PRIMARY AMIDE RAMAN VIBRATIONS AS ENVIRONMENTAL AND STRUCTURAL MARKERS OF GLUTAMINE AND ASPARGINE PROTEIN SIDE CHAINS

by

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University of Pittsburgh, 2016

UV resonance Raman (UVRR) is a powerful spectroscopic technique for the study of protein conformation and dynamics. Excitation at ~200 nm selectively enhances secondary amide vibrations, which are sensitive to the peptide backbone secondary structure and local environment. Primary amide bands are also resonance enhanced in UVRR spectra and could be used to report on glutamine and asparaginese side chains in biophysical studies of proteins and peptides.

IR absorption, visible Raman, and UVRR were used to investigate the small primary amide molecule propanamide. Dramatic spectral changes in the primary amide vibrations were observed upon aqueous solvation. Aqueous solvation impacts the dielectric and hydrogen bonding environment of the primary amide group resonance structures. This leads to a decrease in C-O and increase in C-N bond order of the primary amide group and therefore alters the resonance enhancement and vibrational frequencies of the primary amide vibrations substantially. Due to this significant response, several primary amide bands can be used as sensitive environmental markers for the glutamine and asparagine side chains.

Visible Raman and UVRR spectra of L-glutamine and five derivative molecules, D-glutamine, N-Acetyl-L-glutamine, L-glutamine t-butyl ester, Glycyl-L-glutamine, and L-serine-L-asparagine, were collected and assigned in the 950-1200 cm$^{-1}$ region. The OCCC dihedral angle of each was determined from X-ray crystal structures. An empirical relationship between the AmIII$^P$ vibrational frequency and the OCCC dihedral angle was observed.
This dependence can be explained by hyperconjugation that occurs between the $C_{\beta}-C_{\gamma}$ $\sigma$ orbital and the C=O $\pi^*$ orbital of the primary amide group. This interaction induces an increase in the $C_{\beta}-C_{\gamma}$ bond length. As the $C_{\beta}-C_{\gamma}$ bond length increases, the stretching force constant decreases, downshifting the AmIIIP band. Due to this sensitivity, the AmIIIP can be used as a structural marker diagnostic of the OCCC dihedral angle, such as in the side chains glutamine and asparagine in peptide and protein conformational studies.
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1.0 INTRODUCTION

1.1 MOTIVATION FOR NEW BIOPHYSICAL TOOLS

Polyglutamine expansion in proteins and aggregation into polyglutamine-rich fibril-like aggregates in neuronal cells is associated with several neurodegenerative diseases, including Huntington’s disease and several spinocerebellar ataxias [1–8]. In order to understand the molecular origins of these diseases, the biophysical properties of simple polyglutamine peptides have been a subject of intense study. Fibril aggregation studies have revealed mechanisms of fibril nucleation and growth [9–14]. Models of fibrils structures have been proposed based on X-ray diffraction [15–17] and solid-state NMR (ssNMR) [14, 18–21], but there is as yet no consensus and, until very recently, little was known about the structures of the glutamine side chains. Two distinct populations of glutamine side chains have been observed in fibrils by ssNMR [14, 19, 20, 22]. Sharma et al. interpret X-ray fiber diffraction data to reveal the glutamine side chains in a uncommon bent conformation [17]. Peptide microcrystal studies suggest that the tendency to form fibril polymorphs could possibly be due to side chain interactions [23]. Van der Wel and coworkers observe interdigitated extended side chains in the core of huntingtin exon 1 fibrils using ssNMR [22]. Glutamine side chains are believed to play an important role in the structure and aggregation mechanisms of polyglutamine peptides and could be important for formation or stabilization of fibrils [11, 15, 16, 19, 22, 24].

UV resonance Raman (UVRR) spectroscopy can provide molecular-level insights into protein structures such as polyglutamine fibrils that are insoluble and cannot be crystallized. Primary amide bands are resonance enhanced in UVRR spectra [25] and could act as spectral markers for glutamine side chains. These bands could be used as new spectroscopic tools to
investigate the local environments and structures of glutamine side chains in polyglutamine studies.

1.2 RAMAN SPECTROSCOPY

*The Raman Effect.* Raman scattering is an inelastic light scattering phenomenon in which a molecule is excited and radiates energy at frequencies different from the incident radiation. The differences in energy correspond to the nuclear vibrational motions of the molecule which couple with the electron cloud oscillations created by the incident radiation [26–28]. Raman spectroscopy measures the intensity of the inelastically scattered light and the frequency shifts that occur. This differs from Infrared (IR) spectroscopy, which measures the absorption of different frequencies of light and depends on net changes in the dipole moment as the molecule vibrates. Both vibrational spectroscopies are used to measure molecular vibrations of a sample in order to study the molecular structure, dynamics, and environment.

*UV Resonance Raman Spectroscopy (UVRR).* When excitation occurs within an electronic absorption band of a molecule or specific chromophore in a sample, large enhancement of the scattering intensity is observed for certain vibrations. The enhanced vibrations are those that couple with driven electronic motion in the electronic transition. This is called resonance Raman because the excitation frequency is in resonance with the electronic transition. Figure 1 shows IR absorption, Raman scattering, and resonance Raman scattering.

*Biophysical Applications of UVRR.* UVRR is an excellent technique for the study of proteins conformation and dynamics [29]. The selective enhancement of particular vibrations that are diagnostic of dielectric environment, hydrogen bonding, and structure enables the study of complicated molecules such as proteins in solution and in the solid-state. Excitation at \( \sim 200 \text{ nm} \) selectively enhances secondary amide vibrations [30], which have been shown to depend on hydrogen bonding [31–34] and peptide backbone secondary structure. Asher and coworkers determined a powerful quantitative relationship between the Amide III vibration and Ramachandran \( \Psi \) angle [35–38]. This relationship can be used to calculate \( \Psi \) angle distributions and determine the Gibbs free energy landscape along the \( \Psi \) angle coordinate.
Figure 1: Energy level diagrams of (a) IR absorption (b) Raman scattering (c) resonance Raman scattering.

These studies have made possible remarkable investigations of protein and peptide structure and folding [25, 39–44].

In addition, UVRR can be used to investigate the structure, and hydrogen bonding, and local environment of protein side chains. For example, the hydrogen bonding environment of aromatic amino acids can be determined based on UV resonance Raman cross sections [45]. Takeuchi and coworkers developed an empirical relationship between the W3 band of tryptophan and the $\chi^{21}$ torsion angle [46–48]. Hong et al. [49] found that the hydration state of the side chain arginine is related to the Raman cross section and frequency. Histidine specific vibrations were found to be sensitive to protonation state [50, 51]. A proline tertiary amide vibration, also resonance enhanced near $\sim$200 nm, shows sensitivity to peptide conformation [52–54]. The deep understanding of the relationships between these bands and the structural and environmental characteristics of the backbone and side chains allows for incisive studies of proteins and peptides.
2.0 PRIMARY AMIDE VIBRATION AQUEOUS SOLVATION DEPENDENCE

2.1 INTRODUCTION

The primary amide group is present in the protein side chains glutamine and asparagine. These side chains are of particular interest because of their ability to hydrogen bond with the protein backbone and other side chains, and their presence in disease proteins with elongated polyglutamine segments [1–8] and prions [55]. Primary amide bands are resonance enhanced in UV resonance Raman spectra [25] due the first $\pi \rightarrow \pi^*$ ($NV_1$) transition of the primary amide group and could be used to report on these side chains in biophysical studies of proteins and peptides. Raman and Infrared (IR) spectra were collected of the small molecule propanamide because it is a model of the glutamine and asparagine side chains. A full assignment was completed and large spectral changes were observed upon aqueous solvation. Several bands act as sensitive spectroscopic markers diagnostic of the environment of the primary amide group. These spectral markers will be of use in studies of glutamine and asparagine side chains in peptides and proteins.

2.2 MATERIALS AND METHODOLOGY

2.2.1 Sample Preparation

Propanamide ($\text{CH}_3\text{CH}_2\text{CONH}_2$, 97% purity) was purchased from Acros Organics and used as a solid sample without further purification. The N-deuterated crystalline sample was
obtained by multiple rounds of recrystallization in D$_2$O (99.9% atom D purity), obtained from Cambridge Isotope Laboratories, Inc.

Solutions of propanamide in H$_2$O and D$_2$O were prepared at 30 mM concentrations. In mixtures of acetonitrile (HPLC, far-UV grade), purchased from Acros Organics, and H$_2$O, 30 mM propanamide concentrations were used. As an internal standard, sodium perchlorate (NaClO$_4$, ≥98% purity), purchased from Sigma-Aldrich, was used at 200 mM concentration. N-methylacetamide (CH$_3$CH$_2$CONHCH$_3$, NMA, ≥99% purity) was purchased from Sigma-Aldrich and prepared at 10 mM concentration in H$_2$O.

### 2.2.2 Infrared Spectroscopy

IR spectroscopy was performed on a PerkinElmer Spectrum 100 FTIR with a Universal ATR accessory (UATR). Propanamide crystals were lightly ground with a mortar and pestle for ~30 s, placed on the diamond crystal, and subjected to ~145 N of applied force with the pressure arm. The spectrum was measured in the mid-infrared from 600–4000 cm$^{-1}$ with 1 cm$^{-1}$ resolution.

### 2.2.3 Visible Raman Spectroscopy

Visible Raman spectra were collected on a Renishaw inVia Raman microscope with 633 nm excitation generated by a HeNe laser. Spectra were collected at 2 cm$^{-1}$ resolution from 100–4000 cm$^{-1}$ using a 5× objective lens. The following acetonitrile bands were used for calibration [56]: 380 cm$^{-1}$, 918 cm$^{-1}$, 1376 cm$^{-1}$, 2249 cm$^{-1}$, and 2942 cm$^{-1}$.

### 2.2.4 UV Resonance Raman Spectroscopy

229 nm excitation. UV resonance Raman (UVRR) spectra of crystalline samples were measured using cw 229 nm excitation of a Coherent Innova 300c FreD frequency doubled Ar$^+$ laser [57]. The Raman scattered light was collected and dispersed using a SPEX Triplemate spectrometer that has been adapted for use in the UV. The dispersed light was then detected with a Spec-10 system CCD camera (Princeton Instruments, Model 735-0001) with
Lumagen-E coating. Solid samples were spun on a brass spinning cell to prevent thermal- and photodegradation. The 380 cm\(^{-1}\), 918 cm\(^{-1}\), 1376 cm\(^{-1}\), 2249 cm\(^{-1}\), and 2942 cm\(^{-1}\) bands of acetonitrile were used for calibration.

204 nm excitation. UVRR spectra of solution samples were measured using 204 nm excitation. The 204 nm light was obtained by Raman shifting the third harmonic of a Coherent Infinity Nd:YAG laser with H\(_2\) gas at ~30 psi and selecting the fifth anti-Stokes line. Scattered Raman light was collected and dispersed with double monochromator adapted for use in the UV with a subtractive configuration. The light was then imaged on a Spec-10 CCD. Solution samples were circulated through a temperature-controlled (20 \(^{\circ}\)C) flow-cell to prevent photodegradation products contributing to spectra.

2.2.5 UV Absorption Spectroscopy

UV absorption spectra were collected on a Varian Cary 500 spectrophotometer using a 0.2 mm path length quartz cuvette (Hellma Analytics).

2.3 RESULTS AND DISCUSSION

2.3.1 Vibrational Assignment of Propanamide

In order to complete a full assignment of the vibrational spectrum of propanamide (Figure 2), IR, visible Raman, and pre-resonance 229 nm excitation Raman spectra of solid-state samples and 204 nm excitation resonance Raman spectra of propanamide in solution were collected. These experimental results were interpreted with normal mode analysis completed using new density functional theory DFT calculations. DFT calculations and normal mode analysis were performed by Dr. Zhenmin Hong and Dr. Nataliya S. Myshakina, and analyzed with David Punihaole, as described in detail in [58].

Crystalline Propanamide. Figures 3 and 4 show IR, visible Raman, and 229 nm excitation UVRR spectra of crystalline propanamide. To assist in band assignments, spectra of N-deuterated propanamide, CH\(_3\)CH\(_2\)CODH\(_2\), shown in Figures 5 and 6 were collected [58].
At 229 nm excitation, the UVRR spectra are pre-resonance enhanced, since the excitation wavelength falls within the first $\pi \rightarrow \pi^*$ (NV$_1$) electronic transition absorption band of the primary amide group, at $\sim$180 nm. The geometry is expected to expand along the C–N coordinate within this transition from the ground electronic state to the first excited electronic state [30, 59]. Therefore, bands with significant C–N stretching character are expected to be resonance enhanced.

The IR and visible Raman vibrational frequencies, DFT scaled, calculated frequencies, and vibrational potential energy distributions are listed in Tables 1 and 2. Figure 2 shows the optimized geometry of propanamide, with labels corresponding the potential energy distribution (PED) analysis.

![Propanamide molecule with numbering scheme used in potential energy distributions.](image)

Figure 2: Propanamide molecule with numbering scheme used in potential energy distributions. Adapted with permission from [58]. Copyright © 2015, American Chemical Society.

2000–3500 Wavenumber Region. The 2000–3500 cm$^{-1}$ region is made up of C–H and N–H stretching vibrations. The N–H stretching bands are identified by the significant downshift they undergo upon N-deuteration. The N–H asymmetric stretch is located at $\sim$3355 cm$^{-1}$ and the symmetric stretch at $\sim$3175 cm$^{-1}$. The bands between 2700 and 3100 cm$^{-1}$ do not shift upon N-deuteration, indicating that they are all C–H stretching vibrations.

Note that there are two bands in this region of the spectrum that are not assigned to fundamentals. The band at $\sim$2735 cm$^{-1}$ is not shifted in the spectrum of N-deuterated propanamide. Further, Kuroda et al. [60] show that this vibration does not shift upon
deuteration of the methylene group. This demonstrates that the vibration is not due to an overtone or combination of either the amide or the methylene group. Thus, the $\sim 2735 \text{ cm}^{-1}$ band is assigned to an overtone of the $\sim 1380 \text{ cm}^{-1}$ CH$_3$ symmetric deformation. The band at $\sim 2940 \text{ cm}^{-1}$ is also not shifted in the spectrum of N-deuterated propanamide, but is very strong in the visible Raman and UVRR spectra. This band is assigned to a Fermi resonance between the CH$_3$ stretching mode and the $\sim 1460 \text{ cm}^{-1}$ CH$_3$ asymmetric deformation. This assignment is based on the work of Kuroda et al. \cite{Kuroda60} and Nolin and Jones \cite{Nolin61} and contests the assignment of Nandini and Sathyanarayana \cite{Nandini62}. This band frequency is higher than the typical seen for a CH$_2$ stretching vibration \cite{Nandini63}, which Nandini and Sathyanarayana suggested \cite{Nandini62}.

1500–1800 Wavenumber Region. This region contains more complex vibrations made up of heavy atom stretching and bending motions, especially involving the amide group. The $\sim 1675 \text{ cm}^{-1}$ band is assigned to the Amide I (AmI) vibration, made up of $\sim 75\%$ C=O stretching with $\sim 7\%$ C–N stretching according to the calculations. The Amide II (AmII) band at $\sim 1590 \text{ cm}^{-1}$ band is made up of $\sim 86\%$ NH$_2$ scissoring with a small C–N stretching contribution of $\sim 10\%$. These bands are both well defined in the visible Raman and UVRR spectra, but broad and overlapping in the IR spectrum. This is most likely due to the presence of phonons and differences in phonon selection rules between the two phenomena.

The shifts in vibrational frequencies observed upon N-deuteration support the assignment and PED analysis of the AmI and AmII vibrations. The AmI band downshifts from $\sim 1675 \text{ cm}^{-1}$ to $\sim 1610 \text{ cm}^{-1}$ in the Raman spectra, indicating that the AmI vibration is mostly C=O stretching. In the N-deuterated spectrum, there is no AmII band, rather a band appears at $\sim 1170 \text{ cm}^{-1}$ which is attributed to ND$_2$ scissoring. This supports the idea that the AmII vibration is mostly NH$_2$ scissoring.

1200–1500 Wavenumber Region. Between 1200 and 1500 cm$^{-1}$ there are several bands made up of CH$_3$ and CH$_2$ deformation and bending motions. The vibrations at $\sim 1464 \text{ cm}^{-1}$ and $\sim 1450 \text{ cm}^{-1}$ are assigned to CH$_3$ asymmetric deformations. At $\sim 1380 \text{ cm}^{-1}$ is the CH$_3$ symmetric deformation. The CH$_2$ twisting mode is assigned to the band at $\sim 1260 \text{ cm}^{-1}$.

Within this region there are also bands that contain significant C–N stretching character. Previously, Kuroda et al. \cite{Kuroda60} and Nandini and Sathyanarayana \cite{Nandini62} assigned the vibration at
$\sim1420 \text{ cm}^{-1}$ to a CH$_2$ scissoring/C–N stretching mode and a CH$_2$ bending/CH$_3$ symmetric bending mode, respectively. Nandini and Sathyanarayana [62] assign the $\sim1300 \text{ cm}^{-1}$ band to a vibration made up of C–N stretching/CH$_2$ wagging while Kuroda et al. [60] assign it to mostly CH$_2$ wagging.

The UVRR spectra collected show a resonance enhanced band at $\sim1430 \text{ cm}^{-1}$, which is not resolved in the visible Raman or IR spectra. This analysis suggests that this band be assigned to a complex vibration, $\nu_{12}$, made up of $\sim30\%$ CH$_2$ wagging, $\sim20\%$ C–C stretching, and $\sim19\%$ C–N stretching. The $\sim1420 \text{ cm}^{-1}$ band is assigned to CH$_2$ wagging and the $\sim1300 \text{ cm}^{-1}$ band to another complex vibration, $\nu_{15}$, which contains CH$_2$ wagging and C–N stretching.

700–1200 Wavenumber Region. The 700–1200 cm$^{-1}$ region contains vibrations made up of NH$_2$ rocking, CH$_2$ rocking, CH$_3$ rocking, and C–CH$_3$ stretching. The band at $\sim1145 \text{ cm}^{-1}$ is assigned to NH$_2$ rocking since it downshifts to $\sim940 \text{ cm}^{-1}$ upon N-deuteration. The band at $\sim1070 \text{ cm}^{-1}$ is assigned to $\nu_{19}$, a vibration made of $\sim38\%$ C–CH$_3$ stretching, significant $\sim29\%$ NH$_2$ rocking, and $\sim16\%$ C–N stretching character, which differs from the normal mode analysis of Kuroda et al. [60].
Figure 3: (a) IR (b) visible Raman, and (c) UVRR spectra of crystalline propanamide in the 2000–3500 cm$^{-1}$ region.
Figure 4: (a) IR (b) visible Raman, and (c) UVRR spectra of crystalline propanamide in the 700–1800 cm\(^{-1}\) region.
Table 1: Assignments of IR and Raman Bands of Crystalline Propanamide

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<td>ρ'CH₃ (28), ωCH₂ (19), νC1C2 (18), −ρCH₃ (11), −νC2C3 (9), −ρCH₂ (8)</td>
</tr>
<tr>
<td>22</td>
<td>822</td>
<td>822</td>
<td>853</td>
<td>3.8</td>
<td>ρCH₂ (23), ρ'CH₃ (18), −πC=O (18), ρCH₃ (15), τCH₂ (10), νC2C3 (8)</td>
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<tr>
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<td>νC2C3 (43), −ρCH₃ (14), ρNH₂ (11), −ρCH₂ (7), νC1C2 (6)</td>
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</tbody>
</table>

*a* Infrared and Raman vibrational frequencies reported in wavenumbers (cm⁻¹)

*Δ* = |νobs − νcalc|/νobs × 100 %

*c* ν: stretch; δ: sym deformation; σ: scissoring; δas: asym deformation; ρ: rocking; ω: wagging;

β: in-plane bending; τ: twisting; II: out-of-plane bending

*d* Frequency obtained from 229 nm excitation UVRR data.

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Figure 5: (a) IR (b) visible Raman, and (c) UVRR spectra of crystalline N-deuterated propanamide in the 2000–3500 cm$^{-1}$ region.
Figure 6: (a) IR (b) visible Raman, and (c) UVRR spectra of crystalline N-deuterated propanamide in the 700–1800 cm$^{-1}$ region.
Table 2: Assignments of IR and Raman Bands of Deuterated Crystalline Propanamide

<table>
<thead>
<tr>
<th>ν</th>
<th>Infrared&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Raman&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Calc. ∆&lt;sup&gt;b&lt;/sup&gt; (%)</th>
<th>PED&lt;sup&gt;c&lt;/sup&gt; (≥5% contribution)</th>
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<td>2941</td>
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<tr>
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<td>2912</td>
<td>2900</td>
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</tr>
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<td>1434&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>−</td>
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<tr>
<td>−</td>
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<td>−</td>
<td>770</td>
<td>776</td>
<td>0.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Infrared and Raman vibrational frequencies reported in wavenumbers (cm<sup>−1</sup>)

<sup>b</sup> Δ = |ν<sub>obs</sub> − ν<sub>calc</sub>|/ν<sub>obs</sub> × 100%

<sup>c</sup> ν: stretch; δ: sym deformation; σ: scissoring; δ<sub>as</sub>: asym deformation; ρ: rocking; ω: wagging;
β: in-plane bending; τ: twisting; Π: out-of-plane bending

<sup>d</sup> Frequency obtained from 229 nm excitation UVRR data.

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Propanamide in Aqueous Solution. UVRR spectra of propanamide were collected in H$_2$O and D$_2$O and shifts were observed in the vibrational frequencies of several bands compared to the crystal spectra [58]. Figure 7(a) shows the AmI band of propanamide in H$_2$O at $\sim$1669 cm$^{-1}$, $\sim$7 cm$^{-1}$ downshifted, and the AmII band at $\sim$1610 cm$^{-1}$, $\sim$22 cm$^{-1}$ upshifted from the spectra of the solid-state sample. The $\nu_{12}$ and $\nu_{19}$ bands do not shift significantly, but the NH$_2$ rocking vibration is $\sim$16 cm$^{-1}$ downshifted.

In D$_2$O (Figure 7(b)), the AmI' upshifts to $\sim$1633 cm$^{-1}$ when compared with crystalline propanamide spectra. The ND$_2$ scissoring vibration is found at $\sim$1168 cm$^{-1}$, downshifted from the N-deuterated crystal spectra. The complex vibration, $\nu_{12}$, consisting of $\sim$30% CH$_2$ wagging, $\sim$20% C-C stretching, and $\sim$19% C-N stretching is found at $\sim$1443 cm$^{-1}$ in D$_2$O compared to $\sim$1434 cm$^{-1}$ in the spectra of N-deuterated crystalline propanamide.

Figure 7: UVRR spectra of propanamide in (a) H$_2$O and (b) D$_2$O.
2.3.2 Aqueous Solvation of Primary Amide Vibrations

Upon aqueous solvation, the intensities of UVRR primary amide bands change dramatically [58]. Figure 8 shows large changes in the resonance Raman cross sections and vibrational frequencies of all the bands of propanamide as the solvent transfers from acetonitrile to H₂O. All of the bands show a roughly linear increase in their Raman cross sections as the mole fraction of H₂O increases, except for the AmI band, which shows a decrease in cross section. The AmII and the ν₁₂ band show the largest increases.

![Figure 8: UVRR spectrum of propanamide in mixtures of acetonitrile and H₂O. Adapted with permission from [58]. Copyright © 2015, American Chemical Society.](image)

The vibrational frequencies also show a roughly linear increase in the case of the ν₁₂, NH₂ rocking, and ν₁₉ vibrations. In contrast, the AmI and AmII bands show decreases in vibrational frequency as the mole fraction of H₂O increases.

These observations can in part be explained using observations made on other similar amide molecules. The changes in UVRR spectra upon aqueous solvation have previously been noted in valeramide [25] and N-methylacetamide (NMA) [31, 33, 34, 64, 65]. UV absorption
spectra of NMA were collected to compare with those of propanamide (Figure 9) in order to further understand this behavior [58]. Figure 10 shows the UV absorption spectra of NMA in acetonitrile and water. As the mole fraction of water increases, the maximum absorptivity of the NV₁ transition at ~200 nm, redshifts. Both propanamide and NMA exhibit this redshift in absorptivities, though this is less clear in the case of propanamide for which the NV₁ transition is deeper in the UV (~180 nm). Nielsen and Schellman also observed a redshift of the maximum absorptivity in several primary and secondary amides upon aqueous solvation [66]. It is known that the Raman scattering cross section is proportional to the square of the molar absorptivity. Thus, the changes in the cross sections upon aqueous solvation are due in part to the increase in the molar absorptivity of the NV₁ transition.

However, the dramatic changes in cross sections of the UVRR bands of propanamide are due in larger part to changes in the ground state structure upon aqueous solvation. In the case of secondary amides, the effects of aqueous solvation are attributed to changes in the dielectric and hydrogen bonding on the ground state resonance structures [31, 33, 34, 64, 65]. The dielectric environment and hydrogen bonding of the primary amide group ground state resonance structures are likewise influenced by aqueous solvation.

The primary amide group can act as both a hydrogen bonding donor and as an acceptor with water and itself. The primary amide carbonyl oxygen acts as a hydrogen bonding acceptor while the N–H can act as a hydrogen bonding donor to the oxygen of water. This is possible because water is also both a hydrogen bond acceptor and donor, whereas acetonitrile, being an aprotic polar solvent, can only act as an acceptor. In the case of primary amide group solvated by acetonitrile, the N–H group can act as a hydrogen bonding donor to the nitrogen of acetonitrile, but the carbonyl oxygen is not engaged in hydrogen bonding in this case.

The primary amide group O=C-NH₂ form is more dominant than the −O−C=NH₂⁺ resonance structure in a low dielectric and hydrogen bonding environment like acetonitrile. In aqueous solution, where the dielectric constant is higher and hydrogen bonding is stabilizing, the contribution of the dipolar −O−C=NH₂⁺ form increases. This is because the partial charges on the carbonyl carbon and nitrogen are expected to increase with hydrogen bonding [67]. The dipolar resonance structure will be less favored when the primary amide
group is solvated by acetonitrile because of the lack of hydrogen bonding with the carbonyl oxygen. Thus, upon aqueous solvation the C–O bond order decreases and the C–N bond order increases in the primary amide group [58, 67].

The decrease in C–O bond order and increase in C–N bond order in amides greatly impacts the Raman cross sections. The resonance Raman cross section scales with the square of the displacement along the enhanced vibration normal coordinate between the electronic ground and electronic excited state geometries [68]. In the case of NMA, vibrations with significant C–N stretching character are resonance enhanced [31, 34, 64, 69, 70] because the molecule is displaced along this normal coordinate between the geometries of the electronic ground and excited states [33, 65, 70]. The AmI of NMA however exhibits less enhancement in water due to the fact that there is less displacement along the C–O normal coordinate.

As the C–O bond order decreases in propanamide, the C–O bond elongates, leading to a decrease in the vibrational frequency of the AmI band, which is mostly C=O stretching. This is because an elongation in the C–O bond corresponds to a decrease in the stretching force constant. The resonance Raman cross section decreases as well because expansion of the electronic excited state along this bond is decreased in water.

As the C–N bond order increases, and thus the C–N bond contracts, vibrations with significant C–N stretching upshift as the stretching force constant increases. The C–N bond order increase also explains the increase in the cross sections. The contraction of the C–N bond leads to an increase in the displacement between the electronic ground and excited state along the C–N coordinate. Thus the vibrations with C–N stretch including the AmII, \( \nu_{12}, \nu_{15}, \) NH\(_2\) rocking, and \( \nu_{19} \) vibrations are resonance enhanced in the UVRR spectra.
Figure 9: UV Absorption spectrum of (a) propanamide and (b) N-methylacetamide (NMA) in mixtures of acetonitrile and H$_2$O. Adapted with permission from [58]. Copyright © 2015, American Chemical Society.
Figure 10: UV Absorption spectrum of NMA in acetonitrile and H₂O from 185 – 240 nm.
3.0 PRIMARY AMIDE VIBRATION STRUCTURAL SENSITIVITY

3.1 INTRODUCTION

Structural markers for glutamine side chains would be of great use in investigations of the aggregation mechanisms of polyglutamine-rich peptides and the structures of the fibril-like aggregates they form. It is not yet well understood how the side chains participate in the aggregation process, though their role appears to be significant \([11,15,16,19,24]\). UV resonance Raman (UVRR) spectroscopy has been applied to study the secondary structure and dynamics of proteins in solution using the quantitative relationship between the AmIII vibration and the Ramachandran \(\Psi\) angle \([35-38]\). In addition, several side chain vibrations are diagnostic of the local environment \([25,45-47,49,53,71]\) and in some cases structure \([46-48,52,54]\). Understanding of the structural sensitivity of primary amide vibrations is not as well developed as that of secondary amides. Raman spectra of crystalline L-glutamine and several derivative molecules were collected, and the structures determined by X-ray diffraction. A primary amide Raman band made up of largely \(\text{NH}_2\) rocking, C–N stretching, and C–C stretching shows sensitivity to the OCCC dihedral angle. This novel spectroscopic marker will enable the determination of glutamine and asparagine conformations in peptides and proteins.
3.2 MATERIALS AND METHODOLOGY

3.2.1 Sample Preparation

Glycyl-L-glutamine (Gly-Gln, ≥97% purity), purchased from Sigma-Aldrich, and L-seryl-L-asparagine (Ser-Asn, ≥99% purity), purchased from Bachem, were used without further purification. D-glutamine (D-Gln, 98% purity), purchased from Acros Organics, N-Acetyl-L-glutamine (NAcGln, 97% purity), purchased from Spectrum Chemical Mfg. Corp., and L-glutamine t-butyl ester hydrochloride (GlnTBE, ≥98% purity), purchased from Sigma-Aldrich, were all prepared from drying saturated solutions in H$_2$O (Optima-grade), purchased from Fisher Scientific. L-glutamine (L-Gln, ≥99% purity), purchased from Sigma-Aldrich, was prepared from drying a saturated solution of H$_2$O with 0.1 M concentration NaCl. N-deuterated crystalline samples were obtained by multiple rounds of recrystallization in D$_2$O (99.9% atom D purity), obtained from Cambridge Isotope Laboratories, Inc.

3.2.2 Visible Raman Spectroscopy

Visible Raman spectra were collected as described in Section 2.2.3; however, only the 918 cm$^{-1}$, 1376 cm$^{-1}$ bands of acetonitrile were used for calibration [56].

3.2.3 UV Resonance Raman Spectroscopy

UVRR spectra were collected as described in Section 2.2.4; however, the 801 cm$^{-1}$, 1028 cm$^{-1}$, 2852 cm$^{-1}$, and 2938 cm$^{-1}$ bands of cyclohexane were used to calibrate the 229 nm excitation UVRR spectra.
3.3 RESULTS AND DISCUSSION

3.3.1 Vibrational Assignment of Glutamine and Derivatives

Visible Raman and pre-resonance 229 nm excitation Raman spectra of crystalline L-Gln and five derivative molecules, D-Gln, NAcGln, GlnTBE, Gly-Gln, and Ser-Asn, and 204 nm excitation resonance Raman spectra of L-Gln in solution were collected in order to identify potential spectral markers of conformation of the glutamine side chain. These spectra were assigned in the 950–1200 cm$^{-1}$ region using potential energy distributions obtained from DFT calculations carried out by Dr. Zhenmin Hong and analyzed with David Punihaole, as detailed in [72]. X-ray diffraction of the crystalline samples was performed by David Punihaole and Dr. Steven Geib, described in further detail in [72], providing the molecular structures of the L-Gln and derivative molecule samples (Figures 11 and 12). From these structures, conformational determinants, such as the OCCC dihedral angle, could be obtained.

Crystalline Glutamine and Derivatives. Figures 13, 14, 15, 16, 17, and 18 show the 633 nm visible excitation and 229 nm excitation UVRR spectra of crystalline L-Gln and five derivative molecules: D-Gln, NAcGln, GlnTBE, Gly-Gln, and Ser-Asn. In addition, N-deuterated crystal spectra were collected in order to assist in band assignments, given that the region of interest contains several bands with NH$_2$ and NH$_3$ rocking character. As discussed in Section 2.3.1, bands with significant C–N stretching character are expected to exhibit resonance enhancement in 229 nm excitation Raman spectra.

The visible Raman vibrational frequencies, DFT scaled, calculated frequencies, and vibrational potential energy distributions for each molecule in the 950–1200 cm$^{-1}$ region are listed in Tables 3, 4, 5, 6, 7, 8. The crystal structures of L-Gln and derivative molecules with labels corresponding the potential energy distribution analysis and OCCC dihedral angle can be found in Figures 11 and 12.

L-glutamine and D-glutamine. Ramirez and coworkers [73] have reported spectra of solid-state L-glutamine previously [74]; however, they did not use normal mode calculations to complete assignments. New assignments for L-Gln are reported here using UVRR spectral frequencies and intensities, isotopic shifts, and modern DFT calculations. The ex-
performed experimentally observed Raman frequencies and assignments of crystalline L-Gln are shown in Table 3 and those of D-Gln in Table 4 and are essentially identical. The ~1205 cm\(^{-1}\) and ~1166 cm\(^{-1}\) bands are assigned to \(\text{C}_\beta\text{H}_2\) twisting/\(\text{C}_\alpha\text{C}_\beta\) stretching and \(\text{C}_\alpha\text{H}\) rocking/\(\text{C}_\gamma\text{H}_2\) twisting modes respectively, as they do not shift in the spectra of N-deuterated L-glutamine. In contrast, the bands at ~1135 cm\(^{-1}\) and ~1105 cm\(^{-1}\) are significantly shifted upon N-deuteration. The ~1135 cm\(^{-1}\) band is assigned to \(\text{C}_\beta\text{C}_\gamma\) stretching/\(\text{N}_2\text{H}_2\) rocking and the ~1105 cm\(^{-1}\) is assigned to \(\text{NH}_3\) rocking. The ~1079 cm\(^{-1}\) band shifts in the N-deuterated spectra as well and is assigned to a band made up of \(\text{N}_2\text{H}_2\) rocking, out-of-phase \(\text{C}_\beta\text{C}_\gamma\) stretching, and a significant in-phase \(\text{C}_\delta\text{N}_e\) stretching contribution, called the AmIII\(^P\) due to its similarity to the secondary Amide III vibration. The ~1086 cm\(^{-1}\), ~1052 cm\(^{-1}\), and ~1000 cm\(^{-1}\) bands are composed of mostly \(\text{C}_\alpha\text{N}\) stretching, \(\text{NH}_3\) rocking/\(\text{C}_\alpha\text{N}\) stretching, and \(\text{NH}_3\) rocking/\(\text{C}_\alpha\text{C}_\beta\) stretching respectively.
Figure 12: L-glutamine derivative molecules (a) N-Acetyl-L-glutamine, (b) L-glutamine t-buty1 ester hydrochloride, (c) Glycyl-L-glutamine, and (d) L-seryl-L-asparagine with labeling scheme used in potential energy distributions and OCCC dihedral angle. Adapted with permission from [72]. Copyright © 2015, American Chemical Society.
Figure 13: (a) visible Raman, and (b) UVRR spectra of crystalline L-glutamine in the 1000–1200 cm\(^{-1}\) region. Adapted with permission from [72]. Copyright © 2015, American Chemical Society.
Table 3: Raman Frequencies and Assignments of Crystalline L-Gln

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Calc.</th>
<th>PED(^b) (≥5% contribution)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
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<td>(\tau)CH(_2)/(\nu)C–C</td>
</tr>
<tr>
<td>1166</td>
<td>1153</td>
<td>(\rho)'C(<em>\alpha)H (20), (\tau)C(</em>\gamma)H(<em>2) (17), (\rho)'NH(<em>3) (13), (\tau)C(</em>\beta)H(<em>2) (12), (\nu)C(</em>\alpha)C(</em>\beta) (8), (\rho)C(_\gamma)H(_2) (6)</td>
<td>(\rho)CH/(\tau)CH(_2)</td>
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<td>1122</td>
<td>(\nu)C(<em>\beta)C(</em>\gamma) (34), (\rho)N,H(<em>2) (17), (\nu)C(</em>\alpha)C(<em>\beta) (7), (\nu)C(</em>\alpha)N (6), (\beta)N(<em>{\gamma})C(</em>\gamma) (5)</td>
<td>(\nu)C–C/(\rho)NH(_2)</td>
</tr>
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<td>1097</td>
<td>(\nu)C(<em>\beta)C(</em>\gamma) (26), (\rho)N(<em>{\epsilon})H(<em>2) (26), (\nu)C(</em>\delta)N(</em>{\epsilon}) (13), (\rho)NH(_3) (8)</td>
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<td>1038</td>
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<td>(\nu)C–N</td>
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<td>1003</td>
<td>(\rho)NH(<em>3) (25), (\nu)C(</em>\alpha)N (19), (\rho)C(<em>\gamma)H(<em>2) (14), (\rho)C(</em>\beta)H(<em>2) (14), (\nu)C(</em>\alpha)C(</em>\beta) (7), (\tau)C(_\beta)H(_2) (5)</td>
<td>(\rho)NH(_3)/(\nu)C–N</td>
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<td>(\rho)NH(_3)/(\nu)C–C</td>
</tr>
</tbody>
</table>

\(^a\) Frequencies correspond to visible Raman (633 nm excitation) spectrum.

\(^b\nu\): stretch; \(\delta\): symmetric deformation; \(\delta\): deformation; \(\sigma\): scissoring; \(\rho\): rocking; \(\omega\): wagging; \(\beta\): in-plane bending; \(\tau\): twisting.

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Figure 14: (a) visible Raman, and (b) UVRR spectra of crystalline D-glutamine in the 1000–1200 cm$^{-1}$ region. Adapted with permission from [72]. Copyright © 2015, American Chemical Society.
<table>
<thead>
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<th>PED(^{5}%) contribution</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
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<td>1215</td>
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<td>(\tau CH_2/\nu C-C)</td>
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<td>1153</td>
<td>(\rho C_\gamma H (20)), (\tau C_\gamma H_2 (17)), (\rho NH_3 (13)), (\tau C_\beta H_2 (12)), (\nu C_\alpha C_\beta (8)), (\rho C_\gamma H_2 (6))</td>
<td>(\rho CH/\tau CH_2)</td>
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<td>1134</td>
<td>1122</td>
<td>(\nu C_\beta C_\gamma (34)), (\rho N,H_2 (17)), (\nu C_\alpha C_\beta (7)), (\nu C_\alpha N (6)), (\beta N,C_\gamma C_\gamma (5))</td>
<td>(\nu C-C/\rho NH_2)</td>
</tr>
<tr>
<td>1105</td>
<td>1109</td>
<td>(\rho NH_3 (27)), (\rho C_\gamma H (10)), (\rho N_\epsilon H_2 (10)), (\delta NC_\alpha C(\text{OO}) (9)), (\tau C_\alpha H (7)), (\nu C_\alpha N (7))</td>
<td>(\rho NH_3)</td>
</tr>
<tr>
<td>1096</td>
<td>1097</td>
<td>(\nu C_\beta C_\gamma (26)), (\rho N,H_2 (26)), (\nu C_\beta N_\epsilon (13)), (\rho N_\epsilon H_2 (8))</td>
<td>(\text{AmIII})</td>
</tr>
<tr>
<td>1086</td>
<td>1038</td>
<td>(\nu C_\alpha N (36)), (\nu C_\beta C_\gamma (9)), (\rho C_\beta H_2 (8)), (\rho NH_3 (6)), (\nu C-N)</td>
<td>(\nu C-N)</td>
</tr>
<tr>
<td>1051</td>
<td>1003</td>
<td>(\rho NH_3 (25)), (\nu C_\alpha N (19)), (\rho C_\gamma H_2 (14)), (\rho C_\beta H_2 (14)), (\nu C_\alpha C_\beta (7)), (\nu C_\alpha C (8)), (\nu C_\alpha C_\beta (5)), (\nu C_\alpha C (8)), (\nu C_\alpha C_\beta (7)), (\nu C_\alpha N (6))</td>
<td>(\rho NH_3/\nu C-C)</td>
</tr>
</tbody>
</table>

\(a\) Frequencies correspond to visible Raman (633 nm excitation) spectrum.

\(b\) \(\nu\): stretch; \(\delta\): symmetric deformation; \(\tau\): deformation; \(\sigma\): scissoring; \(\rho\): rocking; \(\omega\): wagging; \(\beta\): in-plane bending; \(\tau\): twisting.

Table 4: Raman Frequencies and Assignments of Crystalline D-Gln

\(N\)-Acetyl-L-glutamine. Table 5 shows the experimentally observed Raman frequencies and assignments of crystalline NAcGln. The band at \(\sim 1180\) cm\(^{-1}\) is assigned to \(C_\alpha-N\) stretching. This is based on the assignment of \(N\)-Acetyl-L-glutamic acid by Kausar et al. \([75]\), who assign a similar band to nearly the same frequency. The band at \(\sim 1138\) cm\(^{-1}\) is assigned to \(C_\alpha-C_\beta\) stretching/\(C_\alpha-N\) stretching. There is no band near \(\sim 1111\) cm\(^{-1}\) in the spectrum of \(N\)-Acetyl-L-glutamic acid \([75]\), so this band is assigned to the \(N_\epsilon H_2\) rocking/\(C_\alpha-C_\beta\) stretching mode. The AmIII\(^P\) vibration is assigned to the shoulder at \(\sim 1071\) cm\(^{-1}\). The \(\sim 1061\) cm\(^{-1}\), \(\sim 1022\) cm\(^{-1}\), and \(\sim 997\) cm\(^{-1}\) bands are assigned to \(CH_3\) rocking, \(C_\beta H_2\) rocking/\(C-H\) rocking, and \(C-CH_3\) rocking of the acetyl group, respectively, as they do not exhibit pronounced shifts upon N-deuteration.
Figure 15: (a) visible Raman, and (b) UVRR spectra of crystalline N-Acetyl-L-glutamine in the 1000–1200 cm\(^{-1}\) region. Adapted with permission from [72]. Copyright © 2015, American Chemical Society.
Table 5: Raman Frequencies and Assignments of Crystalline N-Acetyl-L-Gln

<table>
<thead>
<tr>
<th>Expt. ( ^a )</th>
<th>Calc.</th>
<th>PED ( ^b ) (( \geq 5% ) contribution)</th>
<th>Assignment</th>
</tr>
</thead>
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<tr>
<td>1192</td>
<td>( -\tau C_\beta \text{H}<em>2 ) (32), ( -\tau C</em>\gamma \text{H}<em>2 ) (24), ( -\nu C</em>\alpha C_\beta ) (12), ( \rho C_\alpha \text{H} ) (11)</td>
<td>( \tau \text{CH}_2 )</td>
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<td>1180</td>
<td>( \nu C_\alpha \text{N} ) (46), ( -\delta NC_\alpha C_\beta \text{COO} ) (8), ( \omega C_\beta \text{H}<em>2 ) (7), ( -\sigma C</em>\alpha C_\beta C_\gamma ) (6), ( \nu C_\beta C_\gamma ) (5)</td>
<td>( \nu \text{C–N} )</td>
<td></td>
</tr>
<tr>
<td>1138</td>
<td>( \nu C_\alpha C_\beta ) (22), ( -\nu C_\alpha \text{N} ) (12), ( \nu C_\beta C_\gamma ) (10), ( \rho N_\text{H}<em>2 ) (8), ( -\tau C</em>\gamma \text{H}_2 ) (5)</td>
<td>( \nu \text{C–C}/\nu \text{C–N} )</td>
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<tr>
<td>1111</td>
<td>( \rho N_\text{H}<em>2 ) (20), ( -\nu C</em>\alpha C_\beta ) (15), ( \nu C_\beta C_\gamma ) (14), ( \rho C_\beta \text{H}<em>2 ) (7), ( \rho C</em>\gamma \text{H}<em>2 ) (6), ( \nu C</em>\delta \text{N}<em>\epsilon ) (5), ( -\omega C</em>\gamma \text{H}_2 ) (5)</td>
<td>( \rho \text{NH}_2/\nu \text{C–C} )</td>
<td></td>
</tr>
<tr>
<td>1071</td>
<td>1093</td>
<td>( -\nu C_\beta C_\gamma ) (42), ( \rho N_\text{H}<em>2 ) (24), ( \nu C</em>\delta \text{N}_\epsilon ) (13)</td>
<td>( \text{AmIII}^\text{P} )</td>
</tr>
<tr>
<td>1061</td>
<td>1058</td>
<td>( \rho \text{CH}<em>3 ) (63), ( -\delta</em>\text{sNC'}\text{O'}\text{CH}<em>3 ) (19), ( -\delta</em>\text{as}\text{CH}_3 ) (9), ( -\rho'\text{CH}_3 ) (7)</td>
<td>( \rho \text{CH}_3 )</td>
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<td>1043</td>
<td>( \rho C_\beta \text{H}<em>2 ) (25), ( \rho' C</em>\alpha \text{H} ) (15), ( \rho C_\gamma \text{H}<em>2 ) (14), ( -\nu C</em>\alpha \text{C} ) (8), ( \nu C_\alpha C_\beta ) (8), ( \delta \text{CC}<em>\alpha C</em>\beta ) (5)</td>
<td>( \rho \text{CH}_2/\rho \text{CH} )</td>
</tr>
<tr>
<td>1013</td>
<td>1043</td>
<td>( \rho' \text{CH}_3 ) (58), ( -\nu'\text{C'CH}_3 ) (12), ( \rho \text{CH}<em>3 ) (5), ( -\delta</em>\text{as'}\text{'CH}_3 ) (5)</td>
<td>( \rho \text{CH}_3 )</td>
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<tr>
<td>997</td>
<td>971</td>
<td>( \nu'\text{C'CH}<em>3 ) (28), ( \nu'\text{C'N} ) (16), ( \rho \text{C'O} ) (11), ( -\nu C</em>\alpha C_\beta ) (10), ( -\beta C_\alpha \text{NC}' ) (8)</td>
<td>( \nu \text{C–CH}_3/\nu \text{C–N} )</td>
</tr>
</tbody>
</table>

\( ^a \) Frequencies correspond to visible Raman (633 nm excitation) spectrum.

\( ^b \) \( \nu \): stretch; \( \delta_\text{s} \): symmetric deformation; \( \delta_\text{as} \): asymmetric deformation; \( \sigma \): scissoring; \( \rho \): rocking; \( \omega \): wagging; \( \beta \): in-plane bending; \( \tau \): twisting.

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**L-glutamine t-butyl ester hydrochloride.** The Raman vibrational frequencies and assignments of crystalline GlnTBE are shown in Table 6. The band located at \( \sim 1195 \text{ cm}^{-1} \) is assigned to a mostly C–O stretching/\text{CH}_3 rocking vibration of the butyl ester group. Many of the bands in the spectrum of GlnTBE (Figure 16) shift in the N-deuterated spectra (not shown). Those that do not are assigned to modes that do not contain significant motion of the primary amide N_\epsilon H_2. These are the bands at \( \sim 1043 \text{ cm}^{-1} \) and \( \sim 1030 \text{ cm}^{-1} \), which are assigned to C_\alpha–N stretching/\text{CH}_3 rocking and \text{CH}_3 rocking modes, respectively. The \( \sim 1151 \text{ cm}^{-1} \) band is assigned to C_\alpha–H rocking/\text{NH}_3 rocking. The \text{NH}_3 rocking/C_\beta–C_\gamma and \text{NH}_3 rocking/N_\epsilon \text{H}_2 rocking modes are assigned to the bands at \( \sim 1117 \text{ cm}^{-1} \) and \( \sim 1105 \text{ cm}^{-1} \) because they shift in the N-deuterated spectra. The \( \sim 1082 \text{ cm}^{-1} \) band shifts upon N-deuteration and is assigned to the AmIII^P vibration. The band at \( \sim 998 \text{ cm}^{-1} \) is
assigned to a mostly NH₃ rocking/Cα−Cβ stretching mode.

Figure 16: (a) visible Raman, and (b) UVRR spectra of crystalline L-glutamine t-butyl ester hydrochloride in the 1000–1200 cm⁻¹ region. Adapted with permission from [72]. Copyright © 2015, American Chemical Society.
Table 6: Raman Frequencies and Assignments of Crystalline L-Gln t-butyl ester HCl

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<th>Assignment</th>
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<td>1195</td>
<td>νCO (17), ρCH$_3$ (10), −ρCH$_3$ (9), −νCO (6), −δC'O'CCC (6), δ''C'O'CCC (6), −δ'C'O'CCC (6), ρCH$_3$ (5), ρCH$_3$ (5)</td>
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<td>1161</td>
<td>ρC$<em>\alpha$H (23), ρNH$<em>3$ (19), τC$</em>\gamma$H$<em>2$ (11), −νC$</em>\alpha$C$</em>\beta$ (10), τC$_\beta$H$_2$ (8)</td>
<td>ρCH/ρNH$_3$</td>
</tr>
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<td>1126</td>
<td>−ρNH$<em>3$ (19), −νC$</em>\beta$C$<em>\gamma$ (16), −νC$</em>\alpha$N (14), δNC$<em>\alpha$C$</em>\beta$C (9), −ρN$_e$H$_2$ (5)</td>
<td>ρNH$_3$/νC–C</td>
</tr>
<tr>
<td>1105</td>
<td>1119</td>
<td>−ρNH$<em>3$ (15), ρN$<em>e$H$<em>2$ (14), ρC$</em>\beta$H$<em>2$ (10), νC$</em>\beta$C$</em>\gamma$ (9), ρC$</em>\gamma$H$<em>2$ (7), ρC$</em>\alpha$H (6), δNC$<em>\alpha$C$</em>\beta$C (6)</td>
<td>ρNH$_3$/ρNH$_2$</td>
</tr>
<tr>
<td>1082</td>
<td>1100</td>
<td>ρN$<em>e$H$<em>2$ (34), −νC$</em>\beta$C$</em>\gamma$ (24), νC$_\delta$N$<em>e$ (14), νC$</em>\delta$O$_e$ (5)</td>
<td>AmIII$^p$</td>
</tr>
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<td>1043</td>
<td>1068</td>
<td>−νC$_\alpha$N (17), ρCH$_3$ (13), −ρCH$<em>3$ (11), −ρCH$<em>3$ (11), νC$</em>\beta$C$</em>\gamma$ (10), ρCH$<em>3$ (5), νC$</em>\alpha$C (5)</td>
<td>νC–N/ρCH$_3$</td>
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<td>1058</td>
<td>ρCH$_3$ (27), −ρCH$_3$ (26), −ρCH$_3$ (10), ρCH$_3$ (10), −νCCH$_3$ (6)</td>
<td>ρCH$_3$</td>
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<td>1027</td>
<td>−νC$<em>\alpha$N (24), νC$</em>\beta$C$_\gamma$ (11), ρCH$_3$ (9), ρCH$_3$ (8), −ρCH$_3$ (7), −ρ'CH$_3$ (5)</td>
<td>νC–N/νC–C</td>
</tr>
<tr>
<td>1014</td>
<td>1093</td>
<td>−ρNH$<em>3$ (24), −ρC$</em>\beta$H$<em>2$ (18), −ρC$</em>\gamma$H$<em>2$ (16), νC$</em>\alpha$N (14), −νC$<em>\alpha$C$</em>\beta$ (5), τC$_\beta$H$_2$ (5), −δ$_1$N$<em>e$C$</em>\delta$(O$<em>e$)C$</em>\gamma$ (5)</td>
<td>ρNH$_3$/ρCH$_2$</td>
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<tr>
<td>998</td>
<td>989</td>
<td>ρNH$<em>3$ (35), νC$</em>\alpha$C$<em>\beta$ (25), −νC$</em>\alpha$C (11)</td>
<td>ρNH$_3$/νC–C</td>
</tr>
</tbody>
</table>

$^a$Frequencies correspond to visible Raman (633 nm excitation) spectrum.
$^b$ν: stretch; δ$_s$: symmetric deformation; δ: deformation; σ: scissoring; ρ: rocking; ω: wagging; β: in-plane bending; τ: twisting.
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Glycyl-L-glutamine. Table 7 displays the band assignments of crystalline Gly-Gln. The bands at ~1171 cm$^{-1}$ and ~1139 cm$^{-1}$ are assigned to the mostly C$_\alpha$–N stretching and C$_\alpha$–C$_\beta$ stretching modes, respectively. The first NH$_3$ rocking mode is assigned to the band at ~1124 cm$^{-1}$. The band located at ~1093 cm$^{-1}$ is assigned to the AmIII$^p$ vibration due to its sensitivity to N-deuteration. The ~1027 cm$^{-1}$ is assigned to C$_\beta$H$_2$ rocking. The remaining bands are assigned to vibrational modes that do not significantly involved the primary amide group and are shown in Table 7.
Figure 17: Visible Raman spectrum of crystalline Glycyl-L-glutamine in the 1000–1200 cm$^{-1}$ region. Adapted with permission from [72]. Copyright © 2015, American Chemical Society.
Table 7: Raman Frequencies and Assignments of Crystalline Gly-Gln

<table>
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<th>Calc.</th>
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<td>(\tau\text{CH}_2/\rho\text{CH})</td>
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<tr>
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<td>(\nu\text{C}\text{–N})</td>
</tr>
<tr>
<td>1139</td>
<td>1138</td>
<td>(\nu\text{C}<em>\alpha\text{C}</em>\beta) (26), (-\nu\text{C}<em>\beta\text{C}</em>\gamma) (19), (-\rho\text{C}<em>\beta\text{H}<em>2) (8), (-\rho\text{N}<em>3\text{H}<em>2) (6), (-\delta\text{C}</em>\alpha\text{C}</em>\beta\text{C}) (6), (-\nu\text{C}</em>\alpha\text{N}) (6), (-\omega\text{C}</em>\beta\text{H}_2) (5)</td>
<td>(\nu\text{C}\text{–C})</td>
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<tr>
<td>1124</td>
<td>1128</td>
<td>(\rho\text{N}^\prime\text{H}<em>3) (47), (\tau^1\text{C}</em>\alpha\text{H}<em>2) (18), (-\rho\text{C}</em>\alpha\text{H}<em>2) (13), (-\delta\text{C}^\prime\text{NC}</em>\alpha\text{C}(\text{OO})) (5)</td>
<td>(\rho\text{NH}_3)</td>
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<td>(\rho\text{N}^\prime\text{H}<em>3) (29), (\rho\text{N}<em>3\text{H}<em>2) (12), (-\tau\text{C}</em>\alpha\text{H}<em>2) (7), (-\tau\text{C}</em>\alpha\text{H}<em>2) (6), (\nu\text{C}</em>\alpha\text{N}^\prime) (6), (-\sigma\text{N}^\prime\text{C}</em>\alpha\text{C}^\prime) (5), (-\nu\text{C}</em>\alpha\text{N}) (5)</td>
<td>(\rho\text{NH}_3)</td>
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</tr>
<tr>
<td>1093</td>
<td>1100</td>
<td>(\rho\text{N}_3\text{H}_2) (33), (\nu\text{C}<em>3\text{N}</em>\epsilon) (13), (-\rho\text{N}^\prime\text{H}_3) (12), (-\nu\text{C}<em>3\text{C}</em>\gamma) (6), (\nu\text{C}_3\text{O}) (5)</td>
<td>(\text{AmIII}\text{P})</td>
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<td>1082</td>
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<td>(-\nu\text{C}<em>3\text{N}^\prime) (14), (\nu\text{C}<em>3\text{C}</em>\gamma) (13), (\nu\text{C}^\prime\text{C}</em>\alpha^\prime) (8), (\rho\text{C}<em>\gamma\text{H}<em>2) (7), (\nu\text{C}<em>3\text{C}</em>\beta) (7), (\rho\text{C}</em>\alpha\text{H}) (6), (-\nu\text{C}</em>\alpha\text{N}) (5)</td>
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<td>(-\nu\text{C}_3\text{N}^\prime) (56), (\nu\text{C}_3\text{N}) (9), (-\nu\text{C}<em>3\text{C}</em>\beta) (7), (-\nu\text{C}<em>3\text{C}</em>\gamma) (5)</td>
<td>(\nu\text{C}\text{–N})</td>
</tr>
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<td>1027</td>
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<td>(\rho\text{C}<em>\beta\text{H}<em>2) (28), (\delta\text{C}</em>\alpha\text{C}</em>\beta\text{C}) (9), (\tau\text{C}_\gamma\text{H}<em>2) (8), (-\nu\text{C}<em>3\text{C}</em>\gamma) (7), (\rho\text{C}</em>\gamma\text{H}<em>2) (5), (\delta\text{NC}</em>\alpha\text{C}^\prime) (5)</td>
<td>(\rho\text{CH}_2)</td>
</tr>
<tr>
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<td>946</td>
<td>(\nu\text{C}^\prime\text{C}<em>\alpha^\prime) (19), (-\rho\text{C}</em>\alpha\text{H}<em>2) (10), (\rho\text{C}^\prime\text{O}) (9), (-\beta\text{NC}</em>\alpha\text{C}^\prime) (6), (-\beta\text{C}^\prime\text{C}_\alpha\text{N}) (6), (-\rho\text{N}^\prime\text{H}_3) (5)</td>
<td>(\nu\text{C}\text{–C}/\rho\text{CH}_2)</td>
</tr>
</tbody>
</table>

\(^a\)Frequencies correspond to visible Raman (633 nm excitation) spectrum.

\(^b\): stretch; \(\delta\): symmetric deformation; \(\delta\): deformation; \(\sigma\): scissoring; \(\rho\): rocking; \(\omega\): wagging; \(\beta\): in-plane bending; \(\tau\): twisting.

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**L-seryl-L-asparagine.** Vibrational frequencies and assignments of crystalline Ser-Asn are shown in Table 8. The \(~1188\text{ cm}^{-1}\) band is assigned to a mostly \(\text{C}_\beta\text{H}_2\) rocking/\(\text{NH}_3\) rocking mode and the band at \(~1159\text{ cm}^{-1}\) is assigned to \(\text{C}_\alpha\text{–N}\) stretching. The \(~1121\text{ cm}^{-1}\) and \(~1108\text{ cm}^{-1}\) bands are modes that involve the serine side chain, \(\text{C}_\beta\text{–O}_\gamma\text{H}\) stretching/\(\text{N}_3\text{H}_2\) rocking and \(\text{C}_\beta\text{–O}_\gamma\text{H}\) stretching, respectively. The \(~1051\text{ cm}^{-1}\) band is assigned to the \(\text{AmIII}\text{P}\) vibration due to its shift upon N-deuteration. The remaining peaks are assigned to several mostly \(\text{NH}_3\), \(\text{NH}_2\), and \(\text{CH}_2\) rocking modes as shown in Table 8.
Figure 18: (a) visible Raman, and (b) UVRR spectra of crystalline L-seryl-L-asparagine in the 1000–1200 cm\(^{-1}\) region. Adapted with permission from [72]. Copyright © 2015, American Chemical Society.
Table 8: Raman Frequencies and Assignments of Crystalline Ser-Asn

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<td>$\rho\text{CH}_2/\rho\text{NH}_3$</td>
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<td>$\nu\text{C}-\text{N}$</td>
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<td>1119</td>
<td>$-\nu\text{C}<em>\beta\text{O}\gamma\text{H}$ (17), $\rho\text{N}<em>2\text{H}<em>2$ (13), $\nu\text{C}</em>\alpha\text{C}</em>\beta$ (13), $\nu\text{C}</em>\alpha\text{N}'$ (8), $-\rho'\text{C}_\alpha\text{H}$ (6), $\rho N'\text{H}_3$ (5), $-\rho'N'\text{H}_3$ (5)</td>
<td>$\nu\text{C}-\text{OH}/\rho\text{NH}_2$</td>
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<td>1116</td>
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<td>$\nu\text{C}-\text{OH}$</td>
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<td>$\rho\text{NH}_2/\nu\text{C}-\text{N}$</td>
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<td>$-\rho N'\text{H}<em>3$ (20), $\nu\text{C}</em>\alpha\text{N}'$ (10), $-\nu\text{C}'\text{C}<em>\alpha'$ (9), $\nu\text{C}</em>\alpha\text{C}<em>\beta$ (8), $\rho\text{C}</em>\alpha\text{H}$ (7), $-\nu\text{C}<em>\beta\text{O}\gamma\text{H}$ (5), $\rho'\text{C}</em>\alpha\text{H}$ (5)</td>
<td>$\rho\text{NH}_3/\nu\text{C}-\text{N}$</td>
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<td>1069</td>
<td>$\nu\text{C}<em>\alpha\text{C}</em>\beta$ (23), $-\nu\text{C}'\text{C}<em>\alpha'$ (11), $\nu\text{C}</em>\alpha\text{N}$ (9), $-\delta \text{CC}<em>\alpha\text{N}$ (7), $-\sigma\text{C}</em>\alpha\text{C}<em>\beta\text{C}</em>\gamma$ (5), $-\rho\text{N}_2\text{H}_2$ (5)</td>
<td>AmIII$^\text{P}$</td>
</tr>
<tr>
<td>1019</td>
<td>1023</td>
<td>$-\rho\text{C}<em>\beta\text{H}<em>2$ (23), $\delta\text{C}</em>\beta\text{C}</em>\alpha\text{C}$ (12), $\delta\text{C}'\text{C}<em>\alpha\text{N}$ (8), $-\nu\text{C}</em>\alpha\text{C}$ (7), $\delta\text{C}<em>\alpha\text{COO}$ (6), $\beta\text{C}</em>\alpha\text{N}'$ (6), $\rho'\text{C}_\alpha\text{H}$ (5)</td>
<td>$\rho\text{CH}_2/\delta\text{CCC}$</td>
</tr>
<tr>
<td>1004</td>
<td>981</td>
<td>$\rho\text{C}_\beta\text{H}<em>2$ (28), $\rho'N'\text{H}<em>3$ (25), $-\nu\text{C}</em>\alpha\text{C}</em>\beta'$ (14), $\rho'\text{N}_2\text{H}_2$ (10)</td>
<td>$\rho\text{CH}_2/\rho\text{NH}_3$</td>
</tr>
</tbody>
</table>

$^a$Frequencies correspond to visible Raman (633 nm excitation) spectrum.
$^b$ν: stretch; δ: symmetric deformation; δ: deformation; σ: scissoring; ρ: rocking; ω: wagging; β: in-plane bending; τ: twisting.

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Glutamine in Aqueous Solution. Previously, Ramirez and coworkers [73] assigned L-Gln in solution based on isotopic shifts and DFT calculations. Figure 19 shows the UVRR spectra of L-Gln in H$_2$O and D$_2$O. The vibrational frequencies Ramirez and coworkers report [73] do not match those reported here, and their assignments do not agree with the resonance enhancement seen in Figure 19. Thus, new assignments of L-Gln in solution were made based on more modern DFT calculations using the UVRR spectral frequencies, and previous assignment of propanamide [58]. The experimental UVRR Raman frequencies, DFT calculated frequencies, and assignments of L-Gln in H$_2$O in the 950–1200 cm$^{-1}$ region can be found in Table 9.
There are several shifts in the vibrational frequencies of these bands compared to the crystal spectra, as detailed in [72]. Most notably, in the 950–1200 cm$^{-1}$ region, the AmII$^P$ vibration upshifts from $\sim$1097 cm$^{-1}$ in the solid-state to $\sim$1110 cm$^{-1}$ in H$_2$O.

Figure 19: UVRR spectra of L-glutamine in (a) H$_2$O and (b) D$_2$O.
Table 9: UVRR Frequencies and Assignments of L-Gln in Water

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Calc.</th>
<th>Potential Energy Distribution$^a$ (≥5% contribution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1206</td>
<td>1215</td>
<td>$-\tau C_\beta H_2$ (21), $-\nu C_\alpha C_\beta$ (18), $-\tau C_\gamma H_2$ (16), $-\rho'NH_3$ (13), $\delta NC_\alpha C(OO)$ (5)</td>
</tr>
<tr>
<td>1158</td>
<td>1153</td>
<td>$-\rho' C_\alpha H$ (20), $\tau C_\gamma H_2$ (17), $-\rho'NH_3$ (13), $\tau C_\beta H_2$ (12), $-\nu C_\alpha C_\beta$ (8), $\rho C_\alpha H_2$ (6)</td>
</tr>
<tr>
<td>1130</td>
<td>1122</td>
<td>$\nu C_\beta C_\gamma$ (34), $\rho N H_2$ (17), $-\nu C_\alpha C_\beta$ (7), $\nu C_\alpha N$ (6), $-\beta N_\epsilon C_\delta C_\gamma$ (5)</td>
</tr>
<tr>
<td>1109</td>
<td>1109</td>
<td>$\rho NH_3$ (27), $-\rho' C_\alpha H$ (10), $-\rho N H_2$ (10), $-\delta' NCC(OO)$ (9), $-\rho C_\alpha H$ (7), $\nu C_\alpha N$ (7)</td>
</tr>
<tr>
<td>1110</td>
<td>1097</td>
<td>$\nu C_\beta C_\gamma$ (26), $-\rho N_\epsilon H_2$ (26), $-\nu C_\delta N_\epsilon$ (13), $-\rho NH_3$ (8)</td>
</tr>
<tr>
<td>1038</td>
<td>1038</td>
<td>$\nu C_\alpha N$ (36), $-\nu C_\beta C_\gamma$ (9), $\rho C_\beta H_2$ (8), $\rho' NH_3$ (6), $\rho C_\gamma H_2$ (5), $\rho' C_\alpha H$ (5)</td>
</tr>
<tr>
<td>1078</td>
<td>1003</td>
<td>$\rho NH_3$ (25), $-\nu C_\alpha N$ (19), $\rho C_\gamma H_2$ (14), $\rho C_\beta H_2$ (14), $\nu C_\alpha C_\beta$ (7), $-\tau C_\beta H_2$ (5)</td>
</tr>
<tr>
<td>1006</td>
<td>974</td>
<td>$-\rho' NH_3$ (38), $\nu C_\alpha C_\beta$ (25), $-\nu C_\alpha C$ (8), $-\sigma CC_\alpha C_\beta$ (7), $\nu C_\alpha N$ (6)</td>
</tr>
</tbody>
</table>

$^a \nu$: stretch; $\delta_\alpha$: asymmetric deformation; $\delta_\delta$: symmetric deformation; $\delta$: deformation; $\sigma$: scissoring; $\rho$: rocking; $\omega$: wagging; $\beta$: in-plane bending; $\tau$: twisting.

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3.3.2 Dependance of Primary Amide III Band on Conformation

The experimental and theoretical AmIII$^P$ vibrational frequency exhibits a cosinusoidal dependence (Figure 20(a)) on the primary amide OCCC dihedral angle [72].

**Origin of the AmIII$^P$ Vibrational Frequency and OCCC Dihedral Angle Correlation.** This correlation can be understood in detail by examining the results of DFT calculations carried out on L-glutamine molecules at several fixed OCCC dihedral angles. These calculations show that the AmIII$^P$ vibrational frequency correlates well with the OCCC dihedral angle (Figure 20(a)) [72]. As discussed above in Section 3.3.1, the AmIII$^P$ vibration of L-glutamine is made up of $N_\epsilon H_2$ rocking, $C_\delta-N_\epsilon$ stretching, and $C_\beta-C_\gamma$ stretching. This band composition is subject to change, however, based on the OCCC dihedral angle. For example, at certain angles, $C_\beta H_2$ twisting can also contribute significantly and the contributions of $N_\epsilon H_2$ rocking, $C_\delta-N_\epsilon$ stretching, and $C_\beta-C_\gamma$ stretching can vary.

The $C_\delta-N_\epsilon$ and $C_\beta-C_\gamma$ bond lengths affect the stretching force constants and therefore the AmIII$^P$ vibrational normal mode and frequency. This could account for the changes in normal mode composition of the AmIII$^P$ vibration that occur as the primary amide OCCC angle is varied [72]. The DFT calculated $C_\delta-N_\epsilon$ and $C_\beta-C_\gamma$ atomic distances were plotted against the OCCC dihedral angle (Figure 20). The $C_\beta-C_\gamma$ bond length exhibits the strongest
correlation with the AmIII\textsuperscript{P} vibrational frequency. As the C\textsubscript{\beta}–C\textsubscript{\gamma} bond length increases, the AmIII\textsuperscript{P} vibrational frequency decreases and as the C\textsubscript{\beta}–C\textsubscript{\gamma} bond length decreases, the AmIII\textsuperscript{P} vibrational frequency increases.

Figure 20: Calculated dependence of (a) the AmIII\textsuperscript{P} vibrational frequency and (b) C\textsubscript{\beta}–C\textsubscript{\gamma} and (c) C\textsubscript{\delta}–N bond lengths of L-glutamine on OCCC dihedral angle. Adapted with permission from [72]. Copyright © 2015, American Chemical Society.

The changes in C\textsubscript{\beta}–C\textsubscript{\gamma} bond length as the OCCC dihedral angle is adjusted can be explained by hyperconjugation of the C\textsubscript{\beta}–C\textsubscript{\gamma} \sigma orbital with the C\textsubscript{\delta}=O \pi^* orbital [72]. The C\textsubscript{\beta}–C\textsubscript{\gamma} bond order decreases when the \sigma orbital donates electron density to the \pi^* orbital. As the C\textsubscript{\beta}–C\textsubscript{\gamma} bond order decreases, the bond length increases and the stretching force constant decreases. This C\textsubscript{\beta}–C\textsubscript{\gamma} bond length increase downshifts the AmIII\textsuperscript{P} vibrational frequency.

In Figure 21, the results of the natural bond orbital (NBO) analysis are shown for L-glutamine, which estimates the electron densities of the \sigma and \pi^* orbitals. At an OCCC
dihedral angle of 0° (Figure 21(a)), the $C_\beta-C_\gamma$ bond length is shortest and there is no hyperconjugation because of the antisymmetry of the $\pi^*$ orbital. The $C_\beta-C_\gamma$ bond length is longest when the OCCC dihedral angle is at ±90°, in Figure 21(b), where there is overlap of the $C_\beta-C_\gamma$ $\sigma$ orbital with the $C_\delta=O$ $\pi^*$ orbital. At this angle the AmIII$^P$ vibrational frequency is significantly downshifted, which suggests that the hyperconjugation of the $C_\beta-C_\gamma$ $\sigma$ orbital with the $C_\delta=O$ $\pi^*$ orbital can account for the correlation of the AmIII$^P$ vibrational frequency with the OCCC dihedral angle.

Figure 21: NBO analysis estimates $C_\beta-C_\gamma$ $\sigma$ and $C_\delta=O$ $\pi^*$ molecular orbitals of L-glutamine at OCCC dihedral angles of (a) 0° and (b) ±90°. Adapted with permission from [72]. Copyright © 2015, American Chemical Society.

**Experimental Correlation Between the AmIII$^P$ Vibrational Frequency and OCCC Dihedral Angle.** In Figure 22, the experimental AmIII$^P$ vibrational frequencies from the UVRR and visible Raman spectra are plotted against the OCCC dihedral angles determined from the X-ray structures of L-glutamine and the five derivative molecules.
These data were fit with a sum of cosines:

\[ \nu(\chi) = \nu_0 + A \cos(2\chi) + B \cos(\chi - C) \]  

(3.1)

where \( \chi \) is the OCCC dihedral angle, \( \nu_0 = 1066 \text{ cm}^{-1} \), \( A = 29 \text{ cm}^{-1} \), \( B = 9 \text{ cm}^{-1} \), and \( C = 99^\circ \) with \( r^2 = 0.83 \). Fitting was performed by fixing the ratio of \( A/B \) to the same value obtained by the fit to the theoretical data in Figure 20(a). The fit was obtained by performing a least-square minimization [72]. The terms with one- and two-fold symmetry in Equation 3.1 suggest steric and electronic effects, respectively [76]. Equation 3.1 is the black curve in Figure 22.

Figure 22: Experimental correlation between the AmIII\(^P\) vibrational frequency of L-glutamine and derivative molecules (1) L-Gln, (2) Gly-Gln, (3) D-Gln, (4) GlnTBE, (5) NAcGln, (6) Ser-Asn and OCCC dihedral angle. These data were fit with Equation 3.1 for four different environments of the primary amide group: crystal (black), fully hydrated (blue) low dielectric and hydrogen bonding (red), and unknown (yellow). Adapted with permission from [72]. Copyright © 2015, American Chemical Society.

The dependence of the calculated AmIII\(^P\) vibrational frequency on the primary amide OCCC dihedral angle is asymmetric about 0° (Figure 20) because of the chirality of L-glutamine. This is the reason that the AmIII\(^P\) vibrational frequency dependence on the
OCCC dihedral angle is well modeled by an expression with two cosine terms. Similar calculations were performed on the small, achiral molecule butyramide, a model of the glutamine side chain, that support this claim \cite{72}. Butyramide does not exhibit the same asymmetry and can be represented by a simple cosine equation. D-Gln data was included in the fitting procedure described above despite the difference in chirality. This was due to the fact that the OCCC dihedral angle of D-Gln was close to 0°, mirroring L-Gln almost exactly.

As discussed in great detail in Chapter 2, the dielectric and hydrogen bonding environment can greatly influence the vibrational frequency of primary amide bands \cite{58}. The AmIIIP vibrational frequency of L-glutamine shifts from 1097 cm\(^{-1}\) in the crystalline sample to 1110 cm\(^{-1}\) upon aqueous solvation. In addition, the equilibrium structure OCCC angle in solution was shown to be \(\sim-12.8^\circ\) by Rhys et al. using neutron diffraction \cite{77}. This is very close to the \(-13.54^\circ\) of the X-ray crystal structure \cite{78}. In order to take into account the impact of aqueous solvation on this model, the OCCC dihedral angle was set to \(-13.54^\circ\), the AmIIIP vibrational frequency to 1110 cm\(^{-1}\), and the equation was solved for \(\nu_0\). Thus, when the primary amide group is fully hydrated, \(\nu_{\text{hydrated}} = 1083\ \text{cm}^{-1}\) in Equation 3.1 and the other parameters remain the same. This curve is shown in blue in Figure 22.

The AmIIIP vibrational frequency will also shift in a lower dielectric solvent, such as acetonitrile. Butyramide was used to determine the magnitude of this shift. In H\(_2\)O, the AmIIIP vibrational frequency of butyramide is \(\sim1076\ \text{cm}^{-1}\) and in acetonitrile it downshifts \(\sim15\ \text{cm}^{-1}\) to \(\sim1064\ \text{cm}^{-1}\). Parameter \(\nu_0\) in Equation 3.1 is downshifted \(\sim15\ \text{cm}^{-1}\) to \(\nu_{\text{lowdielectric}} = 1068\ \text{cm}^{-1}\) to account for this, otherwise the remaining parameters remain the same. In Figure 22, this equation is represented by the red curve.

Finally, in the case where the hydrogen bonding and dielectric environment of the primary amide group is not known, an average of the previous two situations can be used. The average of \(\nu_{\text{hydrated}} = 1083\ \text{cm}^{-1}\) and \(\nu_{\text{lowdielectric}} = 1068\ \text{cm}^{-1}\) is \(\nu_{\text{unknown}} = 1076\ \text{cm}^{-1}\). Thus, Equation 3.1 with \(\nu_{\text{unknown}}, A = 29\ \text{cm}^{-1}, B = 9\ \text{cm}^{-1},\) and \(C = 99^\circ\) is used for unknown hydrogen bonding and dielectric environments, shown in yellow in Figure 22.
4.0 CONCLUSIONS

IR, visible Raman, and UVRR spectra of the small primary amide molecule propanamide were collected. The vibrations of propanamide were fully assigned using these data and DFT calculations. Dramatic changes in the primary amide vibrations are observed to occur upon aqueous solvation. The changes are explained in two ways: (i) an increase in molar absorptivity, due to a redshift in the NV$_1$ electronic transition, leads to increases in cross sections and (ii) aqueous solvation alters the dielectric and hydrogen bonding environment of the primary amide group resonance structures. This leads to a decrease in C–O and increase in C–N bond order and therefore alters the resonance enhancement and vibrational frequencies substantially. Due to this significant response, the AmI, AmII, and $\nu_{12}$ bands in particular can be used as sensitive environmental markers for the primary amide group, which is present in the protein side chains glutamine and asparagine.

Visible Raman and UVRR spectra of L-glutamine and five derivative molecules, D-glutamine, N-Acetyl-L-glutamine, L-glutamine t-butyl ester, Glycyl-L-glutamine, and L-seryl-L-asparagine were collected. Assignments were made of the vibrations of these molecules in the 950-1200 cm$^{-1}$ region using DFT calculations. Structures of the crystalline samples were obtained by X-ray diffraction and the OCCC dihedral angles determined. A cosinusoidal dependence of the AmIII$^P$ vibrational frequency on the OCCC dihedral angle was observed. This can be explained by hyperconjugation that occurs between the C$_\beta$–C$_\gamma$ $\sigma$ orbital and the C$_\delta$=O $\pi^*$ orbital. When the OCCC dihedral angle is $\sim \pm 90^\circ$, hyperconjugation results in an increase in the C$_\beta$–C$_\gamma$ bond length. As the C$_\beta$–C$_\gamma$ bond length increases, the stretching force constant decreases, downshifting the AmIII$^P$ band. Due to this sensitivity, the AmIII$^P$ can be used as a structural marker diagnostic of the OCCC dihedral angle in the side chains glutamine and asparagine in peptide and protein conformational studies. For
example, Punihaole et al. [72] used this relationship to determine the mean $\chi_3$ angle of glutamines in a short polyglutamine peptide, $D_2Q_{10}K_2$, in solution to be $-32^\circ$. This work will enable experimental measurements of glutamine and asparagine side chain conformations in samples that are insoluble and cannot be crystallized.

For example, these diagonistic primary amide bands in UVRR spectra can be used to study the environment and structure of the glutamine side chain in polyglutamine fibril-like aggregates. The dependence of primary amide bands on environment and structure can be used to test the models of polyglutamine fibril structures that were previously proposed [14–21]. UVRR could be used to confirm and gain insight into the two distinct populations of glutamine side chains observed in fibrils by ssNMR [14,19,20,22]. Recently, van der Wel and coworkers reported $\chi_1$ ($NC_\alpha C_\beta C_\gamma$) and $\chi_2$ ($C_\alpha C_\beta C_\gamma C_\delta$) dihedral angles of two populations of glutamine side chains in the core of huntingtin exon 1 fibrils using ssNMR [22]. Determination of the glutamine OCCC ($\chi_3$) dihedral angle would complement these results and lead to a better understanding of the glutamine side chains in the fibril core.

Primary amide vibrations can be used to monitor the side chain glutamines and asparagines in conformational studies of peptides and proteins that have a variety of side chains. Interpretation of UVRR spectra of peptides with both backbone secondary amide and side chain primary amides is challenged by the many peaks present in this spectral region. However, this can be overcome with the use of difference spectra. Excitation at $\sim$200 nm selectively enhances backbone secondary amide vibrations [30]. Selective enhancement of side chain primary amide vibrations relative to backbone secondary amide vibrations can be accomplished by excitation closer to $\sim$190 nm [59]. By subtracting the $\sim$200 nm UVRR spectrum of the same sample, one can obtain a difference spectrum made up of primarily the primary amide vibrations [25]. These primary amide vibrations can be used to determine the average OCCC dihedral angle of the ensemble of glutamine or asparagine side chains present in the peptide or protein. If only one glutamine or asparagine residue is present, this allows for the determinations of the average of that residue. The understanding of the dependence of the UVRR primary amide vibrations on the structure and environmental characteristics of the primary amide group will allow for incisive studies of proteins and peptides in solution and in the solid-state.
BIBLIOGRAPHY


