Mathematical Biology and Bioinformatics
2023. V. 18. № 1. P. 105–112. doi: 10.17537/2023.18.105

========== INFORMATION AND COMPUTER ==========
========== TECHNOLOGIES IN BIOLOGY AND MEDICINE ==========

Design of a Molecular Dynamics Model for High-Performance Computing of Conformational Changes in Microtubule Protofilaments Associated with the Anticancer Drug Taxol

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Abstract. Molecular dynamics models of tubulin tetramers in complex with the anticancer drug taxol were created based on high-resolution spatial structures (PDB ID 3J6G). We tested performance of various computational architectures in molecular dynamics calculations of tubulin tetramers. We revealed the optimal computer architecture and carried out three 1 μs molecular dynamic trajectories of taxol-bound tubulin tetramer. We analyzed the conformational flexibility of tubulin tetramers in a complex with taxol, calculated the Euler angles for intra- and inter-dimer interfaces of the protofilament, as well as the degree and direction of protofilament bending. The stiffness of protofilaments was studied using the energy equipartition theorem. The results allowed us to conclude that taxol binding reduces stiffness at both the inter- and intra-dimer interfaces, which may facilitate the process of microtubule assembly.

Key words: *molecular dynamics, microtubules, tubulin, taxol, Euler angles, performance of molecular-dynamics calculations.*

INTRODUCTION

Nowadays molecular dynamics (MD) is a powerful technique to study biomolecular systems and reveal molecular mechanisms underlying their functioning. However, biomolecular systems may comprise over millions of atoms, so MD modeling of such complex systems is still a computationally challenging task. One of the most popular software for MD simulations is GROMACS [1]. To achieve the best performance of MD calculations for taxol-bound tubulin tetramer, it is important to test various configurations of computer architecture combining different variants of the central processing unit (CPU) and graphics processing unit (GPU). Here we extend our previous MD performance benchmark results [2, 3] by testing computer systems based on the latest generation CPUs and GPUs.

The main components of the cytoskeleton are microtubules, which are involved in various processes occurring in the cell, including cell division and intracellular transport. For these processes the key phenomenon is dynamic instability, i.e. spontaneous switching between microtubule assembly and disassembly processes. Dynamic instability results from hydrolysis of GTP in the β-subunits of tubulin dimers in the microtubule body. One of the long-used and effective anti-cancer chemotherapy strategies is the suppression of microtubule dynamics

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during cell division using small molecule inhibitors of tubulin [4]. Even relatively small deviations of microtubule dynamics from the norm as a result of exposure to tubulin inhibitors lead to the impossibility of proper interaction between the division spindle and chromosomes, and stops cell division. This fact has been successfully used in chemotherapy to fight cancer cell division [5]. In particular, dynamic instability is inhibited in the presence of small molecule taxol used in cancer therapy. The simplest system for studying the taxol effect on the tubulin protofilaments is the taxol-bound tetramer. To shed light on molecular mechanisms of dynamic instability and taxol action, in this paper we study in detail the interaction between taxol and tubulin tetramers.

MATERIAL AND METHODS

The procedure of tubulin model creation was described in details in [6]. Briefly, all-atom molecular dynamics (MD) model of taxol-bound tubulin tetramer was based on the cryo-EM structure of GDP-microtubule lattice (PDB ID: 3J6G) [7]. The unresolved amino acids were constructed using Modeller software [8]. To choose the protonation state of ionizable amino acids we used Propka software [9]. For solvation of internal protein cavities, the Dowser program [10] was used. All calculations were carried out with TIP3P water and with 100 mM ionic strength using Gromacs 2022.4 software [1] in CHARMM27 force field [11, 12].

After the steepest descent energy minimization of the assembled system, we performed two-step equilibration calculations at constant pressure and temperature using the Berendsen barostat (time constant 4.0 fs, compressibility 4.5×10^{-5} bar^{-1}) and the Berendsen thermostat: 1) simulation with constrained positions of all heavy protein atoms during 1 ns; 2) simulation with constrained positions of protein backbone atoms during 5 ns. The production MD simulations were performed using the V-rescale thermostat and Parrinello-Rahman barostat with 4 fs time step. The duration of each production calculation was 1 μs.

Analyses of bend and twist angles at tubulin interfaces were carried out with Pymol software in combination with home-made python scripts. Detailed procedure is given in [6]. In short, first a Cartesian coordinate system *x*, *y*, *z* was associated with a fragment of microtubule wall structure (PDB ID 3J6G), and then we aligned the lower reference tubulin monomer onto the microtubule wall fragment. To determine the orientation of the upper tubulin subunit in the examined pair relative to the reference subunit, another microtubule wall fragment was aligned onto the upper tubulin subunit, producing three more orientation vectors: *X*, *Y*, *Z*. The bend and twist angles were then calculated by aligning these two coordinate systems.

RESULTS AND DISCUSSION

Performance tests of molecular dynamics calculation

We tested the performance of MD calculations of tubulin tetramers in explicit solvent using different computational architectures. The MD model of tubulin tetramer in complex with the anticancer drug taxol was created based on high-resolution cryo-EM spatial structures obtained in the laboratory of Eva Nogales in 2014 (PDB ID 3J6G with taxol), that is why we tested the performance of computers released in 2014 and later. For performance tests we chose 5 different personal computer architectures and supercomputer Lomonosov-2 of Lomonosov Moscow State University (assembled in 2014) with two 14-core CPU (Xeon(R) E5-2697 v3) and one GPU (NVIDIA Tesla K40s).

Table 1 shows MD performance results in dependence of the number of Lomonosov-2 supercomputer nodes. As one can see, the more nodes we used, the greater MD calculations performance was observed. Note that the use of 18 participating nodes does not allow to reach performance saturation.

Table 2 summarizes the performance of MD calculations using one personal computer with different architectures. In this study we tested 15 variants of computer configurations with different combinations of CPU and GPU. Represented data was obtained with use of three CPU and six GPU models released in 2014–2022. Note that the use of GPUs significantly, by several times, improves the performance of MD calculations (see the performance of systems 1 and 4 without a GPU in comparison with other tests). System 15, based on the latest generation of CPUs and GPUs, as expected, provides the best result of MD calculations, 130 ns/day. The interesting point is that the installation of the latest GPU into the system based on previous generation CPU (system 9), as well as the use of previous generation GPU in a modern computer (system 11), equally increase performance of MD calculations up to 74–75 ns/day, which is almost two times worse than the performance of the system 15.

Table 1. Performance of MD calculations of tubulin tetramer in explicit solvent on different numbers of Lomonosov-2 supercomputer nodes

Nodes of Lomonosov-2 (2014)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**16**	**18**
Performance, ns/day	16	27	34	43	49	51	53	62	66	75	84	96

The acceleration of calculations using the modern RTX4080 GPU (2022 release date) compared to the RTX2080ti (2018 release date) depends on the CPU. In fact, using the i9-7900X CPU allows to achieve the 1.27 acceleration factor, while for the modern i9-13900KF CPU, this factor is dramatic 1.76. The conclusion is that to unlock the computing potential of modern GPU accelerators in MD calculations, a powerful modern CPU is needed.

Table 2. Performance of MD calculations of tubulin tetramer in an explicit solvent, obtained on personal computers with different architectures

Computer architecture ID	**CPU**	**GPU**	**CPU/GPU release year**	**Performance, ns/day**
1	i7-5820K	no GPU	2014	5
2	i7-5820K	GTX970	2014	14
3	i7-5820K	RTX2080ti	2014 / 2018	35
4	i9-7900X	no GPU	2017	11
5	i9-7900X	RTX3070	2017 / 2020	54
6	i9-7900X	RTX2080ti	2017 /2018	59
7	i9-7900X	RTX3080	2017 / 2020	63
8	i9-7900X	RTX3090	2017 / 2020	67
9	i9-7900X	RTX4080	2017 / 2022	75
10	i9-13900KF	no GPU	2022	21
11	i9-13900KF	RTX2080ti	2022 / 2018	74
12	i9-13900KF	RTX3070	2022 / 2020	77
13	i9-13900KF	RTX3080	2022 / 2020	100
14	i9-13900KF	RTX3090	2022 / 2020	111
15	i9-13900KF	RTX4080	2022	130

Let us compare Tables 1 and 2. One node of the Lomonosov-2 supercomputer, released in 2014, demonstrates slightly higher performance than a personal computer of the same release year (system 2 in Table 2). However, taking into account that the parallel calculation using 18 supercomputer nodes demonstrates a 6 times higher calculation speed, the use of a supercomputer for MD calculations seems to be preferable compared to one 2014 personal computer. Nevertheless, system 15 with the state-of-the-art CPU and GPU is 1.35 times faster than 18 nodes of supercomputer released in 2014.

Analysis of bending and conformational mobility of tubulin tetramers

We carried out a series of computational experiments to obtain three MD trajectories of tubulin tetramer with taxol with a duration of 1 μs each. The tubulin tetramer consists of two dimers, and thus has three interfaces: two intra-dimer (between the monomers of the dimer), and one inter-dimer (between two dimers). To reveal the taxol effect on each type of interface we calculated the Euler angles for intra- and inter-dimer interfaces of the protofilament, which indicate the degree and direction of protofilament bending in each frame of MD trajectories.

Figure 1 represents changes in the inter-dimer bend and twist angles of α-tubulin relative to β-tubulin. As one can see, the bending angle highly fluctuates not exceeding 20 degrees, with a mean value about 8 degree (Table 3). Figure 2 shows the changes in intra-dimer bend and twist angles of β-tubulin relative to α-tubulin in MD trajectories of tubulin tetramers stabilized by taxol. Note that the twist angle of the intra-dimer interface after 500 ns in all calculations fluctuates much less than that of the inter-dimer interface. The same feature we can see in the Figure 3,A for inter- and Figure 3,B for intra-dimer interfaces.

Projections of the unit OZ-vector of the upper subunit onto the *xy*-plane of the lower subunit of each interface in the tetramer are shown in Figure 3. In other words, these projections show the degree of bending and its direction of the upper tubulin monomer in respect to the lower tubulin monomer in the tubulin tetramer during MD simulation. The areas of colored dots for inter-dimer projections (Fig. 3,A) slightly overlap, at the same time overlapping for the intra-dimer interface is more significant (Fig. 3,B). This means that the intra-dimer interfaces are more stable and similar to each other.

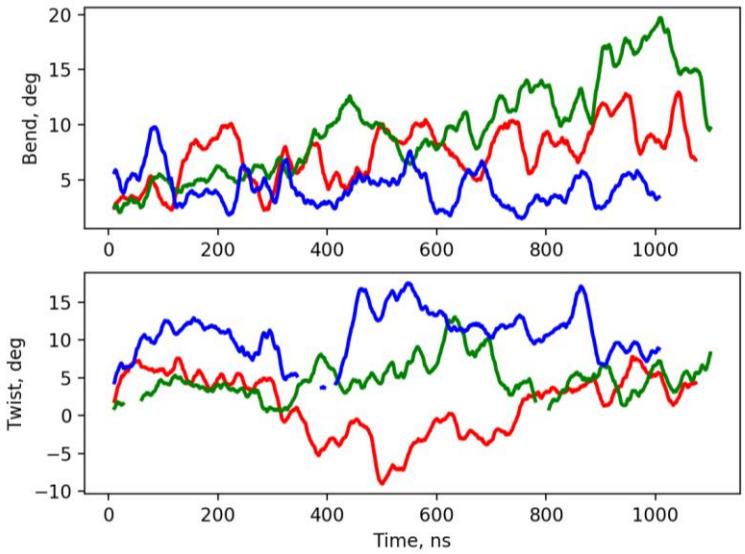

Fig. 1. Changes in the inter-dimer bend and twist angles of α-tubulin relative to β-tubulin in the MD trajectories of free tubulin tetramers with taxol based on the 3J6G structure. The red, green, and blue colored curves represent angles between two dimers in a tubulin tetramer in three different MD trajectories. A 20 ns moving average is shown.

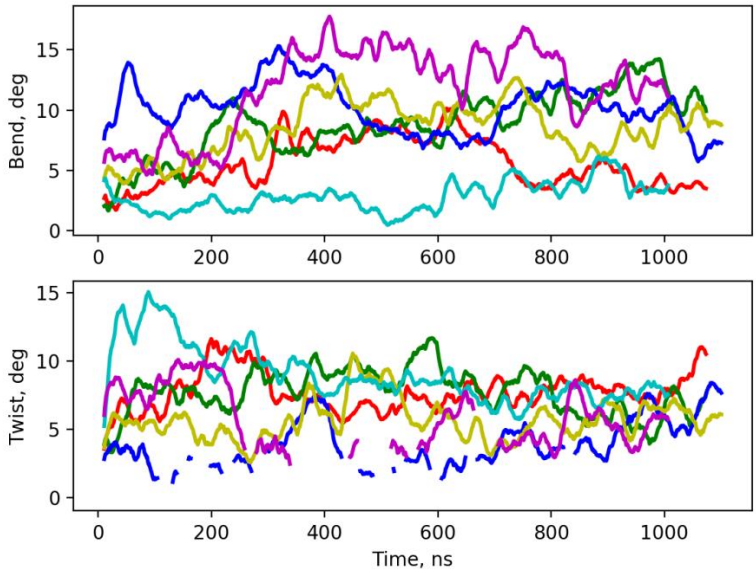

Fig. 2. Changes in intra-dimeric bend and twist angles of β-tubulin relative to α-tubulin in MD trajectories of tubulin with taxol tetramers based on the 3J6G structure. The red, blue and cyan colored curves represent angles in one of the tubulin tetramer dimers in three different MD trajectories. Green, olive and purple colored curves – angles in another tubulin tetramer dimer in the same three trajectories, respectively. A 20 ns moving average is shown.

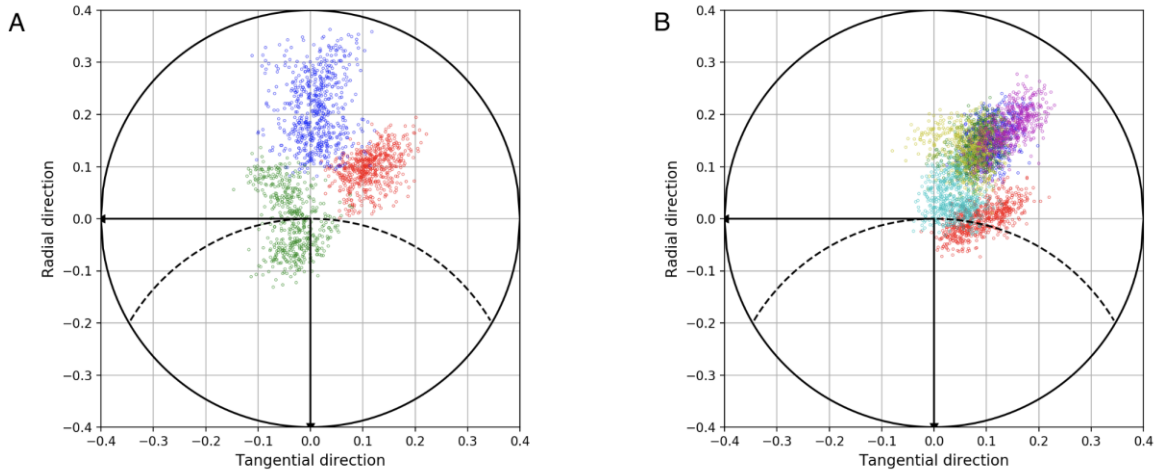

Fig. 3. **A**: Projections of the unit OZ-vector of the α-subunit of the tubulin inter-dimer interface onto the *xy*-plane of the β-tubulin at every nanosecond after the first 500 ns of the simulation. **B**: Projections of the unit OZ-vector of the β-subunit of the tubulin intra-dimer interface onto the *xy*-plane of the α-tubulin at every nanosecond after the first 500 ns of the simulation. The data and color marking of panel A correspond to Figure 1, panel B – to Figure 2. Dashed line schematically shows the circle wall of the microtubule. Horizontal axis is tangential to the microtubule, the vertical axis is directed radially toward the microtubule axis.

Table 3. Parameters of bending of tubulin protofilaments calculated from MD simulations

	3J6F (GDP) [6]		3J6E (GTP) [6]		3J6G (GDP + taxol)	
Interface	**Bend (deg)**	**Twist (deg)**	**Bend (deg)**	**Twist (deg)**	**Bend (deg)**	**Twist (deg)**
inter	5.2 ± 1.7	4.4 ± 3.0	9.1 ± 0.4	7.7 ± 5.6	8.1 ± 3.3	6 ± 4.2
intra	9.4 ± 0.9	5.2 ± 0.9	8.2 ± 0.7	7 ± 1.5	8.5 ± 3.3	6.2 ± 1.5

Based on previously obtained data on the equilibrium structures of free microtubule protofilaments [6] and protofilaments in complexes with taxol, the stiffness of tetramers was studied using the energy equipartition theorem (Table 4). Since taxol is bound to the GDP tetramer, we will first compare its properties with taxol-free GDP-bound tetramer. As we can see from Table 4 taxol makes both inter- and intra-dimer interfaces more flexible compared to taxol-free GDP-bound tubulin. The value of bend stiffness for the inter-dimer interface of the taxol-bound tetramer is slightly lower compared to the intra-dimer interface. The same was also observed for GTP-bound tetramers.

As we previously suggested [6], a decrease in stiffness at the inter-dimer interface affects the microtubule assembly process, thereby facilitating the incorporation of the dimer into the microtubule body. Taxol binding reduces stiffness at both the inter- and intra-dimer interfaces, which may facilitate the process of microtubule assembly.

Table 4. Harmonic stiffness of bend and twist tubulin conformational angles calculated from MD simulations

	3J6F (GDP) [6]		3J6E (GTP) [6]		3J6G (GDP + taxol)	
Interface	**Bend (k_BT/rad^2)**	**Twist (k_BT/rad^2)**	**Bend (k_BT/rad^2)**	**Twist (k_BT/rad^2)**	**Bend (k_BT/rad^2)**	**Twist (k_BT/rad^2)**
inter	1290 ± 510	760 ± 60	350 ± 110	410 ± 70	450 ± 200	260 ± 70
intra	930 ± 120	990 ± 150	1100 ± 120	1160 ± 100	590 ± 140	960 ± 340

ACKNOWLEDGMENTS

Analysis of bending and conformational mobility of tubulin tetramers was supported by the Russian Science Foundation Grant No. 22-74-00119, https://rscf.ru/en/project/22-74-00119/. We acknowledge Scientific and Educational Mathematical Center «Sofia Kovalevskaya Northwestern Center for Mathematical Research» for financial support of performance tests of molecular dynamics calculation (agreement № 075-02-2023-937, 16.02.2023).

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Received 23.03.2023.
Revised 29.03.2023.
Published 02.04.2023.

Создание молекулярно-динамической модели для высокопроизводительных расчетов конформационных изменений протофиламентов микротрубочек, связанных с противоопухолевым препаратом таксол

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Аннотация. Созданы молекулярно-динамические модели тетрамеров тубулина в комплексе с противораковым препаратом таксолом на основании пространственных структур высокого разрешения (PDB ID 3J6G). Было осуществлено тестирование производительности различных вычислительных архитектур в молекулярно-динамических расчетах тетрамеров тубулина. Проведена серия вычислительных экспериментов и получены три молекулярно-динамические траектории длительностью 1 мкс каждая. На основе полученных молекулярно-динамических траекторий проведен анализ конформационной подвижности тетрамеров тубулина в комплексе с таксолом, вычислены углы Эйлера для внутри- и междимерных интерфейсов протофиламента, а также степень и направление изгиба протофиламента.
На основании ранее полученных нами данных о равновесных структурах свободных протофиламентов микротрубочек и протофиламентов в комплексах с таксолом исследована гибкость протофиламентов по теореме равнораспределения энергии, вычислена их жесткость и выявлено влияния таксола на изгибную жесткость протофиламентов микротрубочек.

Ключевые слова: *молекулярная динамика, микротрубочки, тубулин, таксол, углы Эйлера, производительность молекулярно-динамических расчетов.*