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UV resonance Raman study of TrpZip2 and related peptides: π - π interactions of tryptophan

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Abstract

Aromatic interactions are important stabilizing forces in proteins but are difficult to detect in the absence of high-resolution structures. Ultraviolet resonance Raman spectroscopy is used to probe the vibrational signatures of aromatic interactions in TrpZip2, a synthetic β -hairpin peptide that is stabilized by edge-to-face and face-to-face tryptophan π - π interactions. The vibrational markers of isolated edge-to-face π - π interactions are investigated in the related β -hairpin peptide W2W11. The bands that comprise the Fermi doublet exhibit systematic shifts in position and intensity for TrpZip2 and W2W11 relative to the model peptide, W2W9, which does not form aromatic interactions. Additionally, hypochromism of the B_b absorption band of tryptophan in TrpZip2 leads to a decrease in the relative Raman cross-sections of B_b -coupled Raman bands. These results reveal spectral markers for stabilizing tryptophan π - π interactions and indicate that ultraviolet resonance Raman may be an important tool for the characterization of these biological forces.

Keywords

noncovalent interactions; fluorescence; exciton; Fermi doublet; β -hairpin

Introduction

Noncovalent interactions, such as hydrogen bonding, hydrophobic packing, and aromatic interactions, are important forces in biomolecules because these collective interactions contribute to the overall stability of nucleic acids, proteins, and peptides. While hydrogen bonding and hydrophobic packing have been studied and discussed for several decades, cation- π and π - π interactions have become the focus of research only in the past few decades.^[1,2] The majority of efforts have been devoted to cation- π interactions in model systems.^[3–5] In contrast, relatively little is known about π - π interactions between aromatic residues in proteins. This interaction is electrostatic in nature^[6] and plays a critical role in biomolecular stability. For example, aromatic interactions are responsible for base pair stacking in DNA.^[7] In proteins, aromatic amino acids form π - π interactions with other

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Supporting information

Supporting information may be found in the online version of this article.

aromatic residues or substrates in edge-to-face and face-to-face (parallel displaced) geometries which stabilize tertiary structures as well as substrate-protein complexes.^[6]

Aromatic interactions are particularly relevant for tryptophan because the indole ring possesses the largest overall electrostatic potential when compared with the side chains of phenylalanine and tyrosine.^[1] An analysis of the protein structures in the Protein Data Bank indicated that 59% of tryptophan residues participate in aromatic-aromatic interactions, and the edge-to-face geometry was most prevalent.^[2] Identification and characterization of these important interactions in protein structures may aid in understanding key aspects of protein folding, molecular recognition, and drug design.^[8]

Model peptides are ideal for the study of aromatic-aromatic interactions. TrpZip2 is a synthetic, 12-residue peptide with four tryptophan residues that form two edge-to-face aromatic interactions and one face-to-face interaction (Figs 1 and S1, Supporting Information).^[9–11] These tertiary contacts result in the spontaneous formation of antiparallel β -hairpin conformation, with a free energy for unfolding of ~ 1 kcal/mol.^[9] In the W2W11 variant of TrpZip2, tryptophan residues at positions 4 and 9 are replaced with valine residues (Fig. S1). This substitution results in the formation of a single edge-to-face interaction and no face-to-face aromatic interactions. The resulting structure of W2W11 is a β -hairpin, much like the parent peptide TrpZip2 (refer to the overlay of the W2W11 structure with the TrpZip2 structure, Fig. S2). The pairwise interactions that stabilize TrpZip2 and W2W11 provide a simple model to identify the spectral markers that reflect tryptophan π - π interactions. A third variant of TrpZip2, W2W9, serves as a control peptide because the replacement of tryptophan residues at positions 4 and 11 with valine (Fig. S1) results in loss of π - π interactions, causing the peptide to remain in a random coil conformation in aqueous solution.^[12] These peptides, combined with the model tryptophan compound, N-acetyl-tryptophanamide (NATA), provide an opportunity to measure the vibrational structure of tryptophan π - π interactions.

Ultraviolet resonance Raman (UVR) spectroscopy is a sensitive tool for investigating perturbations in the structure and local environment of tryptophan residues. This technique has been used to elucidate conformation, local environment, and hydrogen bonding states of tryptophan on the basis of the measurements of model compounds, peptides, and proteins.^[13–15] Recently, the UVR spectra of a tryptophan model compound was reported in which the indole ring was a participant in a cation- π interaction.^[16] Here, we aim to expand our understanding of noncovalent aromatic interactions of tryptophan by investigating the spectral signatures of the π - π interactions in TrpZip2 and W2W11 peptides.

Materials and methods

Chemicals

TrpZip2, W2W11, and W2W9 were purchased with >98% purity from Genscript (Piscataway, NJ). Potassium phosphate monobasic and dibasic were purchased from Fisher Scientific (Pittsburgh, PA). Ninhydrin and L-serine were purchased from Alfa Aesar (Ward

Hill, MA). NATA was purchased from Sigma Aldrich (St. Louis, MO), and N-acetyl-L-lysine was purchased from Bachem (Torrance, CA). All chemicals were used as received.

Electronic spectroscopy

Absorption spectra were acquired on an Agilent 8453A UV–vis spectrometer using a quartz microcuvette with a path length of 1 cm. Fluorescence spectra were acquired on a Jobin Yvon Horiba Fluorolog-3 spectrofluorometer. The excitation wavelength was 290 nm, and the entrance and exit bandpass were 3 nm. Buffer-only spectra were subtracted from all raw spectra. The extinction coefficients for TrpZip2 and W2W11 were determined using a ninhydrin assay to measure peptide concentrations as described in the Supporting Information.^[17]

UV resonance Raman spectroscopy

A detailed description of the UVRR setup was reported previously.^[18] Briefly, vibrational spectra of tryptophan residues were obtained by setting the fundamental laser wavelength to 868, 880, 912, and 920 nm to generate 217, 220, 228 and 230 nm excitation beams. Unphotolyzed sample (2 ml) was flowed through a 100 μm diameter vertically mounted fused silica capillary at a rate 0.16 ml/min. The UV power was 3 to 4 mW at the sample. Ten one-minute spectra were collected and summed for all samples. Concentrations were chosen to maintain similar scattering intensities from tryptophan residues for all samples: $\sim 25 \mu\text{M}$ for TrpZip2, $\sim 50 \mu\text{M}$ for W2W9 or W2W11, and $\sim 100 \mu\text{M}$ for NATA in 20 mM potassium phosphate buffer. Spectra of phosphate buffer blank solutions were also collected and subtracted from all raw spectra. Overlapping peaks were decomposed into Gaussian bands using a least-squares fitting routine. Raman cross-sections of TrpZip2 relative to those of W2W9 ($\sigma_{\text{TrpZip2}}/\sigma_{\text{W2W9}}$) at each excitation wavelength were calculated using the following equation:

$$\frac{\sigma_{\text{TrpZip2}}}{\sigma_{\text{W2W9}}} = \frac{I_{\text{TrpZip2}}}{C_{\text{TrpZip2}}} \cdot \frac{C_{\text{W2W9}}}{I_{\text{W2W9}}}$$

I_{TrpZip2} and I_{W2W9} are the integrated intensities of TrpZip2 and W2W9 Raman bands of interest, and c_{TrpZip2} and c_{W2W9} are the concentrations of tryptophan residues. An identical analysis was performed for $\sigma_{\text{W2W11}}/\sigma_{\text{W2W9}}$. Peptide concentrations were determined using the Beer–Lambert law and $\epsilon_{280} = 5500 \text{ cm}^{-1} \text{ M}^{-1}$ for each tryptophan residue. We estimate the error in peptide concentrations to be 11% because of uncertainty in the extinction coefficient of the $L_{a,b}$ band (Supporting Information). The intensity of the Raman water bend peak did not change from sample to sample, and error in intensity as a result of self-absorption was negligible because the optical density was a maximum of 0.05 for all excitation and Raman wavelengths. Accuracy and precision of Raman frequencies were determined using ethanol peaks and were found to be $\pm 2 \text{ cm}^{-1}$. The bandpass for the Raman experiment was less than 11 cm^{-1} .

Normal mode calculations

Calculations were performed using the Gaussian 03 W quantum chemistry program operating on a Windows platform.^[19] The geometry and harmonic vibrational frequencies of 3-ethylindole were calculated using DFT with the hybrid B3LYP functional. A 6-31 G(d) basis set was selected for all calculations.

Results

Absorption and fluorescence spectra

The measured extinction coefficients of the $L_{a,b}$ band at 280 nm for W2W9 ($4900\text{ cm}^{-1}\text{ M}^{-1}$) and W2W11 ($5100\text{ cm}^{-1}\text{ M}^{-1}$) are identical within the 11% error and assumed to be $5500\text{ cm}^{-1}\text{ M}^{-1}$, which is the value reported for NATA. We are unable to definitively identify the source of systematic error that results in a measured extinction value to be lower than the expected value; however, the error likely reflects the minor presence of peptide impurities in the sample. The extinction coefficient for TrpZip2 was found to be $4300\text{ cm}^{-1}\text{ M}^{-1}$, and the ~14% decrease relative to the measured extinction coefficients of W2W9 and W2W11 may be a significant result. However, because of the relatively large error associated with the ninhydrin assay and the lack of knowledge of the identity of the impurities in the samples, it is difficult to confirm whether this difference is real. Therefore, we invoke the most conservative assessment that the 280 nm extinction coefficients of all three peptides are identical within error; this assumption ensures that observed differences in the relative Raman cross-sections and B_b extinction coefficient are minimized (see below).

The normalized absorption spectra of TrpZip2, W2W9, W2W11, and NATA are shown in Fig. 2. The tryptophan $L_{a,b}$ band of TrpZip2 is red-shifted relative to W2W9 and NATA, whereas the tryptophan B_b band blue-shifts from 219 nm (220 nm) in W2W9 (NATA) to 218 nm in W2W11 and 217 nm in TrpZip2. Intensity changes are also observed; the B_b band decreases in intensity by ~7% for W2W11 and ~17% for TrpZip2 relative to W2W9 B_b band intensity when the spectra are normalized to the absorbance at 280 nm. The drop in intensity for W2W11 is not significant in the context of the 11% error in extinction coefficients, whereas the decrease in intensity of TrpZip2 is a significant finding that indicates hypochromism of this band. This decreased intensity is especially significant in light of the assumption of identical extinction coefficients at 280 nm; if the TrpZip2 extinction at 280 nm is actually lower than that of W2W9, as suggested by the ninhydrin results, the extent of hypochromism is greater than 17%.

The wavelengths of maximum emission (λ_{max}) for NATA, W2W9, W2W11, and TrpZip2 are summarized in Table 1. Normalized fluorescence spectra are shown as an inset in Fig. 2. Tryptophan fluorescence of TrpZip2 blue-shifts 7 nm (10 nm) relative to the tryptophan fluorescence of W2W9 (NATA). Fluorescence of W2W11 did not shift relative to W2W9. The steady state fluorescence anisotropy of TrpZip2 was observed to increase relative to values obtained for W2W9 and NATA (data not shown).

UV resonance Raman spectroscopy

UVRR spectra were acquired with 217, 220, 228, and 230 nm to probe vibrational modes that are coupled to the tryptophan B_b absorption band (Fig. S3). The labels used to identify bands in the UVRR spectra are the same as those initially described by Harada and coworkers.^[14] These spectra reflect the collective vibrational structures of the four tryptophan residues in the TrpZip2 peptide, two tryptophan residues in the W2W11 and W2W9 peptides, or the single tryptophan moiety of NATA. The Fermi doublet regions with 228 nm excitation wavelength of NATA, W2W9, W2W11, and TrpZip2 are presented in Fig. 3. Additional Fermi doublet regions with 217 and 220 nm excitation of TrpZip2, W2W9, and NATA are presented in Fig. S4. Results for the Fermi doublet with 230 nm excitation are similar to those obtained with 228 nm excitation for each molecule (data not shown).

The Fermi doublet arises from coupling between a combination band and a fundamental mode.^[20,21] The relative intensities, based on peak heights (no decomposition) of the two bands that comprise the Fermi doublet for NATA, are identical to those we reported previously, indicating no measureable aggregation of NATA at 100 μ M.^[13] Three bands are utilized for Gaussian decompositions for this region: one band corresponds to the W8 mode (~ 1305 cm^{-1}), a second band corresponds to the low frequency component of the Fermi doublet (~ 1340 cm^{-1}), and a third band corresponds to the high frequency component (~ 1360 cm^{-1}). In this current report, the Fermi doublet intensity ratio is defined as $R_{\text{FD}} = I_{\sim 1360}/I_{\sim 1340}$, where $I_{\sim 1360}$ and $I_{\sim 1340}$ are the peak intensities of the Gaussian fits to the high and low frequency components, respectively. These intensities reflect the maximum peak heights regardless of band position. In a prior report,^[13] we did not utilize Gaussian decompositions to obtain values for the R_{FD} , and we discuss the reason next. Frequencies and intensity ratios of Gaussian bands are summarized in Tables 1 and S1. For all excitation wavelengths, the R_{FD} values for TrpZip2 and W2W11 are much larger than those measured for W2W9 or NATA. Downshifts of 0–3 cm^{-1} for the low frequency component and 2–6 cm^{-1} for the high frequency component are observed for TrpZip2 and W2W11 relative to analogous peaks in NATA and W2W9. The variations in intensity and peak position in the Fermi doublet region are qualitatively reproduced in the off-resonance Raman spectra (Fig. S5) and are consistent with a prior study of a similar TrpZip peptide.^[22]

Integrated relative Raman cross-sections of TrpZip2 and W2W9 ($\sigma_{\text{TrpZip2}}/\sigma_{\text{W2W9}}$) are presented as a function of excitation wavelength in Fig. 4. In these calculations, concentrations of the peptide were determined using $\epsilon_{280} = 5500$ cm^{-1} M^{-1} for each tryptophan residue. The error bars represent the standard deviation of results for the 228 nm excitation. Raman intensities of TrpZip2 are decreased relative to Raman bands of W2W9, with the exception of the W1 band. The W18, W16, and W3 bands exhibit the most pronounced reduction in intensities, with $\sigma_{\text{TrpZip2}}/\sigma_{\text{W2W9}}$ values of ~ 0.5 to ~ 0.7 . If peptide concentrations based on measured extinction coefficients are utilized, the decrease in relative intensities is further enhanced to ~ 0.4 to ~ 0.6 . W2W11 band intensities are identical to those of W2W9 within error.

Discussion

Electronic spectroscopy of tryptophan

The close proximity of indole side chains of tryptophan residues in TrpZip2 and W2W11 results in exciton coupling of the tryptophan $\pi \rightarrow \pi^*$ transition (B_b band, Fig. 2).^[9,22] This phenomenon leads to a strong circular dichroism (CD) signal^[23] (Fig. S6) and has been utilized to monitor the tertiary structure in TrpZip2 and related peptides.^[11,12,22] W2W9 does not display the negative and positive features at 213–216 and 227–228 nm in the CD spectrum that are indicative of tryptophan π - π interactions. Instead, the W2W9 CD spectrum is characteristic of a random coil secondary structure which was previously confirmed by Nuclear Magnetic Resonance (NMR) and infrared (IR) experiments and indicates that W2W9 does not display π - π interactions.^[12,24]

The red-shift in the $L_{a,b}$ band is consistent with an enhancement of local polarizability and/or hydrophobicity.^[25] However, the B_b band shifts in the opposite direction of what is expected for a hydrophobic environment,^[26] indicating that the blue-shifts of the B_b band in TrpZip2 and W2W11 are a result of the π - π interactions of tryptophan residues.^[27] The observed blue-shift, combined with hypochromism of the B_b band, is consistent with the parallel, stacked orientation of the transition dipole moments that is evident in the structure.^[28] It is somewhat surprising that hypochromism was not detected in W2W11; this finding may indicate that the magnitude of the change is too small for the present experiments to detect and/or that the edge-to-face aromatic interactions in W2W11 do not cause significant hypochromism relative to face-to-face interactions in TrpZip2.

The moderate blue-shift of tryptophan fluorescence of TrpZip2 relative to W2W9, W2W11, and NATA is interpreted in terms of a less polar environment for tryptophan; this partial burial results in decreased rotational freedom, as evidenced by enhanced anisotropy for TrpZip2 (data not shown). However, the $\lambda_{\max}$ value of TrpZip2 is not as blue-shifted as tryptophan residues that are known to be buried in hydrophobic cores of proteins, indicating that the tryptophan residues of TrpZip2 remain solvent-exposed.^[29] D₂O studies support the accessibility of tryptophan residues to solvent; the W17 mode at 882 cm⁻¹ in the UVRR spectrum shifts to 863 cm⁻¹ in D₂O for both TrpZip2 and W2W9 (Fig. S7), as well as for NATA.^[13] In contrast to TrpZip2, the W2W11 peptide does not exhibit a blue-shifted emission maximum despite the presence of the π - π interaction. This finding suggests that face-to-face interactions creates a slightly more hydrophobic environment in TrpZip2 and highlights the subtle effects π - π interactions may have on the emission spectra.

Sensitivity of the Fermi doublet to π - π interactions of tryptophan

The most pronounced UVRR differences that reflect π - π interactions appear in the Fermi doublet region (Fig. 3). This doublet has been shown to correlate strongly with the local environment,^[14,20] and our data suggest it is also sensitive to π - π interactions. R_{FD} values for NATA (230 nm excitation), without Gaussian decomposition, range from 1.1 to 1.7 for aqueous and hydrophobic environments.^[13] Enhancement of R_{FD} is typically accompanied by a fluorescence blue-shift of ~20 nm in a hydrophobic environment. A subtle increase in R_{FD} in the UVRR spectra of TrpZip2 is expected on the basis of the hydrophobic

environment associated with tryptophan π - π interactions, but the high value of ~ 1.8 – 2.0 (228 nm excitation, without Gaussian decomposition) or 2.3 (with Gaussian decomposition) is surprising, given the modest 7 nm blue-shift in fluorescence maximum. Another unusual aspect of the Fermi doublet in TrpZip2 is that it is less resolved than in model compounds or other biomolecules. We attribute this change to a decrease in the intensity of the low frequency combination band and a downshift of the high frequency component, such that the separation between peaks is reduced. In contrast to TrpZip2, W2W9 and NATA exhibit well-resolved Fermi doublet peaks. Because these two peaks are not discernible in the TrpZip2 spectrum, we focus on the R_{FD} values that result from decomposition. The strong similarity between the W2W9 and NATA spectra confirms the lack of aromatic interactions in W2W9.

An enhanced R_{FD} value of 1.8 in the UVRR spectrum of W2W11 is also observed despite the lack of shift in fluorescence. This finding provides additional evidence that the Fermi doublet is sensitive to π - π interactions of tryptophan and that the modulations reported here primarily reflect aromatic-aromatic effects as opposed to local hydrophobicity. The smaller R_{FD} value for W2W11 compared to that of TrpZip2 may reflect the slight difference in local hydrophobicity and/or the effect of a face-to-face interaction that is present in TrpZip2 but not in W2W11.

A description of the modes that give rise to the Fermi doublet is shown^[30] in Fig. 5 and is essential in order to interpret the shifts in the UVRR spectra. The W7 fundamental mode exhibits large in-plane displacements of atoms in the pyrrole ring. The combination band is believed to be comprised of two ring out-of-plane (R-OOP) modes with frequencies of ~ 920 and ~ 420 cm^{-1} .^[20,21] Both R-OOP modes exhibit large out-of-plane displacements of benzyl hydrogen and carbon atoms. In particular, both modes display significant out-of-plane motion of the hydrogen atom (H4) at position C4. The significant contribution of the H4 motion to the combination mode is supported by calculations and prior experiments. The Fermi resonance, and hence doublet, is eliminated in a C4-monodeuterated tryptophan analog because of downshifts in the frequency of the R-OOP modes upon deuteration.^[20]

The observed shifts in the UVRR Fermi doublet likely reflect perturbation of the R-OOP modes. ^1H NMR spectra indicate that H4 is perturbed in trp4 and trp11 of TrpZip2 and in trp11 of W2W11. In TrpZip2, the signal from H4 of trp4 and trp11 is shifted at least 2.0 ppm relative to the same hydrogen in the tryptophan residue of a flexible, random coil peptide H-Gly-Gly-Trp-Ala-OH.^[9,31] The analogous signal from H4 of trp11 in W2W11 shifts 1.95 ppm. The origin of this perturbation is evident in the edge-to-face interaction of tryptophan residues in the NMR structure of both peptides (Figs 1 and S2). H4 atoms in TrpZip2 and W2W11 point towards the π system of the five-membered rings; the distance between C4 of trp11 and the centroid of the pyrrole group of trp2 is ~ 3.6 Å in both peptides. A similar geometry is observed between trp4 and trp9 in TrpZip2, and the analogous distance is ~ 4.1 Å. The orientation of H4 towards the π -cloud likely shifts the vibrational frequencies of the R-OOP modes.

The sensitivity of these R-OOP modes to local environment and hydrogen bonding has been previously reported, and it is generally accepted that these R-OOP modes are the primary

modulators of the relative intensities of the Fermi doublet in different environments.^[20,21,32] The NMR results and current understanding of the Fermi doublet are consistent with our UVRr interpretation: perturbation of R-OOP normal modes that involve H4 alters the observed Fermi doublet. The data presented here augment earlier reports and indicate that π - π interactions, in addition to hydrophobicity, modulate these vibrations. Although this hypothesis is supported by experiments and calculations, the complexity of a Fermi resonance allows for other interpretations. For example, this doublet may also be affected by alterations in the fundamental W7 in-plane pyrrole ring stretching mode upon formation of π - π interactions because the pyrrole ring acts as the π -donor in the edge-to-face and face-to-face interactions in TrpZip2. The observation that TrpZip2 exhibits a more pronounced perturbation of the Fermi doublet ratio relative to W2W11 supports the hypothesis that face-to-face π - π interactions also affect the doublet.

Relative Raman cross-sections of tryptophan

We attribute the decrease in the integrated intensity for Raman bands of TrpZip2 to hypochromism of the B_b band in the absorption spectrum. The trends reported here are similar to those from a UVRr study of DNA,^[33] and suggest predominantly A-term enhancement of some of the UVRr modes. The previously reported differential coupling of normal modes to the excited states further supports this interpretation: W1 is known to be coupled to the L_{a,b} bands and does not show UVRr intensity reduction in the present experiments, whereas the W18, W16, and W3 bands are B_b-coupled bands and exhibit decreased Raman intensities.^[34,35] It is interesting to note that π - π interactions have an opposite effect on cross-sections than hydrophobicity. As shown here, π - π interactions result in decreased Raman cross-sections, whereas hydrophobic environments enhance the cross-sections.^[36]

Conclusions and future outlook

In this work, we report the electronic and vibrational spectra of tryptophan residues of β -hairpin peptides TrpZip2 and W2W11 that participate in stabilizing tryptophan-tryptophan aromatic interactions. The Fermi doublet region and integrated intensities of modes coupled to the tryptophan B_b absorption band are reproducibly altered in the presence of tryptophan π - π interactions. These results suggest that the Fermi doublet region not only reports on local hydrophobicity, as discussed extensively in the literature over the past three decades, but is also sensitive to π - π interactions. In contrast, fluorescence spectra do not show systematic shifts in the presence of aromatic interactions and, instead, reflect changes in local hydrophobicity.

TrpZip peptides provide an opportunity to investigate different types of aromatic interactions in proteins. Other peptides may be studied, such as the W4W9 variant in which the single tryptophan edge-to-face interaction is localized near the hairpin turn.^[12] We anticipate that the spectral features for this peptide will be analogous to those of W2W11 but may be modulated by the distance between the tryptophan residues, as well as the local environment of the turn. UVRr can also be extended to study other aromatic amino acid residues; for example, experiments may be pursued to investigate the spectral signatures of tyrosine-based π - π interactions in TrpZip2.^[11] These experiments, combined with the

results presented here, may provide a more complete set of spectral markers that describe the local environment of aromatic residues in the structures of proteins and peptides.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Tryptophan interactions in the NMR structure of TrpZip2 (Protein Data Bank code: 1LE1).

The atom numbering scheme of the indole ring is shown on trp11. The shortest distance between edge-to-face interacting tryptophan residues is indicated by the solid lines drawn between C4 and the centroid of the pyrrole group. A dashed line between the pyrrole centroids of trp2 and trp9 indicate the face-to-face interaction.

Figure 2. Normalized absorption and fluorescence (inset) spectra of TrpZip2 (red), W2W11 (green), W2W9 (blue) and NATA (black). Absorption spectra are normalized at 280 nm. The excitation wavelengths for UVRF are indicated as vertical arrows. This figure is available in colour online at wileyonlinelibrary.com/journal/jrs

Figure 3.

The Fermi doublet region (including W8) of the UVRR spectrum of NATA, W2W9, W2W11, and TrpZip2. The excitation wavelength is 228 nm. Gaussian decompositions and sums are indicated as dotted bands.

Figure 4.

Relative Raman cross-sections of tryptophan bands of TrpZip2 to W2W9 as a function of the excitation wavelength for six normal modes. FD indicates the Fermi doublet. Error bars represent the standard deviation of the results for the 228 nm excitation. This figure is available in colour online at wileyonlinelibrary.com/journal/jrs

Figure 5. Normal modes of 3-ethylindole. W7 fundamental normal mode with atom numbering scheme of the indole ring (top) and ring out-of-plane (R-OOP) normal modes that comprise the combination band of the Fermi doublet (middle, bottom). Frequencies for normal modes with a hydrogen and deuterium at the C4 position are indicated.

Table 1

Absorbance, fluorescence, and 228 nm excitation UVRR results for NATA, W2W9, W2W11, and TrpZip2

	$\lambda_{\text{max}}^{\text{abs, B}_b}$ (nm)	$\lambda_{\text{max}}^{\text{fluo}}$ (nm)	R_{FD}^a	Band positions (cm^{-1}) ^b	
NATA	220	359	1.3	1339	1362
W2W9	219	356	1.3	1341	1363
W2W11	218	356	1.8	1339	1360
TrpZip2	217	349	2.3	1338	1358

^a Ratio of intensities of the Fermi doublet peaks $I_{\sim 1360}/I_{\sim 1340}$ based on Gaussian decomposition.

^b Positions of Gaussian bands that make up the Fermi doublet. Refer to the text for additional details.