proteins is sought, corresponding to the large-scale collective motions of atoms. Here, the subspace iteration method is applied to all-atom representations of proteins to demonstrate its suitability to protein normal mode analysis. Important properties are that computational cost increases linearly with the required number of lowest eigenpairs and the method is robust computationally. Additionally, the procedure is particularly well suited to cases where numerous analyses are performed for nearby conformational substates, such as in conformational pathway analysis. Finally, the method is amenable to parallel implementation.

Biomolecular NMR Spectroscopy

2101-Pos Board B71

Structure And Alignment Of Membrane-associated Peptaibols By Oriented 15N And 31P Solid-state NMR Spectroscopy

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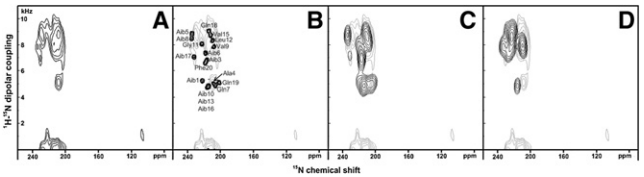
Peptaibol antimicrobial peptides are produced by fungi and are characterized by a high content of hydrophobic amino acids, and in particular alpha-isobutyric acid Aib. Here several peptides from this family were uniformly labeled with ^{15}N, purified and reconstituted into oriented phophatidylcholine lipid bilayers and investigated by ^{15}N and ^{31}P solid-state NMR spectroscopy. Whereas alamethicin (20 residues) adopts transmembrane alignments in POPC or DMPC the much shorter ampullosporin A (15) and zervamicin (16) exhibit comparable configurations only in 'thin' membranes. In contrast the latter compounds are oriented parallel to the surface in 'thick' bilayers indicating that hydrophobic mismatch has a decisive effect. Two-dimensional ^{15}N chemical shift - ^{1}H-^{15}N dipolar coupling solid-state NMR suggests that in their transmembrane configuration ampulosporin and alamethicin adopt mixed alpha-/3_{10}-helical structures due to the restraints imposed by the membranes and the bulky Aib residues. The ^{15}N solid-state NMR spectra also provide information on the helical tilt angles, the details of this analysis depend on the appropriate choice of the ^{15}N chemcical shift tensor.

Figure: PISEMA spectra of alamethicin (A) and simulations of spectra resulting from 3_{10} (B,C) and mixed 3_{10}/α-helical conformations (D).



2102-Pos Board B72

Functional and Shunt States of Bacteriorhodopsin Identified and Characterized by Multidimensional DNP-Enhanced Solid State NMR

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Bacteriorhodopsin (bR) is a 26 kDa archaeal membrane protein that harvests light energy to create an ion gradient across the cell membrane. Photoisomierzation of the retinilydene chromophore is coupled to ion translocation via a sequence of photocycle intermediates. Here we apply selective multidimensional solid-state NMR to uniformly ^{13}C,^{15}N-labeled bR in its native membrane to obtain chemical shifts in the chromophore of cryogenically trapped bR photointermediates. This is made feasible by using 250 GHz radiation to stimulate dynamic nuclear polarization (DNP), whereby the large spin polarization of unpaired electrons in exogenous free biradicals is transferred to nuclei. Subsequent N-C-C transfers in the NMR experiment allow us to distinguish four discrete substates of the L intermediate. Three of these are shunts that revert to the resting state of the protein upon thermal relaxation, while one L substate, labeled as 'persistent L' in our earlier 1D experiments, relaxes to the M state and is therefore deemed functional. Functional L has the strongest counterion, as indicated by its Schiff base (SB) nitrogen chemical shift. It also has a fully planarized 13-*cis* C13=C14 bond, as indicated by the gamma effect on the C12 chemical shift. These results are consistent with indications from time-resolved optical spectrometry and QM/MM studies of multiple barriers on the way to SB deprotonation. On the other hand, they are inconsistent with models in which the C13=C14 bond is twisted until Schiff base deprotonation. The experiments also demonstrate the use of DNP-enhancement at cryogenic temperatures to investigate mixed states of a membrane protein by multi-dimensional NMR. The results presented here would have been impossible without the availability of DNP to enhance spin polarization that is spread over multiple atoms in multiple protein states.

2103-Pos Board B73

C15=N Torsion Measured by DNP-Enhanced Solid State NMR in Bacteriorhodopsin Intermediates

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Bacteriorhodopsin is a 26 kDa light-driven ion pump that establishes an ion gradient across the membrane of *Halobacterium salinarium.* Although it has been characterized extensively by a wide range of techniques, structural details pertinent to its mechanism are still under scrutiny. Of particular interest is chromophore torsion that would orient the protonated Schiff base favorably toward the proton acceptor until proton transfer occurs. Thus, a measurement of the C15=N torsion in L, the intermediate directly preceeding proton transfer, would yield evidence supporting one of the models proposed for the proton transfer. By performing dipolar recoupling between ^{13}C labels at retinal-C14 and Lys-Cϵ, we determined the distance between the labeled sites, and thus the torsion angle around C15=N.

Utilizing the sensitivity available with DNP (Dynamic Nuclear Polarization), only 7.4 hours is needed to record a 2D spectrum from 15 mg of protein, even when the intensities of interest are divided in a roughly 60:40 ratio, corresponding to two different intermediates. This demonstrates the utility of DNP-SSNMR in obtaining precise quantitative measurements in membrane proteins, even in mixed states.

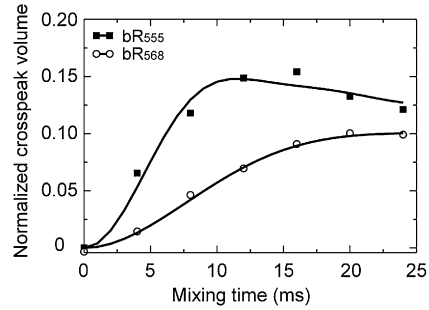

Figure 1. SSNMR recoupling build-up curve of retinal-14C, K216-Cϵ distance in bacteriorhodopsin. Data were fit to yield 3.11 +/− 0.02Å between 14C and Cϵ in bR_{555}, and 3.90 +/−0.08 Å in bR_{568}.

2104-Pos Board B74

Influence of Dynamics on The Analysis of Solid-State NMR Data From Membrane-bound Peptides

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By isotope labeling of membrane-bound peptides, typically with ^{2}H, ^{19}F, or ^{15}N, solid-state NMR experiments can yield data from which the orientation of peptides in a native membrane environment can be determined. Such an orientation is defined by a tilt angle and an azimuthal rotation angle.

Here we show that to obtain correct values of the orientation angles, it is important to include dynamics in the analysis of the NMR data. Nevertheless the effects of dynamics are different depending on the type of isotope labeling and NMR experiment considered.

To analyze the influence of dynamics in detail, we generated virtual NMR observables using a model peptide undergoing explicit Gaussian fluctuations of the orientation angles. For simulated ^{2}H- or ^{19}F-NMR data, even moderate motions were found to have a large influence, as calculated tilt values are consistently much too small, unless dynamics is properly considered. A simple

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.
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[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]
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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