Using super-resolution imaging to understand protein organization within Z-discs of striated muscle.

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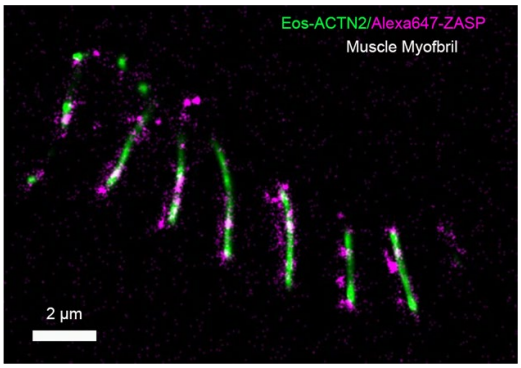
The Z-disc is a complex structure found at the ends of muscle sarcomeres in striated muscle. It is approximately 100nm in width and comprises over 30 different proteins, which play key structural and signaling roles. However, we have little understanding into how these proteins are organized. Electron microscopy approaches have so far been unable to resolve the positions of most of these proteins. Light microscopy approaches are hampered by low resolution, failure of antibodies to penetrate the dense Z-disc structure (Parker et al., 2023), and the large size and flexibility of antibodies is not well suited to super-resolution fluorescence microscopy approaches. Affimers, are small (~10kDa, 3-4 nm in size) non-antibody binding proteins, developed at Leeds, that overcome the challenges of imaging the organization of proteins within the Z-disc (Cordell et al., 2022; Tiede et al., 2017). Their small size means that they penetrate the Z-disc better than antibodies and that they only introduce a small linkage error, as fluorescent dye molecules conjugated to Affimers are close to the epitope of interest.

Here, we have used Affimers to begin to determine protein organization within the Z-disc, using dSTORM. We isolated Affimers against at >10 different Z-disc protein epitopes, targeting titin, α-actinin-2 (ACTN2), ZASP (LIM domain-binding protein 3), myotilin, capping protein, and others. To achieve this, we expressed and purified domains using E.coli from each of these proteins, using a 'BAP' tag (biotinylation site) to ensure that the proteins are biotinylated as they are expressed, and a HIS tag for purification. Purified proteins were used in a phage display library to isolate binders, which were then subcloned, an unique C-terminal cysteine added for subsequent conjugation of the purified Affimers to fluorophores via a maleimide linkage, and the Affimers expressed and purified from E.coli (Tiede et al., 2017). Purified, dye labelled (e.g. Alexa 647 for dSTORM and iPALM) Affimers were used to label myofibrils isolated from mouse or pig hearts and imaged using an Abbelight dSTORM microscope. Alternatively, Affimers were fused to mEos3, expressed and purified and used in labelling. The resulting image datasets (xyz localisation) were analysed using PERPL to gain a better understanding of their organisation with Z-discs (Curd et al., 2015). We have now successfully tested these Affimers in STED, dSTORM and in iPALM, and the results reveal that Affimers penetrate the Z-disc much better than antibodies, and the overall organisation of specific Z-disc proteins with high (~15nm or better), X,Y and Z resolution.

Graphic:



Keywords:

dSTORM, Affimers, super-resolution imaging,

Reference:

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