and mechanochemical properties. The model reproduces key signatures found in optical trapping studies two-kinesin complexes including observations of non-monotonic dependencies of cargo velocities and motor-microtubule unbinding rates on the applied load, and predicts that multiple kinesins have generic difficulties cooperating productively. While such behavior is influenced significantly by extrinsic factors including the spatio-temporal dependence of the applied load, the net-negative cooperative behaviors exhibited by multiple kinesins appear to be directly linked to the efficiency of kinesin's stepping mechanism, and other types of less efficient and 'weaker' processive motors are predicted to cooperate more productively. Thus, mechanochemical efficiencies of different motor types may distinguish how effectively they function as a team, and hence, how motor copy number contributes to the regulation of cargo motion. Finally, the extension of our experimental and computational approaches to studies of multiple motor dynamics inside of living cells will be discussed.

1074-Symp
Dynamics of Pairs of Processive Myosin Motors
Andrej Vilfan, Ph.D.
F5, J. Stefan Institute, Ljubljana, Slovenia.
When two processive myosin motors (myosin V or myosin VI) are coupled together, their dynamics becomes qualitatively distinct from that of a single motor, but also from those of larger ensembles of motors. I will discuss two situations: two identical motors (myosin V, myosin V) or two motors of opposing directionality (myosin V, myosin VI). For identical motors, the dynamics of the pair is determined by an interplay between the randomness of stepping of each motor, the elasticity of the linkage and the low-force region of the force-velocity relation. For two antagonistic motors which engage in a tug of war, the outcome depends on properties of both motors close to the stall force.
Several models for processive myosins describe the dimeric motor as two identical heads, interacting solely through their mechanical connection. These models relate the properties of the dimer (e.g., step size, velocity, processivity, head-head coordination) to those of its individual heads (geometry, elasticity and kinetics). We now extend this approach to relate the properties of a pair of (identical or different) motors to those of their heads, 4 in total.

1075-Symp
Coordination of Multiple Motors Bound to Intracellular Cargos
Adam G. Hendricks[1], Allison L. Zajac[1], Harry W. Schroeder, III[1], Sandra Maday[1], E. Michael Ostap[1], Yale E. Goldman[1], **Erika L. Holzbaur**[2].
[1]University of Pennsylvania, Philadelphia, PA, USA, [2]Department of Physiology , University of Pennsylvania, Philadelphia, PA, USA.
The movement of organelles and vesicles along the cellular cytoskeleton is often driven by multiple motor types, including kinesins, dynein, and myosins. Both in vitro and cellular studies suggest that multiple motors are bound simultaneously to intracellular cargos. For some cargos, such as late endosomes/lysosomes, we find that opposing motors may be active simultaneously, leading to stochastic directional switching best characterized as a tug-of-war. Run lengths are generally short and apparent diffusive movement predominates. For other cargos, such as autophagosomes, both kinesin and dynein motors remain stably bound but motility is highly processive in a single direction, suggesting motor activities are regulated. Intracellular transport is also regulated at the level of the track, as dictated by the complex organization of the intracellular cytoskeleton, characterized by microtubule-microtubule and microtubule-actin filament intersections as well as filament dynamics and filament-binding proteins. By analyzing motility at multiple levels, including: (1) in vitro with purified motors bound to beads at filament intersections; (2) in vitro with motors that co-purify with isolated organelles; (3) in the cell using high resolution tracking of endocytosed quantum dots; and (4) intracellular manipulation of phagocytosed beads using an optical trap, we can investigate the mechanisms that coordinate the interactions of multiple motors in intracellular organelle transport. Supported by NIH GM087253.

Minisymposium: Optical Recording of Ion Channels

1076-MiniSymp
Insights into RyRs Dysfunctions via Studies of Intracellular Calcium Signals
Sergii Kyrychenko[1], Eva Poláková[1], Nina D. Ullrich[2], Ernst Niggli[2], **Natalia Shirokova**[1].
[1]Pharmacology & Physiology, University of Medicine and Dentistry, Newark, NJ, USA, [2]Department of Physiology, University of Bern, Bern, Switzerland.
Duchenne muscular dystrophy (DMD) is a striated muscle disease with severe cardiac manifestations. The *mdx* mouse, an animal model of DMD, develops dilated cardiomyopathy. Several studies associated changes in Ca^{2+} homeostasis and oxidative stress with DMD. In particular, posttranslational modifications of Ca^{2+} release channels (RyRs) increase their sensitivity, leading to augmented Ca^{2+} responses during mechanical challenges, and to cellular and mitochondrial Na^{+} overload, dysfunction and cell death. We examined whether changes in RyR function were causal for or a consequence of cardiac failure and which posttranslational modifications of RyRs drive the development of the pathology. Fluorescent indicators and imaging techniques make it possible to study the function of RyR channels in the natural cellular environment on a near-molecular level. Young *mdx* mice show no changes in cardiac performance, but do so after ~8 months. However, even myocytes from 1 month old *mdx* mice produced exaggerated Ca^{2+} sparks and waves after osmotic shock, and exhibited "hypersensitive" excitation-contraction coupling. Both were nearly abolished by antioxidants and NOX inhibitors and reduced by CaMKII but not by NOS- and PKA-inhibitors. SR Ca^{2+} load, leak or resting $[Ca^{2+}]_i$ were unchanged in young *mdx* cells. However, by the age of 4-5 months and in senescence, load was reduced, leak and resting $[Ca^{2+}]_i$ increased, indicating disease progression. By this age, all agents listed above reduced intracellular Ca^{2+} responses and prevented changes in ECC, Ca^{2+} load and leak. Thus 1) increased Ca^{2+} sensitivity of RyRs precedes and presumably contributes to the development of dystrophic cardiomyopathy and 2) oxidative stress drives its development. RyR oxidation, nitrosylation and phosphorylation, first by CaMKII followed by PKA, lead to even further sensitization. This synergistic sensitization of RyRs by several pathways results in cardiac muscle deterioration and heart failure.

1077-MiniSymp
Optical Recordings of Ca^{2+} Influx via TRPV4 and Voltage-Gated L-type Ca^{2+} Channels in Arterial Smooth Muscle
Luis F. Santana[1], Manuel F. Navedo[1], Rachael Bayle[2], José L. Mercado[1], Joseph Brayden[2].
[1]Department of Physiology & Biophysics, University of Washington, Seattle, WA, USA, [2]Department of Pharmacology, The University of Vermont, Burlington, VT, USA.
In arterial smooth muscle, L-type Ca^{2+} channels play a critical role in multiple physiological processes including excitability, contraction, and gene expression. Recent work suggests that TRPV4 channels are also important contributors to Ca^{2+} influx in these cells. We used optical approaches to record Ca^{2+} sparklets produced by Ca^{2+} influx via TRPV4 and L-type Ca^{2+} channels in arterial myocytes under physiological conditions. We found that TRPV4 and L-type Ca^{2+} sparklet activity varies throughout the sarcolemmal of arterial myocytes. Our data suggest that these regional variations in sparklet activity arise from interactions between channels, the scaffolding protein AKAP150, and associated proteins at only a few sub-sarcolemmal regions in arterial smooth muscle cells. We will present data obtained using biochemical and optogenetic approaches to investigate the mechanisms leading to subcellular variations in TRPV4 and L-type Ca^{2+} sparklet activity as well as the functional consequences of these local Ca^{2+} signals in arterial smooth muscle. These findings will form the basis for a new model for the local control and amplification Ca^{2+} influx via TRPV4 and voltage-gated L-type Ca^{2+} channels in resistance artery smooth muscle.

1078-MiniSymp
Optical Analysis of Ryanodine Receptor Behavior *In Situ*: The Role of FKBP12.6 in Cardiomyocytes
Yan-Ting Zhao[1], Jing-Chao Li[1], Zheng Chen[2], Peng Zhou[1], Qi Yuan[2], Yun-Feng Jiang[1], Guang-Ju Ji[2], **Shi-Qiang Wang**[1].
[1]Peking University College of Life Sciences, Beijing, China, [2]CAS Institute of Biophysics, Beijing, China.
Since the finding of Ca^{2+} sparks (and its equivalents), optical recording has become the most important tool to study intracellular Ca^{2+}-permeable channels *in situ*, which are inaccessible otherwise by electrophysiological means. Due to the mixture of in-focus and out-of-focus events, spontaneous sparks do not quantitatively reflect the ryanodine receptor (RyR) Ca^{2+} release flux (i_{RyR}). To quantify the i_{RyR}, we activated and recorded in-focus Ca^{2+} sparks under the loose-seal patch-clamp condition, and calibrate i_{RyR} with Ca^{2+} sparklets from a single L-type Ca^{2+} channel (LCC). Using this analytical tool, we studied the role of FKBP12.6 in regulating RyR gating behavior in intact cardiomyocytes, which has been highly controversial over the last decade. We found that, in wild-type mouse ventricular myocytes, i_{RyR} exhibited a distribution with periodic quantal peaks, with each quantum of 1.05 pA representing the i_{RyR} of a single RyR. By contrast, in heart cells from FKBP12.6 knockout (FKO) mice, the quantal property of i_{RyR} was eliminated, indicating that

synchronized activation of multiple (2~4) RyRs basically depends on the existence of FKBP12.6. Notably, FKO cells displayed fractional i_{RyR} of 0.3–0.8 pA. This finding provided direct evidence for the partial opening of a single RyR *in situ* in the absence of FKBP12.6. The suppression of fractional i_{RyR} events in wild-type cells demonstrated that FKBP12.6 played a key role in coordinating the allosterism among RyR subunits. Taken together, our study demonstrated for the first time that FKBP12.6 mediates both inter- and intramolecular coordination of RyR gating in intact heart cells.

1079-MiniSymp
Single Molecule Fluorescence Study of the *B. Thuringiensis* Toxin Cry1Aa Reveals Tetramerization
Hugo McGuire, Nicolas Groulx, Raynald Laprade, Jean-Louis Schwartz, **Rikard Blunck**.
Université de Montréal, Montréal, QC, Canada.
Pore forming toxins compose a class of potent virulence factors that attack their host membrane in a 2- or 3-step mechanism. After binding to the membrane often aided by specific receptors, they form pores in the membrane either leading to cytolysis or providing a pathway to introduce enzymes effecting intracellular proteins. The major rearrangements of the toxins upon binding to the membrane are accompanied by oligomerization to the final pore forming construct. However, it remains often unknown how many monomers constitute the pore, if that number is fixed, and whether the toxins enter the membrane in monomeric or oligomeric form. Here, we used *single subunit counting*, to determine the number of oligomers contained in a Cry1Aa toxin as a function of the concentration. Cry1Aa is one of the δ-endotoxins of *B. thuringiensis*, a soil bacteria that is, because of its specificity for certain insect larvae, used as a biological alternative to chemical pesticides. Cry toxins were purified and fluorescently labeled in their monomeric form. Lipid vesicles of various compositions were incubated with labeled toxin monomers, and supported bilayers formed from the mixture. We recorded the fluorescence intensity over time of distinct fluorescence intensities in the supported bilayer and determined their photobleaching behaviour. As single fluorophores bleach in a single step, the number of steps corresponded to the minimal number of monomers in each oligomer. The oligomerization state showed a concentration dependence which was consistent with both a concentration dependent association rate between monomers and a concentration dependent distribution on the lipid vesicles during incubation. Statistical analysis of the results showed that the toxins enter the membrane in their monomeric form and diffuse laterally to form tetramers, which appears to be the pore forming unit.

1080-MiniSymp
Improved Genetically Encoded Voltage Sensitive Optical Probes Detect Action Potentials and Subthreshold Events
Zhou Han[1], Lei Jin[2], Jelena Platisa[1], Julian Wooltorton[3], Brian M. Salzberg[3], Lawrence B. Cohen[2], Vincent A. Pieribone[1].
[1]John B Pierce Laboratory, New Haven, CT, USA, [2]Yale University, New Haven, CT, USA, [3]University of Pennsylvania, Philadelphia, PA, USA.
We improved a genetically encoded voltage sensitive optical probe, a conjugate of the Ecliptic GFP and CiVSP, by modifying the fluorescent protein and its insertion site in the voltage sensitive **phosphatase**. The signal size is increased from −1% to −35% for 100 mV depolarization steps. The improved probes' dynamics is fitted by the double exponential equation, with fast Tau-on of 10 ms and fast Tau-off of 20 ms. The signal size and dynamics enable the probes to detect single action potentials and subthreshold electrical events in individual cultured neurons with high reliability. These probes advance the possibility of fully optical recording and control of neuron activity.

1081-MiniSymp
Using Voltage-Clamp Fluorometry Technique to Study the Mechanism of the Cooperativity Between Hv Channel Subunits
Feng Qiu, Peter Larsson.
University of Miami, Miami, FL, USA.
In the immune system, the activity of voltage-gated proton channels has been shown to play a key role in the charge compensation for the electron extrusion by NADPH oxidase during respiratory burst in phagocytes. In addition, this channel was also found in many other cell types including lung airway epithelia cells, sperms and high metastatic breast cancer cells, in which they have been implicated in the phathology of asthma, pH dependent spermatozoa and tumor metastasis, respectively. It was not until 2006 that two independent groups discovered the genes coding for the voltage-gated proton (Hv) channels. It was shown that the Hv channel is a dimer and that there is a strong coopertivity between two subunits during activation of this channel. However, how the two subunits in Hv channels cooperatively activate the channel and to what extent this coopertivity affect the channel activation are unknown. In this study, we investigate the detailed molecular mechanism of coopertivity in the dimeric Hv channel by using the combination of two electrodes voltage clamp (TEVC) and voltage clamp fluorometry (VCF). We measure the voltage sensor movement and pore opening of each subunit in a linked-dimer that has two subunits with different activation voltage ranges. Thereby we can determine how one subunit affects the other subunit during voltage sensor movement and pore opening.

Platform: Membrane Transporters & Exchangers I

1082-Plat
Intracellular Proton Access Mechanism of the CLC-ec1 Cl^-/H^+ Exchanger
Hyun-Ho Lim, Tania Shane, Christopher Miller.
Brandeis University, waltham, MA, USA.
CLC-ec1 is a bacterial CLC Cl^-/H^+ exchanger that catalyzes 2:1 exchange of Cl^- and H^+. E148 is the pH-dependent external gate of both Cl^- and H^+ transport and E203 mediates proton transport from intracellular solution to the protein interior. Substitution of non-protonatable residues on either glutamate uncouples the exchanger by completely abolishing H^+ transport. However, it is not clear how protons in the intracellular solution gain access to E203, which is buried under a cytoplasmic ''lid'' formed by the protein's N-terminal helix. Truncation of the N-terminal 29 residues removes this lid and preserves 2:1 coupling of Cl^- and H^+ and absolute transport rate. This result implies that proton transport from intracellular solution to E203 is not rate-limiting in the transport cycle, and that protons are somehow facilitated in their movement to the buried E203. In order to discover proton facilitators, we examined polar residues near E203. Nonpolar mutants of Q207, S446 and R403 only slightly inhibit H^+-transport rate. However, mutations on the proximate glutamate, E113, and disruption of a nearby salt-bridge (E117I and R209I) decrease H^+-transport rate ~5-fold. These residues appear to form a water-filled conduit for proton access to E203. Interestingly, nonpolar mutants of E202, located near the dimer interface and near to intracellular solution, show 30-100-fold reduced H^+-transport rates compared to wildtype. All mutations here preserve H^+-coupled Cl^- transport, although those with severely reduced rates display somewhat higher Cl^-/H^+ stoichiometry, indicative of Cl^- slippage. One or two crystallographic water molecules were found between E203 and E202. We propose that these water molecules form an access pathway for H^+ from bulk water, which is blocked by bulky, hydrophobic amino acids at E202.

1083-Plat
Transient Water Chains Connecting the Cytoplasmic and Extracellular Glutamate Gates in CLC
Wei Han, Emad Tajkhorshid.
University of Illinois at Urbana-Champaign, Urbana, IL, USA.
CLC-ec1, a bacterial member of the CLC family, exchanges chloride ions and protons across the cellular membrane. A key step of the transport cycle of CLC- ec1 is the transfer of protons between the extracellular and cytoplasmic gates, E148 and E203, respectively. These residues are 15 Å apart, and in dearth of any intermediate titratable groups needed for proton shuttling. Hence, it is an open question as to how protons shuttle between the two gates. Proton hopping through water chains provides a possible mechanism in this regard, having been investigated in numerous computational studies, given the lack of experimental structural data on water. Two possible chains, either involving Y445 or side-chain rotation of E203, have been proposed based on searching algorithms and short molecular dynamics (MD) simulations. We herein propose another water chain characterized through extended (0.42 μs) MD simulations of CLC-ec1 dimer. The water chain forms frequently (once every 50-100 ns) but transiently (lasting for <1–2 ns). Neither Y445 nor the side-chain rotation of E203 is needed for the water chain. The presence of the water chain, however, coincides with significant side-chain conformational changes of F199 and F357 around the chloride-binding site and F208 and F219 at the dimer interface remote from the ion permeation pathway. We further performed a 0.25 μs simulation of monomeric CLC-ec1, which has been shown to be structurally identical to the dimer, but with a halved activity. We show that water chains don't form as readily in the monomeric simulation as what was observed in the dimer simulation; side-chain conformations of F199, F357, F208 and F219 are also different from the dimer. Our study supports the idea that both local and long-range factors could be important for the CLC-ec1 activity.