BP 23: Single Molecule Biophysics

Time: Thursday 9:30–13:00 Location: BAR 0106

Invited Talk BP 23.1 Thu 9:30 BAR 0106

Conformational dynamics of SARS-CoV-2 spike protein modulates the binding affinity to ACE2 — Fidan Sumbul[1], Claire Valotteau[1], Prithwidip Saha[1], Ignacio Fernadez[2], Annalisa Meola[2], Eduard Baquero[2], Dorota Kostrz[3], James R Portman[3], François Stransky[3], Pablo Guardado Calvo[2], Charlie Gosse[3], Terence Strick[3], Felix Rey[2], and •Felix Rico[1] — [1]Aix-Marseille Univ, CNRS, INSERM, LAI, CENTURI, Marseille, France — [2]Institut Pasteur, Department of Virology, CNRS UMR 3569, Paris France — [3]Ecole Normale Superieure, Institut de Biologie, CNRS, INSERM, PSL, Paris, France

SARS-CoV-2 spike protein (S) interacts with angiotensin-converting enzyme 2 (ACE2) to enter host cells. Protein S forms a homotrimer with three receptor-binding domains (RBD) adopting open and closed conformations. The (un)binding of S to ACE2 may be affected by these conformational dynamics. Here, we used single molecule force spectroscopy to probe the binding strength and affinity of the S-trimer/ACE2 interaction and high-speed atomic force microscopy (HS-AFM) to visualize the RBD opening dynamics of S-trimers. HS-AFM imaging revealed dynamic S-trimers with the three RBDs stochastically and independently switching between open and closed conformations. This modulates binding to ACE2 of S-trimers, but not unbinding. Experimental opening rates and a simple conformational binding model explain the modulation of the binding affinity. Our results shed light on the molecular basis of coronavirus infection.

BP 23.2 Thu 10:00 BAR 0106

ion-specific DNA adsorption at mica mediated by monovalent and divalent metal cations — •Ibrahim Mohd[1], Max Lallemang[2], Bizan N Balzer[2], and Ibrahim Mohd[1] — [1]University of Augsburg, 86159 Augsburg, Germany — [2]universitätsstraße 1

Ion mediated attraction between biomolecules and solid substrates plays a crucial role in a broad range of biotechnological applications. In this work, we combine molecular dynamics simulations and single-molecule atomic force microscopy experiments to characterise the desorption properties of single-stranded DNA at mica surface mediated by the alkali and alkaline earth metal ions Li+, Na+, K+, Cs+, Mg2+ and Ca2+. Our results show that both monovalent and divalent ions induce an attractive interaction between DNA and the negatively charged mica surface. DNA adhesion is caused by two effects: Firstly, ion-specific adsorption of the monovalent cations compensates the negative charge and induces a long-ranged attraction. In addition, the surface adsorbed cations form inner-sphere contacts with the oxygen atoms of the DNA backbone. Both effects depend on the type of cation: Cs+ and K+ ions lead to loosely associated DNA with high surface mobility and low rupture forces. Na+, Li+, and divalent ions lead to a stronger association of DNA with low surface mobility and high rupture forces. By comparing the force-extension curve shapes from experiments and simulations we could provide atomistic insights into the desorption mechanisms and identify the dominant interactions involved in the desorption process.

BP 23.3 Thu 10:15 BAR 0106

Understanding the molecular determinants of chitin-protein interactions in the arthropod cuticle - a single-molecule approach — •Ayesha Talib[1,2], Yael Politi[2], and Kerstin G. Blank[1,3] — [1]Max Planck Institute of Colloids and Interfaces, Potsdam, Germany — [2]Technische Universität Dresden, CMCB, B CUBE, Dresden, Germany — [3]Johannes Kepler Universität, Institute of Experimental Physics, Linz, Austria

In the cuticle of arthropods, structural proteins and chitin fibers form a composite material with anisotropic mechanical properties. The molecular parameters that define the chitin-protein interaction are largely unknown. To answer the fundamental question of what controls cuticle mechanical properties, a molecular strategy is employed that integrates protein engineering with single-molecule force spectroscopy. Chitin binding domains (CBDs) from the spider Cupiennius salei have been identified and expressed recombinantly to qualitatively and quantitatively compare the strength of the protein-chitin interaction. For one CBD present in all spider tissues, we investigated the three partly overlapping consensus motifs RR-1, RR-2 and CB-4. Pull-down assays and single-molecule force spectroscopy suggest that the shortest RR-1 motif does not bind to chitin, whereas similar binding strength is observed for the longer sequences RR-2 and CB-4. We observe a fast dissociation rate, suggesting that CBDs facilitate energy dissipation upon deformation. Our ultimate goal is to correlate molecular properties with the mechanical function of the composite and to synthesize artificial analogues with tunable mechanical properties.

BP 23.4 Thu 10:30 BAR 0106

Complex unfolding, refolding and DNA association of the relaxase TrwC — •César Augusto Quintana-Cataño[1], •Miriam Schramm[1], Ekaterina Vorobevskaia[1], Andreas Hartmann[1], and Michael Schlierf[1,2] — [1]B CUBE - Center for Molecular Bioengineering, TU Dresden, Germany — [2]Cluster of Excellence Physics of Life, TU Dresden, Germany

Referred as "bacterial sex", bacterial conjugation is a process in which a donor cell transfers DNA to a recipient cell. Conjugation is a main driver for spreading of antibiotic resistance genes. The relaxase TrwC associated to the type 4 secretion system is a multi-domain model enzyme essential for bacterial conjugation. It serves multiple purposes: DNA recognition, nicking and unwinding in the donor cell; and religation of ssDNA in the receptor cell. For this last step a mechanical unfoldase denaturates TrwC, which then refolds in the receptor cell. TrwC is a multi-domain protein consisting of 966 amino acids with a 55 amino acids long intrinsically disordered C-terminus. Here, we studied unfolding and folding mechanics of TrwC using single-molecule magnetic tweezers force spectroscopy. We show that the subdomains of TrwC unfold at distinct, different force regimes ranging from ~10 pN to 90 pN and TrwC refolds in a complex multistep reaction. Using our data, we can quantify the folding energetics of TrwC. We have further discovered complex DNA binding events of monomers and dimers using fluorescence correlation spectroscopy, allowing us to build a model of DNA association. We anticipate that our data helps to understand a key enzyme of DNA conjugation and its multifold activities.

BP 23.5 Thu 10:45 BAR 0106

Superpower behavior of the budding yeast kinesin-8 — •Anita Jannasch, Brent Fielden, Michael Bugiel, and Erik Schäffer — Universität Tübingen

Kinesin-8 motor proteins can regulate microtubule dynamics and their length. Furthermore, they can crosslink microtubules and slide them relative to each other. These properties make the motor important for cell division. The budding yeast kinesin-8, Kip3, depolymerizes microtubule in a collective, force and length-dependent manner. The latter is due to the motor's very high processivity. Recently, we found that contrary to the depolymerization activity of multiple motors, a single Kip3 motor stabilizes microtubules. Compared to conventional kinesin, Kip3 is more than 10x slower and can generate about a 5x lower maximum force suggesting that it is a low power motor. Surprisingly, using high-precision optical tweezers, we observed that single Kip3 bound via a nanobody to optically trapped microspheres occasionally moved about 6x faster generating 3x higher forces compared to its usual behavior. This superpower behavior is almost comparable to conventional kinesin, but it is unclear whether it is related to one of its biological functions. The nanobody coupling also reduced the compliance of the system and thereby improved the spatiotemporal resolution. With the improved resolution, we were able to detect 4-nm mechanical substeps of the motor. In the long term, a better understanding of the various talents of Kip3 on the molecular level will have implications for cell division and associated diseases.

BP 23.6 Thu 11:00 BAR 0106

Thermodynamic inference for molecular motor models based on non-invasive conditioned waiting-time measurements — •Benjamin Ertel, Jann van der Meer, and Udo Seifert — II. Institut für Theoretische Physik, Universität Stuttgart, 70550 Stuttgart, Germany

Molecular motors are one example of biophysical systems operating far from equilibrium. For the description of their dynamics in motor-bead assays, three classes of models have been proposed. The first class, discrete Markov networks, emphasizes the configurational changes of the motor where the bead enters the transition rates. The second class of models focuses on the motion of the bead, which is described by

an overdamped Langevin equation. The third class of models, hybrid motor-bead complexes, describes the full dynamics of the motor-bead assay by combining discrete and continuous models from the two previous classes. In this work, we develop a thermodynamic inference scheme that can be used to distinguish qualitatively between the different classes of models. Since this inference scheme is based on the observation of waiting times for conditioned transitions of the bead trajectory only, it is non-invasive and operationally accessible in experiments. Various characteristics of the motor, like its driving affinity, can be inferred without further information. We have obtained these results through analytical derivations and extensive numerical calculations.

15 min. break

BP 23.7 Thu 11:30 BAR 0106

Metal-protein-metal junctions: electron transport and mechanical deformation — •Linda Angela Zotti — Departamento de Física Teórica de la Materia Condensada, Universidad Autónoma de Madrid, 28049 Madrid, Spain

Proteins have proven to be promising candidates for molecular electronics, showing in some cases much higher conductance than one would naively expect from their size. In particular, the blue-copper azurin extracted from Pseudomonas aeruginosa has been the subject of many experimental studies, although the exact electron-transport mechanism is still under debate. Here I will present our efforts towards understanding the origin of such interesting effects from a theoretical perspective, analyzing both the electronic structure and the geometrical arrangement [1-6].

References [1] M. P. Ruiz et al. , J. Am. Chem. Soc. 139, 43, 15337 (2017). [2] C. Romero Muñiz, M. Ortega, J.G Vilhena, I. Diéz Pérez, R. Pérez, J. C. Cuevas, L. A. Zotti, Phys. Chem. Chem. Phys., 20, 30392 (2018). [3] C. Romero Muñiz, M. Ortega, J.G Vilhena, I. Diéz Pérez, R. Pérez, J. C. Cuevas, L. A. Zotti, Biomolecules, 9(9), 506 (2019). [4] C. Romero Muñiz, M. Ortega, J.G Vilhena, I. Diéz Pérez, R. Pérez, J. C. Cuevas, L. A. Zotti, J.Phys.Chem.C 125 (3), 1693 (2021). [5] C. Romero Muñiz, M. Ortega, J.G Vilhena, R. Pérez, J. C. Cuevas, L. A. Zotti, Appl. Sci. 11 (9), 3732 (2021). [6]C. Romero Muñiz, J.G Vilhena, R. Pérez, J. C. Cuevas, L. A. Zotti, Front. Phys. 10:950929. doi: 10.3389/fphy.2022.950929 (2022).

BP 23.8 Thu 11:45 BAR 0106

A variance sum rule and its entropy production estimation — •Ivan Di Terlizzi[1,2], Marta Gironella[3], Marco Baiesi[3], and Felix Ritort[2] — [1]Max Planck Institute for the Physocxs of complex systems — [2]University of Padua — [3]University of Barcelona

Nonequilibrium steady states, from the planetary scale to biological processes, are characterized by entropy production via energy dissipation to the environment, which is often challenging to measure. A novel variance sum rule sets a new resource for exploiting fluctuations to measure physical quantities in stochastic systems. In particular, we focus on the formula it gives for estimating the entropy production rate from trajectories of positions and forces. We describe this method with analytically solvable models and we show its robustness and usefulness in practical applications to experimental data. By introducing a model-dependent fitting procedure, the method is also adapted to deal with conditions where not all degrees of freedom are experimentally accessible. For example, by analysing traces of flickering red blood cells, we obtain estimates of the entropy production rate in line with values obtained with totally different thermodynamic techniques.

BP 23.9 Thu 12:00 BAR 0106

Lattice defect sites accelerate microtubule severing by spastin — •Cordula Reuther[1], Paula Santos-Otte[1], Rahul Grover[1], and Stefan Diez[1,2,3] — [1]B CUBE - Center for Molecular Bioengineering, TU Dresden, Dresden, Germany — [2]Cluster of Excellence Physics of Life, TU Dresden, Dresden, Germany — [3]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Length regulation of microtubules and their organization into complex arrays occurs through the activity of polymerases, depolymerases as well as severing enzymes such as katanin and spastin. The latter hexamerize on the microtubule lattice, pull out single tubulin dimers in an ATP-dependent manner and eventually generate internal breaks in the microtubule. For both enzymes it was shown that the severing activity is regulated by tubulin posttranslational modifications. So far, however, only katanin has been reported to exhibit a lattice-defect- or crossover-sensing activity. Here, we determined whether lattice defects in GMPCPP-stabilized microtubules also affect the severing activity by spastin. In controlled in vitro assays we characterized microtubules with defects next to control microtubules. Defect sites were introduced either through specific polymerization conditions or by end-to-end annealing of microtubules. We found that (i) the presence of defects accelerated the onset of the severing process and (ii) severing occured twice as often in microtubule segments with defect sites as compared to random lattice segments. Furthermore, we quantified the correlation of the fluorescence signal of GFP-labelled spastin along the microtubule lattice to the severing sites as a function of time.

BP 23.10 Thu 12:15 BAR 0106

Keratin filament mechanics and energy dissipation are determined by metal-like plasticity — •Charlotta Lorenz[1], Johanna Forsting[1], Robert W. Style[2], Stefan Klumpp[3], and Sarah Köster[1] — [1]Institute for X-Ray Physics, University of Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany — [2]Department of Materials, ETH Zürich, Vladimir-Prelog-Weg 1-5/10, 8093 Zürich, Switzerland — [3]Institute for the Dynamics of Complex Systems, University of Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany

Cell mechanics is determined by an intracellular biopolymer network, including intermediate filaments that are expressed in a cell-type specific manner. A prominent pair of intermediate filaments are keratin and vimentin as the epithelial-to-mesenchymal transition is associated with a switch from keratin to vimentin. The transition coincides with a change in cellular mechanics, and thus dynamic properties of the cells. This observation raises the question of how the mechanical properties already differ on the single filament level. Here we use optical tweezers and a computational model to compare the stretching and dissipation behavior of the two filament types. We find that keratin and vimentin filaments behave in opposite ways: keratin filaments elongate, but retain their stiffness, whereas vimentin filaments soften, but retain their length. This finding is explained by fundamentally different ways to dissipate energy: viscous subunits sliding within keratin filaments and non-equilibrium α helix unfolding in vimentin filaments.

BP 23.11 Thu 12:30 BAR 0106

The single-strand annealing protein RAD52 can form a stable nucleoprotein filament — •Carolina Carrasco[1], Laura Muras[1], Tobias Jachowski[1], Sivaraman Subramaniam[2], Francis Stewart[2], and Erik Schäffer[1] — [1]Center for Plant Molecular Biology (ZMBP), University of Tübingen, Tübingen, Germany — [2]Department of Genomics, Biotechnology Center, TU Dresden, Dresden, Germany

Genome maintenance requires the repair of DNA double-strand breaks. It can be mediated among others by the single-strand annealing. RAD52 proteins form rings that are thought to promote the annealing. How RAD52 interacts with and anneals DNA strands remains unclear. We have investigated the dynamic interaction of RAD52 with DNA by force spectroscopy using optical tweezers. Upon stretching single DNA molecules in the presence of RAD52, we have observed elongation steps in DNA extension that are consistent with either dissociation, unwrapping, or opening of individual DNA-bound rings. Upon relaxation, reverse steps of similar amplitude were detected. Under constant force, step sizes were uniform. Surprisingly, the disruption forces followed a gamma distribution suggesting that the RAD52-DNA dissociation process consists of multiple stochastic steps. Successive stretch-relax cycles at high forces promoted DNA softening and a melting-force increase because of an intercalation and sealing mechanism on DNA. The final DNA-RAD52 hysteresis-free nucleoprotein filament is consistent with a flexible helical structure in which RAD52 monomers, and not rings, mediate strand annealing.

BP 23.12 Thu 12:45 BAR 0106

Assessing biomolecular interactions across scales using optical tweezers — •Roman Renger, Nicholas Luzzietti, and Philipp Rauch — Paalbergweg 3, 1105 AG Amsterdam, Netherlands

Biological processes involving proteins interacting with nucleic acids, cell membranes or cytoskeletal filaments are key to cell metabolism and hence to life in general. Detailed insights into these processes provide essential information for understanding the molecular basis of physiology and the pathological conditions that develop when such processes go awry. The next scientific breakthrough consists in the direct, real-time observations and measurements of the most fundamental mechanisms involved in biology Single-molecule technologies offer

a powerful opportunity to meet these challenges and to study dynamic protein function and activity in real-time and at the single-molecule level. Here, we present our efforts for further enabling discoveries in the field of biology and biophysics using the combination of optical tweezers with correlative fluorescence microscopy (widefield, TIRF, confocal and STED) and label-free Interference Reflection Microscopy (IRM). We present several examples in which our technology has enhanced the understanding of basic biological phenomena, ranging from protein structure to intracellular organization. Furthermore, we show that advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument that has the ability to open up new avenues in many research areas.