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PhD thesis review

Author: Valerie Siahaan

Title: ‘Tau proteins cooperatively assemble into cohesive envelopes that protect microtubules against severing enzymes’

Tau is a microtubule binding protein found in neurons, and deregulation of Tau has been implicated in numerous neurological disorders. Although Tau has been extensively studied in the past, surprisingly little is known about the molecular underpinnings of Tau function and malfunction. The author took a very original and creative approach to study Tau and its binding kinetics to microtubules in vitro. By performing high-resolution time lapse imaging of purified Tau that binds to microtubules, she uncovered many novel aspects of Tau behavior at the molecular scale. First, the author shows that Tau binds cooperatively to microtubules and this cooperative binding modulates the accessibility of other microtubule binding proteins. Second, she shows that Tau binding compacts the lattice of the microtubule, thereby promoting its own binding. Finally, in the last experimental chapter it is shown how Tau phosphorylation, which has been shown to be important for Tau function in vivo, affects Tau binding in vitro.

The thesis is very clearly written and the author finds exactly the right balance between detail and clarity. The thesis starts with an extensive and well-documented overview of microtubules and their binding proteins, which I warmly recommend to anyone new to the field. The experimental chapters are very concise and describe the key experiments and main messages very well. Finally, in the discussion the author puts her results in a larger perspective and it is exciting to read the enormous potential for follow-up studies.

The work presented in this thesis reports on the discovery of novel molecular mechanisms underlying Tau behavior. Not only are these mechanisms novel, they also address several previous conundrums. Therefore, this work will have a major impact on the field and paves the way for many new research avenues. In line with this assessment is the fact that the candidate is lead author on two publications in top journals, and the third one is on its way. I do have several questions regarding the work (see below) and I am looking forward discussing these with the candidate.

Altogether, the PhD thesis is of extremely high quality. It shows very convincingly that the author 1) is able to conduct complex experiments using various high-end experimental setups, 2) can perform hypothesis-driven research and distill clear conclusions 3) is very well-versed and can communicate her results in a concise and clear fashion, 4) can put her work in a broader perspective. Therefore, I am very happy to approve the thesis and recommend Valerie Siahaan for defending her thesis.

Questions for the author:

- 1) To demonstrate cooperative binding the author measures the levels of Tau on the microtubule at different Tau concentrations. In order to further understand the contribution of diffusive Tau and the Tau in envelopes, could the Hill coefficient be determined separately for both binding modes?
- 2) Above a threshold of Tau concentration, Tau starts forming envelopes on the microtubules which then grow in size. For inferring the Hill coefficient, at which time point, with respect to the onset of envelope formation, did the author measure the levels of MT-bound Tau? Would the timing of the measurement affect the inferred Hill coefficient?
- 3) The author mentions that the cooperativity likely arises due to Tau-Tau interactions, and at the same time, the lattice spacing is a requirement for the cooperative binding mode. If I understand it correctly, there are roughly two explanations for this: 1) The lattice compaction arises as a consequence of cooperative Tau binding, or 2) Tau binding cooperativity arises as a consequence of lattice compaction. To discriminate between these possibilities, did the author extract the Hill coefficient from binding experiments in the presence of GDP-bound microtubules?
- 4) Lattice compaction must inevitably shorten the helical pitch of the microtubule. Can the author speculate on the implications of this? For example, on binding of different molecular motors such as Kinesins or Dyneins?
- 5) Upon wash-out of Tau the envelopes start disassembling mainly from both ends of the envelope and only after a given amount of time do the envelopes start fission. Is there a characteristic time scale as to when the fission starts?
- 6) Related to previous question: Given that Tau in envelopes can exchange freely with tau in solution, can the author speculate why there is not a continuous disassembly of the envelope along its length upon Tau wash-out?

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