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MEETING REPORT

Meeting report – NSF-sponsored workshop 'Progress and Prospects of Single-Molecule Force Spectroscopy in Biological and Chemical Sciences'

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ABSTRACT

The goals of the workshop organized by Piotr Marszalek and Andres Oberhauser that took place between 29 August and 1 September 2019 at Duke University were to bring together leading experts and junior researchers to review past accomplishments, recent advances and limitations in the single-molecule force spectroscopy field, which examines nanomechanical forces in diverse biological processes and pathologies. Talks were organized into four sessions, and two in-depth roundtable discussion sessions were held.

Introduction

Single-molecule force spectroscopy (SMFS) examines the relationships between applied forces and (bio)polymer extensions or the lifetime of bonds between biomolecules being stretched (Makarov and Schuler, 2018). The field originated in the early 1990s following the development of new instruments such as optical tweezers (OTs), magnetic tweezers (MTs) and the atomic force microscope (AFM). These tools realized the unprecedented possibility of visualizing and mechanically manipulating individual molecules under ambient conditions, including in aqueous environments, which is critical for biological studies (Arbore et al., 2019). Over the years, SMFS has facilitated many seminal discoveries. These include capturing the reversible mechanical stretching and unfolding of individual titin molecules that control the passive elasticity of muscle, determining ligand receptor affinities in a totally novel fashion by measuring their mechanical rupture forces, and mechanically following the action of DNA and RNA polymerases to provide new insights into their enzymology under load, to mention just a few areas where SMFS has been employed.

The goal of the workshop, which was divided into six sessions, was to bring together around forty participants, including established researchers – some of whom pioneered SMFS techniques – as well as junior researchers and students focusing on future prospects for the field. Four sessions were devoted to the presentation of new findings on the main SMFS experimental techniques (MTs, OTs and AFM), computational SMFS approaches, and technical advances in SMFS. There were also two roundtable discussions, which allowed researchers to freely converse and exchange ideas on current methodological challenges and limitations in SMFS.

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Below, we highlight meeting presentations grouped thematically rather than chronologically. Owing to space limitations, not all talks could be incorporated into this report.

Cellular nanomechanics

The workshop was opened by Michael Sheetz (University of Texas Medical Branch, Galveston, TX), who presented recent insights into how cells sense the mechanical rigidity of the extracellular matrix (ECM), which is critical for cell growth, death, migration and invasion. By decorating synthetic micrometer-scale pillars (made of materials with different rigidity) with integrin ligands, it was possible to directly measure pillar deformation and, thus, the integrin-mediated pulling forces that cells use to sense substrate rigidity. Cells sense rigidity by contracting the matrix using sarcomeric contractile units and then stabilizing adhesive contacts with the ECM that pull on the matrix. A key finding was that cancer cells, which have many fewer mechanosensing units than normal cells, ignore matrix rigidity, grow on soft substrates and undergo transformation (Sheetz, 2019).

A related talk, about how forces are generated and sensed by cells, was given by Christoph Schmidt (Duke University, Durham, NC). He reported on the exciting development by his group of a dual OT-based microrheology approach (Nijenhuis et al., 2012). The unique feature of this technique is that the cell's response to an oscillatory external force can be measured to determine the elasticity of the cell (active microrheology), while in the same experiment force fluctuations within cells (passive microrheology) can be simultaneously captured. Results were analyzed using a pressurized elastic-shell mode and indicated that both cellular force generation and cellular elasticity are dominated by the actomyosin cortical network.

DNA mechanics

The first talk on this theme was by Laura Finzi (Emory University, Atlanta, GA), who presented recent results from analyses of DNA transcription by RNA polymerase (RNAP) in the presence of various topological obstacles, which used two SMFS approaches – the tethered particle motion (TPM) technique and MTs. These experiments captured a very interesting behavior of single RNAPs. In the presence of roadblocks, in this case the lac repressor protein, an RNAP molecule that has completed a round of elongation has the tendency to return to the obstacle and resume RNA polymerization from that position. In addition, RNAP pausing at obstacles depends not only on the amplitude, but also on the directionality of the tension applied to the DNA template. Finally, she showed that the strength of a protein roadblock is increased if the protein bridges two distant sites, thus mediating a DNA loop (Finzi and Dunlap, 2016). In conclusion, looping might convert a weak protein-binding site into a strong roadblock for transcription, indicating new means of transcription regulation.

The next talk on DNA mechanics was by Keir Neuman (NIH, Bethesda, MD) and focused on a challenging, but extremely promising, combination of two single-molecule techniques, which involve micro-mirror-based total internal reflection fluorescence microscopy (TIRF) and MTs, to study nucleic acids and their interactions with various enzymes (Seol and Neuman, 2018). This combination enabled the simultaneous visualization and mechanical manipulation of single molecules. As presented here, MT-controlled supercoiling of DNA, achieved by rotating a magnetic bead labeled with a fluorescent nanodiamond, could be effectively used to follow a single topoisomerase molecule binding to and relaxing a supercoiled DNA in real time.

Mark Williams (Northeastern University, Boston, MA) continued on the application of SMFS techniques to study DNA–protein interactions. Specifically, he described how optical tweezers can be used to monitor binding and detachment of single-stranded DNA (ssDNA)-binding proteins (SSBs) to and from an ssDNA template. SSB binding has important functions in protecting transient single-stranded DNA segments that appear, for instance, during DNA replication, repair and recombination, and OTs can monitor SSB–DNA interactions as changes in ssDNA length upon binding and DNA wrapping around SSBs (McCauley and Williams, 2011). This approach allowed the recording of different modes of SSB binding, either with simultaneous DNA wrapping or without DNA wrapping when the occupancy of SSBs on the DNA template reached saturation.

Protein mechanics and folding

Hermann Gaub (Ludwig-Maximilians-Universität München, Germany), who developed and applied AFM in many groundbreaking SMFS studies of proteins, sugars and DNA, presented his latest research on the unusually high mechanostability of some proteins and their complexes (Milles and Gaub, 2020). His captivating talk combined AFM-based SMFS with steered molecular dynamics simulations to investigate the molecular mechanisms governing the high resilience of cellulosomal complexes of different cellulolytic microbes. These complexes withstand forces of 600–750 pN, making this one of the strongest bimolecular interactions reported, equivalent to half the mechanical strength of a covalent bond. The detailed understanding of cellulosomal network components might help in the development of biocatalysts for the production of fuels and chemicals from renewable plant-derived biomass.

The use of SMFS to study protein folding was exemplified by Hongbin Li (University of British Columbia, Vancouver, Canada), who presented some fascinating results describing the mechanical unfolding and refolding of the model metalloprotein rubredoxin (RD), obtained using OTs. These measurements were able to capture the detailed mechanism of RD folding, including various routes, either with or without formation of the metal-chelation site. This novel approach provides key insights into how the chemical reactivity of metal centers contributes to the different functions of metalloproteins (Zheng et al., 2015).

Arne Gennerich (Albert Einstein College of Medicine, New York, NY), delivered an inspiring talk addressing how cytoplasmic dynein is able to differentially sense forward and backward load while moving on microtubules. Using OTs and mutagenesis, his work revealed that three structural elements of the motor domain, namely the linker, buttress and stalk, cooperate to collectively regulate directional sensing of tension. In addition, the sliding of the coiled-coil regions of the dynein stalk was found to be responsible for its anisotropic behavior, and conformational changes of dynein's linker and buttress participated in controlling this process, suggesting that significant revisions to the current model of the mechanochemical actions of dynein motors are needed (Brenner et al., 2020).

Christian Kaiser (Johns Hopkins University, Baltimore, MD) presented recent studies that probed the folding status of the nascent polypeptide chain of elongation factor G (EF-G), which matures into a multidomain protein after its folding is complete. He showed a number of interesting properties of EF-G, including the observation that, although the N-terminal domain of the protein folds co-translationally, it can undergo unfolding and further misfolding when allowed to interact with subsequent fragments of the polypeptide chain emerging from the ribosome. These non-native, non-productive interactions are eliminated by a ribosome-associated chaperone, Trigger factor, suggesting that avoidance of early folding defects is crucial, because these can propagate and result in misfolding of the entire protein. These studies further demonstrate the power and advantages of OT-based SMFS techniques (Liu et al., 2019).

Peter Hinterdorfer (Johannes Kepler University, Linz, Austria) described SMFS experiments that directly measured the interaction forces between the serotonin transporter (SERT) and enantiomers of citalopram (a commonly used antidepressant). These experiments provide physical evidence for the existence of two binding sites in SERT, a central (S1) site and a vestibular (S2) site, and that these two sites are allosterically coupled. This exciting approach may thus allow gathering of dynamic information about the function of clinically relevant membrane transporters under physiological conditions, which are inaccessible by X-ray crystallography (Zhu et al., 2018).

Yuri Lyubchenko (University of Nebraska Medical Center, Omaha, NE) focused on the use of AFM-based SMFS in combination with molecular dynamics simulations to characterize the interaction between amyloidogenic proteins. In particular, amyloid beta (Aβ), whose assembly into oligomers is a major cause of neurodegenerative diseases such as Alzheimer's disease (AD) (Banerjee et al., 2020), is an important system for these studies. His group recently discovered that assembly of Aβ dimers stabilizes disease-prone, transient misfolded states of peptides and proteins by several orders of magnitudes. He also discussed the potential of AFM-based force spectroscopy for the study of AD and other protein-aggregation diseases.

Mariano Carrión-Vázquez (Cajal Institute-CSIC, Madrid, Spain) also addressed the use of AFM to study neurodegenerative disorders. His talk focused on the use of AFM-SMFS to investigate conformational polymorphisms of the tau protein fragment that include residues involved in interactions with microtubules. They found that amyloid-promoting factors, such as the tau mutations Δ280K and P301L that cause frontotemporal dementia-17, shift the protein towards non-structured species that are prone to dimerization and amyloid nucleus formation. He also reported on a novel protein engineering strategy (Fernández-Ramírez et al., 2018) that is particularly useful for SMFS measurements of proteins such as tau, which typically are plagued by low resolution because of the small magnitude of the unfolding force, as compared to instrument noise.

Brenton Hoffman (Duke University, Durham, NC) spoke about his ongoing efforts to establish new assays for probing the relationships between key subcellular and protein dynamics (LaCroix et al., 2018). He also discussed the use of biosensors that report the tension across specific proteins in living cells through changes in the color of light they emit, which might allow dynamic

measurements of proteins and subcellular structures that are under load.

Staying on the theme of tension sensing, Hongxia Fu (University of Washington, Seattle, WA) presented the use of a combination of single-molecule fluorescence imaging and microfluidic systems to directly quantify how hydrodynamic forces regulate inter- and intra-molecular interactions in single von Willebrand factor (vWF) molecules and their binding to platelets to form blood clots (Fu et al., 2017). She showed that vWF is first converted from a compact form to a linear form by flow, before being subsequently activated to bind platelet GPIbα in a tension-dependent manner.

Computational SMFS

Robert Best (NIDDK, NIH, Bethesda, MD) discussed how molecular simulations could be used to interpret protein-folding reactions and to investigate whether the ribosome affects the protein-folding pathway. He showed that, for small proteins, the effects are not significant but that the ribosome might exert greater influence on the folding of multidomain proteins (Guinn et al., 2018).

Dmitrii Makarov (University of Texas, Austin, TX) started his presentation by giving an overview of the development of computational algorithms that bridge the gap between computationally accessible and biologically relevant timescales (Makarov and Schuler, 2018). He then described how his approach, of integrating simulations and SMFS experiments, could be used to analyze the conformational transitions that a single protein undergoes during its folding pathway. This methodology is expected to be useful in many future contexts, such as addressing how mutations affect protein structure.

Technical advances in SMFS

Thomas Perkins (JILA/NIST and the University of Colorado, Boulder, CO) presented recent technical developments that have greatly increased the time resolution (by about 100-fold) and force precision (by about 10-fold) of AFM-based SMFS (Edwards et al., 2017). This increase in performance was demonstrated in pulling experiments on individual bacteriorhodopsin (bR) molecules embedded in their native lipid bilayer, which captured unfolding intermediates in unprecedented detail, including those separated by as few as three amino acids (Yu et al., 2017).

Ionel Popa's (University of Wisconsin, Milwaukee, WI) presentation focused on MT instrumentation and operational principles, and illustrated the use of MT-SMFS to study protein dynamics, as well as providing examples of surface chemistry to produce highly robust attachments of proteins to MT instruments (Dahal et al., 2020).

Concluding remarks

Although many aspects of single-molecule biophysics research have been represented before at various conferences, this NSF-sponsored workshop was one of the first meetings devoted solely to SMFS, which is a subfield of the single-molecule biophysics area. The workshop assembled leaders in the field, whose inspiring talks covered the important contributions of SMFS to the study of a wide range of biological systems that either experience or generate mechanical forces, from individual molecules such as bacterial adhesion proteins, nascent polypeptide chains and molecular motors, to individual cells navigating through elastic extracellular networks. The importance of combining force techniques with other assays, such as fluorescence, to expand insights into structure–dynamics relationships was an important theme throughout the workshop. Similarly, close interactions between experimental and computational SMFS approaches for interpreting observations at a sub-molecular level became evident during the workshop. New molecular attachment methodologies proved to be key to increasing a relatively low success rate of SMFS experiments, and new advancements related to force-sensor miniaturization revealed the great potential of SMFS measurements to capture mechanically weak and short-lived structural intermediate states in many systems, including soluble and membrane proteins undergoing unfolding, misfolding and refolding reactions. These advancements will likely promote wider use of SMFS methodologies among molecular and cellular biologists.

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Competing interests

The authors declare no competing or financial interests.

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