DOI: 10.14529/jsfi250201

# Bacterial Mini Microtubule as a Minimal Model System for Exploring Dynamic Instability Using Molecular Dynamics Simulations

*Vladimir A. Fedorov*[1], *Ekaterina G. Kholina*[1], *Nikita B. Gudimchuk*[2,3,4], *Ilya B. Kovalenko*[1]



Large scale computational modeling has been fruitfully applied to explore microtubules – an essential component of the cellular skeleton – for over two decades. In this paper, we describe simulations of a yet computationally unexplored minimalistic system of the bacterial mini microtubule, using the high performance resources of Lomonosov Moscow State University. We highlight similarities between the eukaryotic and bacterial microtubules at the protofilament level, the size and stability of the entire mini microtubule system and the computational benefits of using the bacterial mini microtubule as a minimal model to understand dynamic instability. Our results are discussed in the context of a bigger picture of the evolution of molecular dynamics simulations, aiming to understand microtubules, illustrating how the sophistication and scale of the computational efforts increased over the years.

*Keywords: bacterial microtubule, Lomonosov-2, computational performance, multi-scale simulations, molecular dynamics.*

## Introduction

Tubulins are among the most evolutionarily conserved proteins in eukaryotic cells, from yeast to mammals, which suggests that any considerable changes in their sequence may be lethal to the organism [48]. The reason is that they form essential structures, microtubules, which are critical for numerous intracellular functions, including transport and cell division [6]. Structurally, microtubules are composed of tubulin dimers arranged into linear chains called protofilaments. Typically, 13 protofilaments laterally associate to form a hollow tube (the microtubule), which can elongate by incorporating tubulin dimers at its so-called (+)-end or shorten by loss of subunits. One of the remarkable features of these polymers is their ability to alternate between phases of elongation and shortening, with abrupt transitions between them [29]. This behavior, termed "dynamic instability", is physiologically important for the constant re-modeling of microtubule networks in cells, the continuous search-and-capture of chromosomes during mitosis, and many other essential processes [17]. Due to their dynamic instability, microtubules also act as active force generators within cells [15]. The non-equilibrium behavior of all microtubules is fueled by the energy of guanosine triphosphate (GTP) hydrolysis. GTP molecules bind to tubulin dimers in solution and provide energy for conformational changes within tubulin through the chemical reaction that cleaves a phosphate group from GTP, converting it into guanosine diphosphate (GDP). The precise mechanisms by which this biochemical reaction drives the mechanical destabilization of microtubules and how microtubules generate forces remain long-standing questions in cytoskeletal research [4, 17]. These are some of the problems that have long fascinated scientists and have driven numerous experimental, theoretical, and computational studies. Interestingly, while most bacterial species do not possess microtubules, a unique type has been

[1]Department of Biology, Lomonosov Moscow State University, Moscow, Russia
[2]Department of Physics, Lomonosov Moscow State University, Moscow, Russia
[3]Center for Theoretical Problems of Physicochemical Pharmacology, Moscow, Russia
[4]Pskov State University, Pskov, Russia

identified in *Prosthecobacter* species [22]. These bacterial microtubules, composed of the tubulin homologs BtubAB, consist of only 4–5 protofilaments but display remarkably similar dynamic properties to those of eukaryotic microtubules [5]. Here, we use molecular dynamics simulations to study a short bacterial microtubule and its basic building block – a four-subunit protofilament. Our results show that this system behaves in a way similar to eukaryotic microtubules, making it a useful model for studying dynamic instability.

# 1. Methods

## 1.1. Molecular Systems

Molecular model of the four-stranded mini microtubule (model 2) formed by bacterial tubulin-like BtubAB proteins was based on 5o09 PDB structure [5]. The PDB structure also contained a regulatory BtubC subunit, which we did not include in our simulation. BtubAB proteins were placed in a cubic reaction volume with periodic boundary conditions and 31.72 nm side filled with TIP3P water and ions. The total number of atoms in the system was 3134589 where 110400 atoms belong to tubulin. Additionally, we created the molecular model (model 1) of one protofilament of bacterial mini microtubule based on the same PDB structure 5o09. This model included 544280 atoms where 27599 atoms belong to tubulin in a $16.65 \times 15.3 \times 21.73$ nm cubic reaction volume with TIP3P water and ions. In each model, we added unresolved mobile amino acid chains, using the Modeller program [45]. We used Propka [35] to calculate the degree of protonation of amino acid residues and Dowser [32] to identify and solvate cavities inside the protein. In both models every BtubA and BtubB subunit was bound to a GDP molecule. The size of the reaction volume was set in such a way that the distance from the protein surface to the nearest box boundary was not initially less than two nanometers. The ionic strength 0.1 M of the solution was achieved by adding $K^+$ and $Cl^-$ ions in a way that the total charge of the system was zero.

## 1.2. Molecular Dynamics Simulations

Simulations were performed using hybrid computational architecture and the GROMACS 2022.4 software package [1] with the CHARMM27 force field [25, 26]. The parameters of the GDP molecule were taken from the CHARMM27 force field, and the parameters of their phosphate groups were set in accordance with [37]. The steepest descent algorithm was used to minimize energy of each system. After this, a two-step equilibration was applied: 1-ns-long simulation with constrained positions of all heavy protein atoms at constant pressure and temperature, and 5-ns-long simulation with constrained positions of protein backbone atoms, using the Berendsen barostat and thermostat. The production simulation runs were carried out in the NPT ensemble at 300 K, using the Parrinello–Rahman algorithm [36] and the V-rescale thermostat. Totally we produced three trajectories of the bacterial protofilament and one trajectory of the bacterial microtubule, each trajectory no less than 1 $\mu$s. All-bond P-LINCS constraints and mass rescaling (partial transfer of mass from heavy atoms to bound hydrogens [11]) allowed molecular dynamics simulations with 4 fs time step. Pymol (The PyMOL Molecular Graphics System, Version 2.0 Schrodinger, LLC) was used for visualization.

### 1.3. Analysis

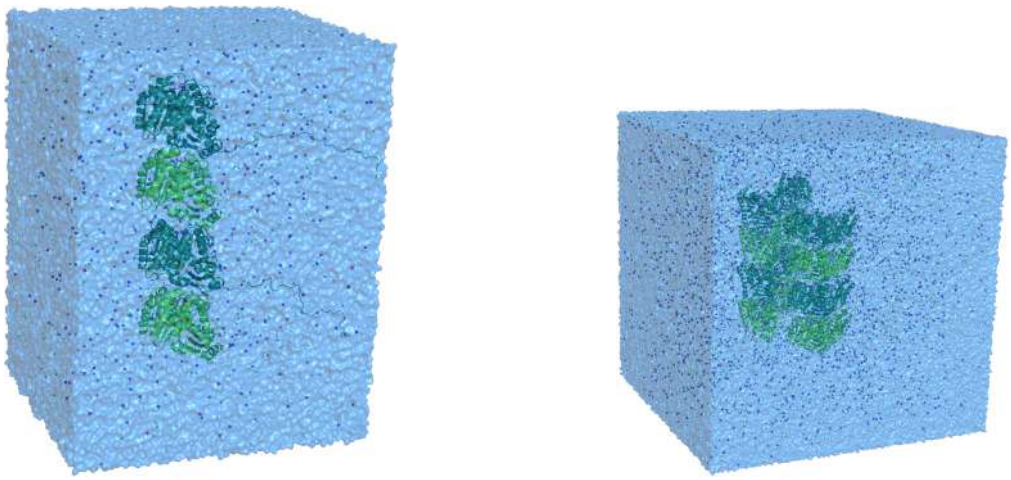
Calculation of bend and twist angles of a tetrameric protofilament of bacterial mini microtubule was made using custom python scripts inside Pymol in the way it had been described in our previous work on eucaryotic tubulins [10]. To do this, a Cartesian coordinate system was associated with a bacterial microtubule structure. Then a bacterial tubulin tetramer was aligned onto the microtubule fragment by its reference subunit. So, the reference subunit became aligned along the microtubule-bound coordinate system. To determine the orientation of the next tubulin subunit relative to the reference subunit, another microtubule fragment was aligned onto the next tubulin subunit, producing another Cartesian coordinate system. After this, we calculated the Euler angles of the rotation of the second coordinate system relatively to the first one.

## 2. Results

### 2.1. Exploring the Behavior of the Bacterial Microtubule System through Molecular Dynamics Simulations

Bacterial microtubules consist of only four protofilaments and are known to display dynamic instability behavior very similar to that of eukaryotic microtubules [5]. Moreover, they offer additional advantages due to the ease of re-engineering and modification, as bacterial systems are generally easier to manipulate genetically.

To examine the behavior of this system *in silico,* we prepared two molecular models: (1) a minimal protofilament, composed of two bacterial tubulin dimers, BtubAB, where each BtubAB monomer contained a GDP molecule in the nucleotide binding site; and (2) a minimal fragment of the entire four-protofilament microtubule, based on the CryoEM structure [5] (Fig. 1). We should note that the CryoEM structure also includes a regulatory BtubC subunit. However, since our goal was to model a minimalistic system, investigating the impact of this regulatory subunit on the microtubule was beyond the scope of the present study, and we therefore excluded it from our simulations.



(a) GDP-bound BtubAB protofilament (b) GDP-lattice mini microtubule

**Figure 1.** Molecular models of the GDP-bound BtubAB protofilament in $16.65 \times 15.3 \times 21.73$ nm$^3$ molecular dynamics cell and the GDP-lattice mini microtubule in $31.72 \times 31.72 \times 31.72$ nm$^3$ molecular dynamics cell filled with TIP3P water and ions. $\alpha$-tubulins are shown in dark green, $\beta$-tubulins in light green

We performed three independent microsecond-long molecular dynamics simulations of molecular model (1) and one such simulation of molecular model (2). These timescales match the current state-of-the-art in computations with eukaryotic microtubules, as we describe in the Discussion section. The simulations have demonstrated that the bacterial GDP-bound protofilaments tend to relax into outwardly curved shapes, very similar to those observed in eukaryotic tubulin (Figs. 2, 3). This result is consistent with the expectation that the mechanism driving the switch of bacterial microtubules to disassembly is analogous to that of dynamic eukaryotic microtubules: they accumulate energy from GTP hydrolysis in the form of mechanical strain, which is released during the depolymerization of GDP-bound subunits.

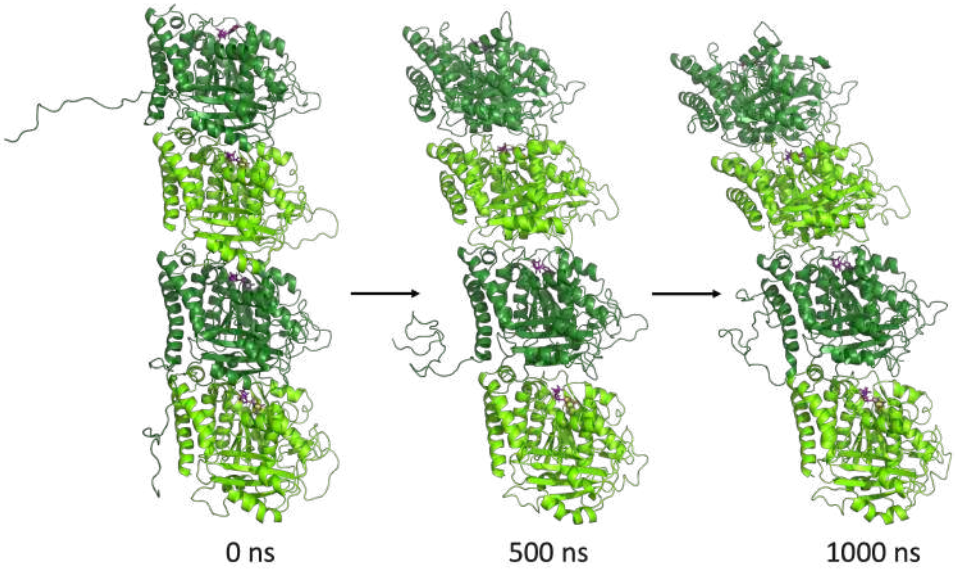



**Figure 2.** Results of molecular dynamics simulations of the GDP-bound BtubAB protofilament. Three snapshots of the protofilament in the onset of the simulation, upon 500 ns of the simulation and upon 1000 ns of the simulation. $\alpha$-tubulins are shown in dark green, $\beta$-tubulins in light green

Interestingly, our simulations of the four-protofilament mini microtubule revealed that it remained stable throughout one-microsecond simulation despite the tendency of individual protofilaments to become curved (Figs. 4, 5). We believe this result is expected, given the much slower rate of microtubule depolymerization observed in experiments [5]. Notably, in analogous simulations of eukaryotic microtubules [21, 47], lateral bonds are lost considerably faster – within one microsecond – suggesting an overly rapid depolymerization. In our opinion, this puzzling behavior has not yet received sufficient attention in the literature.

## 2.2. Computational Efficiency of the Molecular Dynamics Simulations of BtubAB Microtubules

We have previously investigated the computational efficiency of molecular dynamics simulations with GROMACS on diverse computational architectures, such as the Lomonosov-2 supercomputer at Moscow State University [43] and others, using various molecular systems, including tubulins [7–9] (Fig. 6). This analysis yields a simulation efficiency of 1.2 ns/day on one node of the Lomonosov-2 supercomputer for our whole bacterial microtubule system, comprising 3.5 million atoms. Using our newest server with RTX 4090, the performance can be increased by

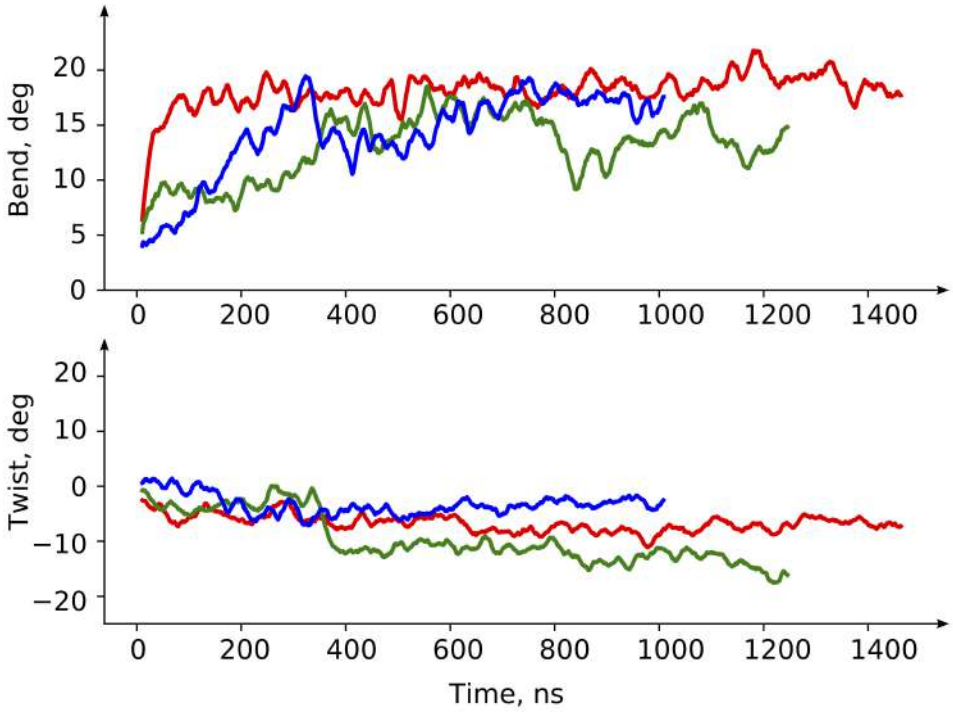



**Figure 3.** Averaged inter dimer bend and twist angles of the BtubAB monomers relative to each other as a function of the molecular dynamics simulation time. Colors show results from independent simulation runs

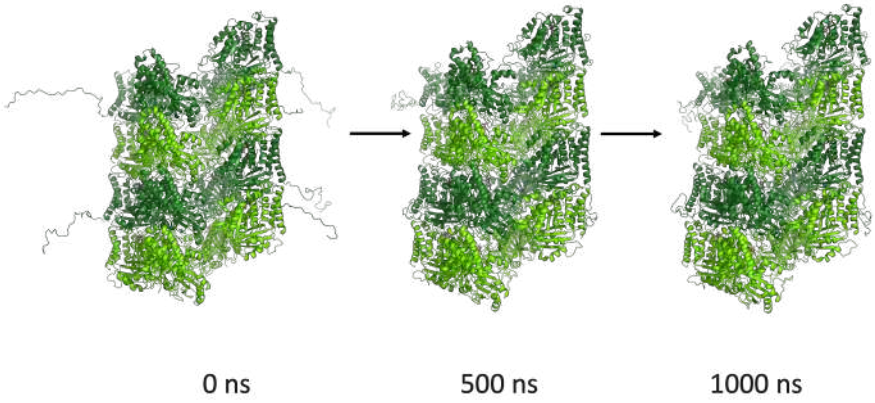



**Figure 4.** Results of molecular dynamics simulations of mini microtubule, containing four laterally attached GDP-bound BtubAB protofilaments. $\alpha$-tubulins are shown in dark green, $\beta$-tubulins in light green

an order of magnitude, up to 12 ns/day. Importantly, the system can be further optimized by reducing its size if simulating the long unstructured tails on the B subunit of bacterial tubulin is not necessary. In that case, a simulation box with dimensions of only $18 \times 18 \times 25$ nm$^3$ may be sufficient, reducing the computational cost by approximately 4-fold.

## Discussion

During the last two decades, multi-scale computational modeling, including efforts from our group, has provided important insights into our understanding of microtubules and their

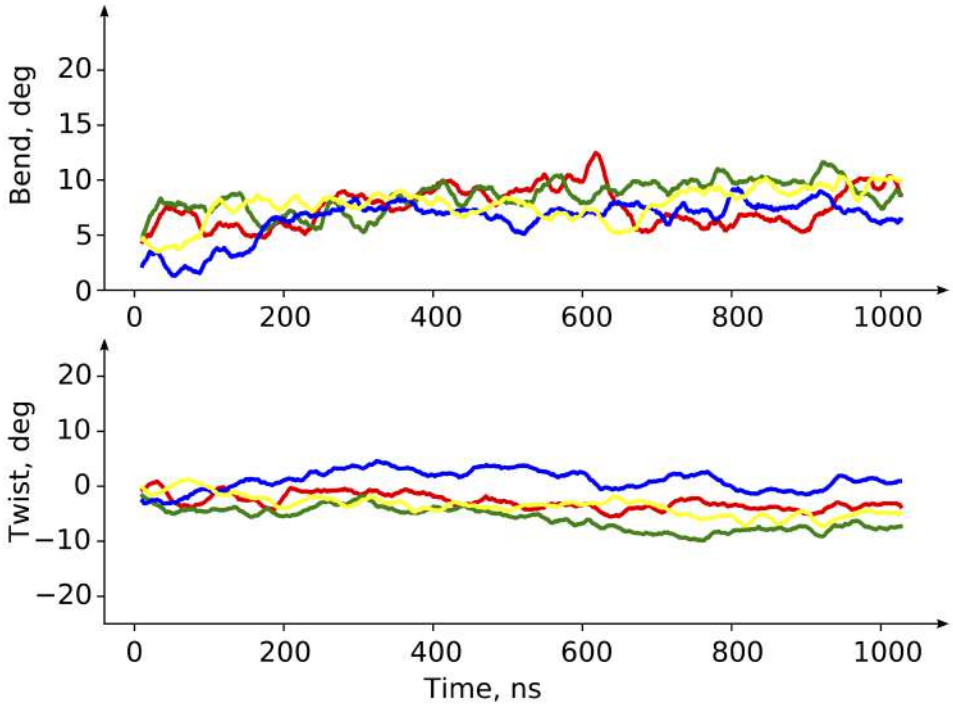



**Figure 5.** Averaged inter dimer bend and twist angles of the BtubAB monomers relative to each other as a function of the molecular dynamics simulation time. Colors show results for each protofilament

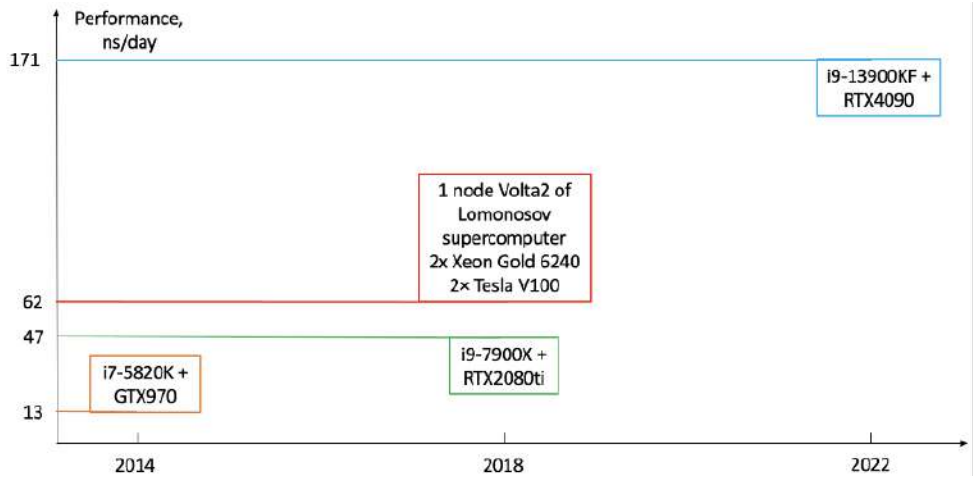



**Figure 6.** Timeline of performance (ns/day) evolution for molecular dynamics simulations on a single-node server for tubulin tetramer depending on various combinations of CPUs and GPUs with 2 fs timestep. The data presented is based on our previous benchmarking studies [7–9]

functioning at the molecular and cellular levels (reviewed in [3, 16, 19]). Molecular dynamics, as one of the most detailed biomolecular simulation techniques, has been particularly important in illuminating the interplay between microtubule structure and dynamics at the nanoscale. This method describes atoms as point masses interacting through a variety of interatomic potentials, collectively called a force field. The first structure of tubulin was obtained in the late 1990s [34]. Pioneering molecular dynamics simulations of tubulin were reported in 2004 by Mitra and Sept, aiming to explore the mechanisms of interaction between tubulin and microtubule targeting agents as potential chemotherapeutics [30]. In 2008, Gebremichael et al. were the first to use

molecular dynamics to address the question of the shape of individual tubulin subunits, providing crucial arguments about the similarity in curvature among the main building elements of microtubules, regardless of their bound nucleotide: GTP or GDP [12]. In the same year, Mitra and Sept developed the first model of six interacting tubulins within the microtubule lattice to gain insights into effects of the important chemotherapy drug paclitaxel on the microtubule [31]. Both studies simulated tubulin systems containing approximately 160000–180000 particles, including protein, solvent, and ion atoms, each covering no more than a few tens of nanoseconds, which was already a significant computational achievement at the time those pioneering simulations were carried out. During the 2010s, molecular dynamics simulations of tubulin already focused on examining the behavior of tubulin protofilaments and small fragments of the microtubule lattice, up to $3 \times 6$ monomers, rather than individual tubulin dimers [10, 13, 14, 20, 33, 38]. The total simulated time reached 1 $\mu$s, typically with 3–5 repetitions to improve sampling. Remarkably, in 2010 the first model of a complete microtubule was constructed by Wells and Aksimentiev [46] to study mechanical response of microtubules to deformations. Given the impressive system size, the simulation timescales, which were achieved, were quite modest, only spanning a few nanoseconds. In 2014, the cryo-EM revolution enabled the first structures of whole microtubules to be resolved at sufficient resolution, allowing for more informed studies of tubulin interactions within the microtubule lattice under different nucleotide states [2, 27, 50, 51]. By the 2020s, advancements in computational power finally enabled the simulation of entire microtubules by several research teams. In 2020 a 16-layer-microtubule model was constructed and simulated by Tong and Voth for about 200 ns [41]. Two years later, a massive computational effort by Igaev and Grubmüller described molecular dynamics simulations of over 15 million particles for a combined time exceeding 5000 ns with each nucleotide [21]. This was sufficient to observe at least partial relaxation of the modeled microtubule tips into flared morphologies, consistent with recent cryo-EM observations. In very recent paper [47] the authors used large microtubule lattice systems comprising ∼21–38 million atoms, and applied their multiscale approach to leapfrog through time and nearly double the computational efficiency in realizing relaxed all-atom conformations of GDP- and GTP-complexed microtubule tips. At the time of writing this manuscript, at least one more research preprint is available on the BioRxiv server, exploring entire microtubule system with simulation times extending up to several microseconds [23]. This clear increase in the simulated timescales and the system sizes, illustrated in Fig. 7, reflects the combined progress in computational algorithms, hardware architecture, and simulation software in recent years, Fig. 6.

Despite the spectacular progress in recent years, it is clear that molecular dynamics simulations of tubulin have reached a point where further advancement becomes more challenging due to the inability to cover the timescales required to observe microtubule dynamics as seen in experiments. While the current molecular dynamics simulations of whole microtubule tips cover only a few microseconds, real microtubules lose layers of tubulin subunits during their rapid depolymerization only once per tens of milliseconds on average [5, 44]. Given the pace of research development, we are confident that this gap will eventually be closed, likely through increases in both the spatiotemporal resolution of experimental methods and the further enhancement of computational resources and algorithms. However, as is often the case with scientific progress, unforeseen breakthroughs or inventions may also help accelerate this development in yet unclear ways. Coarse-graining is one promising pathway, though it inevitably comes at the cost of some loss of information and precision [18, 23, 24, 28, 39, 40, 42, 49]. As a somewhat unconventional

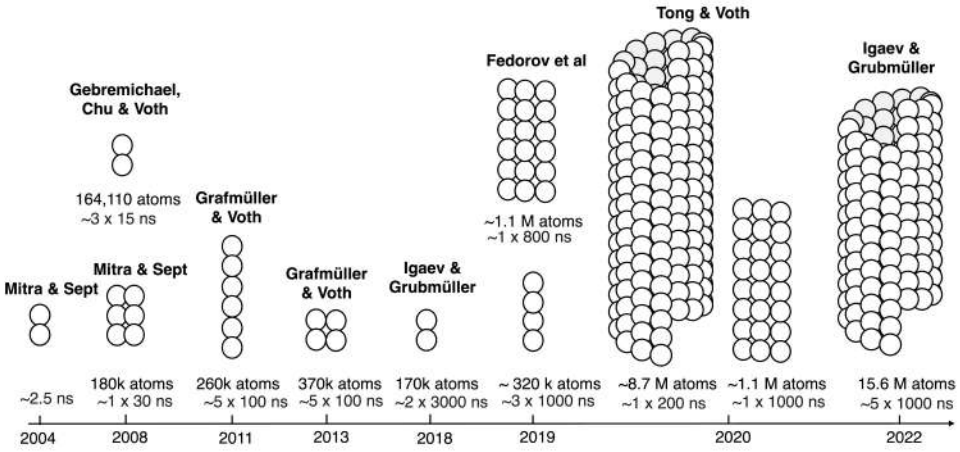



**Figure 7.** Timeline of the molecular dynamics studies of tubulins: from building blocks to whole microtubule

approach to facilitating this work, we have proposed here turning to bacterial microtubules as a more computationally feasible model. We demonstrate that bacterial microtubules share many features with their eukaryotic counterparts, including twist-bending relaxation of their individual building blocks. The computational model can be about an order of magnitude smaller compared to whole eukaryotic microtubule, significantly reducing computational cost and providing a good system for iterative exploration alongside experimental work, given the ease of genetic manipulation and expression of BtubAB mutant genes for in vitro characterization of the modified BtubAB polymers.

## Acknowledgments

Molecular dynamics simulations and data processing of bacterial tubulins were supported by the Russian Science Foundation grant No. 24-74-00002 (https://rscf.ru/project/24-74-00002/). The simulations were carried out using the infrastructure of the shared research facilities of HPC computing resources at Lomonosov Moscow State University. N.G. acknowledges Scientific and Educational Mathematical Center "Sofia Kovalevskaya Northwestern Center for Mathematical Research" for financial support of the analysis of computational performance (agreement № 075-02-2025-1607, 27.02.2025).



## References

1. Abraham, M.J., Murtola, T., Schulz, R., *et al.*: GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX 1, 19–25 (2015). `https://doi.org/10.1016/j.softx.2015.06.001`

2. Alushin, G.M., Lander, G.C., Kellogg, E.H., *et al.*: High-resolution microtubule structures

reveal the structural transitions in $\alpha\beta$-tubulin upon GTP hydrolysis. Cell 157(5), 1117–1129 (2014). https://doi.org/10.1016/j.cell.2014.03.053

3. Bowne-Anderson, H., Zanic, M., Kauer, M., Howard, J.: Microtubule dynamic instability: a new model with coupled GTP hydrolysis and multistep catastrophe. BioEssays 35(5), 452–461 (2013). https://doi.org/10.1002/bies.201200131

4. Brouhard, G.J., Rice, L.M.: Microtubule dynamics: an interplay of biochemistry and mechanics. Nature Reviews Molecular Cell Biology 19(7), 451–463 (2018). https://doi.org/10.1038/s41580-018-0009-y

5. Deng, X., Fink, G., Bharat, T.A., *et al.*: Four-stranded mini microtubules formed by *Prosthecobacter* BtubAB show dynamic instability. Proceedings of the National Academy of Sciences 114(29), E5950–E5958 (2017). https://doi.org/10.1073/pnas.1705062114

6. Desai, A., Mitchison, T.J.: Microtubule polymerization dynamics. Annual Review of Cell and Developmental Biology 13(1), 83–117 (1997). https://doi.org/10.1146/annurev.cellbio.13.1.83

7. Fedorov, V.A., Kholina, E.G., Gudimchuk, N.B., Kovalenko, I.B.: High-Performance Computing of Microtubule Protofilament Dynamics by Means of All-Atom Molecular Modeling. Supercomputing Frontiers and Innovations 10(4), 62–68 (2023). https://doi.org/10.14529/jsfi230406

8. Fedorov, V.A., Kholina, E.G., Kovalenko, I.B., Gudimchuk, N.B.: Performance analysis of different computational architectures: Molecular dynamics in application to protein assemblies, illustrated by microtubule and electron transfer proteins. Supercomputing Frontiers and Innovations 5(4), 111–114 (2018). https://doi.org/10.14529/jsfi180414

9. Fedorov, V.A., Kholina, E.G., Kovalenko, I.B., *et al.*: Update on performance analysis of different computational architectures: Molecular dynamics in application to protein-protein interactions. Supercomputing Frontiers and Innovations 7(4), 62–67 (2020). https://doi.org/10.14529/jsfi200405

10. Fedorov, V.A., Orekhov, P.S., Kholina, E.G., *et al.*: Mechanical properties of tubulin intra- and inter-dimer interfaces and their implications for microtubule dynamic instability. PLoS Computational Biology 15(8), e1007327 (2019). https://doi.org/10.1371/journal.pcbi.1007327

11. Feenstra, K.A., Hess, B., Berendsen, H.J.: Improving efficiency of large time-scale molecular dynamics simulations of hydrogen-rich systems. Journal of Computational Chemistry 20(8), 786–798 (1999). https://doi.org/10.1002/(SICI)1096-987X(199906)20:8<786::AID-JCC5>3.0.CO;2-B

12. Gebremichael, Y., Chu, J.W., Voth, G.A.: Intrinsic bending and structural rearrangement of tubulin dimer: molecular dynamics simulations and coarse-grained analysis. Biophysical Journal 95(5), 2487–2499 (2008). https://doi.org/10.1529/biophysj.108.129072

13. Grafmüller, A., Noya, E.G., Voth, G.A.: Nucleotide-dependent lateral and longitudinal interactions in microtubules. Journal of Molecular Biology 425(12), 2232–2246 (2013). https://doi.org/10.1016/j.jmb.2013.03.029

14. Grafmüller, A., Voth, G.A.: Intrinsic bending of microtubule protofilaments. Structure 19(3), 409–417 (2011). https://doi.org/10.1016/j.str.2010.12.020

15. Gudimchuk, N.B., Alexandrova, V.V.: Measuring and modeling forces generated by microtubules. Biophysical Reviews 15(5), 1095–1110 (2023). https://doi.org/10.1007/s12551-023-01161-7

16. Gudimchuk, N.B., Alexandrova, V.V., Ulyanov, E.V., *et al.*: Modeling microtubule dynamics on Lomonosov-2 supercomputer of Moscow State University: from atomistic to cellular scale simulations. Supercomputing Frontiers and Innovations 11(3), 107–116 (2024). https://doi.org/10.14529/jsfi240307

17. Gudimchuk, N.B., McIntosh, J.R.: Regulation of microtubule dynamics, mechanics and function through the growing tip. Nature Reviews Molecular Cell Biology 22(12), 777–795 (2021). https://doi.org/10.1038/s41580-021-00399-x

18. Gudimchuk, N.B., Ulyanov, E.V., O'Toole, E., *et al.*: Mechanisms of microtubule dynamics and force generation examined with computational modeling and electron cryotomography. Nature Communications 11(1), 3765 (2020). https://doi.org/10.1038/s41467-020-17553-2

19. Hemmat, M., Castle, B.T., Odde, D.J.: Microtubule dynamics: moving toward a multi-scale approach. Current Opinion in Cell Biology 50, 8–13 (2018). https://doi.org/10.1016/j.ceb.2017.12.013

20. Igaev, M., Grubmüller, H.: Microtubule assembly governed by tubulin allosteric gain in flexibility and lattice induced fit. eLife 7, e34353 (2018). https://doi.org/10.7554/eLife.34353

21. Igaev, M., Grubmüller, H.: Bending-torsional elasticity and energetics of the plus-end microtubule tip. Proceedings of the National Academy of Sciences 119(12), e2115516119 (2022). https://doi.org/10.1073/pnas.2115516119

22. Jenkins, C., Samudrala, R., Anderson, I., *et al.*: Genes for the cytoskeletal protein tubulin in the bacterial genus *Prosthecobacter*. Proceedings of the National Academy of Sciences 99(26), 17049–17054 (2002). https://doi.org/10.1073/pnas.012516899

23. Kalutskii, M., Grubmüller, H., Volkov, V.A., Igaev, M.: Microtubule dynamics are defined by conformations and stability of clustered protofilaments. bioRxiv (2024). https://doi.org/10.1101/2024.11.04.621893

24. Kliuchnikov, E., Klyshko, E., Kelly, M.S., *et al.*: Microtubule assembly and disassembly dynamics model: Exploring dynamic instability and identifying features of microtubules growth, catastrophe, shortening, and rescue. Computational and Structural Biotechnology Journal 20, 953–974 (2022). https://doi.org/10.1016/j.csbj.2022.01.028

25. MacKerell, A.D.J., Bashford, D., Bellott, M., *et al.*: All-atom empirical potential for molecular modeling and dynamics studies of proteins. The Journal of Physical Chemistry B 102(18), 3586–3616 (1998). https://doi.org/10.1021/jp973084f

26. MacKerell Jr, A.D., Feig, M., Brooks, C.L.: Improved treatment of the protein backbone in empirical force fields. Journal of the American Chemical Society 126(3), 698–699 (2004). https://doi.org/10.1021/ja036959e

27. Manka, S.W., Moores, C.A.: The role of tubulin–tubulin lattice contacts in the mechanism of microtubule dynamic instability. Nature Structural & Molecular Biology 25(7), 607–615 (2018). https://doi.org/10.1038/s41594-018-0087-8

28. Michaels, T.C., Feng, S., Liang, H., Mahadevan, L.: Mechanics and kinetics of dynamic instability. eLife 9, e54077 (2020). https://doi.org/10.7554/eLife.54077

29. Mitchison, T., Kirschner, M.: Dynamic instability of microtubule growth. Nature 312(5991), 237–242 (1984). https://doi.org/https://doi.org/10.1038/312237a0

30. Mitra, A., Sept, D.: Localization of the antimitotic peptide and depsipeptide binding site on $\beta$-tubulin. Biochemistry 43(44), 13955–13962 (2004). https://doi.org/10.1021/bi0487387

31. Mitra, A., Sept, D.: Taxol allosterically alters the dynamics of the tubulin dimer and increases the flexibility of microtubules. Biophysical Journal 95(7), 3252–3258 (2008). https://doi.org/10.1529/biophysj.108.133884

32. Morozenko, A., Stuchebrukhov, A.: Dowser++, a new method of hydrating protein structures. Proteins: Structure, Function, and Bioinformatics 84(10), 1347–1357 (2016). https://doi.org/10.1002/prot.25081

33. Natarajan, K., Mohan, J., Senapati, S.: Relating nucleotide-dependent conformational changes in free tubulin dimers to tubulin assembly. Biopolymers 99(5), 282–291 (2013). https://doi.org/10.1002/bip.22153

34. Nogales, E., Wolf, S.G., Downing, K.H.: Erratum: Structure of the $\alpha\beta$ tubulin dimer by electron crystallography. Nature 393(6681), 191–191 (1998). https://doi.org/10.1038/34465

35. Olsson, M.H., Søndergaard, C.R., Rostkowski, M., Jensen, J.H.: PROPKA3: Consistent treatment of internal and surface residues in empirical p$K_a$ predictions. Journal of Chemical Theory and Computation 7(2), 525–537 (2011). https://doi.org/10.1021/ct100578z

36. Parrinello, M., Rahman, A.: Polymorphic transitions in single crystals: A new molecular dynamics method. Journal of Applied Physics 52(12), 7182–7190 (1981). https://doi.org/10.1063/1.328693

37. Pavelites, J.J., Gao, J., Bash, P.A., Mackerell Jr, A.D.: A molecular mechanics force field for $NAD^+$ NADH, and the pyrophosphate groups of nucleotides. Journal of Computational Chemistry 18(2), 221–239 (1997). https://doi.org/10.1002/(SICI)1096-987X(19970130)18:2<221::AID-JCC7>3.0.CO;2-X

38. Peng, L.X., Hsu, M.T., Bonomi, M., *et al.*: The free energy profile of tubulin straight-bent conformational changes, with implications for microtubule assembly and drug discovery. PLoS Computational Biology 10(2), e1003464 (2014). https://doi.org/10.1371/journal.pcbi.1003464

39. Sahoo, A., Hanson, S.M.: Martini without the twist: Unveiling a mechanically correct microtubule through bottom-up coarse-graining in Martini 3. bioRxiv (2024). https://doi.org/10.1101/2024.05.29.596440

40. Stewman, S.F., Tsui, K.K., Ma, A.: Dynamic instability from non-equilibrium structural transitions on the energy landscape of microtubule. Cell Systems 11(6), 608–624 (2020). https://doi.org/10.1016/j.cels.2020.09.008

41. Tong, D., Voth, G.A.: Microtubule simulations provide insight into the molecular mechanism underlying dynamic instability. Biophysical Journal 118(12), 2938–2951 (2020). https://doi.org/10.1016/j.bpj.2020.04.028

42. Ulyanov, E.V., Vinogradov, D.S., McIntosh, J.R., Gudimchuk, N.B.: Brownian dynamics simulation of protofilament relaxation during rapid freezing. PLoS ONE 16(2), e0247022 (2021). https://doi.org/10.1371/journal.pone.0247022

43. Voevodin, V.V., Antonov, A.S., Nikitenko, D.A., *et al.*: Supercomputer Lomonosov-2: large scale, deep monitoring and fine analytics for the user community. Supercomputing Frontiers and Innovations 6(2), 4–11 (2019). https://doi.org/10.14529/jsfi190201

44. Walker, R.A., O'Brien, E.T., Pryer, N.K., *et al.*: Dynamic instability of individual microtubules analyzed by video light microscopy: rate constants and transition frequencies. Journal of Cell Biology 107(4), 1437–1448 (1988). https://doi.org/10.1083/jcb.107.4.1437

45. Webb, B., Sali, A.: Comparative protein structure modeling using MODELLER. Current Protocols in Bioinformatics 47(1), 5.6.1–5.6.32 (2014). https://doi.org/10.1002/0471250953.bi0506s47

46. Wells, D.B., Aksimentiev, A.: Mechanical properties of a complete microtubule revealed through molecular dynamics simulation. Biophysical Journal 99(2), 629–637 (2010). https://doi.org/10.1016/j.bpj.2010.04.038

47. Wu, J., Dasetty, S., Beckett, D., *et al.*: Data-driven equation-free dynamics applied to many-protein complexes: The microtubule tip relaxation. Biophysical Journal (2025). https://doi.org/10.1016/j.bpj.2025.01.009

48. Yutin, N., Koonin, E.V.: Archaeal origin of tubulin. Biology Direct 7(1), 10 (2012). https://doi.org/10.1186/1745-6150-7-10

49. Zakharov, P., Gudimchuk, N., Voevodin, V., *et al.*: Molecular and mechanical causes of microtubule catastrophe and aging. Biophysical Journal 109(12), 2574–2591 (2015). https://doi.org/10.1016/j.bpj.2015.10.048

50. Zhang, R., Alushin, G.M., Brown, A., Nogales, E.: Mechanistic origin of microtubule dynamic instability and its modulation by EB proteins. Cell 162(4), 849–859 (2015). https://doi.org/10.1016/j.cell.2015.07.012

51. Zhang, R., LaFrance, B., Nogales, E.: Separating the effects of nucleotide and eb binding on microtubule structure. Proceedings of the National Academy of Sciences 115(27), E6191–E6200 (2018). https://doi.org/10.1073/pnas.1802637115