Super-resolution microscopy for the direct visualization of seeding and toxicity of α-synuclein species in neurons

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In order to develop neuroprotective strategies for neurodegenerative diseases such as Parkinson's disease, it is crucial to understand the molecular details of protein aggregation as they occur *in situ*. The role of alpha-synuclein (AS) aggregation and propagation in the neuropathology of Parkinson's disease has been a subject of recent debate and investigation. In order to elucidate the mechanisms that determine these effects directly in neurons, we have developed and applied an optical nanoscopy assay, based on two-colour super-resolution microscopy with single molecule localisation.

We show that exogenously added α-synuclein seed fibrils primarily elongate by the endogenous α-synuclein, naturally present in neurons (Fig. 1a and c). In contrast, exogenously added monomeric α-synuclein induces nucleation of the endogenous protein and leads to apoptosis (Fig. 1b and e). The latter is rescued by the addition of seed fibrils, suggesting a neuroprotective role of fibrillar species (Fig. 1e). The visualization of these effects at the nanoscale shown here, opens up new avenues for understanding the links between α-synuclein aggregation and neuronal toxicity.

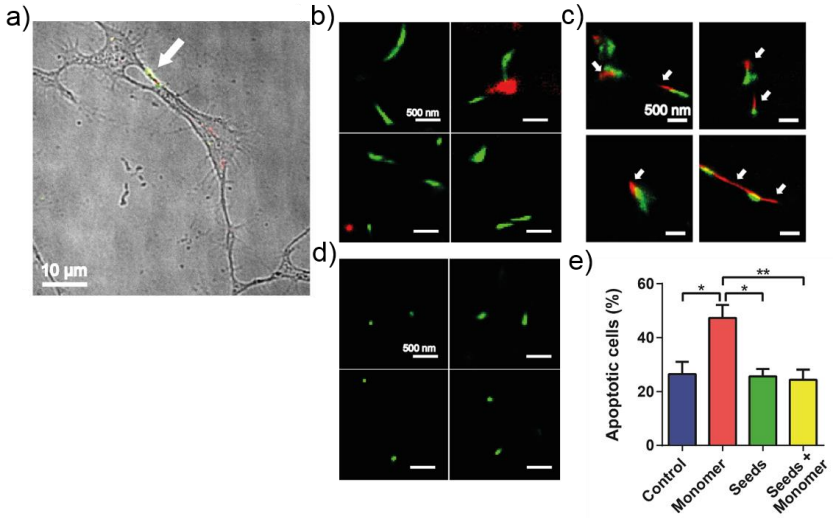

Figure 1: a) Wide-field overlaid with DIC image of ventral-mesencephalic (VM) neuronal cells treated for 1 h with α-synuclein seeds labeled with Alexa Fluor 568 (green), incubated for 24 h in α-synuclein-free medium and immunostained for endogenous α-synuclein (AS), with a secondary antibody tagged with Alexa Fluor 647 (red). b) Zoomed-in dSTORM images showing no co-localisation between the added monomeric protein (red) and the endogenous one (green) c) Zoomed-in dSTORM images of the hetero-fibrils formed from exogenous seeds (green) elongated by the endogenous AS (red). d) Zoomed-in dSTORM images of the endogenous protein in control cells. e) Percentage of apoptotic cells in control cells (blue), cells treated with monomer only (red), cells treated with seeds only (green) and cells treated consecutively with seeds and monomer (yellow).

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