

techniques. TALE proteins are robust, programmable DNA-binding proteins, often fused to a nuclease domain to generate the TALEN system, a leading technology in genetic engineering. Recently, powerful methods for gene editing have been developed, including zinc finger nucleases, the CRISPR/Cas9 system, and TALENs. Despite great promise for treating human disease, however, we still lack a complete understanding of the mechanisms governing TALE search dynamics and the role of off-target binding events threatening to inhibit clinical implementation. Our work aims to develop a molecular-level understanding of TALE binding and target sequence search, which will facilitate the design of new and efficient TALEN systems. To this end we have developed a single molecule assay to directly visualize the binding and 1-D search of TALE proteins along stretched, dual-tethered DNA templates. We implemented an efficient method for specific labeling of TALE proteins using an aldehyde-based bioorthogonal labeling scheme relying on formylglycine generating enzyme. Single molecule data on TALE search dynamics reveal a previously unknown two-state “search and check” model, wherein periods of rapid DNA search are interspersed with stationary local binding. This model reconciles the ability for TALE proteins to locate their target sequence amongst thousands of potential binding sites. We further generated a series of truncated TALE variants and observed the dynamics of these proteins at the single molecule level. In this way, we are able to identify each subdomain’s role in sequence search, thereby further advancing the understanding of TALE search. Overall, our work provides a ‘first-of-its-kind’ view of the 1-D diffusion of TALE proteins on DNA, which will be critically important for engineering improved TALE proteins for precise genomic editing.

#### 1031-Plat

##### Dynamic DNA Target Proofreading in a CRISPR-Cas System

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<sup>1</sup>Institute for Molecular Cell Biology, University of Münster, Münster, Germany, <sup>2</sup>Institute of Biotechnology, Vilnius University, Vilnius, Lithuania. CRISPR-Cas systems provide adaptive immunity against invading genetic elements such as phages and plasmids by degrading the invader DNA. In type I CRISPR-Cas systems the intrinsic RNA component of the Cascade effector complex recognizes a complementary DNA target strand (protospacer) through base pairing while displacing the non-target strand of the duplex. The resulting RNA-DNA hybrid is called R-loop. R-loop establishment recruits the helicase-nuclease Cas3, which subsequently degrades the target DNA. Here we use single-molecule DNA supercoiling experiments to investigate the recognition and verification of the target sequence by Cascade. To this end we carefully explore the effect of mutations across the target. We observe the occurrence of intermediate R-loops which lengths correspond exactly to the distance of the mutation from the start of the target sequence. These intermediate R-loops are unstable and their stability directly correlates with their length. When point mutations are eventually overcome, a full R-loop forms and becomes “locked” through conformational changes of the Cascade complex. These observations provide direct evidence for a directional R-loop formation and suggest a dynamic target scanning by Cascade, mediated through R-loop intermediates. Early encountered mutations challenge R-loop propagation and cause its collapse while distal mutations enable more stable and longer lived R-loops to overcome them. The target validation takes place exclusively at the end of the sequence when full R-loop formation induces conformational changes that initiate DNA degradation. Such a dynamic and directional R-loop propagation scheme offers several advantages for suitable homologous target recognition: (i) the complex spends little time on wrong targets that from the beginning on carry mismatches; (ii) however it supports that point mutations can be tolerated thus precluding invading DNA to escape degradation through protospacer mutations.

#### 1032-Plat

##### Single-Molecule Imaging Reveals Dynamics of SA1-TRF1 Interactions on Telomeric DNA

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<sup>1</sup>Physics, North Carolina State University, Raleigh, NC, USA, <sup>2</sup>Biochemistry and Cell Biology, Rice University, Houston, TX, USA, <sup>3</sup>Division of Biophysics, Universität Osnabrück, Barbarstrasse, Germany, <sup>4</sup>Kimmel Center for Biology and Medicine at the Skirball Institute, Department of Pathology, New York University School of Medicine, New York, NY, USA. The cohesin complex plays a crucial role in accurate chromosome segregation, organization of interphase chromatin, DNA replication, and post-replicative DNA repair in part by promoting DNA-DNA pairing. The core cohesin subunits consist of a tripartite ring and the fourth core subunit Scc3/SA. In

somatic vertebrate cells, SA can be either SA1 or SA2. Recent work indicates that while SA2 is important for cohesion at the centromere, SA1 plays a specific role in sister telomere association. In addition, SA1 directly interacts with shelterin subunits TRF1 and TIN2. While these results demonstrate a unique sister telomere cohesion process depending on the SA1-TRF1 complex, the underlying mechanism is still poorly understood. We applied Atomic Force Microscopy (AFM) and Total Internal Reflection Fluorescence Microscopy (TIRFM) to study the interactions between SA1 or SA1/TRF1 complex and various DNA substrates with or without telomeric sequences. DNA tightrope assays were performed and proteins were visualized by conjugating quantum dots. The data demonstrated that 1) SA1 carried out 1-dimensional diffusion on DNA substrate for searching telomeric DNA sequence; 2) SA1 paused at telomeric DNA sequence, while SA2 did not. Interestingly, the AFM data showed that SA1 further stabilized TRF1 mediated DNA-DNA pairing. These data shed more lights on the process of sister telomere association and segregation.

## Platform: Molecular, Cellular, and Systems Neuroscience: Experimental Approaches, Modeling, and Tools

#### 1033-Plat

##### Electrophysiology-Based Sorting and Screening with Nanowire Electrodes in Microfluidic Devices

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Nanowire electrodes that can measure and manipulate the electrical potential across the cell membrane offer an attractive alternative to conventional intracellular electrodes based on sharp glass pipettes. Unlike glass pipettes, nanowire electrodes can be fabricated over large areas providing a route toward scalable high-throughput electrophysiology that will open to the door to electrophysiological phenotyping and electrophysiology-based cell sorting. To realize the potential of this high-throughput nanowire-based electrophysiology we have developed a suite of integrated microfluidic devices that feature nanowire electrodes that can manipulate and measure the membrane potential as part of a larger lab-on-a-chip concept. With these integrated electrophysiology chips we can rapidly measure and sort cells based on the kinetics of ion channels or the kinetics of voltage sensitive proteins. With this rapid method to quantify the time response of ion channels and proteins we can rapidly screen mutant variants and perform functional cell sorting of primary tissue. In addition to these single measurements we show that integrated nanowire devices can, for the first time, perform electrophysiology in intact whole organisms like the nematode *C. elegans*. The high-throughput capability of our nanowire electrophysiology device allows us to identify mutant strains that show differences in firing rates and action potential waveforms. Overall, by integrating microfluidics with nanowire electrophysiology we believe electrophysiology will stand beside gene sequencing and fluorescence imaging as a complementary high-throughput assay for single cells and whole organisms.

#### 1034-Plat

##### Mechanical Surface Waves Accompany Action Potential Propagation

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The action potential (AP) is the basic mechanism by which information is transmitted along neuronal axons. Although the excitable nature of axons is understood to be primarily electrical, many experimental studies have shown that a mechanical displacement of the axonal membrane co-propagates with the well-characterized electrical signal. While the experimental evidence for co-propagating mechanical waves is diverse and compelling, there is no theoretical consensus either for their physical basis or interdependence with the electrical signal. We present a model in which these mechanical displacements arise from the driving of mechanical surface waves, which we term Action Waves (AWs), in which potential energy is stored in elastic deformations of the neuronal membrane and cytoskeleton while kinetic energy is stored in the movement of the axoplasmic fluid. In our model these surface waves are driven by the traveling wave of electrical depolarization that characterizes the AP, altering the compressive electrostatic forces across the membrane as it passes. Our model allows us to predict, in terms of elastic constants, axon radius and axoplasmic density and viscosity, the shape of the AW that should accompany any traveling wave of voltage, including the AP predicted by the Hodgkin and Huxley (HH)