Both isomers appear to interact with lipid bilayers in a similar fashion. Our results suggest that Aβ mediated disruption of ionic homeostasis may occur by a direct pathway of ion channel formation and may not need to rely on interactions with membrane receptors. Understanding the mechanism of peptide-membrane interaction and insertion at nanoscale resolution is essential for therapeutic design aiming to control and prevent Aβ pore formation. Funded by NCI Contract HHSN261200800001E (RN) and NIH (National Institute on Aging AG028709) extramural program (RL).

2995-Pos Board B765
Scanning Probe Microscopy of Serpin Polymers
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Serpins belong to a superfamily of structurally homologous proteins with metastable native structures. Their metastability is crucial in performing their function as regulators of serine and cysteine protease cascades. However, mutated variants of serpins found in patients with serpinopathies are often prone to polymerization. The most widespread serpinopathy is related to the Z variant of antitrypsin, which forms toxic polymers in the liver leading to liver degeneration. The understanding of polymerization mechanism holds the key to disease prevention, diagnostics and cures. Several models of serpin polymerization have been proposed. Still, the structure of *in vivo* formed polymers is unknown, and the relevance of *in vitro* created oligomers to disease related structures is far from established. Here we employed atomic force microscopy (AFM) to compare topography of *in vitro* and *in vivo* formed antitrypsin polymers and oligomers. We established morphometric features of Z monomers and *in vitro* formed dimers with different types of linkage. Moreover, we found a remarkable heterogeneity of unit types and their arrangement in polymer strands isolated from the Z variant mutant mouse liver. Even within the same strand there were examples of a linear arrangement of monomer units and of compact arrangement of dimers. The *in vitro* formed wild type oligomer preparations contained structures resembling the native Z variant structures from the liver. Still, the partition of types of polymers was strikingly distinct, with compact monomer arrangement and polymerized dimers frequenting the *in vitro* samples. In addition to AFM, we used scanning tunneling microscopy (STM) to study the orientation of units in polymers based on the proteins surface charge. Summarizing, SPM imaging provided the unique data on a high heterogeneity of polymers and pointed at certain types of monomer linkages that might help explain the mechanism of serpin polymerization.

Vibrational Spectroscopy

2996-Pos Board B766
Deep-Uvrr Spectroscopy Studies of Amyloidogenic Transthyretin Fragments
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The protein transthyretin (TTR) has been implicated as the pathogen in several amyloid diseases. Normally a transport protein for thyroxine, amyloidosis occurs when the protein aggregates and deposits in organ tissue as β-sheet structured amyloid fibrils. The formation of amyloid fibril deposits associated with TTR diseases is poorly understood. In order to study amyloid fibril formation several amyloidogenic fragments of TTR have been studied in aqueous solutions. It has been suggested that the amyloidogenic fragments TTR(10-20) and TTR(105-115) contain portions of the protein essential to aggregation and amyloid fibril formation, however, neither have been studied in the presence of cell-like lipid membranes. Here, we present the first studies comparing aqueous and model membrane solution studies of TTR(10-20) and TTR(105-115) via deep-ultraviolet resonance Raman spectroscopy. Initial results suggest a change in peptide secondary structure upon interaction with lipid membranes.

2997-Pos Board B767
In Vivo Molecular Labeling of Halogenated Volatile Anesthetics via Intrinsic Molecular Vibrations using Nonlinear Raman Spectroscopy
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Halogenated volatile anesthetics are frequently used for inhaled anesthesia in clinical practice. No appropriate biological method has been available for visualizing their localization in action. Therefore, despite their frequent use, the mechanism of action of these drugs has not been fully investigated. We measured coherent anti-Stokes Raman scattering (CARS) spectra of sevoflurane and isoflurane, two of the most representative volatile anesthetics, and determined the low-frequency vibrational modes without nonresonant background disturbance. Molecular dynamics calculations predict that these modes are associated with multiple halogen atoms. Because halogen atoms rarely appear in biological compounds, the entire spectral landscape of these modes is expected to be a good marker for investigating the spatial localization of these drugs within the intracellular environment. Using live squid giant axons, we could detect the unique CARS spectra of sevoflurane for the first time in a biological setting.

2998-Pos Board B768
Cell Surface Protein Detection using Surface-Enhanced Raman Scattering (SERS) Gold Nanoparticles
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Surface enhanced Raman scattering (SERS) Au nanoparticles have been used as novel cell surface receptor labels for the identification of markers of interest in chronic lymphocytic leukemia (CLL) and lung cancer. Biocompatibility of the particles was improved using multiple coating and particle protection strategies. Each of these strategies facilitated different methods for the inclusion of Raman active reporter molecules, as well as for different types of targeting moieties. Characterization of the SERS nanoparticles was undertaken including quantification of the number of antibodies bound to the surface. Long-term stability of both the nanoparticle Raman signal intensity and monodispersity was assessed under standard storage conditions, as well as conditions suitable for in vitro biological experiment. The SERS labeling platform has been demonstrated as being compatible with traditional pathology protocols including flow cytometry, and stains such as giemsa. SERS detection using these particles has been adapted to models for both adherent and circulating malignancies, in addition to patient cell samples in the example of CLL. The narrow vibrational spectra of SERS particles used in this study greatly increase the multiplexed labeling potential over traditional fluorescence-based technologies. Preliminary multiplexed labeling of CLL has also been demonstrated.

2999-Pos Board B769
Micro-Raman of Cancer Cells: Toward Label-Free Sorting of Circulating Tumor Cells from Whole Blood
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Even in the early stages of cancer, circulating tumor cells (CTCs) can travel from a primary tumor site to other organs through the blood and lymphatic system. Detecting and isolating CTCs from the blood has great potential for early cancer detection and studies of metastasis. However, they are notoriously rare (a few CTCs per mL of blood) and difficult to distinguish from epithelial non-tumor cells and leukocytes. Attempts to analyze CTC genetic or protein changes in response to treatment have been hampered by the difficulty in isolating intact clonogenic cells. Toward the goal of developing a rapid, non-invasive tool for detecting and isolating live CTCs from the blood, we measured the Raman spectra of live, unlabeled single cells. Using principal components analysis (PCA), we can distinguish WBCs from cancer cells, and tumorigenic ovarian cancer cells (A2780, OVCAR2, CaOV3) from non-tumorigenic ovarian cancer cells (OV429). Adherent cell lines and suspended (laser trapped) cells from cancer patient fluid samples were measured. A microfluidic platform with pressure control has been implemented to transport such cells single-file through the Raman laser focus. Custom microfluidics are in development and will sort the live, unmarked cancer cells into separate reservoirs for cell culture. Each Raman spectrum requires ~1-2 minutes, so we are concurrently developing a faster, coherent anti-Stokes Raman scattering (CARS) microscope for higher throughput analysis. Rapid detection and sorting of live CTCs will give prognostic information and allow for observation of the effect of targeted cancer treatments via a minimally invasive blood test and possibly well before the availability of response data.

3000-Pos Board B770
Study of Energetic Particle Induced Biological Effect through FTIR and Raman Micro-Spectroscopy
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Energetic particles exist ubiquitously in nature and may cause varied biological effects which have been found useful applications such as radiotherapy of

human cancers and mutation breeding of crops and microbes by means of ion-beam technology. However, the underlying mechanism for the energetic particle induced biological effects is still elusive because the interacting system involves radiolysis of different biomolecules such as DNA, proteins, sugar and lipids, and moreover, there are cross-correlations among these varied substances due to their interactions and signal transductions in living cells. A new trend of research is therefore to investigate the interaction between energetic particles and organisms with consideration of the entire cellar micro-environment and the entangled processes occurring in a whole cell, and to scrutinize the changes of cellular structure and compositions on the micro-scale and also monitor the dynamic and kinetic processes for the interactions. In this context, it is very useful and powful to apply micro-spectral imaging technology such as FTIR and Raman confocal microscopy because it can provide not only good time and spatial resolution, but also non-invasive measurement. In this work, we utilized the high spatial resolution FTIR and Raman microscopy to study the cellular changes of some model microbes and cells by mapping and monitoring the fingerprint bands of the cellular components such as lipids, carbohydrates, polyunsaturated fatty acids, proteins and nucleic acids under the irradiation of energetic particles. Correspondingly, the cell activity, the intracellular content of ROS, the level of MDA and GSH, the activity of CAT and SOD were measured to explain the biological effect induced by energetic particles. This work is supported by NSFC (No. 10975152, No. 11175204), CAS Innovative Project (KJX2-YW-N34-1) and Hundred Talents Program of CAS, China.

3001-Pos Board B771

Simulating the Amide I IR Signal of a Peptide in Solution using a Classical Implicit Water Approximation

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Behavior of the amide I IR signal of peptides has been the object of much study because it can provide valuable information about the environments and conformations of peptides in solution. Numerous models have been proposed to predict the amide I band shape, the most successful of which may have limited utility in studying dynamic processes due to high computational cost. Here a new, simple method is employed to predict the effects that the aqueous environment and side chains have on the shape of the amide I IR signal for a polypeptide in water. Using GEPOL to generate a solute cavity, apparent charges at the solvent accessible surface are generated considering a conductor like response from the solvent to partial charges on atoms (AMBER, or CHARMM). Potentials on the atoms in amide groups resulting from the surface charges and from the side groups are then used along with an electrostatic potential map to predict how the amide I signals are perturbed from gas phase signals. Effects of cavity size and atomic partial charges on the integrity of predicted band shapes are examined. The method gives results comparable to much more computationally intensive methods.

3002-Pos Board B772

Measuring Moments of Protein Conformation Distributions using Infrared Spectroscopy

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Infrared spectroscopy is often used to characterize the concentration and secondary structures of proteins in a variety of static and dynamics samples. Our work develops new methods to compare the structure, dynamics and function of nearly identical protein samples, in order to help characterize bio-similar protein therapeutics. We have developed a method to describe protein conformational variations around the average molecular values. By comparing the moments of the protein structural distributions and amide hydrogen/deuterium exchange methods, we explore the relationships between protein stability and dynamics. Examples include lysozyme and albumin in solution, cytochrome c interacting with lipid membranes of varying net-negative surface charge density, and bacteriorhodopsin during its photocycle.

3003-Pos Board B773

Isotope-Edited FT-IR Spectra of Isotopmers of Helical Hexamers of Alpha-Aminoisobutyric Acid

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Isotope-edited FT-infrared spectra of the Amide I region of hexamers of alpha-aminoisobutyric acid (Z-Aib_6-OtBu) have been collected in order to explore the effects of $^{13}C{=}O$ enrichment on the FT-IR spectra in the conformational context of 3_{10} helices. Oligomers of Aib are known to adopt predominantly 3_{10} helical structures, even at short peptide lengths. The Amide I band is sensitive to the details of peptide secondary structure, but the competency of this band to distinguish between alpha- and 3_{10} helical secondary structure remains an open question. The 3_{10} helix is shorter than an alpha-helix of the same number of residues and exhibits an i to i+3 hydrogen bonding pattern, instead of an i to i +4 pattern of the alpha-helix. These differences bring amide oscillators of a 3_{10} helix slightly closer in space and in shorter periodicity of hydrogen-bonding partnership as compared to an alpha-helix. We have collected infrared spectra of isotopomers of hexamers of Aib (e.g., Z-Aib-Aib-Aib-Aib-Aib-Aib-OtBu, Z-Aib*-Aib-Aib*-Aib-Aib-Aib-OtBu, and Z-Aib*-Aib*-Aib-Aib-Aib-Aib-OtBu, Aib* = ^{13}C enrichment at *C=O) in dichloromethane (a nonpolar aprotic solvent) and methanol (a polar, protic solvent) to examine the effects of carbon-13 enrichment on the spectra. Differences among the spectra of peptide isotopomers enriched with one or two ^{13}C labels will be examined, detailing the effects of coupling between pairs of ^{13}C-labeled Amide I oscillators.

3004-Pos Board B774

Under Pressure: Measuring Desolvation of Model α-Helical Peptides

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Model helical peptides have been shown to unfold with temperature, but are stabilized with increased pressure [1-3]. In these peptides, as pressure increases, the solvent environment becomes ordered allowing for an increase of the helical content of the peptide. Our work investigates hydration of the peptide backbone using both temperature and pressure as modifying conditions. The synthetic peptide sequence, $(AAAAK)_3$-AAAAY is well-characterized and ideal for the study of helix properties[3]. To measure the coupling of pressure and temperature, Fourier transform infrared spectroscopy (FTIR) monitors the perturbations in the secondary structure via the amide I' band. FTIR is a sensitive technique for detecting changes in hydrogen bonding and has been used for the estimation of amide photon exchanging with the solvent. Pressure on the peptide is applied using a manually manipulated Diamond Anvil Cell (DAC). Isotopically labeled residues within the peptide have been exploited for probing local interactions due to the shift of the heavier masses of the labeled residues to a lower frequency compared to the global ^{12}C amide I band around 1633 cm^{-1}. Our goal is to compare the location of isotopically edited alanines and the ability to remain desolvated with increasing pressure and temperature. With this technique, it was found that alanines proximal to the lysines were protected from solvent hydrogen interactions due to side chain shielding in the model sequence.

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3005-Pos Board B775

Dependence of Plant Cell Wall Composition and Structure on Cellulose Synthase-Like Knock Out Mutant

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Plant cell walls are a complex mixture of polysaccharides, proteins and the phenolic polymer lignin that have been recently targeted as possible sources of fermentable sugars for the production of biofuels. The development of a biomass-based biofuels industry is partly dependent on genetic engineering and breeding next generation crops containing, among other traits, easily extractable cell wall sugars. Thus, a better understanding of how plants synthesize, deposit and modify their cell walls is necessary for the selection of traits important for biofuel crop improvement. The identification of plants with altered cell wall composition or structure can prove useful in the discovery of novel genes involved in the biosynthesis and modification of the cell wall.
The CELLULOSE SYNTHASE-LIKE 6 (CSL6) gene has been recently shown to mediate the biosynthesis of mixed-linkage glucan (MLG), a cell wall polysaccharide that is thought to be necessary for cell wall expansion in the primary cell wall of young seedlings. A detailed analysis of a loss-of-function MLG rice mutant has been recently conducted revealing surprising results. Though the mutant showed a 99% reduction of MLG content, the rice clsf6 knock out mutant showed only a slight decrease in growth compared to wildtype. The cell wall properties of both mutant and wild type were determined via biochemical and various spectroscopic (Fourier Transform Mid-Infrared spectroscopy)