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
Ademuyiwa Aromolaran[1], William R. Kobertz[2], Henry M. Colecraft[1].
[1]Columbia University, New York, NY, USA, [2]University of Massachusetts Medical School, Worcester, MA, USA.
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
Troy A. Lionberger, Yves Coello, Carlos J. Bustamante.
University of California, Berkeley, Berkeley, CA, USA.
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
Thuy T.M. Ngo, Ruobo Zhou, Jaya Yodh, Taekjip Ha.
University of Illinois at Urbana-Champaign, Urbana, IL, USA.
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
Gerrit Sitters[1], Iddo Heller[1], Onno D. Broekmans[1], Géraldine A. Farge[1], Carolin Menges[2], Wolfgang Wende[2], Stefan W. Hell[3], Erwin J.G. Peterman[1], Gijs J.L. Wuite[1].
[1]Vrije Universiteit Amsterdam, Amsterdam, Netherlands, [2]Justus-Liebig-Universität Giessen, Giessen, Germany, [3]Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.