Acyl capping group identity effects on α-helicity: on the importance of amide•water hydrogen bonds to α-helix stability

Megh R. Bhatt[§], Himal K. Ganguly[§], and Neal J. Zondlo*

Department of Chemistry and Biochemistry

University of Delaware

Newark, DE 19716

United States

[§] These authors contributed equally.

* To whom correspondence should be addressed. email: *zondlo@udel.edu*.

Abstract

Acyl capping groups stabilize α-helices relative to free N-termini by providing one additional C=O_i•••H_{i+4}–N hydrogen bond. The electronic properties of acyl capping groups might also directly modulate α-helix stability: electron-rich N-terminal acyl groups could stabilize the α-helix by strengthening both *i*/*i*+4 hydrogen bonds and *i*/*i*+1 $n\rightarrow\pi^*$ interactions. This hypothesis was tested in peptides X–AKAAAKAAAKAAAAKAAGY-NH_2, X=different acyl groups. Surprisingly, the most electron-rich acyl groups (pivaloyl, *iso*-butyryl) strongly destabilized the α-helix. Moreover, the formyl group induced nearly identical α-helicity as the acetyl group, despite being a weaker electron donor for hydrogen bonds and for $n\rightarrow\pi^*$ interactions. Other acyl groups exhibited intermediate α-helicity. These results indicate that the electronic properties of the acyl carbonyl do not directly determine α-helicity in peptides in water. In order to understand these effects, DFT calculations were conducted on α-helical peptides. Using implicit solvation, α-helix stability correlated with acyl group electronics, with the pivaloyl group exhibiting closer hydrogen bonds and $n\rightarrow\pi^*$ interactions, in contrast to the experimental results. However, DFT and MD calculations with explicit water solvation revealed that hydrogen bonding to water was impacted by the sterics of the acyl capping group. Formyl capping groups exhibited the closest water-amide hydrogen bonds, while pivaloyl groups exhibited the longest. In α-helices in the PDB, the highest frequency of close amide-water hydrogen bonds is observed when the N-cap residue is Gly. The combination of experimental and computational results indicates that solvation (hydrogen bonding of water) to the N-terminal amide groups is a central determinant of α-helix stability.

Introduction

α-Helices are one of the two predominant secondary structures of folded proteins. α-Helices are stabilized by *i*/*i*+4 C=O•••H–N hydrogen bonds between amide groups (Figure 1).[1] The importance of α-helices in protein structure has inspired many detailed studies on the determinants of α-helix stability, including the propensities of individual amino acids for α-helix formation and propagation, the effects of sequence length, the roles of initiation versus propagation, the impacts of helix termini, and interresidue noncovalent interactions, among others.[2-14] In addition, extensive work has examined the use of artificial capping groups or covalent bonds (e.g. stapling) in order to nucleate or stabilize α-helices.[15-18]

Acyl capping groups are typically employed on the N-termini of peptides to more accurately replicate the electronic structure of the peptide compared to that of the same residues within a protein. In addition, in α-helical peptides, acyl capping groups stabilize α-helices by providing one additional C=O•••H–N hydrogen bond. At the N-terminus of capped α-helices, three amide N–H hydrogen bond donors are solvent-exposed and not part of the hydrogen bonding pattern of α-helices (Figure 1b). In proteins, α-helix capping motifs (multiple amino acids N-terminal to the α-helical segment) can function as hydrogen-bond acceptors for these groups, which otherwise interact with solvent water molecules, resulting in substantial stabilization of α-helices in peptides and proteins with α-helix N-capping motifs.[2,12]

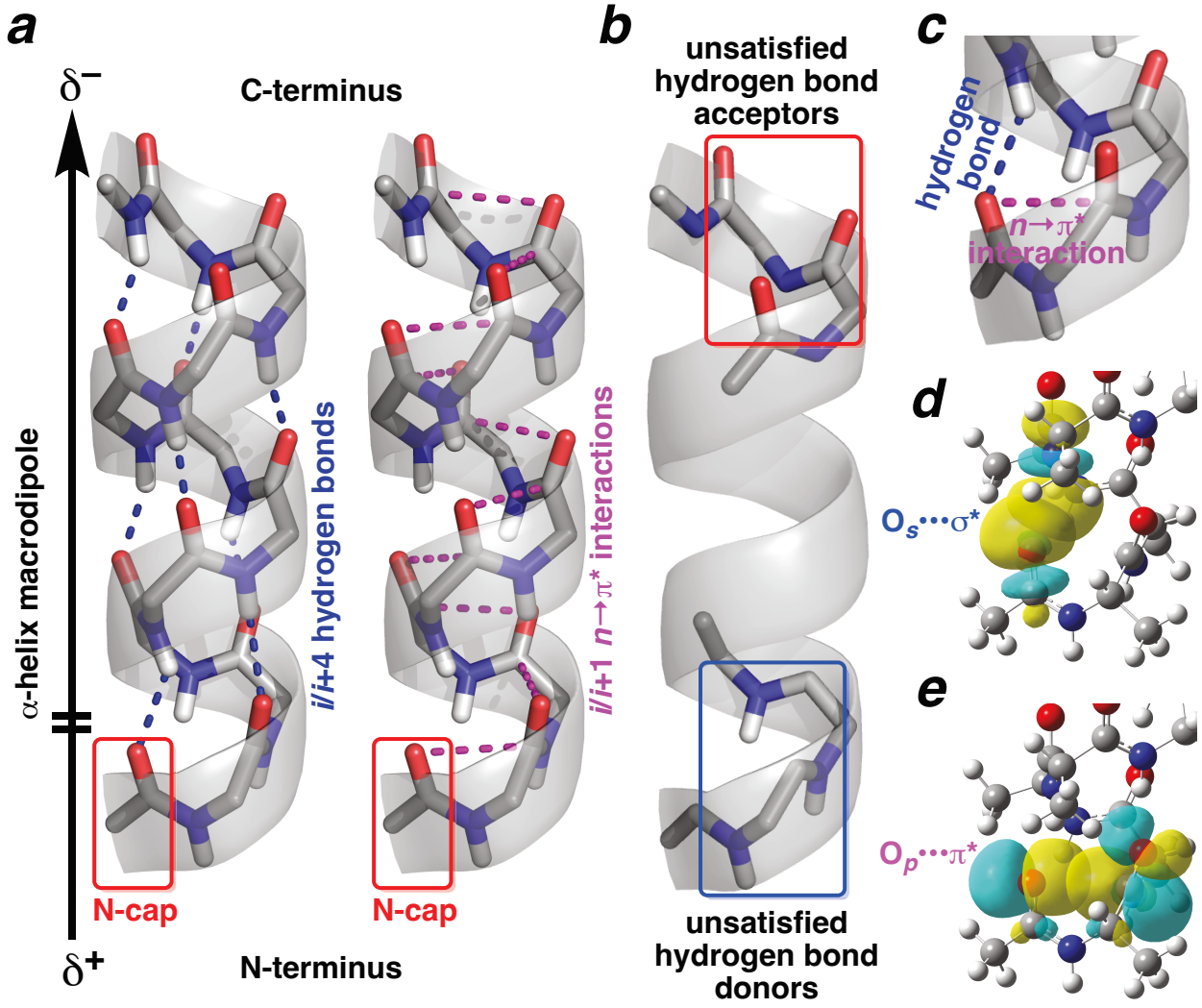


Figure 1. Stabilization mechanisms in the α-helix. (a) Structure of the α-helix, highlighting the alignment of carbonyls, the helix macrodipole, *i*/*i*+4 hydrogen bonds, and *i*/*i*+1 *n*→π* interactions. (b) Structure of the α-helix showing unsatisfied hydrogen-bond donors at the N-terminus and unsatisfied hydrogen-bond acceptors at the C-terminus. (c) Structure of the α-helix N-terminus, showing the acyl capping group and its hydrogen bond and n→π* interaction. (d,e) Natural Bond Orbital (NBO) analysis[19,20] of noncovalent interactions involving the N-terminal acyl capping group, illustrating the orbital overlap (d) between the s-like O lone pair and the σ* orbital of the N-H bond from the *i*+4 residue, and (e) between the p-like O lone pair and the π* orbital of the carbonyl of the *i*+1 residue.

Due to the alignments of the amide groups (N–H groups pointing toward the N-terminus, C=O groups pointing toward the C-terminus), α-helices have a very substantial helical macrodipole, with a large δ^+ at the N-terminus and a large δ^- at the C-terminus (Figure 1a). Thus, α-helices are stabilized by negatively charged amino acids near the N-terminus and by positively charged amino acids near the C-terminus of the α-helix. In contrast, in peptides with uncapped termini, the free amino N-terminus (H_3N^+–) and free carboxylate (–CO_2^-) C-terminus are charged. These charges on the termini add to the magnitude of the helix macrodipole, destabilizing the α-helical/folded structure relative to the disordered/unfolded state. In contrast, capped termini have neutral amide structures, most typically in peptides with an acetylated N-terminus and a C-terminal carboxamide. Interestingly, the majority of eukaryotic proteins (e.g., > 80% of human proteins) are acetylated on the N-terminus, via N-terminal acetyltransferases.[21-23] N-Terminal acetylation of proteins impacts protein processing and other protein functions. In addition, N-terminal acetylation could also directly impact protein structure via the stabilization of α-helices at protein N-termini.[24-27]

Thus, the presence and identity of the N-terminal acyl group can impact the stability of the α-helix directly, via the introduction of hydrogen-bond acceptor groups that can interact with unsatisfied [solvent-exposed] N–H hydrogen-bond donors (Figure 1b).[10,12,28,29] α-Helical capping groups in proteins often interact with sidechain hydrophobic groups to stabilize structure in the first turn of the α-helix.[12]

In addition, acyl capping of the N-terminus can potentially directly contribute to α-helix stability. α-Helicity is significantly dependent on the length of the helix, with short α-helices inherently unstable.[18] The instability of short α-helices is fundamentally due to the energetic cost

of the first turn of the α-helix (initiation of the α-helix), which requires the organization of 3 (3_{10} helix, C=O_i•••H_{i+3}–N) or 4 (α-helix, C=O_i•••H_{i+4}–N) residues in order to form the first helical hydrogen bond (Figure 1c). In contrast, for each additional residue in the α-helix (helix propagation), only one residue needs to be organized to achieve one additional α-helical hydrogen bond. Thus, while α-helix initiation is very unfavorable, α-helix propagation is favorable or neutral for most amino acids. Thus, the incorporation of an acyl capping group at the N-terminus of an α-helical sequence increases α-helix stability by providing one additional carbonyl (C=O) to hydrogen bond to an otherwise unsatisfied amide N–H hydrogen bond donor.

In addition to *i*/*i*+4 C=O•••H–N hydrogen bonds, which utilize the s-like oxygen lone pair as an electron donor, α-helices are also stabilized by intercarbonyl $n \rightarrow \pi^*$ interactions (Figure 1a, 1c). These involve close association between carbonyls of consecutive residues (O_i:•••C_{i+1}=O), with electron delocalization between the p-like oxygen lone pair (*n*) of the *i* residue carbonyl and the π* molecular orbital of the *i*+1 residue carbonyl (Figure 1e), which collectively stabilize the α-helix via these $n \rightarrow \pi^*$ interactions. Thus, both carbonyl oxygen lone pairs stabilize the α-helical structure. Importantly, $n \rightarrow \pi^*$ interactions only require the organization of *one* amino acid, and thus can promote the α-helical conformation at individual residues *prior to* formation of the first hydrogen bond.[30-32]

We recently demonstrated that the α-helix conformation ($\phi,\psi \sim -60^\circ, -40^\circ$) can be stabilized solely through $n \rightarrow \pi^*$ interactions, without the requirement for a hydrogen bond.[33] In a series of molecules X–Hnb-OMe (X = different acyl capping groups, Hnb = the nitrobenzoate ester of the sidechain hydroxyl of 4*R*-hydroxyproline), we observed crystallographically that more electron-rich acyl capping groups promoted closer $n \rightarrow \pi^*$ interactions, with the pivaloyl

group inducing the closest O_i•••C_{i+1}=O distance. The *iso*-butyryl, propionyl, acetyl, chloroacetyl, bromoacetyl, and methoxyacetyl derivatives all adopted the α-helical conformation in the solid state, with O_i•••C_{i+1}=O distances substantially below the 3.22 Å sum of the van der Waals radii of O and C. In contrast, extended conformations were observed for molecules with the more electron-poor fluoroacetyl, formyl, and trifluoroacetyl acyl groups. Both crystallographically and computationally, there was a clear correlation between the electronic properties of the acyl group and the observed conformation and O_i•••C_{i+1}=O distances: more electron-rich acyl capping groups exhibited closer $n\rightarrow\pi^*$ interactions and more compact conformations in ϕ. These results are consistent with more electron-rich carbonyls being better electron donors for $n\rightarrow\pi^*$ interactions, and the ability to electronically tune conformation via the identity of the acyl group.

The polyproline II helix (PPII) conformation, like the α-helix, is also stabilized by $n\rightarrow\pi^*$ interactions.[30,31] We recently tested whether acyl capping group identity can be used to electronically tune PPII conformation, via changes in the strength of $n\rightarrow\pi^*$ interactions.[34] Electronic tuning of PPII conformation was observed in both **X**-PPGY-NH_2 and **X**-APPGY-NH_2 series of peptides, where **X** = a series of acyl capping groups. The pivaloyl group most strongly promoted PPII, while the *iso*-butyryl and propionyl groups also significantly stabilized PPII relative to the standard acetyl N-capping group. In contrast, more electron-poor acyl groups, including the methoxyacetyl and formyl groups, relatively destabilized PPII compared to the acetyl group, though these effects were less significant than those of electron-rich acyl groups. These results indicated that the electronic properties of acyl capping groups can directly impact the conformations of peptides in water, via their relative ability to promote $n\rightarrow\pi^*$ interactions.

The identity of the acyl group at the N-terminus of α-helices could potentially impact α-helicity via electronic effects both on the *i*/*i*+4 C=O•••H–N hydrogen bonds and on the *i*/*i*+1

intercarbonyl $n \rightarrow \pi^*$ interactions that stabilize helical structure (Figure 1cde). For both classes of interactions, a more electron-rich acyl carbonyl would be expected to increase α-helicity, by making the acyl carbonyl C=O a better electron donor (including greater electron density/δ^- on the oxygen), *both* for hydrogen bonds *and* for $n \rightarrow \pi^*$ interactions. Herein, we systematically examine the role of acyl capping group electronic properties on α-helicity.

Results

Effects of acyl capping groups on α-helicity in X–AKAAAAKAAAAKAAGY-NH_2 peptides. A series of standard Baldwin-type alanine-rich peptides was synthesized and purified (Figure 2).[5,35] These peptides were prepared via resin-splitting, and differed only in the identity of the N-terminal acyl capping group, which was added prior to peptide cleavage from resin and side-chain deprotection. Acyl groups with different electronic properties were examined, including pivaloyl, *iso*-butyryl, and propionyl groups that are more electron-rich than the acetyl group; bromoacetyl, chloroacetyl, methoxyacetyl, fluoroacetyl, and formyl groups that are less electron-rich; and the standard acetyl group as a reference, as acetyl is by far the predominant acyl N-terminal capping group used in standard solid-phase peptide synthesis. In all peptides, a C-terminal Tyr was added for concentration determination.

All peptides were analyzed by circular dichroism (CD) spectroscopy (Figure 3, Table 1).[36] Peptides were analyzed at 0.5 °C and 20 °C. The extent of α-helicity was determined primarily by mean residue ellipticity ([θ]) at 222 nm, with secondary (concentration-independent) measures of α-helicity including the ratios of mean residue ellipticity $[\theta]_{222}/[\theta]_{208}$ and $-[\theta]_{190}/[\theta]_{208}$, in which a larger ratio indicates a greater extent of α-helicity. The

thermodynamic effects of different acyl capping groups were determined by helix-coil theory, via Lifson-Roig analysis modified to incorporate N-capping and C-capping.[29,37,38]

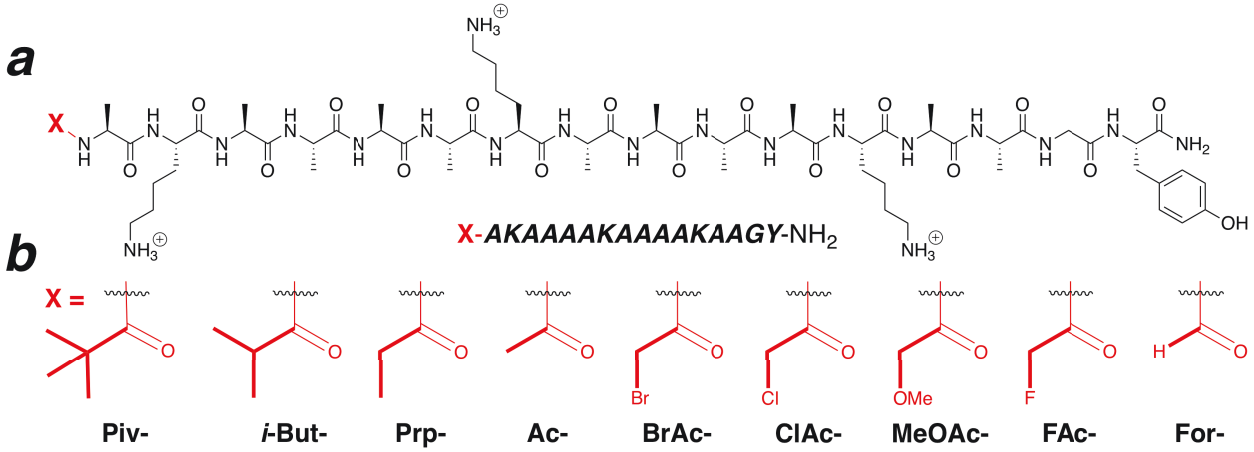


Figure 2. Peptide sequence and structure of acyl capping groups. (a) Baldwin-type α-helix model context of peptides, where X indicates the acyl N-capping group. (b) The acyl N-caps examined include Piv- (pivaloyl), *i*-But- (*iso*-butyryl), Prp- (propionyl), Ac- (acetyl), BrAc- (bromoacetyl), ClAc- (chloroacetyl), MeOAc- (methoxyacetyl), FAc- (fluoroacetyl), and For- (formyl) groups.

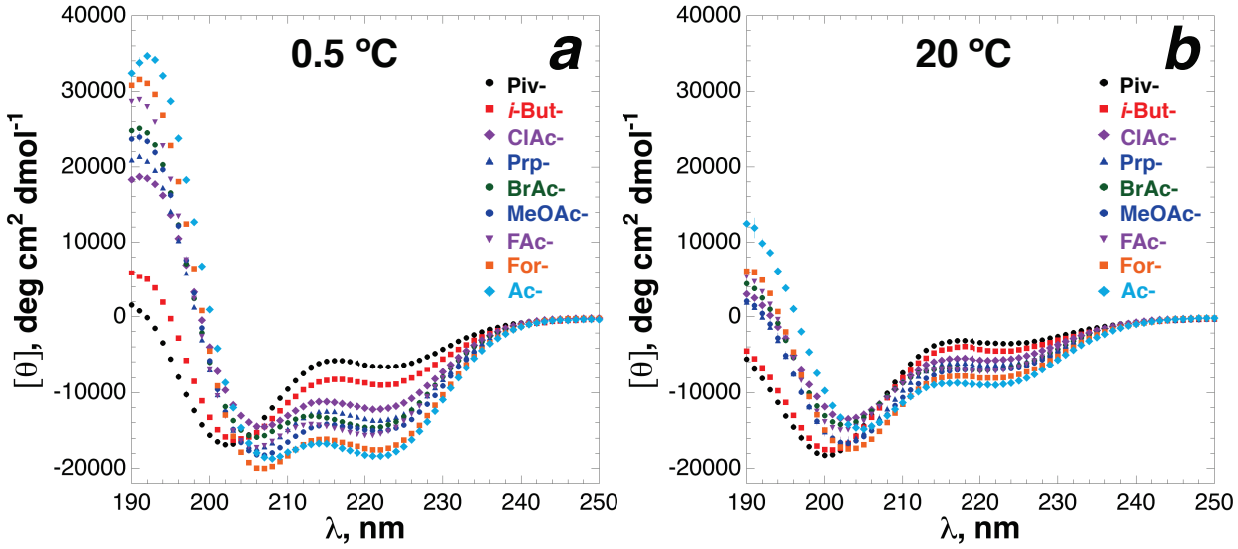


Figure 3. **CD spectra of peptides with N-acyl capping groups**. CD spectra of the peptides X-AKAAAAKAAAAKAAGY-NH_2, X = Piv- (black circles), *i*-But- (red squares), ClAc- (purple diamonds), Prp- (blue triangles), BrAc- (green circles), MeOAc- (blue circles), FAc- (purple inverted triangles), For- (orange squares), and Ac- (cyan diamonds). CD spectra were acquired in solution with 5 mM phosphate buffer pH 7 and 25 mM KF (a) at 0.5 ºC and (b) at 20 ºC. The data are the average of at least three independent trials, with error bars indicating standard error.

Table 1. Summary of CD data for peptides in Figure 3.[a]

peptide, X=	$[\theta]_{222}$[b]	$[\theta]_{208}$[b]	$[\theta]_{190}$[b]	$[\theta]_{222}/[\theta]_{208}$	$-[\theta]_{190}/[\theta]_{222}$	% α-helix	*n*-value[c, d]	ΔG_{Ncap}, $-RT \ln n$, kcal mol^{-1}
Piv	–6520	–12030	1550	0.54	0.24	19.3	n.d.[e]	n.d.[e]
i-But	–8990	–13420	5850	0.67	0.65	26.3	n.d.[e]	n.d.[e]
ClAc	–12190	–14250	18310	0.86	1.50	36.3	~ 0 (< 0.3)[e]	(> +0.6)[e]
BrAc	–12630	–13140	21520	0.96	1.70	37.4	0.1 (< 0.5)[e]	+1 (> +0.4)[e]
Prp	–13660	–16620	20930	0.82	1.53	40.5	0.5 ± 0.4	+0.4 ± 0.5
MeOAc	–15140	–17960	23680	0.84	1.56	44.9	1.1 ± 0.5	–0.05 ± 0.25
FAc	–15470	–16440	28630	0.94	1.85	45.8	1.3 ± 0.5	–0.1 ± 0.2
For	–17570	–19740	30780	0.89	1.75	52.1	2.7 ± 0.9	–0.5 ± 0.2
Ac	–18400	–18750	32390	0.98	1.76	54.5	3.4 ± 1.1	–0.7 ± 0.2

[a] CD data were recorded on solutions at 0.5 °C with 5 mM phosphate buffer pH 7.0 and 25 mM KF. Data at 20 ºC are in Table S2.

[b] $[\theta]$ = mean residue ellipticity (deg cm^2 dmol^{-1}) at the indicated wavelength (nm). The extent of α-helicity and folding can be inferred from the magnitude of the bands at either 222 or 190 nm, or from the concentration-independent ratios $[\theta]_{222}/[\theta]_{208}$ or $-[\theta]_{190}/[\theta]_{208}$, with a larger ratio indicating greater α-helicity. % α-helix was determined here from $[\theta]_{222}$ using the equation: % α-helix = $(100\% \times [\theta]_{222})/(-40000 \times (1-2.5/n))$, where $[\theta]_{222}$ is mean residue ellipticity (deg cm^2 dmol^{-1}) at 222 nm and n (n=16) is the number of residues excluding the N-acyl capping group.[36]

[c] The median *n* value was calculated using the CapHelix program, implementing the Lifson-Roig helix-coil theory with modifications to include N- and C-capping.[29,37] $-RT \ln n$ indicates the free energy of capping (ΔG_{Ncap}) for different acyl N-capping groups relative to that of Ala.

[d] The percent α-helix was examined assuming an estimated total error from all sources of ± 3% α-helix, with the range of *n*-values and free energies determined based on the indicated % α-helix and this range of error.[38]

[e] For the Piv-, *i*-But-, and ClAc- acyl N-capping groups, the program fit the observed % α-helix to a negative *n*-value, which is not thermodynamically plausible. In addition, for the BrAc- group, the lower limit of % α-helix also fit to a negative *n*-value. For the BrAc- and ClAc- groups, the limits of *n*-value ($n <$ indicated value) and free energy ($\Delta G_{Ncap} >$ indicated value) are associated with the maximum % α-helix including the error limits. Doig and Baldwin observed a similar effect of a strongly destabilizing N-cap (calculated $n < 0$) with Gln, in an identical peptide context to that used herein, which those authors interpreted as Gln interacting with the backbone of residues within the α-helix to particularly destabilize the α-helix.[38] Here, we interpret the apparent *n*-values of the Piv-, *i*-But-, and ClAc- groups as indicating substantial disruption of the α-helix through significant destabilization of amide solvation, including at residue 3, which has the potential for additional destabilization of the α-helix that goes beyond the Lifson-Roig model employed herein.

Surprisingly, no clear correlation was observed between the electronic properties of the acyl capping group and the α-helicity of the peptides. The lowest α-helicity was observed in the

peptide with the pivaloyl group. The *iso*-butyryl group induced the second lowest α-helicity. Peptides with the propionyl, bromoacetyl, and chloroacetyl groups exhibited intermediate α-helicities. In contrast, the highest α-helicity was observed with the acetyl group, which is intermediate in its electronic properties among those examined. Moreover, the formyl group induced α-helicity essentially the same as that of the acetyl group, despite the substantially lower electron density on its carbonyl oxygen. Thus, the electronic effects of the acyl capping group on the strength of either the hydrogen bonds or $n \rightarrow \pi^*$ interactions appeared to have little impact on the stability of the α-helix. Indeed, the lowest α-helicity was observed with the most electron-rich acyl capping group, while one of the highest α-helicities was observed with the most electron-poor acyl capping group examined, the formyl group. Similarly, the electronically distinct propionyl, methoxyacetyl, and chloroacetyl groups exhibited relatively similar α-helicities.

However, α-helicity seemed to correlate substantially with the steric properties of the acyl group, specifically with the size of the group on the atom adjacent to the carbonyl. The lowest α-helicities were associated with the most sterically demanding pivaloyl and *iso*-butyryl groups, while the highest α-helicities were observed for the least sterically demanding formyl and acetyl groups. However, examination of a model of an α-helical peptide did not suggest any direct basis by which sterically demanding acyl capping groups could reduce α-helicity - the acyl group is located away from both the backbone and the side chains of the residues in the α-helix, and thus should not directly impact α-helicity via sterics.

Investigation of the effects of acyl capping groups on α-helicity using DFT calculations. Since neither direct steric effects on conformation nor electronic effects on the strengths of

hydrogen bonds or $n \rightarrow \pi^*$ interactions could explain the experimental results, we examined the effects of acyl capping group on α-helicity using computational methods. Because hydrogen bonds and $n \rightarrow \pi^*$ interactions are inherently quantum mechanical in nature, these electronic effects of different acyl groups would be manifested fully only in quantum chemistry-based calculations. Therefore, we used DFT methods to understand how the acyl group identity impacted α-helicity. Peptide models X-Ala_{11}-NHMe were developed and analyzed in an α-helical conformation. For the computational analysis, the Piv-, Ac-, and For- groups were employed as limiting cases of electronic and steric properties. These groups also have the substantial computational advantage of symmetry. All other acyl groups examined experimentally herein have multiple available low-energy conformations of the acyl group. Indeed, preliminary investigations with the propionyl group indicated that the propionyl conformation substantially impacted the observed structure of the α-helical peptides.

The peptides Piv-Ala_{11}-NHMe, Ac-Ala_{11}-NHMe, and For-Ala_{11}-NHMe were subjected to geometry optimization in a fully α-helical conformation using the M11-L DFT functional, which is optimized for computational efficiency in larger molecular systems.[39] Implicit solvation was employed (CPCM), with final optimization using either the Def2TZVP or the 6-311++G(d,p) triple-ζ basis sets (Figure 4).[40-42] Peptides were examined for the lengths of the α-helical hydrogen bonds (particularly the $C{=}O_i{\bullet\bullet\bullet}H_{i+4}{-}N$ distance) and for $n \rightarrow \pi^*$ interactions (using the $O_i{:}{\bullet\bullet\bullet}C_{i+1}{=}O$ distance and pyramidalization at the Ala_1 carbonyl). Notably, using these implicit solvent models, the first α-helical hydrogen bonds were bifurcated, with both *i*/*i*+3 3_{10}-helical and *i*/*i*+4 α-helical hydrogen bonds to the acyl group carbonyl, as is also observed at the N-terminus of α-helices in some proteins.[2,3,43-45]

The results here (Figure 4) were unequivocal: the pivaloyl group exhibited the closest hydrogen bonds to the *i*+3 and *i*+4 amides, the closest C_{acyl}=O•••C_{Ala1}=O $n\rightarrow\pi^*$ interaction distances, and the greatest extent of pyramidalization at the Ala_1 carbonyl. In contrast, the formyl group exhibited the longest hydrogen bonds to the acyl group, longer intercarbonyl distances, and the least Ala_1 carbonyl pyramidalization. Thus, these computational results with implicit solvent matched the expectations of the electronic properties of these groups on α-helicity, that the pivaloyl group should best favor α-helix and the formyl group should be the worst for α-helicity, due to the impacts of these acyl groups on the strengths both of hydrogen bonds and of $n\rightarrow\pi^*$ interactions. However, the computational results in implicit solvent stood in stark contrast to the observed experimental data. Therefore, we considered that the effects of acyl capping group on α-helicity might be due to differences in solvation, in particular effects on the solvent-exposed amide N–H groups on the first three residues of an α-helix.

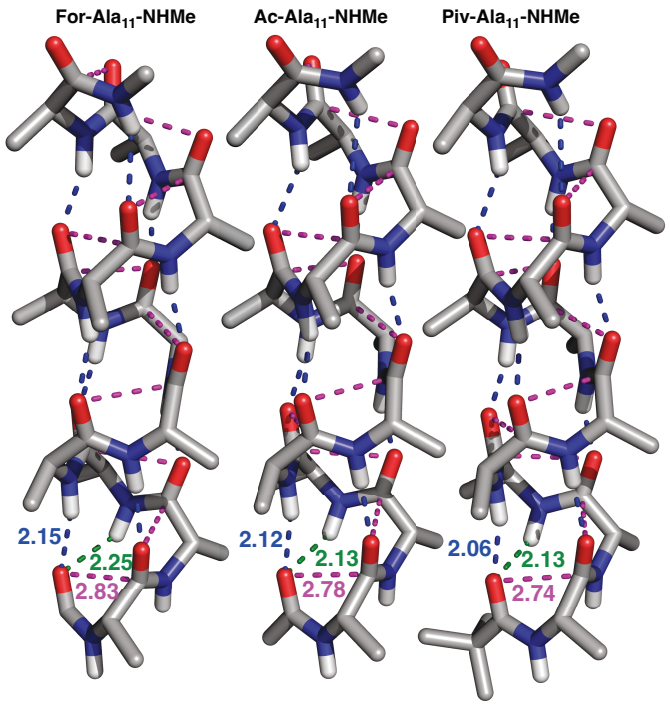


Figure 4. DFT-based computational analysis of the structures of α-helical model peptides with formyl, acetyl, and pivaloyl acyl capping groups and implicit solvation. Geometry optimization calculations were conducted on α-helical model peptides X-Ala$_{11}$-NHMe, where X represents the acyl capping group (formyl, acetyl, and pivaloyl. Calculations were conducted with the M11-L DFT functional and the Def2TZVP basis set in implicit H_2O (CPCM). The α-helical hydrogen bonds (C=O$_i$•••H$_{i+4}$–N distance, Å, blue), *i*/*i*+3 3$_{10}$-helical hydrogen bond to the acyl group carbonyl (C=O$_i$•••H$_{i+3}$–N distance, Å, green), and $n\rightarrow\pi^*$ interactions (O$_i$:•••C$_{i+1}$=O distance, Å, purple) are shown. Similar results were obtained using the 6-311++G(d,p) basis set (Table S3).

Computational investigations of acyl group identity on α-helicity with explicit water. In order to investigate the roles of solvation on α-helicity as a function of acyl capping group, computational investigations were conducted on the peptides Piv-Ala$_{11}$-NHMe, Ac-Ala$_{11}$-NHMe, and For-Ala$_{11}$-NHMe, with 3–6 explicit water molecules on the N-terminus and/or C-terminus (Figure 5).[46,47] Geometry optimization was conducted as described above. In preliminary investigations, it was found that two bridging water molecules at the C-terminus helped prevent fraying of the α-helix there, resulting in typical α-helical geometries. In addition, models with 3

or 4 explicit water molecules at the N-terminus allowed solvation of all amide N–H groups and resulted in canonical α-helical geometry, with the acyl group hydrogen-bonded to only the *i*+4 residue amide hydrogen. These water models at the N-terminus allowed investigation of how the identity of the acyl capping group could impact amide solvation by water.

The computational results with explicit solvation were clear, and were also independent of the basis set employed or the exact number of explicit water molecules in the calculations (Figure 5, Table S3). Hydrogen bonds of the Ala amide N–H groups to water were closest with the formyl group, of somewhat longer distance with acetyl group, and substantially longer with the pivaloyl group. The largest effects were observed at the first and third amide hydrogens (*i*+1 and *i*+3 to the acyl carbonyl), where these acyl methyl groups are closest to the amide hydrogens, and appear to push the water molecules away from ideal hydrogen-bond geometries. In contrast to the results with fully implicit solvation, the acyl group carbonyl hydrogen bonds to the Ala_4 amide N–H were similar for all three acyl groups examined. Explicit solvation also functionally eliminated the 3_{10}-helix-type hydrogen bonding between the acyl carbonyl and the Ala_3 amide N–H, yielding peptides with fully canonical α-helical hydrogen bonding patterns. Notably, even with explicit solvation, the pivaloyl group exhibited the closest $n \rightarrow \pi^*$ interaction and the greatest extent of Ala_1 carbonyl pyramidalization, although the differences between acyl groups were smaller with explicit solvation than with implicit solvation.

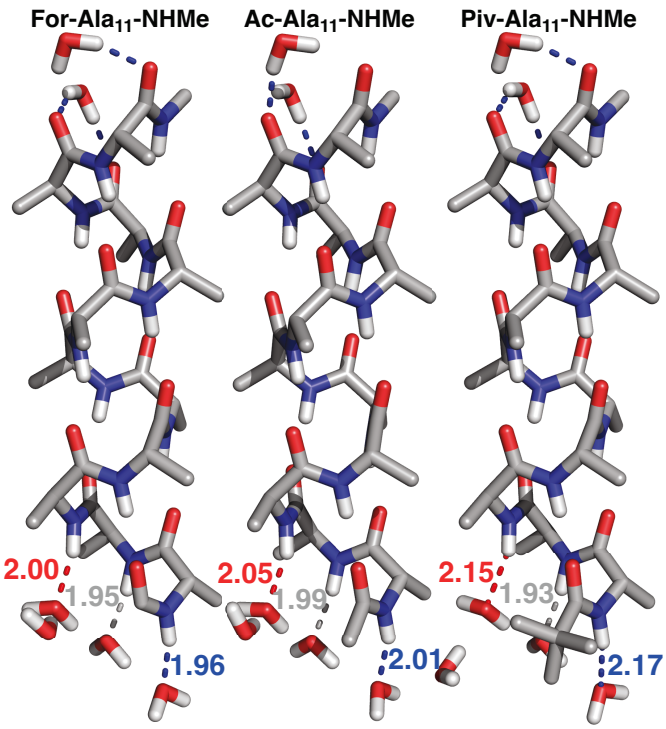


Figure 5. DFT-based computational analysis of α-helical model peptides with formyl, acetyl and pivaloyl N-acyl capping groups with 6 explicit H_2O molecules. Geometry optimization calculations were conducted on minimal α-helical models, X-Ala_{11}-NHMe, where X represents the different N-acyl capping groups (formyl, acetyl, and pivaloyl), with 6 explicit H_2O molecules (2 bridging H_2O molecules on the C-terminus; 4 H_2O molecules on the N-terminus, including one H_2O molecule hydrogen-bonded to each solvent-exposed amide N–H, plus one additional H_2O molecule hydrogen-bonded to the H_2O molecule on the N3 amide). Calculations were conducted using the M11-L DFT functional and the Def2TZVP basis set in implicit H_2O (CPCM). The amide H to water O distances (H_2O•••H–N1 distance, Å, blue), (H_2O•••H–N2 distance, Å, grey), and (H_2O•••H–N3 distance, Å, red) are shown. Similar results were obtained using the 6-311++G(d,p) basis set, or on models with 3 or 4 N-terminal water molecules and no C-terminal water molecules (Table S3).

The computational data with explicit solvation provide an explanation for the experimental data on the effects of acyl capping group on α-helicity. Sterically smaller acyl capping groups (i.e. the formyl group) allow the most favorable hydrogen bonding of water molecules to solvent-exposed amide N–H groups at Ala_1, Ala_2, and Ala_3, with the most dramatic effects due to changes in amide-water hydrogen bond lengths at Ala_1 and Ala_3. As lengths of noncovalent interactions correlate generally with their strength, with shorter distances associated

with stronger interactions, these results suggest that a significant reason why the formyl and acetyl N-capping groups yield peptides with similar α-helicity, despite the substantial difference in the electronic properties of the acyl groups, is due to differences in the hydrogen bonding of the solvent-exposed amide N–H bonds to water that counterbalance the inherent electronic properties of these groups. The least sterically demanding formyl group allows maximal stability in water-amide hydrogen bonds, due to their ability to be geometrically optimized without steric hindrance from the acyl capping group. This effect would be expected to be both enthalpic and entropic, by allowing good hydrogen bonds to the amide N–H with many different water geometries, and with the formyl group allowing the most possible modes for water to hydrogen bond favorably with the N-terminal amide hydrogens.

In contrast, while the acetyl group can afford stronger intrahelical hydrogen bonds, the larger size of a methyl group (acetyl) versus a hydrogen (formyl) results in longer (weaker) water-amide hydrogen bonds due to the greater steric demands of the acetyl group. Finally, the pivaloyl group, while inherently capable of both stronger intrahelical hydrogen bonds and stronger $n \rightarrow \pi^*$ interactions, significantly disrupts the water-amide hydrogen bonds at the N-terminus of the α-helix, due to the size and steric demands of the pivaloyl group. This interpretation also explains the intermediate α-helicity of the peptides with propionyl, bromoacetyl, chloroacetyl, and methoxyacetyl groups: while these groups differ substantially electronically, they are sterically similar α-substituted acetyl groups, and thus similarly impact water hydrogen bonding to the N-terminal amide N–H groups.

These results were confirmed on shorter X-Ala_7-NHMe peptides with 5 explicit water molecules. The shorter sequence allowed investigation with the more computationally rigorous M06-2X DFT functional,[48] in addition to the M11-L functional, with 5 different basis sets

examined for each. Independent of DFT functional or basis set employed, the results were the same as seen above in X-Ala_{11}-NHMe peptides: the formyl group exhibited the closest water-amide hydrogen bonds, while the pivaloyl group exhibited substantially longer water-amide hydrogen bonds (Figure S1, Table S4).

In addition, similar calculations were conducted with only 4 explicit H_2O molecules, removing the water molecule at the N3 amide, with explicit H_2O molecules only at the N1 and N2 amides. Geometry optimization resulted in a bifurcated hydrogen bond between the acyl carbonyl and both the N3 and N4 amide hydrogens (e.g. as was seen in Figure 4). These structures allowed us to quantify the strength of the amide-water hydrogen at residue 3 as a function of acyl cap. By these methods, the amide-water hydrogen bond at the N3 amide was 2 kcal mol^{-1} less stable with the pivaloyl capping group compared to an acetyl or formyl group (Figure S2, Table S6).

Acyl group effects on amide-water hydrogen bonding in model small-molecule amides. In order to further explore the role of acyl capping group identity on solvation, we examined models of X–NHMe, X = Piv-, Ac-, or For-, with hydrogen bonds to 1–3 water molecules, on the amide N–H, on the carbonyl O, or both (Figure 6).[49] These models were subjected to geometry optimization using DFT methods.[48,50] These structures were then subjected to energy calculations to determine water hydrogen bond strengths using the MP2 method with the large aug-cc-pVQZ basis set.[51] The geometry optimization calculations indicated that the pivaloyl group exhibited the most favorable water hydrogen bonds on the carbonyl oxygen, but the most distant water hydrogen bonds on the amide hydrogen. However, the differences in hydrogen bond lengths here were smaller than those observed in calculations on α-helical peptides. Notably, with the pivaloyl group, the N–H•••OH_2 hydrogen bond deviated substantially from linearity (Figure 6c)

due to a steric clash between the water molecule and the pivaloyl group, again indicating that the pivaloyl group sterically disrupts optimal amide•water hydrogen bonding.

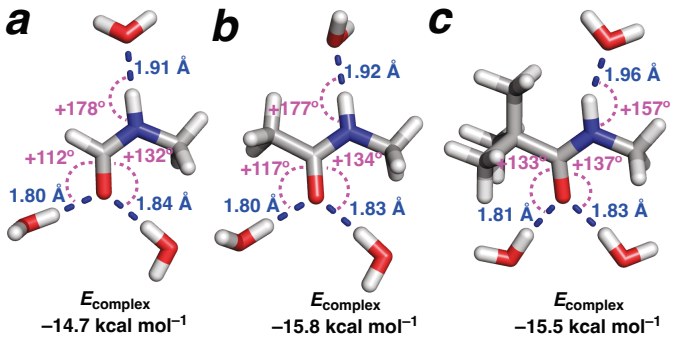


Figure 6. Acyl group effects on amide-water hydrogen bonding in small-molecule amides. Models of X–NHMe, where X = For-, Ac-, or Piv-, are depicted interacting with 3 water molecules. These models were generated via geometry optimization using M06-2X method with the aug-cc-pVTZ basis set in implicit water. Solvation energies were determined by the MP2 method with the aug-cc-pVQZ basis set in implicit water. Distances of hydrogen bonds are indicated in blue. Angles of hydrogen bonds are indicated in magenta. Solvation energies for structures with one, two, or three water molecules for each structure are indicated in Table S7.

However, in these model systems, while the pivaloyl group exhibited the longest (weakest) water hydrogen bonds to the amide N–H groups, energy calculations on complexes with individual water molecules indicated that the pivaloyl group had the strongest water hydrogen bonds to the carbonyls (Figure S3). The opposite was true for the formyl group, with the acetyl group being intermediate in all cases. These results suggest that the electronic effects of the acyl group could directly impact solvation and hydrogen bond strength to water at all hydrogen bonding sites. The more electron-rich pivaloyl group exhibits stronger water hydrogen bonds at its carbonyl but weaker water hydrogen bonds at the amide N–H; the opposite is true for the formyl group. Thus, the low α-helicity of peptides with a pivaloyl acyl capping group could be due to a combination of (1) weaker hydrogen bonds to water at the 3 N-terminal amide hydrogens, due to steric and/or electronic effects of the pivaloyl carbonyl; and (2) stronger

hydrogen bonds to water at the pivaloyl carbonyl oxygen, and thus a greater desolvation energy cost in order for the pivalolyl carbonyl oxygen to hydrogen bond to the N4 amide of the α-helix (though this could be partially or fully compensated by a stronger pivaloyl-amide hydrogen bond and a stronger pivaloyl $n \rightarrow \pi^*$ interaction). Overall, the experimental data, supplemented with the computational data using explicit solvent models, indicate that solvation of the N-terminal amides is a central determinant of the impact of acyl capping groups on α-helicity.

Investigation of acyl capping group identity effects on α-helicity using molecular dynamics calculations. The quantum mechanics-based calculations strongly suggested that the differences in α-helicity of peptides with different acyl capping groups were primarily due to differences in solvation of the amide hydrogens. However, while DFT calculations are highly rigorous in understanding the inherent nature of bonding, they also provide only a static picture, without addressing the inherent dynamics in both peptide structure and in hydrogen bonding to water. Therefore, we conducted molecular dynamics (MD) calculations on X-Ala_{11}-NHMe peptides (X = Piv-, Ac-, For-) in a box of explicit water molecules. These simulations explicitly sample a large number of water hydrogen bonding patterns, in both the folded (α-helical) and unfolded (random coil) states, as well as intermediate states, and thus can address both the enthalpy (lowest energy structures) and entropy (number of possible geometries of interaction) of water-amide hydrogen bonding.

The MD calculations qualitatively matched both the experimental data and the conclusions of the quantum-mechanical computational data (Figure 7). The peptide with a pivaolyl group exhibited substantially lower α-helicity than the other peptides, which had similar overall α-helicity (Figure S4). Examination of the distances of amide hydrogens to water

molecules indicated substantial differences in solvation as a function of acyl capping group (Figure 7). The formyl derivative exhibited both the closest overall amide-water hydrogen bonds, and also the tightest distribution of $H^{N}\bullet\bullet\bullet OH_2$ bond lengths that was the closest to optimal amide-water hydrogen bond lengths. In contrast, the acetyl group exhibited somewhat longer amide-water hydrogen bond lengths and a wider distribution. Dramatically, the pivaolyl group resulted in substantially longer amide-water hydrogen bonds, as well as a distribution that skewed substantially wider and more distant than those of either the acetyl or formyl groups. These MD results corroborate our conclusions from DFT calculations, that the primary effect of acyl groups on α-helicity in peptides is in impacting the structure, geometry, and stability of amide-water ($N–H\bullet\bullet\bullet OH_2$) hydrogen bonds.

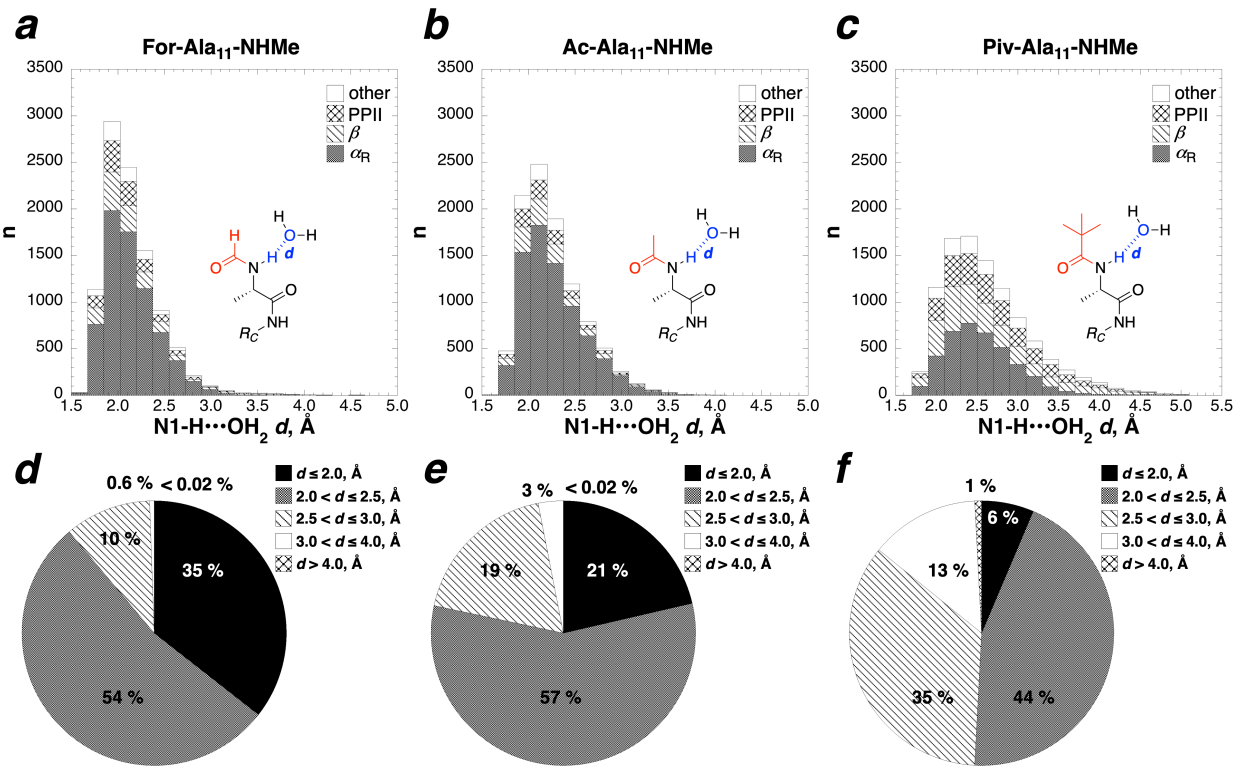


Figure 7. Analysis of amide•water hydrogen bonds in α-helical model peptides via molecular dynamics calculations. (a–c) Histograms of the minimum distance between any water oxygen atom and the amide hydrogen of the first residue (H_2O•••H–N1 distance, *d*). The distribution of secondary structures (α_R, β, PPII, and other) at the first residue is indicated for (a) For-Ala_{11}-NHMe, (b) Ac-Ala_{11}-NHMe, and (c) Piv-Ala_{11}-NHMe. The secondary structures of the residues are defined as follows: α-helical (α_R): ($-110° \le \phi \le -30°$, $-80° \le \psi \le +30°$); β/extended: ($-180° < \phi < -90°$ and [$\psi \le -120°$ or $+180° > \psi \ge +60°$]); and PPII: ($-90° \le \phi \le -40°$ and $+100° \le \psi \le +180°$). (d–f) Analysis of amide-water hydrogen bond distances of peptides in the α-helical conformation. The minimum distances (H_2O•••H–N1 distance, *d*) for hydrogen bonds to amide 1 (N1) with the distances $d \le 2.0$ Å, $2.0 < d \le 2.5$ Å, $2.5 < d \le 3.0$ Å, $3.0 < d \le 4.0$ Å, and $d > 4.0$ Å shown for the peptides (d) For-Ala_{11}-NHMe, (e) Ac-Ala_{11}-NHMe, and (f) Piv-Ala_{11}-NHMe. The analyses are based on geometry-optimized models from Figure 5, which were then subjected to 100 ns MD simulations in GROMACS using the CHARMM36 force field in explicit TIP3P water. Additional details of MD simulations are in the Supporting Information.

Discussion

Herein, we tested the ability of N-terminal acyl capping groups to impact α-helicity in model peptides. More electron-rich acyl groups might be expected to stabilize the α-helix both through stronger *i*/*i*+4 C=O•••H–N hydrogen bonds and through stronger *i*/*i*+1 $n \rightarrow \pi^*$

interactions. However, experimentally, we found that the most electron-rich acyl group examined (pivaloyl) strongly *destabilized* the α-helix. In contrast, the most electron-poor acyl group examined (formyl) induced α-helicity nearly identical to that of the acetyl group.

Thus, while the identity of the acyl capping group can predictably impact the strength of its intrapeptide noncovalent interactions in model compounds and in model peptides of PPII structure,[33,34,52,53] the data herein suggested that acyl capping group identity impacted α-helical structure primarily through mechanisms other than those directly observed within the peptide structure. The N-terminus of an α-helix with an acyl capping group has three solvent-exposed amide hydrogens (Figure 1b). These unsatisfied hydrogen-bond donors interact with water in an isolated α-helix, and thus these amide-water hydrogen bonds do not *directly* contribute to the stability of the α-helix. In proteins, these unsatisfied hydrogen-bond donors are frequently observed to interact with hydrogen-bond acceptors (e.g. Ser/Thr hydroxyls, Asp/Asn/Glu/Gln carbonyls) within the protein, including substantially with α-helix capping motifs that are important to α-helix and protein stability.[2-4,12,38,54] However, it is unclear how these hydrogen bonds substantially stabilize protein structure, as they replace amide-water hydrogen bonds and side chain-water hydrogen bonds present in the unfolded state that should be energetically similar. Indeed, α-helix capping motifs typically include hydrophobic elements to stabilize these intramolecular protein-protein hydrogen bonds, with the hydrophobic effect central to overcoming the entropic cost of adopting defined structures required for these intraprotein hydrogen bonds.[12] However, there are also numerous examples of local capping structures stabilizing α-helicity seemingly primarily through hydrogen bonding to the unsatisfied amide hydrogens, including amino acids with hydrogen-bond donor groups that exhibit higher α-helix propensity in the first turn of the α-helix than they do at other locations in the α-helix.[35,38,54-56]

More generally, in an isolated α-helix, without defined helix-capping motifs or groups, the unfolded and folded states *both* exhibit amide-water hydrogen bonds at the first three residues. Therefore, if the strength of water-amide hydrogen bonds is identical in *both* the unfolded and folded states, then the presence of solvent-exposed amide hydrogens at the N-terminus of the α-helix should *not* be destabilizing.

The combined experimental and computational data above, however, strongly suggest that water-amide hydrogen bonding to the N-terminal amide hydrogens of an α-helix is *not* the same in the unfolded and folded states, but instead suggest that amide-water (N–H•••OH_2) hydrogen bonds are stronger in the unfolded state than in an α-helix. The most compelling data involved comparison between the acetyl and formyl groups, which exhibited nearly identical α-helicity in solution. This similar α-helicity was observed despite the formyl group being a worse electron donor for both the *i*/*i*+4 C=O•••H–N hydrogen bond that stabilizes the first turn of the α-helix and the *i*/*i*+1 O_i:•••C_{i+1}=O $n\rightarrow\pi^*$ interaction that stabilizes the α-helical conformation in both the absence and presence of a hydrogen bond. Thus, based on standard helix-coil theory, the formyl group should induce *reduced* α-helicity via a substantial reduction in its helix nucleation parameter. Moreover, the pivaloyl group should be even better at helix nucleation, due to its greater electron-donor capability at the carbonyl. Instead, the pivaloyl group was dramatically worse in inducing α-helicity, and overall suggested that the steric effects of the acyl capping group overwhelmed the inherent electronic effects of these groups.

The computational data with explicit water (Figure 5, Figure 7) clearly indicated that, in an α-helix, even the acetyl group exhibited steric clashes with water molecules bound to the solvent-exposed amide hydrogens, resulting in longer (weaker) amide-water hydrogen bonds. These effects were seen both in static structures determined by quantum-mechanical calculations

and in dynamic structures determined by MD. The larger the acyl capping group, the greater the disruption of water-amide hydrogen bonding that was observed at the N-terminus of the α-helix. The effects of the steric clash between the acyl group in weakening amide-water hydrogen bonding were likely both enthalpic (weaker [longer] hydrogen bonds) and entropic (greater conformational restriction in water molecules to adopt stable hydrogen-bonded structures). These steric effects of the acyl carbonyl (*i*) on hydrogen bond lengths and geometries were substantial at both the first (*i*+1) and third (*i*+3) amide hydrogens, which are geometrically closest to the acyl group in an α-helix.

Serrano and Fersht examined the impact of Ala versus Gly residues on protein stability at the N-cap position of the two α-helices in barnase.[4,7,57] The N-cap position in these proteins is in a PPII conformation, and thus, Ala should be inherently favored over Gly.[58,59] In contrast, the experimental data demonstrated that Ala destabilized barnase by 0.5–1.2 kcal mol^{-1}. They proposed that Ala destabilized the α-helix primarily via the steric effect of disruption of amide-water hydrogen bonds at the N-terminus of the α-helix. Subsequent analysis of these protein variants by X-ray crystallography indicated that the water molecule bound to residue 3 of the α-helix was substantially longer in the protein with Ala than the protein with Gly (N•••O distances 3.3 Å versus 2.7 Å).[60]

Baldwin and Doig made similar conclusions on the effects of N-cap residues on α-helicity in peptides (of the same sequence as those examined here, but with the acyl (X) group being uncapped amino acids). Peptides with an N-terminal Gly had greater α-helicity than those with Ala, Leu, Ile, and Val (Table 2).[38] They proposed that these observed effects on α-helicity were due to the impact of the side chain of the N-cap residue on amide-water hydrogen bonds. In order to understand whether these proposed steric effects on amide-water hydrogen bonding

were observed generally in proteins, we examined the hydrogen bonds of amides at the N-termini of α-helices to water molecules in ultra-high resolution crystal structures in the PDB. These results (Table 2) strongly support the conclusions of Serrano, Fersht, Doig, and Baldwin: proteins with Gly N-cap residues had the highest percent of structures with bound water molecules within hydrogen-bonding distance (N•••O distance ≤ 3.5 Å), at both the N1 and N3 amides. In contrast, proteins with the more sterically demanding β-branched residues Ile or Val at the N-cap had the fewest crystallographically observed amide-water hydrogen bonds, with substantially longer amide-water distances than in proteins with Gly.

Table 2. Bioinformatics data on water-amide hydrogen bonds on the N-terminus of α-helices in the PDB as a function of acyl N-cap residue, compared to CD data on peptides with the indicated residue as the acyl N-cap.

N-cap residue	N1···OH_2, $d < 3.5$ Å[a]	N3···OH_2, $d < 3.5$ Å[a]	$[\theta]_{222}$[b]
Gly	56%	42%	–17900
Ala	51%	20%	–12900
Leu	46%	23%	–15300
Ile	46%	17%	–14300
Val	45%	20%	–12800

[a] Percent of high-resolution α-helices in structures in the PDB, where Gly, Ala, Leu, Ile, or Val serves as the N-Cap residue, with a minimum distance between either the first amide nitrogen (N1) or the third amide nitrogen (N3) and a water oxygen atom that is < 3.5 Å.

[b] CD data on the peptides XAKAAAAKAAAAKAAGY-$CONH_2$, X= Gly, Ala, Leu, Ile, or Val, from ref. [61]. These peptides have an unmodified (non-acylated) N-terminus, and thus the carbonyl of the first residue functions as the acyl N-cap.

In an alternative approach, Kemp and coworkers developed α-helix nucleation auxiliaries (α-helix templates) based on diproline sequences cyclized with a thioether.[15,62,63] In these structures, the conformational constraint of cyclization aligned the proline carbonyls (residues 1, 2) for direct hydrogen bonding to the *i*+4 (residues 5, 6) amide groups. Thus, the acyl group ("residue" 0, the *i* position/N-cap of the α-helix as defined herein) on the template makes a hydrogen bond to the amide hydrogen of residue 4. Therefore, only the first non-template residue of the α-helix (residue 3) needs to be organized in order to adopt an α-helix. Because residues 1 and 2 are proline, which lacks an amide hydrogen, these templated α-helices only have one solvent-exposed amide hydrogen, at residue 3. The roles of acyl capping groups on α-helicity were examined in these templated structures.[63] Although the work was predominantly focused on the identification of charged acyl capping groups that resulted in the highest α-helicity, in a limited series they also found that α-helicity correlated with the sterics of the acyl capping group, Ac- ≥ For- > ClAc- > Prp- >> Piv-. These α-helix capping groups would also impact the strength of the *i*/*i*+4 hydrogen bond and *i*/*i*+1 $n \rightarrow \pi^*$ interactions, with the pivaloyl group the most favorable and with the formyl group least favorable. The experimental observation of trends that opposed the expected electronic effects of the acyl group on α-helicity, as was observed herein with canonical (non-templated) α-helical peptides, is consistent with the impact of the acyl group on solvation of the single (*i*+3) solvent-exposed amide hydrogen in these templated peptides. Thus, Kemp's data are consistent with our proposal that the identity of the acyl capping group impacts amide-water hydrogen bonding at both the *i*+1 and *i*+3 amide hydrogens.

Solvation of the N3 amide hydrogen can be accomplished three ways (Figure 8): (1) via direct solvation by a water molecule; (2) via a bifurcated hydrogen bond of the carbonyl of the N-cap with both the N3 and N4 amide hydrogens (i.e. both 3_{10}- and α-helical hydrogen bonding patterns); and (3) via an α-helix capping interaction with a side-chain hydrogen-bond acceptor. Analysis of proteins indicates that an oxygen on the side chain of the N-cap residue (e.g. Ser, Thr) most commonly exhibits hydrogen bonds with the N3 amide N–H, and that this interaction is particularly stabilizing.[2-4,12,38,44,57] Bifurcated hydrogen bonds between the N-cap carbonyl and the N3 and N4 amide hydrogens (Figure 8b) are also frequently observed in proteins.[44,45] Thus, the combined experimental, computational, and bioinformatics data suggest that an amide-water hydrogen bond at the N3 residue of an α-helix is significantly weaker compared to an amide-water hydrogen bond in the disordered state of a protein, primarily due to steric effects that prevent ideal amide-water hydrogen bond geometry at this position.

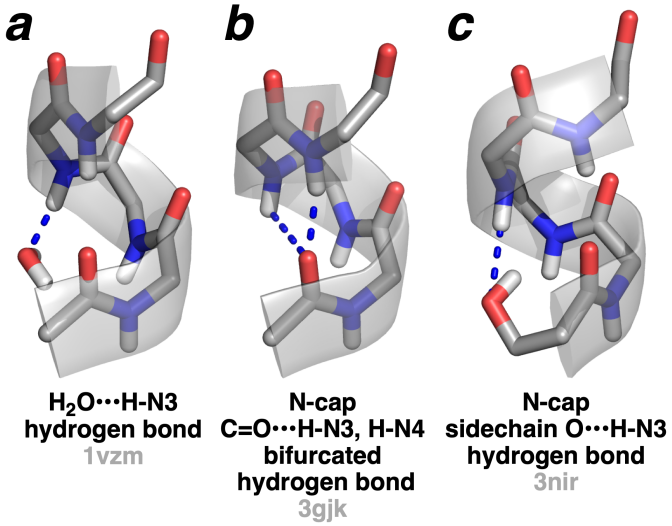


Figure 8. Solvation modes for the N3 amide hydrogen of α-helices. The N3 amide hydrogen can be solvated through (a) direct hydrogen bonding with a water molecule; (b) a dual hydrogen bond involving the N-cap carbonyl, which exhibits a bifurcated hydrogen bond to both the N3 and N4 amide hydrogens (combined 3_{10}-helix and α-helix hydrogen bonding patterns); or (c) an α-helical capping interaction with a side-chain hydrogen-bond acceptor.

The observation that the acetyl group is modestly better than the formyl group in inducing α-helicity, despite its weaker amide-water hydrogen bonding in an α-helix, is consistent with the electronic effects of the acyl group being important in intrahelix noncovalent interactions. However, their overall similarity in α-helicity, and the much worse α-helicity of more electron-rich (but more sterically demanding) acyl groups, indicates the dominance of the strength of amide-water hydrogen bonds in determining α-helicity in acyl groups larger than acetyl.

Solvation effects on α-helix stability have also been previously addressed with denaturants such as urea, or with α-helix-inducing solvents such as trifluoroethanol (CF_3–CH_2–OH, TFE).[64-66] Urea is believed to promote the denatured state in proteins primarily via its hydrogen bonding to backbone carbonyl oxygens and amide hydrogens, with urea exhibiting stronger hydrogen bonds to the backbone than the intramolecular hydrogen bonds (e.g. the *i*/*i*+4 hydrogen bonds of an α-helix) in proteins, resulting in the loss of hydrogen-bonded secondary structure. Alternatively, the ability of TFE to stabilize α-helical structures is primarily due to weaker hydrogen bonding between its O lone pairs (a result of the inductive effect of the fluorines in reducing the basicity of the O) and the amide hydrogens of the backbone.[65] Thus, in water, amide-water NH•••OH_2 and amide-amide NH•••O=C hydrogen bonds are relatively similar in strength, with slightly stronger amide-amide hydrogen bonds being the likely basis for observed α-helix formation in water.[46,49,67] However, in TFE, the amide-TFE NH•••$O(H)CH_2CF_3$ hydrogen bond is substantially weaker, thus promoting the α-helix due to the greater strength of α-helical backbone hydrogen bonds compared to TFE•amide hydrogen bonds. The data herein further support the importance of solvent-amide hydrogen bonds on α-helix stability, and demonstrate that subtle changes in solvent•amide hydrogen bond strength can dramatically

impact α-helical structure. Disruption of optimal hydrogen bonding structure to water at the solvent-exposed amide hydrogens of an α-helix is inherently destabilizing.

This disruption of water-amide hydrogen bonding can be caused even by an acyl group as small as the acetyl group. Within proteins, at the carbon α to the carbonyl, the protein side chain and the continuation of the protein main chain on the N-capping (*i*) residue of α-helices is substantially more sterically demanding that that of a formyl group, comparable to a first approximation to that of the *iso*-butyryl group that was found herein to greatly destabilize α-helical structure. As such, we conclude that the increased stability of hydrogen-bonding N-capping groups in α-helices is due to the relative destabilization of amide-water hydrogen bonds within α-helices compared to in the unfolded state. Protein capping groups are stabilizing in part due to the relative weakness of amide-water hydrogen bonds at the N-terminus of the α-helix compared to in the unfolded state. More broadly, the results herein provide further evidence of the importance of solvent structure and solvent-backbone hydrogen bonds on protein structure.

Methods

Peptide synthesis. Peptides were synthesized by standard methods in solid-phase peptide synthesis. Final acylation was conducted using acid chlorides or using carboxylic acids with an amide coupling reagent. Peptides were purified to homogeneity and characterized by mass spectrometry for identity. Details of peptide synthesis, purification, and characterization are in the Supporting Information.

Circular dichroism. CD experiments were conducted on a Jasco J-810 or J-1500 spectropolarimeter using a 1 mm cell, at 0.5 or 20 ˚C, with peptide concentrations of 50–150 μM, in 5 mM phosphate buffer (at pH 4.0, 7.0, or 8.5, as indicated) containing 25 mM KF. The data

are the average of at least three independent trials, with spectra collected every nm, an averaging time of 8 s and at least three accumulations. Peptide concentrations were determined by UV-Vis spectroscopy. Data were background corrected but not smoothed. Additional details are in the Supporting Information.

Computational chemistry. Calculations were conducted with Gaussian 09.[68] Initial models of the Ac-Ala_{11}-NHMe peptides in an α-helical conformation were generated with amber. These mechanics-based models were then subjected to geometry optimization using DFT methods, using the M11-L DFT functional and implicit solvation (CPCM), with iterative increases in sizes of the basis set in order to achieve greater accuracy in the models.[39,42] The acetyl functional group was also modified to the formyl and pivaloyl functional groups, and these models were also subjected to geometry optimization. Final geometry optimization was conducted on each peptide using the Def2TZVP and the 6-311++G(d,p) basis sets.[40,41] These models then had 1–6 explicit water molecules added, in order to best represent solvation in α-helical peptides and proteins. These models with explicit solvation were similarly subjected to geometry optimization as described above. Similar approaches were applied to geometry optimization of X-Ala_7-NHMe peptides (X = Piv-, Ac-, For-) with 0–5 explicit water molecules, using the M11-L and/or M06-2X[48] DFT functionals.

In addition, simple models of amide solvation were generated, using For-NHMe, Ac-NHMe, and Piv-NHMe structures, in the absence of explicit water and in the presence of 1–3 explicit water molecules on the amide N–H and/or the carbonyl oxygen. After geometry optimization with the M06-2X DFT functional and the aug-cc-pVTZ basis set in implicit water, the energies of these complexes were determined via analysis of the energies in the presence or absence of water, using the MP2 method and the aug-cc-pVQZ basis set in implicit water, which

was used in order to minimize the effects of basis-set superposition error.[42,48,50,51,69] Additional geometric details of all computational models, as well as their relative energies and the coordinates for all models, are in the Supporting Information.

Molecular dynamics. The structures of For-Ala_{11}-NHMe, Ac-Ala_{11}-NHMe, Piv-Ala_{11}-NHMe, determined from geometry optimization using the M11-L functional and Def2TZVP basis set in implicit water as described above, were used as initial models. The models were transformed to mol2 format using AVOGADRO,[70] and corrections were made in the text editor as required. The bonds were sorted using the sort_mol2_bonds.pl script.[71] The processed mol2 files were uploaded to the CHARMM General Force Field server to produce CHARMM topology files, then converted to GROMACS formats using the cgenff_charmm2gmx.py script.[72] The peptides were placed in dodecahedron boxes, distanced 1.0 nm from the edges and solvated using TIP3P water.[73] Energy minimization used the steepest descent with a 10.0 kJ/mol/nm force tolerance and 0.01 nm step for 50,000 steps. Equilibration had two 100 ps phases (NVT and NPT), both at a 2 fs time step, followed by simulation for 100 ns. Temperature and pressure were set to 300 K and 1.0 bar. Data were saved every 10 ps. Analysis was conducted with VMD to determine the dihedral angles of the first three residues.[74] A custom python script with the MDanalysis package was used to measure minimum distances between water oxygens and peptide amide hydrogens.[75] Further details are in the Supporting Information.

Bioinformatics analysis of bound water molecules at the N-terminus of α-helices in the PDB. On January 22, 2024, the Protein Data Bank (PDB) was queried via the PISCES server[76] to identify structures with resolution ≤ 1.6 Å, an R-factor ≤ 0.25, sequence length ≥ 40 residues, and sequence identity ≤ 20%. The search was restricted to only include structures determined by X-ray crystallography. This search yielded a total of 3,054 initial structures. The

dataset was reduced to incorporate only chain A from these structures. Structures with α-helices with a minimum of five residues, inclusive of the N-cap residue, were included, while those lacking a five-residue helix were excluded. Post-filtration, 2,834 PDB files remained, representing a cumulative count of 23,064 α-helices that were at least five residues in length. The N-cap residues in these α-helices were as follows: 2,071 with Gly; 948 with Ala; 979 with Leu; 434 with Ile; and 572 with Val.

Python scripts, utilizing the MDAnalysis package, were used for analysis. These scripts parsed α-helix attributes in PDB files and gathered data including PDB ID, Chain ID, Residue Name, Residue ID, Resolution, and the order of residues within the α-helix. The residue number was defined as 0 for the N-cap residue, followed by 1 through 4 for the subsequent residues in the α-helix. A key metric extracted was the minimum distance (in Å) between an amide nitrogen and the oxygen of the nearest water molecule, analyzed separately for residues 0–4 of the α-helix. These distances, representing the proximity between amide nitrogens and water, were categorized based on their position in the helix: N0•••OH_2 (N-cap), N1•••OH_2 (first residue), N2•••OH_2 (second residue), N3•••OH_2 (third residue), and N4•••OH_2 (fourth residue). Additional details are in the Supporting Information.

Acknowledgements

We thank Professor Andrew Doig for the code for CapHelix and for help in Lifson-Roig analysis. We thank NSF (CHE-2004110, CHE-1412978, and BIO-1616490) for funding. Instrumentation support was provided by NIH (GM110758) and NSF (CHE-1229234).

Supporting Information Available

Details of peptide synthesis, purification, and characterization; additional details of computational investigations; and coordinates of all geometry-optimized structures. This material is available free of charge via the journal web site.

References

(1) Pauling, L.; Corey, R. B.; Branson, H. R. The Structure of Proteins - 2. Hydrogen-bonded Helical Configurations of the Polypeptide Chain. *Proc. Natl. Acad. Sci. USA* **1951**, *37*, 205-211.

(2) Richardson, J. S.; Richardson, D. C. Amino-Acid Preferences for Specific Locations at the Ends of Alpha-Helices. *Science* **1988**, *240*, 1648-1652.

(3) Presta, L. G.; Rose, G. D. Helix Signals in Proteins. *Science* **1988**, *240*, 1632-1641.

(4) Serrano, L.; Fersht, A. R. Capping and Alpha-Helix Stability. *Nature* **1989**, *342*, 296-299.

(5) Marqusee, S.; Robbins, V. H.; Baldwin, R. L. Unusually stable helix formation in short alanine-based peptides. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 5286-5290.

(6) O'Neil, K. T.; Degrado, W. F. A Thermodynamic Scale for the Helix-Forming Tendencies of the Commonly Occurring Amino-Acids. *Science* **1990**, *250*, 646-651.

(7) Serrano, L.; Sancho, J.; Hirshberg, M.; Fersht, A. R. Alpha-Helix Stability in Proteins .1. Empirical Correlations Concerning Substitution of Side-Chains at the N and C-Caps and the Replacement of Alanine by Glycine or Serine at Solvent-Exposed Surfaces. *J. Mol. Biol.* **1992**, *227*, 544-559.

(8) Blaber, M.; Zhang, X. J.; Matthews, B. W. Structural Basis of Amino-Acid Alpha-Helix Propensity. *Science* **1993**, *260*, 1637-1640.

(9) Lyu, P. C.; Wemmer, D. E.; Zhou, H. X.; Pinker, R. J.; Kallenbach, N. R. Capping Interactions in Isolated Alpha-Helices - Position-Dependent Substitution Effects and Structure of a Serine-Capped Peptide Helix. *Biochemistry* **1993**, *32*, 421-425.

(10) Zhou, H. X. X.; Lyu, P. C.; Wemmer, D. E.; Kallenbach, N. R. Alpha-Helix Capping in Synthetic Model Peptides by Reciprocal Side-Chain Main-Chain Interactions - Evidence for an N-Terminal Capping Box. *Proteins* **1994**, *18*, 1-7.

(11) Myers, J. K.; Pace, C. N.; Scholtz, J. M. Helix propensities are identical in proteins and peptides. *Biochemistry* **1997**, *36*, 10923-10929.

(12) Aurora, R.; Rose, G. D. Helix capping. *Protein Sci.* **1998**, *7*, 21-38.

(13) Pace, C. N.; Scholtz, J. M. A helix propensity scale based on experimental studies of peptides and proteins. *Biophys. J.* **1998**, *75*, 422-427.

(14) Schneider, J. P.; DeGrado, W. F. The design of efficient alpha-helical C-capping auxiliaries. *J. Am. Chem. Soc.* **1998**, *120*, 2764-2767.

(15) Kemp, D. S.; Allen, T. J.; Oslick, S. L. The Energetics of Helix Formation by Short Templated Peptides in Aqueous Solution. 1. Characterization of the Reporting Helical Template Ac-Hel_1. *J. Am. Chem. Soc.* **1995**, *117*, 6641-6657.

(16) Schafmeister, C. E.; Po, J.; Verdine, G. L. An all-hydrocarbon cross-linking system for enhancing the helicity and metabolic stability of peptides. *J. Am. Chem. Soc.* **2000**, *122*, 5891-5892.

(17) Chapman, R. N.; Dimartino, G.; Arora, P. S. A highly stable short a-helix constrained by a main-chain hydrogen-bond surrogate. *J. Am. Chem. Soc.* **2004**, *126*, 12252-12253.

(18) Shepherd, N. E.; Hoang, H. N.; Abbenante, G.; Fairlie, D. P. Single turn peptide alpha helices with exceptional stability in water. *J. Am. Chem. Soc.* **2005**, *127*, 2974-2983.

(19) Glendening, C. R.; Landis, C. R.; Weinhold, F. Natural bond orbital methods. *WIREs Comput. Mol. Sci.* **2012**, *2*, 1-42.

(20) Glendening, E. D.; Landis, C. R.; Weinhold, F. NBO 6.0: Natural bond orbital analysis program. *J. Computational Chem.* **2013**, *34*, 1429-1437.

(21) Shemorry, A.; Hwang, C. S.; Varshavsky, A. Control of Protein Quality and Stoichiometries by N-Terminal Acetylation and the N-End Rule Pathway. *Mol. Cell* **2013**, *50*, 540-551.

(22) Varland, S.; Osberg, C.; Arnesen, T. N-terminal modifications of cellular proteins: The enzymes involved, their substrate specificities and biological effects. *Proteomics* **2015**, *15*, 2385-2401.

(23) Drazic, A.; Myklebust, L. M.; Ree, R.; Arnesen, T. The world of protein acetylation. *Biochim. Biophys. Acta Proteins Proteom.* **2016**, *1864*, 1372-1401.

(24) Kang, L. J.; Moriarty, G. M.; Woods, L. A.; Ashcroft, A. E.; Radford, S. E.; Baum, J. N-terminal acetylation of α-synuclein induces increased transient helical propensity and decreased aggregation rates in the intrinsically disordered monomer. *Protein Sci.* **2012**, *21*, 911-917.

(25) Trexler, A. J.; Rhoades, E. N-terminal acetylation is critical for forming a-helical oligomer of a-synuclein. *Protein Sci.* **2012**, *21*, 601-605.

(26) Bartels, T.; Kim, N. C.; Luth, E. S.; Selkoe, D. J. N-Alpha-Acetylation of α-Synuclein Increases Its Helical Folding Propensity, GM1 Binding Specificity and Resistance to Aggregation. *PLoS One* **2014**, *9*.

(27) Dikiy, I.; Eliezer, D. N-terminal Acetylation Stabilizes N-terminal Helicity in Lipid- and Micelle-bound α-Synuclein and Increases Its Affinity for Physiological Membranes. *J. Biol. Chem.* **2014**, *289*, 3652-3665.

(28) Shoemaker, K. R.; Kim, P. S.; York, E. J.; Stewart, J. M.; Baldwin, R. L. Tests of the Helix Dipole Model for Stabilization of Alpha-Helices. *Nature* **1987**, *326*, 563-567.

(29) Doig, A. J.; Chakrabartty, A.; Klingler, T. M.; Baldwin, R. L. Determination of Free-Energies of N-Capping in Alpha-Helices by Modification of the Lifson-Roig Helix-Coil Theory to Include N-Capping and C-Capping. *Biochemistry* **1994**, *33*, 3396-3403.

(30) Bartlett, G. J.; Choudhary, A.; Raines, R. T.; Woolfson, D. N. n->pi* interactions in proteins. *Nat. Chem. Biol.* **2010**, *6*, 615-620.

(31) Newberry, R. W.; Raines, R. T. The n->pi* interaction. *Acc. Chem. Res.* **2017**, *50*, 1838-1846.

(32) Zondlo, N. J. Fold Globally, Bond Locally. *Nat. Chem. Biol.* **2010**, *6*, 567-568.

(33) Wenzell, N. A.; Ganguly, H. K.; Pandey, A. K.; Bhatt, M. R.; Yap, G. P. A.; Zondlo, N. J. Electronic and steric control of n→π* interactions via N-capping: stabilization of the α-helix conformation without a hydrogen bond. *ChemBioChem* **2019**, *20*, 963-967.

(34) Bhatt, M. R.; Zondlo, N. J. Electronic control of polyproline II helix stability via the identity of acyl capping groups: the pivaloyl group particularly promotes PPII. *submitted* **2023**.

(35) Cochran, D. A. E.; Penel, S.; Doig, A. J. Effect of the N1 residue on the stability of the alpha-helix for all 20 amino acids. *Protein Sci.* **2001**, *10*, 463-470.

(36) Chen, Y.-H.; Yang, J. T.; Chau, K. H. Determination of the helix and beta form of proteins in aqueous solution by circular dichroism. *Biochemistry* **1974**, *13*, 3350-3359.

(37) Chakrabartty, A.; Kortemme, T.; Baldwin, R. L. Helix Propensities of the Amino-Acids Measured in Alanine-Based Peptides without Helix-Stabilizing Side-Chain Interactions. *Protein Sci.* **1994**, *3*, 843-852.

(38) Doig, A. J.; Baldwin, R. L. N- and C-Capping Preferences for All 20 Amino-Acids in Alpha-Helical Peptides. *Protein Sci.* **1995**, *4*, 1325-1336.

(39) Peverati, R.; Truhlar, D. G. M11-L: A Local Density Functional That Provides Improved Accuracy for Electronic Structure Calculations in Chemistry and Physics. *J. Chem. Phys. Lett.* **2012**, *3*, 117-124.

(40) Raghavachari, K.; Binkley, J. S.; Seeger, R.; Pople, J. A. Self-Consistent Molecular Orbital Methods. 20. Basis sets for correlated wave functions. *J. Chem. Phys.* **1980**, *72*, 650-654.

(41) Dunning, T. H., Jr. Gaussian basis sets for use in correlated molecular calculations: The atoms boron through neon and hydrogen. *J. Chem. Phys.* **1989**, *90*, 1007-1023.

(42) Tomasi, J.; Mennucci, B.; Cances, E. The IEF version of the PCM solvation method: an overview of a new method addressed to study molecular solutes at the QM ab initio level. *J. Mol. Struct. THEOCHEM* **1999**, *464*, 211-226.

(43) Stickle, D. F.; Presta, L. G.; Dill, K. A.; Rose, G. D. Hydrogen-Bonding in Globular-Proteins. *J. Mol. Biol.* **1992**, *226*, 1143-1159.

(44) Doig, A. J.; MacArthur, M. W.; Stapley, B. J.; Thornton, J. M. Structures of N-termini of helices in proteins. *Protein Sci.* **1997**, *6*, 147-155.

(45) Leader, D. P.; Milner-White, E. J. The structure of the ends of α-helices in globular proteins: Effect of additional hydrogen bonds and implications for helix formation. *Proteins Struct. Funct. Bioinform.* **2011**, *79*, 1010-1019.

(46) Salvador, P.; Asensio, A.; Dannenberg, J. J. The effect of aqueous solvation upon α-helix formation for polyalanines. *J. Phys. Chem. B* **2007**, *111*, 7462-7466.

(47) Marianski, M.; Dannenberg, J. J. Aqueous Solvation of Polyalanine α-Helices with Specific Water Molecules and with the CPCM and SM5.2 Aqueous Continuum Models Using Density Functional Theory. *J. Phys. Chem. B* **2012**, *116*, 1437-1445.

(48) Zhao, Y.; Truhlar, D. G. The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals. *Theor. Chem. Acc.* **2008**, *120*, 215-241.

(49) Guo, H.; Karplus, M. Solvent Influence on the Stability of the Peptide Hydrogen-Bond - a Supramolecular Cooperative Effect. *J. Phys. Chem.* **1994**, *98*, 7104-7105.

(50) Weigend, F.; Ahlrichs, R. Balanced basis sets of split valence, triple zeta valence and quadruple zeta valence quality for H to Rn: Design and assessment of accuracy. *Phys. Chem. Chem. Phys.* **2005**, *7*, 3297-3305.

(51) Frisch, M. J.; Head-Gordon, M.; Pople, J. A. Direct MP2 gradient method. *Chem. Phys. Lett.* **1990**, *166*, 275-280.

(52) Newberry, R. W.; VanVeller, B.; Guzei, I. A.; Raines, R. T. n ->pi* Interactions of Amides and Thioamides: Implications for Protein Stability. *J. Am. Chem. Soc.* **2013**, *135*, 7843-7846.

(53) Costantini, N. V.; Ganguly, H. K.; Martin, M. I.; Wenzell, N. A.; Yap, G. P. A.; Zondlo, N. J. The distinct conformational landscapes of 4S-substituted prolines that promote an endo ring pucker. *Chem. Eur. J.* **2019**, *25*, 11356-11364.

(54) Penel, S.; Hughes, E.; Doig, A. J. Side-chain structures in the first turn of the alpha-helix. *J. Mol. Biol.* **1999**, *287*, 127-143.

(55) Cochran, D. A. E.; Doig, A. J. Effect of the N2 residue on the stability of the alpha-helix for all 20 amino acids. *Protein Sci.* **2001**, *10*, 1305-1311.

(56) Iqbalsyah, T. M.; Doig, A. J. Effect of the N3 residue on the stability of the alpha-helix. *Protein Sci.* **2004**, *13*, 32-39.

(57) Serrano, L.; Neira, J. L.; Sancho, J.; Fersht, A. R. Effect of Alanine Versus Glycine in Alpha-Helices on Protein Stability. *Nature* **1992**, *356*, 453-455.

(58) Rucker, A. L.; Pager, C. T.; Campbell, M. N.; Qualls, J. E.; Creamer, T. P. Host-Guest Scale of Left-Handed Polyproline II Helix Formation. *Proteins* **2003**, *53*, 68-75.

(59) Brown, A. M.; Zondlo, N. J. A Propensity Scale for Type II Polyproline Helices (PPII): Aromatic Amino Acids in Proline-Rich Sequences Strongly Disfavor PPII Due to Proline-Aromatic Interactions. *Biochemistry* **2012**, *51*, 5041-5051.

(60) Harpaz, Y.; Elmasry, N.; Fersht, A. R.; Henrick, K. Direct Observation of Better Hydration at the N-Terminus of an Alpha-Helix with Glycine Rather Than Alanine as the N-Cap Residue. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 311-315.

(61) Chakrabartty, A.; Doig, A. J.; Baldwin, R. L. Helix Capping Propensities in Peptides Parallel Those in Proteins. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 11332-11336.

(62) Kemp, D. S.; Boyd, J. G.; Muendel, C. C. The helical s constant for alanine in water derived from template-nucleated helices. *Nature* **1991**, *352*, 451-454.

(63) Maison, W.; Arce, E.; Renold, P.; Kennedy, R. J.; Kemp, D. S. Optimal N-Caps for N-Terminal Helical Templates: Effects of Changes in H-Bonding Efficiency and Charge. *J. Am. Chem. Soc.* **2001**, *123*, 10245-10254.

(64) Makhatadze, G. I.; Privalov, P. L. Protein Interactions with Urea and Guanidinium Chloride - a Calorimetric Study. *J. Mol. Biol.* **1992**, *226*, 491-505.

(65) Cammers-Goodwin, A.; Allen, T. J.; Oslick, S. L.; McClure, K. F.; Lee, J. H.; Kemp, D. S. Mechanism of Stabilization of Helical Conformations of Polypeptides by Water Containing Trifluoroethanol. *J. Am. Chem. Soc.* **1996**, *118*, 3082-3090.

(66) Guinn, E. J.; Pegram, L. M.; Capp, M. W.; Pollock, M. N.; Record, M. T. Quantifying why urea is a protein denaturant, whereas glycine betaine is a protein stabilizer. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 16932-16937.

(67) Deshmukh, M. M.; Gadre, S. R. Estimation of N-H•••O=C Intramolecular Hydrogen Bond Energy in Polypeptides. *J. Phys. Chem. A* **2009**, *113*, 7927-7932.

(68) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J.: Gaussian 09, Revision D.01. Gaussian, Inc.: Wallingford, CT, 2013.

(69) Simon, S.; Duran, M.; Dannenberg, J. J. How does basis set superposition error change the potential surfaces for hydrogen bonded dimers? *J. Chem. Phys.* **1996**, *105*, 11024-11031.

(70) Hanwell, M. D.; Curtis, D. E.; Lonie, D. C.; Vandermeesh, T.; Zurek, E.; Hutchinson, G. R. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *Cheminform* **2012**, *4*, 17.

(71) Lemkul, J. A. From proteins to perturbed Hamiltonians: a suite of tutorials for the GROMACS-2018 molecular simulation package. *Liv. J. Comput. Mol. Sci.* **2018**, *1*, 5068.

(72) Vanommeslaeghe, K.; Hatcher, E.; Acharya, C.; Kundu, S.; Zhong, S.; Shim, J.; Darian, E.; Guvench, O.; Lopes, P.; Vorobyov, I.; MacKerell, A. D. CHARMM General Force Field: A Force Field for Drug-Like Molecules Compatible with the CHARMM All-Atom Additive Biological Force Fields. *J. Comput. Chem.* **2010**, *31*, 671-690.

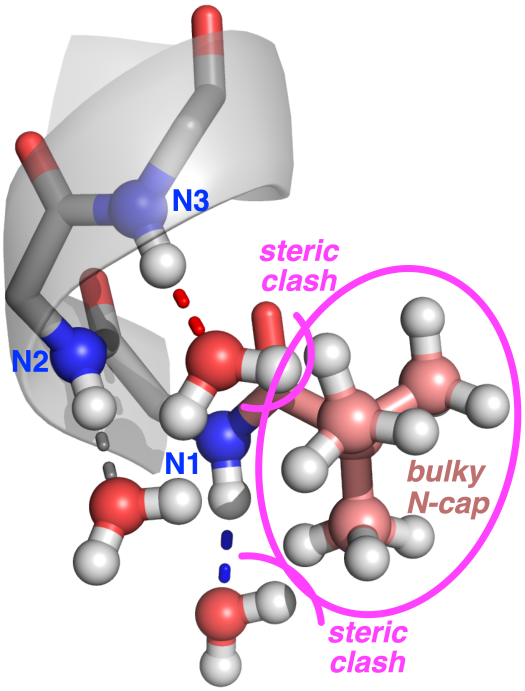
(73) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of Simple Potential Functions for Simulating Liquid Water. *J. Chem. Phys.* **1983**, *79*, 926-935.

(74) Humphrey, W.; Dalke, A.; Schulten, K. VMD: Visual molecular dynamics. *J. Mol. Graph. Model.* **1996**, *14*, 33-38.

(75) Michaud-Agrawal, N.; Denning, E. J.; Woolf, T. B.; Beckstein, O. Software News and Updates MDAnalysis: A Toolkit for the Analysis of Molecular Dynamics Simulations. *J. Comput. Chem.* **2011**, *32*, 2319-2327.

(76) Wang, G.; Dunbrack, R. L. PISCES: a protein sequence culling server. *Bioinformatics* **2003**, *19*, 1589-1591.

Graphical Table of Contents - For Table of Contents Use Only



acyl N-cap disrupts amide·OH_2 hydrogen bonds