doi:10.1017/S1431927620016013
Microsc. Microanal. 26 (Suppl 2), 2020

Super-resolution Microscopy Illuminates Cardiac Structure, Function and Pathology at the Nanoscale

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Cardiac muscle cells have an intricate internal organisation and molecular machinery to tightly regulate rapid calcium fluxes that are critical to controlling their contraction. Light microscopy has been very useful to elucidate this organisation but was until recently limited to diffraction-limited resultion which precluded study of the molecular make-up that biophysical modelling had suggested is critical for calcium signalling. One protein that plays a vital role in cardiac cell calcium handling is the cardiac ryanodine receptor (RyR), a calcium release channel in the sarcoplasmic reticulum of ventricular myocytes. When in close proximity, adjacent RyRs can activate each other so that clustering of RyRs can greatly affect the probability with which calcium trigger signals will activate such clusters. Recently, using the remarkable localization precision of the super-resolution imaging modality DNA-PAINT we visualized punctate labeling within RyR labeling nanodomains, which we confirmed as single RyRs[1]. RyR positions within RyR clusters revealed that clusters are organized randomly into irregular clustering patterns leaving significant gaps occupied by accessory or regulatory proteins. DNA-PAINT super-resolution imaging supports a quantitative imaging mode termed qPAINT (or quantitative PAINT). We used qPAINT to precisely quantify RyR cluster size and determined that, on average, peripheral clusters contain 8.8 ± 3.6 RyRs. Using multi-channel super-resolution imaging we have studied the proximity between RyRs and other accessory cardiac proteins in health and disease[2,3]. Recently, we introduced a new approach to directly study protein-protein interactions in cells that we termed Proximity Dependent PAINT (PD-PAINT)[4]. With this new approach we have investigated the molecular interaction between RyRs and other SR membrane proteins with greatly improved resolution. DNA-PAINT has been an important tool for most of the experiments described above but currently it is generally limited to the study of fixed cell preparations. In collaboration with colleagues at the University of Oslo we have recently begun to correlate local calcium release in live cardiac myocytes with the underlying molecular distribution of RyRs as determined by live cell PALM. I will present initial findings obtained using this new approach and illustrate how this illuminates the molecular structure-function relationships in cardiac myocytes in novel ways. Our observations provide a molecular basis for biophysical models of RyR cluster activation and reveal potential mechanisms underlying pathological changes in cardiac disease.

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