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
Marius Rutkauskas[1], Tomas Sinkunas[2], Maria S. Tikhomirova[1], Virginijus Siksnys[2], Ralf Seidel[1].
[1]Institute for Molecular Cell Biology, University of Münster, Münster, Germany, [2]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
Jiangguo Lin[1], Haijiang Chen[2], Parminder Kaur[1], Wang Miao[1], Preston Countryman[1], Changjiang You[3], Jacob Piehler[3], Yizhi J. Tao[2], Susan Smith[4], Hong Wang[1].
[1]Physics, North Carolina State University, Raleigh, NC, USA, [2]Biochemistry and Cell Biology, Rice University, Houston, TX, USA, [3]Division of Biophysics, Universität Osnabrück, Barbarstrasse, Germany, [4]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
Daniel L. Gonzales, Daniel G. Vercosa, Andrew M. Bell, Benjamin W. Avants, **Jacob T. Robinson**.
ECE, Rice University, Houston, TX, USA.
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
Benjamin B. Machta[1], Ahmed El Hady[2].
[1]Lewis-Sigler Institute, Princeton, Princeton, NJ, USA, [2]Princeton Neuroscience Institute, Princeton, Princeton, NJ, USA.
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)

equations. Our predictions are in line with existing measurements in a range of model systems including the squid giant axon and the garfish olfactory nerve. We expect our model to serve as a framework for understanding the physical origins and possible functional roles of these AWs in neurobiology. See arXiv:1407.7600 for details.

1035-Plat
Penetration of Action Potentials during Collision in the Medial Giant Axon of Invertebrates
Rima Budvytyte, Alfredo Gonzalez-Perez, Lars D. Mosgaard, Soren Nissen, Thomas Heimburg.
Niels Bohr Institute, Copenhagen, Denmark.
It is generally accepted that the collision of two action potentials coming from opposite directions is produced by the mutual annihilation of both signals. The experimental confirmation of this effect was shown by Tasaki in 1949 [1] and experiment is in agreement with the predictions of Hodgkin-Huxley model for action potential propagation [2].
In the current work we performed an analogous experiments to these made by Tasaki but using earthworms Lumbricus terrestris and lobsters Homarus americanus as an animals models. The collision of two simultaneously generated impulses propagating in orthodromic and antidromic directions were investigated. Also, the collision of compound of action potentials in the medial giant axons of sensory nerve bundle present in walking legs of lobster propagating were observed too. The experiments have been performed in the extracted ventral cords and nerve bundles of walking leg of lobster by using external stimulation and recording. The stimulation voltage was used as a tool to selectively stimulate the small neuronal population of giant axons (5 to 6 neurons).
Surprisingly, the collision of two impulses generated simultaneously, does not result in their annihilation. Instead, they penetrate each other and emerge from the collision without material alterations of their shape or velocity [3]. These results were published in Physical Review Letters X (2014) and are not consistent with expectations from the established HH model. But the findings, could be explained if nerve pulses are ''electromechanical'' waves - solitons, as suggested by Heimburg and Jackson [4].
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1036-Plat
Dynamics of Glycine Receptors and their Interactions with Gephyrin Scaffolds Revealed with High-Density Single Particle Tracking and Bayesian Inference
Mohamed El Beheiry[1], Jean-Baptiste Masson[2,3], Charlotte Salvatico[4], Christian Specht[4], Antoine Triller[4], Maxime Dahan[1].
[1]Physical Chemistry, Institut Curie, Paris, France, [2]Physics of Biological Systems, Institut Pasteur, Paris, France, [3]Janelia Farm Research Campus, Ashburn, VA, USA, [4]Ecole Normale Supérieure, Paris, France.
The scaffold protein gephyrin plays a critical regulatory role in the transmission of nerve signals in inhibitory synapses.. Its interactions with receptors of inhibitory neurotransmitters, such as glycine or GABA, are postulated to be a key molecular mechanism of synaptic formation and plasticity. Previous studies have shown that glycine receptors transiently bind to gephyrin scaffolds inside the synapse, however there is limited knowledge of the strength of this interaction. This is due, in part, to the consensus view that the synapse is a complex and dynamic assembly that is sensitive to a host of stimuli including neuronal activity, hormones, and pathological states. Therefore, a beneficial approach to improve our understanding is to reconstitute gephyrin scaffolds and their interactions with receptors in non-neuronal cells. To this end, in a greatly simplified system we use a transmembrane construction that consists of the large intracellular ß-Loop that directly interacts with the gephyrin scaffold, mimicking the actions of the endogenous glycine receptor.
Based on high-density single-particle tracking, we use a robust Bayesian inference approach to spatially map the dynamics inside receptor-scaffold sites in non-neuronal cells. Through adaptive spatial meshing techniques, we are able to conform our maps to highly heterogeneous trajectory and diffusion distributions and, notably, generate whole-cell landscapes of interaction energies. Importantly, we treat the inherently multi-scale nature of receptor motion in a faithful and reproducible manner.
We show that our method allows us to distinguish interactions between different ß-Loop mutants, effectively correlating protein-level modifications to quantifiable metrics of receptor dynamics. This is an important advance that reinforces the treatment of complex biological systems with statistical methods.

1037-Plat
Robust Optical Stimulation of Neuronal Activity using Functionalized Gold Nanoparticles
João L. Carvalho-de-Souza[1], Jeremy S. Treger[1], Bobo Dang[2], Stephen Kent[2], David R. Pepperberg[3], Francisco Bezanilla[1].
[1]Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL, USA, [2]Department of Chemistry, The University of Chicago, Chicago, IL, USA, [3]Lions of Illinois Eye Research Institute, Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, Chicago, IL, USA.
It was recently shown that infrared light pulses can directly stimulate neuronal action potentials by quickly heating the cell membrane and inducing capacitive currents (Shapiro, et al., Nat Commun, 2012). While useful, this technique works by heating the entire aqueous environment around a cell, rendering it nonspecific and incapable of stimulating specific populations of cells. Furthermore, since water absorbs infrared, the light source must be near the target. Here, we sought to stimulate neurons with visible light, using the ability of 20 nm spherical gold nanoparticles (AuNPs) to absorb green light and convert it into heat. By applying the AuNPs to painted lipid bilayers, we first confirmed that, upon 532 nm pulse stimulation, the membrane capacitance increases following the rate of temperature increase. The resulting currents are well-behaved, being linear with respect to both laser power and membrane potential. We next applied AuNPs to cultured dorsal root ganglion (DRG) neurons and demonstrated action potential initiation with 532 nm pulses of $\leq$1 ms. Finally, we obtained a dramatic improvement of this technique by functionalizing AuNPs with high-avidity ligands for DRG neuron membrane proteins. Non-functionalized AuNPs are cleared away from DRG neurons within seconds of starting a perfusion wash, causing the cells to lose optical excitability. However, AuNPs functionalized with Ts1 neurotoxin, or with anti-TRPV1 or anti-P2X3 antibodies, showed substantial resistance to washout; DRG neurons labeled with these functionalized particles remained light-sensitive after more than 20 minutes of perfusion washing. Furthermore, these particles required a lower concentration and less laser power to confer optical excitability. By appropriate selection of AuNP-conjugated ligand, this technique may enable improved cell-type specificity of neuronal photostimulation, such as ganglion cells in diseased retina where photoreceptors are non-functional.
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1038-Plat
Ligand Fingerprinting in the Membrane Dynamics of Single TrkA and P75NTR Neurotrophin Receptors
Stefano Luin[1], Laura Marchetti[1,2], Fulvio Bonsignore[1], Fabio Beltram[1], Antonino Cattaneo[2].
[1]NEST, Scuola Normale Superiore di Pisa and Istituto Nanoscienze – CNR, Pisa, Italy, [2]BioSNS Laboratory, Scuola Normale Superiore di Pisa and Istituto di Neuroscienze – CNR, Pisa, Italy.
We have sought to investigate the responses of Nerve Growth Factor (NGF) receptors TrkA and P75NTR at the plasma membrane of living neuronal cells by single-molecule imaging and tracking. To this purpose we exploit the acyl carrier peptide and some of its shortened versions (A1 and S6 tags, labeled selectively by two different PPTases) to tag human p75NTR and TrkA. These tags were covalently conjugated to the biotin- or fluorophore-substituted arm of a coenzyme A (CoA) substrate.
This approach allows: (i) a precise control of stoichiometry and site of biotin conjugation; (ii) versatility of the tags used; (iii) studying two interacting molecules with orthogonal fluorolabels, at the single-molecule or single-interaction-complex level. This experimental toolbox is completed by fast microscopy (e.g. TIRF microscopy with a fast EM-CCD), and by a semi-automatic algorithm for the analysis of the trajectories. This novel algorithm separates self-similar from multimodal trajectories, divides the last ones in sub-trajectories, and calculates the combined distributions of parameters measuring the diffusivity, the localization or driftness degree, and/or the number of molecules in tracked spots.
We shall present results on the early response of TrkA upon binding different biologically-relevant ligands (including NGF and proNGF): without ligands, TrkA is present mostly as fast-diffusing monomers; ligand binding results in an increasing number of dimers and oligomers, which are typically slower and/or more confined. Each ligand promotes distinct trajectory patterns at the cell membrane, because of different receptor-binding affinities, intracellular effectors recruited and formation of signalling/recycling endosome precursors. We believe that this imaging toolbox and our results pave the way to the quantitative description of the kinetics, dynamics and stoichiometry of any binary or