# Advanced Imaging of amyloid-β protein aggregation

**Meng Lu[1,2], Neil Williamson[2], Ajay Mishra[1,2], Claire H. Michel[2], Clemens F. Kaminski[1,2], Alan Tunnacliffe[1], Gabriele S. Kaminski Schierle[1,2]**

[1]Cambridge Infinitus Research Centre, Department of Chemical Engineering and Biotechnology, University of Cambridge, Cambridge CB3 0AS, United Kingdom
[2]Department of Chemical Engineering and Biotechnology, University of Cambridge, West Cambridge Site, Philippa Fawcett Drive, Cambridge, CB3 0AS, United Kingdom
Mail: ml600@cam.ac.uk

**KEY WORDS:** protein aggregation, amyloid-beta (AB), molecular dynamics, structural model, 3D structure of amyloid aggregates, SIM, Alzheimer's disease, neurodegeneration

The misfolding and aggregation of intrinsically disordered proteins is a hallmark of neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's disease. Although a variety of methods have been applied to study protein aggregation in different conditions, the details of its self-assembly process are largely unknown. A key requirement to understand this is to capture the fast assembly of the aggregation-prone proteins and the heterogeneous structures of the aggregate species at molecular level resolution, notably inside the cell. The rapid development and proliferation of advanced imaging, especially super-resolution imaging, in different areas of cell biology have been revolutionising our observation and understandings of structural organisation, dynamics of intracellular macromolecules and organelles. Here, we constructed stable cell lines expressing aggregation-prone proteins and applied Structural Illumination Microscopy (SIM) to study the kinetics of the protein aggregation and its heterogeneous structural forms in live samples. These studies reveal how proteins aggregate in an unprecedented high spatial-temporal resolution, such as the kinetics of aggregate seeding and expansion, the motions and distribution, and their structural change. Particularly, we identified five development stages of intracellular amyloid β (Aβ) aggregate – oligomers, single fibrils, fibril bundles, clusters and aggresomes – that underline the heterogeneity of these Aβ42 aggregates and represent the progression of Aβ42 aggregation within the cell. In another study based on fast SIM, we applied single particle tracking to analyse the rapid motion of intracellular aggregates and demonstrated that aggresome is chiefly driven by diffusion of small aggregate clusters. These studies uncover the structural progression of protein aggregates in the cell.

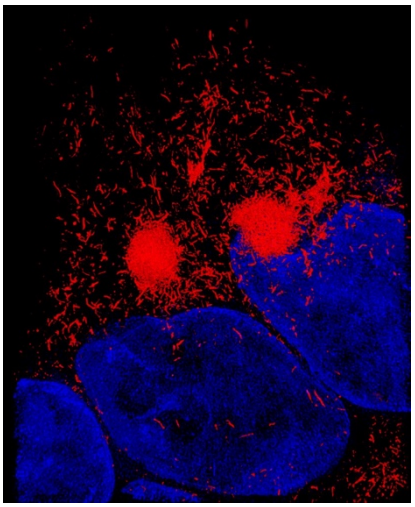

Figure1. Aβ42 Arctic mutant in cell model displays a fast and aggressive aggregation phenotype. Projected views from 3D rendering of cells containing numerous fibrillary fragments, which are in different states of aggregation in the cytosol. Cells are expressing mCherry-Aβ42(E22G) and nucleus are stained with Hoechst 33342.