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Is Raman Optical Activity Spectroscopy Sensitive to β -turns in Proteins? Secondary Structure and Side-Chain Dependence.

Carl Mensch,^[a,b] and Christian Johannessen^{*[a]}

Abstract: β -turns are essential for the structure and function of proteins. The spectroscopic technique Raman optical activity (ROA) has been suggested to be sensitive to such structural elements of proteins in solution. Three spectral features have been reported to mark β -turns in protein ROA spectra: being a negative band at 1220 cm^{-1} , positive intensity around 1290 cm^{-1} and negative intensity around 1340-1380 cm^{-1} . In this work, density functional theory calculations demonstrated that these assignments are inaccurate as these spectral regions are not robust and sense the exact secondary structure surrounding the β -turn as well. Furthermore, it was demonstrated that the amino acid side-chains affect the exact ROA patterns which can direct future research to perform a systematic analysis of the contributions of the side-chains.

1. Introduction

The structural characterization of proteins is a central theme in (bio)chemistry, even more so now that the use of biopharmaceuticals such as therapeutic peptides and proteins have become prevalent.^[1,2] Therefore, techniques that provide fast and detailed information about the solution phase structure of peptides and proteins are required. The field of structural biology traditionally relies on X-ray crystallography and nuclear magnetic resonance (NMR) techniques that provide information at atomic resolution, yet these often need to be supplemented by low-resolution techniques. Moreover, the realization and wide-spread acceptance that proteins that do not have a fixed three-dimensional structure, but rather have a very dynamic and flexible native structure not only exist but furthermore have crucial biological functions, underpinned the need to develop complementary techniques in the field of structural biology even further.^[3–5] Such dynamic proteins are termed intrinsically disordered proteins (IDPs) and receive a considerable amount of scientific interest as they are very challenging to characterise using conventional techniques, yet play central roles in neurodegenerative diseases such as Alzheimer's and Parkinson's.^[6,7]

Raman spectroscopy is very suitable as an experimental technique to study the solution phase structure of biomolecules, as it is non-destructive and can be employed in aqueous solution.^[8] While Raman spectroscopy provides very rich spectra

that contain a plethora of spectral bands arising from the protein's side-chains and backbone, Raman optical activity (ROA), measured as the difference in the right-handed I_R and left-handed I_L circularly polarized components in Raman scattered light, cuts through this complexity in the conventional Raman spectra.^[9,10] Because of the differential nature of ROA and its susceptibility to chirality, only the most rigid and chiral components of the protein are probed. As a result, the contributions of most side-chains cancel out and mainly signals arising from the protein's backbone are recorded.^[9] The ROA spectrum of a protein therefore gives information about the secondary structure of that protein with great sensitivity. While the characteristic patterns for the main secondary structure elements, namely α -helix, β -sheet and disordered structure, can readily be identified in the ROA spectrum of a protein, the detailed structure-spectrum relation is not fully understood.^[9,11,12] Therefore, the past decade, there has been a lot of progress in the development and implementation of the necessary theory and algorithms to routinely calculate ROA spectra.^[13] In 2001 Quinet and Champagne proposed an analytical derivative procedure for evaluating the polarizability tensors at time-dependent Hartree-Fock level.^[14] Subsequent, also schemes for evaluating the analytical derivatives of the electric-dipole/electric-quadrupole and electric-dipole/magnetic-dipole tensors were reported.^[15,16] This was followed by a general Kohn-Sham density functional response-theory scheme for evaluating property tensors, that was published in 2008.^[17] Implementations of the analytical ROA property calculations are available through the Dalton^[16,18] and Gaussian^[19,20] program packages. Based on these developments, a lot of recent work is based on the use of computational approaches in comparison with experiment to study the ROA patterns in more detail. This is a very challenging task and yet it was shown that some of the traditional spectral assignments based on e.g. the comparison of experimental spectra and crystal structures, indeed were inaccurate, which demonstrates the need to further study the ROA patterns of proteins to improve our understanding of the very sensitive structure-spectrum relationship.^[11,21–24] Here, the spectral characteristics of β -turns are investigated in detail as these are crucial structural elements of proteins. The spectral assignment of β -turns is challenging since there are not that many suitable model systems to be studied experimentally, while for example for α -helical structure, many experimental ROA studies of model peptides have been reported.^[11,25,26] In early spectroscopic studies of proteins, some of the observed ROA bands were suggested to arise from β -turns in proteins.^[9] As reviewed by Barron, the extended amide III region (1230-1340 cm^{-1}) contains sharp ROA bands that appear to originate in loops and turns.^[9] By the comparison of a collection of experimental ROA spectra of proteins and their crystal structures, positive intensity in the region 1260-1295 cm^{-1} and negative intensity in the region 1340-1380 cm^{-1} were suggested to arise from β -turns.^[9] Weymuth *et al.* stated that also a negative band around 1220 cm^{-1} that is experimentally observed

[a] Carl Mensch, Prof. Dr. Christian Johannessen
Department of Chemistry,
University of Antwerp
Groenenborgerlaan 171, 2020 Antwerp (Belgium).
E-mail: christian.johannessen@uantwerpen.be

[b] Carl Mensch
Department of Chemistry
Ghent University
Krijgslaan 281 (S3), 9000 Ghent, Belgium

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in the ROA spectra of proteins of which the crystal structures contain β -turns can be assigned to β -turns.^[24] In theoretical work by Weymuth *et al.*, the spectral characteristics of β -turns were studied by using 15 model tetrapeptides.^[24] Using density functional theory (DFT) calculations to calculate the ROA spectra of these models, they concluded that these three assignments in experimental spectra, namely: a negative band around 1220 cm^{-1} , a positive signal between 1260–1300 cm^{-1} and a negative band between 1340–1380 cm^{-1} , were also reproduced by their calculations and can therefore be used to assign β -turns in proteins from the ROA spectra.

In this study, it was further investigated whether the spectral characteristics of β -turns can be differentiated from other secondary structure elements such as the poly-L-proline II (PPII), α -helical and β -strand secondary structures. Furthermore, it was studied how the presence of a β -turn within a sequence affects the spectral patterns of the entire system. To this end, DFT calculations were performed not only on a diverse set of alanine model peptides of variable length, but furthermore on different models including the actual side-chains. While the effect of the side-chains generally is accepted to mostly cancel out, their exact contribution is unknown and could affect the ROA patterns.^[9,21] The spectra are calculated using DFT at the C-PCM/B3PW91/6-31++G(d,p) level of theory that has been shown to provide spectra that reproduce experimentally

observed spectral patterns in good detail (see methodology).^[11,21,27–29]

In the next section, first the classification of β -turns is discussed, which is used as the basis for the construction of the peptide models in this study.

2. β -turn classification and peptide construction

Over the years, different classifications of β -turns have been proposed.^[30–33] Some of these types of β -turn were later excluded again for either being too close to other structural elements (types III, III') or for being rare and having an inaccurate definition (types V, V', VII). For a more complete overview, see the recent publication by de Brevern.^[33] Currently, the standard classification of protein β -turns is based on the definitions by Hutchinson and Thornton (see Table 1).^[31,32] β -turns are identified in protein structures as four consecutive residues with a distance between the C α atoms of residue i and $i+3$ smaller than 7 Å. Next, the β -turns are grouped in seven classes based on the ϕ and ψ torsion angles of residues $i+1$ and $i+2$, as listed in table 1. Furthermore, the $i+1$ and $i+2$ residues must not be helical and a deviation of $\pm 30^\circ$ from the canonical values is only allowed on three of the four central residue angles and a fourth can deviate by $\pm 45^\circ$.^[33]

For each type of β -turn as listed in Table 1, a series of model peptides was constructed and the Raman and ROA spectra were calculated. Type IV was excluded as it is a miscellaneous group that classifies all β -turns that do not fit any of the other categories. Types VI_{a1}, VI_{a2} and VI_b are very rare compared to the other categories and furthermore defined by having a proline at position $i+2$ and can therefore not immediately be compared to the other modelled β -turns here, hence they are also excluded from this study. For the remaining types of β -turn, the model sequence HCO-(Ala)_n-NH₂ was used with the length n defined as 4, 6, 8 or 10. The β -turn angles defined in Table 1 were assigned to the two central residues of each model peptide, while all other backbone torsions were set to $\phi=-75^\circ$, $\psi=145^\circ$ (PPII), $\phi=-66^\circ$, $\psi=-41^\circ$ (α -helix) or $\phi=-140^\circ$, $\psi=135^\circ$ (β -strand). Reference structures were created by setting all torsion angles in additional peptide models (length n) to one of the three latter pairs of ϕ, ψ angles, thereby creating a regular PPII, α -helical or β -strand structure, respectively. Because of steric clashes, the construction of the peptide geometries of β -turn type I' and type II' were not possible for $n>4$ within a PPII backbone and for all lengths of type II' for the β -strand backbone. Since a large collection of modelled spectra was collected as part of this study, only a few are shown explicitly in the main text, while plots of the other spectra can be found in the supporting information. In the following section, firstly the spectra of the small models ($n=4$) are discussed, followed by sections describing the effect of disrupting a longer sequence by a β -turn.

Table 1. Definition of β -turn types based on the backbone angles ϕ and ψ of residue $i+1$ and $i+2$ of the β -turn. β -turns that do not fit any of the criteria are classified as type IV. The types VI_{a1}, VI_{a2} and VI_b are characterized by a cis-proline at position $i+2$. The last column lists the relative occurrence of the β -turns.^[31,33]

Type	ϕ_{i+1}	ψ_{i+1}	ϕ_{i+2}	ψ_{i+2}	%
I	-60	-30	-90	0	38.21
I'	60	30	90	0	4.10
II	-60	120	80	0	11.81
II'	60	-120	-80	0	2.51
VI _{a1}	-60	120	-90	0	0.73
VI _{a2}	-120	-120	-60	0	0.20
VI _b	-135	135	-75	160	0.88
VIII	-60	-30	-120	120	9.84
IV	n.a.	n.a.	n.a.	n.a.	31.72

3. ROA spectra of different β -turn types

In **Figure 1**, the Raman and ROA spectra of the HCO-(Ala)₄-NH₂ peptide model adopting five different types of β -turn are compared for PPII, α -helical and β -strand backbone. These tetrapeptides only differ in the ϕ, ψ of residues *i* and *i*+3. Although the remainder of the geometry is the same, the Raman and ROA spectra are considerably different. The shaded areas are visual aides to identify the three spectral regions that have been proposed to be sensitive to β -turn structure based on experimental ROA spectra (negative ROA around 1220 cm⁻¹, positive ROA 1260-1295 cm⁻¹ and negative ROA 1340-1380 cm⁻¹). Comparing the computed ROA patterns in **Figure 1** with these experimental assignments does not reveal clear trends, which demonstrates that it is unlikely that β -turn structure gives rise to marker bands in the ROA spectra of proteins and that care must be exerted in using these experimental assignments. For example, for the most common type of β -turn (type I), only positive intensity in the 1260-1300 cm⁻¹ is observed in all three spectra, while the other two experimental regions do not fit with all computed spectra. Negative intensity around 1220 cm⁻¹ is noticed in some of the computed spectra, but as the position varies and the intensity is small, the β -turn spectra were compared to reference spectra to establish that it only arises from the β -turn structure itself (see below).

Furthermore, the ROA spectra of the five types of β -turns are very different for all three backbone conformations of residue *i* and *i*+3 (PPII, α -helical, β -strand). Especially the extended amide III region (1240-1340 cm⁻¹) is quite different in all computed spectra. This is a complex spectral region as it arises from coupling between C-N stretching vibrations with in plane N-H deformation and C α -H bending modes.^[9,11] In the different types of β -turn and furthermore with the different torsion angles of residues *i* and *i*+3, the orientations of the N-H and C α -H groups are different relative to each other, which affects the ROA pattern in the amide III region considerably.

The amide I region (1600-1700 cm⁻¹) does however seem to follow the same trend within each set of three spectra of the same type of β -turn. For type I and type II' β -turns, the amide I in the ROA is all positive, while for type II and type I' it is all negative (the prime signifies the mirror image geometry of the β -turn; see **Table 1**). As can be seen for the molecular structures in **Table 1**, the (C=O)_{*i*+1} group of the type I β -turn points away from the reader (likewise for the type II' β -turn), while in the case of the type II β -turn it points towards the reader (likewise for the type I' β -turn). This similar orientation of the (C=O)_{*i*+1} carbonyl relative to the other carbonyls explains the sign dependence of the amide I ROA bands. Type VIII is the only β -turn of the selected five categories that does not have an intramolecular hydrogen bond and its ROA patterns reflect the different orientations of the C=O groups.

As mentioned above, in order to identify the presence or lack of ROA bands that are sensitive to β -turn structure, each individual spectrum was compared to a reference peptide geometry that lacks the β -turn motive in **Figure 2**. The orange spectra were calculated for the five types of β -turn with the residues *i* and *i*+3 set to either a PPII, α -helical or β -strand torsion angles. The spectra of the β -turn geometries are compared to the blue spectra that were calculated with all backbone angles set to

these same respective PPII, α -helical or β -strand angles. This comparison in **Figure 2** supports the conclusion from above that there are no bands robustly marking β -turn structure. Negative bands in the region 1180-1220 cm⁻¹ arise from C α -N stretching vibrations and are for example in the case of the PPII torsion angles (left in **Figure 2**) observed both for the PPII reference structure as well as for the β -turn geometries. In the second region suggested to be sensitive to β -turn structure (1260-1295 cm⁻¹) mostly negative ROA intensity is observed in the calculated spectra, however flanked by positive intensity in the region 1290-1310 cm⁻¹. Even considering that the exact computed wavenumbers might be shifted in comparison with experiment (e.g. due to lacking description of explicit hydrogen bonding with solvent, harmonic approximation, side-chain contributions, etc.), this latter positive band cannot be assigned to β -turn structure for example as it is also noticed for α -helical structure. The third region 1340-1380 cm⁻¹ for PPII and α -helical backbone does not reveal very much difference between the β -turn spectra and the reference spectra and hence again does not show evidence for being sensitive to β -turn structure.

The most reliable marker β -turns for these small peptide models, seems to be the amide I region (1600-1700 cm⁻¹). Although the positive ROA band of the type I β -turn is not distinguishable from that of the PPII band, in the other cases the amide I is significantly different from the reference spectra and even distinguishes some of the types of β -turn, as discussed above.

To conclude this section, the ROA bands suggested by Weymuth *et al.* to arise from β -turns, were shown to not be robust markers for β -turn structure. The C α -N stretching region and amide III region are too sensitive to the exact conformation. Although not uniquely differentiable from all other secondary structures, the amide I region was shown to be the most robust marker of the presence of the β -turn and even could differentiate type I and II' from type II and type I' β -turns in these small peptides.

So far, the Raman and ROA spectra of tetrapeptides have been discussed. In the next section, the effect of disrupting a longer sequence (6, 8 or 10 residues) by a β -turn is discussed.

4. ROA spectra of a β -turn within a longer sequence

To further study the ROA patterns of proteins, here the effect of disrupting a specific secondary structure by a β -turn is studied. In this fashion, not only the spectral patterns of the β -turns are probed, but furthermore the effect of structural disorder or mixing of secondary structure elements on the spectral patterns is assessed. To this end, the modelled structures discussed in the previous section were elongated at both the N- and C-terminus with additional residues having the same backbone torsion angles. Thereby structures of in total 6, 8 or 10 residues were obtained with the central residues adopting the β -turn and the remaining residues adopting a regular PPII, α -helical or β -strand conformation. As the type I β -turn is by far the most common, the results presented here focus on that type specifically. The calculated spectra for the other types of β -turn can be found in

the supporting information (Figure S1-S4). The computed spectra in Figure 3 indeed support the previous conclusions. With increasing chain length, the spectra are dominated by the PPII, α -helical or β -strand spectral patterns, and no marker bands are observed that could be used to discriminate the β -turn structure. In other words, there is no ROA band that appears at a specific position or with a characteristic spectral shape that allows differentiating the β -turn from the bands arising from the other secondary structure elements.

The disruption of the three secondary structures by a β -turn does however affect the overall relative intensities of the ROA bands. The lower wavenumber region (below 600 cm^{-1}) displays a significant decrease in intensities upon interruption of the regular secondary structure and is therefore sensitive to the overall regularity of the protein; mixing a secondary structure with another structural element reduces this spectral region in intensity. The extended amide III region also displays relative intensity differences for all three secondary structures studied here. For α -helical structure the relative intensity of the two positive amide III bands is affected. In the case of the type I β -turn, the band around 1300 cm^{-1} (mainly C α -H bending perpendicular to the C α -N bond)^[11,21,34] has an increased intensity relative to the band around 1340 cm^{-1} (mainly C α -H bending parallel to the C α -N bond). For the other types of β -turn (see Figure S3) this ratio is shown to be either increased or reduced. The exact sensitivity of these two bands has been the matter of debate in scientific literature, but it was previously shown that ratio of these two bands is very sensitive to the exact helical conformation.^[11] These results here again show the difficulty in describing the exact relation between these two bands as the coupling between the different vibrational motions in the extended amide III region is very complex (see above) and lead to a very structurally sensitive spectral region.

The β -strand ROA patterns are very similar to that of the β -turn, again supporting the conclusion made so far that there are no specific bands marking this type of secondary structure. Furthermore, as both the β -turn and β -strand show ROA bands in the three regions suggested to be sensitive to β -turns (grey shaded areas), the assignment of these spectral features to β -strand or β -turn is at least equally likely. As the empirical assignment of the β -turn signatures is largely based on the experimental spectra of proteins containing a major contribution of β -sheet,^[9] this supports the reassignment of these spectral bands to not arise from β -turns, rather from β -strand and β -sheet. The results for the disruption of the PPII backbone by β -turns is very relevant for the understanding of the ROA patterns of IDPs as these adopt an important amount of PPII in their structural ensembles.^[35] The ROA spectra recorded for several IDPs have been reported and are of interest since they seem to be indicative of residual structure as opposed to a fully random coil structure and hence ROA could help elucidating the nature of this intriguing class of proteins.^[6,12,25,36,37] Previous work by our group showed that computed spectra of PPII model peptides with a regular backbone structure fit very well with the experimental spectrum of the XAO peptide that is flexible in solution, yet adopts an important amount of PPII helix in its structural ensemble.^[11,35] The computed spectra reported here

show that if the regular PPII helix is interrupted by a β -turn, the amide III region peak maximum drops slightly in intensity. Although this is only a minor effect in the case of the type I β -turn presented in Figure 3, the spectra calculated for the PPII helix interrupted by the other β -turns show this effect stronger (see Figure S2). In that fashion, the relative ROA intensities could be indicative of the overall extendedness of the PPII structure. For example, if stretches of PPII backbone of an IDP are more disrupted by other secondary structure elements such as β -turns, the amide III intensity is lower relative to the other bands. The best way to quantify this is by looking at the dimensionless circular intensity difference (CID), which is defined as the ratio $(I_R - I_L)/(I_R + I_L)$. As the relative Raman intensity of the amide III region is not affected by the disruption, while the ROA is reduced, the CID will be lower. In this fashion, the CID might be indicative for the PPII content and hence how extended an IDP is. Yet, as discussed above, the amide III is a very complex spectral region having contributions of the entire protein structure. Especially in the context of IDPs, structural disorder has to be considered. The results discussed here, represent a static picture as only a single conformation is considered. However, in the case of an IDP, the structure is dynamic and the backbone angles explore wider regions in the Ramachandran plot. Although our previous work showed that the experimental spectra of a few model peptides (such as the flexible XAO peptide) can very well be reproduced by the calculated spectra of a model peptide with a backbone conformation close to the average backbone torsion angles, further research appears necessary to more deeply understand the influence of structural disorder and dynamics on the ROA patterns and has therefore been initiated in our group.

So far, the results reported above show that the ROA patterns overall are sensitive to β -turns, yet they do not show marker bands. As suggested before, although an ROA spectrum mainly arises from spectral contributions arising from the protein backbone, the side-chains might also affect the exact ROA patterns.^[9,21,23,24] As the contributions of the side-chains to the ROA patterns is not yet fully understood, in the following sections the effect of explicitly including larger side-chains in the calculations is evaluated. Firstly, the effect of the side-chains is calculated for a few examples of β -turns found in the crystal structures of hen egg white lysozyme (HEWL) as this protein is reported in much detail in the Protein Databank Archive (PDB) and furthermore its ROA characteristics can be recorded at high concentration and therefore high spectral resolution.^[38] Next, as an additional example, the effect of the explicit side-chains of a selected β -turn of the so-called tau protein is discussed. This protein is an IDP and receives much scientific attentions due to its involvement in Alzheimer's and Parkinson's disease and the ROA spectrum of one of its isoforms and a mutant have been reported.^[37,39-41]

5. The effect of side-chains

To investigate the effect of the side-chains on the ROA patterns of β -turns, two type I β -turns that are found in the crystal

structures of HEWL were selected. The β -turn sequence Gly54-Ile55-Leu56-Gln57 and Asn74-Leu75-Cys76-Asn77 were selected for this purpose. By keeping the backbone torsion angles exactly the same in the model structures and only varying the torsion angles of the different side-chains of both β -turns, the contributions of the side-chains are averaged out and the pattern arising from the backbone is retained.

Firstly, in Figure 4 the effect of the conformational averaging over 50 different side-chain conformations is demonstrated for the Gly54-Gln57 β -turn (capped by HCO- and -NH_2 groups). The top panels show the separate Raman and ROA spectra of all 50 conformations and show that there is a lot of variation across the entire spectral window. Although averaged over 50 spectra, the average ROA spectrum still retains specific bands in the lower wavenumber region and amide III and amide I region, which suggest that the backbone contributions are retained using this averaging approach. Please note that this conformational averaging is arbitrary and with the specific goal to average out the side-chain contributions as much as possible and might hence not reflect the exact behaviour one would obtain in experiment, as side-chains have distinct conformational preferences depending on the surrounding protein structure.^[42]

Next, to see the average effect of the side-chains on the ROA spectrum, the average ROA is compared to that of a reference geometry with the exact same backbone conformation but with the side chains replaced by a methyl group. In Figure 5, the comparison of both selected β -turns Gly54-Ile55-Leu56-Gln57 and Asn74-Leu75-Cys76-Asn77 is made with the corresponding computed spectrum after removing all side-chains giving a HCO-Gly-Ala₃-NH₂ and HCO-Ala₄-NH₂ reference structure. By replacing the side-chain by a methyl group (hence giving Ala), the chirality of the residue is retained, but the remainder of the side-chain is removed. The averaged Raman spectra compared to the reference spectra shows a lot of differences, which is as expected since there are many Raman bands arising from side-chain modes. However, the sharp amide III band at 1260 cm⁻¹ in the reference spectrum (orange Raman plot on the left in Figure 5) shifts down about 20 cm⁻¹ because of the side-chains. Thus, even after extensive conformational averaging the amide III region is clearly affected by the side-chains. Also in the ROA spectra the amide III is definitely sensitive to the side-chains even after conformational averaging. Furthermore, the skeletal stretch region (870-1150 cm⁻¹) is very dependent on the side-chain.

Comparison of the ROA spectra of the two type I β -turns selected here (Gly54-Gln57 and Asn74-Asn77) support the conclusion made above that the previously assigned ROA spectral markers of β -turns were inaccurate. The similarities of the ROA spectra including the side-chains in Figure 5 suggest that a negative amide III band in the region 1240-1270 cm⁻¹ and a positive amide I band could arise from type I β -turn structure. To further study the side-chain dependence, in the next section a specific β -turn sequence in tau is studied.

6. β -turns in tau protein

As mentioned above, tau is an IDP involved in neurodegenerative diseases. IDPs are characterised by having very flexible and dynamic structures, yet they display specific ROA patterns, which suggests residual structural propensities in said proteins.^[6,12,25,36,37] To structurally describe IDPs that rapidly interconvert between conformations, structural ensembles are used that are obtained via methodologies that combine experimental (e.g. NMR) and computational data (e.g. molecular dynamics).^[43,44] For the IDP tau, structural analysis has indicated that specific sections of its sequence have a high propensity to form β -turns.^[44] Mukrasch *et al.* used NMR to show that the K18 construct of tau in its repeat domains adopts type I β -turns.^[44] Therefore, these β -turns in tau might be ideal model systems to verify the computations done above for HEWL, since the side-chains of tau are likely to adopt different conformations and hence average out. Furthermore, for the two major types of β -turns (type I and type II) the averaged Raman and ROA patterns can be compared to investigate whether ROA can distinguish different types of β -turn. Lastly, the comparison with another structural element, namely PPII, which IDPs have a distinct propensity for, can be made as a final validation of the sensitivity of ROA to β -turns.

Firstly, the averaged Raman and ROA of the Asp314-Leu315-Ser316-Lys317 (DLSK) sequence of tau adopting a type I β -turn are compared to those of the HEWL type I β -turns discussed above. This comparison in Figure 6 demonstrates that a positive amide I band and a negative amide III band in the 1240-1270 cm⁻¹ region are the only common ROA bands among these three type I β -turns consisting of different sequences.

Next, the difference in the Raman and ROA patterns of the Asp314-Leu315-Ser316-Lys317 (DLSK) sequence adopting either a type I or type II β -turn were assessed. As demonstrated in Figure 7, the Raman spectra are very similar for the two types of β -turn, which is reasonable since they comprise the same sequence in a different conformation. In the ROA, which is more conformationally sensitive, the amide I region is positive for the type I β -turn while it is mostly negative for the type II, which is in accordance with the alanine models discussed above. The differences in the amide III region are of the same magnitude as between the different HEWL models discussed above, so hence depend on the specific sequence and conformational averaging. Again, a negative band in the region 1240-1270 cm⁻¹ is observed in the lower part of the amide III region. Therefore, this observation shows that it does not discriminate different β -turns, yet as these are the two most prevalent types of β -turns, it must still be assessed whether this negative amide III can be assigned to β -turn or not.

To establish whether this spectral band can be assigned to the β -turn itself, it needs to be compared to other structural elements. In Figure 8, the Raman and ROA spectra observed for the type I β -turn of DLSK are compared to the same sequence adopting a PPII backbone conformation. The biggest spectral difference in the Raman spectra is found in the amide III region that displays a broad positive band at 1290 cm⁻¹. The amide III is probably sensitive to the hydrogen bond in the β -turn, thereby affecting the vibrational coupling between the N-H and C α -H bending modes and C-N stretching mode involved in the amide III region.

In the ROA, the amide III region is also different for the two backbone conformations and shows two positive amide III bands of the β -turn backbone, while the PPII geometry only shows a single broad positive band. The amide I band is much stronger for the PPII backbone structure, which might be a result of the relative orientation of the amide (C=O) groups or hydrogen bonding in the β -turn. Visual analysis of the vibrational modes in the GaussView 6.0.16 programme (Gaussian, Inc.) reveals that the strong ROA band at 1420 cm^{-1} arises from vibrational motions of the capping group coupling to the lysine side-chain. In the supporting information (Figure S5), this is further supported by the disappearing of this band upon using a different capping group. Nevertheless, the spectra with the different capping group also support that both the type I β -turn and the PPII backbone give rise to a negative band in the lower part of the amide III region ($1240\text{--}1270\text{ cm}^{-1}$). Therefore, it can be concluded that while the overall ROA pattern is sensitive to the backbone conformation, there are no marker bands that can be used to assign β -turn structure in the ROA spectra of proteins. The negative band in the amide III region and the sign of the amide I region are indistinguishable from other secondary structure patterns in the ROA spectra of proteins.

7. Conclusions

In this study, a large set of DFT computed Raman and ROA spectra are reported in order to assess the sensitivity of these spectroscopies to β -turn structure in proteins. In scientific literature, different spectral bands have been suggested that supposedly mark β -turn structure. The data presented here demonstrates that these assignments are inaccurate and that because of the very strong conformational sensitivity of ROA, there are no specific ROA bands that can be assigned to β -turns. The data does show that overall the signals arising from the amide vibrational modes in the backbone are affected by the formation of a β -turn. The relative intensities and exact shapes can be affected by the formation of a β -turn in the backbone. The amide I region was shown to be very sensitive to the orientations of the different amide carbonyl groups and as such could differentiate different types of β -turn in small model systems. In longer peptides and proteins, the signals were shown to be dominated by other secondary structure elements. Furthermore, it was studied here how the side-chains of the different residues affect the Raman and ROA patterns. It was shown that the amide I modes, specifically the negative portion in the window $1230\text{--}1300\text{ cm}^{-1}$ in the extended amide III region can be affected by the explicit side-chains. This supports the previously made suggestion^[9] that the detailed ROA patterns can be affected by the side-chains and demonstrates the need to use a systematic approach study the contributions of the side-chains to the ROA patterns in future research. Furthermore, the contributions of the side-chains strongly depend on their conformational averaging. Therefore, also the effect of conformational averaging on the backbone conformation could have an important influence on the final averaged ROA spectrum. Since both the side-chain contributions and the effect

of conformational dynamics of the backbone on the ROA spectra of proteins are not understood in detail, this is the subject of research currently ongoing in our research group.

Computational methodology

Model peptides were generated by using the Peptide Builder developed by Tien *et al.* that generates the models based on a set of ϕ and ψ torsion angles to define the backbone and a set of χ torsion angles to define the side-chain conformation.^[45] The side-chain χ angles were randomly selected by using combinations of the most common χ angles as determined from the Top8000 database.^[42,46] E.g. most side-chains preferentially take a value near -60° , $+60^\circ$ or 180° .^[42] By randomly selecting combinations of χ side-chain angles, geometrically possible conformations of the side-chains were sampled. For the HEWL β -turns, the backbone ϕ and ψ angles were taken from the PDB crystal structure 1LSE. For tau, the backbone angles (i and $i+3$) were taken from the ensemble reported by Ozenne *et al.* and deposited in the Protein Ensemble Database as entry PED6AAC.^[43,47] All model peptides were capped with a formyl (HCO) at the N-terminus and an amide (NH₂) group at the C-terminus unless stated otherwise. The molecular geometries of the model peptides were optimized using the partial optimization in normal coordinates by Bouř and Keiderling by freezing the normal modes between $i300\text{ cm}^{-1}$ (imaginary) and 300 cm^{-1} , thereby retaining the overall conformation and relaxing the vibrational modes of interest.^[48,49] Note that thus none of the Cartesian coordinates are frozen in this partial optimization scheme, but that by freezing this selection of normal modes, the backbone torsion angles change only within 1° to 2° difference after geometry optimization compared to the originally chosen angles. The Raman and ROA spectra were calculated at the B3PW91/6-31++G(d,p) level of theory for an incident laser wavelength of 532 nm. Full optimization of the geometries often leads to geometries that deviate from the secondary structure conformation of interest (see e.g. fig S6). Both in our previous work^[11] and other reports in scientific literature,^[27–29,50] the partial geometry optimization combined with this level of theory (B3PW91/6-31++G(d,p)) for the calculation of the spectra provided very good calculated spectra in comparison with experiment. Sometimes, a triple zeta basis set is used for both the geometry optimization and property calculations^[51,52] or the rDPS basis set^[53,54] is used only for the property calculations, but we found that these provide very similar results to our choice of basis set (see figure S7 and figure S8). Line broadening was modelled using a Lorentzian band shape with a full width at half height of 20 cm^{-1} and a Boltzmann factor to correct for the temperature (300 K) for each normal mode.^[11,21] The calculated wavenumbers are typically slightly overestimated due to *inter alia* the harmonic approximation. Therefore, the calculated spectra were scaled in the wavenumber dimension by a factor of 0.987 which was found by our group to be appropriate for this level of theory to allow comparison with experimentally observed band positions.^[11] For all DFT calculations, the C-PCM implicit solvent model was used to account for solvent-solute interactions with water. All DFT calculations were performed using the Gaussian16 rev. A.03 programme.^[19]

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Figures

Figure 1. Raman ($I_R + I_L$) and ROA ($I_R - I_L$) spectra of specific β -turn conformations of $\text{HCO}-(\text{Ala})_4\text{-NH}_2$. The backbone angles for the central two residues are set based on the canonical β -turn angles as listed in Table 1. The remaining backbone torsion angles (ϕ , ψ of residues i and $i+3$) are set to $\phi = -75^\circ$, $\psi = 145^\circ$ (blue), $\phi = -66^\circ$, $\psi = -41^\circ$ (red), or $\phi = -140^\circ$, $\psi = 135^\circ$ (green). As an example, the molecular structures of the β -turn model conformations display the effect of the different ϕ and ψ angles of residues i and $i+3$ (colours matches with the spectra). The shaded areas are visual aides to identify the three spectral regions that have been proposed to be sensitive to β -turn structure based on experimental ROA spectra: negative ROA around 1220 cm^{-1} , positive ROA $1260\text{--}1295\text{ cm}^{-1}$ and negative ROA $1340\text{--}1380\text{ cm}^{-1}$.

Figure 2. ROA ($I_R - I_L$) spectra of specific HCO-(Ala)₄-NH₂ β -turn conformations (orange) compared to a reference geometry with repetitive ϕ, ψ angles (blue). For the β -turn conformations, the backbone angles for the central two residues are set based on the canonical β -turn angles as listed in Table 1, while remaining backbone torsion angles (ϕ , ψ of residues i and $i+3$) are set to $\phi = -75^\circ$, $\psi = 145^\circ$ (left), $\phi = -66^\circ$, $\psi = -41^\circ$ (middle), or $\phi = -140^\circ$, $\psi = 135^\circ$ (right). Reference conformations with repetitive backbone angles were created based on the same latter three pairs of ϕ and ψ angles. The shaded areas are visual aides to identify the three spectral regions that have been proposed to be sensitive to β -turn structure based on experimental ROA spectra: negative ROA around 1220 cm^{-1} , positive ROA 1260-1295 cm^{-1} and negative ROA 1340-1380 cm^{-1} . The top three molecular structures represent the reference conformations, the bottom three the type I β -turn conformations. The corresponding Raman spectra are shown in Figure S1.

Figure 3. DFT calculated ROA ($I_R - I_L$) spectra of model peptides of the general formula $\text{HCO}-(\text{Ala})_n-\text{NH}_2$ with n equal to 4, 6, 8 or 10. The backbone angles for the central two residues are set based on the ideal β -turn angles for a type I β -turn as listed in Table 1, the remaining backbone torsion angles are set to $\phi = -75^\circ$, $\psi = 145^\circ$ (left; PPII), $\phi = -66^\circ$, $\psi = -41^\circ$ (middle; α -helix), or $\phi = -140^\circ$, $\psi = 135^\circ$ (right; β -strand). The calculated spectra of these models are given in orange. The reference conformations with repetitive backbone angles were created based on the same latter three pairs of ϕ and ψ angles and shown as the blue spectra. The molecular structures used to compute the ROA spectra with the PPII backbone angles are shown as examples. The blue rectangles highlight the four residues making up the β -turn, the orange shades highlight the PPII backbone angles.

Figure 4. Side-chain averaging: DFT calculated Raman ($I_R + I_L$) and ROA ($I_R - I_L$) of $\text{HCO-Gly-Ile-Leu-Gln-NH}_2$ with 50 different side-chain conformations of the side-chains, while the backbone type I β -turn conformation is retained. The 50 separate spectra are shown on the top, the average spectra are shown in the bottom panels. The molecular structure of a single conformation is shown in comparison to the superimposed 50 structures in stick representation.

Figure 5. Effect of explicit side-chains: Raman ($I_R + I_L$) and ROA ($I_R - I_L$) averaged over 50 side-chain conformations (blue) of the HCO-Gly54-Ile55-Leu56-Gln57-NH₂ and HCO-Asn74-Leu75-Cys76-Asn77-NH₂ type I β -turns compared to the spectra with all the side-chains replaced by a methyl group (to alanine), hence giving a HCO-Gly-Ala₃-NH₂ peptide (left; orange) and a HCO-Ala₄-NH₂ peptide (right; orange).

Figure 6. Effect of explicit side-chains on the type I β -turn: Raman ($I_R + I_L$) and ROA ($I_R - I_L$) averaged over 50 side-chain conformations of HCO-Gly54-Ile55-Leu56-Gln57-NH₂ (GILQ) and HCO-Asn74-Leu75-Cys76-Asn77-NH₂ (NLCN) turns of HEWL compared to the spectra of the HCO-Asp314-Leu315-Ser316-Lys317-NH₂ (DLSK) type I β -turn in tau.^[44]

Figure 7. Effect of explicit side-chains on type I and type II β -turn: Raman ($I_R + I_L$) and ROA ($I_R - I_L$) averaged over 50 side-chain conformations of the HCO-Asp314-Leu315-Ser316-Lys317-NH₂ (DLSK) sequence selected from tau with the β -turn torsion angles set to either a type I or type II β -turn.^[44]

Figure 8. Effect of explicit side-chains on type I β -turn compared to a PPII backbone: Raman ($I_R + I_L$) and ROA ($I_R - I_L$) averaged over 50 side-chain conformations of the HCO-Asp314-Leu315-Ser316-Lys317-NH₂ (DLSK) sequence selected from tau with the angles set to either a type I β -turn (blue) or a PPII backbone (orange; $\varphi = -75^\circ$; $\psi = 145^\circ$).^[44]

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Spectroscopy Sensitive to β -turns in
Proteins? Secondary Structure and
Side-Chain Dependence**Raman optical activity senses β -turns but does not display marker bands.