dynamic model, including a molecular order parameter scaling factor, gives good results only for moderately mobile peptides, while for high mobility cases the correct tilt is only obtained by re-introducing the explicit Gaussian fluctuations in the fitting functions.
In contrast, ^{15}N-NMR data appear to be less sensitive to rigid-body peptide motions, and PISEMA spectra can give correct orientations even for highly mobile peptides, and assuming a static model for the analysis. The differences are due to the different orientation of the tensors of ^{2}H- and ^{19}F-labels, placed on peptide side chains, compared to the orientation of the ^{15}N tensor, placed on amide backbone groups.
We conclude that dynamics should be included in the analysis of solid-state NMR data of membrane-bound peptides. Not only does this give more accurate orientations, but it can also provide information about the dynamics of the peptide.

2105-Pos Board B75
Dynamics of Retinal Studied by ^{2}H NMR Relaxation Sheds New Light on Rhodopsin Activation
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The dynamics of retinal ^{2}H-labeled at the C5-, C9-, and C13-methyl groups have been studied by solid-state deuterium NMR relaxation in the dark, meta I, and meta II states of the G protein-coupled receptor rhodopsin. Relaxation rates and quadrupolar splittings were interpreted in terms of axial rotation and off-axial motion of the methyl groups and revealed interactions between the retinal cofactor and the rhodopsin binding pocket. Surprisingly, in the dark state the crucial C9-methyl group is the most mobile despite its role in stabilizing the polyene chain. The C5-methyl group is slowest which is most likely due to interactions with Glu122 on helix 3. Dynamics of the ligand change significantly after light absorption. However, most of the changes occur between the dark and meta I states, and can be attributed to variations in intra-retinal interactions due to isomerization. Only small changes are observed upon transition from the meta I to meta II state where activation takes place. Overall, the dynamics of the C9- and C13-methyl groups in the meta I and meta II states indicate the absence of significant steric clashes of these groups with the surrounding amino acids. Even more surprising, there is little change in mobility of the β-ionone ring upon light activation. An activation mechanism based on the relaxation data is suggested which assumes that retinal is maintained in a similar environment, and does not experience significant reorientation or displacement upon transition from the pre-activated meta I to the active meta II state.
[1] G. F. J. Salgado *et al.* (2004) *Biochemistry* **43**, 12819.
[2] G. F. J. Salgado *et al.* (2006) *JACS* **128**, 11067.
[3] A. V. Struts *et al.* (2007) *J. Mol. Biol.* **372**, 50.

2106-Pos Board B76
Water Self-Diffusion in Cell Suspensions and Tissues: New PGSE NMR Protocols for Estimating Intracellular Diffusion, the Homogeneous Length Scale, and Membrane Permeability
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Molecular transport by diffusion is a crucial process for the function of biological tissues.[1] By following the self-diffusion of molecules in a cellular system, information about structure and dynamics on the cellular scale can be obtained. PGSE NMR is a powerful method to non-invasively study molecular motion on the micrometer length scale and millisecond time scale.[2] Most of the present day PGSE NMR studies use the same basic experimental design as in the pioneering works of Stejskal and Tanner in the 60's.[3] Here we present new protocols specifically designed for estimating the diffusion in the intracellular medium and the cell membrane permeability for cell suspensions, and the length scale at which an inhomogeneous medium, such as brain tissue, start to appear homogeneous.[4] The new versions are based on a controlled use of deviations from the short gradient pulse approximation,[5] previously considered as an unwelcome experimental artifact, and multiple diffusion periods.[6] Implementation of the proposed protocols in the context of medical MRI is discussed.[7]
(1) Nicholson. *Rep. Prog. Phys.* **2001**, *64*, 815.
(2) Callaghan *Principles of Nuclear Magnetic Resonance Microscopy*; Clarendon Press: Oxford, 1991.
(3) Stejskal, Tanner. *J. Chem. Phys.* **1965**, *42*, 288.
(4) Åslund et al. *J. Phys. Chem. B* **2008**, *112*, 2782.
(5) Malmborg et al. *J. Magn. Reson.* **2006**, *180*, 280.
(6) Callaghan, Furó. *J. Chem. Phys.* **2004**, *120*, 4032.
(7) Le Bihan. *Nat. Rev. Neurosci.* **2003**, *4*, 469.

2107-Pos Board B77
Chemical Structure Effects on Bone Response to Mechanical Loading
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Using solid state NMR (SSNMR) we show that bone mineral and bone matrix both undergo measurable deformations in response to compressive loading. Using bovine cortical bone, load-induced changes in both protein conformation and mineral ion spacings are observed even under sub-physiological loads. Our finding that matrix distortion involves changes in the position of (proline) is not unexpected. Proline and hydroxyproline are the most abundant amino acids in X,Y positions of the repeat gly-X-Y unit of collagen. The local conformation is determined by enthalpic forces stabilizing hydroxyproline and hydrogen bonding stabilizing proline position. Mechanical forces would be expected to be greater, leading to some change in the local orientation. Substitution of another amino acid for glycine, as in most types of osteogenesis imperfecta and in other genetic defects, would have the effect of weakening the stabilizing forces on proline and hydroxyproline, thus allowing greater distortion of the collagen fibrils than would occur in normal bone. In turn, this weakness would contribute to the fragility of the tissue.

2108-Pos Board B78
Helicobacter Pylori: How is Adhesin BabA, a Blood Group Antigen Binding Membrane Protein, Involved in Bacterial Adherence?
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The bacterium *Helicobacter pylori* is the causative agent for peptic ulcer disease. Bacterial adherence to the human gastric epithelial lining, a prerequisite for the pathological action of H. pylori, is caused by its outer membrane proteins. One of their most prominent members is the Lewis B binding adhesin, BabA which interacts with the bloodgroup antigen carbohydrate epitopes. To elucidate the structural basis of Lewis-b antigen recognition by BabA, STD (Saturation Transfer Difference) NMR experiments enabled the specific detection of *Helicobacter*-glycan interactions by using living *Helicobacter* cell suspensions and Lewis B blood group O determinant. In the NMR spectra, one can identify several carbohydrate segments which bind to BabA. This unique setup is ideal for continuing functional analyses of fully functional BabA adhesion protein in its native environment, the bacterial outer membrane.
Further work is using combined liquid/solid state ^{31}P NMR studies to elucidate the variation in membrane lipid compounds arising from outer membrane vesicles (OMV) which the bacterium produce to deliver bacterial virulence factors. Using tailored-made solution NMR (^{1}H, ^{31}P NMR, ^{1}H-^{13}C and ^{1}H-^{31}P correlation NMR spectroscopy) we could identify and quantify various lipids as a function of strain, clinical isolates, mutants and the different membranes. We observed marked differences in the phospholipid composition between inner (IM) and outer membrane (OM) as well as vesicles (OMV).

2109-Pos Board B79
Structure-Activity Relationships in Two Antimicrobial Peptides Based on Chemokine Helical Segments: RP-1 and IL-8α
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Antimicrobial peptides are naturally occurring molecules, part of the innate immune system, and are of high interest as novel antibiotic therapeutics given the increasing resistance of microbes to conventional antibiotics. RP-1 and IL-8α are 18 and 19 amino acid synthetic peptides that were designed based on the sequence of the C-terminal helical segments of two chemokines: platelet factor-4 and interleukin-8. In order to characterize structure-activity relationships and to understand the selectivity of these peptides for bacterial membranes, NMR was used to determine high-resolution structures of both peptides in complex with SDS and DPC micelles. Additionally, solid state NMR experiments in oriented lipid bilayers were performed to assess structure and orientation in a bilayer environment and to indicate the impact of the peptide on bilayer organization. Both peptides structure as amphipathic α-helices with hydrophobic residues on one side and polar and positively charged residues on the opposite side. RP-1 shows very subtle structural differences when in complex with SDS (anionic) versus DPC (zwitterionic) micelles. This suggests that its specificity for prokaryotic versus eukaryotic membranes does not derive from peptide structural differences in the two systems, but rather from differences in the details of the peptide-lipid interactions. The ^{2}H solid state NMR data are consistent with IL-8α associating peripherally with POPC bilayers, and penetrating deeper into POPC/POPG

bilayers. Additional insight is gained from ^{31}P and ^{15}N spectra of IL-8α in these environments.

2110-Pos Board B80

The Orientation and Location of the C-terminal Helix of Surfactant Protein B in Lipid Bilayers as Studied by Solid State NMR

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The lung surfactant protein B (SP-B) is essential for life and plays a critical role in reducing surface tension in the lungs during breathing. An amphipathic helical fragment of SP-B composed of the 16 C-terminal residues, termed SP-B_{CTERM} or SP-B_{63-78}, has a charge of +3 and retains significant bioactivity compared to native SP-B. We have used ^{31}P-, ^{2}H-, ^{13}C-, and ^{15}N- solid state NMR to investigate the insertion of SP-B_{CTERM} into lipid bilayers composed of model lipids, as well as bovine lung extracted surfactant (BLES). When the external magnetic field is parallel to the normal of the oriented lipid bilayers, the ^{15}N chemical shift of the peptide backbone is at ~ 98 ppm, which corresponds to a helical axis alignment of approximately 70° relative to the lipid bilayer normal. The depth of peptide insertion in vesicles was investigated by $^{13}C\{^{31}P\}$ REDOR, as well as by measuring the ^{13}C relaxation time in the absence and presence of the paramagnetic probe Mn^{2+}.

2111-Pos Board B81

Magic Angle Spinning NMR Investigations of the Human Voltage Dependent Anion Channel

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In humans, transport of metabolites through the outer mitochondrial membrane is controlled by a 283 residue, 31 kDa pore-forming protein, the voltage-dependent anion-selective channel (VDAC). In addition to providing a main pathway for metabolite trafficking through the outer membrane, VDAC is postulated to play a critical role in cell apoptosis. VDAC interacts with the Bcl-2 family of pro-apoptotic and anti-apoptotic proteins that control the permeability of the outer membrane to apoptotic signals including the release of cytochrome c.
Recently, the solution NMR structure of human VDAC-1 (hVDAC1) reconstituted in detergent micelles has been reported by two groups, revealing a 19-stranded β-barrel with a short α helix at the N terminus. While these structures are consistent with previous sequence analysis and biophysical studies, there are indications that interactions of the protein with a lipid bilayer are essential for its proper structure as well as function.
Accordingly, we have undertaken studies on hVDAC1 reconstituted in DMPC bilayers with magic angle spinning (MAS) solid state NMR (SSNMR). hVDAC1 forms structurally homogeneous two-dimensional microcrystals as judged by high resolution $^{13}C/^{15}N$ MAS spectra. In addition, uniformly ^{13}C and ^{15}N labeled hVDAC1 in 2D DMPC cyrstals exhibit well resolved multidimensional MAS spectra that allow group assignments, structural analysis, and comparison of chemical shifts with the solution spectra.

2112-Pos Board B82

Ion Interactions of Gramicidin A using Multidimensional Proton Solid State NMR

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Gramicidin A, an enzymatic product from the bacterium *Bacillus brevis*, is a fifteen residue peptide that induces cell lysis by forming a transmembrane channel that conducts monovalent cations and water molecules into a cell. The dimer of gramicidin A forms a right-handed β-helix, creating a pore of 4 A that supports a single column of water molecules. The cation transport properties of the channel formed by the gramicidin A dimer is very similar to physiologically important channels, and therefore makes gramicidin A an attractive model to study.
A two-dimensional heteronuclear correlation (HETCOR) solid state NMR spectroscopy was used to correlate ^{1}H and ^{15}N chemical shifts and to obtain the ^{1}H-^{15}N dipolar couplings of aligned gramicidin A samples in lamellar phase lipid environments. The ^{1}H-^{15}N dipolar splittings obtained from HETCOR are not sensitive to the ^{1}H carrier frequency. The additional ^{1}H chemical shift information may give new insights and therefore offers a great basis for studies looking for new orientation restraints such as ^{1}H chemical shifts.
Ions were introduced to gramicidin A since previous studies have shown ion binding to affect ^{15}N chemical shift tensors, and may affect ^{1}H chemical shift tensors likewise. Initial results yielded a 2 ppm shift in ^{1}H chemical shift upon addition of K^{+} ions at the Trp15 site of gramicidin A. A shift of 1.2 kHz in the ^{1}H-^{15}N dipolar couplings was also observed. Since proton chemical shifts are highly sensitive to the surrounding environment, the 2 ppm difference in ^{1}H chemical shift upon the addition of K^{+} ions suggest a significant change in peptide plane orientation. We will fully report the effects of ions on chemical shift tensors in gramicidin A and demonstrate the ability of the HETCOR experiment to produce high resolution spectra.

2113-Pos Board B83

Biophysical Studies Of The Hn-s Protein From *Xyllela fastidiosa* And Dna

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The nucleoid-associated protein H-NS is a major component of the bacterial nucleoid, involved in DNA compaction and transcription regulation. Extensive studies on the biological functions demonstrated that H-NS plays a role in the regulation of many genes on the Escherichia coli chromosome, and represses the expression of a variety of proteins at the level of transcription, either by binding directly to DNA or through changes in DNA topology. In this work we chose the open reading frame 0749 of the genome of *Xylella fastidiosa*, a bacterium that causes a serious disease of oranges called citrus variegated chlorosis (CVC). The XF0749 codifes a protein of 134 amino acids (15kDa), a predicted H-NS protein. In order to gain insights into its function, we are studying the C-terminal domain (H-NSc), predicted to be involved in DNA binding, by high resolution NMR. The structure was solved and is deposited on the Protein Data Bank under code 2jr1. Other biophysical techniques are being employed to study the differences in DNA binding for the full versus truncated protein in variable conditions. Experiments to test whether H-NSc binds to specific promoters of the Xylella fastidiosa DNA are in progress, as well as relaxation time measurements and calculation of dynamic properties of the structure.