BP 22: Single Molecule Biophysics (joint session BP/CPP)

Time: Wednesday 9:30–13:00 Location: SCH A251

BP 22.1 Wed 9:30 SCH A251

Magnetic tweezers reveal the mechanism of directional transcription termination in human mitochondria — Eugen Ostrofet[1], Flavia Stal Papini[1], Britney Johnson[2], Jamie Arnold[2], Craig Cameron[2], and •David Dulin[1] — [1]Junior Research Group 2, IZKF, FAU Erlangen-Nürnberg, Germany — [2]Department of Microbiology and Immunology, The University of North Carolina Chapel Hill, USA

Transcription termination is essential to synthesize functional RNA and to prevent transcription interference with downstream promoters. Therefor, it must be performed efficiently despite the stability of the elongating RNA polymerase (RNAP) on DNA. One approach adopted by eukaryotic cells is directional transcription termination upon collision of RNAP with a termination factor bound to DNA, as for human mitochondria RNAP (mtRNAP) and Pol I. How the termination factor senses the direction of transcribing RNAP remains to be found. We propose that the termination factor senses DNA unwinding, and consequently terminates transcription directionally. To interrogate this hypothesis, we employed a high throughput magnetic tweezers instrument and a hairpin-based force jump assay to mimic DNA unwinding and look into the human mitochondria transcription termination factor 1 (MTERF1). We found that MTERF1 blocks directionally hairpin opening, explaining directional transcription termination. Performing in situ force calibration, we determined accurately the energy landscape of MTERF1 bound to its termination site.

BP 22.2 Wed 9:45 SCH A251

Magnetic Tweezers Protein Force Spectroscopy — •Jan Lipfert[1], Achim Löf[1], Philipp Walker[1], Steffen Sedlak[1], Sophia Gruber[1], Tobias Obser[2], Maria Brehm[2], and Martin Benoit[1] — [1]Department of Physics, LMU Munich — [2]Department of Pediatric Hematology and Oncology, University Medical Center Hamburg Eppendorf

The physiological function of proteins is often critically regulated by mechanical forces acting on them. Single-molecule manipulation techniques such as atomic force microscopy or optical tweezers have enabled unprecedented insights into the molecular mechanisms underlying such force regulation. However, these techniques have limited throughput and lack resolution at low forces. We have developed a versatile and modular approach for force measurements on proteins in magnetic tweezers [Löf et al. PNAS 2019] that enables ultra-stable ($>$ days) and parallel measurements ($>$ 50) of single molecules in a wide force range including very low forces ($<$1 pN). Leveraging our new assay, we directly probe regulatory low-force transitions within von Willebrand factor, a vascular protein that is activated for its critical role in hemostasis by hydrodynamic forces in the bloodstream. Or results reveal fast (˜250 ms) opening and closing transitions in the dimeric VWF stem at a critical force of 1 pN, which like constitute the first steps in VWF mechano-activation.

BP 22.3 Wed 10:00 SCH A251

Real-time imaging of DNA loop extrusion by condensin and their mutual interactions — •Eugene Kim[1], Jacob Kerssemakers[1], Indra Shaltiel[2], Christian Haering[2], and Cees Dekker[1] — [1]Department of Bionanoscience, Kavli Institute of Nanoscience Delft, Delft University of Technology, Delft, Netherlands. — [2]Cell Biology and Biophysics Unit, Structural and Computational Biology Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany.

How is DNA spatially organized in our cells? By what mechanisms do chromosomes fold over long distances? In this talk, I will discuss our work on understanding the looping structures of DNA using fluorescence imaging assay at the single-molecule level. The major focus is on condensin, that is one of the SMC (Structural Maintenance of Chromosomes) complexes. This ring-shaped protein is the molecular motor that can extrude large loops of DNA, a mechanism thought to be the basis of the chromosome structures at various stages of the cell cycle. I will firstly show how a single condensin can extrude loops at a force-dependent speed of up to 2 kbp/s and it does so in a strictly asymmetric manner. I will then show how these individual condensins can form a dimeric structure by traversing one over the other, in turn forming a novel type of loop structure that we name as Z loop. This condensin dimer can extrude DNA in a symmetric fashion, thus may be able to contribute to chromosomal compaction in a more efficient way. We believe that our work will allow to disentangle the fundamental looping architecture of chromosomes, that is essential to all life.

BP 22.4 Wed 10:15 SCH A251

Magnetic tweezers reveal two coexisting and interconverting bacterial RNA polymerase conformations with different open complex stability — •Subhas Chandra Bera[1], Mona Seifert[1], Santeri Maatsola[2], Eugen Ostrofet[1], Monika Spermann[1], Flavia Stal-Papini[1], Anssi M. Malinen[2], and David Dulin[1] — [1]Junior Research Group 2, Interdisciplinary Center for Clinical Research, Friedrich Alexander University Erlangen-Nürnberg (FAU), Cauerstr. 3, 91058 Erlangen, Germany — [2]Department of Biochemistry, University of Turku, Tykistökatu 6A, 6th floor, 20520 Turku, Finland

To start transcription, the RNA polymerase (RNAP) recognises the promoter, to form the closed complex (CC), and eventually unwinds the DNA to form the open complex (OC), and is then ready for RNA synthesis. OC stability decides the yield of expression in many genes, and is therefore of great importance to regulate expression of a given gene. Using high throughput magnetic tweezers, we investigated OC dynamics. Surprisingly, we observed two OC populations with nearly 10-fold difference in lifetime, where the stability of the OC varies as a function of the nature and the concentration of the anions, as well as the temperature. We further noticed that the RNAP completely dissociates upon return to CC and therefore the two OC populations do not originate from a single interconverting RNAP, but rather from two conformations of RNAP. Our study shows the power of single molecule techniques to resolve two interconverting populations of RNAP that have remained elusive to bulk assays so far.

BP 22.5 Wed 10:30 SCH A251

Towards the Label-free Plasmonic Detection of Single Untethered Proteins — •Martin D. Baaske, Peter S. Neu, Nasrin Asgari, and Michel Orrit — Huygens-Kamerlingh Onnes Laboratory, Leiden University, Postbus 9504, 2300 RA Leiden, The Netherlands

Label-free optical detection schemes so far rely on specific chemical interactions between receptor and target molecules in order to facilitate analyte recognition. Here we present our first step towards the label-free recognition of untethered nanoscale analytes. We show that via a polarization selective technique and careful optimization of a confocal microscope, single gold nanorods, which are commonly used as labels, can be transformed into high-speed nanoscale sensors. We demonstrate the performance of our system by detecting microemulsion nanodroplets which mimic 250 kDA proteins as they diffuse through the near field of a single gold nanorod on nanosecond timescales.

BP 22.6 Wed 10:45 SCH A251

How to gain reliable information from short trajectories — •Marie Schwebs[1], Torsten Paul[2], Marius Glogger[1], Philip Kollmannsberger[2], Markus Engstler[1], and Susanne Fenz[1] — [1]Department for Cell and Developmental Biology, Biocenter, University of Würzburg, Germany — [2]Center for Computational and Theoretical Biology, University of Würzburg, Germany

Trypanosoma brucei expresses a dense coat of GPI-anchored variant surface glycoproteins (VSGs). The fluidity of this coat is fundamental for the evasion of the host's immune system and thus for the survival of the parasite. So far, the VSG dynamics on living trypanosomes has been studied at the micron and second scale for the whole ensemble. In this project, we want to elucidate the dynamics of individual VSGs in relation to the flagellar pocket, the sole site for endo- and exocytosis, with single-molecule fluorescence microscopy. For this purpose, we have recently introduced super-resolution imaging of intrinsically fast-moving flagellates based on cyto-compatible hydrogel embedding. Building on this work, we are now able to track VSG dynamics on living trypanosomes at high spatial (localization precision ˜30 nm) and temporal resolution (f = 100 Hz). The length of gained trajectories is mainly limited by the shape and size of trypanosomes (approx. 18 μm in length and 3 μm in width). Therefore, we use a self-written program based on an approach from Hoze and Holcman [Biophys. J., 2014] to

make reliable statements about local forces and the diffusion tensor. The information is gained from a large number of short trajectories and will be presented in directed motion and diffusion maps.

30 min. coffee break

Invited Talk BP 22.7 Wed 11:30 SCH A251

The mechanical stability of proteins regulates their translocation rate into the cell nucleus — •Sergi Garcia-Manyes — Department of Physics, Randall Centre for Cell and Molecular Biophysics King's College London

The translocation of mechanosensitive transcription factors (TFs) across the nuclear envelope is a crucial step in cellular mechanotransduction. Yet the molecular mechanisms by which mechanical cues control the nuclear shuttling dynamics of TFs through the nuclear pore complex (NPC) to activate gene expression are poorly understood. Here, we show that the nuclear import rate of myocardin-related transcription factor A (MRTFA) * a protein that regulates cytoskeletal dynamics via the activation of the TF serum response factor (SRF) * inversely correlates with the protein*s nanomechanical stability and does not relate to its thermodynamic stability. Tagging MRTFA with mechanically-stable proteins results in the downregulation of SRF-mediated gene expression and subsequent slowing down of cell migration. We conclude that the mechanical unfolding of proteins regulates their nuclear translocation rate through the NPC and highlight the role of the NPC as a selective mechanosensor able to discriminate forces as low as *10 pN. The modulation of the mechanical stability of TFs may represent a new, general strategy for the control of gene expression.

BP 22.8 Wed 12:00 SCH A251

Q band mixing in chlorophyll a - spectral decomposition of Qx and Qy absorption bands — •Clark Zahn[1], Till Stensitzki[1], Angelica Zacarias[2], and Karsten Heyne[1] — [1]Institutfür Experimentalphysik, Freie Universität Berlin, Arnimallee 14, 14195 Berlin, Germany — [2]Max Planck Institute of Microstructure Physics, Weinberg 2, D06120 Halle, Germany and ETSF

Chlorophyll a (Chl a) is one of the most abundant pigments on earth, responsible for the green color of plants. Despite extensive research, the composition of its visible Q absorption band is yet not well understood. Here, we apply polarization resolved femtosecond Vis pump - IR probe spectroscopy, providing a detailed insight into Q band mixing of Chl a. Vis excitation was tuned to various wavelengths scanning the Q band absorption. We show that the dichroic ratio of the keto-C=O stretching vibration at 1698 cm-1 strongly depends on the excitation wavelength. Hence, the angle between the excited electronic transition dipole moment (tdm) and the vibrational keto-C=O tdm changes significantly across the Q band. Tracing the relative angle Θ for different excitation wavelengths allows to determine the Qx contribution along the Q band region. In this way, Qx is found to contribute 40-60% to absorption of the lower energetic peak at 618 nm and to 75-100% to the absorption of the high energy flank at around 580 nm. Complementary measurements on the C=C stretching vibration at 1608 cm-1 provide corroborating evidence for our findings. Further, we show that from our recent results the three-dimensional orientation of the Qx and Qy tdms can be resolved under guidance of quantum chemical calculations.

BP 22.9 Wed 12:15 SCH A251

Power law decays of ligand concentrations in single-molecule kinetic experiments. — •Aykut Erbas[1], Monica Olvera de la Cruz[2], and John F. Marko[2] — [1]Bilkent University- UNAM, Ankara 06800, Turkey — [2]Northwestern University, Evanston 06202, USA

SPR (Surface Plasmon Resonance) or single-molecule kinetic methods rely on the relaxation of initially surface-bound ligands into a confined reservoir to measure the dissociation rates of the corresponding ligands. Similarly, biological processes such as exocytosis (emission of small molecules into the intracellular void for cellular communication) can be considered as a similar relaxation problem. Using molecular dynamics simulations and scaling arguments, we studied a model system closely related to the above cases. In our model, Brownian particles are released from their binding sites into a confined volume. Then, within this volume, we tracked how the concentration of particles throughout the volume changes as a function of time. Our results show that the dissociation process (more specifically rebinding rates of released particles) exhibits various power laws at times longer than the initial exponential decay. Interestingly, the cumulative rebinding number, which is robust against the concentration fluctuations, exhibits a distinct plateau regime as a result of the three-dimensional escape process of the particles from their initial binding sites. Overall our results can be used for new sensor applications to probe molecular kinetics at long tines.

BP 22.10 Wed 12:30 SCH A251

Narrow escape: How long does it take for a camel to go through the eye of a needle? — •Elisabeth Meiser[1], Reza Mohammadi[2], Nicolas Vogel[2], and Susanne Fenz[1] — [1]University of Würzburg, Biocenter: Cell- and Developmental Biology, Würzburg, Germany — [2]Friedrich-Alexander University Erlangen-Nürnberg, Institute of Particle Technology, Erlangen, Germany

The narrow escape problem is a common problem in biology and biophysics. It deals with Brownian particles confined to a given domain with reflecting borders and only a small escape window where particles are absorbed. The mean first passage time (MFPT), the time it takes a set of particles to escape, can be analytically calculated in 2D and 3D for several geometries. It depends on the area of the domain, the size of the escape window and on the diffusion coefficient of the particle. We aim to systematically test the analytical solution of the NEP in 2D by variation of the relevant parameters. Experiments are being complemented by matching random walk simulations. For the experimental test, we prepared micro-patterned phospholipid bilayers from a combination of colloid lithography and vesicle fusion. We imaged fluorescently labeled lipids diffusing in circular membrane patches with diameters of 1-5 um using single-molecule microscopy at 100 Hz and a localization precision of 14 nm. While the area of the membrane was tuned during colloid lithography, the size of the escape window was adjusted in the course of the analysis. We will present our first results on membrane patterning as well as a comparison of our experimental and simulation results with the theoretical prediction for the MFPT.

BP 22.11 Wed 12:45 SCH A251

Probing single molecular surface interactions on electroactive surfaces — •Julia Appenroth, Laura Mears, Pierluigi Bilotto, Alexander Imre, and Markus Valtiner — TU Wien, Applied Physics, Vienna, AT

Adhesive interactions between hydrophobic, charged and electroactive moieties steer ubiquitous processes in aqueous media, including the self-organization of biologic matter and adhesive interfaces in general. Recent decades have seen tremendous progress in understanding these interactions for macroscopic adhesive interfaces. Yet, it is still a challenge to experimentally measure interactions at the single-molecule scale and thus to compare with theory, especially on electroactive surfaces. Here, we directly measure and quantify the sequence dependence and additivity of charge-mediated and electroactive interactions at the single-molecule scale. We combined dynamic single-molecule force spectroscopy with MD simulations and show how electroactive surfaces can be probed with single molecules using force probe techniques.