BP 15: Proteins II

Time: Wednesday 15:00–17:30 Location: H 1058

Topical Talk BP 15.1 Wed 15:00 H 1058

Electron Paramagnetic Resonance in Protein Science — •Malte Drescher — Universität Konstanz, Konstanz, Germany

Electron paramagnetic resonance (EPR) spectroscopy has witnessed tremendous methodological and instrumental developments during the last two decades. These new methods have strong impact on various areas of chemistry, materials science, physics, and especially biophysics. With the advent of site-directed spin-labeling (SDSL) of proteins and DNA or RNA, EPR spectroscopy thus became a valuable technique for obtaining information on structure and dynamics of biomacromolecules.

Intrinsically disordered proteins (IDPs) form a unique protein category characterized by the absence of a well-defined structure and by remarkable conformational flexibility. SDSL EPR is amongst the most suitable methods to unravel their structure and dynamics.

This contribution summarizes methodological developments in the area of SDSL EPR and its applications in protein research. Recent results on the intrinsically disordered Parkinson's disease protein α-Synuclein illustrate that the method gains increasing attention in IDP research.

BP 15.2 Wed 15:30 H 1058

Probing the Ca2+ - switch of the neuronal Ca2+ sensor GCAP2 by time-resolved fluorescence spectroscopy — •Heiko Kollmann[1], Simon F. Becker[1], Javid Shirdel[1], Anna Osterndorp[2], Christoph Lienau[1], and Karl-Wilhelm Koch[2] — [1]Ultraschnelle Nano-Optik, Institut für Physik, Fakultät V, Universität Oldenburg, 26111 Oldenburg, Deutschland — [2]Biochemie, Institut für Biologie und Umweltwissenschaften, Fakultät V, Universität Oldenburg, 26111 Oldenburg, Deutschland

We report fluorescence lifetime and rotational anisotropy measurements of the fluorescence dye Alexa647 attached to the guanylate cyclase-activating protein 2 (GCAP2), an intracellular myristoylated calcium sensor protein operating in photoreceptor cells. By linking the dye to different protein regions critical for monitoring calcium-induced conformational changes, we could measure fluorescence lifetimes and rotational correlation times as a function of myristoylation, calcium and position of the attached dye, while keeping the GCAP2 protein operational. We observe distinct site-specific variations in the fluorescence dynamics when externally changing the protein conformation. A key feature of the dynamics of the protein-dye complex is the up- and down-movement of an α-helix that is situated between the two specific linking positions. Operation of this piston-like movement is triggered by the intracellular messenger calcium.

BP 15.3 Wed 15:45 H 1058

Photo-cycle dynamics of photo-activated adenylate cyclase (nPAC) from the amoeboflagellate *Naegleria gruberi* **NEGM strain** — •Alfons Penzkofer[1], Manuela Stierl[2], Peter Hegemann[2], and Suneel Kateriya[3] — [1]Fakultät für Physik, Universität Regensburg, Universitätsstrasse 31, D-93053 Regensburg, Germany — [2]Institut für Biologie/Experimentelle Biophysik, Humboldt Universität zu Berlin, Invalidenstrasse 42, D-10115 Berlin, Germany — [3]Department of Biochemistry, University of Delhi South Campus, Benito Juarez Road, New Delhi 110021, India

nPAC comprises a BLUF domain (blue light sensor using flavin) and a cyclase homology domain (CHD). The nPAC gene was expressed heterologously in *E.coli* and the photo-dynamics of the nPAC protein was studied by optical absorption and fluorescence spectroscopy. Blue-light exposure of nPAC caused a typical BLUF-type photo-cycle behavior (spectral absorption red-shift, fluorescence quenching, absorption and fluorescence recovery in the dark). Additionally, time-delayed reversible photo-induced one-electron reduction of fully oxidized flavin (Fl_{ox}) to semi-reduced flavin ($FlH^{\cdot}$) occurred. Furthermore, photo-excitation of $FlH^{\cdot}$ caused irreversible electron transfer to fully reduced anionic flavin (FlH^{-}). A photo-induced electron transfer from Tyr to flavin ($Tyr^{\cdot +}$ - $Fl^{\cdot -}$ radical ion-pair formation) caused H-bond restructuring responsible for BLUF-type photo-cycling and permanent protein re-conformation enabling photo-induced flavin reduction by proton transfer. Some photo-degradation of Fl_{ox} to lumichrome was observed. A model of the photo-cycle dynamics of nPAC was developed.

BP 15.4 Wed 16:00 H 1058

A coarse-grained model for protein folding based on structural profiles — •Katrin Wolff[1], Michele Vendruscolo[2], and Markus Porto[3] — [1]SUPA, School of Physics & Astronomy, University of Edinburgh, UK — [2]Department of Chemistry, University of Cambridge, UK — [3]Institut für Theoretische Physik, Universität Köln

We present a coarse-grained protein model based on structural profiles and apply it to the study of protein free energy landscapes and folding trajectories. Our model's two main characteristics are a tube-like geometry to describe the self-avoidance effects of the polypeptide chain, and an energy function based on a one-dimensional structural representation [1]. The latter specifies the connectivity of a sequence in a given conformation, so that the energy function, rather than favoring the formation of specific native pairwise contacts, promotes the establishment of a specific native connectivity for each amino acid. We illustrate our approach by applying the model to the folding of the villin headpiece domain to study its folding behavior and determine heat capacities, free energy landscapes and folding trajectories in various reaction coordinates [1,2]. The results closely resemble those found in extensive molecular dynamics studies and support the idea that coarse-grained models that solely rely on the self-avoidance and the connectivity of a polypeptide chain faithfully reproduce many aspects of the folding behaviour of proteins.

[1] K. Wolff, M. Vendruscolo, M. Porto, Phys. Rev. E **84**, 041934 (2011)

[2] K. Wolff, M. Vendruscolo, M. Porto, EPL **94**, 48005 (2011)

BP 15.5 Wed 16:15 H 1058

Dielectric Relaxation Spectroscopy of Ubiquitin by Poisson-Boltzmann-Monte Carlo Studies — •Stephan Kramer[1], Bartosz Kohnke[1], and Reiner Kree[2] — [1]Institut f. Numerische u. Angewandte Mathematik, Universität Göttingen, Lotzestrasse 16-18, D-37083 Göttingen — [2]Institut f. Theoretische Physik, Universität Göttingen, Friedrich-Hundt-Platz 1, D-37077 Göttingen

To reliably predict the function of ubiquitin it is necessary to know its conformational dynamics on all relevant time-scales. Due to its omnipresence in a multitude of key regulatory processes like molecular recognition or signal transduction these time-scales span from nano- to microseconds. Not all of them are accessible by NMR relaxation dispersion techniques which so far have been the standard way to measure conformational sampling. Only recently [1] dielectric relaxation spectroscopy (DRS) was introduced as further means to measure internal dynamics beyond the temporal resolution NMR is capable of. To get further insight into the measurement process we want to simulate DRS on a single molecule level using standard Monte Carlo techniques for the intramolecular motion. The properties of the ionic solvent and its interaction with the protein are assesed by solving the corresponding Poisson-Boltzmann equation by multigrid methods. For comparison with DRS experiments we compute dielectric loss spectra from the bulk dielectric moment to assess the the interconversion of different comformations of ubiquitin.

[1] David Ban et al. Kinetics of Conformational Sampling in Ubiquitin, Angew. Chem. Int. Ed. 2011, **50**, 11437-11440

BP 15.6 Wed 16:30 H 1058

Origin of decrease in potency against HIV-2 protease by HIV-1 protease inhibitors — •Parimal Kar and Volker Knecht — Max Planck Institute of Colloids and Interfaces, Am Mühlenberg 1, 14476 Potsdam, Germany

The acquired immune deficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV) type 1 and 2 (HIV-1 and HIV-2). An important target for AIDS treatment is the use of HIV protease (PR) inhibitors preventing the replication of the virus. In this work, the popular molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) method has been used to investigate the effectiveness of the HIV-1 PR inhibitors darunavir, GRL-06579A, and GRL-98065 against HIV-2 and HIV-1 protease. The affinity of the inhibitors for both HIV-1 and HIV-2 PR decreases in the order GRL-06579A < darunavir < GRL-98065, in accordance with experimental data. On the other hand, our results show that all these inhibitors bind less strongly to HIV-2 compared to HIV-1 protease, again in agreement with experimental findings. The decrease in binding affinity for HIV-2

relative to HIV-1 PR is found to arise from an increase in the energetic penalty from the desolvation of polar groups (DRV), or a decrease in the size of the electrostatic interactions between the inhibitor and the PR (GRL-06579A and GRL-98065). For GRL-98065, also a decrease in the magnitude of the van der Waals interactions contributes to the reduction in binding affinity. A detailed understanding of the molecular forces governing binding and drug resistance might assist in the design of efficient inhibitors against HIV-2 protease.

BP 15.7 Wed 16:45 H 1058

Dynamics of highly concentrated protein solutions around the denaturing transition — •Tilo Seydel[1], Marcus Hennig[1,2], Felix Roosen-Runge[2], Fajun Zhang[2], Stefan Zorn[2], Maximilian W.A. Skoda[3], Robert M.J. Jacobs[4], and Frank Schreiber[2] — [1]Institut Laue-Langevin, Grenoble, France — [2]Institut für Angewandte Physik, Universität Tübingen, Germany — [3]ISIS, RAL, Chilton, Didcot, UK — [4]CRL, University of Oxford, UK

We discuss the dynamics of highly concentrated aqueous protein solutions around the denaturing transition [1]. For the temperature range $280\text{K} < T < 370\text{K}$, the total apparent mean-squared displacement $\langle u^2 \rangle$ is recorded in solutions of bovine serum albumin by fixed-window neutron scattering. $\langle u^2 \rangle$ increases monotonically with T below and above denaturation, whereas it decreases strongly at the denaturing transition. This observation can be rationalized and modeled as a transition from a liquid protein solution to a gel-like state. Atomic vibrations as well as librations and diffusion of the entire protein contribute to $\langle u^2 \rangle$. The diffusion monitored by quasi-elastic neutron scattering [1,2] is consistent with a significant hindrance due to entanglement of the chains upon denaturing. The related pronounced decrease in $\langle u^2 \rangle$ is analytically separated. Thus, we extract the purely intramolecular dynamics around the denaturing transition of freely diffusing proteins. This analysis introduces a general concept, which is applicable to other colloid systems exhibiting both center-of-mass and internal dynamics [1].

[1] M. Hennig et al., Soft Matter, DOI:10.1039/c1sm06609a;
[2] F. Roosen-Runge et al., PNAS 2011, 108:11815

BP 15.8 Wed 17:00 H 1058

Correlates between Biophysical Dynamics and Sequence Evolution of Proteins — •Kay Hamacher — TU Darmstadt, 64287 Darmstadt, Germany

The evolution of proteins is shaped by two major evolutionary operators: mutation and selection. While entropy concepts from information theory reveal important patterns in the sequence space, the selective advantage of changes reveal themselves in the molecular phenotype. Therefore, to understand protein evolution in more detail, one needs to correlate the results of a sequence analysis with those from biophysical simulations of the expressed proteins. In this talk I will discuss several algorithmic improvements [1-3] and their applications to important proteins in medicinal physics/chemistry, namely the HIV1-protease and the acetylcholinesterase [4-6].

[1] K. Hamacher. Phys. Rev. E 84:016703, 2011
[2] K. Hamacher. J. Comp. Phys. 229:7309-7316, 2010
[3] M. Waechter, K. Hamacher, F. Hoffgaard, S. Widmer, M. Goesele. 9th Int. Conf. on Parallel Processing & Appl.Mathematics, 2011
[4] P. Boba, P. Weil, F. Hoffgaard, K. Hamacher. Springer Communications in Computer & Information Science 127:356-366, 2011
[5] S. Weißgraeber, F. Hoffgaard, K. Hamacher. Proteins 79(11):3144-3154, 2011
[6] K. Hamacher. Gene 422:30-36, 2008

BP 15.9 Wed 17:15 H 1058

Hierarchical Expansion of the Kinetic Energy Operator in Curvilinear Coordinates — Daniel Strobusch and •Christoph Scheurer — Lehrstuhl für Theoretische Chemie, TU München, Lichtenbergstr. 4, 85748 Garching, Germany

Multidimensional nonlinear IR experiments provide detailed dynamical information about peptides on short timescales. The interpretation of these spectra relies heavily on theoretical simulations of anharmonic vibrational systems. A powerful approach to calculate anharmonic vibrational spectra is the vibrational self-consistent field method (VSCF) and its configuration interaction extension (VCI).

Couplings and correlation between different modes can be reduced by employing curvilinear coordinates [1], which is of utmost importance for larger systems. However, these coordinates at the same time introduce a more complex form of the kinetic energy operator T, which has been known for a long time. Its evaluation is involved though, with coordinate dependent reduced masses and kinematic couplings.

A new systematic hierarchical expansion of T is presented, which allows us to judge the quality of simpler ad-hoc approximations [2]. VSCF and VCI calculations for small model systems were performed and the influence of different terms in the kinetic energy operator was studied in detail to allow for efficient approximations.

[1] M. Bounouar and Ch. Scheurer, *Chem. Phys.* 347 (2008), 194
[2] D. Strobusch and Ch. Scheurer, *J. Chem. Phys.* 135 (2011), 124102; *ibid.*, 144101