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Opponent Statement on the Dissertation

Interaction dynamics of cytoskeletal polymers

Jochen Krattenmacher's dissertation presents a thorough investigation into the complex and dynamic interactions within the cytoskeleton, with a specific focus on microtubules and their associated proteins. This work is highly laudable for its in-depth exploration of the molecular mechanisms underlying microtubule stability and regulation, and it significantly advances our understanding of cytoskeletal dynamics.

The dissertation is organized into two major sections, each focusing on a distinct microtubule system. The first section examines the role of the axonal microtubule-associated protein Tau, emphasizing its capacity to form cooperative "islands" that shield microtubules from severing enzymes such as Katanin and regulate the activity of molecular motors like Kinesin-8. These findings offer valuable insights into how axons maintain their structural integrity while supporting intracellular transport. The second section addresses the function of Ase1, a crosslinking protein in mitotic spindles, demonstrating its selective stabilization of antiparallel microtubule overlaps, which is crucial for proper spindle organization and function. Together, these studies provide important contributions to our understanding of how localized protein-microtubule interactions govern the macroscopic organization of the cytoskeleton.

Conceptually, the work is robust, providing clear definitions and a well-established theoretical foundations. The methodological approaches employed in this dissertation are diverse and advanced, encompassing *in vitro* reconstitution experiments, total internal reflection fluorescence (TIRF) microscopy, and mathematical modeling. The choice of methods is well-justified and reflects the author's technical proficiency. The results are presented clearly, supported by thorough data analysis, which adds to the scientific rigor of the work. However, the reliance on *in vitro* approaches raises questions about the physiological relevance of the findings. Although these limitations are acknowledged, further *in vivo* validation would have added significant strength to the conclusions.

The dissertation is well-structured, with a logical flow and clear language, making it accessible to readers. The comprehensive literature review and the identification of specific research gaps demonstrate a thorough understanding of the current state of the field, ensuring the relevance of the research. The results section exemplifies scientific rigor and analytical precision, with findings articulated in a logical sequence and accompanied by a balanced discussion of their broader implications. Jochen's cautious yet optimistic interpretation of the results lends additional credibility to the work, facilitating a nuanced appreciation of the complex interplay among the molecular components under investigation. The discussion section is particularly strong, situating the findings within the broader context of cytoskeletal research and highlighting both their implications and inherent limitations. Jochen demonstrates an admirable capacity for critical engagement with both the literature and his own research, offering thoughtful and well-reasoned interpretations.

In conclusion, Jochen Krattenmacher's dissertation represents a significant contribution to the field of cytoskeletal biology, providing valuable insights into the molecular dynamics of microtubules. Jochen's ability to critically evaluate his own findings, as well as those in the existing literature, underscores an original and independent research endeavor that adheres to high standards of scientific rigor and integrity. This study is a model of academic excellence, characterized by its methodological rigor, conceptual clarity, and insightful analysis. Therefore, I strongly support the award of the doctoral degree to the candidate.

For discussion, I would like to ask the following questions:

1. How do the *in vitro* findings presented in your thesis correlate with the *in vivo* context? Could the labeling of microtubules with Atto-647, which binds to lysines, affect Tau island formation by altering the microtubule surface charge? Furthermore, how might variations in molecular crowding and concentration between in vitro and in vivo environments impact your findings? What strategies would you use to validate these findings *in vivo* or *ex vivo*?

2.The dissertation discusses the role of Ase1 in spindle stabilization. Could you expand on how the regulation of Ase1 activity might vary during different phases of the cell cycle, and what factors might influence its recruitment to specific spindle regions?

3. You propose that diminished Tau island assembly might trigger various downstream pathophysiological effects. Could you provide further insight into this proposed mechanism? Are there any in vivo models that support it?

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