Posters

– 12. Protein misfolding –

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Measuring the prion-like character of tau by TIRF microscopy

F. Kundel[1], B. Falcon[3], L. Hong[1], N. Esteras[2], A. J. Dear[1], A. Y. Abramov[2], T. P. J. Knowles[1], M. Goedert[3], D. Klenerman[1]
[1]University of Cambridge, Dept. of Chemistry, UK; [2]Dept. of Molecular Neuroscience, UCL Institute of Neurology, UK; [3]MRC Laboratory of Molecular Biology, Cambridge, UK

Increasing evidence suggests that neurodegenerative diseases such as Alzheimer's disease share common molecular features with prion disorders. Prions are protein aggregates capable of self-replication, allowing their spread through the brain with fatal outcome. In this study we assessed the ability of the Alzheimer's protein tau to self-replicate in a prion-like manner. Using TIRF microscopy we followed the evolution of single tau aggregates over two months. This revealed a short fibril elongation phase followed by a phase during which existing fibrils undergo slow spontaneous fragmentation. This process increases the concentration of toxic and seeding competent tau species, providing a possible mechanism for the prion-like replication of tau. To corroborate this hypothesis, we determined the rate constants for the elongation and fragmentation of tau and performed simulations of tau aggregate propagation. Importantly, these are able to recapitulate the observed timescales of tau spreading in mouse and human brains and offer an explanation for the slow initiation of AD followed by a rapid decline. This is the first time the key molecular processes of tau replication have been identified and employed to provide a quantitative model for the propagation of tau through the brain.

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Non-perturbative single-molecule imaging of tau aggregates by genetic code expansion

F. Kundel, M. H. Horrocks, Y. Ye, D. Klenerman
University of Cambridge, Department of Chemistry, UK

The aberrant aggregation of tau into intracellular deposits is thought to play a key role in the pathogenesis of Alzheimer's disease and other human tauopathies. Many methods that are used to study protein aggregation *in vitro* and *in vivo* rely on the covalent attachment of a label to the protein of interest. However, amyloid proteins such as tau are highly susceptible to mutations or covalent modifications, necessitating the careful selection of an appropriate labelling strategy to maintain native protein behaviour. Here, genetic code expansion is utilised to introduce a well-tolerated biotin-tag near the N-terminus of a pathological mutant of full length tau. Using a range of single-molecule methods such as smFRET spectroscopy and DNA-PAINT, we demonstrate that this biotin-tag can be used to study different aggregates of full length human tau – such as small oligomeric nuclei or mature fibrils – with unprecedented detail.

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Amyloid β-peptide aggregation and interaction with yeast cells membranes

G. A. Macaluso[1], E. Rao[1], E. Zanni[2], R. Carrotta[3], D. Uccelletti[2], M. Leone[1,4], V. Vetri[1,4]
[1]Department of Physics and Chemistry, University of Palermo, Italy; [2]Department of Biology and Biotechnology "Charles Darvin La Sapienza, University of Rome, Italy; [3]Institute of Biophysics, National Research Council of Italy, Palermo, Italy; [4]ATEN - CHAB,University of Palermo., Italy

Protein misfolding, aggregation and conversion into amyloid fibrils is related to a series of neurodegenerative pathologies as Alzheimer's disease. In particular, interactions of amyloidogenic proteins with cell membranes leading to mutually disruptive structural perturbations are considered key factors in regulating amyloid fibrils formation in cellular environment and related toxicity mechanisms.
Here, we present an experimental study on Amyloid β-peptide aggregation at live Saccharomyces Cerevisiae yeast cell membranes by means advanced fluorescence microscopy techniques. Fluctuation methods are used to analyse the aggregation process in real time quantitatively mapping membrane mediate aggregation and oligomers formation. 2-photon microscopy and Fluorescence lifetime imaging microscopy are used to analyse peptide-membrane interaction and its effects on live yeast cells giving complementary information on their biological response.

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Mechanisms of Amyloid-β 42 oligomer formation from kinetic analysis

T. C. Michaels[1,2], A. Saric[1,5], H. W. Lazell[1], P. Arosio[3], M. Vendruscolo[1], C. M. Dobson[1], S. Linse[4], T. P. J. Knowles[1]
[1]University of Cambridge, UK; [2]Harvard University, USA; [3]ETH Zurich, Switzerland; [4]Lunds Universitet, Sweden; [5]University College London, UK

Alzheimer's disease is intimately connected with the aggregation of the Amyloid-beta 1-42 (Abeta42) peptide into amyloid fibrils. There is increased evidence that low-molecular weight, pre-fibrillar oligomers formed during the early stages of amyloid aggregation, rather than the fibrils themselves, are associated with highest toxicity, yet it has remained challenging to characterize the molecular-level processes through which these species are generated. Here, we present a kinetic analysis of oligomer populations formed during Abeta42 aggregation to shed light on their molecular mechanisms of formation. We show that Abeta42 oligomers, which are predominantly generated through secondary nucleation on the surfaces of existing fibrils, are structurally distinct from fibril nuclei. Moreover, we find that the majority of Abeta42 oligomers do not grow into amyloid fibrils, instead predominantly dissociate back to monomeric form. Our results provide important insights into the physical determinants of the autocatalytic formation of amyloid fibrils, providing fundamentally new targets for interventions to prevent the production of neurotoxic oligomeric species.