3863-Pos
Experimental and Theoretical Spectroscopic Study of 3_{10} Helical Peptides using Isotopic Labeling
Ahmed A. Lakhani.
University of Illinois at Chicago, Chicago, IL, USA.
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
Sayan Bagchi, Michael D. Fayer.
Stanford University, Stanford, CA, USA.
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.

3865-Pos
Low Frequency Vibrations of Biological Solids: Terahertz, FTIR, INS, Raman, DFT, and BOMD Molecular Dynamics of the L-Serine Crystal
Robert W. Williams[1], Edwin J. Heilweil[2].
[1]Uniformed Services University, Bethesda, MD, USA, [2]National Institute of Science and Technology, Gaithersburg, MD, USA.
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
Lin Xu.
Boxer lab, Stanford Univeristy, Stanford, CA, USA.
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
Adam E. Colbert, Gina MacDonald.
James Madison University, Harrisonburg, VA, USA.
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
Karsten Heyne[1], Yang Yang[1], Martin Linke[1], Janina Hahn[2], Peter Schmieder[2].
[1]Freie Universität Berlin, Berlin, Germany, [2]Leibniz-Institut für Molekulare Pharmakologie, Berlin, Germany.
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

and allows a deeper insight into structural rearrangements during the first isomerization step.

3869-Pos

Effects of Hydration Levels on the Bandwidth of Microwave Resonant Absorption Induced by Confined Acoustic Vibrations

Tzu-Ming Liu, Hung-Pin Chen, Shih-Chia Yeh, Chih-Yu Wu, Chung-Hsiung Wang, Tang-Nian Luo, Yi-Jan Chen, Shen-Iuan Liu, Chi-Kuang Sun.
National Taiwan University, Taipei, Taiwan.

The vibration modes of molecules can be revealed by infrared absorption spectroscopy if their displacements change the dipole moments of molecules. Depending on the bonding strength, the mass of atoms, and the types of vibrations, the resonant absorption frequencies of molecules range from hundreds of terahertz (THz) to several THz. For collective vibrations of macromolecules like proteins or virions, the corresponding resonant frequencies will be around THz and could be probed by the THz or microwave absorption spectroscopy. However, in this frequency range, the periods of vibrations are close to or above the persistence time of hydrogen bonding of water molecules. If the surface to volume ratio of macromolecules is large, surrounding water molecules will overdamp the vibrations and smear the resonant absorption feature. Recently, we demonstrated that confined acoustic vibrations (CAV) of viruses can modify dipole moments and result in microwave resonant absorption (MRA) (Liu *et al.*, 2009). The resonant absorption frequencies correspond to those of dipolar active [SPH, l=1] modes. The activation of the resonant coupling relies on the core-shell charge structures, which are inherent on the capsid surfaces. Such characteristic absorption peak is rarely found in THz spectroscopy on solvated proteins and the actual mechanism worth a further investigation.

In this study, by decreasing the pH value of solution dwon to 5.2 or inactivating viruses, we enhanced the surface hydrophilicity and increased the magnitude of surface potentials. Both of these surface manipulations raised the surface affinity to water molecules, provide better acoustic confinements, and narrowed the bandwidths of CAV-induced MRA. Our results indicate that the viscoelastic transition of hydration shells play a critical role in the THz or microwave vibration spectroscopy.

T.-M. Liu *et al.*, *Appl. Phys. Lett.* **94**, 043902 (2009).

3870-Pos

Raman Spectroscopic Detection of an Optically Trapped Single DNA Molecule

Satish K. Rao, Saurabh Raj, Dmitri Petrov.
ICFO - The Institute of Photonic Sciences, Castelldefels, Spain.

Optical trapping has opened up a number of biophysical fields because of its ability to hold and manipulate single cells and molecules. In addition, the force sensitivity of an optical trap has allowed for a number of studies in to the mechanics of the most basic biological systems such as DNA. However, the majority of these experiments are based on a measured force correlated to a detected displacement or extension of the molecule in question. Due to the low optical cross section of a single DNA molecule, for example, interacting light directly with the structure, in order to obtain a detailed spectrum, has not been possible.

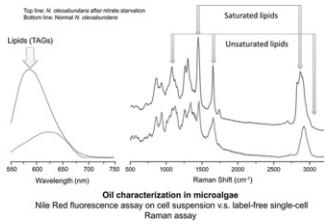
In this work, we present a measurement of a Raman spectrum from a single DNA molecule that is attached to two optically trapped dielectric microspheres. The scattering cross section in this instance is enhanced by the injection of nanosized silver colloids to the solution that adsorb on to the DNA. A near-infrared beam is used for excitation and Raman bands of DNA are obtained that agree with those from previous studies of DNA-metal colloid solutions. The presence of just one DNA molecule is verified by measuring the well-established force-extension curve. The adsorbed nanometer sized silver structures do not greatly affect the overall elasticity of the DNA, however the mechanical response at low to medium range forces seems to be altered. The addition of Raman spectroscopy to existing force spectroscopy methods could provide new information about the mechanochemical makeup of a structure through a correlation of the two methods.

3871-Pos

Single-Cell Diesel Mining on Microalgae: Direct and Quantitative Monitoring of Microalgal Oil Production In Vivo by Raman Spectroscopy

Huawen Wu, Joanne V. Volponi, Seema Singh.
Sandia National Laboratories, Livermore, CA, USA.

Microalgae, known for their rapid growth and high lipid content, became a promising candidate for the next generation feedstocks for liquid biofuels. Traditionally, instead of living organisms, they were treated as lifeless biomass in bulky, lyophilized or extracted forms, making it difficult to understand the fundamental biological processes in play. Labeling algae with fluorescent probes can be a potential high-throughput method but it provides little chemical information and is limited by impermeability, toxicity and specificity. In this work, we focus on in situ, in vivo and label-free Raman characterizations of single living green algae of several species. Our study has demonstrated that single-cell laser-trapping confocal Raman spectroscopy can directly obtain quantitative information of the lipids produced inside individual algae. Information critically related with the quality of derived biodiesel, such as lipid unsaturation and melting temperature can be obtained at single-cell level. Meanwhile, lipid triggering effect by nitrate starvation was characterized in vivo on single cells. Our real-time in vivo "diesel mining" on individual microalgae cells enables the possibility of researching and engineering of the best conditions and species for algal oil production.



Imaging & Optical Microscopy IV

3872-Pos

How to use Confocal Microscopy in Search of a Highly Resolved Hologram

Aura I. Moreno-Vega[1], Carlos Saldaña[2], Veronica Morales-Tlalpan[3], Mauricio Diaz-Muñoz[4], Victor M.Castaño Meneses[5].
[1]Centre of Applied Physics and Advanced Technology Universidad Nacional Autónoma de México , Campus, Juriquilla, Querétaro 76230, QRO, México., Queretaro, Mexico, [2]Biomedicine Department, Universidad Autónoma de Querétaro. Qro-México., Queretaro, Mexico, [3]High Specialty Regional Hospital of the Bajio. León Guanajuato, México, Leon, Guanajuato, Mexico, [4]Department of Molecular and Celular Neurobiology, UNAM, Campus, Juriquilla, Querétaro 76230, QRO, México., Queretaro, Mexico, [5]Centre of Applied Physics and Advanced Technology) Universidad Nacional Autónoma de México, Campus, Juriquilla, Querétaro 76230, QRO, México., Queretaro, Mexico.

Objective: To apply confocal microscopy and non-conventional holographic techniques, for the three-dimensional reconstruction of cancer cell endomembranes.

Both confocal microscopy (CM) and holography (H) allow the capture of high quality images for their 3D reconstruction, while each technique varies in the way light is captured and processed. Combining both techniques with electron microscope grids of different sizes will hence allow a 3D reconstruction of higher quality and fidelity.

We hypothesize that, by placing grids of differently sized holes in our cell preparations, they will acts as multiple pinholes, increasing image resolution for its 3D construction as a digital hologram. The hologram produced would have higher spatial precision, due to wave optics phenomena.

Preliminary results of images captured with grids of differently sized holes (100, 50, 40 & 30μm) have shown a differential pattern in the fluorescence intensity. Additionally, image resolution distributes itself as a Gaussian. This may be due to the bar thickness of the grid interfering with the capturing of light. So far, these results show two important aspects: 1) The fluorescence intensity obtained is not proportional to the mesh size and 2) Image resolution behaves in a normally distributed way against the grid hole size.

Our prospects are therefore to use grids with specific characteristics (hole size and bar thickness) to create higher quality images and so more precise 3D reconstructions.

3873-Pos

Imaging Contrast and Biomechanics using Optical Coherence Tomography to Sense Superparamagnetic Iron Oxide Labeled Platelets

Amy L. Oldenburg, Thomas H. Fischer, Caterina M. Gallippi.
University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

Optical coherence tomography (OCT) provides 3D tissue imaging by contrasting light backscattering. OCT also senses nanoscale motions from optical phase shifts. We employ temporally modulated magnetic field gradients to mechanically displace superparamagnetic iron oxide nanoparticles (SPIOs). By locking in to the modulation frequency, SPIOs are contrasted in OCT, dubbed magnetomotive OCT (MMOCT).