BP 22: Molecular Machines

Time: Thursday 9:30–13:15 Location: H43

Invited Talk BP 22.1 Thu 9:30 H43

DNA-based molecular machines and synthetic biology — •Friedrich Simmel — Department Physik, LMU München, Geschwister-Scholl-Platz 1, 80539 München

The unique biophysical and biochemical properties of DNA molecules can be utilized to artificially construct machine-like molecular devices. These devices can perform simple mechanical tasks, controllably bind and release molecules, they can be used as autonomous biosensors, or as active units of switchable materials.

A 'natural' way to control DNA-based devices is to utilize RNA effector molecules rather than DNA. These RNA effectors can be transcribed from artificial 'genes' which in turn can be put under the control of gene regulatory elements. This allows for the construction of genetic control circuits for DNA devices. For example, the action of molecular machines can be made dependent on environmental stimuli by use of appropriate transcriptional logic gates. Coupling DNA devices to transcription also opens up the possibility to realize molecular machines which respond to the presence of naturally occuring RNA molecules.

BP 22.2 Thu 10:00 H43

Bidirectional cargo transport by two species of molecular motors — •Melanie Muller[1], Stefan Klumpp[2], and Reinhard Lipowsky[1] — [1]Max Planck Institute of Colloids and Interfaces, Potsdam-Golm, Germany — [2]Center for Theoretical Biological Physics, University of California San Diego, USA

Long-range intracellular transport is based on molecular motors that pull cargos along cytoskeletal filaments. One type of motor always moves in one direction, e.g. conventional kinesin moves to the microtubule plus end, while cytoplasmic dynein moves to the microtubule minus end. However, many cellular cargos are observed to move bidirectionally, involving both plus-end and minus-end directed motors. We present a stochastic 'tug-of-war' model for which motors work independently and are only coupled via the mechanical interaction with their common cargo. Depending on the motor parameters (such as microtubule affinity or stall force), we obtain three distinct types of motility behaviour of the cargo: no significant motility, stochastic switching between fast plus and minus end motion, and stochastic switching between all three types of motion. In the parameter range which leads to switches between fast plus and minus end motility, the motors appear to act in a cooperative way in spite of the underlying tug-of-war.

BP 22.3 Thu 10:15 H43

Microtubule crosslinking triggers the directional motility of Kinesin-5 — Lukas C. Kapitein[1], Benjamin H. Kwok[2], Tarun M. Kapoor[2], Erwin J.G. Peterman[1], and •Christoph F. Schmidt[1,3] — [1]Department of Physics and Astronomy and Laser Centre, Vrije Universiteit, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands — [2]Laboratory of Chemistry and Cell Biology, The Rockefeller University, New York, NY 10021, USA — [3]III. Physikalisches Institut, Fakultät für Physik, Georg-August-Universität, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany

Tetrameric kinesin-5 motor proteins are needed for eukaryotic cell division. Assembly and maintenance of the spindle is a highly controlled process. Some kinesins have been found to be cargo-activated, but for a tetrameric motor such was Eg5 it is not obvious how a corresponding mechanism could function. Here we examine factors that influence the switching of Eg5 between the a directional and a diffusive mode, varying buffers and microtubule-binding geometries. We found that at moderate ionic strength, Eg5 moves directionally. In contrast, at higher ionic strength Eg5 diffuses along microtubules without directional bias. Remarkably, under these conditions Eg5 still moves directionally when bound between two microtubules. In the spindle, this functional specialization might allow Eg5 to diffuse on single microtubules without hydrolyzing ATP until the motor gets activated by binding another microtubule.

BP 22.4 Thu 10:30 H43

Kinesin's network of chemomechanical motor cycles — •Steffen Liepelt and Reinhard Lipowsky — Max-Planck-Institute of Colloids and Interfaces, Science Park Golm, 14424 Potsdam

Using the chemical energy released by the hydrolysis of ATP, the molecular motor kinesin moves processively along microtubules. A single motor step is caused by the coupling of conformational changes and filament-binding that is described by the chemomechanical cycle.

We will discuss a general network theory that is based on the distinct chemical states of the motor and on the recent observation that stepping occurs as a single event and is not built up by sub-steps. The necessity of a network theory including several motor cycles comes with the fact, that kinesin is able to walk backwards even at small concentrations of the ATP hydrolysis product ADP, which is inconsistent with the conventional picture of a single chemomechanical cycle.

In our theory, the motor's behavior is governed by the competition of two chemomechanical motor cycles which determine the motor's stall force. A third cycle becomes important for large ADP concentrations. The theory provides a quantitative description for the functional dependencies of different motor properties as observed in single molecule experiments.

BP 22.5 Thu 10:45 H43

Optimal flexibility for conformational transitions in macromolecules — •Richard Neher[1], Wolfram Möbius[1], Erwin Frey[1], and Ulrich Gerland[2] — [1]Arnold-Sommerfeld-Center for Theoretical Physics, LMU München — [2]Institute for Theoretical Physics, Universität zu Köln

Conformational transitions in macromolecules often involve the rotation of extended lever-like structures. We show, that the transition rate is drastically accelerated by a flexible hinge in the lever and that the transition is fastest at an optimal stiffness of the hinge. Near the optimal stiffness, the rate decreases only weakly when cargo is attached to the lever, which might be exploited by molecular motors. To describe our simulation data, we generalize the Kramers-Langer theory to configuration dependent mobility matrices.

BP 22.6 Thu 11:00 H43

Spontaneous wave propagation in muscle fibres. — •Stefan Günther[1,2] and Karsten Kruse[1,2] — [1]Universität des Saarlandes, Theoretische Physik, 66041 Saarbrücken, Germany — [2]Max-Planck-Institut für Physik komplexer Systeme, 01187 Dresden, Germany

Coupled to an elastic element, molecular motors can spontaneously oscillate. In cilia and flagella an ensemble of such oscillatory elements leads to regular wave patterns. In order to gain insight into the mechanism generating these waves, we study chains of sarcomeres. A sarcomere is the elementary force generating unit of a muscle and contains motors as well as elastic elements. Single sarcomeres have been found to oscillate spontaneously [1] and waves of contraction are generated in sarcomere chains [2]. We analyse the dynamics of such chains by using a microscopic sarcomere model. Using parameter values obtained from single molecule experiments, we find quantitative agreement between our calculations and the experiments. By coarse-graining our description we can relate the parameters of a phenomenological description to the microscopic parameters.

[1] Yasuda, Shindo, and Ishiwata, Biophys. J. 70 (1996)
[2] Sasaki et al, J. Muscle Res. Cell Motil. 26 (2005)

15 min. break.

Invited Talk BP 22.7 Thu 11:30 H43

From biological towards artificial Molecular Machines — •Thorsten Hugel — Biophysics (E22) and Institute for Medical Engineering (IMETUM), Technical University Munich, Garching, Germany

A thorough understanding of nature's fascinating molecular machines will not only help to develop better drugs, but should also guide the construction of man-made Nanosystems, especially if they have to function in physiological conditions. I will report on latest insights into DNA-packaging by the bacteriophage Phi29 portal import motor [1] and on the mechanism of the molecular chaperone HSP90 [2]. Most experiments were performed on the single molecule level, especially by single-molecule force spectroscopy and single-molecule fluorescence. In addition, I will demonstrate how peptide-based synthetic material is capable of energy conversion at the molecular level and could therefore be the basis for biomimetic molecular machines.

[1] T. Hugel, et al., PLoS Biol 5(3): e59 (2007)
[2] H. Wegele, et al., Rev. Physiol. Biochem. Pharmacol 151, 1 (2004)

BP 22.8 Thu 12:00 H43

Single-molecule fluorescence resonance energy transfer studies of RNA polymerase II — •Jens Michaelis[1,2], Joanna Andrecka[1], Florian Brückner[3], and Patrick Cramer[1,3] — [1]Ludwig-Maximilians-Universität München, Department Chemie und Biochemie, Butenandstr.11, 81377 München — [2]Center for Nanoscience, CeNS — [3]Ludwig-Maximilians-Universität München, Gene Center

The crystal structure of the elongation complex of the complete 12 subunit RNA polymerase II (Pol II) reveals incoming template and non-template DNA, a seven base pair DNA/RNA hybrid, and three nucleotides each of separating DNA and RNA. Albeit, longer oligomers were used in preparation, the exit pathway of the nascent RNA could not be observed, presumably due to the inherent flexibility.

To determine the position of the nascent RNA, we have measured the distances between several known points on the Pol II elongation complex and the RNA using single pair fluorescence resonance energy transfer (sp-FRET). For a given position on the RNA we have measured three distances to known positions within the elongation complex, in order to map the unknown RNA position by triangulation. We have determined the position of the end of a 17-nt, 20-nt and 23-nt RNA thus mapping the exit pathway of the RNA product. As the RNA grows longer, we observe binding of to the dock domain and dynamical repositioning of the RNA.

BP 22.9 Thu 12:15 H43

Stepsize of the two rotary motors of FoF1-ATP synthase monitored by single-molecule FRET — Monika Düser, Nawid Zarrabi, Rolf Reuter, Dongmei Ji, Frank-Mario Boldt, and •Michael Börsch — 3. Physikalisches Institut, Universität Stuttgart, Pfaffenwaldring 57, 70550 Stuttgart

FoF1-ATP synthases are the membrane-embedded enzymes in mitochondria, chloroplasts and bacteria which supply cells with the chemical 'energy carrier' adenosine triphosphate, ATP. The enzyme consists of two coupled counteracting rotary motors. The Fo motor is driven by the electrochemical potential difference of protons across the membrane and has 10 proton binding sites. However, the isolated F1 motor can be driven by ATP hydrolysis and rotates in 120° steps in opposite direction. We investigate the elastic energy storage within FoF1 caused by the symmetry mismatch of the two motors (10-step versus 3-step per 360° turn) using time resolved single-molecule Förster resonance energy transfer, FRET. Therefore we have introduced pairs of two fluorophores at several positions within the Escherichia coli enzyme using eGFP-fused subunits and specific amino acid labeling. To switch-on and control the ATP production of an individual FoF1-ATP synthase we recently developed local electrochemical proton generation at the tip of a nanoelectrode in the confocal detection volume. Multiparameter FRET data provide insights into the varying stepsize of the Fo motor during ATP synthesis, and into the action mode of the non-competitive inhibitor aurovertin which modulates the rotary motion during ATP hydrolysis.

BP 22.10 Thu 12:30 H43

Stochastic thermodynamics of the single F_1-ATPase molecules — •Alexander Kovalev[1], Florian Werz[1], Michael Börsch[1], Dirk Bald[3], Tim Schmiedl[2], Udo Seifert[2], Jörg Wrachtrup[1], and Carsten Tietz[1] — [1]3rd Institute of Physics, Stuttgart University, Stuttgart, Germany — [2]II. Institute for Theoretical Physics, Stuttgart University, Stuttgart, Germany — [3]Department of Structural Biology, Vrije Universiteit Amsterdam, Amsterdam, Netherlands

A water soluble part of the whole transmembrane protein F0F1-ATP synthase, F1-ATPase, is a rotary motor driven by ATP hydrolysis. The back rotation of F1-ATPase induces ATP synthesis. Due to the 3 fold symmetry the central subunit of F1-ATPase rotates in three steps pausing during ATP-binding. The single molecule study allows us to reconstruct the distribution of the rate constants, which seems to be higher compared to ensemble measurements. We have determined different statistical quantities characterizing dynamical properties of F1-ATPase transition rates between its three states. A fluctuation theorem relating the forward and backward steps was verified on single trajectories using the 3-state model. That gives us opportunity to describe F1-ATPase behaviour using stochastic thermodynamics theory. The time dependent conditional probabilities for F1-ATPase to be in a certain state were compared with solution of the master equations. The 3-states model trajectories were simulated to estimate the statistical errors.

BP 22.11 Thu 12:45 H43

Data Analysis with Hidden Markov Models on a single rotary motor FoF1-ATP synthase — •Nawid Zarrabi, Monika Düser, and Michael Börsch — 3. Physikalisches Institut, Pfaffenwaldring 57, Universität Stuttgart, 70569 Stuttgart

The formation of ATP from ADP and phosphate is the major reaction that provides the 'chemical energy' for living organisms. This reaction is performed by a stepwise internal rotation of subunits of the enzyme FoF1-ATP synthase. We modeled the stepwise subunit rotation of the ATP synthase with Hidden Markov Models (HMM) and evaluated those models with confocal single-molecule fluorescence resonance energy transfer (FRET) data [1,2]. To monitor the capability of the HMM approach we generated single molecule data of freely diffusing enzymes in liposoms by a Monte-Carlo-simulation. Thereby we included the intensity fluctuations due to Brownian motion. The rotary catalysis of the ATP synthase was described by a Markov process with predefined rates for forward and backward steps. The aim of the data analysis method was the determination of dynamic parameters of ATP synthase, i.e. the occurrence of substeps depending on the ATP concentration.

References: [1] B. Zimmermann, N. Zarrabi, M. Diez, P. Gräber, M. Börsch, (2005) EMBO J. 24:2053-2063. [2] M. G. Düser, N. Zarrabi, Y. Bi, B. Zimmermann, S. D. Dunn, M. Börsch (2006) Proc. of SPIE 6062:89-104.

BP 22.12 Thu 13:00 H43

Regulation of a chimeric Eg5head/DmKHC tail motor protein — •Stefan Lakämper[1], Mikhail J. Korneev[2,3], Stefanie Reiter[1], Lukas C. Kapitein[3], Erwin J.G. Peterman[3], and Christoph F. Schmidt[1] — [1]III. Physikalisches Institut, Georg-August-Universität, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany — [2]ASML, De Run 6501, 5504 DR Veldhoven, The Netherlands — [3]Department of Physics and Astronomy and Laser Centre, Vrije Universiteit, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands

We have constructed a chimeric motor protein using the head and neck portion of the mitotic Eg5 kinesin and the tail protion of a kinesin 1 (DmKHC). This chimeric motor maintains characteristic features of the slow mitotic Eg5, but it is dimeric instead of tetrameric and the regulation by the opposing pair of heads is eliminated. This allowed us to study directly the inhibition of the dimer function by the small drug monastrol.