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Microtubule dynamics: moving toward a multi-scale approach

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Microtubule self-assembly dynamics serve to facilitate many vital cellular functions, such as chromosome segregation during mitosis and synaptic plasticity. However, the detailed atomistic basis of assembly dynamics has remained an unresolved puzzle. A key challenge is connecting together the vast range of relevant length–time scales, events happening at time scales ranging from nanoseconds, such as tubulin molecular interactions (Å–nm), to minutes–hours, such as the cellular response to microtubule dynamics during mitotic progression (μm). At the same time, microtubule interactions with associated proteins and binding agents, such as anti-cancer drugs, can strongly affect this dynamic process through atomic-level mechanisms that remain to be elucidated. New high-resolution technologies for investigating these interactions, including cryo-electron microscopy (EM) techniques and total internal reflection fluorescence (TIRF) microscopy, are yielding important new insights. Here, we focus on recent studies of microtubule dynamics, both theoretical and experimental, and how these findings shed new light on this complex phenomenon across length–time scales, from Å to μm and from nanoseconds to minutes.

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Introduction

Microtubules (MTs), filamentous structures self-assembled from αβ-tubulin heterodimers, constitute an essential part of the cytoskeleton of eukaryotic cells. Microtubules perform diverse cellular functions such as cell division, mitosis, and motor-mediated organelle transport, and in general, help establish cellular structure and enable motility. They accomplish subcellular reorganization through their 'dynamic instability' behavior, defined as stochastic switching from growth to shortening and back again (referred to as 'catastrophe' and 'rescue,' respectively) [1,2]. The guanosine nucleotide state in the β-tubulin subunit has proven to be an important mediator of microtubule assembly dynamics. Whereas guanosine triphosphate (GTP)-tubulin is believed to establish a protective cap at the growing ends of microtubules by being more stable in the microtubule lattice, guanosine diphosphate (GDP)-tubulin is believed to act as a destabilizing factor [3–6]. However, some conflicting views exist regarding the GTP-cap being mainly responsible for stabilization [7,8]. In addition, microtubule associated proteins (MAPs), and microtubule-targeting agents (MTAs) interact with polymerized and un-polymerized tubulin, and consequently influence the dynamic cycle, making them potential pathways for treating cancer and neurodegenerative diseases [3,9–12]. Considering the fundamental role of microtubules in the cell, recent studies have aimed at understanding how their atomistic-level structure impacts dynamic instability and function. This review focuses on novel computational and experimental approaches that probe the dynamic behavior of microtubules and their interactions with various MAPs and MTAs.

Computational modeling from angstroms to micrometers, and nanoseconds to minutes

Microtubules have been a significant subject for computational modeling at different length–time scales. Their dynamic behavior is dependent on hydrolysis of the GTP nucleotide in β-tubulin. This atomic-scale change is believed to be responsible for the ultimate dynamic instability of microtubules [6]. Considering this small-scale event, microtubules and their building blocks, αβ-tubulin dimers, are prominent targets to be modeled at different scales to understand the underlying mechanism of their behavior and the effect of their interactions with MAPs and MTAs. Starting from the atomic-scale, experimental techniques have been notably improved to obtain high-resolution X-ray crystal and, more recently, cryo-electron microscopy (cryo-EM) structures of tubulin, making atomic-resolution modeling possible. These studies provided tubulin structure both when polymerized in a microtubule lattice [13], and when un-polymerized in solution bound to MAPs [14–16]. Of particular note, Zhang *et al.* [17••], focused on the structural transitions that occur upon hydrolysis. In contrast to previous studies that noted lateral contacts as the difference between polymerized GDP-tubulin and GTP-tubulin [18], they

concluded that GDP-MT and GTPγS-MT lattices are more compact around the longitudinal interface compared to the extended GMPCPP-lattice, confirming the results of their previous study [19]. The end-binding protein EB3 was also shown to have a binding preference for the compacted GTPγS-MT, as the intermediate state of the two other nucleotides. The surge of recent cryo-EM structures has helped the field to improve our understanding of the microtubule interaction with several MAPs that regulate the dynamic behavior such as EB proteins, molecular motors kinesin and dynein, and tubulin tyrosine ligase-like (TTLL7) enzyme [20•].

These three-dimensional structural investigations have provided insight into binding mechanisms in addition to establishing a firm basis for molecular-scale modeling, which can be employed to study the dynamic behavior of tubulin movement, deformations and energetics in an aqueous solution with salts and other interacting molecules. All-atom molecular dynamics (AA-MD) simulations, now extending out to microseconds with the help of parallel graphical processing unit (GPU) computing, have been useful in obtaining an accurate estimate of free energies of binding, solvation, and conformational energies, although they are still computationally expensive [21]. On the other hand, protein–ligand docking studies, trading off speed versus accuracy, have grown noticeably, especially in drug discovery and mutation probing [22–24]. Tripathi *et al.* [25•] investigated leucine point mutations (L215H, L217R, and L225M), known for paclitaxel resistance in cells, using molecular docking, MD simulations and approximate binding energy calculations [26]. They concluded that effects of mutations on M-loop flexibility play a key role in mediating paclitaxel binding strength to tubulin. In another docking modeling and MD simulation study [27••], differential binding affinity of tubulin isotypes $\alpha\beta_{I}$, $\alpha\beta_{III}$ and $\alpha\beta_{IV}$ for a colchicine analogue, DAMA-colchicine, was monitored. It was revealed that $\alpha\beta_{IV}$ has the highest binding energy for the drug among the three isotypes, although it should be noted that the binding free energy was estimated without taking into account the entropic cost of binding. One of the drawbacks of free energy estimates that neglect full entropy costs is the inability to directly relate these computed energy landscapes to larger-scale coarse-grained simulations, which can capture a wider range of dynamic time-scales and length-scales. It remains to be demonstrated that these relative docking calculations are sufficiently accurate to predict the interaction differences that influence large-scale events such as tubulin–tubulin bond formation.

In parallel with structural studies and MD simulations, Brownian dynamics and thermo-kinetic simulations have grown rapidly, capturing the kinetics, thermodynamics and mechanics of tubulin addition and loss at microtubule ends at nano-scales and micro-scales. Different pioneering models vary in their assumptions and parameter space, all trying to provide a framework for explaining microtubule catastrophe, rescue and other related features of dynamic instability. Zakharov *et al.* [28••] employed a coarse-grained Brownian dynamics simulation, incorporating thermal fluctuations as well as fluctuations in protofilament tip shapes, beyond those contained in previous simpler thermo-kinetic models [29]. Variations in the extent of protofilament curling at the microtubule tip were identified as the dominant factor causing catastrophe, rather than the loss of GTP-tubulin cap. Moreover, they reported that microtubule aging is traced back to slow evolution of molecular events at the microtubule tip rather than an accumulation of specific permanent defects in microtubule tip or wall. Although emphasizing the critical role of bond energies and elastic deformation, GDP-state and GTP-state tubulin were assumed to have similar energetics in their model, both having an energy barrier in their potential of interaction, the justification of which has not been confirmed by molecular simulations. In addition, the GTP-hydrolysis rate was increased 6–18-fold to be able to observe microtubule catastrophe within the model, which deviates from a physiologically relevant value of $\sim 1\ s^{-1}$. In another study, Piedra *et al.* [30••], favoring the role of a GTP-cap in microtubule stabilization and using a simplified thermo-kinetic model similar to [18], found that adding GDP-to-GTP exchange on terminal subunits results in less frequent catastrophe, an observation that was verified using *in vitro* assays. In their model, they assumed a trans-acting nucleotide effect, where the nucleotide state of the tubulin subunit underneath the terminal subunit determines the terminal subunit's energetics, rather than the nucleotide state of the terminal subunit itself (cis-acting nucleotide). This model originates from the finding that previous studies failed to find a structural change in soluble tubulin as a function of nucleotide-state [31,32,15]. Additionally, a trans-acting nucleotide effect is consistent with the previously noted observation of lattice compaction upon hydrolysis [17••]. Still, the question of how nucleotide-state induced conformational changes in tubulin and upon its neighbors translate to changes in kinetics and thermodynamics remains to be clarified.

Recently, by integrating updated thermo-kinetic modeling, previously successful in recapitulating experimental observations of dynamics [29,33], and fluorescent microscopy, the mechanisms of action of two important MTAs, paclitaxel and vinblastine, were described in the context of kinetic and thermodynamic fundamentals [34••]. Consequently, the employed methodology relates micrometer-scale behavior to nanometer-scale computational modeling parameters, such as hydrolysis rate and bond energy weakening or strengthening. Although, the model could be improved with a more precise knowledge of the tubulin–tubulin interaction energies and the energetic