# Biomolecular NMR Spectroscopy

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**Bone Regeneration Studied by μMRI and Solid-State NMR Spectroscopy**

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Bone substitutes are increasingly used in orthopedic interventions. Currently, there is a high interest to optimize the bone scaffolding materials for optimal healing. Using a tibial head defect we investigated bone regeneration using biodegradable poly(lactic-co-glycolic acid) (PLGA) scaffolds, providing a macroporous three-dimensional carrier. Cylindrical scaffolds with similar porosity but different pore sizes of 100-300, 300-500, or 500-710 μm were implanted into a tibial defect of a rat model. Two or four weeks after implantation, the scaffolds were monitored by μMRI and solid-state NMR. In particular, the molecules of the regenerated extracellular matrix (collagen and apatite) were quantitatively studied. Using μMRI, the implanted PLGA scaffolds were clearly visible and a homogeneous generation of ECM was obvious. The regeneration of the collagen moiety was studied by $^{13}$C CPMAS NMR. The total amount of collagen synthesized in the scaffolds depended on the pore size of the scaffolds, best results were obtained for the matrix with 300-500 μm pores. Order parameter measurements of the collagen amino acids showed already very good agreement with those from the natural bone. The inorganic ECM component of the *de novo* formed bone was investigated by $^{31}$P CPMAS NMR. It could be shown that hydroxyapatite was synthesized in the implant by the chondrocytes. The amount of hydroxyapatite increased significantly towards the end of the 4 week animal study indicative of progressed biomineralization. In all experiments, a pore size of 300 to 500 μm turned out to be most effective. From our molecular assessment, both concentration and molecular dynamics of the *de novo* formed ECM was already very close to that of native bone. However, as the μMR images revealed, the macroscopic trabecular bone structure in the implants was isotropic as oppose to the anisotropic structure in healthy bone.

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**Stabilization and Structure Determination of the Recombinant C-Terminal Domain of Nephila Clavipes Dragline Silk**

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Spider silk, as simple as it may look, holds a secret which lies in the processing of the silk proteins from a highly concentrated soluble state to the insoluble fiber. The conserved C-terminal domain of MaSp1, the dragline silk main protein, appears to play a key role in the control of solubility and fiber formation initiated by changes in ionic composition, mechanical stimuli or pH variations along the secretory gland ducts. Therefore, the study of its structure is necessary to understand this remarkable molecular behaviour. We have established a purification protocol to prevent the aggregation of the recombinant *Nephila clavipes* MaSp1 C-terminal domain. We have also investigated the structure of the protein using solution nuclear magnetic resonance (NMR), dynamic light scattering (DLS) and fluorescence spectroscopy. NMR diffusion and DLS experiments provide information about the oligomerization and aggregation of the protein. Further NMR experiments with the doubly $^{13}$C/$^{15}$N labelled protein will lead to the complete determination of the protein structure.

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**Probing the Molecular Architecture and Assembly of Synthetic and Fungal Melanins with Solid-State NMR**

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Melanins are ubiquitous natural pigments that are associated with biological roles in protection against sunlight, camouflage, free radical scavenging, and microbial virulence. However, elucidating structural characteristics of these enigmatic pigments remains a challenging task because of their insolubility and heterogeneous properties. High-resolution solid-state NMR spectroscopy provides a unique tool to unravel the mysteries of melanin structure, and melanogenesis in *Cryptococcus neoformans* (CN) requiring exogenous obligatory catecholamine precursors such as L-dopa offers unprecedented opportunities to probe melanin biosynthesis and assembly in fungal cells. 1D solid-state $^{13}$C NMR has been used to assess the structural differences among CN melanins produced by systematic variation of catecholamine precursors and in cell-free media. The precursor preferences of CN melanogenesis have been demonstrated by competition studies in which the cells were provided with equimolar mixtures of catecholamines, and a mechanistic scheme adapted from the Mason-Raper pathway has been proposed to rationalize the possible intermediates for biosynthesis of indole-based melanin polymers. $^{13}$C and $^{15}$N solid-state NMR, including 2D through-space and through-bond correlation spectra, have been implemented to elucidate the ultimate molecular framework of the pigments and melanization in CN with exogenously $^{13}$C-enriched L-dopa and/or [U-$^{13}C_6$]-glucose supplied to the growth media. Findings include: (1) the main aromatic components are located spatially within 5-7 Å of the long-chain aliphatics; (2) glucose scrambles metabolically to appear in oxygenated- and long-chain aliphatic structures; (3) some aromatic, oxygenated- and long-chain aliphatic moieties are attached covalently to carboxyl groups of the pigment; (4) the pigment is likely to be covalently bound to the polysaccharides of fungal cell wall. The molecular architecture of natural melanin revealed by the solid-state NMR study can guide the design of new drugs against microbial virulence and novel therapeutics for melanoma tumors.

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**Improved Identification of Organic Phosphorus Species in Tropical/ High Latitude Soils and Aquatic Ecosystems by Advanced Liquid and Solid State NMR Approaches**

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Phosphorus (P) availability is limiting plant growth in many of the worlds biomes. Recent predictions suggest that rock P exploitation peaks within 35 years with severe impacts on future global food production[1]. In the soil much of mineral P is transformed to organic P species which are unavailable for plants. There is a lack of knowledge about the molecular processes controlling the reactivity of organic P species and their bioavailability. Our aim is to develop solution and solid state $^{31}$P NMR techniques to identify P species in soils and water; information ideal for correlating different organic P species to plant and soil processes[2]. However, NMR studies on soil P are complicated by serious line broadening caused by paramagnetic ions. For liquid-state NMR, soil organic P is commonly extracted by a NaOH-EDTA method, leading to co-extraction of heavy metal. In order to avoid paramagnetic line broadening these ions have to be physically removed. We find that sulfide precipitation removes Fe and Mn ions without affecting the P-composition. It dramatically reduces the line widths from over 100 Hz down to 2 Hz on soil extracts and allows 2D $^{1}$H, $^{31}$P NMR to be applied[3]. Using 2D $^{1}$H-$^{31}$P NMR resolves the highly crowded spectral region where abundant monoester P appears. By exploiting 2D $^{1}$H-$^{31}$P correlations in the NMR spectra of soil extracts, we can identify individual organic P species (including unknowns) by a combination of P and H chemical shifts and coupling constants[3,4]. Studies using passive sampling with ion exchange resin analyzed with solid state $^{31}$P MAS NMR have the potential to analyse the reservoir of organic P-species in aquatic systems e.g. streams.

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**Hydration-Mediated Slow Dynamics in Phospholipid Membranes**

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Biological activities of integral membrane proteins depend on the nature of the surrounding lipid bilayer [1]. The dynamic organization of lipid bilayer systems spans a wide frequency range encompassing individual fast acyl motions, molecular rotations, lipid protrusions, and collective bilayer fluctuations [2]. Time scales of these motional modes typically span a large range from subpicoseconds up to milliseconds, and can be investigated using solid-state NMR spectroscopy. To correlate structural changes of lipid bilayers mediated by osmotic stress [3] with biological function and molecular dynamics, we measured $^{2}$H longitudinal ($R_{1Z}$) and transverse ($R_2^{CP}$) relaxation rates in the liquid-crystalline phase of DMPC-$d_{54}$ membrane bilayers at various hydration levels. The $R_{1Z}$ experiments used a conventional inversion-recovery pulse sequence, while $R_2^{CP}$ rates were measured from quadrupolar-echo intensities using a Carr-Purcell-Meiboom-Gill pulse train. By Fourier transforming individual echoes with different pulse spacings we map the frequency dependence of relaxation rates and motional modes of individual acyl segments.