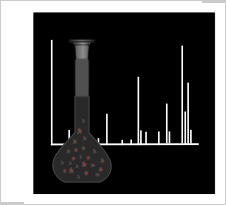
doi:10.2533/chimia.2023.874 Chimia 77 (2023) 874



Highlights of Analytical Sciences in Switzerland

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Fluorescently Labelled Tau Protein

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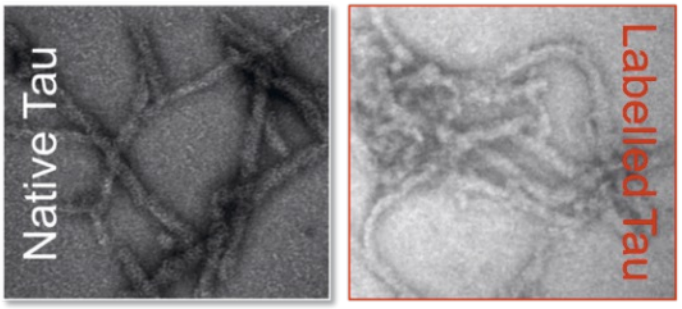
Keywords: Alzheimer's disease · Sortase A · Tau aggregation

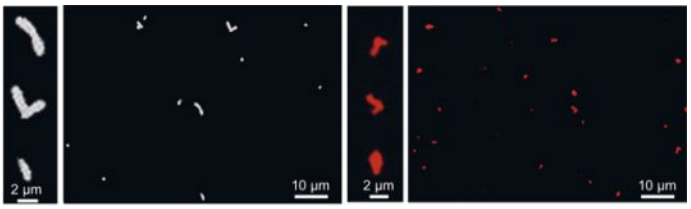
Aggregation of microtubule-associated protein Tau (MAP-Tau) is involved in the progression of Alzheimer's disease and other neurodegenerative disorders. Collectively, these diseases are termed tauopathies and affect millions of people worldwide. Therefore, monitoring the aggregation of Tau protein is critical for a better understanding of the pathological mechanisms of neurodegenerative disorders. In this framework, single-molecule fluorescence microscopy provides a compelling tool for studying the formation of Tau aggregates. One challenge, however, is that labeling Tau monomers with a fluorescent dye may interfere with the regions involved in aggregation. The two hexapeptide hydrophobic regions in the microtubule-binding repeats R2 and R3, which are responsible for Tau aggregation, and the cysteine residue Cys-322, which is involved in the formation of dimers that putatively drive the formation of fibrils, should not be perturbed. Conventional approaches for preparing fluorescent derivatives of Tau protein are, however, targeted to cysteine or lysine residues and may hence perturb these essential regions of Tau.

Here, we designed a strategy to label Tau at its C-terminus, using an approach mediated by the enzyme Sortase-A. We added a short peptide sequence (LPETGG) to the C terminus of full-length Tau, which enables Sortase-A to bind to the threonine (T), forming a thioester. Then, a histidine-tagged fluorophore (GGGH6C-Alexa647) is conjugated to Tau, with the terminal glycine replacing the thioester with an amide bond and cleaving off Sortase-A. We chose the fluorophore Alexa647 for its photostability and for its strong fluorescence emission in the far-red region of the visible spectrum, which prevents its excitation and emission spectra to overlap with those of Thioflavin T (ThT), a commonly used fluorophore for monitoring Tau aggregation. Results from circular dichroism (CD) spectroscopy and from monitoring ThT fluorescence revealed that these Alexa647-labeled Tau monomers have similar secondary structures to native Tau and that the presence of the fluorophore exhibits minimal effect on aggregation kinetics. Characterization of the morphology of Tau fibrils by transmission electron microscopy (TEM) and atomic force microscopy in a liquid cell revealed no difference between fibrils from native (wildtype) Tau and the C-terminally-labelled Tau introduced here.

This approach provides an effective way to prepare fluorescently labeled Tau protein while minimizing possible effects on its aggregation kinetics and fibril morphology. Since site-specific labeling by Sortase-A can also be carried out inside living cells, the resulting Alexa647-labeled Tau protein may be useful for studying Tau aggregation *in vivo* and for carrying out single-molecule fluorescence microscopy studies.



Transmission electron microscopy images of amyloid fibrils formed by native and Alexa647-labelled Tau protein. Sortase-mediated C-terminal labeling of Tau does not affect the overall morphology of amyloid fibrils.



Fluorescence microscopy images of amyloid oligomers, protofibrils and fibrils formed by native and labelled Tau protein. Left: Native Tau protein labeled with thioflavin T (ThT) in solution. Right: Alexa647-labeled Tau protein without ThT in solution.

Received: November 6, 2023

L. Bryan, S. Awasthi, Y. Li, P. N. Nirmalraj, S. Balog, J. Yang, M. Mayer, *ACS Omega* **2022**, *7*, 47009. https://doi.org/10.1021/acsomega.2c06139.

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