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Abstract OR-5: Prediction of Relaxed Conformations for Tubulin Dimers: from Cryo- Electron Microscopy to Molecular Dynamics

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Background: Tubulin microtubules are essential cytoskeletal filaments, with diverse and critical functions at all stages of the cell cycle. Tubulin dimers, which serve as main building blocks for microtubules, can self-assemble and disassemble, depending on the phosphorylation state of associated nucleotide. Tubulins bound to guanosine triphosphate (GTP) make stable microtubule lattice, but when the GTP molecules lose their γ-phosphates, microtubules become prone to depolymerization. The relationship between tubulin conformation and GTP hydrolysis is still poorly understood. Recent progress in cryo-electron microscopy and related data analysis methods have brought new critical insights into the nucleotide-dependent structure of microtubule lattice (Alushin et al., Cell, 2014; Manka et al., Nat. Struct. Mol. Biol., 2018; Zhang et al., PNAS, 2018). However, supplementary methods are needed to bridge static cryo-electron microscopy-based structures with microtubule dynamic instability. Here we use all-atom molecular dynamics (MD) simulations to examine relaxed conformations and mechanical properties of tubulin dimers, extracted from cryo- electron microscopy-based tubulin structures of different generations. This analysis enables us to draw conclusions about the mechanism for microtubule dynamic instability.

Methods: Molecular models of straight GDP-bound tubulin and Mg-GTP-bound tubulin structures (dimers and tetramers) were based on 3j6f, 6dpv and 3j6e, 6dpu PDB structures of tubulin lattice (Alushin et al., Cell, 2014; Zhang et al., PNAS, 2018). 3j6e and 6dpu structures contained non-hydrolyzable GTP analog (GMPCPP) in the exchangeable site of β-tubulin. GMPCPP was converted into GTP by replacing the carbon atom between α- and β-phosphate with an oxygen atom, and the new bond lengths and angle relaxed to their equilibrium values during minimization. Simulations were performed using the GROMACS 5 software package, which allowed parallel computing on hybrid architecture, with the

CHARMM27 force field (Abraham et al., SoftwareX, 2015). Parameters for MD-preparation and simulations were set according to our previous work (Fedorov et al., Supercomput. Front. Innov., 2018).

Results: Tubulins were extracted from cryo electron microscopy-based structures of microtubule lattice and subjected to one-microsecond-long MD simulations. Overall, we carried out 12 MD simulation runs, covering total simulation time of six 6 microseconds for each nucleotide. Relaxed conformations of tubulins were twisted and non-radially bent at the intradimer interfaces, regardless of the bound nucleotide type. Both low (Alushin et al., Cell, 2014) and high (Manka et al., Nat. Struct. Mol. Biol., 2018) resolution cryo-electron microscopy-based model structures converged to similar shapes, which closely resembled structures, obtained with X-ray crystallography method (Ravelli et al., Nature, 2004).

Conclusion: A combination of cryo-electron microscopy and molecular dynamics data suggest that the GTP hydrolysis does not change the conformation of the intradimer tubulin interface, implying that microtubule dynamic instability may rather be explained by the modulation of lateral bonds or interfaces between adjacent tubulin dimers.

Key Words: microtubules • tubulin • cryo-electron microscopy • conformation

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