XXI Discussions in Structural Molecular Biology and 8th User Meeting of the Czech Infrastructure for Integrative Structural Biology

Meeting of the Czech Society for Structural Biology

Conference Centre of the Czech Academy of Sciences, Nové Hrady, March 20 - 22, 2025

Organisers:

Jan Dohnálek, Jarmila Dušková, Jan Stránský, Kristýna Adámková, Radek Kužel

The event is organized by the Czech Society for Structural Biology, the Czech Infrastructure for Integrative Structural Biology, and the Institute of Biotechnology of the Czech Academy of Sciences.

Thursday, March 20, Session I

PhD Thesis Award

L1

Tau PROTEINS COOPERATIVELY ASSEMBLE INTO COHESIVE ENVELOPES THAT PROTECT MICROTUBULES AGAINST SEVERING ENZYMES

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Tau is a microtubule-associated protein that is preferentially found in the neuronal axons. In neurodegenerative diseases, collectively termed tauopathies, malfunction of tau and its detachment from axonal microtubules, often associated with abnormal phosphorylation of tau, are correlated with axonal degeneration and loss of microtubule mass [1]. Tau can protect microtubules from microtubule-degrading enzymes such as katanin [2] and regulate transport by molecular motors along the microtubule [3,4]. However, how tau carries out these regulatory functions is still unclear. Using in vitro reconstitution and TIRF microscopy, we show that tau molecules can bind to microtubules in two distinct modes: either as (i) single tau molecules independently diffusing on the microtubule surface, or (ii) cooperatively-bound tau that form a cohesive tau "envelope" enclosing the microtubule lattice [5-8]. We found that tau envelope formation alters the spacing of tubulin dimers within the microtubule lattice, where envelope formation compacted the underlying lattice, and lattice extension induced tau envelope disassembly [7]. Tau envelopes form a selectively permissible barrier that inhibits kinesin-1 motors while allowing dynein movement, and protects microtubules against the activity of microtubule severing enzymes such as katanin [5]. Tau envelopes itself are regulated by tau phosphorylation, where phosphorylation of tau leads to destabilization of "healthy" non-phosphorylated tau envelopes and reduced protective functionality of the envelopes [8]. Combined, our data reveals the microtubule-dependent cooperative binding mode of tau that can constitute an adaptable protective layer on the microtubule surface. The subtle change in the microtubules lattice structure can differentially affect the affinities of other microtubule-binding proteins to the microtubule surface, thus potentially dividing microtubules into functionally distinct segments. Finally, our data suggests that a reduction in microtubule mass linked to tau hyperphosphorylation in neurodegenerative diseases, could be explained by the destabilization and impaired functionality of the tau envelopes upon tau phosphorylation.

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Thursday, March 20, Session I

L2

SEQUENCE-BASED POLYMORPHISM OF Tau PROTEIN AMYLOID FIBRILS

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Thanks to advancements in cryo-electron microscopy, the structure and sequence composition of tau amyloid fibrils from different diseases are well-established [1]. However, the exact mechanisms underlying disease-specific mechanisms, spreading patterns, and resulting disease-specific fibrils morphology remain elusive, hindering the development of specific anti-tau therapies. Previously validated amyloid motifs in the tau protein sequence are regarded as carriers of tau amyloidogenicity because of their intrinsic beta propensity and ability to assemble into stable amyloid fibrils core [2-3]. Together with environmental factors and posttranslational modifications (PTMs), these short amyloid-nucleating motifs contribute to the formation of different protofilaments interface, which underpin the amyloid fibrils polymorphism. One of the most common PTMs that plays a key role in the pathogenesis of Alzheimer's disease is truncation, which promotes the self- assembly of tau monomers by exposing regions that are prone to aggregation in Alzheimer's disease [4]. In some cases, however, truncations in amyloid-prone regions can impair further amyloid aggregation. In our study, five variants of different lengths of tau involving different amyloid motifs were used, including two experimentally tested PHF6(306-311) and PAM4(350-362) and predicted G(326-331) and L(341-449) to demonstrate the sequence-dependent aggregation mechanism. All tau variants were truncated at residue 391 from the C-terminus and at the different sites from N-terminal part of the protein (297-391, 306-391, 316-391, 321-391, 326-391). Propensity toward amyloid aggregation of five tau variants was evaluated by ThT fluorescence assay, and the presence of polymorphic amyloid fibrils was confirmed by atomic force microscopy under four aggregation conditions – with or without heparin and DTT. Tau321-391 exhibits the highest amyloid robustness across all tested conditions, being the only tau variant to produce filaments under physiological conditions represented by pure PBS buffer. On the other hand, tau326-391 variant failed to produce the fibrils even in the presence of heparin, indicating the crucial role of 321-325 for tau monomers self-assembly.

Molecular dynamics simulations revealed an increased propensity of 321-325 sequence toward beta-structures in all atom simulations but a rather helical propensity in coarse-grained simulations. Tau321-391 monomers interacted through a helical interface, which was already featured for other amyloid proteins in early stages of aggregation. MD simulations successfully replicated sequence-specific beta-sheet propensity in agreement with computationally predicted and experimentally established amyloid-nucleating motifs.

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Part of the research results was obtained using the computational resources procured in the national project National competence centre for high perFormance computing (project code:311070AKF2) funded by European Regional Development Fund, EU Structural Funds Informatization of society, Operational Program Integrated Infrastructure. This research was funded by the European Union's Horizon Europe program under the grant agreement No. 101087124. This work was also funded by the grants APVV 21-0479, APVV-22-0598 and Vega 2/0125/23, 2/0141/23 and 2/0141/25. This research has received funding from the European Union's Horizon 2020 research and Innovation programme under the Marie Skłodowska-Curie grant agreement No 873127. This research was further funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00623.