(CaM), circular-permuted fluorescent protein (cpEGFP or others) and the light-chain myosin kinase peptide RS20. Although genetically engineering has led to a broad variety of different GECIs and also red probes suitable for optogenetics, there are only few variants with fast kinetics to monitor action potentials [1]. We have performed mutations on two red fluorescent GECIs, jRCaMP1a and jRGECO1a [2], to weaken the interaction between CaM and the RS20 target peptide, to fasten the kinetics of these slow probes.
In total we made and biophysically characterized 17 variants. At physiological ionic strength and 20°C, jRCaMP1a shows a biexponential fluorescence rise with rates 52 ± 1 (47%) and $2.3 \pm 0.1\ s^{-1}$ (53%) and a dynamic range of 6.8 ± 0.3. Fluorescence decay is biexponential with a fast ($2.2 \pm 0.1\ s^{-1}$ (17%)) and slow phase (2.2 ± 0.1 (17%) and $0.32 \pm 0.01\ s^{-1}$ (83%)). The variant termed $jRCaMP1a_{fast}$ has similar rise kinetics to jRCaMP1a with rates 15 ± 1 (47%) and $2.1 \pm 0.1\ s^{-1}$ (53%) but decays 21-fold faster ($6.9\ s^{-1}$) with dynamic range of 4.5 ± 0.2.
jRGECO1a (dynamic range 12.3 ± 1.2) has single exponential kinetics with rise and decay rates $150 \pm 3\ s^{-1}$ and $4.3 \pm 0.1\ s^{-1}$, respectively. The variant termed $jRGECO1a_{fast}$ has a similar rise rate ($150 \pm 3\ s^{-1}$) with a 5-fold faster bi-phasic decay (k_{off}: 25 ± 2 (85%) and $1.5 \pm 0.1\ s^{-1}$ (15%)) and dynamic range (12.9 ± 0.8). The variant termed $jRGECO1a_{ultrafast}$ has slower rise kinetics ($53 \pm 2\ s^{-1}$) but a 100-fold faster decay (k_{off}: 515 ± 80 (80%) and $7 \pm 1\ s^{-1}$ (20%)) and a dynamic range of 6.9 ± 0.1.
The faster kinetics and preserved dynamic ranges of the novel red GECIs make them useful imaging tools.
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2380-Plat
Particle-Based Approaches to Clearing Calcium: a Protein Landscape Model of the Sarco/Endoplasmic Reticulum Calcium-ATPase (SERCA) Pump for Sub-Cellular Stochastic Models

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The Sarco/Endoplasmic Reticulum Calcium-ATPase (SERCA) is a membrane-protein that completes the cycle of Excitation-Contraction Coupling; achieving cardiomyocyte ‘‘relaxation’’ by importing Ca^{2+} to the Sarcoplasmic Reticulum (SR). SERCA associates with many small molecules and ions during the pumping mechanism. Existing models of the SERCA pump mechanism reduce complexity of intermolecular reactions by combining discrete binding and unbinding steps, effectively reducing the total number of states. To be able to more-closely understand how small perturbations in variables like Ca^{2+}, ATP, and H^{+} concentrations affect the mechanism of SERCA, we developed an explicit-particle based approach to modeling the SERCA-2a pump. Our protein-landscape model features twelve discrete states and explores the effects of pH on the rate of calcium clearing.

2381-Plat
Ca^{2+} Signals Originate from Immobile IP_3 Receptors at ER-PM Junctions

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Inositol 1,4,5-trisphosphate receptors (IP_3Rs) are ubiquitous intracellular Ca^{2+} channels, which upon activation by IP_3 and Ca^{2+}, release Ca^{2+} from the endoplasmic reticulum (ER). Regulation of IP_3Rs by Ca^{2+} allows regenerative propagation of Ca^{2+} signals, generating a hierarchy of Ca^{2+} release events. The spatial and temporal organization of these Ca^{2+} signals contributes to their capacity to selectively regulate diverse biological functions. The spatial arrangement and dynamics of IP_3Rs are important for producing these regenerative Ca^{2+} signals. To study the distribution and dynamics of native IP_3Rs, we used transcription activator-like effector nucleases (TALENs) to tag endogenous IP_3R1 of HeLa cells with EGFP. EGFP-IP_3Rs were functional and formed small clusters within ER membranes. Most IP_3R clusters were mobile, but some were immobile over protracted periods; with minimal mixing of the mobile and immobile IP_3Rs. Single-particle tracking revealed that IP_3Rs move by diffusion, and along microtubules by both kinesin-1 and dynein motors. Within both mobile and immobile IP_3R puncta, some IP_3Rs were tightly packed but others were too far apart for their association to be mediated by direct interactions between IP_3Rs. Simultaneous visualization of EGFP-IP_3Rs and Ca^{2+} signals showed that Ca^{2+} signals, whether evoked by photolysis of caged IP_3 or activation of endogenous receptors that stimulate IP_3 formation, originate from immobile IP_3Rs at ER-plasma membrane (PM) junctions. These Ca^{2+} release sites closely apposed the ER-PM junctions where stromal-interaction molecule (STIM), the ER Ca^{2+} sensor that stimulates store-operated Ca^{2+} entry (SOCE), accumulated after depletion of ER Ca^{2+} stores. Our results show that IP_3-evoked Ca^{2+} signals are initiated by immobile IP_3R clusters tethered near the ER-PM junctions at which SOCE occurs. We suggest that this organization may both optimize delivery of IP_3 to IP_3Rs and allow effective regulation of SOCE by local depletion of Ca^{2+} stores.

2382-Plat
Characterization of Different Localized Ca^{2+} Signals in Skeletal Muscle Fibers

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Discrete localized Ca^{2+} signals (LCS) occur in many cell types. In skeletal muscle fibers they have been shown e.g. to be elicited as a result of an osmotic shock or mechanical skinning of isolated muscle fibers. Indicating their possible pathophysiological importance, spontaneous LCS have been observed in the mdx mouse model of human Duchenne muscular dystrophy and in wild type mouse muscle fibers after severe mechanical stress. In addition, there is a variety of different types of intricate subcellular Ca^{2+} signaling in micro-domains of mammalian skeletal muscle fibers. However, global Ca^{2+}-transients from the sarcoplasmic reticulum often mask these localized Ca^{2+} signals (LCS). In our study we have focused on the localization of nuclear Ca^{2+} signals which are thought to be involved in the regulation of gene expression and that, among other functions, have been shown to play a role in tumor growth in non-muscle cells. We have identified nucleus-localized and perinuclear-localized Ca^{2+} signals observed under hypertonic stimulation conditions. The Ca^{2+} signals are in the micrometer range, with full width at half maximum at 2.75 ± 0.27 µm and 2.55 ± 0.17 µm for nuclear and perinuclear LCS respectively. Types of Ca^{2+} channels involved in formation of nuclear and perinuclear LCS were investigated utilizing channel blockers. Dantrolene (RyR blocker), nifedipine (DHPR blocker) and Xestospongin C (IP_3R blocker) have been used. We have observed the incidence of LCS associated with nuclei in the presence of each blocker. STIM channel blockers may provide further insight on the nature of the intranuclear Ca^{2+} signals which may shed light to new signals influencing a variety of nuclear processes.

2383-Plat
Role of ORAI Proteins in Activation of Endogenous TRPC1-Composed Channels

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Depletion of intracellular calcium stores activates store-operated channels. This process induces numerous intracellular signaling events. The most studied store-operated channels are CRAC channels. Other channels are believed to consist of TRPC and Orai proteins. However, their role in molecular composition of endogenous store-operated non-CRAC channels remains obscure. One of the main questions to ask is how TRPC channels are activated after store depletion. Most studies have used whole-cell patch clamp or calcium imaging techniques. To discriminate different types of store-operated channels, we used single-channel patch-clamp recordings in HEK293 cells. We showed that in experiments with dominant-negative mutant ORAI E106Q endogenous TRPC1-composed channels were not sensitive to store depletion, but they were activated by other pathways. In cells overexpressing STIM2 proteins endogenous TRPC1 channels were activated with a delay (similarly to ORAI channels activated by STIM2). In summary, we propose that (i) ORAI does not serve as a pore forming subunit of endogenous TRPC1 channels, but it is necessary to maintain sensitivity to calcium stores and (ii) TRPC1 channels are activated downstream to ORAI channels after store depletion. This study was supported by the Russian Scientific Foundation, Project 14-14-00720 (to E. K., D.K. and A.S.); and the Russian Foundation for Basic Research Project 16-04-01792 (A.S. and L.G.).

2384-Plat
The Two-Pore Domain K^{+} Channel TALK-1 Provides a Countercurrent that Facilitates Endoplasmic Reticulum Ca^{2+} Leak

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The two-pore domain K^{+} (K2P) channel TALK-1 modulates insulin secretion by limiting β-cell electrical excitability and cytosolic Ca^{2+} (Ca^{2+}_{c}) influx.