These results clearly demonstrate the role of *in vitro* NMR spectroscopy in differentiating the biochemical differences in non-involved and malignant breast tissues which could be used for characterization of breast malignancy as an adjunct to histopathology.

748-Pos 3D solution structure of the C-terminal Chromodomain of the Chloroplast Signal Recognition Particle

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Board B592

Chloroplasts use chloroplast signal recognition particle (cpSRP) pathway to import important cargo like light harvesting chlorophyll protein (LHCP). cpSRP is unique among SRPs in being devoid of RNA. cpSRP consists of an evolutionarily conserved 54-kDa subunit (cpSRP54) and an unique 43-kDa subunit (cpSRP43). cpSRP43 subunit has four-ankyrin repeat domain at the N terminus and a C-terminal chromo domain (CD). The C-terminal CD of cpSRP43 has been shown to provide interaction sites for the cpSRP54 subunit. In addition, the chromodomain in the cpSRP43 subunit is also believed to be important for the formation of the transit complex with LHCP. In this context, we embarked on the structural characterization of the C-terminal CD using a variety of biophysical techniques including multidimensional NMR spectroscopy. Far UV circular dichroism spectrum of CD shows that the backbone of the protein is predominantly in the helical conformation. ^{1}H-^{15}N HSQC spectrum of CD is well- dispersed suggesting that the protein is structured. Complete resonance assignments (^{1}H, ^{15}N and ^{13}C) in CD have been accomplished using a variety of triple resonance experiments. Chemical shift index plots show that CD is an $\alpha + \beta$ protein. A detailed analysis of the three-dimensional solution structure of CD will be presented. The three-dimensional solution structure of CD provides valuable insights into the molecular mechanism underlying the post-translational transport and integration of LHCP on the thylakoid membrane.

748.01-Pos S100A1 Binds The Calmodulin Binding Site Of RyR1 And Positively Regulates EC Coupling In Skeletal Muscle: Structural Studies

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Board B593

S100A1, a small acidic Ca^{2+} binding protein, is an enhancer of cardiac contractility and a potential therapeutic agent for the treatment of cardiomyopathy. However the precise molecular mechanism underlying S100A1 modulation of sarcoplasmic Ca^{2+} release in striated muscle has not been fully elucidated. Here, we show structural interactions between S100A1 and RyR that underlie a physiological role of S100A1 in excitation contraction coupling in skeletal muscle. We show that the absence of S100A1 leads to depressed sarcoplasmic reticulum Ca^{2+} release following electrical excitation in murine FDB fibers. Through competition assays and fluorescence experiments we identify a novel S100A1 interaction site on the cytoplasmic face of the intact ryanodine receptor that is conserved throughout striated muscle and corresponds to a previously identified calmodulin binding site. Using a 12-mer peptide of this putative binding domain, we demonstrate low micromolar binding affinity to S100A1. NMR spectroscopy reveals this peptide binds within the Ca^{2+} dependent hydrophobic pocket of S100A1. Here we present the NOE- and RDC-based solution structure of S100A1 bound to this peptide, termed RyRP12.

Solid-State NMR

749-Pos Oxidative Modification Of Histidine Residues In Cu,znsod Induced By Bicarbonate-stimulated Thiol Oxidase And Peroxidase Activities: *ENDOR, Pulsed EPR And NMR Studies.*

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Board B594

Increasing evidence suggests that elevated oxidative stress in *sporadic* ALS (90–95%) and *familial* ALS (5–10%) causes extensive damage to several proteins, including both *wild type* and *fals*-mutant SOD1. The histidine-rich SOD1 is prone to undergo oxidative damage both at the active site and also away from it. Many histidine mutants, *viz*., H43R, H46R, H48Q, H80R *etc* are associated with ALS pathogenesis. In this study, we investigated the oxidative modifications induced by peroxidase and thiol oxidase activities of SOD1 using ENDOR, pulsed EPR and NMR spectroscopy. ENDOR and ESEEM/HYSCORE of SOD1 treated with H_2O_2/Cys in the absence of HCO_3^- revealed that the coordinated nitrogens and distal nitrogens of the His-46 and His-48 at the Cu(II) active site were oxidized; these modifications were absent in the presence of bicarbonate. Additionally, 1D NMR and 2D-NOESY were also used to investigate the oxidative damage at the Zn(II) and Cu(II) active sites as well as at histidines away from the active site. Results indicate that during SOD1 treatment with H_2O_2/Cys in the absence of HCO_3^-, both exchangeable and non-exchangeable protons were affected. Both His-46 and His-48 of Cu(II) active site residues were totally oxidized based on the disappearance of NOESY cross peaks between CH and NH of the imidazole rings. The His-71 of Zn(II) site, closer to His-46, was also damaged. However the presence of

bicarbonate protected the active site His residues. Instead, resonances assigned to His-43 residue, away from the Cu(II) site, were completely eliminated during both bicarbonate stimulated peroxidase and thiol oxidase activities. Plausible mechanisms for selective oxidation of histidines in SOD1 in the presence and absence of bicarbonate will be presented.

750-Pos Following Fungal Melanin Biosynthesis with Solid-state NMR: molecular structures and connections to cell-wall polysaccharides

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Board B595

Melanins serve a variety of protective functions in plants and animals, and in fungi such as *Cryptococcus neoformans* they are also associated with virulence. Despite their importance, the chemical structures of these pigments have remained elusive because of their insolubility and amorphous nature. We report an investigation of the molecular species involved in fungal melanin biosynthesis using a recently developed solid-state NMR strategy, including incorporation of obligatory exogenous precursors enriched with ^{13}C at particular sites followed by spectroscopy of both powdered and solvent-swelled melanin ghosts. The sidechain of an L-dopa precursor was shown to cyclize and form a proposed indole structure in *C. neoformans* melanin, and modification of the aromatic rings revealed possible patterns of chain elongation and cross-linking within the biopolymer. Mannose supplied to the growth medium was retained as a β-pyranose moiety in the melanin ghosts even after exhaustive degradative and dialysis treatment, suggesting direct incorporation into the polysaccharide fungal cell walls and covalent binding to the pigment. In contrast, glucose was scrambled metabolically and incorporated into both polysaccharide cell walls and aliphatic chains present in the melanin ghosts, consistent with a metabolic role as a cellular nutrient as well as covalent attachment to the pigment of interest. The prominent aliphatic groups reported previously in several fungal melanins were identified as triglyceride structures that may have one or more sites of chain unsaturation. These results establish that *C. neoformans* melanin contains chemical constituents derived from sources other than L-dopa polymerization and suggest that covalent linkages may attach L-dopa-derived products to the cell-wall polysaccharide components.

751-Pos Characterization of Vancomycin-Susceptible Enterococci

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Board B596

Solid-state NMR has been used to characterize the peptidoglycan structure of intact whole cells in vancomycin-susceptible Enterococcus faecium (ATTC 49624). The organisms were grown in a defined media containing 13C and 15N labels of specific amino acids incorporated into various positions of the pentapeptide stem of the cell wall. Rotational-echo double resonance (REDOR) NMR experiments have been used to measure the percentage of cross-links and bridge-links as 48% and 61% respectively. In addition the number of stems terminating in D-ala-D-ala has been determined to be 7%, in contrast with a 46% value observed in another major Gram-positive pathogen, S. aureus. This in comparison suggests that carboxypeptidase is highly active even though the organism is vancomycin-susceptible, which is contrary to the notion that the level of carboxypeptidase activity determines the extent of glycopeptide-resistance in E. faecium. Since the degree of bridge-links, cross-links, and pentapeptide stems significantly differ from those of S. aureus, it is likely that the mode of action of vancomycin and its analogues is affected. The lower level of D-ala-D-ala in E. faecium means that vancomycin has fewer binding sites in the cell wall, allowing more to pass through to the bacterial membrane and target full stems associated with nascent peptidoglycan critical to peptidoglycan biosynthesis.

752-Pos Solid State NMR Study of DNA packaging in bacteriophage T4

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Board B597

In the late lytic cycle of virulent bacteriophage T4, phage DNA is packaged to fit in the protein capsid . When fully extended, the phage T4 DNA molecule, with about 170,000 base pairs, is 54 micrometers long. The capsid is about 80 nm in diameter and 100 nm in length. Both counterions and capsid proteins are hypothesized to compensate the charge-charge repulsions between the negatively charged tightly packed DNA phosphate groups. We have used both uniform ^{15}N labeling and 6-^{15}N-lysine labeling of bacteriophage T4 to determine the extent of phosphate charge balance by proteins and polyamines. $^{31}P\{^{15}N\}$ rotational-echo double resonance (REDOR) solid state NMR shows that packaged DNA has a B-form conformation and that both lysine-rich proteins and polyamines are in close contact with DNA phosphate groups. The distance between the nitrogen atoms of nearest-neighbor lysine side-chains and the DNA phosphate groups is 3.5 Å, which implies that the interaction is via hydrogen bonding. REDOR-determined distances between DNA phosphate groups and amino-nitrogens of polyamine are 3.5 Å and 5.5 Å. This result suggests that some amino groups of polyamine provide full charge balance while others are more distant and provide partial charge balance.

753-Pos Solid-state Nmr Study Of The Membrane Interaction Of A 21-mer Cytotoxic Model Peptide

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Board B598

We have investigated the membrane interaction of a 21-mer cytotoxic model peptide that acts as an ionic channel by solid-state NMR spectroscopy. The amphipathic helical peptide is an oligomer of a repeating unit of leucines and phenylalanines bearing crown ethers. To shed light on its mechanism of membrane perturbation, we have performed ^{31}P and ^{2}H NMR experiments on the 21-mer peptide-containing bicelles composed of DHPC and DMPC or DPPC. ^{31}P NMR results indicate that the 21-mer peptide stabilizes the bicelle structure and orientation in the magnetic field, and perturbs the lipid polar headgroup conformation. On the other hand, ^{2}H NMR spectra reveal that the 21-mer peptide orders both the DMPC and DPPC acyl chains upon binding. ^{15}N NMR experiments performed in DMPC bilayers stacked between glass plates also reveal that the 21-mer peptide remains at the bilayer surface. ^{15}N NMR experiments in perpendicular DMPC bicelles indicate that the 21-mer peptide does not show a circular orientational distribution in the bicelle planar region. Finally, ^{13}C NMR experiments were used to study the 21-mer peptide dynamics in DMPC multilamellar vesicles. By analysing the ^{13}CO spinning sidebands, the results show that the 21-mer peptide is immobilized upon membrane binding. In light of these results, we propose a model of membrane interaction for the 21- mer peptide where it lays at the bilayer surface, which is the energetically most favourable state, and perturbs the lipid headgroup conformation.

754-Pos Diving into the Spectral Depths: Torsion angle mapping as a probe for transmembrane helix uniformity

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Board B599

The hydrocarbon core of lipid bilayers provides a low dielectric environment that dramatically influences the molecular interactions within membrane proteins and stabilizes transmembrane helical structures in comparison to helices within water soluble globular proteins. Through an examination of integral membrane protein crystal structures and solid state NMR PISEMA data and simulations we describe the biophysical foundations for the remarkable uniformity of transmembrane helices that results from the distinct molecular interactions within lipid bilayers. In fact, the characteristic uniformity of transmembrane helices leads to unique spectroscopic opportunities allowing for ϕ,ψ torsion angles to be mapped directly onto solid state NMR PISEMA spectra. Results from spectral simulations, solid state NMR PISEMA data, the solid state NMR structure of the M2 proton channel transmembrane domain from Influenza A, and high resolution crystal structures of 27 integral membrane proteins demonstrate that transmembrane helices tend to be more uniform than previously thought. The results are discussed through the definition of a preferred range of backbone ϕ,ψ torsion angles for transmembrane α-helices and presented with respect to improving biophysical characterizations of integral membrane proteins.

755-Pos Probing Molecular Interactions In Biological Membranes By Solid-state NMR

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Board B600

The interaction between integral membrane proteins and the lipid bilayer is vital in the regulation of many biological processes. Here we report preliminary solid-state NMR studies on two systems where interactions at the lipid/protein interface play an important role in the regulation and trafficking of integral membrane proteins.

The anaesthetic octanol has been proposed to mediate its effects on the nicotinic acetylcholine receptor (nAChR) through interactions that may occur at the lipid/protein interface. To characterize the nature of these putative interactions we are currently developing solid-state NMR methods which will enable us to map out the interaction between octanol and the receptor at the lipid/protein interface. Preliminary ^{1}H magic angle spinning NMR studies in conjunction with molecular dynamics simulations have enabled us to localize the octanol within the lipid bilayers and these methods are currently being applied to nAChR enriched membranes.

The interaction of proteins with their lipid environment has been proposed to play an important role in protein trafficking within the cell. Here, we are investigating how the lipid bilayer affects Fukutin, a protein implicated in Fukuyama muscular dystrophy, and the role lipid/protein interactions may have on trafficking in-vivo. Early work has lead to the establishment of an expression system for the trans-membrane domain of the protein, and work is underway to characterize the conformation of the protein in a range of lipid environments.

756-Pos Binding Interface of Phospholamban and Ca2+-ATPase (SERCA) in Lipid Bilayers by Solid-State NMR

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Board B601

Phospholamban (PLN), a 52-residue integral membrane protein, resides in the sarcoplasmic reticulum (SR) of cardiac myocytes and inhibits the SR Ca-ATPase (SERCA).

Although PLN is in equilibrium between a monomeric and pentameric form, the monomer is responsible for SERCA inhibi-

tion. Using a monomeric PLN mutant (C36A, C41F, C46A), we have reconstituted PLN in the presence and absence of SERCA in 4/1 DOPC/DOPE lipid bilayers, conditions in which the ATPase is fully functional. We have studied this interaction using both oriented and magic-angle-spinning (MAS) NMR spectroscopy.

For oriented solid-state NMR, we performed the (^{1}H,^{15}N) 2D PISEMA experiment, which correlates an anisotropic ^{15}N chemical shift with a ^{1}H-^{15}N dipolar coupling, allowing the determination of structure and topology within fully fluid lipid bilayers. In the absence of SERCA, we report the structure of PLN to be *L-shaped* where the cytoplasmic domain helix is in contact with the surface of the lipid bilayer. In the presence of SERCA, we report subtle topological changes for the transmembrane and cytoplasmic domains, but important changes in PLN dynamics upon binding the ATPase.

In addition, we also reconstituted PLN and SERCA in multilamellar vesicles and performed MAS experiments to monitor changes in secondary structure upon binding SERCA ($^{13}C_\alpha$ and $^{13}C_\beta$ chemical shifts). Mn^{2+} dephasing experiments were also used to ascertain the insertion depth of PLN in the presence and absence of SERCA.

All of our results indicate that in the absence of SERCA, PLN assumes an *L-shaped* geometry, in which the cytoplasmic helix is in contact with the surface of the lipid bilayer. While the *L-shaped* geometry is not significantly perturbed upon binding SERCA, there is a substantial change in PLN dynamics and side chain interactions.

757-Pos The Effect of C-terminal of Surfactant Protein B (SP-B) on Orientated Lipid, Characterized by Solid State 2H-, and 31P-NMR

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Board B602

Interactions between lung surfactant protein B (SP-B) and lipids play a critical role in lung surfactant, which is itself essential for life. A helical fragment of SP-B composed of its C-terminal 16 residues, termed SP-BCTERM, retains significant activity as compared to native SP-B. We have studied the interactions between SP-BCTERM and oriented phospholipid bilayers using 2H and 31P solid-state NMR. From the magnitude of deuterium quadrupole couple splittings of the 2H-NMR spectra, it appears that SP-BCTERM affects the dynamic motion of orientated lipid bilayers, even as low as 1.0 % (by weight) of SP-BCTERM added. Due to the positive charge of SP-BCTERM, the interaction between protein and lipid bilayers is expected to be strongly dependent on the electrical charge of lipids. The 2H NMR spectra indicate that the effect of SP-BCTERM on the orientation of zwitterionic 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) is greater than on negatively charged 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-rac-1-glycerol (POPG). In the absence of SP-BCTERM, the 31P spectra show that the phospholipid bilayers are well aligned. When SP-BCTERM is added, bilayer lipid order and orientation are perturbed and fragmented lipid structures are formed

758-Pos Orientation of Membrane Bound Peptides Studied with Solid State NMR

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Board B603

The tilt angles of transmembrane model peptides have been subject of debate; especially has MD simulations given larger tilt angles for WALP model peptides than previously published values calculated from ^{2}H-NMR. We show that the values calculated from ^{2}H-NMR data depend on the model used and for large rotational motion around the peptide, tilt angles close to those from MD simulations can be obtained. PISEMA NMR experiments on ^{15}N-labeled WLP peptides are performed to resolve the ambiguity.

The influence of the dynamics model used on the calculated orientation from ^{2}H-NMR data on 3,3,3-^{2}H-alanine labeled WALP and PGLa peptides is investigated by comparing models of increasing complexity. In the simplest model only a tilt angle (τ) and an azimuthal angle (ρ) are used. Considerably improved fits are obtained when dynamics in included as an order parameter which scales all data with a factor between 0 and 1. To better model peptide mobility, additional parameters were used.

(i) Peptide mobility around the peptide axis was modeled with a Gaussian distribution of ρ angles, centered at ρ_0 and with width σ_ρ.
(ii) A corresponding distribution of τ angles was introduced.
(iii) Simultaneous distributions of both τ and ρ were considered.

From calculations using these models on ^{2}H-NMR data, it was found that there is an ambiguity of the tilt angle of peptides in a transmembrane orientation when a distribution of azimuthal angles is introduced. This is explained by the form of the helical wave being the same for different sets of parameters. The ambiguity is not seen for peptides in a surface-bound or slightly tilted state, where the orientation is well-defined independent of the dynamics model. A distribution of tilt angles does not give similar effects.

759-Pos M2 Proton Channel from Influenza A Virus Studied by Solution and Solid State NMR Spectroscopy

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Board B604

M2 protein of influenza A virus forms a homo-tetrameric proton channel involved in modifying virion and trans-Golgi pH for virus infection and inhibited by influenza drugs Amantadine and Riman-

tadine. The aim of this research is to study the correlation of structure and function of intact M2 proton channel, implications of amphipathic helices on structural stability and mechanism of inhibition by influenza drugs. In order to obtain high resolution structural information in native environment, we have successfully expressed and purified M2 protein from E. Coli. membrane in non-denaturing mild conditions and reconstituted in a lipid bilayer. We have applied a range of NMR approaches to study the structure of M2 protein in apo state as well as complexed with amantadine. Solid State NMR experiments such as PISEMA were performed on uniform 15N labeled as well as amino acid specific labeled M2 protein reconstituted in DMPC:DHPC (Q=3.2) bicelle and DMPC: DMPG (4:1) bilayers uniformly aligned with respect to external magnetic field. 31P NMR spectroscopy has been performed on pure bicelle and bicelles containing M2 protein in order to optimize the sample preparation protocol and experimental conditions for uniform alignment in magnetic field. Solution NMR spectroscopy is performed on 15N and [2H,15N,13C] M2 protein reconstituted in detergent micelles including sequential resonance assignment and residual dipolar coupling measurements in weakly aligned samples. Preliminary results suggest that helical tilt angle of transmembrane domain with respect to bilayer normal in intact M2 protein is smaller compared to that of isolated transmembrane peptide and oligomeric state of the channel is stabilized due to interactions of amphipathic helices and soluble domains.

760-Pos Nonambiguous Orientation and Dynamics of Membrane Peptides from PISEMA NMR Spectra

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Board B605

In the smectic phase of liquid crystal lipid bilayers, embedded peptides exhibit orientational order which can be characterized by a number of spectroscopic methods. Molecular dynamics (MD) simulations suggest that such a peptide orientation is highly fluctuating and the corresponding dynamics affects experimental measures, like 2H NMR splittings (S. Esteban-Martin and J. Salgado, Biophys J., in press). In this work, we investigate the influence of broad distributions of the orientational parameters, tilt and polarity (self-axis rotation), of helical peptides in membranes, on 1H-15N dipolar couplings and 15N CSA. Both observables are usually registered simultaneously as 2D PISEMA spectra, where they form wheel patterns from which the tilt and self-rotation of the peptide can be determined. We calculate NMR parameters from idealized peptide systems with Gaussian fluctuations of the tilt and self-rotation (no structural fluctuations allowed), and find that such a peptide whole-body dynamics affects the simulated PISEMA spectra. However, as a difference with respect to the 2H NMR splittings, nonambiguous orientations can still be determined, additionally yielding the standard deviation of the corresponding orientational distributions. For reported experimental PISEMA of oligomeric peptides, a re-analysis including dynamics gives tilt and rotation angles comparable with the values from a static model, although accompanied by distributions of a small width. In contrast, theoretical spectra expected for highly dynamic monomeric peptides cannot be described with a static model, although a dynamic model provides both, the orientational parameters and the width of their distributions. Experimental measurements on the WLP23 model peptide, thought to be monomeric, are being performed to test our predictions.

761-Pos Mechanism Of Action Of The Antibiotic Lipopeptide Daptomycin

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Board B606

Daptomycin is a potent antibiotic that displays rapid bactericidal activity and has recently been approved by the FDA to treat complicated skin infections. It is a cyclic anionic tridecapeptide, with a number of D-amino acids (D-asparagine, D-alanine, and D-serine), 3 uncommon amino acid residues (ornithine, (2S,3R)-3-methyl-glutamic acid and kynurenine), and a N-terminus that is acylated with a n-decanoyl fatty acid side chain. It functions in a calcium-dependent manner.

Many aspects of daptomycin's mode of action remain to be determined. We will present some recent NMR and other biophysical data which:

1. demonstrates that daptomycin forms a micellar structure in solution when Ca^{2+} is present in a 1:1 Ca^{2+}/daptomycin ratio and
2. that this binding event, as well as binding to lipid membranes is not accompanied by as significant a structural change as originally postulated (Jung et al., *Chem Biol.* 2004 Jul;***11*** (7):949–57).

Through the examples shown, we will demonstrate how structure refinement and modelling can be used to obtain structural information for a system for which only a limited number of NOE restraints are available. Finally, the implication of the presented results for the mechanism of action of daptomycin will be discussed.

762-Pos Magic-Angle Spinning Solid-State NMR Spectroscopy of Nanodisc-Embedded Human CYP3A4

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Board B607

Cytochrome P450 (CYP) 3A4 contributes to the metabolism of approximately 50% of commercial drugs by oxidizing a large number of structurally diverse substrates. Like other endoplasmic reticulum-localized P450s, CYP3A4 contains a membrane-anchoring N-terminal helix and a significant number of hydrophobic

domains, important for the interaction between CYP3A4 and the membrane. Although the membrane affects specificity of CYP3A4 ligand binding, the structural details of the interaction have not been revealed so far because x-ray crystallography studies are available only for the soluble domain of CYP3A4. Here we report sample preparation and initial magic-angle spinning (MAS) solid-state NMR (SSNMR) of CYP3A4 (Δ3–12) embedded in a nanoscale membrane bilayer, or Nanodisc. The growth protocol yields ~2.5 mg of the enzymatically active, uniformly 13C, 15N-enriched CYP3A4 from a liter of growth medium. Polyethylene glycol 3350-precipitated CYP3A4 in Nanodiscs yields spectra of high resolution and sensitivity, consistent with a folded, homogeneous protein. CYP3A4 in Nanodiscs remains enzymatically active throughout the precipitation protocol as monitored by bromocriptine binding. The 13C line widths measured from 13C-13C 2D chemical shift correlation spectra are ~0.5 ppm. The secondary structure distribution within several amino acid types determined from 13C chemical shifts is consistent with the ligand-free x-ray structures. These results demonstrate that MAS SSNMR can be performed on Nanodisc-embedded membrane proteins in a folded, active state. The combination of SSNMR and Nanodisc methodologies opens up new possibilities for obtaining structural information on CYP3A4 and other integral membrane proteins with full retention of functionality.

Imaging and Optical Microscopy - I

763-Pos Optical nanometer scale gap sensing based on surface Plasmon Resonance (SPR)

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Board B608

Surface plasmon resonance (SPR) can be used to measure the gap distance of thin film, due to its highly sensitive optical properties. SPR is an effective method of detecting the gap distance between the sample and the substrate surface. In this study, we introduce a technique for detecting a nanometer scale gap distance based on SPR. Depending on the gap distance between the sample and the substrate surface, reflectivity is investigated as a function of the incident angle of optical radiation around the SPR angle, which is related to the resolution limit and the propagation length of the plasmons. This method can facilitate the study of searching the mechanism for the contact configuration between a living cell and a substrate surface in vitro and in real time.

764-Pos Precision of Localization Methods for Individual Fluorescent Probes

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Board B609

A fluorescent probe observed through a microscope appears as a diffraction limited spot. It is well known that the Rayleigh criterion is too conservative for determining microscope resolution. The center of a spot can be estimated with a precision that increases infinitely with the number of photons producing the spot; see [Ober et al., 2004 Biophys. J., *86*: 1185–1200; Michalet X., Weiss S., 2006 Proc. Natl. Acad. Sci. USA, *103*: 4797–4798] and references therein. In practice the finite supply of photons limits this precision. So does the choice of statistical estimator. We discuss the relative virtues of estimators and demonstrate the differences experimentally. A popular estimator employs a least-squares fit of a 2D Gaussian to the photon distribution in the image of a spot [Thompson et al., 2002 Biophys. J., *82*: 2775–2783]. We demonstrate that the experimentally observed point-spread function neither is a 2D Gaussian nor the classical Airy point-spread function. We give the theory for the correct function. Then we show that the maximum likelihood estimator based on the experimentally correct point-spread function has only half the variance of the 2D Gaussian estimator, i.e., it is twice as efficient in its use of available photons.

765-Pos Study on the Quantitative Classification for Exfoliate Cells of Lung Cancer in Sputum Smears Stained by Pap Test

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Board B610

Aim: to quantitatively discriminate the lung cancer cells from normal cells in sputum smear stained by Pap test.

Methods: 160 cases sputum samples were collected, include 60 normal cases consisted of 360 cells of columnar and squamous epithelium cells and histiocytes, 100 lung cancer cases consisted of 592 cells from 30 cases of squamous cell carcinoma (SqC), 30 adenocarcinoma (AC), 30 small cell carcinoma (SCC), and 10 large cell carcinoma (LCC). The sputum were smeared and stained by Pap test. Technique of compute image analysis was applied to test the chromatics parameters of R, G, and B and their coefficient, to test morphometry parameters of the cells and the nuclears. Discriminating analysis was done to establish the discriminants.

Results: The test results to the parameter of chromatics and morphometry were given. And the discriminated functions to discriminate the lung cancer cells from normal cells, to discriminate the different subtypes of lung cancer cells, to discriminate the different normal cells were set up based on the parameter of