ULTRA-HIGH-THROUGHPUT SINGLE MOLECULE STUDIES OF EARLY-STAGE AGGREGATION EVENTS IN ALZHEIMER'S DISEASE

Alastair Magness[1], Bogachan Tahirbegi[2], Keith Willison[2], Thomas Knopfel[3], David Klug[2], Liming Ying[1,2]

[1]Molecular Medicine, National Heart and Lung Institute, Imperial College London

[2]Institute of Chemical Biology, Imperial College London

[3]Department of Medicine, Imperial College London, London

Email: l.ying@imperial.ac.uk

The molecular species that damages brain function in Alzheimer's Disease is known to be low-order oligomers of amyloid beta (Aβ), and we are currently exploring the hypothesis that Aβ ligation to critical metal ions in the synaptic cleft during neurotransmission is an important accelerant of amyloid beta oligomer formation and thus a key determining mechanism in the amyloid cascade hypothesis of Alzheimer's Disease. Recently, through a combination of experimental kinetics studies and coupled reaction-diffusion simulations, we predicted that Cu(II) rather than Zn(II) plays an important role in the very early stages (i.e. dimer formation) of Aβ aggregation in the synapse [1]. To verify this experimentally we have developed an automated, ultra-high-throughput protocol for single molecule optical photobleaching experiments, which enables us to rapidly extract stoichiometric information about the aggregation state of tens of thousands of Aβ oligomers attached to a protein-immobilising substrate. This allows us to not only measure the static distribution of oligomers, but by following the system through time, directly probe the aggregation kinetics of amyloid beta at the level of individual oligomeric species. Our high sampling rate allows us to overcome the deleterious effects of molecular noise and measure subtle shifts to aggregated states at previously unobservably low concentrations of amyloid beta (picomolar to nanomolar scale).

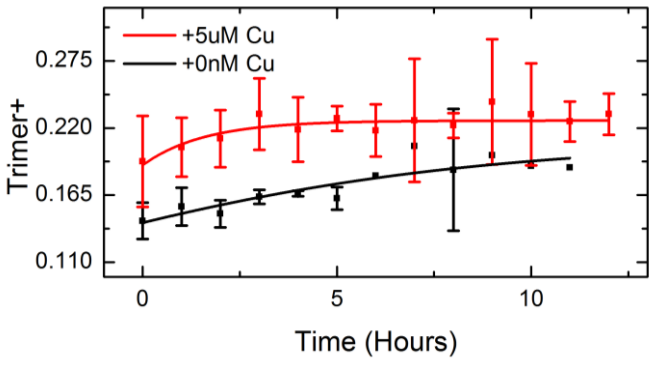

Figure 1: The kinetics of amyloid oligomer growth in the presence of copper is accelerated with respect to the case without this key neurometal.

[1] T.Branch, M. Barahona, C.A. Dodson, L. Ying, "Kinetic Analysis Reveals the Identity of Aβ-Metal Complex Responsible for the Initial Aggregation of Aβ in the Synapse", *ACS Chem. Neurosci.*, **8 (9)**, pp 1970–1979 (2017)