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Modeling Microtubule Dynamics on Lomonosov-2 Supercomputer of Moscow State University: from Atomistic to Cellular Scale Simulations

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Cytoskeletal polymers of tubulin, the microtubules, are critically important for cellular physiology. Their remarkable non-equilibrium dynamics and unusual mechanical properties have nurtured interest in exploring microtubules with diverse experimental methods and modeling their properties at different scales. In this work, we overview the studies of microtubules from the atomistic level of detail to the cellular dimension, focusing on the computational modeling work that has been carried out by our group on Lomonosov-2 supercomputer of Moscow State University since 2015. Our computational efforts have been aimed at understanding of microtubules through a set of models at multiple spatial and temporal scales, starting from examining the properties of tubulin dimers, as the building blocks, and further elucidating how those properties enable more complex assembly/disassembly and force-generation behaviors of microtubules, emerging at larger scales. Our methodology includes different approaches, from atomistic molecular dynamics to more coarse-grained techniques, such as Brownian dynamics and Monte Carlo simulations. We describe the motivation and the context for each model, overview the major conclusions from the simulations, which we believe were instrumental in building an integrative understanding of these polymers. We also discuss some technical aspects of the modeling, such as the computational performance of different types of simulations, current limitations and potential future directions for description of the microtubule dynamics, using the multi-scale approach.

Keywords: microtubule, Lomonosov-2, computational performance, multi-scale simulations, molecular dynamics, Brownian dynamics, kinetic Monte Carlo simulations.

Introduction

Microtubules are essential biopolymers that form a core component of a system of cytoskeletal fibers in eukaryotic cells, alongside actin and intermediate filaments [9]. Tubulin monomers, the building blocks of microtubules, are globular proteins approximately 4 nm in diameter. Alpha and beta tubulins, associate into heterodimers, which then polymerize into hollow cylindrical microtubules with a helical lattice, typically composed of thirteen protofilaments. Due to their high flexural rigidity, microtubules can span entire cell, sometimes reaching lengths of tens of micrometers. A remarkable feature of microtubules is their dynamic assembly/disassembly behavior. These polymers undergo extended phases of elongation and shortening [26], with stochastic transitions between these phases termed catastrophes and rescues. This energy-consuming process allows microtubules to continuously explore the interior of the cells and rebuild their network. Intriguingly, they can even turn the energy of their growth and shortening into useful work, producing significant forces within cells (reviewed in [19]). These multi-tasking polymers are involved in numerous critical processes, including intracellular transport, maintenance of cell

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shape, building the cell division apparatus for correct segregation of chromosomes, transporting chromosomes and remodeling membranes, etc. [20].

To explain the mechanism underlying the transitions of microtubules between elongation and shortening, the idea of the guanosine triphosphate (GTP) cap has been put forward about four decades ago [2], and further refined in the late 1980s and 1990s, into a widely accepted model. The key postulates of this model can be formulated as follows:

1. During microtubule assembly, tubulin dimers incorporate into the microtubule end from the solution in the GTP-bound form.
2. GTP-bound tubulins exhibit a relatively 'straight' equilibrium curvature in the dimeric and oligomeric form, allowing them to incorporate into the microtubule without significant strain.
3. After incorporation into the microtubule lattice, GTP hydrolysis is catalyzed, converting tubulin dimers to the GDP-bound form. Thus, the majority of tubulins in the microtubule lattice are GDP-bound, while only the freshly polymerized layers at the growing microtubule end are GTP-bound. These layers are termed the 'GTP cap'.
4. The GTP cap protects the growing microtubule from depolymerization. The stochastic loss of the GTP cap from the microtubule tip triggers a catastrophe – a transition from growth to rapid shortening.
5. GDP-bound tubulins have 'curved' conformations, resulting in strain within the microtubule lattice, and accumulating the energy of mechanical deformation. During microtubule depolymerization, this energy is released as the protofilaments lose their lateral bonds and 'unzip' from the tip.
6. Stochastic regain of the GTP cap enables the microtubule rescue – a transition from depolymerization to growth.

Together, these six postulates summarize an effective conceptual model for the dynamic instability of microtubules, successfully describing key aspects of microtubule behavior. However, like any model, it simplifies reality. Recent observations on microtubule dynamics and structure have revealed inconsistencies, both quantitative and qualitative, with some predictions of this model (reviewed in [8, 20]). Although seemingly subtle, these new observations reveal important details, which are essential for eliciting the mechanisms of microtubule control through different factors, including: (i) associated regulatory proteins, (ii) scaffolds that couple microtubule ends to various structures in order to transmit mechanical forces, (iii) tubulin targeting drugs, representing a major type of anticancer chemotherapeutics, and (iv) tubulin modifications, such as point mutations, or post-translational changes.

Here we describe our use of the multi-scale modeling, as a powerful approach to integrate the complexity of accumulating new multi-faceted and sometimes contradictory data about microtubules into a comprehensive framework, necessitating revisions to the traditional GTP cap model. The systems simulated in this study span three orders of magnitude on the spatial scale, from tens of nanometers to micrometers, and over eight orders of magnitude on the temporal scale, from nanoseconds to hours (Fig. 1). Typical particle numbers and temporal and spatial scales for all models are summarized in Tab. 1. The simulations were conducted using the high-performance computing resources of the Moscow State University, primarily the Lomonosov-2 supercomputer [34].

The article is organized as follows. Section 1.1 is devoted to molecular dynamics simulations of tubulin dimers and oligomers. Section 1.2 describes Brownian dynamics simulations of entire

microtubule tips. Section 1.3 reviews Monte Carlo simulations of microtubule dynamic instability at the scale of tens of micrometers in length. The final section of this study summarizes the findings and suggests directions for future research.

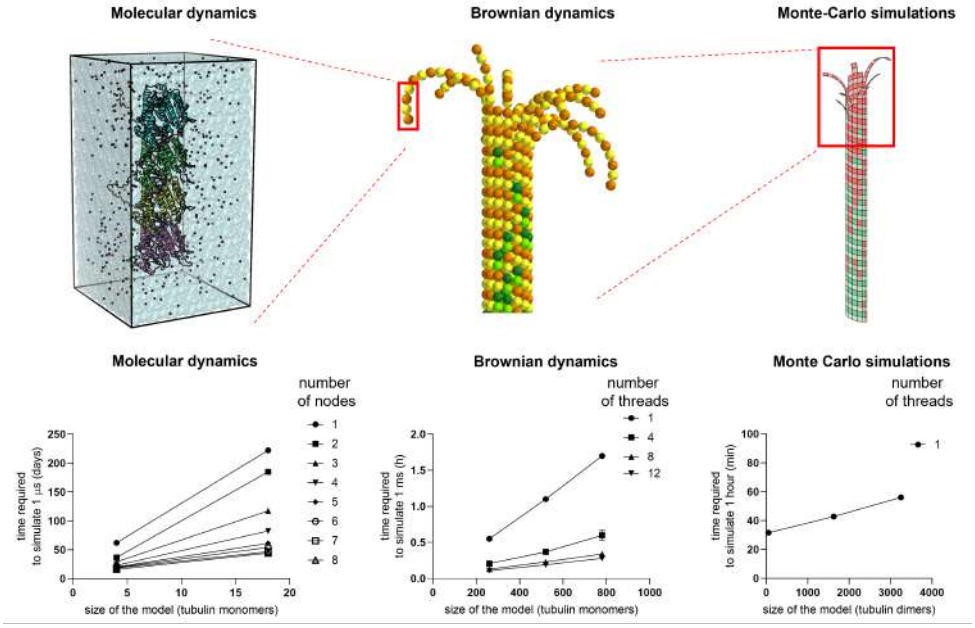

Figure 1. Multi-scale models of microtubules and computational performance of the simulations on Lomonosov-2 supercomputer of the Moscow State University. The upper row of images provides schematics of molecular models at different spatiotemporal scales. The graphs below show the performance of the simulations on Lomonosov-2 (CPU: 2Intel Xeon E5-2697, GPU: Nvidia Tesla K40) as a function of the size of the modeled system and the number of nodes/computing threads. The analysis of MD simulation performance is based on data from [13]

Table 1. Typical spatiotemporal scales covered in each type of simulation

Model type	Typical model size (tubulin subunits)	Typical model size (number of particles)	Spatial scale	Temporal scale
Molecular dynamics (MD)	2–18 monomers	200,000–1,100,000 atoms	∼10 nm	∼1 μs
Brownian dynamics (BD)	100–200 monomers	100–200 tubulin monomers	∼100 nm	∼1 s
Monte Carlo (MC) simulations	10–3000 dimers	100–3000 tubulin dimers	∼10 μm	∼1 h

1. Results and Discussion

1.1. Molecular Dynamics Simulations of Tubulin Dimers and Oligomers

Recent structural and biochemical findings about tubulins and microtubules have challenged some of the postulates of the classical GTP cap model (reviewed in [11, 20]). Specifically, several studies have reported that GTP-bound tubulins in solution exhibit curvature similar to that of GDP-bound tubulins [2, 6, 27, 29]. In addition, a growing body of literature (reviewed in [23, 32]) has suggested that microtubule dynamics in cells are affected by posttranslational modifications

of the non-globular, intrinsically disordered tails – polypeptide regions that are neglected by the classical model.

These observations motivated our investigation of individual tubulin dimers and oligomers at the nanoscale using atomistic molecular dynamics (MD). We aimed to address two questions: (1) Is GTP-bound tubulin significantly less curved than GDP-bound tubulin? (2) Are the unstructured charged tails of tubulins involved in the microtubule assembly/disassembly process? Atomistic MD is a computational technique that represents individual atoms as particles, described by Newtonian equations of motion [31]. The forces acting on these particles are derived from a force-field – a set of pre-calibrated parameters describing bonded and non-bonded interactions between any set of atoms. Due to high-frequency bond-angle vibrations involving hydrogen atoms, the positions and velocities of atoms in protein simulations need to be updated with very short timesteps, typically about 1–2 femtoseconds (fs) or slightly longer [16]. The MD method is widely used in numerous laboratories worldwide to simulate dynamics in various molecular systems, including proteins, at the resolution of individual atoms and on timescales up to tens of microseconds. Several major software packages have been developed for efficient MD simulations. GROMACS is one of the leading packages, optimized for computations using hybrid architectures and employing multi-level parallelism [1, 28].

We created several molecular models, including (i) models of tubulin dimers, as the smallest building blocks of microtubules, (ii) models of tubulin tetramers, as the shortest possible oligomers, and (iii) models of tubulin octadecamers, representing small fragments of the microtubule wall (three laterally connected tubulin hexamers). All modeled systems included the unstructured charged regions of beta and alpha tubulins. The tubulins were solvated in water and charge-neutralized with K^+ and Cl^- ions (Fig. 1).

Analysis of the MD simulation trajectories, collectively spanning approximately 30 microseconds, revealed that the overall curvatures of tubulin dimers and small oligomers were very similar in both the GTP and GDP states [15]. This finding contrasts with the postulate of the classical model but aligns with recent structural and biochemical data. Thus, it is not the curvature of tubulin that is modulated by the nucleotide to switch its properties from polymerization-competent to polymerization-incompetent. Rather, we found that the nucleotide likely alters the flexibility of the tubulin interface between tubulin dimers, making GDP-bound protofilaments softer and, therefore, easier to straighten and incorporate into the microtubule lattice.

Examination of the behavior of the disordered tails in the simulations has revealed the potential for direct interaction between the negatively charged amino acid residues of the alpha-tubulin tail and the positively charged amino acid residues on the longitudinal polymerization interface of the tubulin dimer [7]. This suggests that the tail could occlude the polymerization interface, thereby downregulating the rate of incorporation of tubulin dimers into the growing microtubule tip. This modeling result is in good agreement with experimental observations of faster microtubule growth in tubulin mutants with deleted alpha-tubulin tails [7]. Together, these theoretical and experimental data identify a direct role of unstructured charged regions of tubulin in the modulation of microtubule assembly, opening new possibilities for controlling microtubule dynamics by modifying the interactions of the tails with tubulins.

A typical MD simulation trajectory was 1 microsecond long and required approximately 10–30 days of computer time on 8 nodes of the Lomonosov-2 supercomputer, depending on the model system's size (Fig. 1). The computational performance of MD simulations of tubulins and other molecular systems has been extensively explored in a series of dedicated publications

in this journal. That analysis included assessments of the performance of MD simulations on various computational architectures and offered practical suggestions [12–14].

1.2. Brownian Dynamics Simulations of Microtubule Tips

Due to the high computational complexity of MD simulations, it has not been possible to simulate whole microtubule tips at the necessary timescale of at least about a second, which is required to describe microtubule assembly, disassembly, and force generation. To address this limitation, we employed the Brownian dynamics (BD) approach [21, 25, 33, 36]. In our super-coarse-grained model, each tubulin monomer was represented as a hard sphere with four interaction centers on its surface. The interactions between these centers were described by empirical energy potentials, comprising a potential well and an activation energy barrier. Quadratic energy potentials were used to describe the bending of tubulin protofilaments. The model consisted of two layers. In the fast ('dynamic') layer, the updated positions of each tubulin monomer were calculated using the Ermak–McCammon algorithm for solving the overdamped Langevin equation, with a computational step of 50–100 ps [10]. In the slow ('kinetic') layer, new tubulins were stochastically added to the tip of the microtubule with a certain probability once per millisecond, ensuring microtubule elongation without explicitly considering the arrival of tubulin dimers from the solution. The slow layer also enabled the implementation of GTP hydrolysis as the key event switching the properties of tubulin dimers, eventually triggering an abrupt transition from assembly to disassembly. These transitions could be explored at the timescale of about a second, which was feasible with this type of modeling (Fig. 1).

The main questions we addressed with the BD model were: (i) What is the mechanism of microtubule elongation, given the curved shape of GTP-tubulin dimers and oligomers? (ii) How much force can be generated by the microtubule tip, and how does this force depend on parameters of tubulin-tubulin interactions?

The first question was partially motivated by computational and experimental data described in the previous section, including our own MD simulations. Additionally, structural findings using cryo-electron tomography from our group and others provided new evidence for the presence of curved protofilaments at the growing microtubule ends under a wide range of conditions, in various species, and in purified in vitro systems [21, 22, 24, 25]. The second question pertained to the ongoing debate on the mechanisms of force production by microtubules and the coupling between shortening microtubule tips and chromosome-associated kinetochore proteins – one of the longstanding problems in the biology of mitosis [5, 18, 30, 35].

The modeling offered several insights that allowed us to conceptualize a revised model of microtubule assembly. Central to this model is the computational observation that the thermal fluctuations of curved tubulin protofilaments are frequent enough to permit the straightening of protofilaments necessary for establishing lateral contacts. This reconciles the seemingly inconsistent properties of tubulin protofilaments: their curved shape and their ability to elongate microtubules. According to the model's prediction, even relatively stiff protofilaments are expected to fluctuate sufficiently to form lateral bonds [21]. Protofilament stiffness and the activation barriers for lateral tubulin-tubulin interactions were predicted to be the two key factors responsible for the efficient conversion of the free energy of GTP hydrolysis into mechanical work and the ability of curling protofilaments to exert forces on cargoes [19, 21].

Our BD model was implemented in C++ and parallelized using OpenMP technology. In this implementation, the computational performance scaled linearly with the system size, as shown

in Fig. 1. We routinely used 14–28 simulation threads per simulation, determined by the number of cores in one Lomonosov-2 node. The use of multiple nodes, typically 20–30, of Lomonosov-2 was critical for obtaining the required statistics to extract ensemble-averaged characteristics of the simulated microtubule tips and enable comparison with experimental data.

1.3. Monte Carlo Simulations of Microtubule Dynamic Instability

Stochastic transitions between microtubule growth and shortening phases, termed catastrophes and rescues, are the most striking and captivating aspects of microtubule behavior. In cells, these transitions are essential, and their frequency is tightly controlled throughout the cell cycle. For example, the frequencies of catastrophes increase and the frequencies of rescues decrease by an order of magnitude when the cell enters the mitotic division stage.

Despite considerable theoretical and experimental work, our understanding of catastrophes and rescues remains incomplete. Recent observations suggest that factors beyond the GTP cap contribute to these transitions [11]. Computational modeling of microtubule catastrophes and rescues at the level of tubulin subunits (reviewed in [37]) usually involves kinetic Monte Carlo simulations, because the MD and BD models, such as described above, fail to achieve the necessary spatiotemporal scale of micrometers and minutes, dictated by the frequencies of catastrophes and rescues, observed in cells and in purified systems in vitro. Efficient algorithms for such simulations, pioneered by Gillespie, rely on calculating the time to the next reaction and randomly selecting the reaction to occur based on its relative probability [17].

Our motivation for creating a new Monte Carlo model of microtubule dynamics was two-fold. First, we aimed to determine whether the novel mechanism of microtubule assembly conceptualized through MD and BD simulations was consistent with the phenomenology of microtubule transitions and whether it could offer new insights into dynamic instability. Second, we sought to resolve long-standing questions about the origin of microtubule 'aging' and clarify the mechanism of microtubule rescue.

We developed a model that introduces two structural states of tubulin, 'curved' and 'straight', in addition to the two biochemical states, 'GDP-bound' and 'GTP-bound' [3]. Previous Monte Carlo models had only considered the two biochemical states (reviewed in [37]). This extension allowed us to incorporate information about the curved structures of GTP- and GDP-bound tubulins in solution and at dynamic microtubule ends, thereby providing a continuous description of microtubule behavior across multiple scales. Our 'four-state' Monte Carlo model enabled an improved description of the dependence of microtubule catastrophe frequencies on soluble tubulin concentration and microtubule polymerization time, also known as microtubule 'aging'. Additionally, the model provided insights into the possible role of lattice defects and their repair by GTP-bound tubulins, which we further confirmed experimentally [3, 4].

The four-state Monte Carlo model was implemented in Matlab 2021a. Generally, the time step in this type of simulation depends on the specific kinetic rates. In the fully parameterized four-state model of the microtubule, the average time step was approximately equal to 1/300 of a second. Our most efficient implementation, which was not parallelized, required approximately equivalent to or even shorter clock time to simulate a second of model time (Fig. 1). This computational efficiency allowed us to collect substantial statistics within a reasonable timeframe by running several simulation trajectories in parallel or sequentially on a single node of the Lomonosov-2 supercomputer. The simulations easily covered timescales of hours in model

time, enabling the observation of repeated transitions from growth to shortening within a single simulation run.

Conclusion

The multi-scale simulations discussed here have facilitated the construction of a coherent, integrated model of microtubule dynamics, consistent with a broad spectrum of experimental observations, including recent structural data on the conformations of tubulin dimers and oligomers in solution and at dynamic microtubule ends. The new perspective on microtubule polymerization and dynamics that emerged from the modeling has been instrumental in guiding experiments to address long-standing and newly conceived puzzles in the field. These include the mechanisms of microtubule force generation, catastrophes, aging, rescues, and the roles of unstructured tubulin regions in the control of tubulin polymerization. Collectively, this work represents a significant computational effort made possible by the high-performance supercomputing resources, and a continuous support from the Moscow State University computational facilities.

Over the past decade, there has been a dramatic increase in computational capabilities, enabling simulations of biological systems at an unprecedented scale. We anticipate that future developments in hardware architecture, and more importantly, in the development of more accurate and efficient biomolecular simulation techniques – potentially enhanced by rapidly developing machine learning approaches – will open up exciting possibilities. These advancements will enable the creation of not only qualitatively consistent but quantitatively predictive models, covering spatiotemporal scales from atoms to entire cells, and describing processes ranging from nanoseconds to hours in duration.

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