constructed by sandwiching a polymer spacer between two infrared transparent windows, creating a closed channel system. The channels in the spacer were laser cut to establish the layout of the mixer. The mixer takes advantage of hydrodynamic focusing with two side flow channels, squeezing a middle sample channel into a thin jet and initiating fast mixing through diffusion and advection. The mixing region is probed with a laser source in the mid-infrared region, then magnified and imaged on a focal plane array detector for absorption measurements. The mixer was experimentally calibrated in order to determine the amount of time per pixel in the detector. A pD jump mixing experiment of an adenosine monophosphate solution was employed in order to establish a mixing time on the order of 350 μs. Finally, a flow study with H2O and D2O was completed in order to compare experimental results with simulation. With the established mixing time and use of vibrational spectroscopy, this system can be applied to the study of protein and enzymatic reactions.

3097-Pos Board B789

Ultra Fast Raman Hyperspectral Imaging using Bragg Tunable Filters and a High Performance Emccd Camera

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Because of its high specificity to a variety of molecular processes and its low sensitivity to the presence of water, Raman hyperspectral imaging is regarded as a very promising technique to help pathologists improve the accuracy of medical diagnostics when compared to conventional histopathological analysis. However, since on average approximately one photon per million undergoes Raman scattering, acquisition time per hyperspectral image is very long, typically of about 6 hours. This significantly reduces the appeal of this technique for ex-vivo diagnostics and makes in-vivo applications impracticable. To increase acquisition speed, a Raman hyperspectral imager based on holographic Bragg tunable filters was used and images of carbon nanotubes could be acquired 30 times faster than with a conventional confocal microscope optimised for fast mapping. This speed gain over traditional methods was further enhanced when also using a low-noise EMCCD camera, resulting in measurements performed 150 times faster. These results thus indicate that in vivo and ex vivo applications of wide-field Raman hyperspectral imaging are now at reach, hence paving the way for real time tumor detection during surgery.

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Difference FT-IR Studies on the Effects of Buffers on Nucleotide Binding to Reca

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The *Escherichia coli* protein RecA catalyzes DNA strand exchange and plays a role DNA repair and genetic recombination. Nucleotide binding influences RecA oligomerization and its affinity for DNA. Previous studies in our lab have shown buffer-specific changes in RecA stability and unfolding transitions. Past circular dichroism (CD), infrared (IR), and fluorescence studies suggest only minimal buffer dependent changes in nucleotide binding and secondary structure that did not explain the large buffer dependent differences in RecA stability and unfolding profiles. These observations led to further investigations of how the four common biological buffers Tris, HEPES, MES, and PO_4 alter RecA structure and nucleotide binding. Here we have employed difference infrared spectroscopy utilized in conjunction with caged nucleotides to generate RecA-ADP *minus* RecA difference infrared spectra in each of the four buffers. These higher resolution studies are aimed at detecting if the buffers alter nucleotide binding to RecA. Preliminary results show that ADP binding results in perturbations in Gln, Glu, Asp, Asn, Tyr, and Lys residues and secondary structural changes. Initial comparisons of difference spectra obtained in the four buffers show some similar changes but also show some differences. These differences between RecA-ADP *minus* RecA difference spectra will be discussed.

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FT-IR Spectroscopy and Density Functional Theory Calculations of Carbon-13 Isotopologues of the Helical Peptide Z-AIB(6)-OTBU

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Isotope-edited FT-IR spectroscopy is a combined synthetic and spectroscopic method used to characterize local (e.g., residue-level) vibrational environments of biomolecules. We have prepared the 3_{10} helical peptide Z-Aib$_6$-OtBu and seven ^{13}C-enriched analogues which vary only in the number and position(s) of ^{13}C=O isotopic enrichment. FT-IR spectra of these eight peptides solvated in the nonpolar aprotic solvent dichloromethane have been collected and compared to frequency, intensity, and normal mode results of DFT calculations. Single ^{13}C enrichment of amide functional groups tends to localize Amide I vibrational eigenmodes, providing residue-specific information regarding the local environment (e.g., hydrogen bonding or solvent exposure) of the peptide bond. Double ^{13}C enrichment of Z-Aib$_6$-OtBu allows for examination of inter-amide coupling between two labeled amide functional groups, providing experimental evidence of inter-amide coupling in the context of 3_{10} helical structure. Although the calculated and observed inter-amide couplings of Z-Aib$_6$-OtBu are a few cm^{-1} and less, the eight peptides exhibit distinct infrared spectra, revealing details of inter-amide coupling and residue level vibrational environments.

3100-Pos Board B792

Ulrtrafast Water Dynamics in Bacteriorhodopsin

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Protein bound water molecules play an important role for protein function. In the trans-membrane proton pump bacteriorhodopsin (BR) water molecules in the proton transport channel and in the retinal chromophor binding pocket have been shown by means of infrared vibrational spectroscopy to participate in the light driven proton transport mechanism (Garczarek et al., Nature 2006, 439, 109). Static low temperature FTIR experiments strongly suggest that the pentagonal water cluster involving three water molecules W401, W402 and W406 as well as the retinal Schiff-base, Asp85 and Asp212, is perturbed when the ground state BR570 is photoconverted to the K610 state (H. Kandori et al., J.Phys.Chem.B 1998, 102, 7899). We have now investigated the spectral range above 3.3 μm by means of femtosecond time resolved transient mid-IR spectroscopy. Here we find a broad IR absorption band which bleaches within (or even faster than) retinal isomerization, i.e. 0.5 ps. This finding is strongly corroborated by quantum-chemical QM/MM calculations that attribute this continuum band to a polarization coupling between the protonated retinal Schiff-base N-H stretch and water W402 in BR570 (M. Baer et al., ChemPhysChem 2008, 9, 2703). Our results indicate that the pentagonal water cluster is heavily perturbed already on this time scale.

3101-Pos Board B793

A New Method for Analysis of Temperature Dependent IR Amide I Spectra of Peptides and Proteins

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Changes in the amide I' IR band with temperature are widely used for studies of structural transitions in peptides and proteins. Since amide I' exhibits inherent temperature dependent frequency shifts, standard mixture analysis methods are not applicable. To reliably extract the true thermodynamic states, frequency shifts of the component spectra must be explicitly taken into account. New methods termed Shifted Multivariate Spectra Analysis (SMSA) and parametric SMSA (pSMSA) were developed. SMSA uses no specific functional form for the transition (soft modeling), while the parametric variant (pSMSA) assumes a thermodynamic model (hard modeling). The implementation is optimized specifically for amide I' IR in that it takes advantage of known, linear dependence of the frequencies, as well as intensities, on temperature. The methods are first tested on sets of synthetic data with varying amounts of noise as well as on a real experimental amide I' data for the thermal unfolding of an α-helical peptide. The synthetic data tests demonstrate that the methods very reliably recover the correct parameters, although the non-parametric SMSA is subject to the rotational ambiguity. Application to the peptide experimental amide I' data illustrates additional complications encountered with the analysis of real systems, namely the correction for the side-chain spectral bands and interference of spectral shape changes. Finally the pSMSA is applied to the analysis of site-specific thermal unfolding of two small α-helical proteins from sets of multiple ^{13}C isotopically edited amide I' spectra.

3102-Pos Board B794

Computing Theoretical Circular Dichroism of Proteins using the Dipole Interaction Model (DINAMO) with a United Atom Approach

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The dipole interaction model is a classical electromagnetic theory for calculating the π-π^* transitions of peptides and proteins. DInaMo reduces all amide