storage, and release of the neurotransmitter acetylcholine and its postsynaptic activation of the nicotinic acetylcholine receptor. Many lines of evidence support the idea that in addition to acetylcholine, additional neurotransmitters and/or neuromodulators are also released from cholinergic synapses. Utilizing a single-vesicle imaging approach, we have found that individual synaptic vesicles from the electric ray possess neurotransmitter transporters for glutamate, ATP, and acetylcholine. In addition to those transporters, cholinergic synaptic vesicles from the electric ray also possess an orphaned transporter from the bile acid transport family, SLC10A4. We are now expanding our research to identify the molecules transported by SLC10A4 using multiple biochemical approaches and a mouse knockout model.

Magnetic Resonance Spectroscopy, Imaging, and EPR Spectroscopy

3101-Pos Board B531

Multi-Component Water Dynamics and Exchange in Brain Cortical Tissue Probed via In-Vitro D-T2 2D Correlation NMR

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Water self-diffusion in brain cortical tissue has been shown to have two components by diffusion NMR measurement. This phenomenon is tissue microstructure-related and is essential for understanding the diffusion NMR signals' changes associated with some physiological processes, such as acute stroke, neuronal activation, etc. However, the biophysical mechanisms explaining multi-component diffusion inside cortical tissue and possible exchange among components are still poorly understood.

Here we propose using a Diffusion (D) – Transverse Relaxation (T_2) 2D correlation NMR method to characterize the multi-component water dynamics in the biological tissue. In the first dimension, the diffusion is measured by a spin echo in a strong static magnetic field gradient, which can efficiently shorten the diffusion time to be less than 1.6ms, making the effect of exchange negligible. In the second dimension, a CPMG pulse sequence is used to measure the T_2 of each diffusion component. Here, exchange will affect the NMR signal evolution during this long T_2 encoding period. In this 2D D-T_2 NMR method, each dynamics-different water component can be characterized by its specific (D, T_2) combination and the exchange process among different components can also be quantified simultaneously. Another unique aspect of this work is that our NMR data is obtained using a single-sided NMR system with permanent magnet.

Furthermore, temperature-dependent D-T_2 experiments were performed on cortical tissue from fixed rat brain. The self-diffusion constant of both the fast and slow diffusion components and the exchange between them are slowed as the temperature decreases, whereas the slow diffusion component shows higher translational activation energy. More interesting and surprising, the relative fraction of each component is also observed to have a strong dependence on the temperature, which indicates this bi-component diffusion is diffusion-distance dependent.

3102-Pos Board B532

Probing the Secondary Structure of Membrane Proteins with the Pulsed EPR Technique: Electron Spin Ehco Envelope Modulation (ESEEM)

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Despite the importance and large number of membrane proteins, relatively limited structural information is known about them. New biophysical techniques are needed to probe their structural properties. Their hydrophobic nature and low expression yields cause difficulties for traditional structural techniques such as x-ray crystallography and solution NMR. ESEEM spectroscopy indirectly observes NMR transitions through an electron spin coupled to a nearby NMR active nucleus. ESEEM can detect weak dipolar interactions between a NMR active nucleus and a spin label out to a distance of approximately 8 Å. The modulation depth for weakly coupled nuclei is scaled by $1/r^6$. A novel approach is being developed to probe the secondary structure of membrane proteins and peptides qualitatively utilizing the three-pulse Electron Spin Echo Envelope Modulation (ESEEM) pulse sequence. In order to demonstrate the practicality of this biophysical technique, the M2delta subunit of AChR (α-helical) and KIGAKI(β-sheet) peptides were incorporated into phospholipids bicelle to probe their secondary structure with ESSEM spectroscopy. Utilizing site-directed spin-labeling (SDSL) coupled with deuterated amino acid labeling of the peptides, the corresponding ESEEM spectra reveal characteristic patterns for α-helix and β-sheet structures. This ESSEM secondary structural approach can be used with different deuterated amino acids and provide pertinent qualitative structural information on membrane proteins in a short period of time (10 minutes) with small amounts of sample (30 μg).

3103-Pos Board B533

Spin-Labeled Uni-Lamellar Vesicles as an Oxygen Sensitive Analyte for Measurement of Cellular Respiration using Rat Dopaminergic Neuronal Cells

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Small uni-lamellar vesicles were prepared from 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) with 1% spin label of 1-palmitoyl-2-(16-doxylstearoyl) phosphatidylcholine (16-PC). POPC membranes are in the fluid phase in the physiological temperature range, which ensures high solubility of oxygen in the membrane center where the 16-PC nitroxide moiety is located. The nitroxide moiety is isolated from cellular reductants and paramagnetic ions that might interfere with spin-label oximetry measurements. This analyte is stable in cell suspensions and can be used in studies as long as 400 min. The saturation recovery EPR approach, possibly for the first time, was used to study cellular respiration of rat dopaminergic neuronal cells. It is widely accepted that this approach is the most direct way to carry out oximetric studies. The preliminary experiments were carried out at X-band for closed chamber geometry. Oximetric measurements were performed for cell concentrations from 750 to 5000 cells in one μL of culture media. Results confirmed that the oxygen consumption rate was linearly proportional to the number of cells. Furthermore at the lower cell concentration, we found a breaking point in the rate which is not due to diffusion limitation but is due to the change in cell metabolism because there is not enough oxygen, consistent with Michaelis-Menten kinetics. In the developed analyte, the same hydrocarbon environment, which dissolves oxygen very well, always surrounds nitroxide moieties of spin-label molecules. Therefore, the partial pressure of oxygen influence the EPR spectrum of spin-labels in the analyte. Such microscopic analytes are readily and uniformly distributed within the sample, thus giving a rapid response to changes in oxygen partial pressure. The improved oximetry methodology developed here presents a high impact opportunity for non-invasive assessment of disease mechanisms and response to therapeutic intervention.

3104-Pos Board B534

Probing the Protein-Protein Interactions between KCNQ1 and KCNE1 using Electron Paramagnetic Resonance (EPR) Spectroscopy

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KCNQ1 is a membrane-bound protein composed of 676 amino acids with a molecular weight of 75 kD and contains 6 transmembrane helices. KCNQ1 is a voltage-gated potassium channel primarily involved in the transport of potassium in the human heart. The activity of KCNQ1 is modulated by the integral membrane protein KNCE1 (also known as MinK). This potassium modulation is critical for repolarization in the heart, specifically in the left ventricle. Mutations in the KCNQ1 and KCNE1 genes have been linked to genetic disorders such as long QT syndrome, sudden infant death syndrome (SIDS), as well as deafness. However, most current studies between KNCQ1 and KCNE1 have been from an electrophysiological or functional standpoint, which leaves structural and dynamic information on the proteins unknown. EPR spectroscopy is a powerful structural biology tool that can be used to probe both protein structures as well as protein-protein interactions between KCNQ1 and KCNE1. Currently, we have overexpressed full length KCNE1 and the C-terminal domain of KCNQ1. In this study, we used both continuous-wave (CW) EPR and the pulsed EPR technique of double electron-electron resonance (DEER) to probe the structural and dynamic parameters of the interaction of KCNQ1 and KCNE1.

3105-Pos Board B535

Characterization of a Bifunctional Spin Label for the Structure and Dynamics of a Membrane Protein using CW-EPR Spectroscopy

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Electron Paramagnetic Resonance (EPR) spectroscopy is a powerful structural biology tool when combined with site-directed spin labeling (SDSL) to study the structure and dynamics of peptides and proteins. The most widely used spin label for SDSL is methanthiosulfonate (MTSL), however the flexibility of this spin label introduces greater uncertainties in the EPR measurements obtained for determining structure, side chain dynamics, and backbone motion of membrane protein systems. Most recently, a new bifunctional spin label (BSL) 3,4-Bis-(methanethiosulfonylmethyl)-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-1-yloxy, has been introduced to overcome some of the limitations associated with the MTSL spin label and has been invaluable in determining protein dynamics and inter-residue distances due to its restricted internal motion and fewer size restrictions. While BSL has been successful in