ILLUMINATING THE SELF-ASSEMBLY OF ALPHA-SYNUCLEIN AMYLOID FIBRIL POLYMORPHS

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Amyloid fibril formation of alpha-synuclein is a major pathological hallmark of Parkinson's disease (PD). These fibrils exhibit structural polymorphism. There is an increasing evidence implicating different amyloid polymorphs resulting in distinct disease phenotypes [1]. It is therefore imperative to have a detailed mechanistic understanding into the amyloid self-assembly process and structure.

To this end, we used fluorogenic amyloid binding dyes for PAINT imaging in single molecule localization microscopy (SMLM)[2]. Sequential localization of each fluorogenic binding event affords visualization of amyloid fibrils with an unprecedented spatial resolution of approx. 15 nm. Analyzing the polarization signature of each binding event enables us to visualize fibril ultrastructure to distinguish different fibril polymorphs. We then extended our imaging assay to visualize the self-assembly process of single amyloid fibrils in real-time. Performing such multiparametric imaging allows us to describe fibril growth kinetics with respect to its underlying structure.

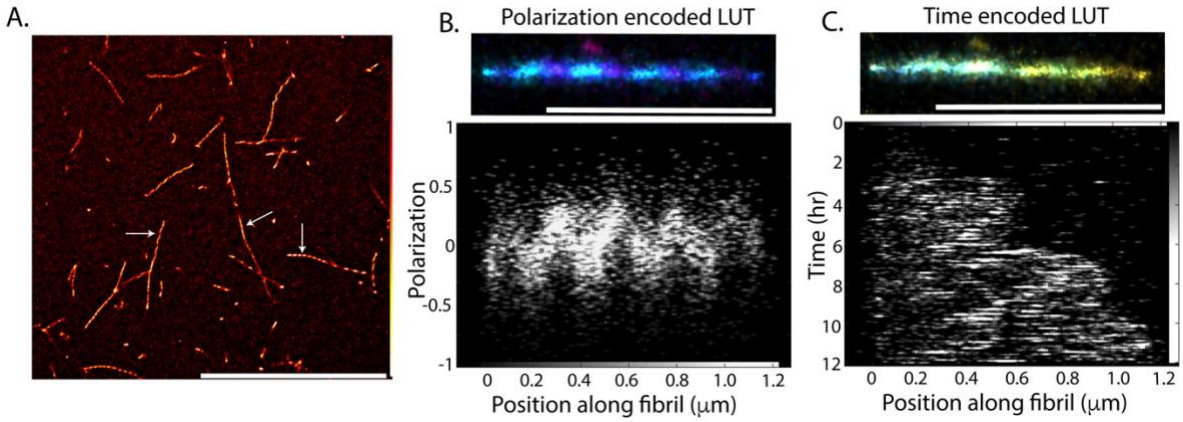

Figure 1. A) Differences in localization decoration of fibrils within the same field of view (indicated by white arrows). Scale bar 10 μm. **B)** Localizations on a fibril colour encoded according to their respective polarization. Plotting each localization's calculated polarization against its respective position along the fibril provides additional information on the underlying fibril structure. **C)** The same fibril as in B) with its localizations colour encoded according to time (blue - early, red - late). Plotting each localization's position along the fibril against time enables the generation of a kymograph to describe single fibril growth kinetics. Scale bar 1 μm.

[1] Qiang, W et al. *Nature* (2017). doi:10.1038/nature20814
[2] Ries, J et al. *ACS Chem Neurosci* (2013). doi:10.1021/cn400091m