Isotope-Edited Amide II Mode: A New Label for Site-Specific Vibrational Spectroscopy

Supporting Information

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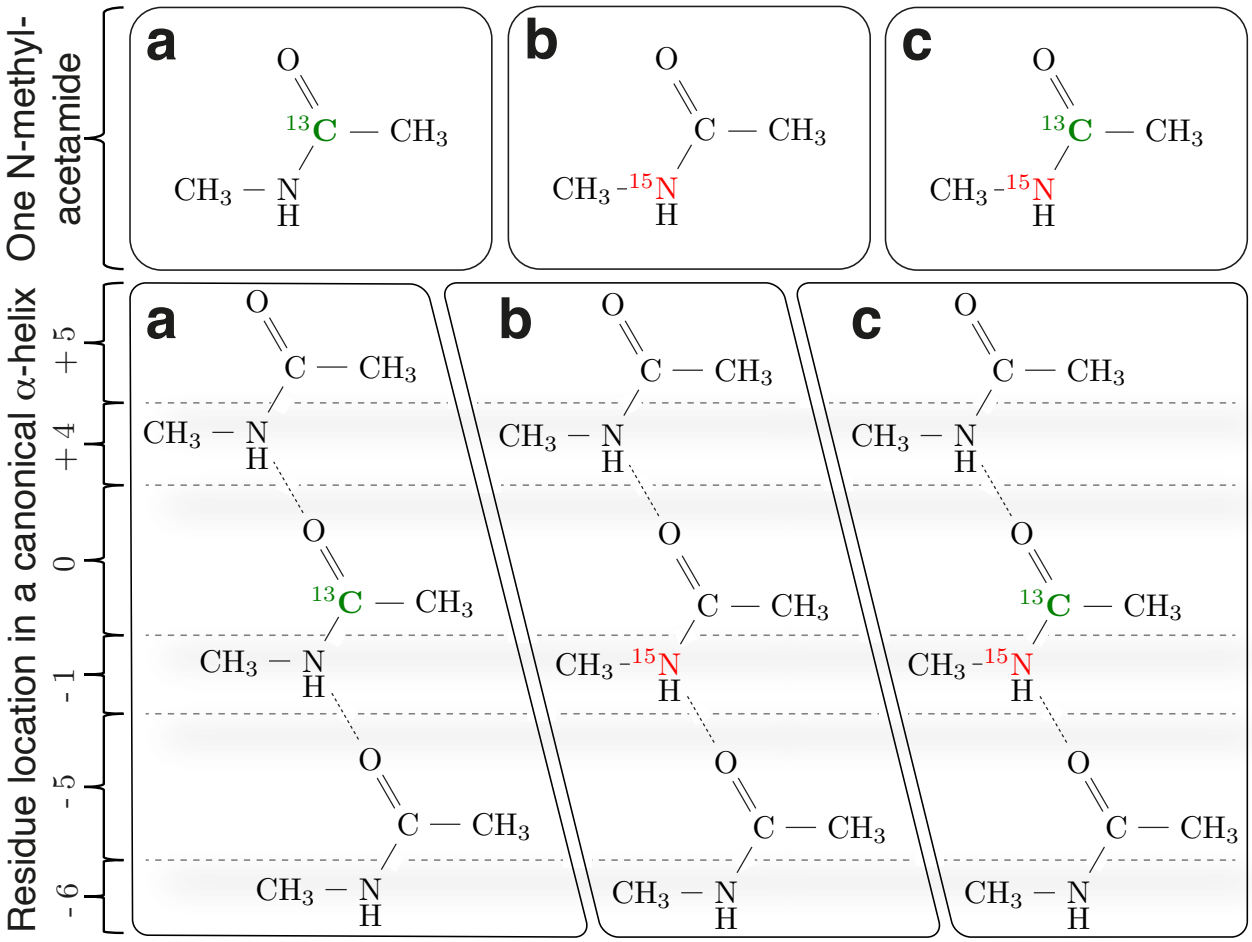
Additional figure



Supporting Figure 1: Molecular mimetics used in the DFT quantum chemical calculations. Two systems were used: a single N-methylacetamide (top), representing one peptide bond, and three N-methylacetamides positioned according to the coordinates of a model helix at the exact location of residues involved in canonical H-bonding: i, $i-4$, and $i+4$ (bottom). For each system three different configurations were used (a-c) distinguished by the presence of isotopic atoms, colored in blue (^{15}N) or green (^{13}C). The horizontal, dotted lines correspond to different locations in a canonical α-helix ($i-6$ to $i+5$), as indicated.

Experimental methods

Sample preparation

The labeled and unlabeled WALP19 peptides[1] were synthesized separately employing standard f-moc chemistry on a solid support. The sequence of these peptides is GWWLALALALALALALWWA. For the isotope edited peptide, L8, L10, and L12 are 1-^{13}C=^{16}O labeled, and A9, A11, and A13 are ^{15}N labeled (Cambridge Isotope Laboratories, Inc., Tewksbury, MA).

Each of these peptides were purified on a reverse phase 20 ml Jupiter 5 µm C4 300 Å HPLC column (Phenomenex, Torrance, CA). The column was pre-equilibrated with 80% water, 12% isopropanol, and 8% acetonitrile, where each of these solvents has 0.1% trifluoroacetic acid (Merck, Darmstadt, Germany). Once the peptide is on the column, the solvents were adjusted using the VWR Hitachi Chromaster 5160 Pump to remove all water, while retaining the isopropanol to acetonitrile ratio of three to two. Since the WALP19 peptide has tryptophans, the peptide fraction was visualized at 280nm via the VWR Hitachi Chromaster 5410 UV detector. Once the peptide was eluted from the column, 1 µl of 33% HCl was added to the fractions to remove the trifluoroacetate adduct, and the peptide in isopropanol and acetonitrile was frozen in liquid nitrogen and lyophilized overnight.

Membrane reconstitution was achieved by dissolving each peptide in 1 ml of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (Merck, Darmstadt, Germany) and co-solubilization with 10 mg of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (Avanti polar lipids, Alabaster, AL). The sample was roto-evaporated until all HFIP was removed. Spontaneous vesicle formation containing the WALP19 peptide was induced by adding water.

Spectra collection

Spectra were obtained using an attenuated total internal reflection (ATR) unit (Specac, Orpington, UK) within a Nicolet iS10 FTIR Spectrometer, with a mercury cadmium telluride

detector (Thermo Scientific, Madison, WI), cooled with liquid nitrogen. The ATR unit reflects the infrared beam 25 times along the germanium trapezoid ATR plate (50 mm × 2 mm × 20 mm) (Wilmad, Vineland, NJ) with a 90° incident IR beam hitting the 45° face angle of the plate. For the background and sample spectra, the air within the FTIR machine was purged of water and carbon dioxide. 1000 FTIR scans were sampled with a data spacing of 0.241 cm^{-1}, 2 levels of zero filling, N-B strong apodization, and Mertz phase correction. Background spectra of the clean Ge crystal were subtracted from each of the labeled and unlabeled sample spectra.

DFT calculations

DFT model structures were created using the VMD[2] molefacture plugin. An ideal α-helical structure of glycines was created. The atoms seen in Fig. 3 were retained from the protein structure, but all others were deleted. Any missing hydrogens were added using molefacture. Structure optimization and frequency shifts were calculated as described previously.[3] Optimization until convergence was performed with the Q-Chem software package[4] using the aug-cc-pvdz basis set[5,6] and the B3LYP method,[7,8] followed by frequency calculations with the isotopic schemes seen in Fig. 3. Frequency shifts of the amide I and amide II vibrational modes were calculated with respect to the unlabeled structure and recorded in Table 1.

References

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