BP 2: Proteins

Time: Monday 9:30–12:45 Location: H44

Topical Talk BP 2.1 Mon 9:30 H44

Energy conversion mechanisms of heat shock proteins — •Thorsten Hugel — Physik Department and IMETUM, Technische Universität München, Boltzmannstr.11, 85748 Garching, Germany

Single molecule methods allow real time observation of molecular machines at work. We have utilized single molecule Förster Resonance Energy Transfer (smFRET) to decipher the mechano-chemical cycle of the heat shock proteins yeast Hsp90 [1] and bacterial Hsp90 [2]. Although they are homolog we observe significant differences in domain movement and in their mechanism of energy conversion.

To further elucidate the structure-function relationship in these Hsp90s we use optical tweezers and a smFRET based nanopositioning system. Our in vitro results are mostly consistent with the crystal structure of yeast Hsp90 [3], but show some significant deviations in the N-terminal domain.

Finally, these methods are not only suited to determine the structure and function of isolated single proteins, but yield valuable insights into their interplay with other proteins.

[1] Ratzke et al., PNAS (2012) [2] Ratzke et al., JMB (2012) [3] Ali et al., Nature (2006)

BP 2.2 Mon 10:00 H44

Peptide with a trigger: The aggregation and refolding of pH sensitive peptide GALA in solution and at interfaces — •Denise Schach, Christoph Globisch, Adrian Fuchs, Clemens K. Weiss, Christine Peter, Mischa Bonn, and Tobias Weidner — Max-Planck-Institut für Polymerforschung, Ackermannweg 10, D-55128 Mainz

GALA is a 30 amino acid synthetic peptide consisting mainly of the Glu-Ala-Leu-Ala repeat motif. Originally it was designed to act as a shuttle across lipid membranes in drug or gene delivery systems. Importantly, structure and function can be triggered by pH. The triggering mechanism relies on the conversion from a random coil to an amphipathic α-helix when decreasing the pH from 7 to 5. The helix formation is driven by protonation, i.e. neutralization, of the Glu side at pH 5, which makes a folded structure energetically more favorable. The repetition of hydrophobic and hydrophilic residues is responsible for the amphipathic peptide properties in the helical state. In its amphipathic state, GALA is also likely to aggregate. Near a lipid membrane the aggregation leads to cell penetration, pore formation and membrane leakage. To better understand the fundamental mechanisms of refolding and aggregation in bulk solution and at interfaces, we probe structural details with Fourier-transform infrared spectroscopy and sum frequency generation. Moreover, we apply Brewster angle microscopy and light scattering to follow aggregation. The interpretation of the experiments is complemented by molecular dynamics simulations of GALA refolding and aggregation.

BP 2.3 Mon 10:15 H44

Structure and dynamics of the iron binding protein Lactoferrin studied with neutron scattering — •Clemens Sill[1], Ralf Biehl[1], Bernd Hoffmann[2], and Dieter Richter[1] — [1]JCNS-1 & ICS-1: Neutron Scattering, Forschungszentrum Jülich, Germany — [2]ICS-7: Biomechanics, Forschungszentrum Jülich, Germany

The understanding of the functionality of proteins started with a rigid model, namely the Lock and Key analogy. Meanwhile, a more dynamic and flexible picture of these macromolecules has evolved to explain protein function. The importance of thermodynamically driven, internal motions for the functioning of proteins is subject of ongoing research.

Lactoferrin is an iron-binding protein with antimicrobial activity as a part of the innate immune system. It consists of two binding sites located in a cleft of the two main domains, each is capable of binding and releasing one iron ion. A combined approach of Small Angle Neutron Scattering for structural characterization and Neutron Spin Echo spectroscopy to elucidate the dynamic properties of different binding states was undertaken to enlighten the influence of iron binding on large scale protein dynamics. A comparison of the SANS data with 3D structures (crystallography and homology models) proved that the binding sites are closed when occupied by iron and open otherwise. In combination with normal mode analysis it was found from the NSE measurements that the internal dynamics are dominated by fluctuations of the main domains relative to each other. Stretching and twisting motions can describe the found dynamics, and their occurrence is independent whether the domains are open or closed.

BP 2.4 Mon 10:30 H44

Influence of surface and subsurface properties on the structure and activity of adsorbed lysozyme — •Christian Spengler[1], Stéphane Mesnage[2], Hendrik Hähl[1], Peter Loskill[1], Simon J. Foster[2], and Karin Jacobs[1] — [1]Saarland University, Experimental Physics, 66041 Saarbrücken — [2]University of Sheffield, The Krebs Institute, Department of Molecular Biology and Biotechnology, Sheffield S10 2TN, United Kingdom

Protein adsorption is the first step in biofilm formation: Protein films serve as a conditioning layer that enables and affects the attachment of bacteria and other organisms. Hence, the understanding and control of protein layers is an important task that is relevant to life sciences and engineering. Previous studies revealed that the structure of adsorbed proteins and the adhesion force of bacteria depend on both the surface properties and the subsurface composition of the adsorbent material [1,2]. These findings raise the question whether or not the activity and effectivity of adsorbed proteins are also influenced by the properties of the underlying material. In this study, we investigate how the activity -the bactericidal effect- of adsorbed lysozyme is affected by surface and subsurface properties. The activity is thereby characterized by measuring the turbidity of a very sensitive protein assay containing purified peptidoglycan.

[1] Hähl et al., Langmuir 28 (2012) 7747-7756
[2] Loskill et al., Langmuir 28 (2012) 7242-7248

BP 2.5 Mon 10:45 H44

Integrating Genomic Information with Molecular Simulation for Protein Dynamics — •Alexander schug[1], Hendrik Szurmant[2], Martin Weigt[3], and Abhinav Verma[1] — [1]Karlsruhe Institute of Technology — [2]The Scripps Reserach Institute — [3]Université Pierre et Marie Curie

Protein function often requires a protein to form a complex or adopt multiple conformations during its function cycle. Structural characterization of these states is experimentally difficult as they are typically stabilized by transient interactions. Here, we demonstrate how a mixed theory approach can predict such structures on the example of two-component signal transduction systems (TCS), a ubiquitous signal response system. We predicted the TCS complex structure in high agreement (3.5 RMSD) with concurrent experimental work [1] by combining molecular dynamics [2] and statistical genomic analysis [3]. Similarly, we were able to predict the active conformation occurring during autophosphorylation by identifying co-evolving interdomain amino acid pairs in agreement with biochemical mutagenesis data [3]. We can now simulate the conformational transition between active and inactive conformations, quantify its free-energy barrier and its change as reaction to transmembrane forces exercised by the sensor domain. [unpublished data]

[1] Schug A et al., PNAS (2009) 106, 22124-22129
[2] Schug A and Onuchic J, Curr Opin Pharm (2010) 10, 709-714
[3] Weigt M et al., PNAS (2009) 106, 67-72
[4] Dago A et al., PNAS (2012), 109, E1733-42

15 min break

BP 2.6 Mon 11:15 H44

Urea's effect on protein secondary structures — •Beate Moeser and Dominik Horinek — Institut für Physikalische und Theoretische Chemie, Universität Regensburg, 93040 Regensburg

Proteins in cells are surrounded by a large variety of chemical compounds (as e. g. metabolites, messenger substances, and osmoregulators). Some of these cosolutes denature proteins and others (over-)stabilize their native fold. This is due to the fact, that they interact differently with different protein secondary structures. The molecular origin thereof, however, is not yet fully understood.

We developed a simulation setup [1] for molecular dynamics simulations, which allows us to quantitatively investigate cosolute effects on various secondary structural elements and provides insight into the molecular forces at play.

Here, we present the influence of the denaturant urea on homopep-

tides in four distinct conformations: extended strand, 3_{10}-helix, α-helix, and β-sheet. Furthermore, we check, whether the strength of urea's effect on a given structure is proportional to the solvent accessible surface area of the conformation. This is one of the main assumptions of the group transfer model (TM), which is widely used to predict cosolute effects on proteins. Our quantitative checks allow for a detailed validation and assessment of implementations of the TM and its conclusions concerning the role of the backbone and sidechains in urea denaturation.

[1] Horinek, D., Netz, R.R. 2011. *J Phys Chem A 115(23)*, 6125-6136

BP 2.7 Mon 11:30 H44

Internal protein dynamics - a study on fully deuterated cyano phycocyanin by 2H NMR experiments and random-walk simulations — •Kerstin Kämpf, Beke Kremmling, and Michael Vogel — TU Darmstadt

Although possessing an ordered structure, proteins exhibit a versatile but common internal dynamics. The precise nature and geometry of this motion remains, however unclear. In order to investigate this, a combined approach of solid state 2H NMR and random-walk simulations (RWS) is used. Solid state 2H NMR is sensitive to the time scale as well as the geometry of motion[1]. It has been applied to samples of fully deuterated c-phycocyanin (hydration h= 0 g/g,h= 0.3 g/g). Suppressing the contribution of the fast methyl groups, we find that the protein backbone exhibits a temperature dependent small amplitude motion. The NMR parameters of the backbone motion are calculated by RWS for two limiting cases: A heterogeneous scenario with temperature dependent correlation times and a homogeneous scenario, in which the amplitude of motion increases with temperature. The RWS show that the existence of a T dependent amplitude of the motion is a main feature of internal protein dynamics. Nevertheless a single T dependent angle, increasing from 0°-15° for 200<T<300 K, cannot explain all experimental observations. A distribution of angles is required for a good description of the observations in 2H NMR. Thus, the present study reveals that internal protein dynamics is a complex motion with an amplitude that strongly depends on temperature.

[1]Lusceac, BBA, (2010), 1804, 41-48.

BP 2.8 Mon 11:45 H44

Effects of ligand binding on the mechanical properties of ankyrin repeat proteins — •Giovanni Settanni[1], David Serquera[2], Piotr Marszalek[3], Emanuele Paci[4], and Laura Itzhaki[5] — [1]Physics Department, Johannes Gutenberg University, Mainz, Germany — [2]Hutchison/MRC Research Centre, Cambridge, UK — [3]Duke University, Durham, NC, USA — [4]University of Leeds, UK — [5]University of Cambridge, UK

Ankyrin repeat proteins are elastic materials that unfold and refold repeat by repeat, under force. Herein we use atomistic molecular dynamics to compare the mechanical properties of the 7-repeat protein Gankyrin in isolation and in complex with its binding partner S6-C. We show that the bound S6-C greatly increases the resistance of Gankyrin to mechanical stress. The effect is specific to those repeats of Gankyrin directly in contact with S6-C. A consequence of the localized nature of ligand binding is that it impacts on all aspects of the protein's mechanical behavior, including the order of repeat unfolding, the diversity of unfolding pathways, the nature of partially unfolded intermediates, the forces required and the work transferred to the system to unfold the whole protein and its parts. Stepwise unfolding thus provides the means to buffer repeat proteins and their binding partners from mechanical stress in the cell. Our results illustrate how ligand binding can control the mechanical response of proteins. The data also point to a cellular mechano-switching mechanism whereby binding between two partner macromolecules is regulated by mechanical stress.

BP 2.9 Mon 12:00 H44

Regulatory mechanism of the light-activable DNA-binding switch LOV-TAP : A computer simulation study — Emanuel Peter, Bernhard Dick, and •Stephan A Baeurle — Institute of Physical and Theoretical Chemistry, University of Regensburg, D-93040 Regensburg, Germany

The spatio-temporal control of gene expression is fundamental to elucidate cell proliferation and deregulation phenomena in living systems. Novel approaches based on light-sensitive multi-protein complexes have recently been devised, showing promising perspectives for the reversible modulation of the DNA-transcriptional activity in vivo. This has lately been demonstrated in a striking way through the generation of the artificial protein construct light-oxygen-voltage (LOV)-tryptophan-activated protein (TAP), in which the LOV2-Jalpha photoswitch of phototropin1 from Avena sativa (AsLOV2-Jalpha) has been ligated to the tryptophan-repressor (TrpR) protein from Escherichia coli. Here, we elucidate the early stages of the light-induced regulatory mechanism of LOV-TAP at the molecular level, using the non-invasive molecular dynamics simulation technique [1]. More specifically, we find that Cys450-FMN-adduct formation in the AsLOV2-Jalpha-binding pocket after photoexcitation induces the flexibilization through unfolding of a hairpin-like helix-loop-helix region interlinking the AsLOV2-Jalpha- and TrpR-domains, ultimately enabling the condensation of LOV-TAP onto the DNA surface.

[1] E. Peter, B. Dick, S.A. Baeurle, Prot. Struct. Funct. Bioinf., in press (2012); doi:10.1002/prot.24196

BP 2.10 Mon 12:15 H44

Zinc Finger Proteins and the 3D Organization of Chromosomes — •Dieter Heermann[1], Christoph Feinauer[1], Sebastian Goldt[2], Andreas Hofmann[1], Lei Liu[1], and Gabriell Mate[1] — [1]Institut für Theoretische Physik, Universität Heidelberg, Philosophenweg 19, 69120 Heidelberg — [2]Fitzwilliam College, Cambridge University, Cambridge, England

Zinc finger domains are one of the most common structural motifs in eukaryotic cells. These DNA-binding proteins contain up to 37 zinc finger domains connected by flexible linker regions. They have shown to be important organizers of the 3D structure of chromosomes and as such are called the master weaver of the genome.

Our results indicate that the binding affinity is increased by the flexible linkers by several orders of magnitude. Moreover, the binding map for proteins with more than one domain exhibits interesting structures which, having been neither observed nor described before can be interpreted to fit very well with existing theories of facilitated target location.

We have developed a methodology to characterize these flexible proteins. Employing the concept of barcodes we propose a measure to compare such flexible proteins in terms of a similarity measure. This measure is validated by a comparison between a geometric similarity measure and the topological similarity measure that takes geometry as well as topology into account.

BP 2.11 Mon 12:30 H44

Stochastic dynamics of direct and hierarchical virus capsid assembly — •Heinrich Klein[1], Johanna Baschek[1], and Ulrich S. Schwarz[1,2] — [1]Institute of Theoretical Physics, University of Heidelberg — [2]Bioquant, University of Heidelberg

In order to replicate within their host, many viruses have developed self-assembly strategies for their capsids which are sufficiently robust as to be reconstituted in vitro. Models for virus self-assembly usually assume that the bonds leading to cluster formation have constant reactivity over the time course of assembly (direct assembly). In some cases, however, binding sites between the capsomers have been reported to be activated during the self-assembly process (hierarchical assembly).

Here we present a computational approach to study assembly of icosahedral viruses based upon the overdamped Langevin equation (Brownian dynamics)[1]. Hard spheres covered by reactive patches (patchy particles) serve as fundamental building units for the capsid. Hierarchical assembly is implemented by a switching in reactivity upon the formation of pentameric and hexameric rings. These substructures are considered key intermediates during the assembly.

Using computer simulations, we compare the efficiency of direct versus hierarchical assembly as function of association and dissociation rates. Our analysis shows for which molecular parameters hierarchical assembly schemes can outperform direct ones and suggests that viruses with high bond stability might prefer hierarchical assembly schemes.

[1] Johanna E. Baschek, Heinrich C. R. Klein and Ulrich S. Schwarz. BMC Biophysics 5:22, 2012.