**Board B589**

The electrophysiological and oligomeric properties of active Sec61 complexes in the endoplasmic reticulum (ER) membrane of canine pancreas- and yeast-cells were investigated by the planar bilayer technique and with high resolution confocal fluorescence laser spectroscopy in artificial horizontal bilayers. After activation of the Sec61 complexes by various substrate polypeptides, displayed a large variety of open channel states with two regimes of transient channel openings. These corresponded to internal mean pore diameters of 1.2 and 2.2 nm. The various substrate polypeptides determined the respective pore size distribution and selectivity of the channel. Thus the Sec61 complex contains a highly dynamic substrate-activated channel that, once active, fluctuates between two distinct conformations. In the one state small pores exist, in the other state, a single channel pore with roughly doubled pore size is formed. The size of this channel pores are only compatible with an oligomeric structure of the Sec61 complex. This is in line with the FIDA (fluorescence intensity distribution analysis) of the labeled Sec61 complex in artificial horizontal bilayers which indicated that the tetrameric state of the heterotrimer constituted the most prominent oligomeric fraction of the complex. In addition, the channel is regulated by ribosomes on the cytosolic face and calcium-calmodulin and by BiP on the lumenal face of the membrane. We propose that these interactions are important factors of the regulation in preventing the uncontrolled efflux of calcium and other small ions or non charged solutes from this calcium storage compartment.

**Solution NMR**

## 746-Pos Study of Biochemistry of Muscle Disorders using *in-vitro* Proton NMR Spectroscopy

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**Board B590**

The metabolism in muscle disorders was investigated using *in vitro* proton NMR spectroscopy. Muscle tissues were obtained by open surgical biopsy procedure under regional anesthesia from patients with Mitochondrial Myopathy (MM), Duchenne Muscular Dystrophy (DMD), Limb Girdle Muscular Dystrophy (LGMD) and controls (from orthopaedic surgery). Histological and histochemical methods were used for diagnosis of myopathy. Perchloric acid extracts of muscle tissues were prepared and various 1D and 2D NMR experiments were carried out for assignment of the different metabolite resonances and concentration of metabolites was determined.

Significantly higher levels of Lac, Gln and Ala was observed in MM patients compared to controls which could primarily be due to

(i) accelerated rate of anaerobic glycolysis to maintain ATP concentrations resulting in high Lac, and
(ii) defective oxidative metabolism due to mitochondrion abnormalities.

Higher concentrations of Gln and Ala indicate their utilization to meet the additional glucose requirement of cells through gluconeogenic pathway in MM. However, patients with LGMD and DMD showed lower concentration of Lac compared to controls indicating the reduced rate of glycolysis and energy deficit in these patients. The concentration of Ala, Glu+Gln, GPC and Cho was also lower in LGMD and DMD patients suggesting altered metabolism and degeneration of muscle tissue. Between patients of various muscle disorders, significantly reduced concentration of Lac, Glu+Gln and GPC was observed in LGMD, DMD patients compared to patients with MM indicating underlying metabolic differences in these muscle disorders.

Present results demonstrate the potential usefulness of *in vitro* MRS in understanding the metabolism of muscle disorders and indicate the role of *in vitro* MRS to complement the histological methods in the diagnosis of various muscle disorders.

## 747-Pos Biochemical Characterization of Involved and Non-involved Tissue from Breast Cancer Patients using *in vitro* NMR Spectroscopy

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**Board B591**

Breast cancer is the commonest cancer among women worldwide. Results of the comprehensive biochemical characterization of malignant breast tissue and its comparison with the non-involved tissue to determine biomarkers for diagnosis of breast cancer and to understand the metabolism are presented. Malignant (n=15) and non-involved (n=22) breast tissue were surgically obtained from breast cancer patients who underwent either modified radical mastectomy or breast conservation surgery. Histopathological evaluation of tissue was carried out to determine malignancy. 1D and 2D proton MR spectra of the perchloric acid extract of the lymph nodes were recorded at 400 MHz. Forty metabolites including amino acids, organic acids, carbohydrates and membrane components were assigned and absolute concentration of 14 metabolites were determined. Kruskal-Wallis and Mann-Whitney U test were used to compare the concentration between malignant and non-involved tissue.

Malignant tissues showed significantly higher concentration of Lac, Ala, Lys, Ace, Glu, Gln, PCr/Cr, Cho, GPC, mI, Tyr and Phe in comparison to the non-involved tissues. The increase in the concentration was of the order of 5-10 times in ceratin metabolites. Higher concentration of Ala, Gln, Glu and Lys are attributed to increased protein synthesis in fast growing tumor cells, as amino acids serves as building blocks for proteins. Higher concentration of Lac could be due to high glycolytic activity in tumor cells. Higher levels of GPC and Cho characterize the rapidly proliferating nature of tumor cells.

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