3863-Pos
Experimental and Theoretical Spectroscopic Study of 3_{10} Helical Peptides using Isotopic Labeling
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Experimental and theoretical studies of IR, VCD, and Raman spectra have been performed on synthesized peptides (*i*PrCO-Aib-L-Ala-Aib-L-Ala-L-Ala-Aib-NH*i*Pr) having a 3_{10}-helical conformation. These sequences vary only due to isotopic labeling (^{13}C=O) of the L-Ala on the relative (i , i +1), (i, i + 2), and(i, i + 3) positions. Di-alkyl substitution on the α-carbon of Aib restricts the rotational freedom of the backbone torsional angles (f, ψ) and favors the formation of 3_{10}-helices [1]. Theoretical IR, VCD and Raman simulations were performed on sequences identical to the synthesized ones but constrained (in terms of f, ψ torsional angles) to an ideal 3_{10}-helical geometry (-60, -30) and fully optimizing all the other coordinates. All calculations were performed for peptides in vacuum using the DFT BPW91/6-31G* level of theory. The simulations predicated the relative separations of ^{13}C=O and ^{12}C=O features and their dependence on conformation as seen experimentally, with the exception that end effects caused a change in diagonal force field not well represented in the theoretical modeling. Experimental spectra for longer sequences and singly labeled variants confirmed the source of deviation for the i,i+1 and i,i+3 models. Comparison of IR and VCD intensity patters helped sort out the vibrational coupling constants sensed in the ^{13}C=O modes. The isotopic labeled group vibrations are coupled to each other most strongly when degenerate and are effectively uncoupled from those of the unlabeled groups.
[1] Wang et al., Peptide Science, 2009, 92, 452-456.

3864-Pos
Ultrafast Interconversion between Protein Conformational Substates: Directly Observed by 2D IR Vibrational Echo Spectroscopy
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Conformational dynamics of flexible biomolecules play an important role in the function and stability of proteins. Folded proteins can exist in multiple conformational substates, where each substate has a distinct structure and corresponds to a local minimum on the free energy landscape. Transitions from one minimum to another correspond to dynamical changes in the structure of the protein. By using 2D IR spectroscopy, conformational interconversion between these well defined substates of a myoglobin double mutant is observed on picoseconds timescale. The conformational dynamics are directly measured through the evolution of cross peaks in the 2D IR spectra of CO bound to the heme active site. The conformational switching changes the CO frequency, as detected by the waiting time dependence of the 2D IR vibrational echo spectrum. This result is an example where conformational switching between protein substates occurs on very fast time scales. Moreover, this myoglobin mutant shows enzymatic activity upon substrate binding, which makes it an excellent system to study the influence of substrate binding on structural dynamics.

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Low Frequency Vibrations of Biological Solids: Terahertz, FTIR, INS, Raman, DFT, and BOMD Molecular Dynamics of the L-Serine Crystal
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Molecular dynamics simulations provide our most realistic description of biological events at the molecular level. Motions below 200 wave-numbers are of particular interest since they contribute most of the vibrational entropy and probably infuence many biological processes. Measurements of the vibrational spectrum in this region yield direct information about the potential energy hyper-surface, and these measurements can be used to refine molecular mechanics potential functions. We present here a vibrational analysis of polycrystalline L-serine using experimental vibrational spectra,calculated inelastic neutron scattering (INS), and Born-Oppenheimer molecular dynamics (BOMD) simulations. Corrections are made to density functional theory (DFT) calculations for van der Waals interactions.Assignments and potential energy distributions are included for all 3N=336 normal modes of an eight molecule super-cell, including those for 48 non-bonded whole molecule translating and rotating vibrations,of which 3 are acoustic modes, usually not considered. Calculated and observed frequencies differ by an average 3 wave-numbers (s=4).The INS spectrum of these modes below 100 wave-numbers, calculated from energy second derivatives, show a remarkable similarity to the experimental 10K spectra. The calculated low frequency modes are insensitive to small changes in cell parameters and geometry.Power spectra of 13 ps BOMD trajectories at classical temperatures of 20K, 400K, and 500K are markedly similar to the experimental terahertz spectra at 77K and 298K.

3866-Pos
Studying Electrostatic Fields in Human Aldose Reductase with New Inhibitor as Probe
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Our research focuses on electrostatics in the system of human aldose reductase (hALR2), a 36 kDa aldo-keto reductase, which plays an important role in diabetes control. Vibrational Stark effect (VES) spectroscopy is utilized to measure the electrostatic fields near the active site of hALR2, using nitrile-containing inhibitors as the probe. Herein, a new hARL2 inhibitor was synthesized and bound to wild type hALR2 (wt_hALR2) with binding constant of 200 nM. Two vibrational absorption peaks were observed in the nitrile region when it bound to wt_hALR2, indicating the probe was experiencing two different environments. To explore the source of the two peaks, electrostatics calculations were performed based on crystal structures of hALR2 bound with similar inhibitors. The calculated projection of the protein electrostatic field also had a two-peak distribution. Analysis of trajectories suggests that they might be correlated with a possible hydrogen bond between the nitrile probe and a nearby residue threonine 113. To further test this assumption, IR spectra of the inhibitor bound to a series of mutants were taken; especially, the inhibitor bound to mutant hALR2_T113A had a single peak, which was also confirmed by simulations. This approach provides precise local information on electrostatic fields.

3867-Pos
Using Difference Infrared Spectroscopy to Investigate the Effects of pH on PGK-Substrate Complexes
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Yeast phosphoglycerate kinase catalyzes the reversible phosphate transfer in the reaction: ADP +1,3-bis-phosphoglycerate ↔ ATP + 3-phosphoglycerate. Prior research indicates a hinge-bending mechanism occurs during catalysis to bring the substrates into closer proximity. Domain closure is only initiated in ternary complexes, in which both substrates are simultaneously bound to the enzyme. The activity and conformation of PGK is directly influenced by substrate and salt concentrations as well as pH. For example, activity assays confirm that PGK activity increases from pH 6.5 to 7.5. To determine the effects of pH on the conformational changes of PGK, we used difference Fourier transform infrared spectroscopy (FTIR) in conjunction with caged nucleotides. Difference infrared data associated with nucleotide (ATP or ADP) binding to PGK or PGK-3PG complexes was compared at pH 5.5, 6.5 and 7.5. Circular dichroism was also used to study PGK secondary structure at the aforementioned pH conditions. Comparison of the difference FTIR data allowed the isolation of pH dependent vibrations that arise from protein conformational changes induced by substrate binding. We have identified multiple vibrations that are associated with the PGK ternary complex and are influenced by pH. Difference FTIR studies resulted in the identification of specific changes within amino acid side chains and protein secondary structures that are altered by pH and associated with ternary complex formation.

3868-Pos
Determination of the Isomerization Dynamics and Transient Ring D Orientation Changes of the Phytochrome Pfr form in Solution by Polarization Resolved Femtosecond VIS Pump - IR Probe Spectroscopy
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We demonstrate the use of polarization resolved femtosecond VIS pump - IR probe spectroscopy in determining the isomerization dynamics of Pfr to lumi-F in isotopically labelled phytochrome Cph1d2. In ^{13}C and ^{15}N labelled Cph1d2 protein we identified the C=O stretching vibrations of ring A and ring D of the unlabelled chromophore in the Pfr form. Time resolved transients show a broad red shifted aborption band of the C_{19}=O vibration in the electronic excited state decaying on a 1 ps time scale. The blue shifted C_{19}=O absorption of lumi-F product displays a rise time of some ps. By polarization resolved measurements the relative angles of the C_{19}=O vibrational transition dipole moment (tdm) with respect to the electronic tdm were determined for Pfr and lumi-F for the first time. This approach provide us with structural information on the chromophore ring D orientation in real time