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Assessment of the Thesis

INTERACTION DYNAMICS OF CYTOSKELETAL POLYMERS

by

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Microtubules represent a fundamental component of the cytoskeleton, shaping essential cellular processes such as intracellular transport and cell division. The key to a mechanistic understanding of the diverse functions of microtubules in cell biology lies in decoding their interactions with other cellular components.

The central theme of this thesis is thus a thorough analysis of the interaction dynamics between microtubules and two selected microtubule-associated proteins (MAPs), Tau and Ase1. Methodologically, the author takes advantage of a well-established reductionist system of *in vitro* reconstitutions of purified proteins. The dynamics of the reconstituted components are imaged by TIRF microscopy, which provides a robust readout with superior spatiotemporal resolution. This enabled a number of interesting observations. First, the author significantly contributed to several original findings on the interaction of the neuronal protein Tau with reconstituted microtubules: (i) Tau exhibits two distinct binding modes to microtubules, a diffusive mode and a cooperative mode that leads to the formation of protective "Tau islands or envelopes". (ii) These islands significantly enhance the stability of microtubules, offering protection against severing by enzymes such as Katanin. (iii) The research demonstrates that Tau islands can modulate the activity of a molecular motor kinesin-8. Second, the author reveals that a protein Ase1 (i) selectively stabilizes antiparallel microtubule overlaps, critical for the mitotic spindle. (ii) Ase1 accumulates at depolymerizing microtubule ends, suggesting a herding effect that promotes stability. (iii) Mathematical modeling suggests that this herding effect can be explained solely by obstructing the dissociation of terminal tubulin dimers by Ase1.

The text is exceptionally well-structured and displays a consistent and logical flow. The comprehensive introduction is a thorough summary of the current knowledge, well balanced between a general overview and a focus on the topical matter. The aims are clearly framed, and the methods, results, discussion and conclusion are detailed and well formulated. A particularly notable strength of the thesis lies in its visual presentation, which is enriched by an array of high-quality graphics and figures.

The thesis is supported by two published, high-impact research papers, which serve as a testament to the author's exceptional productivity. This achievement underscores the high quality, and integrity of the work.

Based on the content of the thesis, I propose a few topics for further discussion:

The thesis provides a thorough insight into the interaction of microtubules with Tau and Ase1 at the biophysical level. However, it will be of great interest to frame the findings in a broader cellular context.

- Tau island formation depends on several parameters, such as tau concentration or microtubule curvature; can we discuss how this relates to the general morphological organization of neurons and microtubule structure? Are Tau islands expected to form in a particular neuronal structure, and can this be related to neuronal function?

- What is the role of variability in the cellular environment, such as varying molecular crowding or ionic strength, in the mikrotubule - MAPs interaction?

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