stretch and returned to pre-stretch values during relaxation (Figure 1B). Thus, X-ROS signaling and the consequent stretch-dependent Ca^{2+} release "tuning" found in ventricular myocytes is also operative in atrial myocytes. We will present data on the importance of NOX2, TTs and other components of the X-ROS signaling pathway in health and disease in atrial myocytes.

1075-Plat
Differential Impact of Distinct Long Qt Syndrome-1 (LQTS-1) C-Terminus Mutations on KCNQ1-KCNE1 Channel Trafficking and Gating
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KCNQ1/KCNE1 subunits together generate the slowly activating, delayed rectifier potassium current, I_{Ks}, important for human cardiac repolarization. Mutations throughout KCNQ1 lead to long QT syndrome-1 (LQTS-1), which predisposes patients to lethal ventricular arrhythmias. Many LQTS-1 mutations map to KCNQ1 C-terminus, but in many cases the mechanistic bases for their pathophysiological effects in heart are unknown. We compared the functional impact and mechanistic bases of six LQTS-1 mutations in distinct regions of KCNQ1 C-terminus that have been implicated in: PIP_2 or calmodulin binding domains (R555H, L619M). Optical assays for channel surface density and electrophysiological recordings were carried out in Chinese Hamster Ovary (CHO) cells. All KCNQ1 mutants, except L619M, displayed a significant decrease in channel surface density, which was either partially (R366W, V524G, R539W, G589D) or fully (R555H) rescued by wild-type KCNQ1. G589D and L619M alone yielded no currents, while all other mutations moderately reduced current amplitude. When co-expressed with wild-type KCNQ1, G589D current amplitude was fully rescued whereas L619M exerted a dominant negative effect. When co-expressed with KCNE1: all mutants displayed reduced current amplitude compared to control, albeit to different extents; all except L619M displayed the slowly activating signature of I_{Ks}; three mutants displayed a large rightward shift in the activation curve which was either partially (R366W, R555H) or fully (R539W) recovered with wild-type KCNQ1. The data reveal a surprising heterogeneity of trafficking and gating mechanisms underlie KCNQ1 C-terminus mutations. Such information is essential for developing potential targeted therapies for LQTS-1 patients. We further introduce the first study of L619M, an unusual KCNQ1 mutant that trafficks normally to the cell surface, but is essentially non-functional and exerts a strong dominant-negative effect on wild-type KCNQ1 channels.

1076-Plat
The Distal Kcne1 C-Terminus is Crucial for Yotiao Mediated Pka-Dependent Phosphorylation of KCNQ1
Meidan Dvir[1], Dana Shaham[2], Inbal Ben-Tal Cohen[1], Roi Strulovitz[2], Yoni Haitin[1], Carmen Dessauer[3], Robert Kass[4], Joel Hirsch[2], **Bernard Attali**[1].
[1]Sackler Medical School, Tel Aviv University, TelAviv, Israel, [2]Faculty of Life Sciences, Tel Aviv University, TelAviv, Israel, [3]University of Texas Health Science Center, Houston, Houston, TX, USA, [4]Columbia University, New York, NY, USA.
The assembly of KCNQ1 with KCNE1 produces the I_{KS}potassium current that is crucial for the late repolarization of the cardiac action potential. Mutations in either KCNQ1 or KCNE1 genes produce the long QT or short QT syndromes, which are genetically heterogeneous cardiovascular diseases, characterized by ventricular or atrial arrhythmias. The scaffolding A-kinase anchoring protein (AKAP) Yotiao brings together PKA, PP1, PDE4D3, AC9, and the I_{KS} channel complex to achieve regulation following beta adrenergic stimulation.
We recently showed that the distal KCNE1 C-terminus interacts with the coiled-coil helix C of KCNQ1 C-terminus. Here we examined the effect of LQT mutations located at this C-terminal interface of the two subunits. Four KCNQ1 LQT mutations located at helix C, S546L, K557E, R555C, and R562M impaired the interaction with KCNE1 C-terminus and produced a drastic reduction in I_{KS} current density mostly caused by a right-shift of the voltage-dependence of channel activation. A much weaker PIP2 binding paralleled the decrease in I_{KS} current density. The KCNE1 LQT mutation, P127T, situated at the distal C-terminus weakened the interaction with KCNQ1 helix C and caused a 40% decrease in I_{KS} current density but with no shift of the voltage-dependence of channel activation. Interestingly, the KCNE1 mutant P127T markedly reduced the cAMP-dependent Yotiao-mediated upregulation of I_{KS} current.
While the P127T mutation did not alter the ability of KCNQ1 to interact with Yotiao, it strongly disrupted KCNQ1 phosphorylation of S27, in the presence of Yotiao and cAMP. Similar results were found with a deletion of the distal KCNE1 C-terminus.(del 109-129). These results suggest that the distal KCNE1 C-terminus, probably via its interaction with the coiled-coil helix C, is a crucial determinant for the functional modulation of KCNQ1 by Yotiao-mediated PKA phosphorylation.

Platform: Single Molecule Techniques I

1077-Plat
Positional Imprinting of Optical Traps Corrects for Mechanical Drift in High-Resolution Instruments
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The performance of high-resolution optical traps typically depends on the passive mechanical stability of several critical optical components. Passive stability demands stringent requirements for the laboratory environment to limit instrumental drift arising from vibrations, acoustics, and temperature variations. To follow the real-time position of biological motors taking base pair steps along DNA, angstrom resolution is needed. Limiting the positional drift of an optical trap to one angstrom requires that a collimated beam be held angularly stable to 40 nanoradians. To minimize instrumental drift, our lab uses a dual-beam setup wherein two traps are used to perform measurements in solution, isolated from the microscope stage. Consequently, angular drift from optical elements interacting with both beams is correlated and therefore cancelled. Even in this dual-beam configuration, however, four optical elements can introduce relative angular drift between the beams, resulting in uncorrelated motions that are indistinguishable from single molecule activity. We have identified this necessary portion of all dual-beam instruments as the primary source of relative positional drift between the traps. Unfortunately, even stringent environmental controls are expected to be insufficient to prevent nanoradian-level motions of these optical components. We therefore seek instead to measure and correct for drift that inevitably occurs. Here, we present a new advance in trap design that enables us to correct for the relative positional instability of dual optical traps in real-time using a novel positional imprinting technique. We show that our new optical trap design is capable of virtually eliminating mechanical drift, consistently yielding noise characteristics under high-noise conditions that are comparable to the quietest conditions we can achieve in our laboratory. This technology will potentially reduce the engineering controls necessary for high-resolution optical trapping, making the technique available to research labs lacking specialized facilities.

1078-Plat
Asymmetric Unwrapping of Nucleosome Revealed by Single Molecule Fluorescence-Force Spectroscopy
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The nucleosome is the fundamental unit of eukaryotic chromatin structure which compacts DNA in the nucleus. It is a stable yet dynamic complex which is significant to transcriptional regulation. In cells, DNA is likely to be under tension to varying extents. For example enzymes such as RNA polymerase and chromatin remodelers can generate force on nucleosomes. Thus it is important to understand the dynamics of nucleosomes under mechanical constraints. We are utilizing a cutting-edge single-molecule optomechanical technology which combines fluorescence with optical tweezers (aka 'fleezers') to probe the conformational transitions of nucleosomes under force. Our hybrid fleezers instrument provides fluorescence readouts with nm resolution under pN levels of force applied in order to locally monitor conformational dynamics of nucleosome at various coordinates. Here, we report the first observation of force-dependent gradual unwrapping and rewrapping of the nucleosome using fluorescence reporters. Moreover, we observed force-induced two-state hopping corresponding to the opening and closing of the outer DNA wrap as well as more complex transitions for the inner wrap. Interestingly, our fluorescence probes at various locations report significant asymmetric unwrapping behavior. In our pulling experiments, it takes less than 6 pN to unwrap the outer DNA at the 3' end of the 601 positioning sequence but takes up to ~15-17 pN to unwrap the DNA from the 5' end. Asymmetric unwrapping may have implications for how enzymes such as chromatin remodelers interact with nucleosome substrates.

1079-Plat
STED Nanoscopy Combined with Optical Tweezers Reveals Spatial Dynamics of Proteins on Densely Covered DNA
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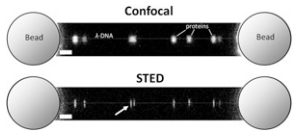
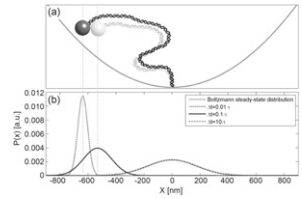
Dense coverage of DNA by proteins is a ubiquitous feature of cellular processes such as DNA replication, transcription, repair, and compaction. We present a single-molecule manipulation and visualization approach capable of studying individual DNA-protein interactions in the presence of a high density of proteins on the DNA and in solution. This approach combines optical tweezers with multicolor confocal fluorescence microscopy and STED nanoscopy. We demonstrate visualization of proteins on DNA with a spatial resolution of 48 nm, about six-fold better than with traditional wide-field microscopy. The resolution enhancement along the direction of the DNA can be seen in the figure below (scale bar 1μm). Two proteins positioned within the diffraction limit can clearly be distinguished in the STED image. In combination with fast confocal line scanning, STED allows real-time imaging of DNA-protein dynamics with a temporal resolution better than 50 ms. The individual trajectories of proteins translocating on DNA can be distinguished at high protein density and tracked with enhanced localization precision. This unique multimodal approach allowed us to visualize, in real time, the assembly of dense nucleoprotein filaments on DNA with unprecedented spatial resolution.



1080-Plat
Routine and Timely Sub-Piconewton Force Stability and Precision for Biological Applications of Atomic Force Microscopy
Allison B. Churnside[1], Ruby May A. Sullan[1], Duc M. Nguyen[1], Matthew S. Bull[1], Gavin M. King[2], **Thomas T. Perkins**[3].
[1]Univ. of Colorado, Boulder, CO, USA, [2]Univ. of Missouri, Columbia, MO, USA, [3]NIST & CU-Boulder, Boulder, CO, USA.
Force drift is a significant, yet unresolved, problem in atomic force microscopy (AFM). We show that the primary source of force drift for a popular class of cantilevers is their gold coating, even though they are coated on both sides to minimize drift. Drift of the zero-force position of the cantilever (z_0) was reduced from 900 nm for gold-coated cantilevers to 70 nm (N =10; rms) for uncoated cantilevers over the first 2 hours after letting the tip; a majority of these uncoated cantilevers (60%) showed significantly less drift (12 nm, rms). Removing the gold also led to ~10-fold reduction in reflected light, yet short-term (0.1-10s) force precision improved. Moreover, improved force precision did not require extended settling; most of the cantilevers tested (9 out of 15) achieved sub-pN force precision (0.54 ± 0.02 pN) over a broad bandwidth (0.01-10 Hz) just 30 min after loading. Finally, this precision was maintained while stretching DNA. Hence, removing gold enables both routine and timely access to sub-pN force precision in liquid over extended periods (100 s). We expect that many current and future applications of AFM can immediately benefit from these improvements in force stability and precision.

1081-Plat
A Hybrid Tirf-Magnetic Tweezers Instrument for Studying Force-Induced Conformational Changes in Proteins
Samuel M. Leachman, Christian A.M. Wilson, Bernardo Cervantes, Athena Ierokomos, Susan Marqusee, Carlos Bustamante.
University of California, Berkeley, Berkeley, CA, USA.
Optical tweezers are a useful research tool for applying forces to single proteins and measuring the resulting changes in extension, but they can only observe conformational changes along the axis of force application and only if the accompanying changes in extension are on the nanometer scale. Our experimental setup skirts this limitation by measuring distance changes using single-molecule Förster resonance energy transfer (smFRET) produced from a total internal reflection fluorescence (TIRF) microscope incorporating magnetic tweezers. Individual protein molecules are conjugated to FRET-paired fluorescent dyes and functionalized DNA handles using disulfide and click chemistry. These handles tether each molecule between a glass coverslip on the TIRF microscope and a paramagnetic bead. An external magnet applies a uniform field that exerts a force on each molecule tethered to the surface. Simultaneous recording of the intensities of the donor and acceptor fluorophores and the magnet position enables direct observation of force-induced conformational changes. The use of triplet-state quenchers and oxygen scavengers extends the lifetime of tethers into the minute timescale, producing single-molecule trajectories exhibiting hundreds of transitions. Here we present our latest results from this new setup.

1082-Plat
A Novel Method for Dynamic Analysis of Single-Molecule Experiments in Trapping Potentials
Yuval Garini[1], Guy Nir[1], Anat Vivante[1], Ian T. Young[2], Moshe Lindner[1].
[1]Bar Ilan University, Ramat Gan, Israel, [2]Delft University of Technology, Delft, Netherlands.
We present a novel method for analyzing the dynamics of tethered particles in single-molecule experiments such as optical and magnetic tweezers, and tethered particle motion.
By using the Smoluchowski equation and its solution, that was not yet fully explored, we developed a method for analyzing the same data being currently measured in the single-molecule experiments mentioned above. We demonstrate the power of the method by analyzing a nano-bead tethered to a single DNA molecule. It allows one to simultaneously extract all the parameters that describe the system, namely, the diffusion coefficient and the restoring-force constant without having to plug-in prior values.
The method is based on the extraction of the moving particle probability distribution function in its complete phase-space. It improves the accuracy of the parameters that are extracted in the experiment, and makes them more robust.



Evolution of the particle distribution function as a function of time, providing the basis for the new analysis method we describe www.igc.ethz.ch and www.gromos.net.

1083-Plat
Detection and Mapping of 5-Methylcytosine and 5-Hydroxymethylcytosine in Short Strands of ssDNA using Nanopore Sequencing with MspA
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University of Washington, Seattle, WA, USA.
Epigenetic modifications of cytosines, such as 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC) in CpG sites of DNA, are both, inheritable and influenced by the environment. These nucleotide modifications have been shown to be important in gene regulation, cell programming, and carcinogenesis. It is therefore imperative that next-generation sequencing techniques are able to detect epigenetic modifications. Conventional methods for detection of 5-mCpG and 5-hmCpG, require chemical conversion of 5-mC to uracil or are limited to bulk analysis of relative ratios of C to 5-mC to 5-hmC. Here we demonstrate a technique for mapping individual 5-mCpG and 5-hmCpG sites within single molecules of ssDNA. We used nanopore sequencing whereby the phi29 DNA polymerase draws ssDNA through the porin MspA. An ion current passing through the pore directly detects and maps the location of such modifications in single molecules. We will present data on specific detection of both 5-mCpG and 5-hmCpG sites based on comparisons with unmodified sequences.

1084-Plat
Nanopore Sequencing using a Hidden Markov Model for Base-Calling
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Nanopore-based DNA sequencing has many features which recommend it over current state of the art sequencing by synthesis methods; increased read length, reduced sample requirements, and increased speed to name a few. However, the accuracy of base-calling using the electrolytic current has thus far been relatively limited, from both solid-state and biological nanopores. Though part of this is due to the low signal-to-noise ratio, another significant contribution is the base resolution of the nanopore - it does not interrogate a single base at a time, rather the current is influenced by multiple bases at the same time.
We suggest using this multi-base interrogation as an advantage rather than a disadvantage - each base in the DNA strand is read multiple times in this circumstance, allowing for improved accuracy. We have implemented a method to decode the electrical signature of 3bp resolution nanopore electrical measurements into a DNA sequence using a hidden Markov model. We produced simulated ionic current for all 64 possible triplets using atomic-resolution Brownian dynamics (BD).
Using simulated current signatures, we have been able to demonstrate 98.3% base-calling accuracy for λ DNA, a substantially increased value compared to using only a single-base method (47.1%). When applied to 50kb fragments of the human genome, we found a similar median accuracy level (98.2%). Furthermore, we determined that there is a correlation between the local complexity of the sequence, as measured by Shannon's entropy, and the error rate; lower complexity sequence is more error-prone. Longer sequenced fragments have lower error rate - the more information input into the Markov model, the more effective the algorithm is at decoding the DNA sequence; this is in marked contrast to the dephasing problems endemic to current sequencing by synthesis methods.