Measuring the prion-like character of tau by TIRF microscopy

Franziska Kundel[1], Benjamin Falcon[2], Liu Hong[1], Noemi Esteras Gallego[3], Alexander Dear[1], Andrey Y Abramov[3], Tuomas PJ Knowles[1], Michel Goedert[2], David Klenerman[1]

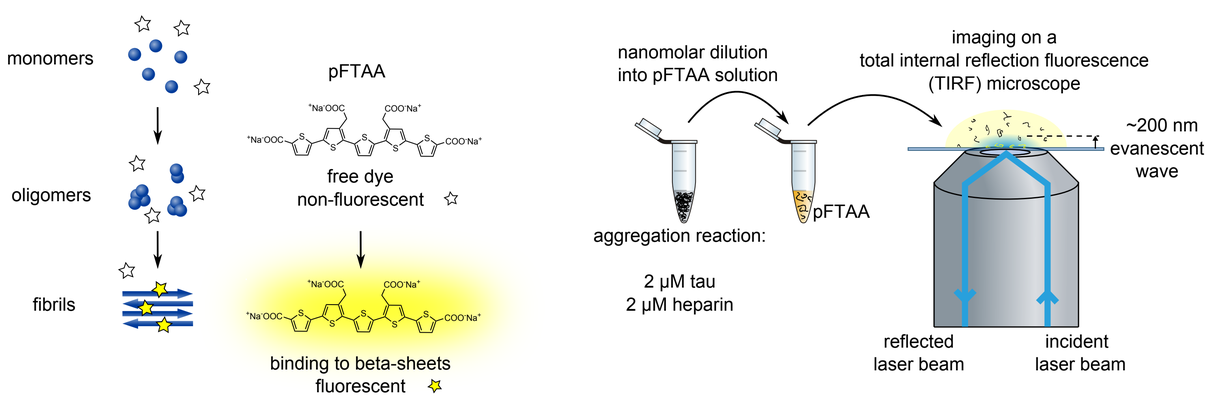
1 Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK.
2 Medical Research Council Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, UK.
3 Department of Molecular Neuroscience, UCL Institute of Neurology, London, WC1N 3BG, UK.

fk278@cam.ac.uk

Introduction

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.The replication of prions is thought to occur by fragmentation[Ref1]. In this study we assessed the ability of the Alzheimer's protein tau to amplify using SAVE imaging - a recently developed approach to visualise single unlabelled protein aggregates[Ref2].

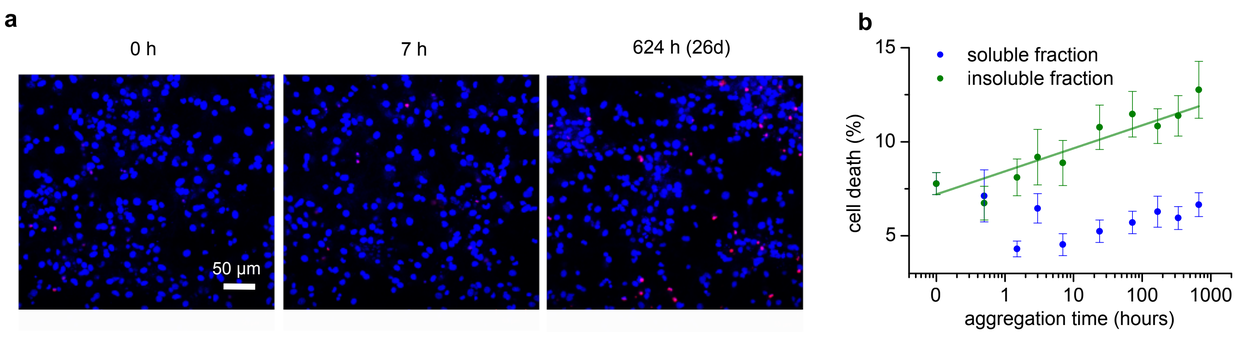
Method - SAVE imaging (Single Aggregate Visualisation by Enhancement)[Ref2]



In order to detect amyloid species evolving during the aggregation of full-length tau, we take aliquots from the aggregation mixture and dilute into a solution of the dye pentameric formyl thiophene acetic acid (pFTAA). Upon binding to beta-sheet rich aggregates the pFTAA fluorescence is strongly enhanced. This enables the ultrasensitive detection of single aggregates on a TIRF setup without the need of covalent fluorescent labelling.

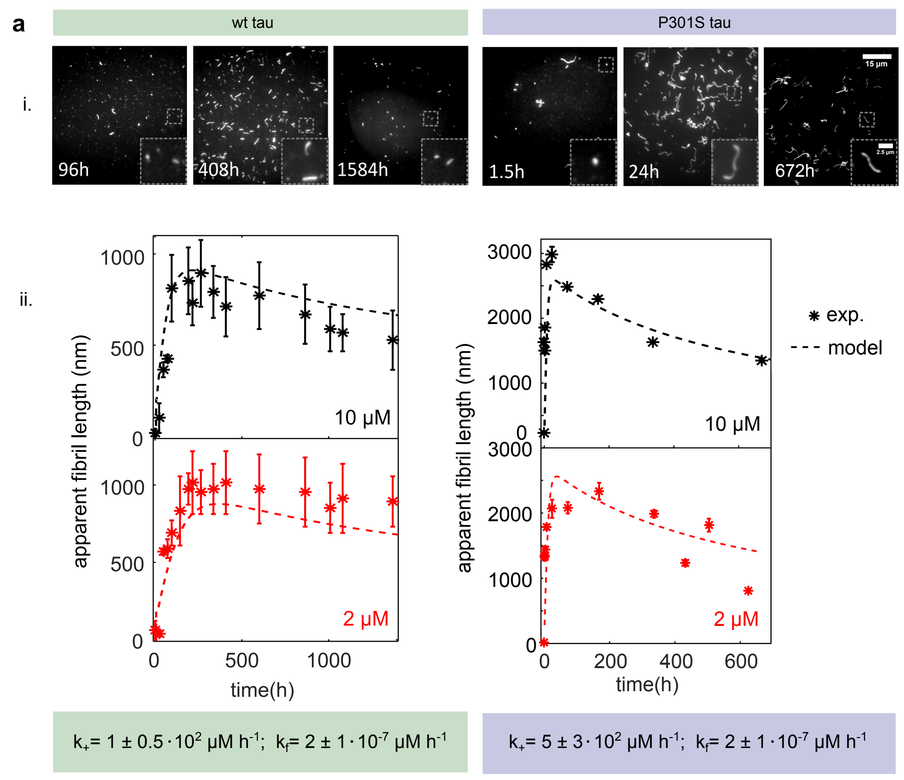
Build-up of toxic fragments

Aliquots were taken from the aggregation mixture at indicated time points (x-axis) and centrifuged to separate soluble and insoluble aggregates. These were added to the extracellular medium of primary cortical rat neurons and astrocytes and cell death was measured by propodium iodide staining.
a) Representative images (N=3). DAPI: blue, propidium iodide: red.
b) Toxicity of tau aggregation mixture increases as a function of aggregation time (N=3, error bars s.e.m.)

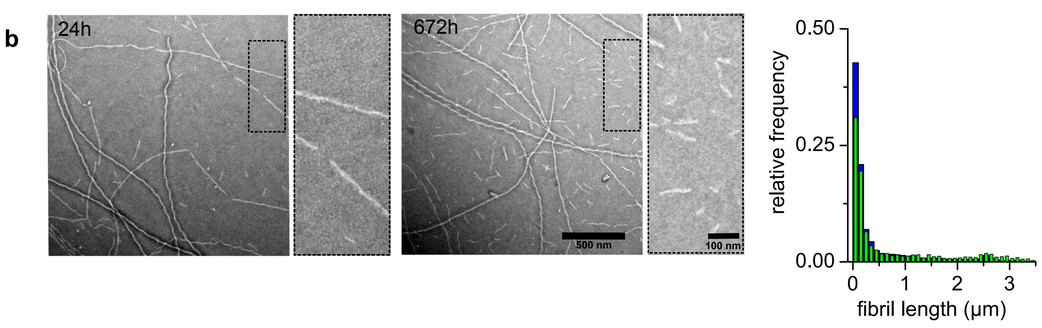


Quantitating fibril elongation and fragmentation

a) Following the aggregation of tau by SAVE imaging. Tau aggregation is first dominated by a rapid elongation phase (0-24h) followed by a fragmentation phase.
The apparent average fibril length was then fitted using a classical nucleation-elongation kinetic model[Ref3] including fibril fragmentation to determine the elongation rates (k_+) and fragmentation rates (k_f) of wt and mutant tau.



b) Electron micrographs of P301S tau fibrils after 24h (max. length) and 672h (min. length).



Summary and Discussion

- We can follow the aggregation of tau on a TIRF microscope "SAVE imaging".
- The aggregation of tau is first dominated by rapid fibril elongation and then by slow fragmentation.
- We could fit this data to a simple kinetic model of breakable fibril assembly to obtain the kinetic rates for fibril elongation and fragmentation. This allowed us to make simulations of fibril amplification in a cell and derive the doubling times of wt tau (440 h) and P301S tau (84 h).

Let's assume fragmentation is the underlying mechanism for tau propagation...

We can calculate the time it takes to form a certain number N of fibrils from a single fibril:

$$N = 2^{(t/t_2)}$$

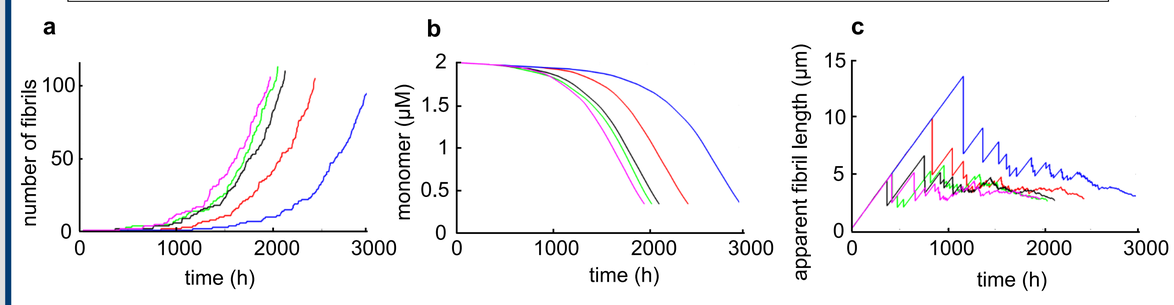
N	*t*
$7*10^7$ (e.g. number of cells in mouse brain)	16 months for wt tau 3 months for P301S tau
$9*10^{10}$ (e.g. number of cells in human brain)	10 years for wt tau

→ This is in good agreement with experimental data from mouse models[Ref4]!

Simulations of fibril amplification in a cell

Typical stochastic trajectories for tau aggregation and amplification in a cell-like volume (10 µm^3) starting from a single tau fibril. **a)** Number of fibrils. **b)** Remaining monomer concentration. **c)** Apparent fibril lengths during fragmentation process

The doubling time t_2 of a tau fibril depends on elongation rate k_e, fragmentation rate k_f and the concentration of free monomers m: $t_2 \propto \left(k_e k_f \frac{m}{1 + m / K_m} \right)^{-1/2}$



→ mean doubling times:

	wt tau	P301S tau
t_2	440 h	84 h

References

1. Tanaka, M., Collins, S. R., Toyama, B. H. & Weissman, J. S. The physical basis of how prion conformations determine strain phenotypes. Nature 442, 585–589 (2006).
2. Horrocks, M. H. et al. Single-Molecule Imaging of Individual Amyloid Protein Aggregates in Human Biofluids. ACS Chem. Neurosci. 7, 399–406 (2016).
3. Knowles, T. P. J. et al. An analytical solution to the kinetics of breakable filament assembly. Science 326, 1533–1537 (2009).
4. Clavaguera, F., Hench, J., Goedert, M. & Tolnay, M. Invited review: Prion-like transmission and spreading of tau pathology. Neuropathol. Appl. Neurobiol. 41, 47–58 (2015).

UNIVERSITY OF CAMBRIDGE

Frances and Augustus Newman Foundation & European Research Council

erc