Protein Structures

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Amide-to-Ester Substitution in Coiled Coils: The Effect of Removing Hydrogen Bonds on Protein Structure**

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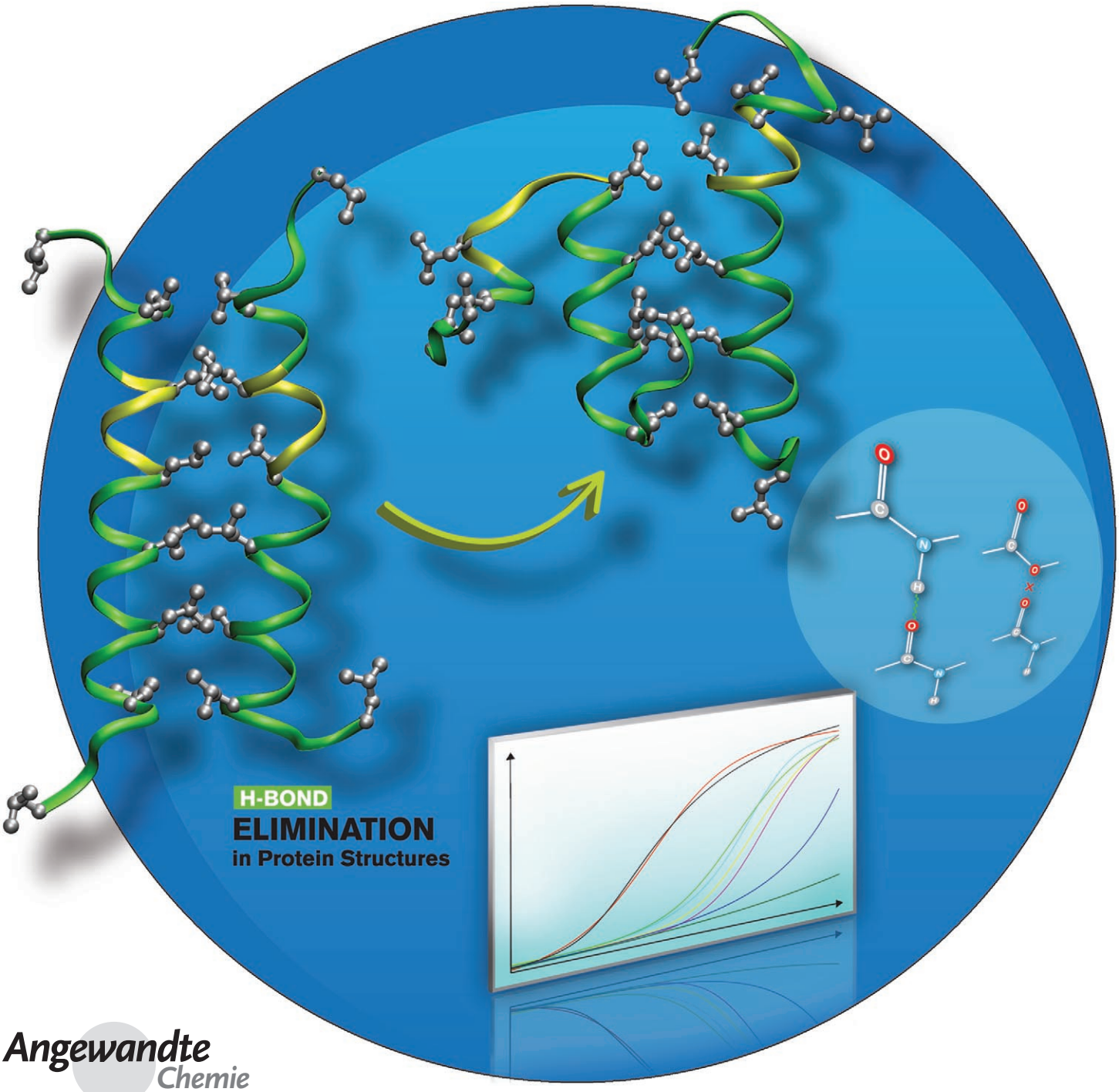

Deciphering the effect of backbone H bonding is of paramount importance for predicting protein structures and folding patterns.[1–5] "Amide-to-ester" substitutions have been proven as a versatile tool for investigating the effect of backbone H bonds on the structure formation and stability of proteins.[6–16] We have chosen a hybrid approach of experimental techniques and molecular dynamics (MD) simulations to study the environment-dependent contribution of backbone H bonding to overall structure formation.

The introduction of an α-hydroxy acid into a peptide sequence results in the formation of an ester bond, also called a depsipeptide bond. Such a replacement of an H-bond-donating NH group by the oxygen atom of an ester makes it possible to selectively remove specific backbone H bonds in peptides and proteins (Figure 1 a). Moreover, α-hydroxy acid building blocks are isostructural to α-amino acids.[8,9] Thus, they maintain the basic structural properties, and no major structural perturbations unrelated to backbone H bonding are exerted.

In previous studies either monomeric α helices,[7–10] β sheets,[11,12] or complex proteins[13–16] were employed as test systems to investigate the impact of certain H bonds on folding and stability. In one of those studies the hydrophobic core of an α-helical coiled coil was used as environment to prove the impact of a certain H bond on protein structure.[6] In the α-helical coiled coil the dielectric character of a protein environment is mimicked perfectly as it provides both solvent exposition of amino acid residues and an extended hydrophobic core. Moreover, peptides following the characteristic coiled-coil heptad repeat predictably fold into stable helical structures already at an overall length of about 20 amino acids. Therefore, the coiled-coil motif is ideal for a direct comparison between the impact of backbone H bonds in hydrophobic and solvent-exposed areas, respectively, on folding and stability. Taking advantage of these features, we have generated a small library of peptides that contain amide-to-ester substitutions in hydrophobic as well as solvent-exposed positions.

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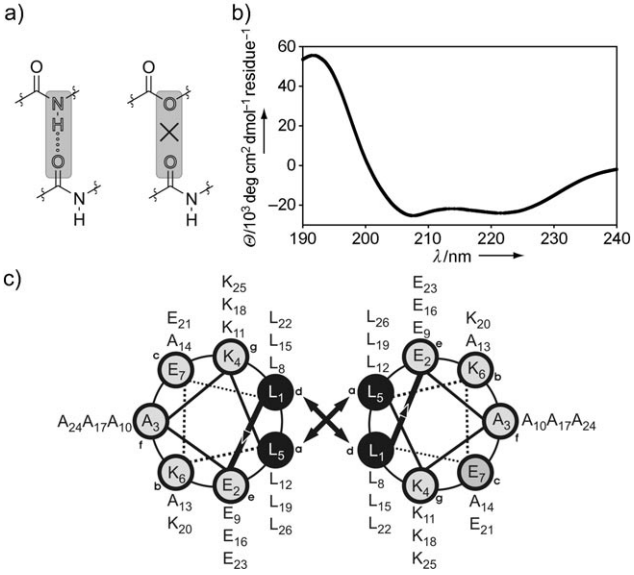

Figure 1. a) The introduction of an ester bond results in the removal of an H bond. b) CD spectrum of the parent peptide pp. c) Helical wheel representation of the homodimeric parent peptide.

The α-helical coiled coil is a common and well-studied structural motif composed of α helices that wrap around each other to form a supercoil.[17–20] The primary structure is organized as a heptad repeat $(a\text{-}b\text{-}c\text{-}d\text{-}e\text{-}f\text{-}g)_n$, where positions a and d are occupied by hydrophobic amino acids that form the hydrophobic core by "knobs-into-holes" packing of the side chains (Figure 1 c). The solvent-exposed positions b, c, e, f, and g are occupied mostly by polar or charged amino acids.

The 26 amino acid long parent peptide (pp, Table 1), which is the basis for our test system, is a de novo designed homodimeric α-helical coiled coil with parallel alignment. We systematically introduced amide-to-ester substitutions by solid-phase peptide synthesis (SPPS) and investigated the folding stability by CD spectroscopy and MD simulations.

The CD spectrum of pp shows the expected α-helical conformation (Figure 1 b). Size exclusion chromatography shows only one peak which indicates the exclusive formation of a coiled-coil homodimer (see the Supporting Information). We introduced single amide-to-ester substitutions at different positions (Table 1): 1) in the N- and C-terminal regions (L5λ and L22λ) and 2) in the middle of the sequence in either a hydrophobic (L12λ) or solvent-exposed (A10α, K11κ, and A13α) environment. Furthermore, we introduced amide-to-ester substitutions in two consecutive ester bonds (L12λA13α). Stabilities of the different variants were monitored by CD spectroscopy under denaturating conditions (Figure 2 a).

In the variants L5λ and L22λ the substitutions are five residues away from their respective peptide termini. In the absence of the denaturating agent guanidine hydrochloride (GuHCl) both variants display show a α-helical coiled-coil structure similar to that of pp (Figure 2). While L5λ with the N-terminal substitution is as stable as pp, the stability of peptide L22λ with the C-terminal substitution is distinctly decreased by 1.4 kcal mol^{-1} (Table 2) relative to pp The MD

Table 1: Sequences of the peptides investigated.

Peptide[a]	Sequence[a]
pp	H_2N-LEAKLKELEAKLAALEAKLKELEAKL-COOH
L5λ	H_2N-LEAKλKELEAKLAALEAKLKELEAKL-COOH
A10α	H_2N-LEAKLKELEαKLAALEAKLKELEAKL-COOH
K11κ	H_2N-LEAKLKELEAκLAALEAKLKELEAKL-COOH
L12λ	H_2N-LEAKLKELEAKλAALEAKLKELEAKL-COOH
A13α	H_2N-LEAKLKELEAKLαALEAKLKELEAKL-COOH
L22λ	H_2N-LEAKLKELEAKLAALEAKLKELEAKλ-COOH
L12λ A13α	H_2N-LEAKLKELEAKλαALEAKLKELEAKL-COOH

[a] The one-letter code of the α-hydroxy acids are lowercase Greek letters and correspond to the one-letter code of the analogous α-amino acid.[12,14]

Table 2: Relative thermodynamic stabilities determined by denaturation with GuHCl. Negative $\Delta\Delta G_U^{H_2O}$ values indicate stabilities lower than that of pp.

Peptide	$D_{50\%}$[a]	m[b]	$\Delta G_U^{H_2O}$[c]	$\Delta\Delta G_U^{H_2O}$[c]
pp	3.62	0.92	3.32	0
L5λ	3.57	1.09	3.90	−0.05
A10α	2.13	0.76	1.63	−1.25
K11κ	1.76	0.73	1.28	−1.53
L12λ	0.35	n/a	n/a	n/a
A13α	1.53	0.81	1.25	−1.81
L22λ	2.04	0.83	1.69	−1.38

[a] In M GuHCl. [b] In kcal mol^{-1} M^{-1}. [c] In kcal mol^{-1}.

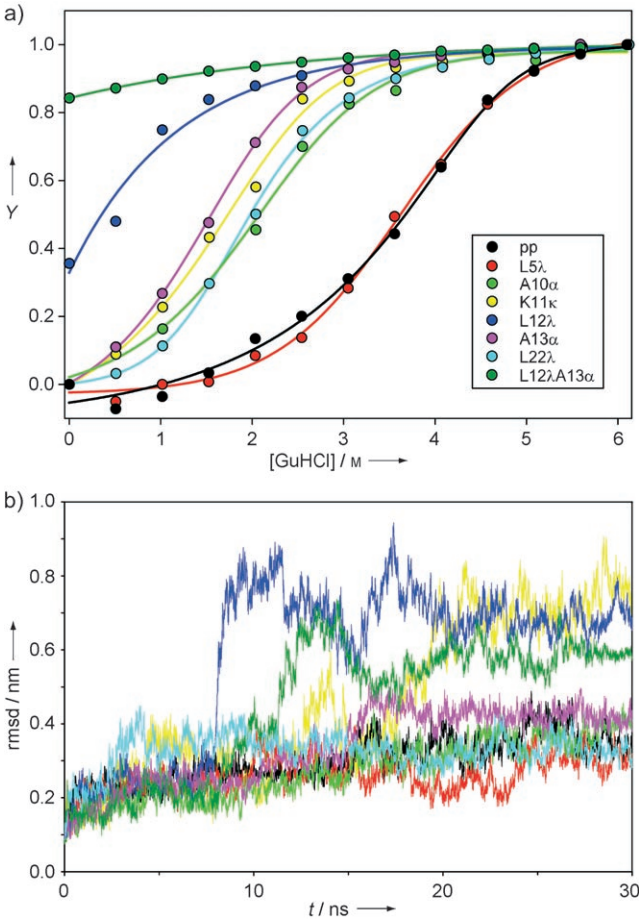

Figure 2. Correlation of experimental results with the MD simulation. a) CD spectra for the unfolding of pp and the depsipeptides; y = fraction unfolded. b) rms deviations for backbone positions during the MD simulation (same color code as in part a).

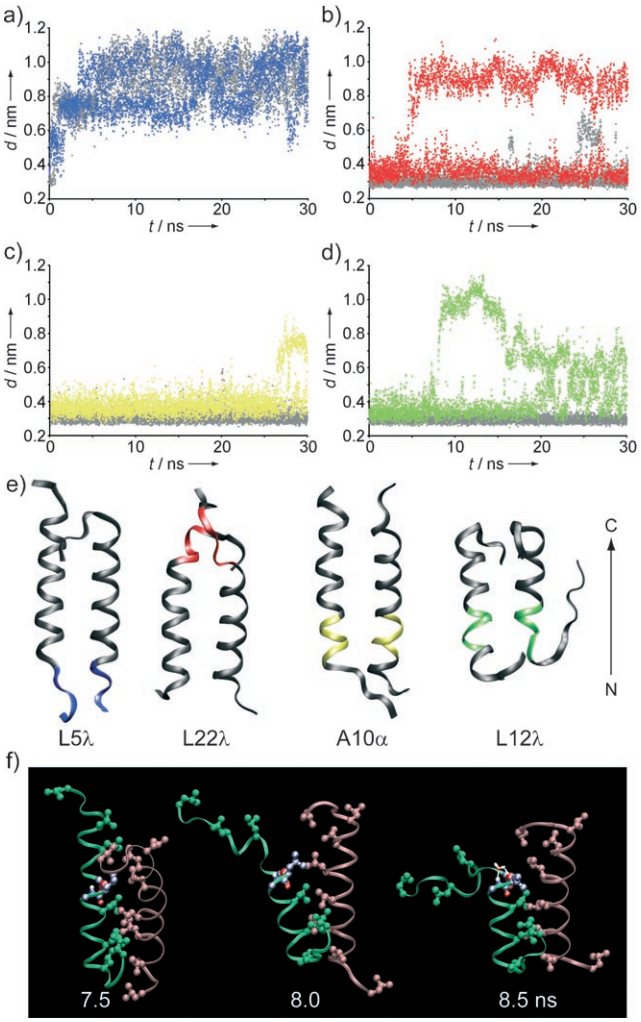

Figure 3. Distances *d* between the amide N and carbonyl O atoms involved in H bonds in pp (gray) and the corresponding distances between the depsi O to carbonyl O atoms in the variants L5λ (blue, a), L22λ (red, b), A10α (yellow, c), and L12λ (green, d). e) Representative snapshots from the trajectories. f) Snapshots from the MD simulation of L12λ.

simulations are consistent with the experimental data. Figure 3a shows the high flexibility of the N terminus of pp. Therefore, the H bond between the carbonyl function of residue L1 and the amide hydrogen of L5 is not formed. Thus, the amide-to-ester substitution in position 5 has no influence on the stability relative to the pp. The plot in Figure 3b shows the high conservation of the H bond between positions L22 and K18 of pp. Thus, we observe significant structural differences in the MD simulations in contrast to the behavior of L5λ. In the case of L5λ the H bond points outwards from the helix along the helix axis. Therefore, this H bond is of limited importance for the additive character of helical H bonding and also is only rarely formed. In contrast, the amide group of L22 is the first H-bond donor of a series and

thus, of great importance for the overall helical H-bonding pattern as well as for the dipolar character of the helix.[21] In both cases the substitution deletes an H-bond donor. However, in the case of L5λ the acceptor is not oriented to recognize this lack, while the acceptor of the amide H of L22 is rigidly placed.

The variant A10α exemplifies solvent-exposed substitutions, like K11κ and A13α, while substitution L12λ is in the hydrophobic core of the coiled coil. The CD spectrum of A10α is typical for an α-helical coiled coil, while that of L12λ indicates a slightly lower helical content in the absence of GuHCl. Denaturation curves show a significantly decreased stability of A10α of 1.3 kcal mol^{-1} (Table 2). Since the unfolding curve of L12λ (Figure 2a) does not show a lower plateau, an exact value for $\Delta\Delta G_U$ is not available (Table 2), but the plots in Figure 2a clearly reflect the significantly decreased stability of the coiled-coil structure. Corresponding MD simulations show that the distances of the amide and carbonyl groups involved in H bonds are well conserved at around 3 Å (Figure 3c and d) in pp. Interestingly, substitution A10α in the solvent-exposed face shows a similar conserved behavior with a slightly increased distance around 4 Å between the introduced ester oxygen and the corresponding carbonyl function. In contrast to the stability of the coiled-coil structure of A10α, which persists over the entire 30-ns simulation time, L12λ already unfolds after 7 ns; this is evident from the significant increase in the distance between the depsi O and the carbonyl atoms (Figure 3d). The packing of the hydrophobic core becomes distorted, and the N-terminal part of the helix unfolds.

The reason for the different behavior of peptides with amide-to-ester substitutions in the hydrophobic core and those with substitutions in the solvent-exposed face is based on the environment-dependent strength of H bonds.[14,22] H bonds in the solvent-exposed side have an intrinsically lower contribution to the stability owing to the shielding effect of the solvent.[23] The slight repulsive effect between the partially negatively charged ester and the corresponding carbonyl oxygen[11] is reduced by both the general dielectric properties of the solvent and by explicit interactions with water molecules. This does not hold for the substitution in the hydrophobic core. In the case of substitutions in the hydrophobic core, alternative interactions to water molecules are not possible, and owing to its low dielectric constant the hydrophobic environment does not shield the repulsive interactions between the partially negatively charged oxygens. Additionally, the lack of a hydrogen atom in the depsipeptides perturbs the dense packing of the hydrophobic core. This leads to an irreversible, rapid unfolding because of the resulting saturation of the ester and carbonyl oxygen atoms of the depsi peptide backbone by interactions with water molecules in the unfolded state (Figure 3f). Ester-to-amide substitution in two positions (L12λA13α) results in a complete loss of helicity even in the absence of denaturant (Figure 2).

In conclusion, we find a clear environmental dependency for the impact of H bonding on protein folding. The effect results primarily from a drastically reduced influence of solvent in the nonaccessible regions of peptides and proteins. Our results illustrate that H bonds are not the driving force of protein or peptide folding but contribute substantially to the maintenance of a given fold.

Experimental Section

The parent peptide and depsipeptides were synthesized following the Boc strategy by automated SPPS. The purity and identity of the peptides were proven with HPLC and mass spectrometry. All CD spectra were measured at peptide concentrations of 6 mg mL^{-1} in 100 mM sodium phosphate buffer at pH 7.4 and 20 °C. Unfolding of the peptides was induced by addition of the denaturating agent GuHCl. The MD simulations were carried out with the Gromacs suite[24] using the Gromos 53a6 force field.[25] The coiled-coil dimers were solvated in dodecahedric boxes with around 9500 simple-point charge (SPC) water molecules. Further details can be found in the Supporting Information.

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[1] K. A. Dill, *Biochemistry* **1990**, *29*, 7133.
[2] E. Lacroix, A. R. Viguera, L. Serrano, *J. Mol. Biol.* **1998**, *284*, 173.
[3] N. Ferguson, J. R. Pires, F. Toepert, C. M. Johnson, Y. P. Pan, R. Volkmer-Engert, J. Schneider-Mergener, V. Daggett, H. Oschkinat, A. Fersht, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 13008.
[4] M. Gruebele, *Curr. Opin. Struct. Biol.* **2002**, *12*, 161.
[5] R. L. Baldwin, *J. Biol. Chem.* **2003**, *278*, 17581.
[6] J. W. Blankenship, R. Balambika, P. E. Dawson, *Biochemistry* **2002**, *41*, 15676.
[7] A. S. Cieplak, N. B. Surmeli, *J. Org. Chem.* **2004**, *69*, 3250.
[8] I. L. Karle, C. Das, P. Balaram, *Biopolymers* **2001**, *59*, 276.
[9] T. Ohyama, H. Oku, M. Yoshida, R. Katakai, *Biopolymers* **2001**, *58*, 636.
[10] J. T. Koh, V. W. Cornish, P. G. Schultz, *Biochemistry* **1997**, *36*, 11314.
[11] Y. W. Fu, J. M. Gao, J. Bieschke, M. A. Dendle, J. W. Kelly, *J. Am. Chem. Soc.* **2006**, *128*, 15948.
[12] S. Deechongkit, P. E. Dawson, J. W. Kelly, *J. Am. Chem. Soc.* **2004**, *126*, 16762.
[13] M. Wang, T. E. Wales, M. C. Fitzgerald, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 2600.
[14] E. T. Powers, S. Deechongkit, J. W. Kelly, *Adv. Protein Chem.* **2005**, *72*, 39.
[15] X. Y. Yang, M. Wang, M. C. Fitzgerald, *Bioorg. Chem.* **2004**, *32*, 438.
[16] J. K. Myers, C. N. Pace, *Biophys. J.* **1996**, *71*, 2033.
[17] J. M. Mason, K. M. Arndt, *ChemBioChem* **2004**, *5*, 170.
[18] Y. B. Yu, *Adv. Drug Delivery Rev.* **2002**, *54*, 1113.
[19] Á. Piñeiro, A. Villa, T. Vagt, B. Koksch, A. E. Mark, *Biophys. J.* **2005**, *89*, 3701.
[20] K. Pagel, K. Seeger, B. Seiwert, A. Villa, A. E. Mark, S. Berger, B. Koksch, *Org. Biomol. Chem.* **2005**, *3*, 1189.
[21] J. J. Dannenberg, *J. Mol. Struct.* **2002**, *615*, 219.
[22] H. Umeyama, K. Morokuma, *J. Am. Chem. Soc.* **1977**, *99*, 1316.
[23] F. M. Dicapua, S. Swaminathan, D. L. Beveridge, *J. Am. Chem. Soc.* **1991**, *113*, 6145.
[24] E. Lindahl, B. Hess, D. van der Spoel, *J. Mol. Model.* **2001**, *7*, 306.
[25] C. Oostenbrink, A. Villa, A. E. Mark, W. F. Van Gunsteren, *J. Comput. Chem.* **2004**, *25*, 1656.

