Jochen Krattenmacher – Outline of thesis "Interaction dynamics of cytoskeletal polymers"

The cytoskeleton, a network of interlinking protein filaments, plays a critical role in organizing and maintaining cellular architecture, as well as in facilitating intracellular transport. One major component of the cytoskeleton, microtubules, are highly dynamic structures that are involved in a wide range of cellular functions. Microtubules form diverse arrays in different cellular environments, such as long, stable axonal microtubules in neurons or the highly organized antiparallel microtubule arrays in mitotic spindles. To understand how microtubules are stabilized and spatially organized, this dissertation investigates how microtubule-associated proteins (MAPs) contribute to these processes through their interactions with microtubules.

The aim of this thesis is to advance our understanding of the ways in which microtubules interact with MAPs to give rise to emergent phenomena, such as stabilization and dynamic regulation in different cellular environments. Using a minimal *in vitro* reconstitution approach, this work seeks to isolate key components and understand their roles in microtubule stabilization and organization, without interference from other cellular factors. The thesis focuses on two MAPs — Tau and Ase1 — both of which are known for their involvement in microtubule organization, but in different cellular contexts. Tau is primarily associated with neuronal axonal microtubules, while Ase1 is involved in mitotic spindle dynamics. By studying these two proteins, this work contributes to a deeper understanding of how MAPs function within their respective systems to organize microtubules and support their cellular roles.

The main experimental method employed throughout this study was total internal reflection fluorescence (TIRF) microscopy, which selectively excites fluorophores near the glass/water interface. To conduct our microscopy, we implemented a setup in which stabilized microtubules were immobilized on the glass coverslip via antibodies bound to a functionalized surface. Fluorescently-labeled proteins of interest, such as Tau or Ase1, were added to the experimental buffer, allowing their interactions with the microtubules to be visualized. This approach minimized background fluorescence by limiting excitation to fluorophores in close proximity to the glass surface, thereby improving the signal-to-noise ratio in the

assay. The combination of this precise illumination and immobilization technique provided high-resolution insights into how MAPs interact with microtubules under various conditions.

The first set of experiments investigates Tau, a protein known for its role in stabilizing axonal microtubule arrays. Here, we found that full-length Tau, when present in the assay buffer above a certain concentration, forms high-density "islands" on paclitaxel-stabilized microtubules. These islands assembled from their ends, and likewise disassembled from their ends upon removal of Tau from solution, indicating that islands formed via cooperative binding of Tau. Indeed, when we examined Tau binding at the single-molecule level, we observed individual Tau molecules within islands to be stationarily-bound, while Tau molecules outside of islands displayed rapid diffusion. To further characterize these two populations of Tau on the microtubule, we performed fluorescence recovery after photobleaching (FRAP) experiments, which revealed that Tau molecules within island regions have a lower unbinding rate than Tau molecules outside of islands. Interestingly, when Tau was removed from solution, the unbinding rate of Tau from island regions was reduced by orders of magnitude, indicating a strong concentration-dependence of this unbinding-rate which suggests that cooperatively-bound tau interacts multivalently with the microtubule lattice. We further found that Tau islands have a density of 0.26 Tau molecules per tubulin dimer, suggesting that each Tau molecule binds to 4 tubulin dimers. While at higher concentrations of Tau in the assay buffer the density of Tau within island-covered regions was higher than 0.26 Tau molecules per tubulin dimer, a removal of Tau from solution resulted in a rapid drop to this characteristic density, revealing that the now-unbound Tau molecules were only bound diffusively to the microtubule. When varying the ionic strength of the assay buffer, islands were most stable at intermediate ionic strengths, indicating that their binding to the microtubule partially depends on hydrophobic interactions - as opposed to the diffusive binding mode, which was strongest at lower ionic strengths. Further, we found Tau islands to effectively protect microtubules from severing by the microtubule-severing enzyme Katanin. Even at conditions where Tau binding to the microtubule was saturated, where the density of Tau was comparable inside and outside of island-covered regions, we found microtubules to only be protected within the island-covered regions, confirming that there is a qualitative difference between the binding mode which gives rise to islands and the diffusive binding mode of Tau. We also found islands to interact with the molecular motor kinesin-8 (Kip3), slowing it down and reducing its rate of binding to the microtubule. In turn, kinesin-8 motors, when present in sufficient number, were capable of disassembling Tau islands. Finally, we found that Tau was also stationarily bound to highly-curved microtubule regions, and that Tau islands did not form in such regions, suggesting that the cooperative binding mode of Tau prefers either expanded or compacted microtubule lattices.

The second set of experiments focuses on Ase1, a homodimeric MAP which diffuses on microtubules and crosslinks them, preferably in antiparallel fashion. Here, we used GMPCPP-stabilized microtubule seeds immobilized on the coverslip in combination with free tubulin and GTP in the assay buffer, which allowed us to observe dynamic microtubule extensions

growing from the stabilized seeds. We found that Ase1 selectively stabilizes antiparallel microtubule overlaps, such as those found in the mitotic spindle, greatly increasing their lifetimes as compared to the lifetimes of parallel microtubule overlaps. We found that Ase1 does so by increasing the frequency of microtubule rescue events of antiparallel microtubules in a concentration-dependent manner, while having no significant effect on microtubule polymerization speed and frequency of catastrophe events. In addition to this selective stabilization, we also observed Ase1 to slow down microtubule depolymerization for all types of microtubules in a concentration-dependent manner, with the strongest effect on antiparallely crosslinked microtubules. We also observed a phenomenon known as "herding" of Ase1 molecules at depolymerizing microtubule ends which correlated with the slow-down of depolymerization. We created a simple mathematical model which assumes an ability of Ase1 to reduce tubulin subunit detachment when bound at the tip of a protofilament of a given microtubule. This model successfully captured key features of Ase1's behavior, including its accumulation at shrinking microtubule ends and the concomitant decrease in depolymerization velocity. The model's quantitive fit with our observed data increased when extended by an assumption that Ase1 molecules also affect the dissociation rates of the terminal tubulin dimers of the two neighbouring protofilaments of a given protofilament, and assumption which appears justified given that Ase1 likely bridges protofilaments.

Collectively, the results of this thesis highlight how distinct MAPs stabilize and regulate microtubule dynamics in different cellular environments, through unique binding modes and interaction patterns. In axons, Tau's cooperative binding and interaction with severing enzymes plausibly plays a key role in enabling the formation of stable microtubule arrays that resist severing. In mitotic spindles, Ase1's ability to stabilize antiparallel microtubule arrays may contribute to the structural basis for accurate chromosome segregation. The experimental approach used in this thesis, which isolates key components of these systems, provides a clearer understanding of the biophysical mechanisms that potentially underly the emergent patterns observed in living cells. Furthermore, these results may have implications for understanding neurodegenerative diseases linked to Tau dysfunction, as well as the organization of microtubule arrays in mitosis, especially for less complex organisms.

The sources used in this thesis include a review of relevant literature on microtubules and the MAPs explored in this thesis as well as experimental work performed by me or collaborators (as indicated in the thesis). The work conducted for this thesis resulted in two publications — "Kinetically distinct phases of tau on microtubules regulate kinesin motors and severing enzymes," published 2019 with Nature Cell Biology, and "Ase1 selectively increases the lifetime of antiparallel microtubule overlaps," published 2024 with Current Biology.