Coupled vibrations in peptides and proteins: Structural information using 2D-IR spectroscopy

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Salt Bridges in Peptides Have Two Geometries

Salt bridges play an important role in protein folding but they are difficult to detect and characterize in solution. Here we use 2D-IR spectroscopy to investigate the salt bridges between glutamate (Glu^-) and arginine (Arg^+) of three different peptides in solution whose structure is stabilized by these type of salt bridges: a β-turn, an α-helical peptide, and a coiled coil. We detect a bidentate salt bridge in the β-turn and a monodentate one in the α-helical peptide, whereas the coiled coil shows signatures of both types of salt bridges.

5.1 Introduction

Salt bridges are hydrogen-bonded ion pairs that play a fundamental role in inter- and intramolecular self-assembly processes. In supramolecular chemistry, salt bridges are one of the interactions used to design new systems.[44,45,58] The intramolecular self-assembly or folding of proteins is influenced, among others, by long-range or tertiary interactions (those between non-adjacent residues). Salt bridges are a special case among these interactions because in many cases it is still not clear whether or not they are stabilizing for proteins. There is widespread evidence for the presence of salt bridges in stable conformations,[46,48,55,59] but the salt-bridge interaction is often found to be energetically unfavorable.[46,60] More importantly, salt bridges of-

ten influence the dynamics and functionality of proteins.[47,61–63] Therefore, a probe of salt bridges in solution would be a valuable tool to clarify the role that they play in the structure and dynamics of proteins. NMR is the standard method to determine the existence of salt bridges in solution,[64] but the time resolution of NMR is generally not sufficient to resolve protein dynamics, and structural information is not easy to obtain. Here we demonstrate how 2D-IR spectroscopy can be used to detect salt bridges in solution by investigating the interaction between vibrational modes of arginine (Arg^+) and glutamate (Glu^-). As a result of the spatial proximity of these amino acids when they are forming a salt bridge, the local vibrational modes of their side chains are coupled, and this coupling is visible as cross peaks in their 2D-IR spectra. We investigate systems where the formation of salt bridges between Arg^+ and Glu^- has structural relevance. We study a β-turn peptide that is held folded by an intramolecular salt bridge between Arg^+ and Glu^-, an α-helical peptide that is stabilized by three $Glu^- \cdots Arg^+$ salt bridges, and an α-helical coiled coil that is kept together by salt bridges. From the 2D-IR response of these peptides, we find that they have different salt-bridge conformations.

5.2 β-Turn

The first peptide that we study is a β-turn that is held together by a salt bridge between Arg^+ and Glu^- when solvated in DMSO.[65] The peptide has the amino acid sequence

$$\mathrm{Ac-\underbrace{Arg-Ala-Gly-Glu}_{\text{salt bridge}}-NHEt.} \tag{5.1}$$

As shown in Chapter 4, when one of the hydrogens of Gdm^+ is substituted by a carbon atom, the D_3 symmetry of the molecule is broken, and a solvent-independent frequency splitting between the two vibrational modes is induced, as seen in the FTIR spectrum of methylguanidinium, shown in Figure 5.1B. An analogous frequency

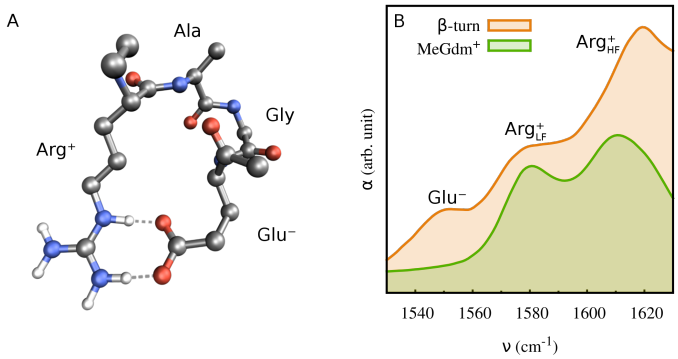

Figure 5.1: (A) Molecular representation of the β-turn. (B) FTIR spectrum of $MeGdm^+$ and the β-turn in deuterated DMSO (solvent subtracted).

splitting occurs in the isolated Arg^+ amino acid, resulting in two IR-active modes centered at 1578 cm^{-1} (Arg^+_{LF}) and 1608 cm^{-1} (Arg^+_{HF}).[16] In the β-turn, where Arg^+ forms a salt bridge with the side chain of Glu^- (illustrated in Figure 5.1A), the frequency splitting between Arg^+_{LF} and Arg^+_{HF} is larger, as can be seen in the FTIR spectrum of the β-turn shown in Figure 5.1B. This larger frequency splitting is an indication of the existence of a coupling between the vibrational modes of the side chains of Arg^+ and Glu^- in the β-turn. The 2D-IR spectrum unambiguously confirms the existence of a coupling, and consequently of a salt bridge, between the side chains of Arg^+ and Glu^-. The 2D-IR spectra of the β-turn are shown in Figure 5.2. Along the diagonal the parallel- and perpendicular-polarization spectra, the bleach

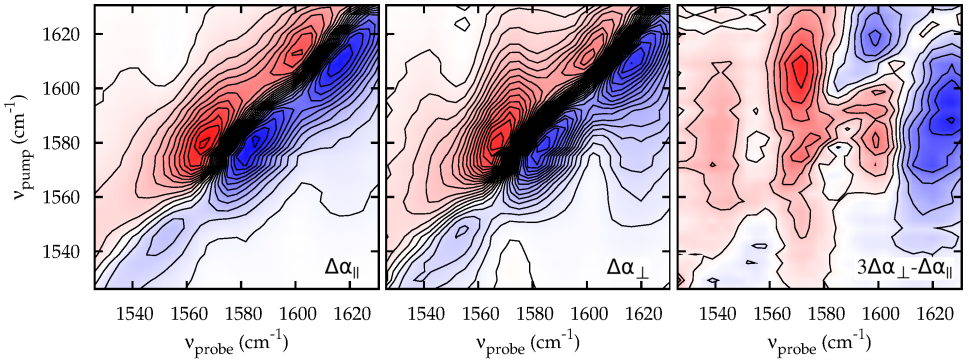

Figure 5.2: 2D-IR spectra of the β-turn for the (A) parallel, (B) scaled perpendicular polarizations of pump and probe pulses, and (C) weighted difference. The contour intervals are 0.4, 0.1 and 0.15 mO.D. The delay between pump and probe pulses is 1 ps.

and induced absorption of the Glu^- mode and the two Arg^+ modes are clearly visible. Their inhomogeneous linewidths, manifest as the elongation along the diagonal of the 2D-IR spectra, are larger than in the $Gdm^+\cdots Ac^-$ dimer investigated in chapter 4, which is an indication of a larger conformational variation for the β-turn, probably as a result of the heterogeneity in its backbone conformation. In the off-diagonal region there are clear cross peaks between the two Arg^+ modes, and also between each of these modes and the Glu^- mode. Figures 5.3A shows cross sections for $\nu_{pump} = \nu_{Glu^-}$ for both parallel and perpendicular polarizations of pump and probe pulses, which shows the cross peaks between Glu^- and both of the Arg^+ modes more clearly. A cross section for ν_{pump} resonant with Arg^+_{HF} is shown in Figure 5.3. The presence of cross peaks between the Glu^- mode and both of the Arg^+ modes shows that there is a coupling between them, and the fact that we are able to measure this coupling in the β-turn shows that we can detect $Glu^-\cdots Arg^+$ salt bridges in peptides in solution. In the previous chapter, we showed that there are two possible bidentate salt-bridging geometries between Arg^+ and Glu^-, and each of them shows a distinctive 2D-IR response (see section 4.3). The 2D-IR response that we observe for the β-turn, shows only one such response, which indicates that only one bidentate salt bridge geometry occurs.

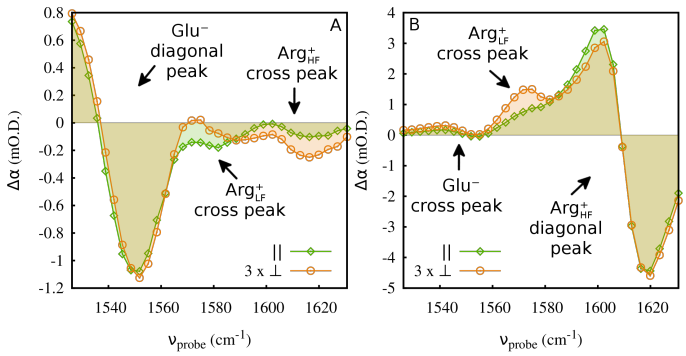

Figure 5.3: Cross sections of the 2D-IR spectra for parallel and (scaled) perpendicular polarizations of pump and probe pulses for ν_{pump} resonant with (A) Glu^- and (B) Arg^+_{HF}.

5.3 α-Helical Peptide

To investigate whether 2D-IR is sensitive to the spatial arrangement of salt bridges in solution, we compare the 2D-IR response of the β-turn with that of an α-helical peptide that is stabilized by three $Glu^- \cdots Arg^+$ salt bridges.[66] The amino acid sequence of this α-helical peptide is

$$\mathrm{Ac-Ala-(\underbrace{Glu-Ala-Ala-Ala-Arg}_{\text{salt bridge}})_3-NH_2}, \tag{5.2}$$

and its molecular structure is illustrated in Figure 5.4A. The FTIR spectrum of the α-helical peptide in the 1550-1670 cm^{-1} region is shown in Figure 5.4B. The lowest-frequency band is due to the Glu^- residue. The bands at 1585 and 1605 cm^{-1} are due to the Arg^+_{LF} and Arg^+_{HF} modes, respectively. The band centered at 1630 cm^{-1} is the amide I′ band, whose central frequency is sensitive to the α-helical content of the peptide. The Glu^- band is at a higher frequency than the one of the $Gdm^+ \cdots Ac^-$ dimer and the β-turn because of the different solvent (water instead of DMSO).[16] The

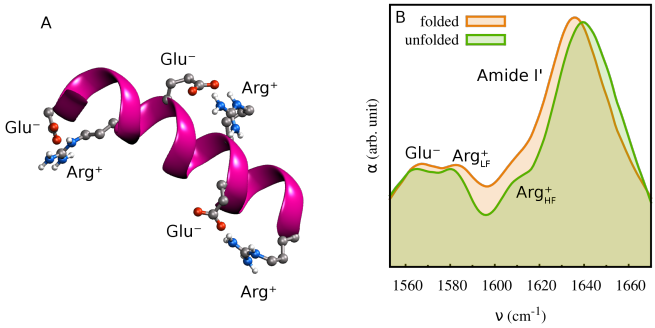

Figure 5.4: (A) Molecular representation of the α-helical peptide. The side chains of Arg^+ and Glu^- are drawn explicitly. (B) FTIR spectra of the folded and unfolded α-helical peptide in D_2O solution.

2D-IR spectra of this peptide are shown in Figure 5.5A-C. The cross peaks between

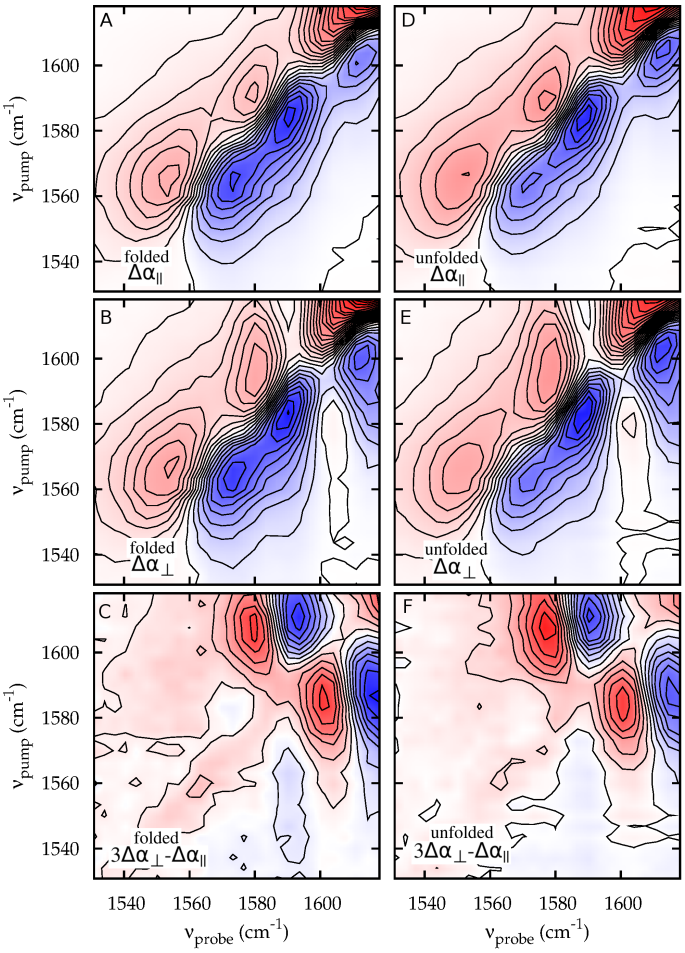

Figure 5.5: 2D-IR spectra of the folded α-helical peptide for (A) parallel and (B) perpendicular polarizations of pump and probe pulses, and (C) weighted difference. The contour intervals are 0.2, 0.06 and 0.08 mO.D. (D), (E) and (F) are the analogous to (A), (B) and (C) for the unfolded α-helical peptide upon addition of 6 M NaCl. The delay between pump and probe pulses is 1 ps.

the two Arg^+ modes are evident, but the cross peaks between these modes and the Glu^- mode are difficult to see in the 2D-IR spectrum. However, in a cross section for ν_{pump} resonant with Arg^+_{LF}, shown in Figure 5.6A, an off-diagonal response is observed at the frequency of the Glu^- mode. This response, although small, has the characteristic cross-peak line shape, and the polarization dependency of its intensity clearly indicates that it is a cross peak. No cross-peak is however observed for ν_{pump} resonant with the Arg^+_{HF} mode, which indicates that the Glu^- mode is only coupled to the Arg^+_{LF} mode. This result suggests that this peptide shows a different salt-bridge geometry than that of the dimers of Chapter 4 and the β-turn, where the COO^- mode was coupled to both Gdm^+, $MeGdm^+$ or Arg^+ modes. To confirm that the Glu^- cross peak arises from salt bridge formation, we added 6 M NaCl to the system, which is known to screen the salt-bridge interaction and disrupt the α-helical structure of the peptide.[66] Figure 5.4B shows the FTIR spectrum of the unfolded peptide. The amide I′ band undergoes a blue shift that is indicative of the loss of α-helical structure,[67] the Arg^+_{LF} and the Glu^- modes redshift, and the Arg^+_{HF}

mode remains almost unchanged. The off-diagonal region of the 2D-IR spectra in the salt bridge region, which is shown in Figure 5.5D-F, provides the explanation for these shifts. The coupling between Glu^- and Arg^+_{LF} that was detected for the folded peptide has vanished. This is clearly seen in the cross section for ν_{pump} resonant with the Arg^+_{LF} mode, shown in Figure 5.6B, in which the cross-peak feature at the Glu^- frequency has disappeared. As a result of the loss of coupling with the Glu^- side chain, the Arg^+_{LF} undergoes a frequency redshift. However, the Glu^- mode also redshifts, but probably because of hydrogen bonding with the surrounding water. These observations prove that the cross peak between Arg^+_{LF} and Glu^- in the folded conformation of the α-helical peptide arise because of a salt bridge. The fact that the Glu^- mode is only coupled to the Arg^+_{LF} mode suggests that the salt bridges of the α-helical peptide have a different geometry than the one in the β-turn. To understand this difference, we determined the number and relative probability of side-chain rotamers that allow the formation of $Glu^- \cdots Arg^+$ salt bridges in the α-helical peptide.[63] We find that there are three different accessible rotameric isomers in which salt bridge formation is possible. All three conformations involve only one of the NH groups of the side chain of Arg^+ binding to Glu^- in a monodentate manner. This finding strongly suggests that the salt bridge that we detect with 2D-IR spectroscopy for the α-helical peptide is a monodentate one, with a characteristic coupling of the Glu^- mode to only the Arg^+_{LF} mode. Our result suggest that we can differentiate between bidentate and monodentate $Glu^- \cdots Arg^+$ salt bridges by determining whether the Glu^- mode is coupled to only the Arg^+_{LF} mode or to both Arg^+ modes.

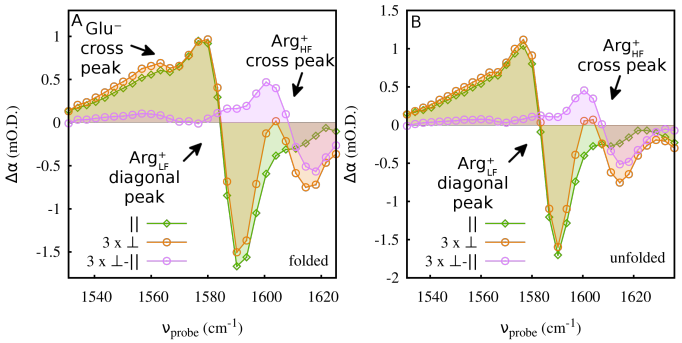

Figure 5.6: (A) Cross sections of the 2D-IR spectra for parallel and (scaled) perpendicular polarizations of pump and probe pulses, and weighted difference for ν_{pump} resonant with Arg^+_{LF} (1587 cm^{-1}). (B) is analogous to (A) for the unfolded α-helical peptide upon addition of 6 M NaCl.

5.4 Coiled Coil

The coiled coil is a structural motif of proteins that consists of two or more α-helical peptides that are wrapped around each other in a superhelical fashion.[68,69] Coiled coils are amongst the most ubiquitous folding motifs found in proteins and have not only been identified in the structure of proteins, but they are also involved in vari-

ous intracellular regulation processes.[13] They are also used as model system to study protein folding and stability due to their small size and because they have both short range interactions, which stabilize the monomeric α-helices, and long range interactions, responsible of the oligomeric packing. The coiled coil peptide that we investigated was designed de novo and characterized before,[70] it is highly α-helical and forms 100% dimers in solution under physiological conditions. Its folded conformation is stabilized by a complex network of inter- and intrahelical salt bridges, the most important ones being between Arg^+ and Glu^-. The peptide has the sequence

$$\mathrm{Suc-D\underset{(i+3)}{\underline{E}L\overset{(i+4)}{\overbrace{\underline{E}RR}}IR}\underset{(i+4)}{\underline{E}LEAR}IK-NH_2}, \tag{5.3}$$

where 'Suc' at the N-terminus stands for succinylation, an addition of a succinyl group (-CO-CH_2-CH_2-CO-) to a lysine residue. In Figure 5.7A we show the three-dimensional structure of this peptide obtained with X-ray crystallography.[70] Note that under crystalline conditions, the peptide packs into coiled-coil trimers rather than dimers. In this figure, we show explicitly the side groups of $Arg6^+$ and $Glu11^-$ (underlined in equation 5.3), which are involved in the interhelical salt bridge that stabilizes the coiled coil (for both dimers and trimers). From this structure it was

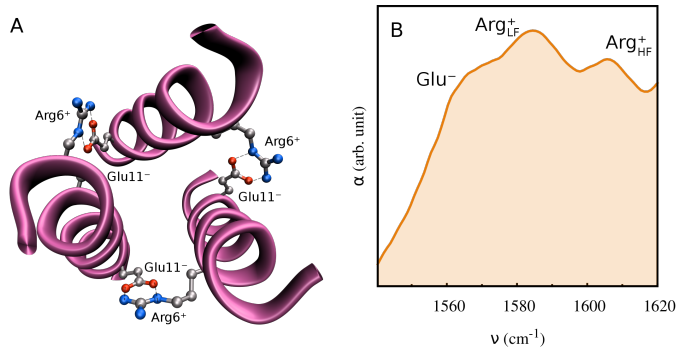

Figure 5.7: (A) Molecular representation of the α-helical coiled coil obtained with X-ray crystallography.[70] The side chains of $Arg6^+$ and $Glu11^-$ are drawn explicitly. (B) FTIR spectrum of the coiled coil in D_2O solution.

found that the intrahelical salt bridge network shows more variability, with many salt bridges between Arg^+ and Glu^- being possible. However it was found before that intrahelical salt bridges between Glu^- and Arg^+ at a distance of four and three residues (i+4 and i+3) are in general more favorable in α-helices than those between Arg^+ and Glu^- at those same distances.[66] Therefore, in the sequence of equation 5.3, we only indicate the possible $Glu^-\cdots Arg^+$ salt bridges with (i+4) and (i+3) spacing. These intrahelical salt bridges are most likely monodentate ones, like the ones of the α-helical peptide investigated in the previous section. The $Arg6^+\cdots Glu11^-$ interhelical salt bridge might have a different geometry than the intrahelical salt bridges, and therefore a different 2D-IR response. A monodentate geometry is suggested by the three-dimensional structure of Figure 5.7A. The FTIR spectrum of the coiled coil

in D_2O is shown in Figure 5.7B, which as before consists of three bands. The lowest frequency band arises from the antisymmetric CO stretch of Glu^-, and the other two bands are from Arg^+.

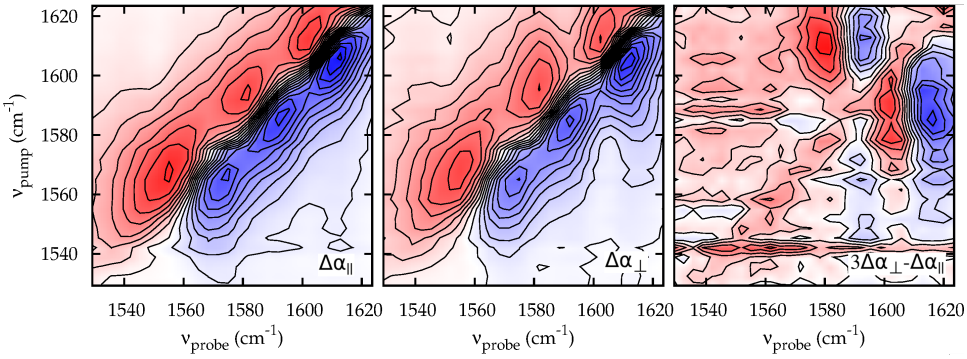

Figure 5.8: 2D-IR spectra of the coiled coil for the (A) parallel, (B) scaled perpendicular polarizations of pump and probe pulses, and (C) weighted difference. The contour intervals are 0.17, 0.06 and 0.05 mO.D. The delay between pump and probe pulses is 1 ps.

The 2D-IR spectra of this peptide are shown in Figure 5.8. Along the diagonal the spectra are composed of the three bands that are visible in the FTIR spectrum, and some response is seen in the off-diagonal region. The cross peaks between the two Arg^+ modes are clearly seen in the weighted difference spectrum of Figure 5.8C, but cross peaks between these two modes and the Glu^- band are better appreciated in the cross sections shown in Figure 5.9. Figures 5.9A and B show cross sections of parallel and perpendicular polarizations of pump and probe pulses, and for the weighted difference for ν_{pump} resonant with Arg^+_{LF} and Arg^+_{HF}, respectively. The cross section for ν_{pump} resonant with Arg^+_{LF} shows two distinctive cross peaks for the Glu^- mode frequency (labeled Glu^-_1 and Glu^-_2, as indicated in the figure). The cross section for ν_{pump} resonant with Arg^+_{HF} however shows cross peaks only with the Glu^-_1 mode. This observation seems to suggest that the there are two different salt-bridge geometries between Arg^+ and Glu^- in the coiled coil, which give rise to the two

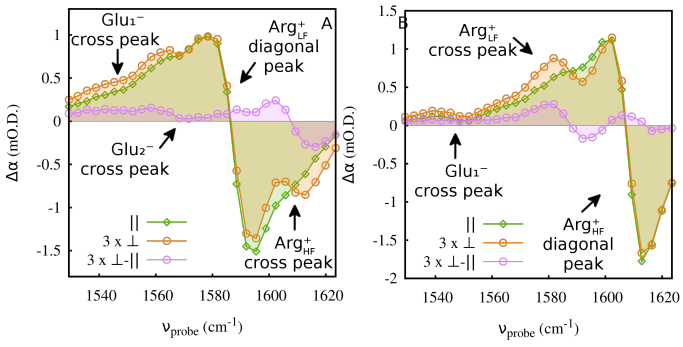

Figure 5.9: Cross sections of the 2D-IR spectra of the coiled coil for parallel and (scaled) perpendicular polarizations of pump and probe pulses for ν_{pump} resonant with (A) Arg^+_{LF} and (B) Arg^+_{HF}.

types of 2D-IR response that we detect. The two different Glu^- modes cannot be distinguished along the diagonal because their separation is about 15 cm^{-1}, which is most likely smaller than their inhomogeneous widths. In analogy to the previous two systems that we showed above, a salt bridge in which Glu^- couples to only the Arg^+_{LF} mode would be a monodentate one (very much like the α-helical peptide presented in the previous section), whereas a salt bridge in which Glu^- couples to both Arg^+ modes would be a bidentate one (shifting to a lower frequency due to the stronger coupling, similarly to what we observed for the β-turn). The Glu^-_2 mode is only coupled to the Arg^+_{LF}, indicative of a monodentate salt bridge. This salt bridge is very likely one of the three possible intrahelical $Glu^- \cdots Arg^+$ salt bridges highlighted in equation 5.3 that would adopt a monodentate geometry due to sterical constraints imposed by the helical structure of the backbone, very similar to the restrictions encountered for the α-helical peptide. The Glu^-_1 mode of Figure 5.9, which is coupled to both Arg^+ modes, would then be part of a bidentate salt bridge. The interhelical $Arg6^+ \cdots Glu11^-$ salt bridge is thus most likely the bidentate one, in agreement with the molecular representation of Figure 5.7A.

5.5 Conclusion

We have shown that 2D-IR spectroscopy is a robust probe of salt bridges in peptides in solution. We use this probe to detect salt bridges in solution between Arg^+ and Glu^- in a β-turn, and α-helical peptide and a coiled coil. We can qualitatively differentiate salt bridges with different geometries: bidentate geometry is present in the β-turn; a monodentate geometry is present in the α-helical peptide, and both geometries are present in the coiled coil. The monodentate geometry arise most likely due to sterical constraints that restrict the intrinsically preferred rotameric conformations of the side chains involved in salt bridge formation. The results that we present here show a method for probing salt bridges in solution with picosecond time resolution, that is ideally suited for time-resolved experiments to investigate systems in which salt bridges have structural relevance. This method is applicable to larger peptides and proteins, since intrinsic complications such as spectral crowding can be overcome by using isotopic labels: ^{15}N for Arg^+ (~40 cm^{-1} redshift)[71] and ^{18}O for Glu^- (~10 cm^{-1} redshift).[26] Such detection and characterization of salt bridges in solution should bring new insights into the controversial role that they play in the structure, folding dynamics, and biochemical activity of proteins.