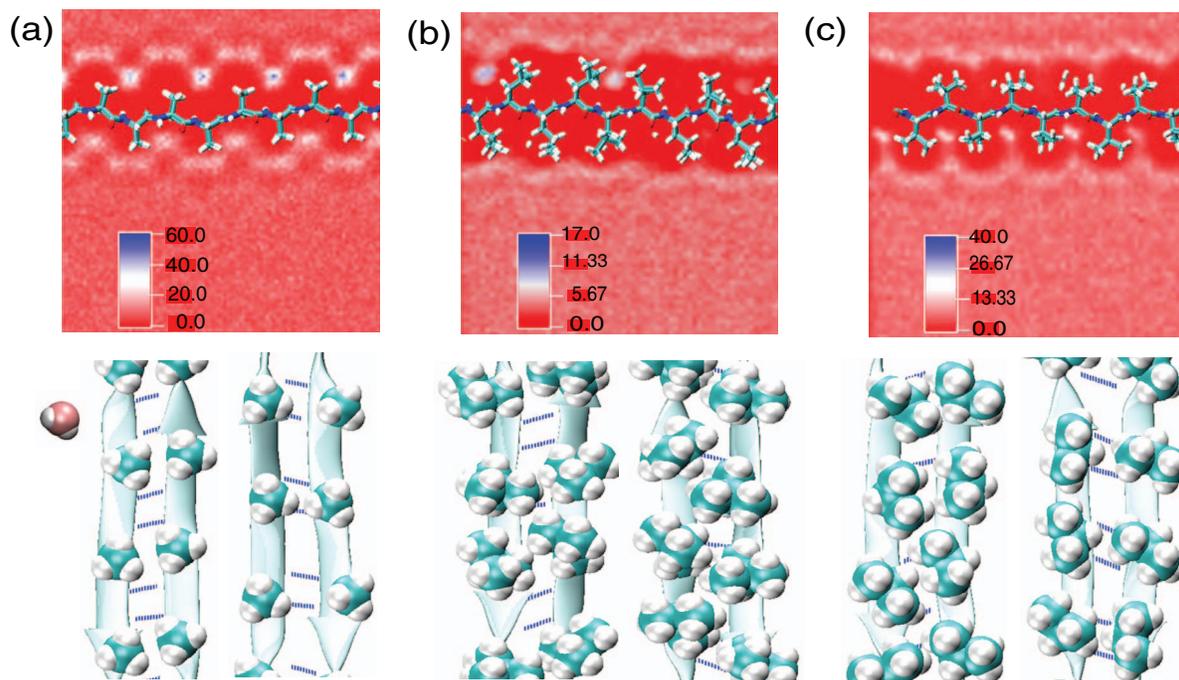


FIG. 5. (Top) Spatial distribution function of water in the plane perpendicular to the two peptides and located midway between them for (a) alanine, (b) leucine, and (c) valine. NHB and HB side chains are pointing to the top and bottom of the page, respectively. Different color scales are used for alanine, leucine, and valine. (Bottom) NHB (left) and HB (right) side chains for (a) alanine, (b) leucine, and (c) valine. Hydrogen bonds are represented by dotted blue lines.

minimum of the RDF for N_b which is located at $\xi_m = 3.3 \text{ \AA}$. By contrast, along β -strands water can not penetrate between side chains of valine and leucine residues—see Fig. 5. This accounts for pronounced minima in RDFs of valine and leucine (Fig. 3) which is absent in RDFs of alanine.

B. Solvation of side chains

Upper panels in Figure 5 illustrate the spatial distribution function of water at HB and NHB faces of the peptide. Distributions of water around HB side chains are clearly not symmetric compared to distributions around NHB ((below and above) peptides in Fig. 5). For alanine we observe a high concentration of water at the NHB face. These water molecules are localized in the space between side chains just above the exposed polar groups of the backbone. In the HB face of alanine, water molecules are less localized. For leucine, the space between adjacent NHB side chains alternates between being occupied and non-occupied by water, whereas the HB face of leucine is not solvated. Surprisingly, valine has an opposite behavior. Its NHB face is dry while its HB face is solvated.

To rationalize these asymmetries in hydration we show characteristic configurations of the peptides at NHB (left) and HB (right) faces in the lower panels of Fig. 5. Alanine's side chain is small and at both NHB and HB faces water can penetrate in the space between side chains. However, at the NHB face polar groups of the backbone are exposed to water creating a more favorable environment for the solvent compared to the HB face for which polar groups are protected by side chains. This rationalizes the high presence of water at NHB faces in alanine peptides.

The more extended side chain of leucine (Fig. 5(b)) allows the formation of contacts not only between residues i and j that are facing each other but also between i and $j \pm 2$. At the NHB face, residues i and j are close to each other and they are naturally in contact (distance between C_β atoms is 3.9 \AA ^{31,43}). Additional stability can be gained with C_γ and C_δ side chain atoms forming a dry hydrophobic core involving residues i , $i + 2$, j , and $j + 2$. However, to form these long range contacts (distance between C_β atoms of side chains i and $i+2$ is $\sim 7 \text{ \AA}$ ³²), all side chain atoms of residues i , $i + 2$, j , and $j + 2$ have to commit to the dry core at the expense of breaking bonds between residues $i, i - 2$, and $j, j - 2$. This rationalizes the pattern observed in the spatial distribution function of leucine at the NHB face (Fig. 5(b)) which alternates between being solvated and dry. At the HB face, residues i and j are further apart (distance between C_β atoms is $\sim 5.6 \text{ \AA}$ ^{31,43}) and the formation of contact requires contribution from C_γ and C_δ side chain atoms. This leads to the side chain zigzag shaped pattern in Fig. 5(b) which impedes water from penetrating the space between side chains.

Side chains of valine are shorter compared to leucine. As a result, valine cannot form strong long range interactions which impairs closed packed configurations as the ones observed for leucine. At the NHB face where residues i and j are close to each other and C_β atoms are naturally in contact, side chains cooperate to interact with $i \pm 2$ and $j \pm 2$. This accounts for a pattern where side chains are stretched along the axis of the β -sheet. In contrast at the HB face where residues i and j are farther apart, side chains of residue i have to point towards residue j to form contact. The side chain pattern that emerges at the HB face has enough empty space to allow the

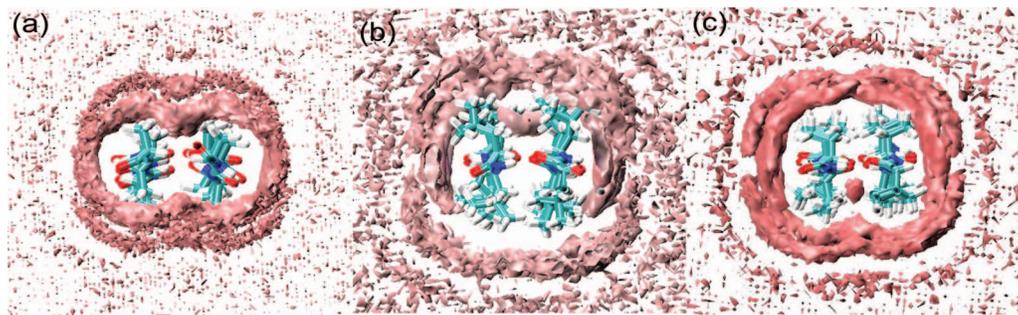


FIG. 6. Isosurface of water around β -sheets made of (a) alanine, isovalue of 14.65, (b) leucine, isovalue of 5.37, and (c) valine, isovalue of 9.10. NHB and HB side chains are pointing to the top and bottom of the page, respectively.

presence of water and this rationalizes the spatial distribution function in Fig. 5(c).

Three main factors account for the penetration of water in the space between side chains: (i) formation of side chain patterns to maximize the amount of contact between hydrophobic side chains; (ii) the presence of exposed polar groups of the backbone at NHB faces which accounts for increased peptide-water attraction; and (iii) steric restraints.

In Fig. 6, we illustrate the solvation asymmetry at HB and NHB faces by plotting the iso-surface of water density at a cross-section along the axis of the β -sheet. Compared to Fig. 5 this new plot gives a better three-dimensional perspective of the distribution of water around peptides. For alanine and leucine, we observe a higher concentration of water in space between sidechains at the NHB face compared to the HB one. By contrast, the NHB is dry for valine while water solvates HB side chains.

IV. CONCLUSION

In this work we studied the distribution of water around anti-parallel β -sheets. We find that more water molecules can be accommodated in the first shell around oxygen than around nitrogen atoms of the backbone. This result can be explained by taking into account the hydrogen donor and acceptor nature of nitrogen and oxygen atoms, respectively, as well as the directionality of the hydrogen bond interaction. Because of this directionality, water molecules around nitrogen atoms are more localized in the plane formed by the backbone of the two peptides. We show that changing side chain size from valine to leucine does not have a strong effect on the first hydration shell around polar backbone atoms. However, in the case of alanine water can penetrate in the space between its side chains affecting the first minimum of the RDF. Distributions of water around alanine, leucine, and valine side chains depend strongly if these amino acids are hydrogen-bonded or non-hydrogen-bonded to each other. For alanine pairs, the space between side chains will have a higher concentration of water if they are located in the NHB face of the β -sheet as opposed to the HB face. For leucine residues, the HB face is found to be dry while the space between side chains at the NHB face alternates between being occupied and non-occupied by water. For valine residues, the NHB face is dry, whereas the HB face is occupied by water. Driving forces accounting for these differences are discussed in the text.

Around non-polar solutes, water prefers to adopt a reduced number of states that are optimized for hydrogen bonding and, thus, have low energy. This reduced number of conformations accounts for a low entropy that is responsible for the unfavorable solvation free energy of non-polar solutes.⁴⁷ Moreover, pairing of non-polar solutes reduces the solvent accessible surface area which leads to a decrease in the solvation free energy. This is known as the hydrophobic interaction. Here, we found that hydrophobic pairings of alanine-alanine residues in NHB faces are more exposed to the solvent when compared to pairings at HB faces. Idem for leucine-leucine pairing. Thus, our results suggest that alanine-alanine and leucine-leucine pairs at solvent exposed HB faces are more favorable than their counterparts in solvent exposed NHB faces. The opposite trend is expected for valine. Extension of the current work to other amino acid pairs and to include free energy calculations⁴¹ could be used to rationalize propensity scales for β -sheet formation. For example, it is still puzzling why mutations of an alanine-alanine pair at a solvent exposed HB face to an alanine-leucine pair is unfavorable ($\Delta\Delta G = -0.24$ kcal/mol), whereas mutations to an alanine-valine pair is found to be favorable ($\Delta\Delta G = 0.17$ kcal/mol).¹⁴

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