BP 28: Focus session: Intracellular Spectroscopy

Organized by Vinod Subramaniam and Malte Drescher, this focus session addresses the biophysical challenges to study biological macromolecules using spectroscopic approaches, in particular in their natural intracellular environment.

Time: Thursday 9:30–13:00 Location: H44

Topical Talk BP 28.1 Thu 9:30 H44

Advanced Fluorescence Methods for Investigating the Life-cycle of Viruses — •Don C. Lamb — Department of Chemistry, Ludwig-Maximilians-Universität München, Munich, Germany

Advances in fluorescence spectroscopy and microscopy make it possible to perform quantitative experiments on biological systems. One of the goals within my group is to develop and apply methods to quantify the details of viral entry and assembly in live cells. To look at the early interactions of the structural HIV protein Gag in the cytosol, we combined pulsed interleaved excitation (PIE) with Raster Image Correlation Spectroscopy. Gag was labeled using fluorescent fusion proteins and PIE-RICS measurements were performed. A slower then expected diffusion of the Gag protein in the cytosol was observed even though no significant oligomerization of Gag was detected. To investigate the origin of the slow diffusion behavior, we measured the mobility of a number of mutant Gag molecules where different interaction sites have been altered. To investigate the fusion of viral particles, we have combined the advantages of single-particle-tracking with image correlation spectroscopy. Individual viruses are tracked in three-dimensions and the signal from the different channels in the volume surrounding the particle are cross-correlated. Using the TRacking Image Correlation analysis (TRIC), we detected multiple fusion events of Foamy Virus. The analysis revealed a novel intermediate step during the fusion process where the envelope and capsid are still connected although they are separated by several hundred nanometers.

BP 28.2 Thu 10:00 H44

Quantifying the milieu of organelles in living cells via fluorescence lifetime imaging — Nisha Pawar, Saeedeh Aliaskarisohi, and •Matthias Weiss — Experimental Physics I, University of Bayreuth, Germany

Compartmentalization is a hallmark of eukaryotic cells. A diverse set of organelles is dynamically maintained by eukaryotes to provide biochemical reaction vessels with a distinct milieu. Prominent examples are the endoplasmic reticulum (ER) and the Golgi apparatus, both of which are major constituents of the cell's secretory pathway. Along this pathway, about 10,000 different protein species are sorted and eventually sent to their final destination. It has long been hypothesized that the lumenal milieu of the ER and the distinct cisternae of the Golgi apparatus provide important cues for these sorting processes, yet assessing their milieu in a living cell has been a challenge. Using fluorescence lifetime imaging (FLIM), we have monitored the milieu of the Golgi apparatus and the ER under various conditions. We find a significant change in the Golgi milieu towards that of the ER when adding perturbative drugs. Moreover, following the trajectory of proteins from the ER through the Golgi, we can attribute distinct milieus to the individual sub-compartments along the secretory pathway, e.g. Golgi cisternae.

BP 28.3 Thu 10:15 H44

Protein motion in crowded solutions on fast time scales: diffusion and internal dynamics — •Felix Roosen-Runge[1], Marcus Hennig[1,2], Fajun Zhang[1], Tilo Seydel[2], and Frank Schreiber[1] — [1]Institut für Angewandte Physik, Universität Tübingen — [2]Institut Laue-Langevin, Grenoble, France

Protein function depends on the complex interplay of structure, dynamics and the aqueous, but crowded cellular environment. We present a comprehensive experimental study accessing the full hierarchy of protein dynamics in solutions, e.g. vibrations, interdomain motions and diffusion of the entire protein. Quasi-elastic neutron and dynamic light scattering experiments have been performed and compared to theoretical predictions. In crowded solutions, both self diffusion D_s and collective diffusion D_c of protein solutions are well described by colloidal concepts, with D_s reduced to 20% at $\approx$ 20% volume fraction [1,2]. Separating the motion of the entire protein molecule, the internal motions are accessed under native conditions [3]. We studied the dynamics before, during and after thermal denaturation, supporting the notion of protein unfolding with subsequent chain entanglement. In the denatured, gel-like state, long-range motions are strongly *reduced*, while the local flexibility is *enhanced*. Using the analysis frameworks, neutron backscattering is well-suited to address the relation of protein function and dynamics under native conditions at fast time scales.

[1] F. Roosen-Runge, M. Hennig et al., PNAS 108 (2011) 11815; [2] M. Heinen, F. Zanini et al., Soft Matter 8 (2012) 1404; [3] M. Hennig, F. Roosen-Runge et al., Soft Matter 8 (2012) 1628

BP 28.4 Thu 10:30 H44

Labelfree coherent Raman scattering microspectroscopy: A new intracellular spectroscopy tool in lipidomic research — •Gregor Hehl[1], Margot Grandl[2], Alexander Kovalev[1], Markus Peer[2], Gerhard Liebisch[2], Gerd Schmitz[2], and Andreas Volkmer[1] — [1]3rd Institute of Physics, University of Stuttgart, 70569 Stuttgart, Germany — [2]University Hospital Regensburg, 93053 Regensburg, Germany

A series of technological advances have made coherent Raman scattering (CRS) microscopy a highly sensitive and chemically selective imaging tool for the noninvasive analysis of biological cells. To facilitate a spectral identification and quantitative analysis of intracellular lipid species of a priori unknown composition by CRS microscopy, we developed the technique of hyperspectral coherent anti-Stokes Raman scattering (CARS) imaging. Here, we report on its exemplifying applications in lipidomic research. We investigated the uptake mechanism of atherogenic model lipoproteins into THP-1 macrophages. We observed a decrease of the degree of acyl unsaturation within individual lipid droplets in those cells, which have been loaded with oxidized lipoproteins, compared to the unperturbed cell. Furthermore, we addressed the question: Can an intracellular Raman-based spectroscopy selectively identify and detect minor molecular structure differences between the different lipid oxidation pathways in the living cell? Corresponding mass spectra of the respective cellular lipid contents were compared and correlated with our results obtained from Raman and CRS microscopy, and will also be presented.

BP 28.5 Thu 10:45 H44

Mapping lipid membrane molecular properties via vibrational imaging of vesicular systems — •Hilton B. de Aguiar and Andreas Volkmer — 3. Physikalisches Institut, Universitaet Stuttgart, Pfaffenwaldring 57, 70569 Stuttgart

Probing the physical properties and chemical composition of lipid membranes is of fundamental importance to understand their role on biological cell functioning. Unlike in conventional fluorescence-based microscopies of membranes, which require specific labeling and may perturb the molecular properties of interest, Raman microspectroscopy directly probes the intrinsic vibrational spectral response of the membrane lipids offering the advantage of both chemical specificity and sub-micron spatial resolution in a noninvasive manner. Here, we report on the spatially resolved Raman spectroscopy of vesicular model membranes (Giant Unilamellar and Multilamellar Vesicles). We observe distinct Raman image patterns of an individual vesicle for distinct vibrational modes. We successfully model this sensitivity of the Raman image contrast to the physical properties of the lipids by taking the higher order optical nature of Raman scattering into account. Remarkably, the image patterns turn out to be very sensitive on molecular orientation and order parameters offering the opportunity of membrane structure analysis by label-free Raman microscopy. Furthermore, we demonstrate how the Raman spectral response can be readily used to distinguish between thermodynamic phases (liquid/gel state) and domain composition of membrane lipids.

BP 28.6 Thu 11:00 H44

Plasmonic Nanoantennas for SERS on Supported Membranes — •Paul Kühler, Theobald Lohmüller, and Jochen Feldmann — Chair for Photonics and Optoelectronics, LMU München

We utilize plasmonically coupled gold triangles for Surface enhanced Raman scattering (SERS) measurements of a fluid lipid bilayer. First, large arrays of plasmonic nanoantennas made of gold triangles are pre-

pared on a solid support by an improved colloidal lithography technique. Then, a fluid supported membrane is formed on the intervening glass substrate by vesicle fusion. We demonstrate the applicability of this platform for spectroscopic investigations by performing SERS measurements of molecules that are constituents of a fluid supported phospholipid membrane. Our method offers a tool to analyze lipid membranes and membrane components under physiological conditions without fluorescent labeling or static entrapment of the membrane molecules.

15 min break

Topical Talk BP 28.7 Thu 11:30 H44

Looking at proteins inside live cells with atomic resolution: Science fiction or science reality? — •Phil Selenko — FMP Berlin

A protein's structure and its dynamic behavior are inherently dependent on its physiological environment. While most proteins function inside live cells, our knowledge about them is largely based on experiments that bear little resemblance to the physical and biological properties of a cell. A new approach in liquid-state NMR spectroscopy, in-cell NMR, now offers convenient means to directly study proteins at atomic resolution inside live cells. In this lecture, I will introduce the basic concepts behind in-cell NMR spectroscopy and provide examples of the structural and dynamic characteristics of a human amyloid protein in five different mammalian cell types, including dopaminergic neurons.

BP 28.8 Thu 12:00 H44

Inhibition of amyloid formation is impeded at the phospholipid interface — •Michael Schleeger[1], Corianne van den Akker[2], Maarten Engel[2], Tobias Weidner[1], Gijsje Koenderinke[2], and Mischa Bonn[1] — [1]Max Planck Institute for Polymer Research, Ackermannweg 10, 55128, Mainz, Germany — [2]FOM Institute AMOLF, Science Park 104, 1098 XG Amsterdam, The Netherlands

Several severe human diseases (including Alzheimer's, Parkinson's disease and type 2 diabetes mellitus) are characterized by the deposition of proteins as insoluble fibrils, also called amyloids. Amyloids fibrils are formed by self-assembly of peptide or protein precursors, which aggregate spontaneously into highly well-defined polymeric structures. The formation of amyloids can be inhibited by polyphenols, as has been previously shown in bulk studies. However, recent studies have shown that amyloid fibrils assemble and exert their cytotoxicity at cellular membranes, rather than in bulk solution. We therefore investigated the inhibitor activity specifically at the phospholipid membrane interface. We show, using surface-specific sum frequency generation (SFG) spectroscopy, that the commonly used amyloid inhibitor epigallocatechin gallate (EGCG) is a much less efficient inhibitor of amyloid formation at a lipid interface than in bulk solution. Moreover, we demonstrate that EGCG is not able to disaggregate existing amyloid fibrils at a lipid interface, in contrast to its behavior in bulk. Clearly, inhibitors are much less effective at membrane surfaces, which should be considered during the design and testing of novel amyloid inhibitors.

BP 28.9 Thu 12:15 H44

Calculation of the CD spectrum of a flexible peptide — •Zlatko Brkljača[1], Karmen Čondić-Jurkić[1,2], Momir Mališ[2], David M. Smith[2,3], and Ana-Sunčana Smith[1] — [1]Institut für Theoretische Physik, Universität Erlangen-Nürnberg, Erlangen, Germany — [2]Ruđer Bošković Institute, Zagreb, Croatia — [3]Computer Chemie Centrum, Universität Erlangen-Nürnberg, Erlangen, Germany

Circular dichroism (CD) spectroscopy is a standard experimental method employed in the structural characterization of optically active chiral molecules, such as proteins and peptides. Because the CD spectrum of a molecule is an ensemble average over its entire conformational phase space, the interpretation of the experimental data is challenging. This is particularly true for highly flexible peptides, where the spectra cannot be unambigously interpreted without theoretical modelling. However, so far there has been no accurate theoretical approach to predict peptide CD spectra. We address this problem by developing a method that combines replica exchange molecular dynamics to generate the conformational phase space, and TDDFT to calculate the electronic transitions. Thereby we systematically treat the solvent induced polarization of the peptide. We validate our method on an example of the CD spectrum of the opioid growth factor (Met-enkephalin). On the level of the single conformation we compare TDDFT with high level ab-initio calculations (RI-CC2), while on the level of the ensemble we make a comparison with the experimentally measured spectrum. Good agreement is obtained in both cases.

BP 28.10 Thu 12:30 H44

CD-Spectroscopic Assessment of Potential Antigenicity of Negatively Charged Biopharmaceuticals — •Sven Brandt[1], Krystin Krauel[1], Kay Gottschalk[2], Christiane Helm[3], Andreas Greinacher[4], and Stephan Block[1] — [1]ZIK HIKE - Humorale Immunreaktionen bei kardiovaskulären Erkrankungen, Fleischmannstr. 42-44, D-17475 Greifswald, Germany — [2]Institut für Experimentelle Physik, Universität Ulm, D-89069 Ulm, Germany — [3]Institut für Physik, Ernst-Moritz-Arndt Universität, Felix-Hausdorff-Str. 6, D-17487 Greifswald, Germany — [4]Institut für Immunologie und Transfusionsmedizin; D-17475 Greifswald, Germany

Platelet factor 4 (PF4), a protein with a high positive surface charge forms complexes with natural or artificial polyanions (PA). It is known that such complexes exhibit an antigen of the adverse drug reaction heparin induced thrombocytopenia (HIT) and that their antigenicity is three fold: (i) the molar ratio between PF4 and the PA, (ii) the charge density of the PA, and (iii) the chain length of the PA. To the best of our knowledge, we show for the first time by circular dichroism (CD) spectroscopy that the secondary structure of the protein is altered when complexes which are known to be antigenic are formed. Here we correlate the changes in the proteins secondary structure determined using CD spectroscopy with HIT antigenicity determined by ELISA. This allows us to assess potential antigenicity of negatively charged biopharmaceuticals without the necessity of in vivo studies or the use of antibodies isolated from immunized patients specific for the antigenic epitopes.

BP 28.11 Thu 12:45 H44

Ribonuclease A: A model system to study intrinsically unfolded proteins — •Jennifer Fischer[1], Ralf Biehl[1], Bernd Hoffmann[2], and Dieter Richter[1] — [1]ICS-1, FZ-Jülich, Jülich, Germany — [2]ICS-7, FZ-Jülich, Jülich, Germany

Structure and dynamics play the key role in protein function, but roughly 30% of eukaryotic proteins are partially or even completely unfolded [1]. Nevertheless, intrinsically unfolded proteins are functional and involved in several biological processes. To get further insight into disordered structures and their dynamics, we use Ribonuclease A (RNase A) as a model system, as it is a well known protein denaturing reversibly upon heating. Additionally, by varying the buffer conditions by different pH values, several states can be prepared. A detailed study of the structure and dynamics using Small Angle Neutron and X-ray Scattering (SANS, SAXS) as well as Neutron Spin Echo Spechtroscopy (NSE) and Circular Dichroism Spectroscopy is presented. The combination of these techniques allows us to observe large-scale internal dynamics of subdomains or of unfolded protein strands that operate on the same length scale as rotational diffusion. However, the timescale can be different and depends on the protein structure and internal interactions. [1] A. L. Fink, Current Opinion in Structural Biology 2005, 15:35-41