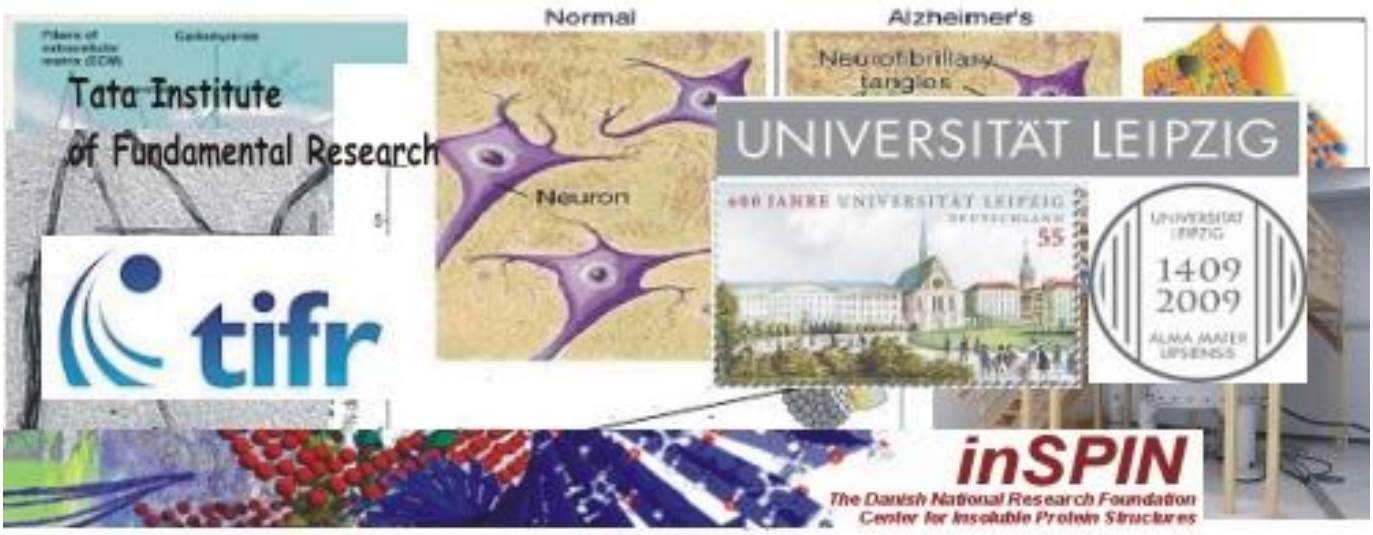

NMR Meets Biology: An Interaction Week

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Overview of Solid-State NMR Methods for Recoupling and Decoupling

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This talk will review advances made in the field of decoupling and recoupling in solid-state NMR. Such methods find use in obtaining high-resolution spectra and geometry information. Strategies for experimental realisation will be discussed with reference to magic-angle spinning frequency conditions. Adaptability of these schemes to the study of membrane proteins and fibril systems will also be highlighted.

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Dynamic Aspects of Membrane Proteins

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In spite of the recent success in the structure determination of membrane proteins, we are just in the beginning of understanding the structure-function relationship of these complicated and extremely interesting molecules. An important factor is the molecular dynamics of membrane proteins, which is usually not subject of many structural studies. Also, crystal structure determination of membrane proteins is typically only possible after comprehensive mutagenesis, antibody binding, or thermostabilization of the membrane proteins, which abolishes much of the molecular mobility of these molecules. As it turns out, membrane proteins show a surprising variety of molecular dynamics within a large correlation time window. In the talk, I shall discuss examples of membrane protein dynamics including membrane toxins and G protein-coupled receptors. We will also discuss important aspects of the solid-state NMR methodology, which holds great potential for the comprehensive dynamical characterization of these molecules.

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Mechanistic Insights into Water−Protein Interactions of Filamentous Bacteriophage

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Water plays a major structural and functional role around proteins. In an attempt to explore this mechanistic structural aspect of proteins, we present site-specific interaction of hydration water with the major coat protein subunit of filamentous virus Pf1 by magic angle spinning (MAS) solid-state NMR. The interaction of surrounding water with 36 MDa Pf1 virion is investigated in uniformly ^{13}C, ^{15}N isotopically labeled; polyethylene glycol precipitated fully hydrated samples by solid-state nuclear magnetic resonance spectroscopy. Dipolar edited two-dimensional (2D) $^{1}H-^{15}N$ heteronuclear correlation (HETCOR) experiments lead to unambiguous assignments of cross-peaks originating exclusively from ^{1}H resonances of water molecules correlating to the protein amide nitrogen. An enhanced resolved ^{1}H chemical shift dimension in these experiments also precludes the need of perdeuteration. We report seven residues spanning the 40-residue continuous α-helical conformation assembly of Pf1 interacting with surrounding water. It shows a highly hydrated inner core inside this viral filamentous assembly. The results obtained also suggest the first evidence of a water-mediated interface cluster formed at the site of Arg44 with the single-stranded DNA genome of the filamentous phage supramolecular assembly.

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Some Studies on Long Lived States and Coherences

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There is a constant pursuit to increase the lifetimes of states and coherences as spectral resolution is directly related to them. Longlived states (LLS) [1, 2] and coherences (LLC) [3], with lifetimes longer than the norm, were introduced recently in NMR. In this talk we will discuss some of our recent studies on LLSs and LLCs.

Storage of the LLS could play a vital role in their use for structural and dynamical studies of molecules. Earlier, windowless pulse schemes such as CW, WALTZ16, shaped and adiabatic pulse schemes were explored to store these states over the larger bandwidths. Windowed pulse schemes such as CarrPurcell and Uhrig dynamic decoupling (UDD) [4] sequences were explored only for onresonance storage of LLS. The results of our extensive study on the use of UDD and a number of phase variants of UDD [5] for storing LLS will be presented. [6] Our results indicate that UDDp7 outperforms CW and other windowed pulse schemes for storing LLS and is comparable to WALTZ16 sequence over a large radiofrequency offset range both in the small and large chemical shift difference regimes.

The lifetime of LLC is longer than the “normal” transverse spin coherence lifetime and is limited by the interaction of nuclei with other species. It is well known that unpaired electron spins provide an efficient mechanism for nuclear spin relaxation. Earlier studies indicate that paramagnetic species relax LLS inefficiently in comparison with longitudinal magnetization. We attempt to answer the question “Like LLSs, are LLCs relatively immune to paramagnetic ions?” [7] The addition of Cu^{2+} and Mn^{2+} on the relaxation behavior of the LLC in 2,3,6trichlorobenzaldehyde is studied and compared with other common NMR relaxation rates. We find that LLC are hypersensitive to paramagnetic relaxing agents with the effect increasing with the number of unpaired electrons. Quantitative analysis of paramagnetic relaxation rate of the LLC is performed by an external random field model within Redfield relaxation theory.

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Atomic-Resolution Structural Characterization of Native, Unfolded and Intermediate States of a Protein

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To understand phenomenon of protein-folding, complete characterization of all the states of the protein present along the folding funnel is essential. For this purpose NMR spectroscopy has a significant advantage over other techniques in providing atomic-resolution structural and dynamics information for all states along the folding funnel. We report here unfolding study of a RNA recognition motif (RRM) of a human RNA binding protein involved in alternate splicing. We have observed simultaneous existence of two major individual species '*native like*' intermediate as well as unfolded in solution at various urea concentrations during. An ensemble of '*native like*' intermediate at different urea concentrations has shown loosing of tertiary structure but having almost all secondary structural elements intact. The swollen *'native like*' ensemble is a semi-dry molten globule like state having higher solvent accessibility of the core. As the urea concentration is increased there is steady increase in concentration of unfolded species and decrease in the intermediate. The completely unfolded ensemble at 10 M urea is highly dynamic and has native as well as non-native structural propensities. We have also used a combination of time-resolved and steady-state fluorescence spectroscopy to gain complementary information.

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T1 Mapping Performed by Optimized Frequency Selective Pulses as a Tool for Fat Localization and Quantification in Tissues

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MR imaging is the method of choice for investigating the fat distribution in organisms in vivo. Common methods in MRI for fat quantification are point resolved spectroscopy (PRESS) and 2-point Dixon imaging, either with limitations. Another popular method is the usage of frequency selective pulses, mostly based on pulses with non optimized shape profiles. Here we describe an experiment for visualizing and quantifying fat in tissues based on pulses optimized by the Shinnar-Le-Roux Algorithm [1]. Excitation profiles of typical pulses like Sinc or Gauss are predicted by Fourier-Transform leading to imperfections at higher flip angles. In contrast SLR is entirely based on the Bloch equations which results in more accurate frequency selectivity. These selective pulses do not rely on specific MRI pulse sequences but can be combined with a variety of readouts. Here a RARE (Rapid Acquisition with Refocused Echoes) sequence is used for imaging. On this basis, frequency selective, pixelwise T1 relaxation measurements for fat and water will be performed. The acquired T1 values will be used as fitting parameters in order to obtain a quantitative image of the fat distribution in tissues. This idea is going to be tested on zebrafish fed with different diets. In the future we are going to extend this method to analyze lipid disorders in organs like the hepatic steatosis.

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Benefits of Fractional Calculus for Protein Diffusion in Gels Measured by PFG-NMR

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Engineered materials are increasingly used in orthopedic interventions. Nevertheless, a high interest to optimize these materials for optimal healing in early stages and critical size defects – considering especially the use in avascular tissues - remains. The proliferation should be enhanced by surface coating with growth factors or ECM components. The controlled release of the coating materials is of major impact and guided by diffusion. Hence, we studied the free and hindered diffusion of interleukin-8 (IL-8) (18 kDa protein dimer) and Bovine serum albumin (BSA, 66 kDa) in Agarose and Collagen gels by pulsed field gradient (PFG) NMR. As expected, hindered diffusion clearly depends upon the gel concentration and thus should be carefully taken into account to ensure an optimal coating of the used materials for further experiments.

Interestingly, a non-regular decay of the intensity-course was observed necessitating the usage of a stretched exponential fit introducing an additional fitting parameter alpha. This concept is derived from fractional calculus of the Bloch-equations and well known to better resemble the situation in fractal networks and complex biological tissues. However, we did not observe alternating of alpha for different gel concentrations but constant alphas for each protein independently of the surrounding gel. Thus, we conclude that the protein shape, e.g. spherical or more ellipsoidal, influences the course of the diffusional probability distribution which is described by alpha. Towards the characterization of engineered materials we are at the moment investigating if non-specific protein binding to the matrix is also able to influence this parameter in a detectable amount.

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Membrane Proteins: Doorkeepers in Health and Disease

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Biological membranes represent complex two-dimensional, non-covalent, anisotropic assemblies of a diverse variety of lipids and proteins. Membrane proteins are workhorses of the cellular machinery, and are crucial components of biological membranes. About 30% of all proteins are predicted to be membrane proteins and ~50% of all proteins are membrane proteins for eukaryotic cells. They control vital cellular functions such as transport of material into and out of the cell, cell-cell communication, signal transduction, infection and immunity, and pathogen entry, besides others. It is becoming increasingly clear that membrane proteins and receptors need to be viewed in the context of membrane lipids surrounding the protein. Crystallization efforts of membrane proteins in their native conditions are often complicated, and pose considerable challenge due to the intrinsic dependence of membrane protein structure on surrounding membrane lipids. Approaches based on NMR and fluorescence spectroscopy have proved useful in elucidating the organization, topology and orientation of membrane proteins and peptides. An additional advantage of spectroscopic approaches is that the information obtained is dynamic in nature, necessary for understanding membrane protein function. Importantly, membrane proteins represent major drug targets for a variety of diseases. In this lecture, I will provide a brief overview of membrane proteins combining physicochemical and biomedical aspects.

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Solid State NMR Experiments for the Partially Ordered and the Fully Rigid Systems – Need for Improved and Sensitive Methods for the Study of Structure and Dynamics

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In our laboratory, over the years we have developed several solid state NMR methods for the study and understanding of partially ordered systems. While a variety of systems such as membranes and membrane proteins and oriented polymers fall within this category, our focus has been on liquid crystals and their different phases. Liquid crystals are easily oriented by the magnetic field and provide a rich spectrum that is influenced by the anisotropy of the interactions and is highly useful for structural and dynamics study of these systems. One of the challenges in the study of such systems is the assignment of the resonances in the ordered phase. The resonance lines are shifted away significantly by the anisotropy of the chemical shift (CSA) and the line positions are influenced by both CSA and the local dynamics represented by the order parameter. While many techniques exist for assigning spectra in the solution phase, this is not the case for spectra in the partially ordered phase. Consequently methodologies need to be developed for this purpose. A method we have utilized recently is the use of proton double quantum (DQ) coherences correlated to carbon single quantum (SQ) coherences. Since the proton DQ coherences can result from both J and dipolar couplings, the method is highly useful in both assigning the spectral lines and delineating the carbon skeleton of the molecule. However one of the problems of the technique is sensitivity arising out of the low efficiency of simultaneous DQ excitation for several pairs of protons having different dipolar couplings and the low sensitivity of carbon detection. Therefore it will be useful to examine ways of improving sensitivity for such systems by utilizing novel developments in NMR. Recently, we have been implementing such DQ-SQ correlation experiments in several small rigid molecules in powder form in the solid state with magic angle spinning. Such systems which are of significant chemical and biological interest adopt a variety of conformations in the solid state which is different from the ones observed in solution. In view of their possible application as drug molecules, it is useful to understand the detailed structural aspects of such molecules. Examples of results obtained for such systems and the current limitations of applicability of the technique will be presented. Benefits of high sensitivity detection in such cases will be highlighted.

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GPCR-Cholesterol Interaction: A Multidimensional Approach

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G protein-coupled receptors (GPCRs) are the largest class of molecules involved in signal transduction across membranes, and represent major drug targets in all clinical areas. The serotonin$_{1A}$ receptor is an important neurotransmitter receptor of the GPCR superfamily and is implicated in the generation and modulation of various cognitive, behavioral and developmental functions. We previously demonstrated that membrane cholesterol is necessary for ligand binding, and G-protein coupling of serotonin$_{1A}$ receptors. Interestingly, recently reported crystal structures of GPCRs have shown structural evidence of cholesterol binding site(s). In this context, we reported the presence of cholesterol recognition/interaction amino acid consensus (CRAC) motifs in the serotonin$_{1A}$ receptor. We also showed that the receptor is more stable and compact in the presence of membrane cholesterol. Our recent results utilizing coarse-grain molecular dynamics simulations to analyze the molecular nature of receptor-cholesterol interaction offer interesting insight in cholesterol binding site(s) in the receptor and oligomerization of the receptor. We showed utilizing homo-FRET that the serotonin$_{1A}$ receptor is constitutively oligomerized in live cells, with the possibility of higher order oligomers of the receptor. Progress in deciphering molecular details of the nature of GPCR-cholesterol interaction in the membrane would lead to better insight into our overall understanding of GPCR function in health and disease.

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Solid-state NMR structural studies of an amphiphilic n-type nanotube

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Structural studies of one-dimensional n-type organic nanotubes, which have been shown to facilitate rapid energy migration and have potential applications as components of light-harvesting devices, are being done here using solid-state NMR as the primary technique. The nanotubes are formed by the rapid self-assembly in water of bolaamphiphilic molecules consisting of a hydrophobic napthalenetetracarboxylic acid diimide moiety flanked by hydrophilic L-lysine head-groups.Electron microscopy, atomic force microscopy and X-ray powder diffraction data suggest that nanotube assembly proceeds via 'nanoring' type structures, composed of ~100 bolaamphiphile monomers and stabilized through a combination of electrostatic and π-π stacking interactions, that further stack on top of one another.[1] Previously we have used 1D and 2D magic-angle spinning (MAS) solid-state NMR to perform the initial characterization of nanotubes containing uniformly 13C,15N-labeled lysine head-groups. This preliminary NMR analysis allowed complete resonance assignments to be established for both lysines and to differentiate between head-groups located on the inner and outer nanotube surfaces (labeled K1 and K2, respectively, in Figure).[1] Most significantly, the solid-state NMR data demonstrated an exceptional degree of conformational homogeneity in the bolaamphiphile molecules making up the nanotubes, which is likely responsible for the favorable energy transfer properties of these materials.

Here initial NMR studies are being extended, with the ultimate goal of deriving a high-resolution structural model for the nanotubes that can aid in understanding their excited state characteristics. To achieve this we determined a set of intramolecular ^{13}C-^{15}N and ^{13}C-^{13}C distances in the ~3-5 Å regime for the K1 head-group, using z-filtered transferred-echo double resonance (ZF-TEDOR)[2] and rotational resonance width (R2W)[3] methods and 'diluted' nanotubes formed from a mixture of ^{13}C,^{15}N-labeled and natural abundance monomers; only one such distance could be obtained for the K2 head-group due to limited spectral sensitivity caused by its dynamic nature.[1] We also probed intermolecular ^{13}C-^{15}N contacts using samples formed from a mixture of ^{13}C-and ^{15}N-labeled monomers. A summary of the distances is given in the top panel of the figure. Structure calculations using Xplor-NIH were done with the restraints observed from the electron microscope studies, which gave the approximate inner and outer diameter. As restraints the NMR distances observed were also being put in as potentials which sufficient error range. Finally the probable structural models were being subjected to X-Ray simulations and from these results we can propose a structural model for these nanotubes, which is shown in lower panel of the figure below. DFT calculations are being carried out to investigate the charge transfer processes.

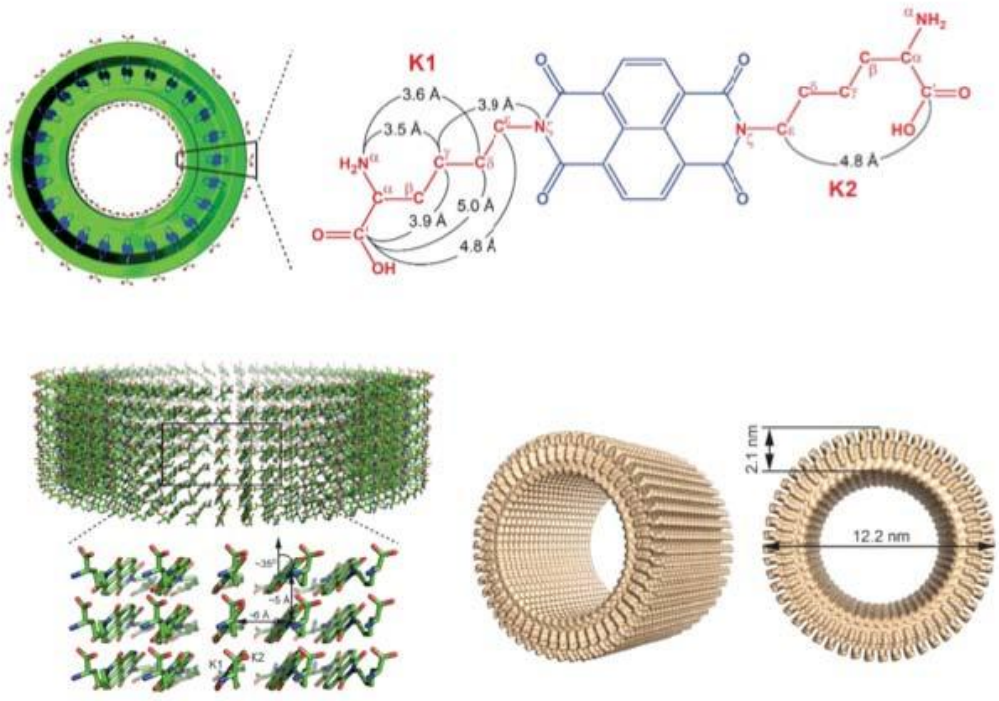

Figure: Summary of the structural characterization of bolaamphiphile A nanotubes by magic-angle spinning solid-state NMR spectroscopy. Top panel shows the summary of the distance obtained from solid-state NMR whilst the bottom panel shows the plausible structure of the nanotubes obtained using Xplor-NIH with restraints obtained from NMR and TEM.

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Structure of Transient Membrane-Active Amyloid-β Oligomers Probed in Physiological Conditions by Sequential Fluorescence and Solid-State NMR Spectroscopy

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Small oligomers of Amyloid-β (Aβ), rather than the monomers or the fibrils, are suspected to initiate Alzheimer's disease (AD). However, their low concentration and transient nature in physiological conditions have made structural investigations difficult. Here we develop a method for addressing such problems by combining rapid fluorescence techniques with slower two-dimensional solid-state NMR (ssNMR) methods. We probe the smallest $A\beta_{40}$ oligomers which demonstrate a potential sign of toxicity, namely, an enhanced affinity for cell membranes. We find that the two hydrophobic regions (residues 10-21 and 30-40) have already attained the conformation observed in the fibrils. However, the turn region (residues 22-29) and the N-terminal tail (residues 1-9) are strikingly different. Notably, 10 of 11 known Aβ mutants linked to familial AD map to these two regions. Our results provide specific structural assay for AD therapeutics, and also suggest a general method for determining transient protein structures.

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Local Interactions Influence the Fibrillation-Kinetics, Structure and Dynamics of Amyloid β Peptides but Leave the General Fibril Structure Unchanged

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The protein folding pathway to the highly ordered and biologically active form is determined by intramolecular interactions and the individual 3D structure of a given protein is encoded in the amino acid sequence. In addition another thermodynamically stable conformation can be formed, e.g. during pathological processes, which results in the formation of amyloid fibrils. Here, intermolecular interactions between the polymer chains dominate the formation of the cross-beta structure. More evidence accumulates that amyloid structures represents a common motif of proteins irrespective of their amino acid sequence. One underlying question for understanding the fundamental physical basics in this context is: How can local interactions influence the fibril forming kinetics, structure and dynamics?

We study this question in model fibrils of Aβ(1-40), where amino acid mutations introduce local physical forces. We investigate how flexibility, electric charges, the replacement of a strong hydrophobic contact with a salt bridge and the effect of an introduced electrostatic repulsion influence the amyloid fibrils. Investigations of structure and dynamics were performed using solid-state NMR. In our Aβ(1-40) model peptides, four amino acids where ^{13}C labeled as probes of local structure and dynamics. The isotropic chemical shifts of the Cα and Cβ of each amino acid depend strongly on the secondary structure. Therefore the ^{13}C cross-polarization spectra indicated local secondary structure elements. For dynamical studies DIPSHIFT experiments were performed, in order to measure ^{1}H-^{13}C dipolar couplings and determine order parameters. For almost all investigated peptide mutations fibril formation was observed with somewhat altered kinetics. Also subtle dynamical differences were observed. Only one peptide in which a hydrophobic contact was replaced by a salt bridge resulted in no fibrils at all. Although several local structure changes were observed, the overall cross-beta structure turned out to be rather robust.

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Efficient Heteronuclear Cross Polarization Technique for Solid State NMR and its Applications

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A highly efficient approach for heteronuclear coherence transfer in solid-state NMR spectroscopy under high speed spinning conditions is presented along with its adiabatic derivative. $^{RESPIRATION}CP^{1}$ has been shown to be a very simple experiment with an easy set up and significantly higher robustness towards imperfections such as rf inhomogeneity, maladjustments and sample induced variations. Also it requires only a short pulse per rotor period on one of the channels, therefore capable of an efficient polarization transfer from spin-1 nuclei (^{2}H, ^{14}N) to spin-1/2 (^{13}C, ^{1}H) without causing unwanted interaction between rf pulses and strong quadrupolar interactions. The same characteristic makes it suitable for polarization transfers involving ^{19}F nucleus, which otherwise is difficult to handle due to its high CSA. The numerical and experimental results showing that this rotor synchronized set up leads to significantly higher transfer efficiencies than state-of-the-art techniques including ramped and adiabatic cross-polarization experiments, will be presented.

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The Structure of Neuropeptide Y bound to its G protein-coupled Y2 receptor

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In order to influence the numerous pharmacological important signal transduction pathways involving G protein-coupled receptors (GPCRs), agonists or antagonists with high specificity and selectivity have to be developed to avoid adverse reactions. This is accomplished by large scale screening or considerably more efficiently by structure based drug design. The latter requires detailed information about the atomistic structure and dynamics of the natural ligand in its receptor bound state. NMR spectroscopy is an excellent method to study ligand/receptor interaction in their native lipid environment.

Here, we determined the structure and binding sites of the 36 amino acids comprising Neuropeptide Y (NPY) bound to the Y2 receptor. The interaction of NPY with the Y2 receptor plays, among others, an essential role in food intake and in the regulation of the circadian rhythm. Our approach was applying solution as well as solid-state NMR spectroscopy as complimentary methods. While backbone angle information were obtained from 13C-13C correlation MAS NMR spectra, interaction sites could be identified by comparing 1H-15N chemical shifts of NPY in presence and in the absence of the receptor, acquired from HSQC solution NMR spectra.

The required milligram amounts of the Y2 receptor were obtained by recombinant expression in *E.coli* as inclusion bodies and subsequent refolding of the GPCR into lipid membranes. Full functionality of the prepared receptor was shown by ligand binding and G protein activation. Several variants of NPY with four 15N/13C labeled amino acids each for easy signal assignment were produced by solid-phase syntheses.

Using the obtained restraints from NMR measurements, the structure of receptor bound NPY was calculated and modeled into the receptor binding pocket. The result revealed a structural change for the last five C-terminal amino acids of NPY from α–helix to random-coil upon receptor binding. The known C-terminal binding site could be confirmed. Further, a second binding site was identified, that interacts with the second extracellular loop of the Y2 receptor, which was finally proven in cell culture assays.

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Structure and Dynamics of Membranes and Membrane Proteins

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The lipid membranes of living cells are very flexible, and the flexibility of both the lipids and embedded proteins plays an important role in the many vital processes occurring across the cell membrane. We have pursued several routes to characterise both structure and dynamics features of lipids and membrane proteins by combining 15N, 13C, and 2H oriented solid-state NMR with other biophysical techniques and molecular dynamics (MD) simulations. By calculating the nuclear spin interaction parameters for the individual snapshots in MD simulations and do appropriate time- and ensemble averaging, we can obtain precise and detailed structures and dynamics information on both the lipids and membrane proteins in agreement with all experimental data.

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A Dynamic Protein Complex Helps in Cytoadherence During Malaria Infection

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Plasmodium falciparum infected erythrocytes (iRBCs) display membrane knobs that are essential for their adherence to vascular endothelia and for prevention of clearance by the spleen. This process of cytoadherence is mediated by *P. falciparum* erythrocyte membrane protein–1 (PfEMP1), which is tethered in clusters to these knobs. The basic knob associated histidine rich protein (KAHRP) is indispensable to knob formation and has been implicated in the participation of this tethering process by binding the cytoplasmic domain of PfEMP1, termed VARC. The interaction of the two proteins occurs electrostatically and the complex as a whole is tethered to the RBC cytoskeleton. Using solution-state nuclear magnetic resonance spectroscopy, we have obtained sequence-specific resonance assignments for the first of two tandem repeats (termed K2A1) in the central lysine-rich K2A region of KAHRP and concluded that K2A is an intrinsically disordered protein (IDP). We also mapped the perturbations in chemical shift on interaction with VARC to gain insight into the molecular mechanism of the clustering of PfEMP1 on knobs. Corroborated by calorimetry and transmission electron microscopy, our data provides evidence of four positively charged, linear sequence motifs of high intrinsic mobility on K2A1 that interact with VARC in solution to form a fuzzy complex. The inherent dynamism of this complex enables it to oligomerize to form spherical structures of ~15 nm diameter thus laying the basis for crosslinking of VARC by KAHRP. We have also used confocal laser-scanning microscopy to observe the co-localization of KAHRP and PfEMP1 to membrane knobs of *P. falciparum* infected erythrocytes.

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Investigations of the Structure and Dynamics of Different Ceramide Species in Models of the Stratum Corneum using Solid-State NMR and MD Simulations

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Ceramides are special lipids showing significant structural differences compared to other groups of lipids. They naturally occur in the Stratum corneum (SC) where they account for up to 40% of the SC lipids. Hence, they are the most abundant and structurally most important component of the epidermal barrier, playing a substantial role in maintaining the protective function of the SC. However, it is widely unknown how these dermatologically interesting lipids influence the structure and thereby the physiological function of the outer layer of the epidermis. So far, only few studies concerning phase characteristics and mixing behavior of physiologically relevant SC lipid systems have been established.

It is our goal to investigate the structure and dynamics of different types of ceramides in the SC. Since there are several theoretical models describing lipid organization in the SC, we are using ^{2}H-solid-state NMR supported by MD simulations to verify these assumptions. Mixing ceramides, free fatty acid and cholesterol provides a physiologically relevant model of the SC and offers an ideal opportunity to study each labeled component by ^{2}H-solid-state NMR spectroscopy.

We have investigated ceramides CER[NP]-C24, CER[NS]-C24, CER[NS]-C16 and CER[NdS]-C24. Analysis of the recorded spectra showed phase separation for the free fatty acids and for CER[NP]-C24 and CER[NS]-C24. CER[NS]-C16 spectra rather demonstrated a mix of several phases maybe due to the shorter acyl chain. Analysis of the CER[NdS]-C24 spectra shows an isotropic behavior.

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Structural Investigation of Glycosaminoglycan Binding to the Immune-Regulatory Protein Interleukin-10

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The immune response against microbial infections bears the intrinsic risk of an immune-mediated inflammatory damage to the host tissue. The cytokine interleukin (IL)-10 is a key regulator of the innate and adaptive immune system, which prevents an overwhelming immune reaction and tissue damage[1]. IL-10 inhibits the synthesis of pro-inflammatory cytokines (e.g. IFNγ, TNFα) and of cell surface molecules (e.g. MHC class II proteins). Thereby, cellular immune responses mediated by macrophages or T cells are inactivated. IL-10 has mostly paracrine functions and acts over short distances within the tissue[2]. In this context, glycosaminoglycans (GAGs) of the extracellular matrix have been suggested as important binding partners of IL-10 that restrict the protein to the vicinity of the secreting or targeting cell[3]. GAGs are a class of highly sulfated carbohydrate molecules that are known to bind and regulate a number of distinct proteins, including chemokines, cytokines, growth factors and adhesion molecules[4].

Here, we have analyzed the molecular interactions between IL-10 and different GAG oligosaccharides by NMR spectroscopy. Chemical shift perturbations from [^{1}H-^{15}N]-HSQC spectra and were used to identify the GAG binding site within IL-10. Binding occurs mainly at the DE-loop, which forms a crevice between both subunits of the IL-10 dimer. Additional contacts are made with residues of the N- and C-terminus. Furthermore, saturation transfer difference (STD) NMR spectroscopy revealed the important carbohydrate binding epitopes and could quantify binding affinity of different GAG oligosaccharides. In particular, N-sulfation of the GAG hexosamine unit is critical for interaction with IL-10. Upon binding the carbohydrate adopts a slightly changed conformation as indicated by transfer NOESY NMR experiments. For heparin, additional NOEs are observed between proton H2 of glucosamine and H1 of the respective preceding iduronic acid unit. In the near future we also plan to use paramagnetic NMR label, which can be attached to IL-10 or the carbohydrate, respectively. In particular, paramagnetic relaxation enhancements (PRE)[5] and pseudo contact shifts (PCSs)[6,7] will be used to characterize the molecular complex. At the end, our data will help to understand how biological functions of IL-10 are related to its interaction with extracellular matrix GAGs.

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The Orientaion and Dynamics of Estradiol and Estradil Oleate in Lipid Membranes and HDL Disc Models

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Estradiol (E_2) and estradiol (E_2) oleate associate with high-density lipoproteins (HDL). Their orientation in HDL is unknown. We studied the orientation of E_2 and E_2 oleate in membranes and reconstituted HDL. E_2 and E_2 oleate are membrane-associated and highly mobile. A model of the orientation of the molecule predicts the interaction of E_2 and E_2 oleate with bilayers and HDL discs. Our study predicts that the long axis of E_2 assumes a parallel, perpendicular, or anti-parallel orientation relative to the membrane's z-direction. The perpendicular orientation is preferred. In this orientation, E2 strongly favors a particular roll angle, facing the membrane with carbons 6, 7, 15, and 16, while carbons 1, 2, 11, and 12 point towards aqueous phase. In contrast, the long axis of E_2 oleate is almost exclusively perpendicular to the z-direction. In such an orientation, the oleoyl chain is firmly inserted into the membrane. Thus, E_2 and E_2 oleate have a preference for an interface localization in the membrane. This orientation was also found in HDL discs suggesting that only lipid-E_2 interactions determine the localization of the molecule. The structural mapping of E_2 and E_2 oleate may provide a design platform for specific E_2-HDL targeted pharmacological therapies.

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Predominant Role of Water in Native Collagen Structure, Stability and Functions Revealed by Solid – State NMR Spectroscopy

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Water plays a major structural and functional role around proteins. Collagen being a most abundant protein has been subject of several structural studies in last few decades. Most of these studies were performed on extracted form of collagen proteins, which is different from its native states. We present here ^{1}H detected solid-state NMR (ssNMR) experimental studies of truly native collagen proteins in intact bones. Various ^{1}H-detected ssNMR have helped in identifying high-resolution structural changes due to dehydration and H/D exchange. Our results clearly show that native collagen structure is significantly different from extracted form of collagen proteins. Further, dehydration of collagen proteins makes triple helical fiber to come closer whereas H/D exchange relaxes fiber packing. Together, these results give mechanistic insight about reduction in bulk mechanical property of native collagen proteins due to dehydration and H/D exchange.

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Basic Concepts of DNP Spectroscopy of Solids

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During my presentations I will try to present our present understanding of the nuclear enhancement process during Dynamic Nuclear Polarization (DNP) experiments on static and rotating solids. Continuing the work of the pioneers of DNP and the many people who contributed significantly to this field, I will discuss the theoretical building blocks that explain a large part of the experimental observations obtained in different laboratories and in ours. Subjects that will be discussed are the well-known Solid Effect and Cross Effect. The interplay between these effects as a function of radicals, temperature and microwave power will be examined and the question where the Thermal Mixing description can support our understanding of the enhancement process. Dipolar assisted DNP and DNP assisted Spin Diffusion will be taken into account as well as the effects of Spectral Diffusion between the unpaired electrons in the samples. Even the classical Overhauser effect observed in solids will be mentioned. The discussion will be accompanied with experimental observations and their interpretation. No specific applications of DNP will be discussed.

A distinction will be made between DNP on static and rotating samples and the differences between their enhancement mechanisms will be explained. Part of our conclusions will be complemented with results from MAS-DNP experiments.

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New Aspects of Cellular Function Studied with NMR: Rapid Dissolution ^{13}C DNP, and Enantiomeric Discrimination with Stretched Hydrogels

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Rapid-dissolution dynamic nuclear polarization (**RD-DNP**) with ^{13}C NMR decreases the time in which metabolic events can be studied with ^{13}C-labelled metabolites[1-3]. Emphasis has been given to enzyme-catalyzed reactions in pathological states of cells, and in a small number of clinical trials for the detection of neoplasms. The range of rapid biochemical reactions that are amenable to study by RD-DNP is limited; and area that has been less explored is membrane transport of rapidly exchanging solutes. Some molecules have ^{13}C NMR chemical shifts that differ inside and outside cells thus affording an NMR-based means of measuring their transmembrane exchange. One such molecule is **^{13}C-urea**. We studied the kinetics of its exchange in human erythrocytes on the sub-minute timescale using ^{13}C-RD-DNP[4]. *Mathematica* was used to describe the exchange and NMR-relaxation kinetics. Increasing awareness of the presence of **D-amino acid** residues in proteins in the dominant 'L-chiral biosphere', and of the preferential use of one enantiomer of a metabolite versus the other is largely due to recent developments in rapidly-applicable, analytical-chemical methods; and the **stretched hydrogel** NMR method is one of these[5-9]. Fully differentiated mammalian erythrocytes have no sub-cellular organelles and yet they display compartmentation of lactate depending on whether it is generated by glycolysis or the glyoxalase pathway. Thus operational compartmentation exists based not on the chemistry of the groups in lactate but its stereoisomerism; we call this 'chiral compartmentation'[10]. We confirmed that the glyoxalase pathway yields D-lactate with ^{1}H and ^{2}H NMR spectroscopy and stretched gelatin gels providing weight for the new concept, and pointing the way methodologically to its discovery in other cases of parallel metabolic pathways.

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Structure and Dynamics of Ghrelin Bound to Membranes and its Receptor

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The peptide hormone ghrelin is the endogenous ligand of the growth hormone secretagogue receptor (GHS-R1a) stimulating growth and appetite. The highly conserved 28 amino acid peptid is mainly secreted from the stomach and requires a posttranslational octanoylation at Ser3 for full activity.[1] The peptide is highly flexible in solution, but CD spectroscopy and molecular dynamics simulation show some helical content in presence of organic solvents and micelles.[2,3]

Since ghrelin addresses a transmembrane GPCR, we aim to characterize the structural and dynamical properties of the peptide backbone as well as the octanoyl moiety bound to lipid vesicles. We synthesized ghrelin peptides with varying $^{13}C/^{15}N$ labeled amino acids covering 17 out of 28 residues and a peptide with a perdeuterated octanoyl chain.

We have studied the membrane integration of the lipid modification of ghrelin by recording ^{2}H NMR spectra of vesicles containing perdeuterated DMPC and with and without associated ghrelin and a spectrum of the perdeuterated octanoyl chain in the presence of the membrane. We could show, that ghrelin binds acidic phospholipid membranes with its hydrophobic moieties as well as electrostatic interactions.

^{13}C NMR spectra under magic angle spinning conditions and measurements of the motional averaged dipolar couplings allowed the determination of backbone torsion angles and molecular dynamics, indicating a highly flexible conformation of the whole peptide.

Using $^{1}H/^{15}N$ heteronuclear single quantum correlation NMR spectra of ghrelin in solution in the presence of recombinantly expressed and refolded ghrelin receptor reconstituted in bicelles, we could identify the amino acid residues among the peptide interacting with the receptor. Solid-state 13C/13C correlation NMR experiments of ghrelin bound to GHS-receptor reconstituted in lipid bilayers reveal the peptides secondary structure in its functional state.

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Optimization of NMR experiments for Oriented and Magic Angle Spinning Experiments on Membrane Proteins

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Magic angle spinning (MAS) and oriented solid-state NMR (O-ssNMR) techniques can be used in tandem to solve the high resolution structures, orientations, and interactions of transmembrane proteins. To maintain native-like environments, however, high lipid-to-protein ratios are required for sample preparations. The latter represents a significant challenge for NMR spectroscopists, since it limits the effective protein concentration in the samples. In this presentation, we will describe our recent progress in the optimization of solid-state pulse sequences for both MAS and O-ssNMR techniques. These new experiments enable one to increase both the sensitivity and resolution of membrane protein samples reconstituted under functional conditions and at physiological temperatures, where the bilayers are in liquid crystalline phase. We will explain the theory behind these experiments, demonstrate their application to small and medium size proteins, and describe the technical details for setting up these new experiments on the new generation of NMR spectrometers.

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GCAP-2-New Insights into the Mechanism of Membrane Binding

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Guanylate cyclase-activating proteins (GCAPs) are neuronal Ca^{2+}-sensors playing a central role in light adaptation through the Ca^{2+}-dependent regulation of the retinal guanylate cyclase. GCAPs are N-terminally myristoylated, membrane-associated proteins. Although, the myristoylation of GCAPs plays an important role in light adaptation, the structural and therefore the physiological role is not yet clearly understood. The myristoyl moiety can in principle be buried inside the hydrophobic core or in a hydrophobic pocket of the protein and have a structure-stabilizing role. But more frequently, it serves as a membrane anchor and is therefore exposed. Additionally, the related protein Recoverin shows a so called Ca^{2+}-myristoyl switch mechanism. The Ca^{2+}-free form has the myristoyl moiety buried inside the hydrophobic core, but Ca^{2+}-binding leads to the exposure of the myristoyl moiety and hence to the membrane binding of the protein. Currently, only the structures of Ca^{2+}-bound, nonmyristoylated GCAP-2 (solved by solution-state NMR spectroscopy) and Ca^{2+}-bound, myristoylated GCAP-1 (solved by X-ray crystallography) are available. The crystal structure of myristoylated GCAP-1 shows the myristic acid inside the hydrophobic core of the protein; therefore, a structure-stabilizing function of the myristic acid was proposed. In contrast, we performed ^{2}H solid-state NMR investigations on the deuterated myristoyl moiety of GCAP-2 in the presence of liposomes and proofed that the myristoyl moiety is inserted into the lipid bilayer of the membrane. We now succeeded with the backbone assignment (H^{N}, N^{H}, Cα, C') of myristoylated, Ca^{2+}-bound GCAP-2. Therefore, we were now able to compare the myristoylated and the non-myristoylated form of GCAP-2 and test the structural influence of the myristoyl moiety in different environments (solution, micelles, bicelles and membranes) by solution and solid-state NMR. From these data we hypothesize that, in solution, the myristoyl moiety is buried inside a hydrophobic pocket, similar as shown in the crystal structure of GCAP-1 and that in the presence of membranes (liposomes) or membrane mimics (e.g. bicelles and micelles) the myristoyl moiety is exposed from the protein and inserted into the lipid bilayer, bicelle or micelle.

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Interaction of Gα protein peptides with Y2 receptor studied with NMR spectroscopy

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The human Y2 receptor (Y2R) is a rhodopsin-like (class A) G protein coupled receptor (GPCR). Its extracellular ligands are tyrosine rich peptides which belong to the neuropeptide Y (NPY) family comprising NPY, pancreatic peptide (PP) and peptide YY (PYY). Binding of these stimuli is coupled to the activation of complex cytosolic signaling via G proteins. This action of Y2R is involved in the inhibition of neurotransmitter release, the regulation of memory retention, circadian rhythm and angiogenesis making it an interesting drug target.

With the help of NMR spectroscopy we aim to understand the function and dynamics of Y2R with respect to the intracellular G proteins. G proteins are heterotrimers consisting of Gα-, Gβ- and Gγ-subunits of which the C-terminus of the Gα protein forms the most prominent interaction site with the receptor. In the past it was shown that a peptide with only eleven C-terminal amino acids can stabilize the activated form of the receptor. Such an eleven residue long peptide was synthesized with isotopically labeled amino acids, incubated with Y2R and investigated with solution NMR. First results indicate that it is possible to distinguish between different forms of the Gα peptide in dependency of the presence of the extracellular ligand NPY. Thus, we are able to characterize the interactions and dynamics of the Gα peptide bound to Y2R. Furthermore, solid-state NMR will provide information about the secondary structure of the Gα peptide.

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Understanding the Link Between Structure, Dynamics and Toxicity of α-Synuclein and its Mutants in PD

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A generic pathological feature among neurodegenerative diseases is the excessive accumulation of aggregated proteins in the brain. In Parkinson's disease, α-synuclein (AS), a natively unfolded 140 amino acid long cytoplasmic protein, misfolds and is found in intracytoplasmic neuronal inclusions termed Lewy bodies, the pathological hallmark of the disease. The proteinaceous plaques containing amyloid fibrils of AS were thought to be toxic species in neurodegeneration but recent investigations on amyloid intermediates suggest that it is not the insoluble aggregates but the soluble oligomeric species that are lethal to neurons. Despite of their immense importance in curing neurodegeneration, little has been understood about the relationship between structures and toxicity. This is primarily because of their transient nature. Here, we have used NMR methods along with other biophysical techniques to investigate the structural and motional features of disease-related mutants of alpha-synuclein. Our findings seem to indicate a tight link between structural features and neuro-toxicity

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On the Way to Understanding Amyloid Plaques- Solid-State NMR Investigations of Different Structures of Amyloid β Along the Fibrillation Process

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Plaques of amyloid β (Aβ) peptides are one hallmark of Alzheimer's disease (AD). Since it was found that not these plaques of mature fibrils but their transient intermediate species (oligomers, protofibrils) are the cell toxic species during the development of AD, therapeutic approaches try to interfere in the early stages of the fibrillation process and target these intermediate or the amyloid precursor protein (APP). Therefore, substantial knowledge about the molecular structure, dynamics and interactions of these molecules is necessary.

Using ^{13}C CP MAS NMR spectroscopy we investigated the structure and dynamics of Aβ protofibrils. In comparison to the known structure of mature Aβ fibrils, several differences are observed: First, the two well-known β-strands are already present in protofibrils but they are shorter then in mature fibrils. Second, there is evidence that the hydrogen bonds between the β-strands are still intramolecular in protofibrils and not intermolecular like in mature fibrils. These differences are mirrored in the molecular dynamics, which were measured via C-H dipolar couplings in DIPHSIFT experiments. Overall, protofibrils are structurally closer related to oligomers than to mature fibrils with whom the share a similar morphology. Therefore, a structural remodeling has to take place during the conversion from protofibrils to mature fibrils.
Furthermore, we investigated the interaction of the peptide C99, a fragment of APP, with cholesterol in lipid bilayers. Using ^{13}C CP MAS NMR and ^{1}H PFG MAS NMR significant changes in the molecular dynamics and lateral diffusion of cholesterol were observed. These changes are a result of the specific binding of cholesterol to C99, which could be shown introducing a mutation in C99.

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Structure and Function of 7-Helical Membrane Proteins Investigated by NMR Spectroscopy

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Membrane proteins form one of the most important types of biomolecules but remain structurally underrepresented. 7-helical membrane proteins represent a highly interesting group and include the G protein-coupled receptors (GPCRs) – a class that accounts for 40% of current drug targets. NMR offers a tool to look not only at the structures, but also the dynamics of these proteins.

Following the structure determination of the 7TM receptor pSRII by solution NMR spectroscopy, efforts in our lab have been concentrating on the investigation of functional aspects of such membrane proteins. Recent applications to microbial rhodopsins and GPCRs will be presented. New developments in NMR methodology aiming at making membrane protein studies more accessible will be discussed, focusing primarily on pseudocontact shift based information and data reconstruction using
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Resolving the Functional Mechanism of Membrane Proteins by High-Field and DNP-Enhanced Solid-State NMR

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MAS NMR at high fields allows site-resolved structure and dynamic data of membrane proteins embedded within lipid bilayers to be obtained. In addition, dynamic nuclear polarisation, usually applied at lower fields under cryogenic conditions, provides orders of magnitude better sensitivity enabling experiments, which could not be done otherwise. Here, the combination of both approaches will be introduced and demonstrated for proteorhodopsin, which is the most abundant microbial retinal protein found on earth. It occurs in bacteria near the ocean's surface and show a high level of adaptation to their local environment, especially in terms of light absorption. Mutation-induced colour tuning effects and their dynamic, functional and structural basis will be explained. Furthermore, the use of DNP-enhanced MAS-NMR to probe protein-protein interfaces within the membrane will be demonstrated. New methodological developments, challenges and opportunities will be discussed.

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Real-time Homodecoupled Pure-shift NMR Methods for Medium-size Molecules and Small Secondary Structures

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Unambiguous spectral assignments in ^{1}H solution state NMR are central, for accurate structural elucidation of complex molecules, which are often hampered by signal overlap, primarily due to scalar coupling multiplets, even at very high magnetic fields. The recent advances in homodecoupling methods have shown powerful means of achieving high resolution pure-shift ^{1}H NMR spectra by effectively collapsing the multiplet structures. In this regard, we will discuss the developments made in our laboratory, for through–bond and through-space correlation studies, wherein we employ homonuclear broadband as well as band-selective decoupling methods. For example, the power of pure-shift ROESY sequence will be demonstrated for two different organic molecules, wherein complex conventional ROE cross-peaks are greatly simplified with high resolution and sensitivity. The enhanced resolution allows to derive possibly more number of ROEs with better accuracy, thereby facilities accurate means of structural characterization of medium-size molecules.

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NMR Methods for Rapid Resonance Assignments of Challenging Proteins

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Challenging systems such as large, intrinsically disordered or membrane proteins require high dimensional NMR methods for efficient resonance assignments and structure determination. A downside of this is the long 'minimal' measurement time required for acquiring the NMR spectra. We have developed several new NMR methods which address this problem. The methods combine the different approaches for fast NMR data acquisition such as projection NMR spectroscopy, non-uniform sampling and multiple NMR receivers to increase the efficiency and provide unambiguous assignments. Additionally, we have developed new isotope labeling schemes combined with a set of 2D NMR experiments which facilitate rapid sequential assignments. Thus the combination of fast NMR methods and isotope labeling schemes promise new avenues in protein resonance assignment and structure determination. In this presentation, application of these methods to some challenging proteins studied in our laboratory will be presented.

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Characterization of Structure and Dynamics of Bacterial Peptidyl-tRNA hydrolases and Eukaryotic ADF/Cofilins

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Using NMR spectroscopy, we have characterized structures and dynamics of bacterial peptidyl-tRNA hydrolases (Pth) and eukaryotic ADF/cofilins. Peptidyl-tRNA hydrolase is an essential enzyme of bacteria and is a validated target for design of new anti-microbial agents. Its substrate is the peptidyl- or N-protected amino-acyl-tRNA. 5'-phosphate and 1-72 base-pairing of tRNA are also important determinants for Pth activity. ADF/cofilins depolymerize and weakly or strongly sever actin filaments and their activity is dependent on pH, PIP2, and phosphorylation.

Among the bacterial Pth proteins, we have determined the structures of Pth from *Mycobacterium tuberculosis* H37Rv (MtPth), *M. smegmatis* (MsPth), and *Vibrio cholerae* (VcPth). Among the eukaryotic ADF/cofilins, we have determined the structures of ADF/cofilins from *Leishmania donovani* (LdCof), *Toxoplasma gondii* (TgADF), and *C. elegans* (Unc-60A and Unc-60B).

We'll discuss the various insights that we've gained from these structures and the future work that we need to plan in order to address pertinent questions of biological relevance.

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Exploring Protein Energy Landscapes by NMR

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The accessible free energy landscape is a generic property of proteins, which determines both their protein folding pathways and their biological function. Several examples will be presented, how NMR can contribute to explore these landscapes and which structural rearrangements are related to the transitions between different protein states. Examples include the metallochaperone SlyD, where real-time NMR during actual catalysis of protein substrates allowed a molecular interpretation of the Michaelis-Menten parameter K_M and k_{cat} and a structural characterization of the transient enzyme/substrate complex. For efficient catalysis, a dynamic cross-talk between two domains of SlyD is required, which can be followed at various time scales and directly correlated with the function.

A second example is $p19^{INK4d}$, which negatively regulates the human cell cycle by inhibiting CDK4/6. The binding affinity is regulated by conformational selection induced by phosphorylation. This *in vitro* result could be confirmed by *ex vivo* NMR by using crude cell extracts from various cell lines to correlate cell biology methods with structural biology.

A third example is the cross-β structure formation in amyloid fibrils, which is difficult to follow in molecular detail. We employed H/D exchange in mature fibrils to label amide protons involved in hydrogen bonds and detect these amides in the disassembled peptide in DMSO. This allows to compare on a residue level fibrils grown under physiological and non-physiological conditions.

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Role of Transthyretin's Backbone Conformational Flexibility in Amyloid Fibril Formation

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Transthyretin (TTR) is a 55 kDa thyroxine transporter protein that is responsible for a large number of amyloidoses. The wild type protein is implicated in senile systemic amyloidosis whereas its mutants are responsible for neuropathies, cardiomyopathy, ocular, leptomeningeal and other forms of amyloidosis (1). Biophysical characterization of full length, wild type and mutant forms of TTR by various groups have revealed that protein aggregation is preceded by tetramer dissociation to monomers which then proceeds to form fibrils through a non-native or intermediate state. Mutations that destabilize the protein have higher fibril forming propensity and *vice versa*. Although conformational dynamics has been indicated to be a key factor facilitating fibril formation (2), this has not been investigated in details before. Here, we probe the backbone dynamics of TTR spanning multiple time scales and combine them with molecular dynamics (MD) simulations to gain insights into the process of fibril formation. Results of backbone ^{15}N R_1, R_2 and $\{^1H\}$-^{15}N NOE spin relaxation measurements on wild type TTR at 40 ˚C and pH 5.8 suggests that the molecule remains a tetramer with an estimated rotational correlation time of 21 ns and an isotropic diffusion tensor. Estimation of modelfree order parameters suggests that most of the residues that are dynamic in this regime (i.e. ps – ns timescales) reside in the unstructured regions in the periphery of TTR's structure while the hydrophobic core is virtually intact and is in agreement with MD calculations performed in our group. In addition, we also probed the existence of slower timescale (~ms) motions using ^{15}N C.P.M.G. relaxation dispersion NMR on backbone amide spins of TTR. Here, we found that the dynamic residues are predominantly located in the β-strands of TTR's hydrophobic core that also forms the interface between multiple subunits. Fitting the set of contiguous, dynamic residues to a 2-site exchange model (3-ste exchange model did not significantly improve the fit), we found that approximately 10 % of the ensemble exists in chemical exchange with a minor conformer. Similar experiments on a set of stabilizing and destabilizing (pathogenic) mutants of wild type TTR has given crucial information in the modulation of backbone dynamics by these mutations and provide a model for the role of conformational flexibility in the initiation of fibril formation.

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Structural Characteristics of Conserved C-terminal Segment of Eukaryotic Acidic Ribosomal P2 proteins: Functional Implications

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Ribosome structure has been solved by X-ray crystallography several years ago, but to date, very little is known about the ribosomal stalk, which in the ribosome is a supramolecular assembly of proteins. During protein synthesis, the stalk is known to adopt different conformations at different steps of the elongation cycle. Eukaryotic stalk is much more complex than prokaryotic stalk. It consists of acidic proteins known as P proteins (P0, P1, P2), which are organized as a complex assembly, $P0(P1\text{-}P2)_2$. However, the individual proteins are known to self-associate under physiological conditions.

The P2 protein is quite conserved across several eukaryotic species, particularly, its C-terminal domain. Within the C-terminal domain also, the last 11 residues are even more conserved. It has been argued that multiple copies of this C-terminal sequence protrude outwards from the ribosome to the cytoplasm, function to fetch the elongation factors and draw them into GTPase-associated center. In order to understand structure function relationships of this domain, we have carried out investigations on several constructs of the domain (5kDa) from different eukaryotic species (human, *P. falciparum, T. gondii*). MALDI indicated that the protein is a monomer and Circular Dichroism (CD) and NMR studies showed that this domain is intrinsically disordered. Fluorescence spectroscopy revealed that this domain interacts with GTP and elongation factor (eEF2). It also interacts with the TCS (trichosanthin) ribosome inactivating protein, which has been characterized by NMR. The binding site on P2 has been mapped by chemical shift perturbation. Binding is seen to be conserved throughout the species. Further, it seems that TCS occupies the same site as eEF2 and this may be responsible for translational inhibition. The C terminal domain also seems to show phosphorylation as post translation modification. Further investigations are in process to unravel more details.

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Allosteric Regulation of the Sarcoplasmic Reticulum Ca^{2+}-ATPase by Phospholamban and Sarcolipin using Solid-State NMR Spectroscopy

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The membrane protein complexes between the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) and phospholamban (PLN) or sarcolipin (SLN) control Ca^{2+} transport in cardiomyocytes, thereby modulating cardiac muscle contractility. Both PLN and SLN are phosphorylated upon b-adrenergic-stimulated phosphorylation and up-regulate the ATPase via an unknown mechanism. Using solid-state NMR spectroscopy, we mapped the interactions between SERCA and both PLN and SLN in membrane bilayers. We found that the allosteric regulation of the ATPase depends on the conformational equilibria of these two endogenous regulators that maintain SERCA's apparent Ca^{2+} affinity within a physiological window. Here, we present new regulatory models for both SLN and PLN that represent a paradigm-shift in our understanding of SERCA function. Our data suggests new strategies for designing innovative therapeutic approaches to enhance cardiac muscle contractility.

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