

Correlative 2-color STED/AFM for mechanistic studies of synaptic vesicle and α -synuclein fibril interactions

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The misfolding of alpha-synuclein (α -syn) is considered to be closely related to the death of neurons in Parkinson's disease. Alpha-synuclein is a presynaptic protein and is proposed to have a physiological role in synaptic vesicle release, recycling and homeostasis. In PD models synaptic vesicle (SV) numbers are often depleted, SVs are more immobile and less neurotransmitter is released. We therefore investigated whether a pathological role of α -syn could be responsible for the destruction of the vesicles. Misfolded α -syn could result in a change of interactions with the vesicle membrane, rupture these and cause a malfunction of signal transfer, which still needs to be further investigated. It was shown before, that the N-terminus of α -syn is interacting with lipids and the C-terminus with vesicles upon calcium binding, indicating a double-anchor mechanism. [1] To study these effects, we demonstrate a method which merges the advantage of chemical specificity of super-resolution fluorescence microscopy with label free topography measurements. [2][3] By combining STED nanoscopy and atomic force microscopy (AFM) we show firstly that the co-localisation of vesicles (mCLING) and α Syn (Atto594) fibrils increases over time and secondly, we investigate the mechanical stiffness of vesicles and α -syn on the nanoscale to determine whether vesicle destruction occurs (see Fig.1 (a), (b)).

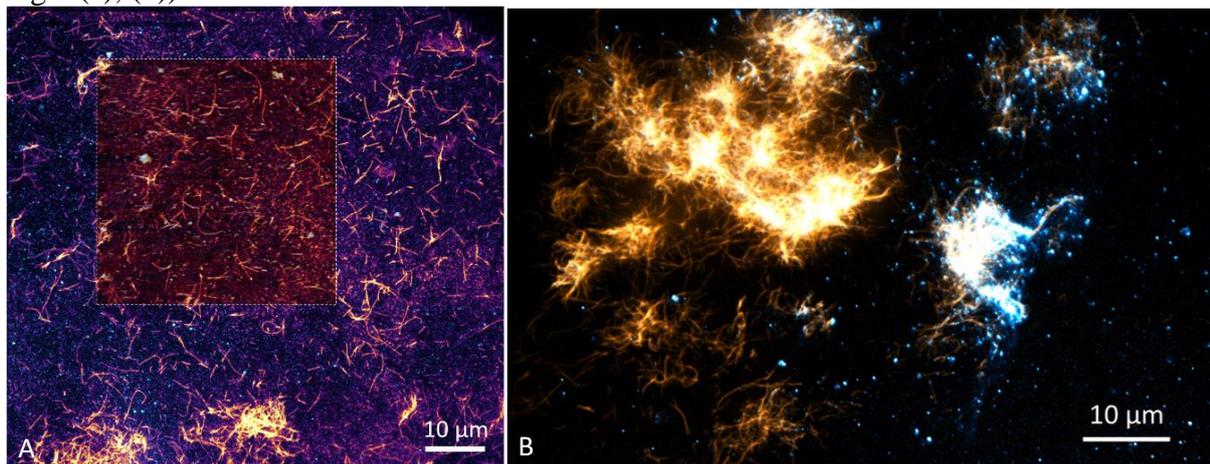


Figure 1: (a) Large FOV correlative 2-color STED image with AFM insert; (b) Super-resolved clusters of synaptic vesicles on fluorescently labelled α -synuclein fibrils

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