Do we know why microtubules grow and break?

Subnanometer mechanics of microtubule dynamic instability

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In Short

- Microtubules are key components of the cell cytoskeleton and play a central role in cell division/development as well as in various diseases.
- Despite decades of intensive research, the mechanism of how they polymerize and depolymerize and, hence, how they are regulated by other proteins remains elusive because dynamics information at the atomistic scale is missing.
- In this project, we aim at revealing the subnanometer dynamics of microtubules by a combined cryo-EM and biomolecular simulation approach and probing the interaction of regulatory proteins with microtubules, such as the neuron-specific Tau protein.

Microtubules (MTs) are cellular filamentous assemblies of $\alpha\beta$-tubulin heterodimers stacked head-to-tail as polar protofilaments and folded into hollow tubes via lateral interactions (Fig. 1). MTs grow stochastically and are able to switch between phases of growth and shrinkage. This property is called *dynamic instability* and allows the cell to rapidly remodel MTs in response to internal and external signals [1].

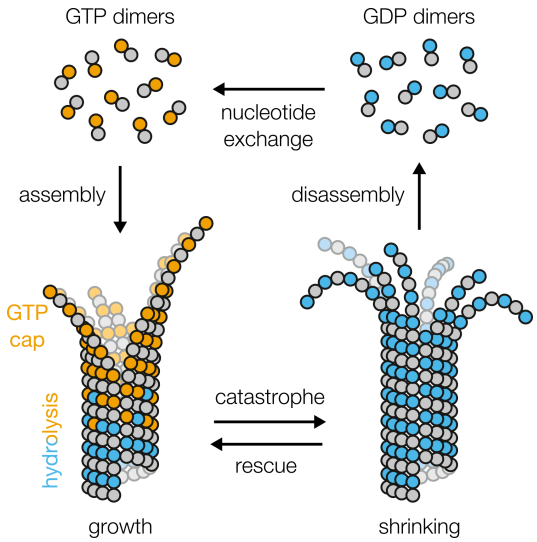

Figure 1: *Microtubule life cycle. Tubulin dimers circulate through four different states fueled by GTP binding and hydrolysis.*

Disclosing the MT life cycle has been a long-standing problem for cell scientists. Both assembly and disassembly of MTs operate via changing the shapes of individual tubulin dimers triggered by GTP binding and hydrolysis, ultimately facilitating the addition of new dimers to growing MT ends or driving already existing MTs beyond their stability threshold. The latter process is termed *catastrophe* and characterized by the explosive dissociation of dimers from shrinking MT ends. However, it is still elusive how and where these shape changes contribute to the mechanisms of MT growth and catastrophe. This is mainly due to the lack of atomistic knowledge about the dynamics of tubulin dimers in solution and when locked in the MT. Understanding these mechanisms is crucial because they define how MTs are controlled in the cell by a variety of regulating proteins, many of which are involved in serious diseases such as Alzheimer's [2].

In this project, we study the subnanometer dynamics of MTs by atomistic molecular dynamics (MD) simulations and cryo-electron microscopy (cryo-EM), which allows us to optimally combine high-resolution but static structural data with dynamic information obtained from simulations. The project encompasses several research directions, some of which have been successfully completed and published (Fig. 2). First, we focused on the dynamics of individual tubulin dimers in solution (not MT-integrated) to understand how GTP or GDP binding is linked to tubulin's conformation (Fig. 1, top, right-to-left transition; Fig. 2, top). Based on the results of extensive MD simulations, a novel mechanism has been proposed in which tubulin's bending flexibility controlled by the bound nucleotide is the driving force for MT assembly [3]. We then switched to the nucleotide-dependent dynamics of tubulin in the MT (Fig. 1, bottom transitions). However, no accurate structures of entire MTs were available. We therefore developed, implemented and tested a MD-based method for automated refinement of atomic models into cryo-EM density data [4]. The method has allowed us to set up and perform refinements of custom-built MT models against the recent high-resolution cryo-EM densities of complete MTs [5,6] (Fig. 2, bottom).

Current work builds upon the obtained MT models in both GTP- and GDP-bounds states. Here, our goal is to gain access to the mechanism of MT catastrophe. Recent experimental data suggest that subnanometer changes in the shape of tubulin dimers triggered by GTP hydrolysis in the MT precede the catastrophe event, but it is unclear how. Simply put, we investigate the energetic cost of such tubulin changes and how they contribute to an overall destabilization of the MT using extensive and large-scale MD simulations. Another point of our ongo-

ing simulation studies is the recently published cryo-EM structure of MTs coated with Tau proteins [7]. The MT-associated Tau protein regulates the neuronal MT network and is involved in the development of several neurodegenerative diseases such as Alzheimer's [2]. The interaction of Tau with the MT surface is extremely challenging due to the disordered nature of Tau (few to no tertiary structure). With the Tau-MT structure available, we now aim to directly perform interaction simulations to get atomistic insights into the mechanism of Tau (un)binding.

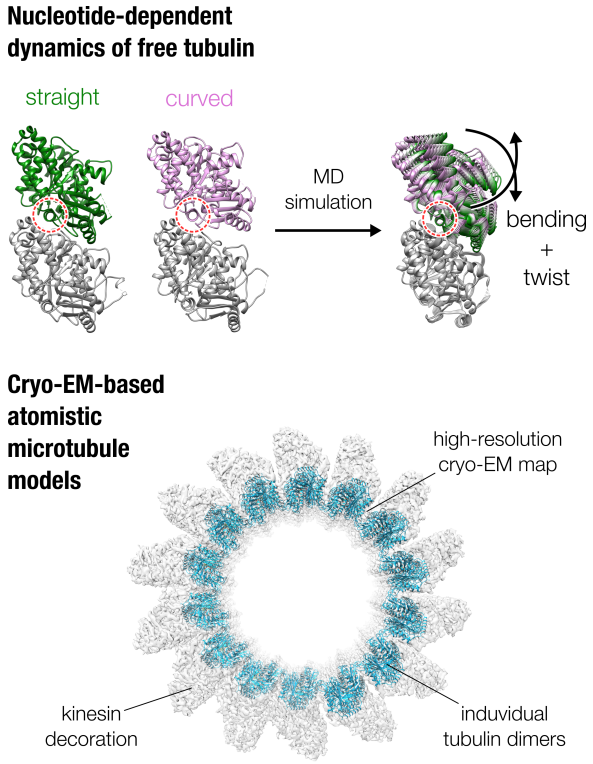

Figure 2: *Research directions. (top) Bending dynamics of free tubulin and its dependence on the bound nucleotide has been one of the major challenges for understanding the mechanism of assembly. We have studied the dynamics and energetics of this process using recently published high-resolution tubulin structures. (bottom) The dynamics of tubulin when locked in the lattice is, in turn, key to the mechanism of MT catastrophe. Investigating these requires both considerable computational resources and atomistic models of entire MTs. Here, we have developed and implemented an automated structure refinement method to derive accurate MT models from high-resolution cryo-EM reconstructions.*

WWW

https://www.mpibpc.mpg.de/grubmueller/microtubules
https://www.mpibpc.mpg.de/grubmueller/densityfitting

More Information

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