microscopy to the analysis of the lacunar-canalicular network in demineralized bovine tibia. Results from our study showed that

1. the canalicular diameter, d = 419 ± 113 nm;
2. the canalicular porosity, e = 0.051 ± 0.018;
3. the canalicular channels have a specific morphology and location with respect to lamellae and lacunae;
4. the canalicular wall is made of globular structures; and
5. the canalicular depth appears constant over high and low lamellae.

From this study, we conclude that the integrity of the lacunar-canalicular network is preserved after demineralization and that AFM and confocal microscopy are powerful tools in high-resolution structural analysis of bones. The acquired results allow us to calculate diffusivities and tortuosity, which should facilitate microfluidics simulations under various mechanical stress stimuli.

854-Pos Protein Adsorption and Platelet Adhesion on Polyurethane Biomaterial Surfaces

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Board B699

Understanding the surface properties influencing thrombus formation is a key to the development and application of new biomaterials in the long term use of blood-contacting medical devices. In this study we utilized a series of segmented polyurethane (PU) biomaterials with different soft segments chemistries - polycarbonate (PC), polytetramethylenoxide (PTMO), and polydimethylsiloxane (PDMS), to produce a variety of surface chemistries. Atomic force microscopy (AFM) was used to characterize the polymer surface microphase separation structure of PUs and to identify adsorbed fibrinogen on the surfaces so that relationship between biomaterial surface chemistry, fibrinogen adsorption, and platelet adhesion could be addressed.

AFM phase images show that PDMS-PUs undergo strong phase separation and suggest three phases (soft, intermediate and hard domains) present in structure, while PC-PU and PTMO-PU appear to have two-phase structures. Platelet adhesion was measured optically on the PDMS-PU surfaces and found to increase with hard segment content from the range of 35% to 52% hard segment. Fewer platelets were observed on PC-PU and PTMO-PU. Protein adsorption studies were carried out by incubating polymers in mixed protein solutions of BSA and fibrinogen for 10 min. The amount of fibrinogen adsorbed on the surface was detected through antibody recognition measurements using AFM probes modified with a polyclonal anti-fibrinogen antibody. Results show that fibrinogen adsorption was roughly correlated to platelet adhesion on the PDMS-PUs, however, more fibrinogen was measured on PC-PU and PTMO-PU surfaces despite the fact that fewer platelets were observed. This work suggests that platelet adhesion is not necessarily determined by the amount of fibrinogen, but is likely related to the activity of fibrinogen, most likely correlated to the availability of the platelet binding site in the fibrinogen γαμμα- chain dodecapeptide (γαμμα400–411).

Vibrational Spectroscopy

855-Pos Kinetics of Electron Transfer to Cytochrome c Oxidase by Time-Resolved Surface Enhanced ATR-FTIR Spectroscopy

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Board B700

Cytochrome c oxidase (CcO) from R. sphaeroides genetically engineered with an his-tag on SU II was immobilized in a protein-tethered bilayer lipid membrane (ptBLM) with the cytochrome c binding site directed towards the electrode. The immobilization was followed by an in-situ reconstitution into a bilayer lipid membrane. Electron transfer was enabled by direct electronic wiring to the gold film deposited on the silicon crystal of the IR setup in an ATR configuration. The kinetics of electron transfer to the CuA, the heme a and a3 center of the enzyme was investigated by time-resolved surface enhanced ATR-FTIR spectroscopy.

Rate constants of electron transfer are obtained by a periodic application of potential pulses and analyzing the difference spectra of amide I bands assigned to the respective redox centres as a function of time. These spectral changes are monitored by surface enhanced ATR-FTIR spectroscopy. Assignment of site specific vibrational modes was facilitated using phase sensitive detection. Rate constants were measured under different conditions (pH value, anaerobic, aerobic) and compared to those obtained by electrochemistry.

The wild type enzyme engineered with a his-tag is investigated compared to the N139C

Mutant. Mechanistic implications of the results are presented.

856-Pos The Investigation of the Effects of Simvastatin on Rat Skeletal Muscle by Spectroscopic and Electrophysiological Techniques

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Board B701

Statins are widely used for the treatment of hypercholesterolemia which have some adverse effects on skeletal muscles. This study

aims to investigate the effects of chronic simvastatin administration on different rat skeletal muscles. Simvastatin (50 mg/kg/day) was given to male rats (n=10 in each group) by gavage for 1 month. Extensor digitorium longus (fast twitch, glycolytic, Type II), soleus (slow twitch, oxidative, type I) and diaphragm (mixed fibers) muscles were then dissected. Contractile properties of muscles were evaluated by recording electrical field stimulation-induced contractions. Electrophysiological properties were studied by recording resting membrane and action potentials by glass microelectrodes in diaphragm muscles. Fourier Transform Infrared Spectroscopy (FTIR) was used which is an excellent technique to detect molecular changes [1]. Simvastatin treatment caused a decrease in the body weight, serum total cholesterol levels and food intake ($P<0.05$). Although contraction strength of single twitches were unaltered, force-frequency relationship of all muscles were decreased at frequencies >40 Hz ($P<0.05$). Muscles isolated from treated animals displayed increased action potential amplitude, duration and decay-time ($P<0.05$). Spectroscopic studies revealed significant decrease in lipids including unsaturated lipids, protein, glycogen and nucleic acid content in simvastatin-treated muscles, indicating an increase in lipid peroxidation, and degradation of these macromolecules. Significant changes in protein secondary structure and conformation and an increase in membrane fluidity were also observed in treated samples. These findings showed that chronic high-dose simvastatin treatment impairs contractile properties of skeletal muscle without affecting excitability, possibly due to structural, functional and compositional changes in macromolecules. Prolongation of action potential duration may be due to inhibition of K^+ channel activity in order to compensate for decrease in contractility.

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References

[1]. Cakmak G., Togan I., Severcan F., (2006) Aquatic Toxicology, *77*:53–63

857-Pos Temperature Dependence of the Amide I spectra of Peptides in Solution

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Board B702

Amide I frequencies and intensities are widely used to monitor structural changes during thermal unfolding of peptides and proteins. However, in addition to the spectral changes arising from the structural transitions, the amide I peak frequencies and intensities of peptides in solution also exhibit intrinsic temperature dependence. For example, in D_2O, the amide I' of an alpha-helical oligopeptide, the random coil poly-L-lysine as well as the simplest amide N-methyl acetamide (NMA) exhibit very similar frequency shifts and intensity variations with increasing temperature. Similar temperature dependence of the NMA amide I frequency and intensity is observed in D_2O and in organic solvents both polar (acetonitrile and DMSO) and non-polar (1,4-dioxane), ruling out hydrogen bonding strength as the cause of these effects. In order to understand this intrinsic amide I temperature dependence, we have analyzed the NMA amide I spectra using a simple theory based on the Onsager reaction field with temperature-dependent solvent dielectric properties and a solute molecular cavity. DFT-level calculations (BPW91/cc-pVDZ) for NMA with an Onsager reaction field are used to provide independent insights into the effects of the temperature-dependent solvent properties on the NMA amide I spectra. We demonstrate that the Onsager model can accurately explain the observed experimental amide I frequency shifts and intensity changes with temperature in the studied solvents. The success of this simple model is encouraging as it can provide an understanding of the solvent effects on the vibrational spectra based only on macroscopic solvent properties: dielectric constant, index of refraction and density.

858-Pos Structural Rearrangements on the Pathway to Human-γC-Crystallin Aggregation

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Board B703

The aggregation of Crystallin proteins in eye lenses, which can occur as a result of a genetic mutation or external factors such as oxidative stress, leads to Cataract formation, the leading cause of blindness worldwide.

The aggregation of Human-γC-Crystallin (HγC-Crys) has been studied using infrared (IR) spectroscopy, a powerful tool for structural analysis of proteins. The amide I band, due primarily to the C=O stretching vibration, is particularly sensitive to the strength and position of the hydrogen bonds which define secondary structure. Our experiments show that as HγC-Crys aggregates, a conversion occurs from soluble globular β-sheet (characterized by an IR absorbance at 1636 cm^{-1}) to fibrous β-sheet (1615 cm^{-1}), with the amount of disordered protein remaining constant.

Here we report on equilibrium and kinetic studies of this aggregation. Temperature, concentration and pH all play a role in HγC-Crys aggregation: At pH 7 no aggregates are observed at temperatures up to 65°C; however, at pH3 (50 mM citrate buffer) aggregates are detected in solution at a temperature which decreases with increasing protein concentration. Interestingly, the total amount of fibrous β-sheet following an anneal cycle to 65°C is approximately 15%, regardless of concentration. Kinetic studies showed that rate of aggregation increases with temperature and concentration, though the total amount of aggregate ultimately formed is remarkably independent of either. Seeding experiments provide further insight: aggregated HγC-Crys, added to the native protein at 37°C, acted as a template for the misfolding of native HγC-Crys promoting aggregation that was not observed in unseeded experiments at this temperature.

These results, together with fluorescence measurements characterizing native and partially unfolded states, and turbidity measurements that detect the presence of larger aggregates, allow us to build

up a comprehensive picture of the unfolding and misfolding pathways in HγC-Crys aggregation.

859-Pos FTIR Micro-spectroscopy Identifies Symmetric PO_2^- Modifications As A Marker Of The Putative Stem Cell Region Of Human Intestinal Crypts

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Board B704

Complex biomolecules absorb in the mid-infrared ($\lambda = 2$–20 μm) giving vibrational spectra associated with structure and function (Walsh et al., Biochem Biophys Res Commun. 2007; *352*; 213–9). We employed Fourier transform infrared (FTIR) micro-spectroscopy to "fingerprint" locations along the length of human small and large intestinal crypts.

Paraffin-embedded slices of normal human gut were sectioned (10-μm thick) and mounted to facilitate infrared (IR) spectral analyses. IR spectra were collected employing globar (15 μm × 15 μm aperture) FTIR microspectroscopy in reflection mode, synchrotron ($\leq$10 μm × 10 μm aperture) FTIR microspectroscopy in transmission mode, or near-field photothermal micro-spectroscopy (PTMS). Dependent on the location of crypt interrogation, clear differences in spectral characteristics were noted. Epithelial-cell IR spectra were subjected to principal component analysis to determine whether wavenumber-absorbance relationships expressed as single points in "hyperspace" might on the basis of multivariate distance reveal biophysical differences along the length of gut crypts.

Following spectroscopic analysis, plotted clusters and their loadings plots pointed towards symmetric (ν_s) PO_2^- (1080 cm^{-1}) vibrations as a discriminating factor for the putative stem cell region; this proved to be a more robust marker than other phenotypic markers such as β-catenin or CD133. This pattern was subsequently confirmed by image mapping and points to a novel approach of non-destructively identifying a tissue's stem cell location. $\nu_s PO_2^-$, probably associated with DNA conformational alterations, might facilitate a means of identifying stem cells, which may have utility in other tissues where the location of stem cells is unclear (Walsh et al., Stem Cells. In Press).

860-Pos Conformational Study Of Different Beta-turns Coupled To A Cyclic Disulfide Bond To Model The Turn Roles In β-hairpin Stability

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Board B705

Turns and loops are often classified as the third type of secondary structural element due to their role as the connecting unit between α helices and β sheets in globular proteins. The most common turns connecting adjacent β-sheet strands are β turns. Cyclic peptides are useful structural models for the study of β turn conformation, because they can be easily synthesized and characterized by several spectroscopic methods. We synthesized a selection of cyclic peptides of the general formula Cyclo[Ac-Cys-Val-Xxx-Gly-Lys-Cys-NH_2] which were cyclized by a disulfide bond between the two cysteines. Their steric structures were characterized by NMR spectroscopy and restrained MD simulations. Furthermore, we compared their thermal stability using optical(IR, ECD and VCD) spectra and extended the result of this study to aid understanding of the folding energetics of a 12-residue β-hairpin model-Trpzip2. Additional CD and IR studies, based on variation of the Trpzip2 turn sequence from -Asn-Gly- to -^{D}Pro-Gly-, show that the more stable -^{D}Pro-Gly- turn causes the hairpin to follow a different unfolding mechanism for secondary and tertiary structure. Trp residues at non-H-bonded positions on different strands facilitate hydrophobic interactions and consequently formation of the Trpzip β-hairpin and that mutation to residues promoting formation of a more stable turn will additionally stabilize Trp interaction between two strands.

861-Pos Determination of the electronic transition dipole moment orientation of Chlorophyll A

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Board B706

The orientation of the electronic transition dipole moment of the Qy band within the molecular structure of Chlorophyll A is the basis for simulating many properties of photosynthetic systems. Static properties such as the formation of excitons for strongly coupled chromophores in light harvesting complex and reaction centre are of outmost importance for understanding the dynamics of energy transfer and charge separation. Determinations thus far of the electronic transition dipole moment orientations of Chlorophyll A were made on oriented samples with steady state spectroscopy [1], and with a combination of steady state anisotropy experiments [2]. These experiments yielded angles for the orientation of the Qy electronic transition dipole moment with respect to the x-axis varying from 70° to 90°. Using polarization resolved VIS pump - VIS probe and VIS-pump - IR-probe experiments we reinvestigated this issue [3].

References

[1]. M. Fragata, B. Nordén, T. Kurucsev, Photochemistry and Photobiology *47* (1988) 133–143
[2]. M.A.M.J. van Zandvoort, D. Wróbel, P. Lettinga, G. van Ginkel, Y.K. Levine, Photochemistry and Photobiology *62* (1995) 299–308
[3]. V.V. Nechaev, K.V. Berezin, Molecular Spectroscopy *96* (2004) 251–254

862-Pos Photocromic Intrinsic Fluorescent Proteins: a Raman Study of the Cromophore States

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Board B707

Intrinsically fluorescent proteins (IFPs) of the green fluorescent protein family are extensively used in molecular and cellular biology as genetically encoded fluorescent markers for monitoring protein dynamics and interactions. Specific mutations make it possible to tailor the protein structure and consequently their chemical and photophysical properties[1–3]. Raman spectroscopy is a powerful method to investigate selectively conformational changes in active domains of these proteins. Indeed by exciting under pre-resonance conditions it is possible to measure the vibrational spectrum of the chromophore without the need of crystallization. This is extremely helpful to enable rational protein engineering. Moreover, Raman is a non-destructive technique that allows one to monitor on-the-flow the products of photoconversion.

We will discuss the use of this technique for studying photochromism in two cases: a blue variant with highly-stable states, and a green mutant whose photochromism is fully reversible with negligible loss of active protein. Theoretical and experimental results on chemically synthesized model chromophores under different protonation and/or isomerization states will be presented: a very good agreement with calculations based on time-dependent density functional theory will be shown. This will allow us to clarify the nature of the detected vibrational modes and to link the latter to the different ground state configurations. Based on this knowledge, we shall discuss the chromophore state when in the protein. These results allow us to discriminate between the effect of *cis-trans* isomerization and of different protonation of the chromophore in the photoproducts of these proteins.

References

References:[1]. R. Bizzarri *et al.*, *Biochemistry* ***46***, 5494 (2007).
[2]. D. Arosio *et al.*, *Biophys. J.*, ***93***, 232 (2007).
[3]. S. Habuchi *et al.*, *J. Am. Chem. Soc.* ***127***, 8977 (2005)

863-Pos Robust measurements of water using Wavelength Modulation Spectroscopy(WMS)

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Board B708

Based on the Wavelength Modulation spectroscopy(WMS) and direct detection schemes, we developed a robust measurement scheme which can precisely detect water vapor concentration independent of the scattering due to the dust inside the observing humidity chamber. The relative water vapor concentration was measured by use of 938nm Distributed Feed-Back Diode Laser at different temperature using wavelength modulation technique. These relative water vapor concentration is calibrated to absolute water vapor concentration and we confirmed that the experimental results are consistent for those of different temperature. Our measurement system gives the same water concentration in any case of high or low scattering due to the non-water dust inside the observing humidity chamber.

864-Pos Vibrational Dynamics, Mode Coupling, and Anisotropy of Tyrosine Side Chains in Peptides

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Board B709

Through noncovalent interactions, aromatic side chains of Phe, Trp and Tyr play an important role in many biophysical processes such as molecular recognition, and folding and stabilization of proteins. Detailed knowledge of ultrafast side chain dynamics and interactions will contribute to fundamental understandings of these biophysical and chemical processes. To this end, we have investigated vibrational energy relaxation, mode coupling, and anisotropy of the tyrosine side chains in tyrosine and tyrosine-containing peptides using femtosecond infrared transient grating and two-dimensional infrared (2D IR) spectroscopy. Two tyrosine ring modes (mostly C=C stretch motion localized on the phenol ring) around 1515 and 1615 cm^{-1} as well as amide-I and -II modes are examined. The lower frequency ring mode is used to measure the anisotropy of the phenol ring, which reflects overall rotational diffusion of the tyrosine side chain and fast orientational fluctuations, on a picosecond time scale. Vibrational mode coupling between two tyrosine ring modes is clearly seen in the 2D IR spectra. Intramolecular vibrational energy transfer among tyrosine ring modes and amide-I modes is also observed in the 2D IR spectra with different waiting times. Anisotropy, mode coupling and energy transfer measured for different tyrosine peptides are compared. Spectral diffusion of tyrosine ring modes is investigated in neat solvents and membranes to reveal the influence of local environments on side chain conformational fluctuations.

Monday, February 4, 2008

Symposium 7: Translation and the Translocon

865-Symp Sec61-Mediated Membrane Protein Integration

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Aquaporin water channels comprise a conserved family of six-spanning homotetrameric membrane proteins that utilize at least two distinct pathways for ER membrane insertion. Protease protec-