A superresolution method to quantify the association of alpha synuclein with synaptic organelles in the study of neurodegeneration

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Alpha-synuclein (a-syn) is a small protein whose dysfunction is associated with Parkinson's Disease (PD) and is primarily localized at the pre-synapse of neurons. In this study we have used single-molecule localisation microscopy to improve on previous evidence linking a-syn to synaptic signalling by clarifying its relationship to SNARE/clathrin-mediated (CM) exo/endocytosis.

The pre-synapse contains a complex array of tightly-packed vesicles which interact with synaptic proteins at nanometric scales, requiring super-resolution microscopy to quantify the morphology and distribution of different protein populations in single synapses. Using synaptosomes from rat brains as a model, we labelled trafficked vesicles with the membrane marker, mCLING[3], and a-syn and vesicle-associated membrane protein 2 (VAMP-2) with antibodies. We addressed the challenges in labelling synaptic proteins for three-colour dSTORM[2], correcting for chromatic aberrations, and analysing high-density single-molecule data sets. We also developed software for the automatic detection of synaptosomes in super-resolution images, and applied coordinate-based colocalization methods to quantify the relationships between three colour channels.

We found that the colocalization of VAMP-2 and mCLING was strongly temperature-dependent, indicating that, as expected, VAMP-2 is associated with SNARE/CM exo/endocytosis. Interestingly, the colocalization of a-syn and mCLING was temperature-independent, which suggests that a-syn is associated with non-canonically trafficked synaptic vesicles.

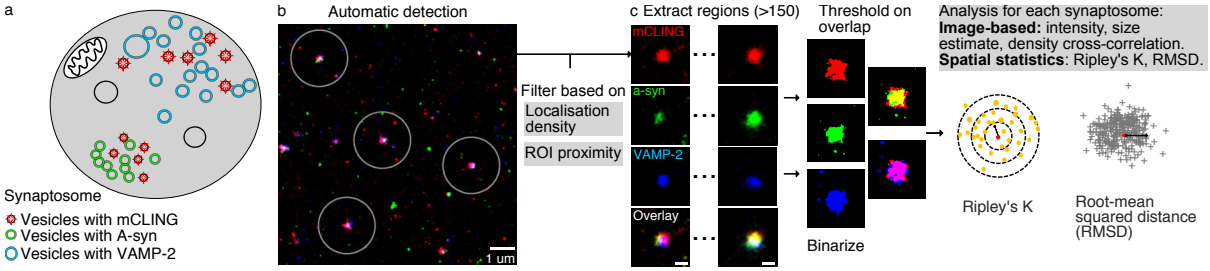

Figure 1: a) Illustration of a synaptosome with labelled synaptic proteins and trafficked vesicles. b-c) Workflow of the synaptosome analysis software, from the automatic detection in reconstructed super-resolution images, to the filtering and analysis of extracted regions of interest (scale bar in c: 200 nm).

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