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Alpha- and beta-polypeptides show a different stability of helical secondary structure

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Abstract—β-Polypeptides are known to adopt helical secondary structure in organic solvents, even for rather short chain lengths. It is investigated whether a short α-polypeptide with amino-acid side chains that enable β-peptides to adopt helical structures, can maintain or adopt stable helical structure in methanol or in water. The molecular dynamics simulations do not predict a particular fold, which indicates an essential role for the additional methylene moiety in the backbone of β-peptides regarding helix stability.

1. Introduction

In aqueous solution proteins, that is, long polypeptide chains of a particular composition of α-amino acid residues, generally adopt a specific tertiary structure or fold. Shorter α-polypeptides in the range of 10–30 residues may adopt secondary structure, such as α-helices or β-sheets, but their fold generally becomes less stable the shorter the polypeptide chain. In contrast, polypeptides made up of β-amino acids are known to adopt rather stable helical or β-sheet structures even for very short chain lengths of 4–7 residues, in particular when solvated in an organic solvent such as methanol.[1,2] Accordingly, short β-polypeptides are suitable molecules to investigate polypeptide stability and folding mechanism.[3–5] Yet, one may ask why short α-polypeptides do not adopt stable secondary structure. Is this due to their different backbone composition compared to β-peptides, or to the solvation effects of water compared to methanol, or to differences in side-chain sequences of the α- and β-polypeptides studied experimentally? Here, we address this question through molecular dynamics (MD) simulation of a 7-residue α-peptide (Val, Ala, Leu, Aib, Ile, Met, Phe) solvated both in methanol and in water. The α-amino acid composition has been proposed by our colleagues Jaun and Seebach in analogy to the β-amino acid sequence (Val, Ala, Leu, di-Ala, Val, Ala, Leu) of a 7 residue β-peptide that exhibits a rather stable 3_{14}-helical fold in methanol.[6] In this β-heptapeptide all side chains are at the β-carbon and the central residue is substituted with a methyl group at the α-carbon in addition. In order to facilitate the interpretation of NMR spectra to be measured all residues were chosen to be different. The central Aib residue should promote helix formation.[7] We investigate whether this α-heptapeptide that was designed to adopt a helical fold, will indeed maintain or adopt an α-helical conformation in methanol or in water.

The α-heptapeptide has been simulated for 26 ns in methanol and for 7.8 ns in water, both starting from an ideal α-helical initial structure and starting from a wholly extended backbone structure (Fig. 1). Because of the higher density of interacting atoms, the simulation in water is three

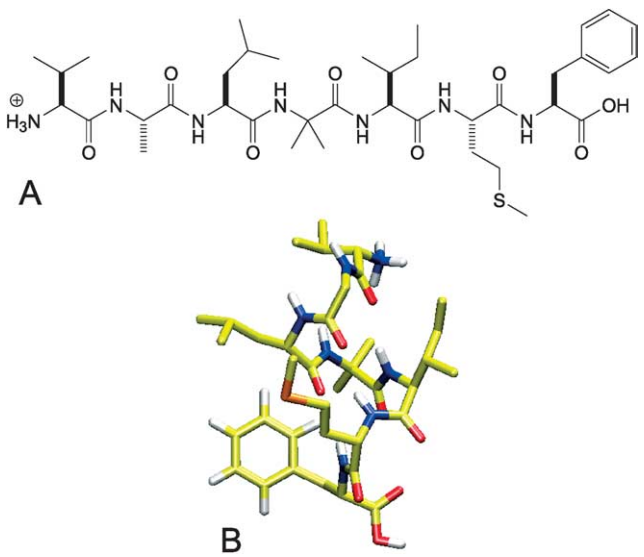

Figure 1. Panel A. Chemical formula of the α-heptapeptide studied. Panel B. α-Helical conformation of the peptide.

Keywords: Alpha-peptide; Beta-peptide; Molecular dynamics simulation; Peptide folding; Conformation analysis.
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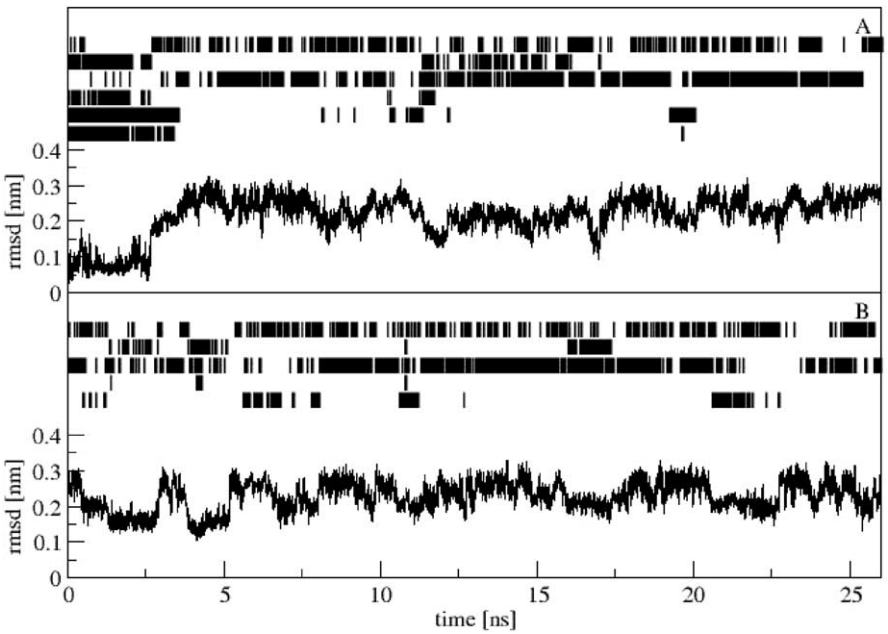

Figure 2. MD simulations of the α-heptapeptide in methanol starting from an α-helical initial conformation (Panel A) and from an extended initial conformation (Panel B). The evolution of all intramolecular hydrogen bonds with an occurrence larger than 5% is displayed together with the backbone (atoms N, C_α, C, O of residues 2–6) atom-positional root-mean-square deviation of the trajectory structures from an ideal α-helical structure. The hydrogen bonds are from top to bottom: NH(4)–O(2), NH(5)–O(2), NH(5)–O(3), NH(6)–O(2), NH(6)–O(3) and NH(7)–O(3).

to four times more expensive than that in methanol. This is why the water simulations are rather short compared to the methanol ones. The simulations were carried out using the GROMOS biomolecular simulation software[8,9] and the GROMOS biomolecular force field with parameter set 45A3.[8,10] This force field has been shown to accurately reproduce folding equilibria of a number of α- and β-polypeptides in agreement with experimental NMR data, as a function of amino-acid composition and solvent composition.[4,11–21] Therefore, one may expect this force field to be able to correctly predict the folding equilibrium of the α-heptapeptide, in methanol as well as in water.

2. Results

In Figure 2 the root-mean-square difference (rmsd) between the trajectory structures of the MD simulations of the α-heptapeptide in methanol and an ideal α-helical model structure is shown as a function of time together with the occurrence of intra-solute hydrogen bonds that occur for more than 5% in the simulations. Starting from an α-helical initial structure (Panel A), the helix is lost after 2.5 ns and is not formed again over the remaining 23.5 ns. Starting from an extended initial structure (Panel B), no α-helix is formed within 26 ns, although the molecule comes close after about 4 ns (rmsd 0.10 nm). As can be seen from Table 1, both simulations in methanol show the same dominant hydrogen bonds, which indicates a reasonable degree of convergence of the simulations. However, no NH(*i*)–O(*i*-4) hydrogen bonding (with residue number *i*=5, 6 or 7) characteristic for an α-helix is observed. This is also true for the corresponding simulations of the α-heptapeptide in water. Starting from an α-helical initial structure (Fig. 3A), the helix is only maintained for 1.5 ns and is not formed again in the remaining 6.3 ns. Starting from an extended initial structure (Fig. 3B), no helix is formed within 7.8 ns. We note that the hydrogen bond patterns observed in both water simulations are rather different, which indicates that 7.8 ns is not sufficient to sample the conformational ensemble of the solute in water at 300 K and 1 atm.

Table 1. Occurrence (%) intramolecular hydrogen bonds

Simulation initial structure	In methanol		In water	
	Helical	Extended	Helical	Extended
NH(4)–O(2)	8	4	3	1
NH(5)–O(2)	5	3	21	15
NH(5)–O(3)	30	26	12	38
NH(6)–O(2)	3	1	6	0
NH(6)–O(3)	12	7	17	0
NH(7)–O(1)	0	0	1	16
NH(7)–O(3)	6	0	5	0

Only hydrogen bonds occurring in more than 5% of the analysed conformations of a simulation have been considered. The residue sequence numbers of the atoms are indicated in parentheses. A hydrogen bond is considered to exist when the donor–hydrogen–acceptor angle is larger than 135° and the hydrogen–acceptor distance is smaller than 0.25 nm.

The conformational space that is sampled in a MD simulation and the degree of conformational overlap between two ensembles or simulation trajectories can be analysed using conformational cluster analysis. Such an analysis groups the structures of a trajectory or of combined trajectories into clusters of similar structures according to the atom-positional rmsd-value of the backbone (N, C_α, C, O) atoms (excluding the first and last residue) between pairs of structures from the trajectory or combined trajectories. In Figure 4 the results of the cluster analysis of the combined

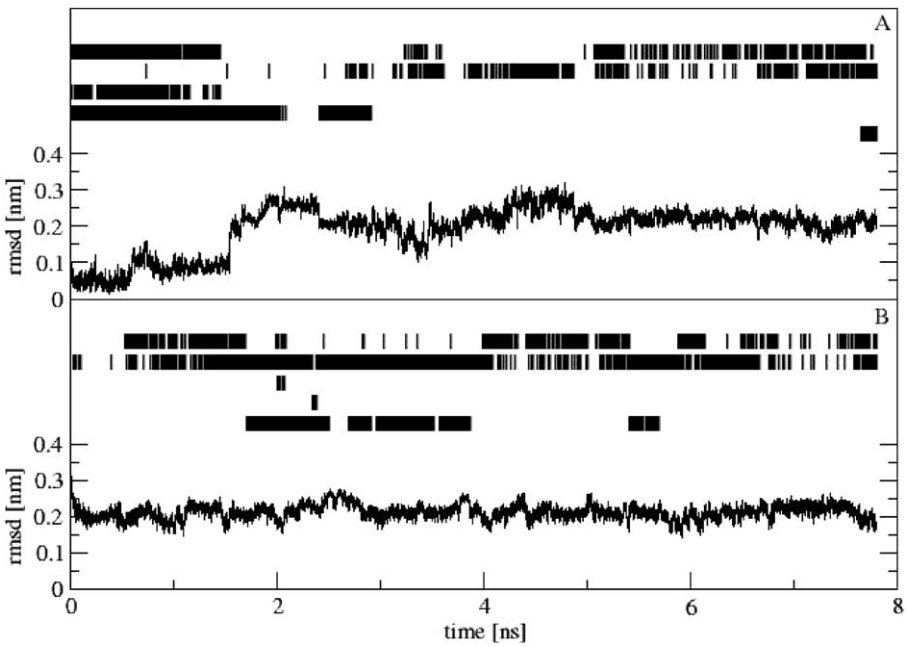

Figure 3. MD simulations of the α-heptapeptide in water starting from an α-helical initial conformation (Panel A) and from an extended initial conformation (Panel B). The evolution of all intramolecular hydrogen bonds with an occurrence larger than 5% is displayed together with the backbone (atoms N, C_α, C, O of residues 2–6) atom-positional root-mean-square deviation of the trajectory structures from an ideal α-helical structure. The hydrogen bonds are from top to bottom: NH(5)–O(2), NH(5)–O(3), NH(6)–O(2), NH(6)–O(3) and NH(7)–O(1).

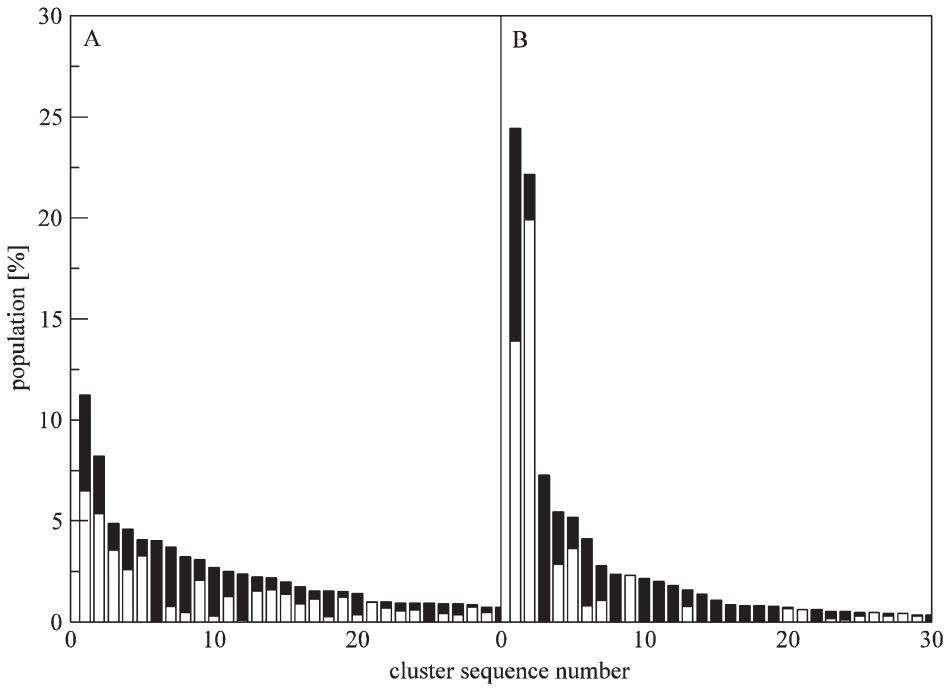

Figure 4. Conformational cluster analysis of pairs of MD trajectories of the α-heptapeptide. The plots show the population (in %) per cluster and the portion of structures per cluster that belongs to each of the two trajectories. Panel A. MD in methanol, starting from an ideal α-helix (black) or from an extended structure (white). Panel B. MD in water, starting from an ideal α-helix (black) or from an extended structure (white).

(starting from the helical and extended initial structures) trajectories of the α-heptapeptide in methanol (Panel A) and of the molecule in water (Panel B) are shown. A rmsd similarity criterion of 0.08 nm was used. In methanol both simulations sample the same set of conformers, of which none is very dominant (largest population 12%). The results for the simulations in water confirm that the sampling of the conformational space is not yet complete: both simulations sample partially different conformations. In Figure 5 the simulations in different solvents starting from identical initial structures are compared. Starting from an α-helical structure the α-heptapeptide seems to sample comparable parts of conformational space in methanol and in water. Starting from an extended structure (Panel B) more different

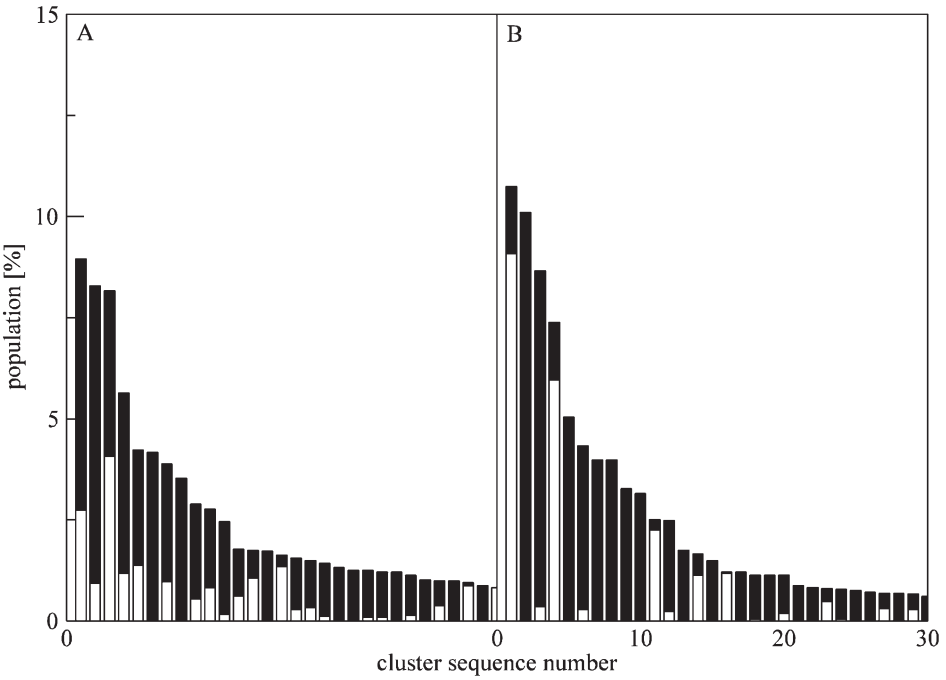

Figure 5. Conformational cluster analysis of pairs of MD trajectories of the α-heptapeptide. The plots show the population (in %) per cluster and the portion of structures per cluster that belongs to each of the two trajectories. Panel A. MD starting from an ideal α-helix in methanol (black) or in water (white). Panel B. MD starting from an extended structure in methanol (black) or in water (white).

conformations are visited in methanol compared to water. This is due to the lack of convergence of the water simulation.

In Figure 6 the central member structures of each of the three most populated clusters from each of the four simulations are shown together with their percentage population and dominant hydrogen bonds. The lowly populated structures are of irregular character, showing a variety of intramolecular hydrogen bonds. They have no helical character. However, many of the most populated conformers do show the NH(5)–O(3) hydrogen bond, particularly in methanol.

3. Conclusions

The four MD simulations of the α-heptapeptide shown in Figure 1, in methanol and in water, and starting from an α-helical and from an extended initial structure, predict that the α-heptapeptide will neither maintain nor adopt an α-helical conformation in methanol or in water. The conformational sampling over 26 ns in methanol seems to be rather complete, whereas in water 7.8 ns is not sufficient to obtain a converged ensemble of conformations. The conformers that dominate the conformational ensemble do not exhibit any particular secondary structure character. Although the α-heptapeptide was designed by Jaun and Seebach to maximize its tendency to adopt an α-helical conformation, simulations based on the GROMOS biomolecular force field do not predict any particular fold. This points at an essential role for the additional methylene moiety in the backbone of β-peptides regarding helix stability. We look forward to experimental (NMR) data that will confirm or contradict this prediction.

4. Methods

The MD simulations were carried out using the GROMOS software[8,9] and the GROMOS biomolecular force field,[8] parameter set 45A3.[10] Aliphatic CH_n groups were treated as united atoms, both in the peptide and in the solvent methanol.[22] For water the simple-point-charge (SPC) model[23] was used. Two initial α-heptapeptide structures were used: an α-helical one and an extended structure with $\varphi=\psi=180°$ for all residues. These peptide structures were placed in a truncated octahedron such that the minimum distance of a solute atom to one of the square walls was 1.5 nm. The remaining empty space in the truncated octahedron was filled with methanol or water molecules taken from an equilibrated configuration of these liquids. Four systems were generated: the peptide in the α-helical conformation with 633 methanol molecules and in the extended conformation with 1312 methanol molecules, and the peptide in corresponding conformations with 1396 and 2930 water molecules, respectively. Periodic boundary conditions were applied. After relaxation of the systems using steepest descent energy minimization the four MD simulations were started by taking the initial atomic velocities from a Maxwell distribution at low temperature followed by a gradual heating of the system till 300 K while position restraining the peptide atoms. Bond-lengths in the solute and all internal degrees of freedom of the solvent molecules were kept rigid using the SHAKE algorithm[24] with a geometric tolerance of 10^{-4}. Solute and solvent were separately coupled to a temperature bath at 300 K and with a relaxation time of 0.1 ps.[25] The pressure was calculated with a molecular virial and held constant at 1 atm using an isothermal compressibility of 4.575×10^{-4} $(\text{kJ mol}^{-1}\,\text{nm}^{-3})^{-1}$ and a relaxation time of 0.5 ps.[25] The equations of motion were integrated using the leap-frog algorithm and a time step of 2 fs. The non-bonded

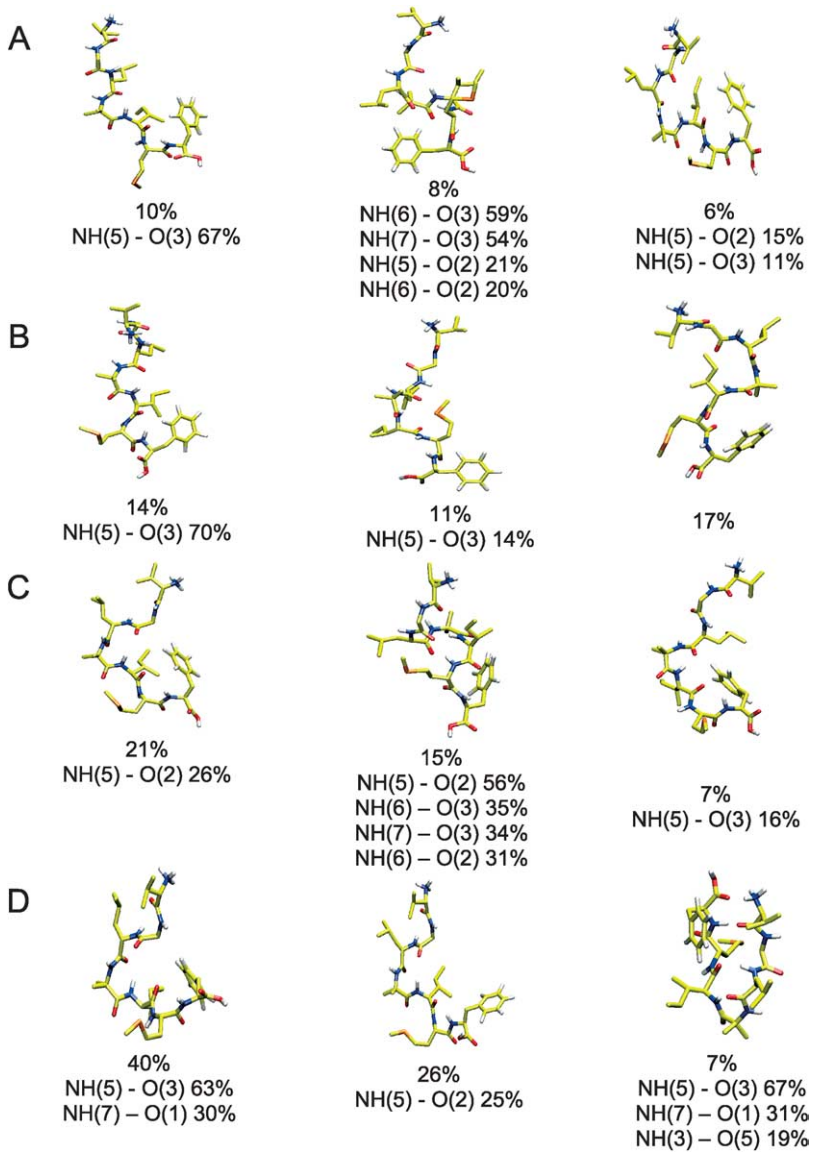

Figure 6. The three most populated conformers (central structures of the three most populated clusters using a backbone (N, C_α, C, O of residues 2–6) root-mean-square-difference (rmsd) criterion of 0.08 nm) observed in the four MD simulations. For each conformer, its corresponding population and occurrence of its most dominating hydrogen bonds is given. MD simulations in methanol (Panels A and B) and in water (Panels C and D) starting from an α-helical conformation (Panels A and C) or from an extended conformation (Panels B and D).

interaction between atoms grouped into so-called charge groups[8] was calculated according to a triple-range cut-off scheme: short-range van der Waals and electrostatic interactions were evaluated at every time step from a charge-group pair list that was generated with a short-range cut-off radius of 0.8 nm between the centres of geometry of the solute charge groups and the oxygen atoms of the methanol or water solvent molecules. Longer-range van der Waals and electrostatic interactions, between pairs at a distance longer than 0.8 nm and shorter than the long-range cut-off radius of 1.4 nm, were evaluated every tenth time step, at which point the pair list was also updated, and were kept unchanged between these updates. A Poisson–Boltzmann reaction-field[26] force was used to approximate electrostatic interactions due to the medium outside the long-range cut-off radius. The dielectric permittivity for the continuum outside the long-range cut-off radius was 66. Centre of mass translation and rotation of the whole system, peptide plus solvent, was eliminated every 10 time steps.

The two MD simulations in methanol were run for 26 ns, saving configurations every 0.4 ps for analysis. The two MD simulation in water were run for 7.8 ns, saving configurations every 0.2 ps. Least-squares translational and rotational superposition of trajectory structures for the calculation of atom-positional root-mean-square differences (rmsd) between pairs of structures was based on the backbone atoms (N, C_α, C, O) of residues 2–6. The conformational clustering analysis was performed as described by Daura et al.[27] on the sets of 3250 peptide structures from the methanol simulations and the sets of 975 peptide structures from the water simulations taken at 8 ps intervals. As similarity criterion the value of 0.08 nm for the mentioned backbone atom-positional rmsd was used, a value commonly used for β-hexapeptides.[12,17] Hydrogen

bonds were defined using a geometric criterion: a minimum donor–hydrogen–acceptor angle of 135° and a maximum hydrogen–acceptor distance of 0.25 nm.

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