SAS-Chicago

Poster Session on Spectroscopy

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University of Illinois at Chicago, Student Learning Center in the SES

Graduate

Spectroscopic Studies of Beta-lactoglobulin with Model Membrane Vesicles
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Bovine beta-lactoglobulin (•LG) is a lipocalin protein found in mammalian milk. In the native state, its secondary structure is dominated by beta-sheet, though it has the propensity to form • helices based on secondary structure predictions. We have shown that •LG can adopt a significant fraction of alpha-helical conformation upon mixing with synthetic phospholipid vesicles. The thermodynamic and kinetic aspects of interaction between •LG and lipid vesicles have been previously studied. However, the function of •LG is still not clear. In this work, CD and fluorescence measurements have been done to further study the interaction between •LG and phospholipids vesicles.

Towards Comprehensive Histopathologic Analyses in Breast Cancer with Mid-IR Spectroscopic Imaging
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Widespread screening leads to large numbers of breast biopsies, which require expensive and time-consuming pathology assessments. Current histopathologic analysis, composed of tissue staining and manual recognition, may not be optimal to meet the growing demand for rapid tissue evaluation. Accurate automated techniques for breast biopsy examination could improve cancer diagnosis efficiency by reducing materials, time, and human interpretation required for preliminary histopathology analyses. Fourier transform infrared (FT-IR) molecular spectroscopy, extensively used in chemical analysis, may be applicable for breast pathology. An IR spectrum gives a quantitative measure of molecular composition, which can be numerically related to identify important types of breast tissue. Recent instrumentation advances permit the combination of FT-IR molecular spectroscopy with optical microscopy to obtain morphologic and spectroscopic information in the form of FT-IR images. This technique involves only non-perturbing light, and produces tissue image datasets without the use of molecular probes or contrast agents. Therefore, FT-IR imaging can be directly applied to fixed tissue sections prior to

conventional staining procedures. In this study we employ tissue microarrays (TMAs), which offer a high-throughput approach to collect data and build a prediction algorithm from a large selection of cancer and normal tissue samples. FT-IR TMA datasets are automatically classified by supervised pattern recognition to provide false-color images comparable to stains used in conventional breast cancer diagnosis. We demonstrate that a single classified FT-IR image dataset can provide a histopathology evaluation similar to a panel of traditional immunohistochemical stains. Classification accuracy is assessed by receiver operating characteristic (ROC) analysis and validated on biopsy surgical resections. Results demonstrate high accuracy for an automated diagnostic tool and help establish FT-IR imaging as a novel technology for breast cancer diagnosis and research.

Structure and orientation of hydrocarbon adsorbates at catalytically relevant oxide surfaces studied by sum frequency generation

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The chemical environment of an adsorbate controls its orientation and structure on oxide surfaces. We determined the orientation and structure of cyclic and acyclic hydrocarbons at catalytically relevant surfaces, in various interfacial environments. Using sum frequency generation, (SFG) which is sensitive to vibrational mode orientation at surfaces, we directly compared hydrocarbons adsorbed at liquid/α-Al_2O_3 and vapor/α-Al_2O_3 interfaces. Cyclic and acyclic alkanes lay flat at the liquid/solid interface but adopt more upright orientations at vapor/solid interfaces. At liquid/solid interfaces, interactions between adsorbates and solvent may play a primary role in directing adsorbate structure and orientation. To investigate adsorbate-solvent interactions, we studied a polar adsorbate, 1-hexanol, in liquid cyclohexane solution at the liquid/α-Al_2O_3 interface. Hexanol forms ordered adlayers on α-Al_2O_3. The ordering of hexanol is accompanied by solvent molecule reorientation in the interfacial region. We also present preliminary results in which we demonstrate detection of liquid hydrocarbon adsorbates on 2 nm palladium clusters prepared on α-Al_2O_3 by atomic layer deposition (ALD). Future work will focus on adsorbate identity, structure and orientation on palladium-ALD clusters when they are used as catalysts for selective alcohol oxidation. These results provide comparisons of liquid, gas, and solvated adsorbate orientation and structure, and will be applied to assess the role of adsorbate-molecule and adsorbate-surface interactions in directing reactivity and selectivity at catalytic oxide interfaces.

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Laser Induced Breakdown Spectroscopy (LIBS) is a powerful and universal analytical technique for the identification of atomic species. This poster will present a means for producing more usable spectra known as Polarization Resolved LIBS using an aluminum sample. Other important experimental conditions are explored such as the difference between using nanosecond and femtosecond pulses as well as proposing a mechanism for continuum background which plagues many LIBS spectra.

Finding the Right "Mis'match: Millisecond conformational dynamics of MutS-DNA complex during DNA damage recognition

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The 3-D structure of biological macromolecules (proteins, DNA and RNA) is held together by many interactions (ionic, hydrophobic, van der waals, hydrogen bonds) which can be disrupted by thermal fluctuations, leading to fluctuations in the conformations accessible to the macromolecule. These conformational fluctuations are central to molecular recognition, in which two or more macromolecules (protein-DNA, protein-RNA, protein-protein) interact with each other to form higher order complexes. Our research group studies the underlying energetics of these binding interactions in protein-DNA complexes by measuring the dynamics of conformational fluctuations that lead to the formation of a specific complex. Conformational dynamics in DNA-protein complexes are believed to occur on timescales of microseconds to milliseconds, and our group has pioneered the application of laser temperature-jump (T-jump) fluorescence spectroscopy techniques to probe these dynamics with microsecond time-resolution on these timescales.

Current research in our group is directed towards an understanding of the mechanism by which proteins recognize a specific site in DNA and then bind to it. This recognition could occur by specific contacts between DNA bases and protein amino acids, or by sensing local variations in the mechanical properties of DNA such as "bendability" of DNA. Our interest lies in probing the role of DNA bendability in the recognition mechanism, particularly for proteins which are known to bend DNA in complex. The MutS protein recognizes errors in DNA such as mismatches and bulges and initiates repair by recruiting other proteins to the mismatch site. While it is known that MutS bends DNA by 45-60^{o} while interacting with it, the role of DNA bending in the recognition process has not been clearly understood. We have carried out equilibrium and kinetic measurements on DNA labeled with 2-aminopurine (2AP) adjoining a T-bulge, in complex with prokaryotic MutS and its eukaryotic homolog MutS**.**. Equilibrium fluorescence measurements as a function of increasing temperature reveal a sharp increase in 2AP fluorescence near the optimal physiological temperature ~40°C for MutS**.**, and at ~70°C for *Taq* MutS. Relaxation kinetics monitored with 2AP fluorescence in response to a rapid T-jump show relaxation kinetics in *Taq* MutS near 70°C, occurring with a time constant of a ~10 milliseconds. This rapid phase has not been observed in previous dynamic studies, and illustrates how laser T-jump spectroscopy can be applied to probe the dynamics of mismatch recognition in DNA by DNA mismatch repair proteins.

Dynamics of DNA-Bending in Binding Site Recognition by IHF

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We present recent progress in monitoring the DNA bending dynamics in site-specific recognition by IHF, an architectural protein from *E. coli* that recognizes several sites on phage • DNA, primarily by indirect readout. IHF bends the DNA at its cognate site by nearly 180° over ~35 bp, creating two kinks in DNA stabilized by intercalation of conserved proline residues located on

two •-ribbon arms that wrap around the DNA.

Previous stopped-flow and laser T-jump measurements on IHF binding to its cognate H' site revealed that DNA bending in the complex occurs on ~1-10 ms, similar to the time-scales for thermal disruption of a single base pair in B-DNA. Here we find that inserted mismatches that increase the DNA flexibility at the site of the kinks accelerate the bending rates by nearly the same factor as the corresponding increase in binding affinity. On the other hand, modifications in DNA away from the site of the kink, as well as mutations in IHF, designed to perturb specific protein-DNA contacts, leave the bending rates unchanged despite a ~60-100-fold decrease in the binding affinity. These results support our earlier conclusion that in the transition state ensemble separating the nonspecific from the specific complex the DNA is bent/kinked, but protein-DNA interactions that stabilize the complex have not yet been made.

Our measurements also reveal a rapid (~100 microseconds) phase in the bending kinetics. In contrast to the relaxation rates for the slow phase, which are affected by modifications in the DNA at the site of the kinks, the relaxation rates for the fast phase appear to be unaffected. This rapid phase may correspond to the wrapping/unwrapping of the •-arms of the protein in a nonspecific binding mode, as IHF scans potential binding sites on genomic DNA.

Inter-residue Coupling of Model PPII Helices Using ^{13}C Isotopic Labeling

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Characterization of poly-proline II (PPII) conformation on a site-specific basis has importance in developing a model for structure and stability in these systems. Coupling of selected residues for a series of related peptides having predominantly PPII conformations were measured using VCD and IR spectra of selected variants that were doubly labeled with ^{13}C on the amide C=O. The characteristics of the ^{13}C=O component of the amide I' IR band and their sensitivity to the local structure of the peptide are compared to predictions based on DFT level calculations for related structures and used to determine coupling between C=O groups along the backbone of this helical structure. Doubly labeled peptides have spectral shifts reflecting the mass change in addition to coupling between residues. In the PPII case the coupling is relatively weak, yet by combining IR and VCD along with DFT level calculations, we have been able to determine its coupling constants. Comparison of PPII structures with "random coils" can be done by comparing all Proline, mixed Ala-Pro and Lys-rich sequences. The shifts and couplings reflect the computations in all cases. The distinct vibrational coupling patterns of the labeled sites based on this structure are also well matched by *ab initio* DFT-level calculations of their IR and VCD spectral patterns.

Modeling, Data Visualization and Histopathology using Fourier Transform Infrared (FT-IR) Spectroscopic Imaging of Human Tissue Specimens

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Fourier transform infrared (FT-IR) spectroscopic imaging is an emerging technique that provides both chemically and spatially resolved information. The rich chemical content of data may be

employed for computer-aided determinations of structure or pathologic state of biological specimens. An exciting emerging avenue is the use of one such technique, Fourier transform infrared (FTIR) spectroscopic imaging for determining structure and disease within tissue (histopathology). A major problem in this approach is whether the numbers of spectra, samples and patients employed to construct computer algorithms influences the prediction performance of the developed algorithms. In this work, we demonstrate theoretically and experimentally the effects of these parameters on histologic classification of prostate tissue. Results indicate that a small number of samples, carefully tested and identified with appropriate biologic significance, can be employed to provide robust classification schemes. It is also shown that a relatively small number of pixels are sufficient to capture information about the entire dataset and a statistical analysis of the intra-person and inter-person variance in spectral metrics (features) is presented to understand and explain these results. The number of spectral metrics required for classification and the effects of signal to noise ratio on classification are also discussed.

Data visualization and representation in biomedical spectroscopic imaging involves the measurement of data ranging from micron-to-millimeter length scales. Organization of materials from various sources, patients and locations within organs adds another layer of complexity. Currently, there is no general framework for the organization and visualization of these datasets. Here, we describe a system for graphically displaying data to convey the organizational complexity inherent in the data, estimate the reduced dimensionality of acquired data seta, results of statistical processing or classification and relationships between features in complex spectral patterns. This format would help both experts and non-experts visualize complex relationships in data. FT-IR spectroscopic imaging data from a large data set of prostate cancer patients is visualized to determine expression patterns for different tissue histologies. The methods developed are applicable to vibrational spectroscopic imaging in particular and chemical imaging methods in general.

Identification of a low molecular weight ligand for N^5-carboxyaminoimidazole ribonucleotide mutase (Class I PurE), an essential bacterial enzyme

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PurE, N^5-carboxyaminoimidazole ribonucleotide mutase, is an enzyme in the *de novo* purine biosynthetic pathway. The octomeric recombinant protein, PurE from *Bacillus anthracis* (138.6 kDa), was expressed in *E. coli* cells and purified with aid of an N-terminal GST tag. This protein is an antibiotic target for structure based drug design due to a unique step of the *de novo* purine pathway in prokaryotes. Step 6 of the *de novo* purine pathway differs in mechanism between higher eukaryotes and lower eukaryotes as well as prokaryotes. Higher eukaryotes convert aminoimidazole ribonucleotide (AIR) to carboxyaminioimidazole ribonucleotide (CAIR) directly with the second substrate CO_2 by the Class II PurE. In contrast, lower eukaryotes and prokaryotes achieve this conversion through a two-step mechanism, with AIR first converted to N^5-carboxyaminoimidazole ribonucleotide (N^5-CAIR) by PurK, followed by Class I PurE conversion of N^5-CAIR to CAIR. The monomer (17.3 kDa) of PurE contains a single tryptophan residue at position 15, near the binding site. Taking advantage of this residue, ligand binding to PurE was characterized by fluorescence intensity emission, with excitation at 295 nm and

measuring λ_{max} at 346 nm. Computational modeling identified several potential low molecular weight ("fragment") hits. Experimental screening identified a binding ligand with a K_d of 74 ± 21 μM, through a titration assay with fluorescence detection. This lead is being assessed for further optimization. The assay and binding characterization will be described.

Interaction of ethylene and nitrogen atoms on the Pt(111) surface

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The selective catalytic reduction (SCR) of NO_x by hydrocarbon on noble metals is critically important to the implementation of leaner burning more fuel efficient processes. Understanding the reaction mechanisms and pathways is essential for designing an effective catalytic system. As one small part of this effort, we focus on the interaction of nitrogen atoms and simple alkenes such as ethylene on Pt(111) surface under UHV conditions in an effort to understand the potential intermediates in NOx reduction. The adsorption of ethylene on nitrogen pre-covered Pt (111) surface has been investigated using by reflection absorption infrared spectroscopy (RAIRS) and thermal desorption spectroscopy (TDS) as well as density functional theory calculations. Two interesting observations have been made. First, we observe the presence of • -bonded ethylene below 220 K. indicating a switch in the preferred binding site for ethylene on N-Pt (111) as compared to the clean surface. Second, above 500 K,CN coupling occurs and ammonia is seen to desorb. The appearance of ammonia is believed to be the result of a reaction between N atoms (or NH) with coadsorbed ethylidyne.

The L49F Mutation in Erythroid Alpha Spectrin Induces Local Disorder in the Tetramer Association Region: Fluorescence and Molecular Dynamics Studies of Free and Bound Alpha Spectrin

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The bundling of the N-terminal, partial domain helix (Helix C') of human erythroid • -spectrin (• I) with the C-terminal, partial domain helices (Helices A' and B') of erythroid • -spectrin (• I) to give a pseudo spectrin structural domain (triple helical bundle A'B'C') has long been recognized as a crucial step in forming functional spectrin tetramers in erythrocytes. We have used fluorescence studies to obtain apparent polarity and Stern-Volmer quenching constants of Helix C' of • I bound to Helices A' and B' of • I. These properties were used to guide us in homology modeling with a previous NMR structure as the template. The homology models then became input structures for molecular dynamics simulations for both wild type (WT) and an • I clinical mutant Spectrin Lyon (L49F). The simulation output structures show a stable helical bundle for WT, but not for L49F. In WT A'B'C', four critical interactions were identified: two hydrophobic clusters and two salt bridges. However, in L49F, the C-terminal region of Helix C' was unable to assume a helical conformation and one critical hydrophobic cluster was disrupted. Other molecular interactions critical to the WT helical bundle were also weakened in L49F. We suggest that these conformational changes lead to a lower tetramer levels observed in Spectrin Lyon patients. (Funded by NIH GM68621 and AHA 0350617Z).

Low Flow Push-pull Perfusion Sampling of subtle gray mouse brain
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The xCT(cystine-glutamate) transporter proteins are thought to be involved in maintaining the levels of the extracellular glutamate in the nervous system of the brain. To explore this hypothesis, low flow push-pull perfusion sample collection and capillary electrophoresis sample analysis is used to quantitate extracellular glutamate in a xCT mutant mouse model. Low Flow Push Pull Perfusion system has previously been used for *in-vivo* sampling of rat brain. LFPS is modified for the mouse model, where xCT transporter protein expression has been eliminated. LFPS sampling is performed from the striatum of the mouse brain of both control and mutant mice. Amino acid composition is determined by CE-LIF. LFPS for this study utilizes a miniaturized probe of 170 micron (o.d). Standard flow rates of 40-50nL/min saline are pushed to the probe tip as aspirated through the withdrawal line to collecting 500nL of samples. Laser-induced fluorescence detection is employed to observe fluorescently labeled amino acids separated by capillary electrophoresis. Tagging was performed with fluorogenic agent fluorescamine. Reaction optimization of pH has been performed. The extracellular levels of glutamate for both males and females of control and mutant show statistically significant differences between control and mutant for both males and females. There is a significant difference in the extracellular glutamate level between males and females. The efficiency in placing the probe at the striatum is greatly complicated by the small size of mouse brain.

Undergraduate

Nitrate/Nitrite concentration change after capillary flow through.
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Nitrate and nitrite are commonly measured as the stable metabolites of NO. The limits of detection of these analytes are limiting for analysis from biological samples. In this work we study a concentration effect of prepared mixtures, after they flow under pressure through a capillary segment. To determine concentrations of nitrate and nitrite in the solutions, capillary electrophoresis (CE) apparatus with UV-VIS were used. Various sizes of inner diameter capillary were used (10, 20, and 50• m in i.d.) The solutions were collected using a vacuum pump at constant flow rates of 20.0 and 50.0 nL/min. Different concentrations of nitrate and nitrite were prepared in KRB, such as: 2.5, 5, 10, 20, and 40 • M. Calibration curves were prepared to monitor the effects of analytes under different conditions. The effects of inner diameter, the flow rate and the pH were studied for nitrate and nitrite. After passing known amounts of nitrate/nitrite mixture, there has been a noted significant increase of concentration of nitrate, and a significant decrease in concentration of nitrite. With smaller concentrations of nitrate, the percentage of increase is greater than for larger concentrations. With larger concentrations of nitrite, the percentage of decrease is large than for smaller concentrations. The increase in nitrate and decrease in nitrite concentration trend was seen in capillaries of all sizes and with different flow rate. Different KRB solutions where prepared acidic, basic, and normal. The biggest percentage of increase in nitrate level and the largest decrease in nitrite level was

observed in monobasic KRB solution (acidic). Results indicate that the concentration change is dependent on capillary's inner diameter, different starting concentrations, different starting volumes, different flow rates and different sample's pHs. The results show an ability to manipulate nitrate and nitrite concentration that may be exploited to improve bioanalysis.

PeakFit; A Unique Method to Identify Various Sulfonate Groups on Heparin

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Heparin is an acidic glycosaminoglycan with a diverse number of biological functions, the main one being anticoagulant. The quantification of sulfonates on heparin facilitates in depth understanding of the diversity and functionality of this heteropolysaccharide.

Fourier transformed infrared Spectroscopy (FTIR) is useful in identifying band areas originating from various covalent bonds and thus characterizing different molecules. FTIR spectroscopy methods are not yet refined enough to quantify band areas of closely related chemical groups. For example, heparin has 6 different sulfonates, whose S=O stretching vibration absorbs infrared light in the 1280 to 1200 cm^{-1} region without actually differentiating them.

PeakFit is a software designed to analyze and identify hidden peaks in spectroscopy, chromatography and electrophoresis graphs. It was used in this study to characterize infrared bands of S=O sulfonates of heparin before and after chemical N-desulfonation.

The FTIR spectrum of 3-O-hexanoylated heparin analyzed by PeakFit shows 6 peaks in the 1280-1200 cm-1 region. When the same experiment was carried on a chemically N-desulfonated-3-O-hexanoylated heparin, the band at 1242.80 cm^{-1} was not detectable, indicating that it originated from the N-bonded sulfonates (SO_3^{1-}).
These results show that PeakFit is indeed a useful tool to separate spectroscopy bands, and thus identify different sulfonates, which may be quantified if needed. PeakFit proved to be a simple technique to resolve different S=O (SO_3^{1-}) bands in heparin. The method may be used for other groups like the Amide I band of proteins.