An integrated approach to the study of cellular interactions with amyloid

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Introduction

The formation of insoluble amyloid fibrils is associated with a spectrum of human disorders, the amyloidoses, which include Alzheimer's, Parkinson's, type 2 diabetes and dialysis related amyloidosis (DRA). In these disorders the formation of amyloid fibrils is associated with cellular dysfunction and tissue destruction. Yet despite decades of research the culprit species and mechanisms of amyloid toxicity remain poorly understood.

Our goal is to determine how the structure and physical properties of amyloid affects cellular physiology and viability. This involves a multidisciplinary approach in which information obtained by NMR, atomic force microscopy, electron microscopy, photo-crosslinking, mass spectrometry and fluorescence based spectroscopic techniques is integrated with analyses of cell function and viability. We are studying the oligomeric assembly intermediates, fibrils and fibril-derived oligomers formed by an array of amyloidogenic precursors, including α-synuclein (Parkinson's), amyloid-β (Alzheimer's) and β_2-microglobulin (DRA). Experimental approaches used to analyse the interactions and effects of these amyloid species on cells include plate-based assays for cell viability and metabolism, live cell confocal microscopy microscopy, flow cytometry, subcellular fractionation and proteomics. In addition, we are exploring approaches for the delivery of amyloid aggregates into the cytoplasm of single cells with colleagues in the Schools of Biomedical Sciences and Electrical and Electronic Engineering.

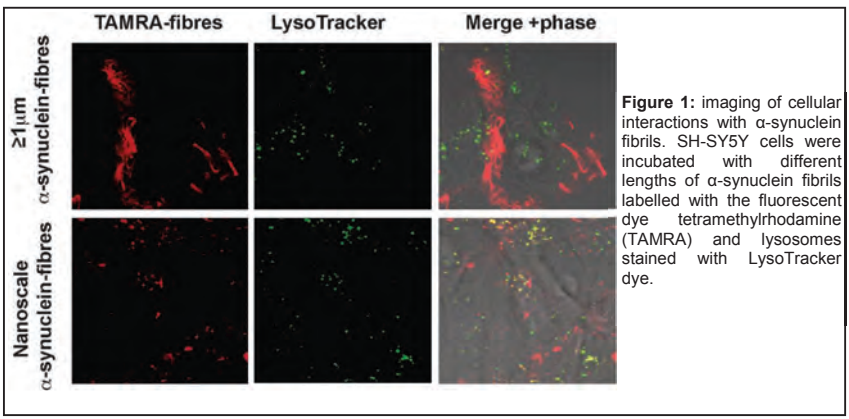

Figure 1: imaging of cellular interactions with α-synuclein fibrils. SH-SY5Y cells were incubated with different lengths of α-synuclein fibrils labelled with the fluorescent dye tetramethylrhodamine (TAMRA) and lysosomes stained with LysoTracker dye.

Publications

Jackson M.P. & Hewitt E.W. (2016) Cellular proteostasis: degradation of misfolded proteins by lysosomes. *Essays Biochem.* **60**:173-180.

Pashley C.L., Hewitt E.W. & Radford S.E. (2016) Comparison of the aggregation of homologous beta(2)-microglobulin variants reveals protein solubility as a key determinant of amyloid formation. *J. Mol. Biol.* **428**:631-643.

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