OPTICAL NANOSCOPY APPLIED TO THE CHARACTERIZATION OF MATURATION AND LOCALIZATION DEFECTIVE DYSTROGLYCAN MUTANTS

Wolfgang Hübner[a] , Manuela Bozzi[b], Saskia Bannister[a], Giulia Signorino[b], Jana-Freja Frommann[a], Alice Wilking[a], Viola Mönkemöller[a], Francesca Sciandra[b], Andrea Brancaccio[b] and Thomas Huser[a]

[a]Biomolecular Photonics, Dept. of Physics, Bielefeld University, 33615 Bielefeld, Germany
[b]ICRM - CNR c/o Università Cattolica del Sacro Cuore, Roma 00168, Italy
whuebner@physik.uni-bielefeld.de

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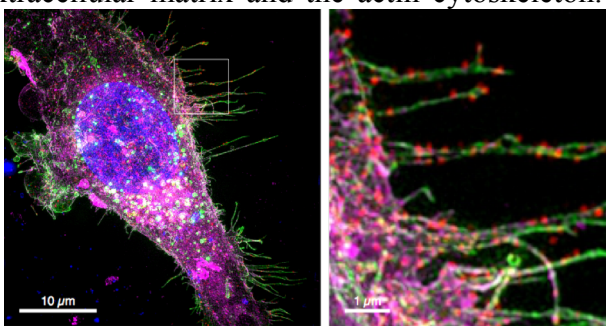
The dystroglycan adhesion complex is part of the dystrophin-associated complex. Dystroglycan is ubiquitously expressed as a pro-peptide that is undergoing a yet poorly understood molecular maturation and cellular trafficking pathway. The precursor molecule is cleaved in the endoplasmic reticulum into an alpha (extracellular) and beta (transmembrane) subunit. Multiple glycosylation processes take then place within the golgi apparatus
Located at the plasma membrane, the alpha and beta subunits plays a crucial role in muscle stability by providing the link between the extracellular matrix and the actin cytoskeleton. Several autosomal recessive neuromuscular disorders such as severe congenital (Muscle-Eye-Brain and Walker-Warburg syndrome) or limb-girdle muscular dystrophies (LGMD2P) are attributed to dysfunctional dystroglycan complexes. Secondary dystroglycanopathies are mostly caused by genetic abnormalities of glycosyltransferases leading to hypoglycosylated alpha subunits and therefore reduced affinity towards the extracellular matrix protein Laminin-2. But, in recent years, primary dystroglycanopathies involving direct missense mutations of the dystroglycan core proteins were identified.



SR-SIM resolves extracellular alpha-dystroglycan from transmembrane beta-dystroglycan subunit. A U2OS cell expressing wild-type dystroglycan is shown on the left, boxed area is magnified on the right. Alpha subunit is immunolabelled (red) while the beta is labelled intracellularly with EGFP (green). The membranes are stained post fixation with cellmask (magenta) and the nucleus with DAPI (blue).

Based on our multidisciplinary approach in establishing models for the analysis of primary dystroglycanopathies, we present here the use and combination of several super-resolution fluorescence microscopy techniques. Specific labeling techniques permit us to discern and track the alpha and beta core protein subunits of dystroglycan separately. Mutated constructs expressed in selected cell lines are analysed via nanoscopy for their subcellular localization of both separate subunits. This detailed imaging approach illustrates the severe molecular and trafficking defects in dystroglycan mutations involved in LGMD2P and muscle-eye-brain disease.
We believe that our experimental work can be crucial to understand the structural-functional relationships of the dystroglycan precursor molecule and that it may therefore have an invaluable biomedical impact, influencing the design of future therapeutic or diagnostic strategies.