**909-Pos**

**Separated Local Field $^{13}C$ NMR Spectroscopy Reveals Lipid Order Fluctuations**

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Solid-state NMR and small angle X-ray scattering (SAXS) are currently among the most widely used experimental methods for investigating structural and dynamic properties of phospholipid bilayers [1]. However, SAXS measurements provide information about membrane dynamics only at very high-resolution, whereas $^{2}H$ NMR has a prerequisite of isotopic enrichment of the phospholipid. An alternative is magic-angle spinning (MAS) solid-state $^{13}C$ NMR spectroscopy, where direct magnetic-dipolar couplings and nuclear spin-lattice relaxation times are measured at natural isotopic abundance [2]. This method provides structure and dynamics for the headgroup, glycerol backbone, and acyl chains in one experiment. As proof of principle, we measured the magnetic-dipolar couplings and nuclear spin-lattice relaxation rates in the liquid-crystalline state for the homologous series of phosphatidylcholine lipids (DLPC, DMPC, DPPC). The quasi-static dipolar lineshapes exhibit segmental order parameters that can be compared with previously measured $^{2}H$ couplings. In terms of dynamics, correlation of the effective segmental order parameters with the nuclear spin-lattice relaxation rates shows that the square-law scaling for the $^{13}C$ and $^{2}H$ systems is universal. Moreover, $^{13}C$ measurements provide valuable information about the highly dynamic headgroup region that is not commonly observed in $^{2}H$ NMR experiments. Combination of solid-state $^{2}H$ and $^{13}C$ NMR methodologies provides greater insight into the structure and dynamics of membranes than would be determined from either technique alone [3]. This study establishes further precedent for the use of solid-state $^{13}C$ NMR for research into complex biological membranes and biomaterials at natural isotopic abundance. [1] H.I. Petrache and M.F. Brown (2007) *Methods in Membrane Lipids*, Humana Press, 339-351. [2] J.D. Gross *et al.* (1997) *JACS***119,** 796-802. [3] M.F. Brown and S.I. Chan, *Encyclopedia of Nuclear Magnetic Resonance*, Wiley, New York 1996, 871-885.

**910-Pos**

**Solid-State NMR Investigation of Membrane-Associated Peptides and Proteins**

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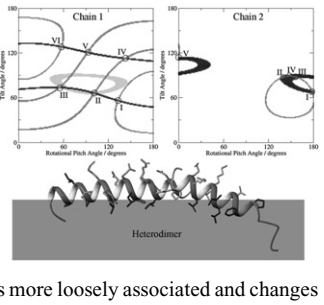
Solid-state NMR spectroscopy has a proven record during the investigation of the structure and dynamics of membrane-associated polypeptides. In particular from oriented samples considerable details on the structure and topology of the protein is obtained. A solid-state NMR approach which allows for the accurate determination of the tilt and rotational pitch angles of peptides reconstituted into uniaxially oriented membranes will be presented. Proton-decoupled 15N and 2H solid-state NMR spectroscopy have been used to characterize the tilt and rotational pitch angle of several peptides in considerable detail. Furthermore, valuable information on the rotational diffusion constants in membranes and thereby the size of peptide complexes is obtained.




Here we present solid-state NMR results that have been obtained from the heterodimeric antimicrobial peptide distinctin as well as from the two chains independently. The data indicate that upon membrane insertion the protein unfolds, that the amphipathic helix of chain 2 orients parallel to the membrane surface and stably anchors the polypeptide into the membrane. In contrast chain 1 remains more loosely associated and changes its alignment by about 5 degrees when in contact with chain 2. Figure from PNAS, 106, 16639 (2009)

**911-Pos**

**Time-Resolved Dehydration-Induced Structural Changes in An Intact Bovine Cortical Bone Revealed by Solid-State NMR Spectroscopy**

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Understanding the structure and structural changes of bone, a highly heterogeneous material with a complex hierarchical architecture, continues to be a significant challenge even for high-resolution solid-state NMR spectroscopy. While it is known that dehydration affects mechanical properties of bone by decreasing its strength and toughness, the underlying structural mechanism at atomic-level is unknown. Solid-state NMR spectroscopy, controlled dehydration, and H/D exchange are used for the first time to reveal the structural changes of an intact piece of bovine cortical bone. Proton spectra are used to monitor the dehydration of bone inside the rotor and high-resolution $^{13}C$ chemical shift spectra obtained under magic angle spinning are used evaluate the dehydration-induced conformational changes in bone. Experiments reveal the slow denaturation of collagen while the *trans*-Xaa-Pro conformation in collagen is unchanged due to dehydration. Our results suggest that GAGs in the collagen fiber and mineral interface may chelate with a $Ca^{2+}$ ion present on the surface of the mineral through sulfate or carboxylate groups. These results provide insights into the role of water molecules in the bone structure and shed light on the relationship between the structure and mechanics of bone.

**912-Pos**

**Solid-State NMR Structural Studies of Alzheimer's Disease Aβ(1-42) And Aβ(1-40) In Phospholipid Bilayers**

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Amyloid-β peptides, Aβ(1-42) and Aβ(1-40) are believed to cause loss of nerve cell function in individuals suffering from Alzheimer's disease, where evidence suggests that interaction with the cell membrane correlates strongly with cytotoxicity. Previous studies report a range of different but plausible structures, which depend on the molecular environment of the peptide. This sensitivity to sample preparation suggests that the structure relevant to disease is found in lipid bilayer membranes. While model membrane vesicles are too large to be studied by conventional solution NMR, solid-state NMR is one of the few technologies available to study such systems. We present recent magic angle spinning NMR measurements which suggest a novel peptide structure in a lipid bilayer environment: (i) rotational-echo double-resonance (REDOR) distance measurements between selectively enriched 13C-carbonyl and 15N-amide positions constrain dihedral angles of intervening residues and suggest that at least part of the Aβ(1-42) peptide folds into a β-sheet like conformation, in contrast to the helical and coiled structures in previous reports; and (ii) double quantum filtered DRAWS measurements indicate that the extended strand does not assemble into an in-register parallel sheet as reported for amyloid fibrils. Additional static NMR experiments (sensitivity enhance PISEMA) of 15N labelled Aβ(1-40) in mechanically aligned phospholipid bilayers and magnetically oriented bicelles indicate that the peptide has multiple conformations in model membranes.

**913-Pos**

**Structural Studies of a Novel Zinc Finger Domain Required for Recognition of the Cytoplasmic Polyadenylation Element Within the 3′ UTR of MRNA**

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Cytoplasmic polyadenylation element-binding protein (CPEB) plays an important role in interacting with mRNA, specifically in the translation regulation of oogenesis, cellular senescence and synaptic plasticity, by regulating poly(A) tail elongation via interactions with several other proteins. CPEB binds to mRNA at a specific region, the cytoplasmic polyadenylation element (CPE). The carboxy terminal region of CPEB contains six cysteine and two histidine residues that are conserved among all species suggesting a zinc finger structure with two zinc ions. This novel zinc finger region of CPEB is required for recognition of the uracil-rich cytoplasmic polyadenylation element within the 3′ UTR of mRNA and binding is dependent on the presence of zinc. We have purified the zinc finger domain alone and as a fusion protein with the immunoglobulin binding domain of streptococcal protein G (GB1) expressed in an E. coli host using ion exchange or metal affinity chromatography. Preliminary results of the structure determination by nuclear magnetic resonance (NMR) spectroscopy will be presented. Structural studies, in conjunction with mRNA binding assays, will lead to a better understanding of translational regulation and the regulatory role of CPEB in cellular development and synaptic plasticity.