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

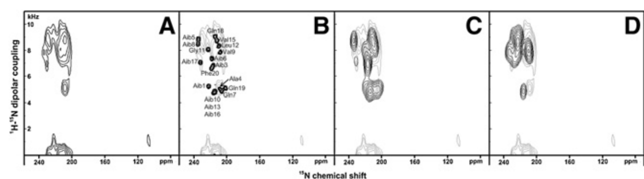
2101-Pos Board B71

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

Evgeniy Salnikov[1,2], Herdis Friedrich[3], Xing Li[4], Philippe Bertani[1], Siegmund Reissman[3], Christian Hertweck[3], Joe O'Neil[4], Tatiana Ovchinnikova[5], Sergei Dzuba[2], Jan Rapp[6], **Burkhard Bechinger[1]**.
[1]University Louis Pasteur, Strasbourg, France, [2]Russian Academy of Sciences, Novosibirsk, Russian Federation, [3]University of Jena, Jena, Germany, [4]University of Manitoba, Winnipeg, MB, Canada, [5]Russian Academy of Sciences, Moscow, Russian Federation, [6]University of Leiden, Leiden, Netherlands.

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

Melody L. Mak-Jurkauskas[1], Vikram S. Bajaj[1], Marina Belenky[2], Judith Herzfeld[2], Robert G. Griffin[1].
[1]Massachusetts Inst Technology, Cambridge, MA, USA, [2]Brandeis University, Waltham, MA, USA.

Bacteriorhodopsin (bR) is a 26 kDa archaeal membrane protein that harvests light energy to create an ion gradient across the cell membrane. Photoisomerization 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

Alexander B. Barnes[1], Melody L. Mak-Jurkauskas[1], Yoh Matsuki[2], Johan Lugtenburg[3], Judith Herzfeld[2], Robert G. Griffin[1].
[1]Massachusetts Institute of Technology, Cambridge, MA, USA, [2]Brandeis University, Waltham, MA, USA, [3]Rijksuniversiteit te Leiden, Leiden, Netherlands.

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.

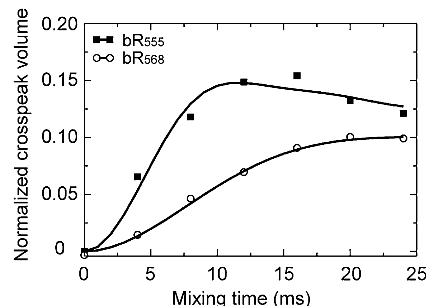


Figure 1. SSNMR recoupling build-up curve of retinal-14C, K216-Cϵ distance in bacteriorhodopsin. Data were fit to yield 3.11 +/− 0.02Å between 14C and Cϵ in bR_{555}, and 3.90 +/−0.08 Å in bR_{568}.

2104-Pos Board B74

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

Erik Strandberg[1], Santi Esteban-Martin[2], Jesús Salgado[2], Anne S. Ulrich[1,3].
[1]Forschungszentrum Karlsruhe, Eggenstein-Leopoldshafen, Germany, [2]Universitat de Valencia, Valencia, Spain, [3]University of Karlsruhe, Karlsruhe, Germany.

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