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

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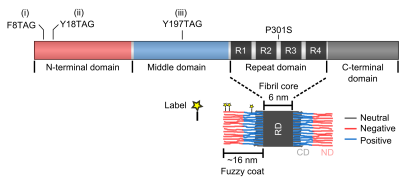
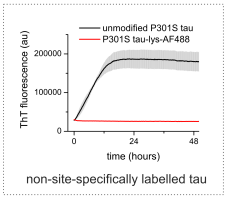
Introduction

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[Ref1] 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 cTCCD[Ref2] and DNA-PAINT[Ref3], 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.

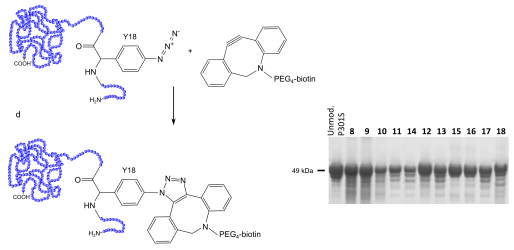
Site-specific labelling of full length P301S tau

Full length P301S tau (0N4R) domain structure and labelling sites (i-iii)





Incorporation of azido-phenylalanine[Ref1] and subsequent click chemistry

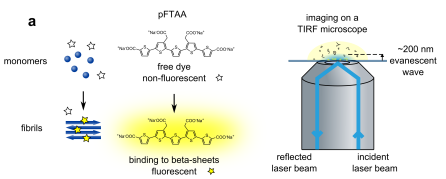


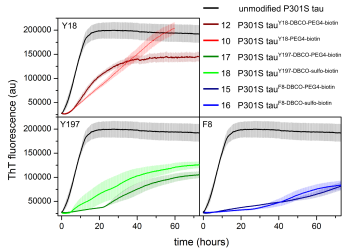
P301S tau conjugates produced

8	P301S tau$^{Y18\text{-}atto488}$
9	P301S tau$^{Y18\text{-}atto594}$
10	P301S tau$^{Y18\text{-}PEG4\text{-}biotin}$
11	P301S tau$^{Y18\text{-}DBCO\text{-}PEG4\text{-}atto488}$
12	P301S tau$^{Y18\text{-}DBCO\text{-}PEG4\text{-}biotin}$
13	P301S tau$^{Y18\text{-}DBCO\text{-}sulfo\text{-}biotin}$
14	P301S tau$^{Y18\text{-}DIBO\text{-}AF488}$
15	P301S tau$^{F8\text{-}DBCO\text{-}PEG4\text{-}biotin}$
16	P301S tau$^{F8\text{-}DBCO\text{-}sulfo\text{-}biotin}$
17	P301S tau$^{Y197\text{-}DBCO\text{-}PEG4\text{-}biotin}$
18	P301S tau$^{Y197\text{-}DBCO\text{-}sulfo\text{-}biotin}$

Testing self-assembly behaviour of tau conjugates

a+b) Morphology of aggregates: Stain aggregates with fluorescent dye pFTAA and image on a total internal reflection fluorescence (TIRF) microscope.
c) Aggregation kinetics: Monitoring fibril formation using the reporter dye Thioflavin-T (ThT)

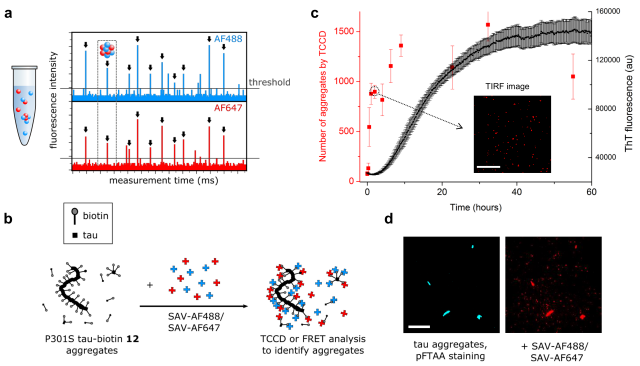




➤ only P301S tau-biotin 12 aggregates similar to the unmodified P301S tau protein!

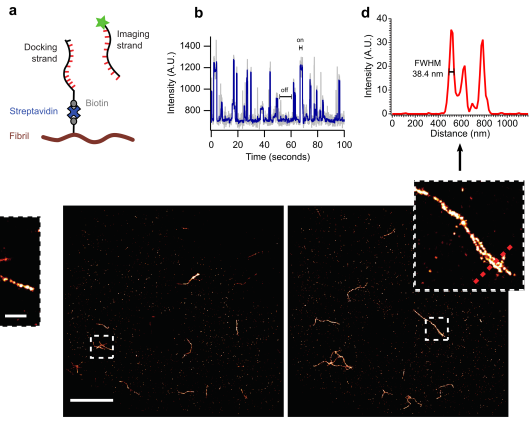
Biotinylation of tau allows sensitive detection of early soluble tau oligomers

a) Typical cTCCD spectrum obtained from dual-labelled protein aggregates on a confocal setup[Ref2].
b) In-situ labelling of biotinylated tau with streptavidin-AF488 and streptavidin-AF647.
c) Observation of early non-ThT active tau species by cTCCD and TIRF microscopy.
d) In-situ labelling of biotinylated tau with streptavidin-AF488 and streptavidin-AF647 allows detection of small oligomeric aggregates by smFRET which are not stained by pFTAA.



Imaging tau aggregates beyond the diffraction limit using DNA-PAINT

a) DNA-PAINT principle[Ref3]. A single stranded DNA strand is attached to tau via a biotin-streptavidin-biotin sandwich. The imaging strand is added to the sample and binds transiently to the docking strand.
b) The imaging strand readily paints biotinylated tau - representative intensity time trace (grey: raw trace; blue: Chung-Kennedy filter).
c) Representative diffraction-limited and SR images of tau fibrils.
d) Intensity trace across a bundle of two fibrils.



Summary and Conclusion

- The self-assembly behaviour of full length tau is highly susceptible to covalent modifications of the protein
- Site-specific biotinylation close to the N-terminus of tau yielded an aggregation competent tau conjugate
- The biotin-tag enables the highly sensitive detection of oligomeric tau species and can be used to obtain super-resolved images of tau aggregates
- This method is a good alternative labelling approach for proteins sensitive to modifications such as amyloid proteins

References

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Frances and Augustus Newman Foundation & European Research Council