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STORM-based Quantitative Assessment of Sodium Channel Localization Relative to Junctional Proteins Within the Cardiac Intercalated Disk

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Computer models suggest that ephaptic coupling in the heart is feasible given close apposition (<30 nm) between sodium channel ($Na_v1.5$) -rich membranes of adjacent myocytes.(1-3) Such close apposition of membranes only occurs in some parts of the intercalated disc (ID). We previously demonstrated 5-10 nm intermembrane distance within the perinexus, a gap junction (GJ) -adjacent ID nanodomain.(4) Further, gSTED super-resolution microscopy suggest enrichment of $Na_v1.5$ within the perinexus. Moreover, experiments disrupting the close apposition of membranes within the perinexus slowed conduction and precipitated arrhythmias. Therefore, we sought to quantify the localization of $Na_v1.5$ and its auxiliary subunit β1 (SCN1b) relative to connexin43 (Cx43) and N-Cadherin (N-Cad), which respectively served as markers of ID interplicate and plicate regions.

Confocal micrographs of guinea pig ventricular myocardium revealed enrichment of $Na_v1.5$ and β1 at the ID. Next, we analyzed STORM images of guinea pig ventricular sections immunolabeled for $Na_v1.5$ / β1 along with Cx43 and N-cadherin (N-Cad) using custom algorithms. Briefly, the STORM data, comprised of the precise 3D locations of individual fluorophore molecules, was subjected to 3D particle density-based cluster detection. Convex hulls were fit to each cluster and the following parameters evaluated for each cluster of a given protein: 1) degree of overlap with a cluster of the co-labeled protein, and, 2) surface-to-surface distance to the nearest cluster of the co-labeled protein. Overall, less than a quarter of the Cx43 and $Na_v1.5$ clusters overlapped each other. Where overlap occurred, it accounted for less than a quarter of either cluster's volume, suggesting tangential contact (figure 1). However, over half of the Cx43 clusters identified had $Na_v1.5$ located less than 200 nm away, within the previously reported extent of the perinexus. Interestingly, a second population of $Na_v1.5$ was identified in regions of high N-Cadherin density where intermembrane spacing exceeds 50 nm.(5) In short, the data suggests two ID-localized populations of $Na_v1.5$, one adjacent Cx43 aggregates, in regions corresponding to the perinexus, and the other co-distributing with N-Cadherin. In contrast, β1 was preferentially enriched adjacent Cx43 with less than 10% co-distributing with N-Cadherin.

Taken together these data suggest that there may exist two pools of $Na_v1.5$ within the ID: One co-distributed with β1 adjacent Cx43, where intermembrane distances can be <= 10 nm. The second was not co-distributed with β1 and was located in N-Cadherin-rich regions where membrane spacing exceeds 50 nm. Thus, the former $Na_v1.5$ pool may be preferentially able to support ephaptic coupling. This hypothesis is further strengthened by previous reports that β1 can cluster $Na_v1.5$ and also act as a cell adhesion molecule.(6) Therefore, these data suggest a role for β1-mediated adhesion in modulating ephaptic coupling.

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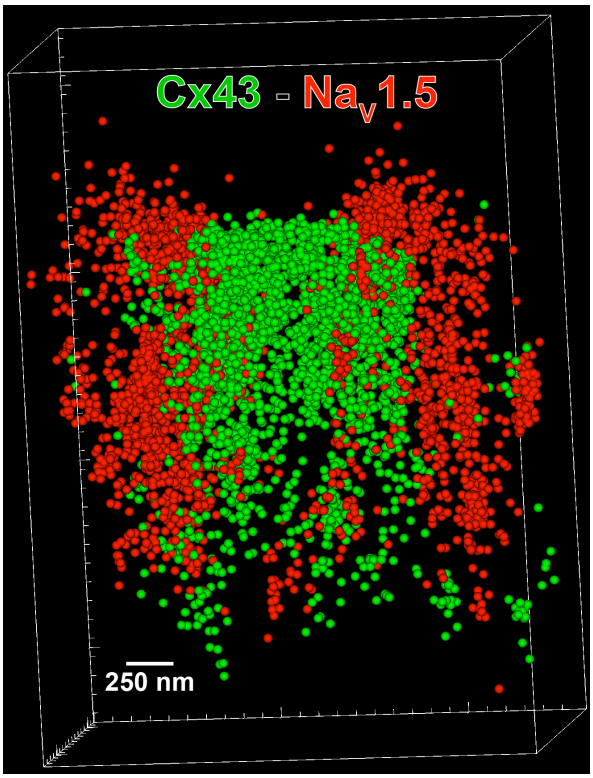

Figure 1. A 3D rendered view of representative STORM data showing a cluster of fluorophores corresponding to Cx43 (green) flanked by clusters of $Na_v1.5$ (red) on either side. Each sphere represents an individual fluorophore molecule localized.

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