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Teacher's evaluation of the dissertation: Valerie Siahaan - "Tau Proteins Cooperatively Assemble into Cohesive Envelopes that Protect Microtubules Against Severing Enzymes"

Tau is a microtubule-associated protein found in neuronal axons. In many neurodegenerative diseases, malfunction of tau and its detachment from axonal microtubules, associated with abnormal phosphorylation of tau, is correlated with axonal degeneration and loss of microtubule mass. The molecular mechanism, explaining how tau protects microtubules, remained elusive.

In her doctoral thesis, Valerie Siahaan, found two distinct modes of tau association with microtubules: tau molecules either diffuse individually or cooperatively assemble into cohesive patches enveloping the microtubule surface. Using purified isolated components, Valerie reconstituted these processes *in vitro*. Employing a combination of total internal reflection fluorescence microscopy with single molecule resolution and interference reflection microscopy, complemented by optical trapping, Valerie explored and described (a) the process of envelope formation and how tau cooperative binding effects other microtubule associated proteins, for example by blocking of the microtubule severing enzyme katanin (b) the effect of tau envelope formation on the structure of the underlying microtubule, finding that tau cooperativity specifically affects tubulin conformation allosterically and (c) that tau phosphorylation hinders cooperativity, which results in the release of tau molecules from microtubules.

During her doctoral studies, Valerie mastered the experimental approaches required by the project, ranging from protein biochemistry and single molecule fluorescence microscopy to the advanced biophysical technique of optical trapping. Valerie demonstrated her ability to conduct independent experimental work and her thorough approach resulted in the publication of two first-author papers in high-ranking journals, with a third first-author manuscript currently being in revision. Valerie further co-authored a paper (likewise published in a high-ranking journal) on a topic unrelated to tau cooperativity. The presented thesis comprehensively summarizes Valeries's doctoral work on





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tau proteins, demonstrating her thorough insight into the subject, as well as her ability of independent scientific work. In the light of her achievements, it is my pleasure to approve the thesis by Valerie Siahaan and recommend it for further proceedings of the thesis defense.

Marcus Braun

