clusters (~50 nm dia). The mouth of the t-tubules were often very heavily labeled for 2-3 μm into the cell.

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Superresolution Microscope Image Reconstruction by Spatiotemporal Ob-ject Decomposition and Association: Application in Resolving T-Tubule Structure in Skeletal Muscle
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One key factor that limits resolution of single-molecule superresolution microscopy relates to the localization accuracy of the activated emitters, which is usually deteriorated by two factors. One originates from the background noise due to out-of-focus signals, sample auto-fluorescence, and camera acquisition noise; and the other is due to the low photon count of emitters at a single frame. With fast acquisition rate, the activated emitters can last multiple frames before they transiently switch off or permanently bleach. Effectively incorporating the temporal information of these emitters is critical to improve the spatial resolution. However, majority of the existing reconstruction algorithms locate the emitters frame by frame, discarding or underusing temporal information. Here we present a new image reconstruction algorithm based on tracklets, short trajectories of the same objects. We improve the localization accuracy by associating the same emitters from multiple frames to form tracklets and by aggregating signals to enhance the signal to noise ratio. We also introduce a weighted mean-shift algorithm (WMS) to automatically detect the number of modes (emitters) in overlapping signal clusters. Addition of external Ca2+ ions flowing through voltage-controlled isolated skeletal muscle fibres from mice and zebrafish larvae. Depolarizing pulses of increasing amplitudes evoked L-type Mn2+ currents associated with for a-2 quenching signals displaying comparable voltage-dependences. In the presence of external Ca2+ L-type currents were totally abolished whereas substantial quenching signals remained associated with voltage pulses. The voltage-dependence and reactivation kinetics of the quenching signals were comparable in the presence of Ca2+ and in its absence. In zebrafish fibres, neither voltage-activated L-type current nor voltage-activated quenching signal was observed. Taken together, these data suggest that the L-type current is the only contributor to voltage-activated Ca2+ entry in skeletal muscle. Calibration of the quenching signal in mouse fibres indicated that an influx producing a current of 0.3 A/F developed during a train of action potentials (AP). Prolongation of AP induced by tetraethylammonium led to an increase in the quenching signal evoked by a train of AP. A series of experiments using FluoS5N to monitor sarcoplasmic reticulum (SR) Ca2+ changes in voltage-controlled mouse fibres showed that increasing the L-type current amplitude by doubling external Ca2+ concentration led to an acceleration of the recovery phase of SR Ca2+ signals. Addition of external Mn2+ led to opposite effects suggesting that Mn2+ ions flowing through L-type channels have access to the SR lumen and quench FluoS5N fluorescence. These results strongly suggest that Ca2+ influx through L-type channels significantly contribute to replenish the SR and in this way helps to preserve muscle performance during prolonged activity.

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Contribution of L496-L500-W503 Motif of DHPR-β1a Subunit to Skeletal-Ty E-C Coupling
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The β1a subunit is a cytoplasmic component of the dihydropyridine receptor (DHPR) complex that plays an essential role in skeletal muscle excitation-contraction (EC) coupling. Here we investigate the role of the carboxyl terminal end of this auxiliary subunit in the functional and structural communication between the DHPR and the Ca2+ release channel (RyR1). Progressive truncation of the β1a C-terminus showed that deletion of amino acid residues 489-Q to -W503 resulted in loss of depolarization-induced Ca2+ release, severe reduction of L-type Ca2+ currents and lack of tetrad formation as evaluate by freeze-fracture analysis. However, deletion of this domain did not affect expression/targeting or density (Qmax) of DHPR-β1a subunit to the plasma membrane. Within this region are found residues L496, L500 and W503, which are thought to mediate direct β1a-RyR1 interactions. Disruption of the motif L496-L500-W503 by a triple alanine substitution weakened EC-coupling but it did not replicate the truncated phenotype. These data demonstrate that an amino acid segment comprising sequence 489-Q to 503 of β1a contains critical determinant(s) for the physical link of DHPR and RyR1, further confirming a direct correspondence between DHPR positioning and DHPR/RyR functional interactions. In addition, our data strongly suggest that motif L496-L500-W503 within the β1a C-terminal tail plays a non-essential role in bi-directional DHPR/RyR1 signaling that supports skeletal-type EC-coupling. Supported by NIH Grants 5K01AR54818, R03AR066359 (to CDF), R37-HL-048992 (to EFA) and Miller Fund/Harvard Medical School (to CFP).

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FRET-Based Structural Analysis of the Skeletal Muscle DHPR using Biarsenical Labeling
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The z1s skeletal muscle dihydropyridine receptor (DHPR) subunit triggers muscle contraction during excitation contraction (EC) coupling. The intracellular loops of this voltage-gated calcium channel have diverse functions, though the spatial organization of these loops relative to each other and to other DHPR subunits, is poorly understood. We employed a FRET-based assay to determine the proximity of these intracellular loops relative to the N-terminus of the z1s DHPR subunit and then determined how these proximities changed upon binding of the β1a DHPR subunit. Recombinant z1s DHPRs were expressed in HEK293T cells and then FRET was measured from YFP fused to the DHPR N-terminal to the bisarsenical reagent ReAsH targeted to a tetracysteine (Tc) tag inserted into each of the DHPR intracellular loops. Energy transfer was highest to ReAsH bound to a Tc tag placed adjacent to the N-terminally fused YFP (E~50-50%) whereas low FRET (E~10%) was observed for a negative control construct lacking a Tc tag. Placement of Tc tags in each of the DHPR intracellular loops resulted in intermediate FRET efficiencies whereas insignificant energy transfer was observed between the DHPR N- and C-termini. Addition of purified recombinant DHPR β1a subunit resulted in significant enhancement of energy transfer between the N-terminal YFP and ReAsH targeted to the DHPR z1s II-III loop, a major EC coupling determinant. Our data demonstrate that FRET measurements using biarsenical labeling reagents offer an effective approach to study DHPR structure since disruption of native conformation is minimal. In addition, these studies should allow for subsequent measurements of changes in DHPR structure that occur during EC coupling. Supported by NIH grants R01AR059126 (to JDF) and R03AR066359 (to CFP).

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Dependency of Ca2+ ω Alternans on Ion Channel Localization in Human Atrial Cells
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Introduction: Localization of ion channels to t-tubules (TTs) comprises a critical part of excitation-contraction coupling in cardiomyocytes. Recent studies have shown that TT organization varies greatly among atrial cells, which have different ion channel distribution than ventricular cells. Ca2+ transient (CaT) alternans are known to occur in atrial cells with fewer TTs, but the ion channels which contribute to this are unknown.