Molecular mechanism of microtubule dynamic instability [1]

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Summary

Microtubules are a major component of the cell cytoskeleton. Utilizing the tremendous computational power of Blue Waters, we are investigating how nucleotide and anticancer drugs binding regulate the microtubule dynamics. The atomic model of microtubule was obtained using our Molecular Dynamics Flexible Fitting method, which combines information from X-ray crystallography and electron microscopy through computational approach. The simulation of microtubule in its GTP binding state has been carried out for over 1 μs. An intrinsic curvature of the protofilament was observed at the growing end of the microtubule. We will investigate the microtubule dynamics in its GDP binding state in the next allocation cycle. The results will allow us to compare the effect of different nucleotide binding (GTP *vs.* GDP) on the microtubule dynamic instability. The system of the microtubule, containing ∼10 million atoms in total, represents one of the largest biological protein complexes studied through molecular dynamics (MD) simulation.

Introduction

Microtubules are a major component of the cell cytoskeleton, important for maintaining cell structure, intracellular transport, and cell division [1, 2]. A microtubule consists of two subunits, α- and β-tubulin (Fig.1A), which assemble into a tube structure in an alternating fashion [3–5], as shown in Fig.1B. Dynamic instability is a property of microtubules which allows them to switch between phases of assembly and disassembly [5–7], thereby playing a key role in the process of cell division [2]. Microtubules have long been considered an ideal drug target to stop the unwanted cell division in the case of cancer [8, 9].

In the current project, we seek to explore through MD simulations how nucleotide binding and subsequent hydrolysis lead to microtubule assembly and disassembly, respectively [5, 7], and how the anticancer drug, Taxol, stabilizes microtubules against hydrolysis-induced depolymerization [9]. The results will not only shed light on the molecular mechanism of the stabilizing effect of anticancer drug Taxol, but also help in the battle against the ever-increasing drug resistance in chemotherapy, leading to better designs of next generation of anticancer agents [10].

MD simulations of microtubules have been limited to individual or several subunits so far due to restrictions on available structural data and computational resources [11–15]. Our collaborator, Prof. Eva Nogales (Univ of California, Berkeley and Howard Hughes Medical Institute), has recently obtained high-resolution (4.7 Å) cryo-EM structures of a microtubule [16]. Based on the cryo-EM data and crystal structure of α- and β-tubulin, we derived reliable atomic models for microtubules in different nucleotide binding states (Fig.1B). The system of the microtubule, including surrounding water and ions, contains ∼10 million atoms in total,

[1] **This report includes confidential information that not yet published.**

representing one the largest MD simulation to date. Blue Waters is one of the few computing systems in the world that can accommodate such large-scale MD simulations and can carry out the calculation efficiently. The proposed questions can only be tackled by combining the computational power of Blue Waters and the extreme scalability of the simulation software NAMD [17], developed in our lab. Our benchmark has shown that MD simulations of microtubule systems can scale up to 1000 nodes efficiently using NAMD [17].

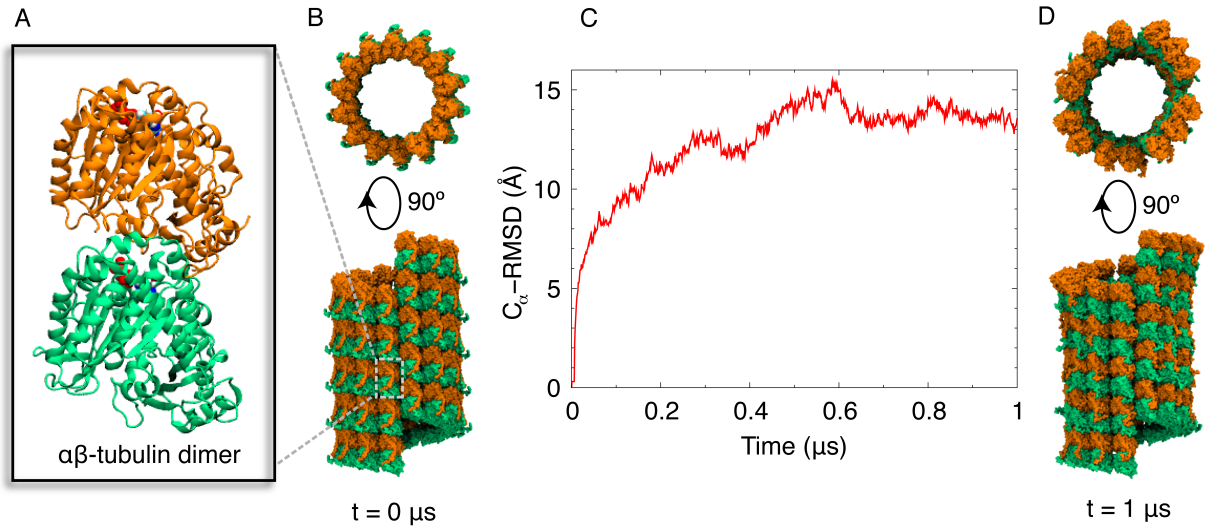

Figure 1: (A) Crystal structure of α(green)β(orange)-tubulin dimer, which is the building block of microtubule. (B) Atomic model (both top view and side view) of a segment of microtubule, containing 70 $\alpha\beta$-tubulin dimers. The model has ∼10 million atoms including proteins, water molecules, and ions. The model was obtain using our Molecular Dynamic Flexiable Fitting (MDFF) method. (C) Time evolution of root mean square deviation (C_α-RMSD) of microtubule compared to initial MDFF-derived structure in the GTP binding state. (D) A snapshot of microtubule (both top view and side view) after 1-μs equilibration in molecular dynamics simulation using the Blue Waters Professorship allocation.

Results

With the help of the Blue Waters professorship allocation, we have built atomic models for microtubules in different nucleotide (GTP and GDP) binding states. The modeling was achieved by using our Molecular Dynamics Flexible Fitting (MDFF) method [18,19], which integrates the crystal structures of α- and β-tubulin and cryo-EM data of assembled microtubules through MD simulation. Starting from the MDFF derived atomic model of microutuble in GTP binding state (Fig.1B), we carried out equilibrium MD simulation for 1 μs. The root-mean-square deviation along the trajectory compared to the initial structure is shown in Fig.1C. The equilibrated structure at the end of the simulation is shown in Fig.1D. The microtubule remains intact as a large protein complex in the simulation, inferring a reliable initial model obtained from MDFF. A certain degree of curvature is seen at the growing end of the microtubule, a observation consistent with previous electron tomography data. We are currently in the process of analyzing the simulation to pin down the atomic interactions that drive the curvature formation. A manuscript reporting the modeling and the simulation results is in preparation.

Future work

Although we have built two atomic models for microtubules (GTP and GDP binding states, respectively), only the GTP binding model was simulated on Blue Waters using the available computational resource last year. With the new allocation this year, we will extend the dynamic simulation of microtubule in GDP binding state. It is predicted that GTP hydrolysis will destabilize the microtubule, therefore, we expect to see the curvature formation at the growing end of microtubule to a larger degree in GDP binding state than in GTP binding state [5, 7, 16]. The microtubule in GDP binding state will be simulated for 1 μs, as same as in GTP binding state we already obtained. The comparison of the two trajectories will reveal the molecular mechanism of microutuble dynamic instability and how nucleotide binding controls the balance between microutuble assembly and disassembly. We are also actively developing force field parameters in MD simulation for the anticancer drug, Taxol. The goal is to eventually incorporate Taxol in our simulation to understand its stabilizing effect on microtubules [10].

Allocation Request

The proposed microtubule system has been benchmarked on Blue Waters. The simulation scales up to 1024 nodes efficiently using GPU (XK node), as shown in Fig.2, giving a performance of 23 ns/day (or 0.00093 ns/node-hour). A 1-μs simulation of microtubule in GDP state will require $\frac{1{,}000\,ns}{23\,ns/day} \times$ **24 hours** $\times$ **1,024 nodes** $=$ **1,068,521 node-hours.** Our system is ready to be simulated on Blue Waters; it is likely that we will use all of the allocated resource in the first two quarters (Q1: 50%, Q2: 50%, Q3: 0%, Q4: 0%). Once the Taxol parameters are ready in the next few months, we will request a supplemental allocation for the third and fourth quarters.

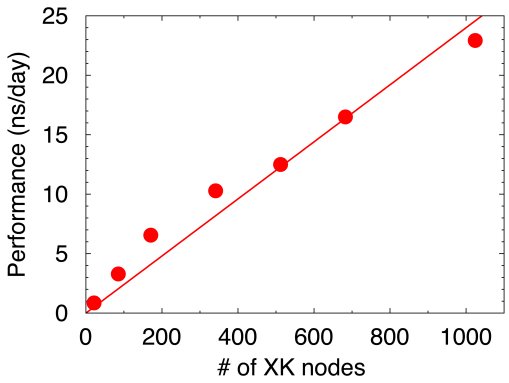

Figure 2: Simulation benchmark of the proposed microtubule system using XK nodes

Snapshots of the MD simulation need to be frequently saved in order to capture rare events in a dynamic process. We are currently saving the frame every 0.01 ns along the trajectory, generating data at a rate of 10.3 GB/ns. We are also requesting additional **1,000 ns** $\times$ **10.3 GB/ns** $=$ **10 TB** of disk space at NCSA Nearline storage.

The proposed work will employ the programs NAMD, developed and distributed free of charge for nearly two decades by the PI's NIH Center. NAMD (http://www.ks.uiuc.edu/Research/namd) is a parallel molecular dynamics code designed for high performance simulation of large biomolecular systems [17]. The libraries and software required by NAMD include CUDA, FFTW, Tcl, and Charm++. All proposed simulations have been tested and shown to run within the available memory on either XE6 or XK7 nodes.

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