NEXT GENERATION, REPRODUCIBLE AND QUANTITATIVE SUPER-RESOLUTION IMAGING OF CARDIAC RECEPTOR CLUSTERS.

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Modern single molecule localisation microscopies, such as PALM (Photoactivated Localisation Microscopy) or STORM (Stochastic Optical Reconstruction Microscopy) rely on the photo-switching of individual fluorophores. This typically results in non-recoverable photobleaching over the course of image acquisition, restricting such experiments to a single attempt. More recent single molecule methods using DNA, such as DNA-PAINT (Points Accumulation Imaging in Nanoscale Topography) [1], rely on the brief and reversible binding of oligonucleotides with attached fluorophores in free solution. The ability to image the sample for an extended duration results in an improved spatial resolution, with approximate single figure nanometre localisation precision. This improvement has enabled resolving the arrangement patterns of single ion channel proteins at an unprecedented level of spatial detail. This is exemplified by the visualisation of ryanodine receptor calcium channels (RyRs), within dense protein clusters in cardiomyocytes, to our knowledge for the first time using light microscopy. Due to the predictable nature of this approach, quantitative measurements of receptor numbers in clusters were made by the process of in-situ calibrated qPAINT [2]. Additional confidence can be taken in these ultra-resolved images, with repeat measurements yielding reproducible results of marker positions, which confirms robust structure determination at the nanometre scale. Exchange-PAINT was used to determine co-clustering with other proteins with single-receptor resolution in this complex cell type.

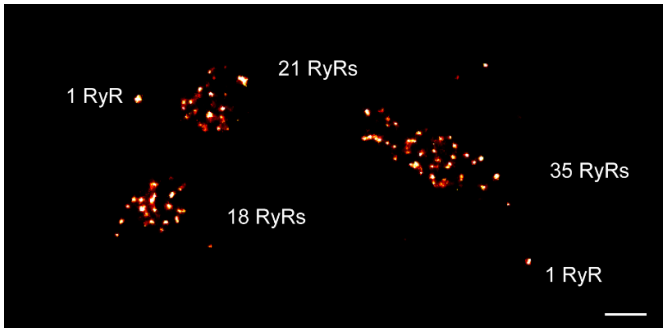

Figure.1: Using in-situ calibration with single receptors, appearing as isolated puncta, qPAINT analysis was used to accurately determine receptor numbers in RyR clusters. Scale bar: 200 nm.

[1] R. Jungmann, M. S. Avendaño, J. B. Woehrstein, M. Dai, W.M. Shih & P. Yin; "Multiplexed 3D cellular super-resolution imaging with DNA-PAINT and Exchange-PAINT", *Nat. Methods*; **11**, 313-318(2013)
[2] R. Jungmann, M. S. Avendaño, M. Dai, J. B. Woehrstein, S. S. Agasti, Z. Feiger, A. Rodal & P. Yin; "Quantitative super-resolution imaging with qPAINT", *Nat. Methods*; **13**, 439-442(2016).