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

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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+}.

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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.

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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.

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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.