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 (Naᵥ1.5) -rich membranes of adjacent myocytes. 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. Further, gSTED super-resolution microscopy suggest enrichment of Naᵥ1.5 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 Naᵥ1.5 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 Naᵥ1.5 and β1 at the ID. Next, we analyzed STORM images of guinea pig ventricular sections immunolabeled for Naᵥ1.5 / 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 Naᵥ1.5 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 Naᵥ1.5 located less than 200 nm away, within the previously reported extent of the perinexus. Interestingly, a second population of Naᵥ1.5 was identified in regions of high N-Cadherin density where intermembrane spacing exceeds 50 nm. In short, the data suggests two ID-localized populations of Naᵥ1.5, 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 Naᵥ1.5 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 Naᵥ1.5 pool may be preferentially able to support ephaptic coupling. This hypothesis is further strengthened by previous reports that β1 can cluster Naᵥ1.5 and also act as a cell adhesion molecule. Therefore, these data suggest a role for β1-mediated adhesion in modulating ephaptic coupling.

References:

Figure 1. A 3D rendered view of representative STORM data showing a cluster of fluorophores corresponding to Cx43 (green) flanked by clusters of Na$_v$1.5 (red) on either side. Each sphere represents an individual fluorophore molecule localized.