CPP 28: Poster: Biopolymers and Biomaterials

Time: Wednesday 17:30–19:00 Location: Poster C

CPP 28.1 Wed 17:30 Poster C

Knots in viral DNA and proteins: A Monte Carlo test with simplified models — •Daniel Reith[1], Thomas Wüst[2], Peter Cifra[3], Daniel Bölinger[4], and Peter Virnau[1] — [1]Institut für Physik, Johannes Gutenberg-Universität Mainz, 55099 Mainz, Germany — [2]The Center for Simulational Physics, The University of Georgia, Athens, GA 30602-2451, USA — [3]Polymer Institute, Slovak Academy of Sciences, 842 36 Bratislava, Slovakia — [4]Max Planck Institute of Neurobiology, 82152 Martinsried, Germany

We investigate the influence of chain stiffness on self-entanglements of a single polymer chain with Monte Carlo simulations. Surprisingly, the number of knotted chains in our equilibrium ensemble increases manifold if a small chain stiffness is imposed and stays large if the stiffness is increased to a point at which the polymer is coiled up inside the capsid. Implications on the internal structure of DNA in viral capsids are discussed. In the second part, we test the influence of sequence on single globular heteropolymers. The entanglements of these states turn out to be very similar to those observed in homopolymers of comparable density. As knots are abundant in globular homopolymers these simulations cannot solve the conundrum why only few knots are observed in globular protein structures[1,2].

[1] P. Virnau, Y. Kantor, and M. Kardar, J. Am. Chem. Soc 127, 15102 (2005).
[2] P. Virnau, L. A. Mirny, and M. Kardar, PLoS Comput. Biol. 2, e122 (2006).

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Modification of casein films by rennin enzyme action — •Ronald Gebhardt[1,2], Ulrich Kulozik[1], Christian Riekel[2], Manfred Burghammer[2], and Peter Müller-Buschbaum[3] — [1]TU München, Chair for Food Process Engineering and Dairy Technology, Weihenstephaner Berg 1, 85354 Freising-Weihenstephan, Germany — [2]European Synchrotron Radiation Facility, Grenoble, France — [3]TU München, Physik Department E13, Garching, Germany

The aim of our research is the development of new functional materials on the basis of natural products. As a model system we investigated films of casein micelles whose properties were consciously changed. Beside imaging techniques like AFM, we used grazing incidence small angle X-ray scattering with a micrometer-sized X-ray beam (so called microGISAXS) to investigate the micro- and nanostructure of protein films. The method provides information about the multilevel-structure of casein micelles with high positional accuracy and statistical relevance. We used two different approaches in order to modify the casein film structure by rennin. When added to casein solution prior to film preparation, rennin generated homogeneous particle films by transforming casein micelles into para-casein micelles. Contrary, the coating of casein films by rennin led to a surface near transformation into para-casein-micelles only, while casein micelles inside the film kept their native structures. The latter results will be explained on the basis of a diffusion model (1). (1) R. Gebhardt, M. Burghammer, C. Riekel, U. Kulozik, P. Müller-Buschbaum: Dairy Sci. Technol. (2009) accepted

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Mineral Capsules via Mineralization of Emulsions — •Alexander Schulz[1], Bernhard Blümich[2], and Alexander Böker[1] — [1]Lehrstuhl für Makromolekulare Materialien und Oberflächen, DWI an der RWTH Aachen e.V., RWTH Aachen University, Germany — [2]Lehrstuhl für Makromolekulare Chemie, RWTH Aachen University, Germany

Mineral capsules of hydroxyapatite with diameters in the range of 10 to 300 μm are prepared by mineralization of a protein-stabilized oil-in-water emulsion.

An oil-in-water emulsion is prepared via vigorous shaking and stabilized by a protein. Subsequently, the water phase is replaced by a supersaturated solution of calcium and phosphate in water, with citrate as an additive, at a slightly alkaline pH. This solution is exchanged frequently by a fresh supersaturated solution, prepared in the same way. After several days, mineral capsules are obtained. The size varies with the parameters of the preparation, as the structure of the mineral and the number of capsules also do. The analysis of the capsules is performed via SEM-imaging of multiple samples taken at different times of mineralization. The mineral is characterized by EDX-spectra taken from the SEM-samples. Thus, optimum parameters for the mineralization procedure could be identified. Further characterization, like crystal structure and porosity of the material, is carried out by X-Ray diffraction (XRD), electron diffraction and NMR.

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Silkworm Silk - A High Pressure SAXS/WAXS Study — •Christina Krywka[1], Igor Krasnov[1], Florian Kunze[1], and Martin Müller[2] — [1]Christian-Albrechts-Universität zu Kiel, Institut für Experimentelle und Angewandte Physik, Leibnizstraße 19, D-24098 Kiel — [2]GKSS Forschungszentrum Geesthacht, Max-Planck-Straße 1, D-21502 Geesthacht

Due to the remarkable mechanical properties of natural silks numerous potential applications exist. However, the nanoscopic structure is still a matter of debate and none of the synthetic silk fibers exhibit mechanical properties comparable to those of the bio-spun fibers.

Degummed silkworm silk (*Bombyx Mori*) is a pure protein fiber consisting only of fibroin. The fiber is a semicrystalline nanocomposite with beta-sheet nanocrystals embedded in a soft amorphous matrix. There is indication for a self-modulation of the fibroin within the silk fiber, leading to a phase-separation between those fibroin residues that can form crystallites and those that can't. If this bimodal nanostructure is a result of a self-arrangement of the different domains of the fibroin molecule, this separation should be able to be disrupted by denaturating conditions, such as high pressure or strong denaturants.

Small and wide-angle X-ray scattering (SAXS and WAXS, resp.) methods with *in situ* high hydrostatic pressure (up to 5 kbar) were employed to investigate the impact of strong denaturants (urea) and undirected stress (pressure) on the crystalline and nanoscopic fiber structure and to gain a clearer view onto the mesoscopic structure of silk fibroin.

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Hierarchies in the structural organization of spider silk - A quantitative combined model — •Roxana Ene, Periklis Papadopoulos, and Friedrich Kremer — Institut für Experimentelle Physik I, Leipzig, Germany

Combined time-resolved mechanical and polarized Fourier-transform infrared measurements allow us to determine the interconnection of the nanocrystal and amorphous phases in major ampullate spider silk in the native and supercontracted state [1]. Crystal stress can be measured from the frequency shift of main-chain vibrations. The results show that in both states of silk a serial arrangement between the crystalline and amorphous phase dominates the nanostructure. However, supercontracted silk shows a different behavior before being stretched because a hydrogen-bonded network is formed in the amorphous phase, due to release of pre-stress and hydrophobic effects. A three-component combined model of crystals in serial arrangement with amorphous chains and a fraction of chains bypassing them can describe all states of spider silk, assuming hydrogen bonding of worm-like chains at low pre-strain [2]. Additionally water permeability of dragline silk is studied by measuring changes in amide deuteration [3]. The results show that the chemical exchange of amide hydrogen occurs in a large fraction of amino acids, including b-sheeted alanine residues, suggesting that also the crystalline regions are accessible to water. [1] P. Papadopoulos, R. Ene, I. Weidner, F. Kremer Macromol. Rapid Commun 30, 851-857 (2009). [2] R. Ene, P. Papadopoulos, F. Kremer, Soft Matter 5, 4568-4574 (2009) [3] R.Ene, P. Papadopoulos, F. Kremer (in preparation)

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2H NMR Studies on Acylated Transmembrane Fusion Peptides — •Anja Penk[1], Matthias Müller[1], Holger Scheidt[1,2], Dieter Langosch[3], and Daniel Huster[1] — [1]Institut für Medizinische Physik und Biophysik, Universität Leipzig, Leipzig, D — [2]Institut für Biochemie/Biotechnologie, Martin-Luther-Universität Halle-Wittenberg, Halle, D — [3]Lehrstuhl Chemie der Biopolymere, Technische Universität München, Freising, D

Fusion of biological membranes is mediated by integral membrane proteins, often modified by covalent attached hydrocarbon chains. Previously, a series of de novo designed alpha-helical peptides with mixed

Leu/Val sequences was presented, mimicking fusogenic transmembrane segments. From this series, we have investigated the peptide LV16 (KKKWL VLVLV LVLVL VLVLV KKK), which was synthesized presenting either a free N-terminus or an N-acylation of 2, 8, 12, or 16 carbons. We used 2H and 31P NMR to investigate the structure and dynamics of these peptide lipid chains in POPC and DLPC bilayers and compared them to the hydrocarbon chains of the surrounding membrane. Except for the C-2 chain, all peptide acyl chains were found to insert well into the membrane. This can be understood from the high local lipid concentration, which the N-terminal lipid chains experience. The insertion of these peptides did not influence the membrane structure and dynamics. Although the longer acyl chains insert into the membrane, there is no length adaptation. In spite of the significantly different lengths of the acyl chains, the fraction of gauche defects in the inserted chains is constant, suggesting similar chain entropies.

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Structure and Dynamics of the Myristoyl Lipid Modification of a Src Peptide Determined by 2H Solid-State NMR Spectroscopy — •Holger A. Scheidt[1,2] and Daniel Huster[1] — [1]Institut für Medizinische Physik und Biophysik, Universität Leipzig, Leipzig, Deutschland — [2]Institut für Biochemie/Biotechnologie, Martin-Luther-Universität Halle-Wittenberg, Halle, D

Lipid modifications of proteins are widespread in nature and play an important role in numerous biological processes. The nonreceptor tyrosine kinase Src is equipped with an N-terminal myristoyl chain and a cluster of basic amino acids for the stable membrane association of the protein. We used 2H NMR spectroscopy to investigate the structure and dynamics of the myristoyl chain of myr-Src(2-19) and compare them with the hydrocarbon chains of the surrounding phospholipids in bilayers of varying surface potential and chain length. The myristoyl chain of Src is well inserted in all bilayers investigated. In zwitterionic DMPC membranes, the myristoyl chain of Src is significantly longer and appears 'stiffer' than the phospholipid chains. This is explained by an equilibrium between the attraction due to the insertion of the myristoyl chain and the Born repulsion. In a DMPC/DMPS membrane, where attractive electrostatic interactions come into play, the differences between the peptide and the phospholipid chain lengths are attenuated and the molecular dynamics of all lipid chains is similar. In a much thicker DPPC/DPPS/cholesterol membrane, the length of the myristoyl chain of Src is elongated nearly to its maximum and their order parameters are comparable to those of the surrounding membrane.

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Automated solvent artifact removal and base plane correction from multidimensional NMR protein spectra by Auremol-SSA — •Wilhelm Massimiliano Malloni[1], Silvia De Sanctis[1], Elmar W. Lang[1], Klaus-Peter Neidig[2], and Hans Robert Kalbitzer[1] — [1]Institut für Biophysik und physikalische Biochemie, Universität Regensburg, Germany — [2]Bruker Biospin GmbH, Software Abteilung, Germany

Strong solvent signals lead to a disappearance of weak protein signals close to the solvent resonance frequency and to base plane variations all over the spectrum. AUREMOL-SSA method provides an automated approach for solvent artifact removal from multidimensional NMR protein spectra. Its core algorithm is based on singular spectrum analysis (SSA) in the time domain and it is combined with an automated base plane correction in the frequency domain. The SSA technique is an extension of the PCA applied to a time lagged data set that embeds each FID separately in an M-dimensional vector space. The performance of the method has been tested on synthetic and experimental spectra including a two-dimensional NOESY spectrum and a three-dimensional 1H,13C-HCCH-TOCSY spectrum. An extension of the FLATT algorithm for base points selection has been introduced after the solvent removal. Those pure baseline regions are then linearly interpolated and subtracted from the original spectrum. The advantage for practical applications is the complete automation that includes the SSA followed by filtering Fourier transformation, phase correction related to the group delay management and baseline correction.

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AUREMOL: Automatic protein structure determination from NMR data — •Harald Donaubauer[1], Tobias Harsch[1], Willhelm Malloni[1], Silivia de Sanctis[1], Bärbel Kieninger[1], Nina Danilova[1], Konrad Brunner[1], Wolfram Gronwald[1], Jochen Trenner[1], Klaus-Peter Neidig[2], and Hans-Robert Kalbitzer[1] — [1]University of Regensburg — [2]Bruker BioSpin

Several approaches to the problem of automated protein structure determination from NMR data exist. The concept for automation is a molecule centred approach where all of the available a priori information is used to eliminate as many free parameters as possible and reduce the amount of information and experimental data. Using a starting structure and as much additional information as possible, like predicted chemical shifts and backbone torsion angles, the assignment and the structure itself are refined in an iterative process. Therefore we are developing AUREMOL, which goal is the reliable and automatic structure determination of biological macro molecules such as proteins from NMR data(1). In AUREMOL included modules are RELAX, which uses complete relaxation formalism to calculate a simulated NOESY NMR spectrum, SIBASA (simulated based sequential assignment), which bases on simulated annealing to determine the chemical shifts of these signals, KNOWNOE, REFINE, which calculates intermolecular distances from a NOESY spectrum and the structure evaluation RFAC. (1)Gronwald, W. and Kalbitzer, H.R. Automated Structure Determination of Proteins by NMR Spectroscopy. 2004, Progr. NMR Spectr. 44, 33-96

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Development of an Integrated System for High-Pressure NMR Spectroscopy on Proteins — •Werner Kremer, Markus Beck Erlach, Claudia E. Munte, T. Ernst, Rainer Hartl, M. Arnold, Dörte Rochelt, Dieter Niesner, and Hans Robert Kalbitzer — Institute of Biophysics and Physical Biochemistry, Regensburg

High hydrostatic pressure can induce multiple effects on proteins including denaturation, depolymerization, and changes of side chain protonation state. Pressure induced structural changes can be investigated with high pressure NMR spectroscopy, because different conformers in the energy-landscape of proteins are accessible via their different specific volume. Therefore static pressure in the range from 4-200 MPa has been applied to proteins and peptides. In addition the application of pressure jumps with a microprocessor controlled on-line pressure system has been performed in order to analyze possible structural intermediates which are not accessible by the utilization of static pressure. Quartz, sapphire or ceramic cells are used to handle the proteins in aqueous solutions during the experiment. The best results can be obtained with ceramic cells because they can withstand high pressures and can be easily handled. A completely new autoclave for these ceramic cells has been constructed, including an improved method for pressure transmission, an integrated safety jacket and a fast closing emergency valve.

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A NMR Bioreactor Setup for 5 mm High-Resolution Probes at 800 MHz — •Paul Ramm[1,2,3], Werner Kremer[1], Ulrich Bogdahn[2], Ludwig Aigner[3], and Hans Robert Kalbitzer[1] — [1]Department of Biophysics and Biophysical Chemistry, University of Regensburg — [2]Department of Neurology, University of Regensburg — [3]Institute of Molecular Regenerative Medicine, Paracelsus Medical University, Salzburg

NMR spectroscopy (MRS) of cells and tissues provides a quantitative insight into cellular composition and metabolism. A common approach is MRS of cell extracts, which can be used to distinguish e.g. different neural cell types [1]. MRS of living cells more closely resembles the in vivo situation, but is restricted due to unphysiological conditions in a NMR tube. Therefore, we are realizing a cell perfusion setup for a Bruker Avance 800 MHz spectrometer equipped with a 5 mm cryo probe. Immobilized cells can then be perfused with variably composed media saturated with a tunable gas mixture of N_2, O_2, and CO_2. Highly-resolved ^{1}H-NMR spectra (FWHM < 3 Hz) can be acquired with a temporal resolution < 5 min. Based on our former investigations on stem cell-specific biomarkers of neural progenitor cells (NPCs) [2], MRS of NPCs under controllable cell culture conditions will be the next step towards a metabolic profiling and biomarker screening of neurogenesis, leading to both a better understanding of stem cell metabolism and to a possible detection of neurogenesis in humans.

[1] Urenjak J et al., J Neurosci 13 (3), 1993
[2] Ramm P et al., Stem Cells 27 (2), 2009

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Xenon-Binding Studies of the Thermophilic Enzyme HisF from Thermotoga maritima Using NMR-Spectroscopy — •Christoph Liebold[1], Felix List[1], Hans Robert Kalbitzer[1],

Reinhard Sterner[1], and Eike Brunner[2] — [1]Institute of Biophysics and Physical Biochemistry, University of Regensburg, 93040, Germany — [2]Department of chemistry and food chemistry, Dresden University of Technology, 01062,Germany

The hydrophobic noble gas xenon is known to interact with hydrophobic cavities of macromolecules. Important functional entities of enzymes such as substrate tunnels or the active site often exhibit hydrophobic properties. Therefore, Xe can serve as a probe to explore these entities. [1] Moreover, Xe-binding sites are thought to be centres of increased flexibility, thus providing the possibility of conformational changes required for the function of the enzyme. We evaluated the influence of Xe-binding upon the enzyme HisF from the thermophilic bacterium Thermotoga maritima using ^{1}H-^{15}N HSQC spectra and detected xenon-induced conformational changes mainly for hydrophobic residues located around preexisting cavities within the molecule.This behavior indicates that xenon indeed binds into the aforementioned cavities. Biological implications of our observations are discussed and compared with the results of substrate-binding experiments.

[1] Rubin, S. M.; Spence M. M.; Goodson B. M.; Wemmer D. E. Pines A. Proc. Natl. Acad. Sci. U.S.A, 2000, 97, 9472-9475