

Dynamic Simulation and Mechanical Properties of Microtubules

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ABSTRACT

This work is conducted to obtain mechanical properties of microtubule. For this aim, interaction energy in alpha-beta, beta-alpha, alpha-alpha, and beta-beta dimers was calculated using the molecular dynamic simulation. Force-distance diagrams for these dimers were obtained using the relation between potential energy and force. Afterwards, instead of each tubulin, one sphere with 55 KDa weight connecting to another tubulin with a nonlinear connection such as nonlinear spring could be considered. The mechanical model of microtubule was used to calculate Young's modulus based on finite element method. Obtained Young's modulus has good agreement with previous works. Also, natural frequency of microtubules was calculated based on finite element method.

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1 INTRODUCTION

MICROTUBULES (MTs) are the active filaments, play important roles in cellular functions [1]. MTs are protein filaments of the cytoskeleton [2] which are composed of alpha and beta tubulins assembled into linear protofilaments and form a closed tube [3]. Every tubulin is composed of nearly 4300 atoms and has a mass of 55 kDa [4].

The basic structural and geometrical properties of these tubular shape filaments (the number of protofilaments, the helical pitch, etc.) have been well determined by electron microscopy [5,6].

Different MT configurations exist based on the number of protofilaments. The number of protofilaments in a MT observed in-vivo and in-vitro conditions varies widely from 8 to 19 [7]. However, the majority of these structures have a size of 13 protofilaments.

In microtubules the protofilaments bind together laterally and generate a spiral with a pitch of 2, 3, or 4 monomers' length [7]. The mechanics of MT is complicated due to its helical lattice structure composed tubulin monomers α , β . [8-10].

Finding mechanical properties of microtubules has attracted special attention of scientists in recent decade. Pablo probed the local mechanical properties of microtubules at the nanometers scale by radial indentation with a scanning force microscope (SEM) tip [11]. Janosi and co-workers [12] simulated microtubule as a homogeneous sheet of elastic material with a curved structure.

Modelling protein structures and studying their dynamic behaviors using computational methods and molecular dynamics simulation (MD) have been used in recent years [13]. MD simulations have become an important tool in

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studying the physical basis of the structure and function of biomolecules since the first simulation work was published about three decades ago [14]. SEPT [3] used the package APBS and molecular dynamic to find the lateral and longitudinal bonds along protofilaments. Zeiger also applied molecular mechanics approach to perform tensile tests on individual tubulin monomers and determined values for the axial and circumferential moduli for all currently known complete sequences [15]. Bekir et al. [16] found mechanical responses of isolated microtubules. Kis et al. [17] investigated anisotropic properties of single MTs and they found that the shear modulus is two orders of magnitude lower than elastic modulus. Kasas et al. [18] studied on mechanical properties of MTs with finite element method.

In this research, the bond-related inter-atomic interactions of alpha-beta, beta-alpha, beta-beta and alpha-alpha dimers are replaced by connection and spring elements, in the structural model, where the interaction will be obtained using molecular dynamic and GROMACS [19] package. The structural model was modeled in finite element software and mechanical properties of microtubule were obtained.

2 MATERIALS AND METHODS

Microtubules are biopolymers built from globular proteins (alpha and beta monomers) with $46 \times 65 \times 40 \text{ \AA}$ dimensions [20] bound together to form protofilaments [21], which are aligned in parallel mode to generate the microtubule.

Protein structures are usually achieved by two methods: X-ray crystallography and nuclear magnetic resonance (NMR). From the statistics of the protein data bank (PDB) (<http://www.rcsb.org/pdb/>), approximately 88113 X-ray structures and 10435 NMR structures have been deposited at this date.

Because of experimental limitations, the number of protein-protein complexes solved and deposited in the PDB is rather low compared with the number of freeform structures. So, theoretical methods to study protein complexes have been well developed during the past few years. There are now a number of programs performing “ab initio” protein-protein docking [20, 21]. Most of these programs use the same approach: one protein is fixed in space and the second one is rotated and translated around the first one. For each new configuration, a score is calculated on the basis of various terms such as surface complementarities, electrostatic interactions, van der Waals repulsion, and so forth [22].

In this work, alpha-alpha, alpha-beta, beta-alpha and beta-beta dimers have been studied to find their structures using HADDOCK (High ambiguity driven docking approach). HADDOCK [22] makes use of biochemical and/or biophysical interaction data such as, for example, chemical shift perturbation data obtained from NMR titration experiments or mutagenesis data, to find proteins’ structures. After calculation, the structures are ranked according to their intermolecular energy, that is, sum of electro-static, van der Waals, and AIR (ambiguous interaction restraints) energy terms we found the best solutions generated by HADDOCK, that is, the structures with the lowest intermolecular energy term.

After finding atomic structure of four dimers, molecular dynamic simulation should be used to estimating potential energy between monomers. For this, GROMACS 4.5.3 software [23] with the GROMOS96 43a1 force field was used to perform the simulation. GROMOS96 has been developed for the dynamic modeling of biomolecules using the methods of molecular dynamics, stochastic dynamics, and energy minimization [24].

The potential energy function used in GROMOS96 is as:

$$V(\vec{r}(t); \lambda, \mu) = V^{phys}(\vec{r}(t); \lambda, \mu) + V^{special}(\vec{r}(t)) \quad (1)$$

where, the standard physical atomic interaction is:

$$\begin{aligned} V^{phys}(\vec{r}(t); \lambda, \mu) &= V^{bon}(\vec{r}(t); \lambda, \mu) + V^{nonb}(\vec{r}(t); \lambda, \mu) = V^{bond}(\vec{r}(t); \lambda, \mu) + V^{angle}(\vec{r}(t); \lambda, \mu) + \\ &V^{har}(\vec{r}(t); \lambda) + V^{trig}(\vec{r}(t); \lambda, \mu) + V^{nonb}(\vec{r}(t); \lambda, \mu) = \mu[V^{bond}(\vec{r}(t); \lambda) + V^{angle}(\vec{r}(t); \lambda) + \\ &V^{trig}(\vec{r}(t); \lambda)] + (1 - \mu)[V^{bond}(\vec{r}(t); \lambda) + V^{angle}(\vec{r}(t); \lambda) + V^{trig}(\vec{r}(t); \lambda)] + V^{har}(\vec{r}(t); \lambda) + \\ &V^{nonb}(\vec{r}(t); \lambda, \mu) \end{aligned} \quad (2)$$

and the nonphysical terms is:

$$V^{special}(\vec{r}(t)) = V^{pr}(\vec{r}(t)) + V^{dr}(\vec{r}(t)) + V^{dtr}(\vec{r}(t)) + V^{Jr}(\vec{r}(t)) + V^{le}(\vec{r}(t)) + V^{fdr}(\vec{r}(t)) \tag{3}$$

The superscript 4D over r indicates that the position vector r is to be taken as a 4-dimensional vector. Likewise, the superscript 3D over r indicates that only the 3-dimensional part(x -, y -, z -components) of the position vector r is to be taken. If only the 4th dimensional (w) component of the position vector is meant, this is indicated by the superscript 4thD over r .

The various terms of V are as: λ is the coupling parameter used in free energy perturbation calculations and μ is an additional parameter involving the coupling between the 3D (x,y,z) dimensions and the 4th (w) dimension. Here, the superscripts are:

Bon (bonded interaction), Nonb (non-bonded interaction), Angle (bond angle), Har (harmonic or improper dihedral angles), Trig(trigonometric dihedral angles), Pr (Position Restraining), Dr (Distance Restraining), Dlr (Dihedral Angle Restraining), Jr (J-Coupling Constant Restraining), Le (Local Elevation), and Fdr (Fourth Dimension Restraining) [23].

Table1. provides the parameters for microtubules, including the parameters of the atomistic potential and the volume densities of all type of atoms in tubulin monomer that were used for calculating Lennard-Jones interaction potential(non-bonded interaction) [23].

$$U(r_{ij}) = 4\epsilon_{ij} \left(\frac{\sigma_{ij}^{12}}{r_{ij}^{12}} - \frac{\sigma_{ij}^6}{r_{ij}^6} \right) \tag{4}$$

σ, ϵ are Lennard-Jones parameters and r is interatomic distance.

Table 1
Lennard-Jones Parameters' value for calculation energy [23].

$\epsilon_{ij}(\text{kcal / mol})$	C	H	N	O	P
C	0.1094	0.04144	0.1364	0.1516	0.1654
H	0.04144	0.01570	0.05166	0.05742	0.06265
N	0.1364	0.05166	0.1700	0.1889	0.2062
O	0.1516	0.05742	0.1889	0.2100	0.2291
P	0.1479	0.05604	0.1844	0.2049	0.2236
C	0.3816	0.3308	0.3732	0.3569	0.3908
H	0.3308	0.2800	0.3224	0.3061	0.3400
N	0.3732	0.3224	0.3648	0.3485	0.3824
O	0.3569	0.3061	0.3485	0.3322	0.3661
P	0.4008	0.3500	0.3924	0.3761	0.4100
$\rho(\text{nm}^3)$	C	H	N	O	S
	19.07	37.10	5.026	9.444	0.3961

Cut-offs of 1 nm was used for non-bond interactions (van der Waals and electrostatic). Moreover, the time step was set to 2 fs for all MD simulations.

2.1 Interaction energy between monomers

As the next step, pulling molecular dynamic simulation was performed for 200 ps duration. The distance between two monomers varies around 0.01 nm/ps and the interaction energy was extracted for them. During the pulling step, different configurations of dimer structure were picked up based on the monomer distance and each one was equilibrated for 100 ps to obtain appropriate interaction energy. Using this method, potential energy of $\alpha\beta, \beta\alpha, \alpha\alpha$ and $\beta\beta$ tubulin versus distance (d) was obtained and the data were plotted in Fig. 1 and fitted with a third order polynomial function that approximates potential energy as a function of distance (d).

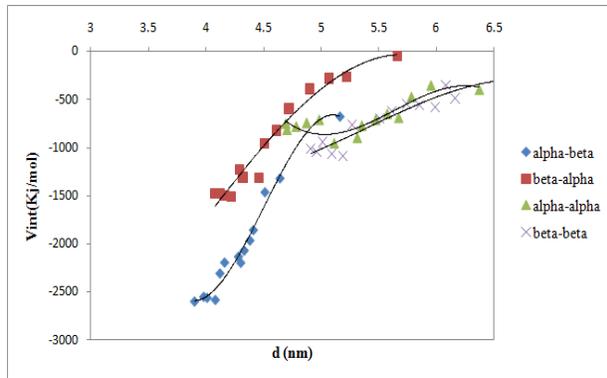


Fig.1
Interaction energy between $\alpha\beta$, $\beta\alpha$, $\alpha\alpha$ and $\beta\beta$ tubulins versus distance (d).

Furthermore, the difference in potential energy between two points (point A and B) is the work required to move against the force[25]:

$$V(B) - V(A) = - \int_A^B F(x).dx \quad (5)$$

Knowing that the change in potential energy is as the change of an object in its location, the nature of the force responsible in this mechanism can be determined as:

$$-\frac{V(B) - V(A)}{X(B) - X(A)} = \text{Force} \quad \text{or} \quad -\frac{\partial V}{\partial x} = F \quad (6)$$

So, derivative of the energy function, the force-displacement can be obtained which plotted in Fig. 2.

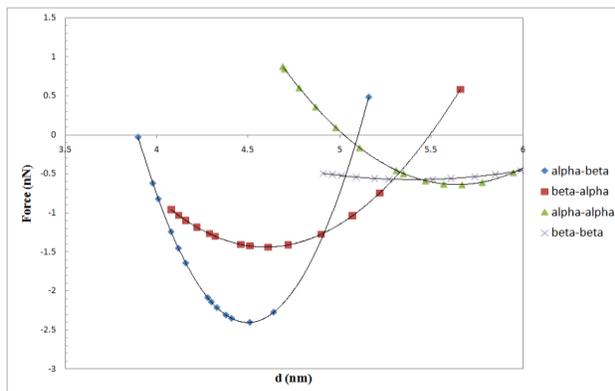


Fig.2
Force-displacement diagram between $\alpha\beta$, $\beta\alpha$, $\alpha\alpha$ and $\beta\beta$ tubulins.

2.2 Structural molecular mechanic

Each alpha or beta tubulin has nearly 4300 atoms. In a microtubule with 13 protofilaments and $0.1 \mu\text{m}$ length, there are more than 100 tubulins. So, molecular dynamic simulation of a microtubule will be a very difficult task.

Then, it would be better to build a structural mechanic model which has rather similar properties with microtubule. The first and most important step for this process is obtaining the interaction force between tubulins, which were calculated earlier. Therefore, instead of the alpha and beta tubulin we can consider two spheres with 55 KDa weight that connect with a nonlinear connection such as nonlinear spring (Fig. 3). The mechanical properties of nonlinear connector are shown in Fig. 2.

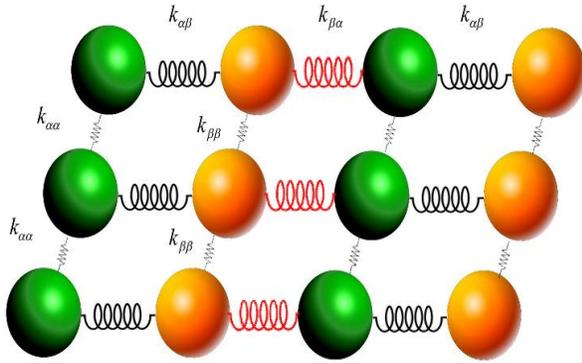


Fig.3
Structural model of tubulins connected by springs.

2.3 Finite element analysis and mechanical properties

Microtubule can be treated as a frame-like structure with their bonds as nonlinear spring members and tubulins as joints. At this level, the interaction between individual tubulins can be described using the force-distance diagrams which show in previous session. For tubulins, solid element and for interaction nonlinear spring element has been used. One side of MT is fixed in all direction and the other side is pulled with force.

A Microtubule with 13 protofilaments and 3 start-helix was considered. For calculating Young modulus, an axial load pulled MT from one side while the other side was constrained.

Using Hook’s law, Young’s modulus of MT can be calculated [26]:

$$\sigma = E \varepsilon \tag{7}$$

Substituting $\sigma = \frac{F}{A}$ and $\varepsilon = \frac{\Delta L}{L}$:

$$\frac{F}{A} = E \frac{\Delta L}{L}, \tag{8}$$

which F is pulled force, A is section area, and L is length of MT.(Fig. 4)

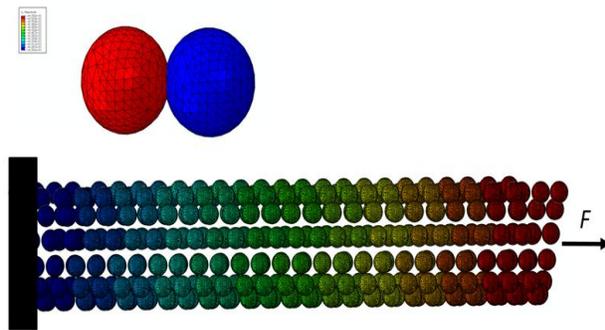


Fig.4
Finite element model of a dimer and a microtubule.

After calculating, Young’s modulus of MT obtained about 1GPa. This value has good agreement with previous work. Frederick and co-workers [27] found Young’s modulus of MT about 1.2 Gpa. They measured it from thermal fluctuation in shape. Brian also [28] found it 1.4 Gpa.

The Differences between this research and other ones comes from the differences between simulation and experimental research.

2.4 Natural frequencies

The natural frequency is the frequency that system oscillates when it is disturbed. In this section natural frequency of microtubule is achieved using finite element method (Fig. 5). Schematic of 6 mode shapes shown in this Fig. 6.

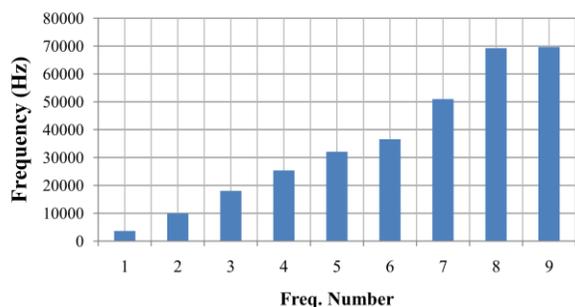


Fig.5
Natural frequency of MTs.

And

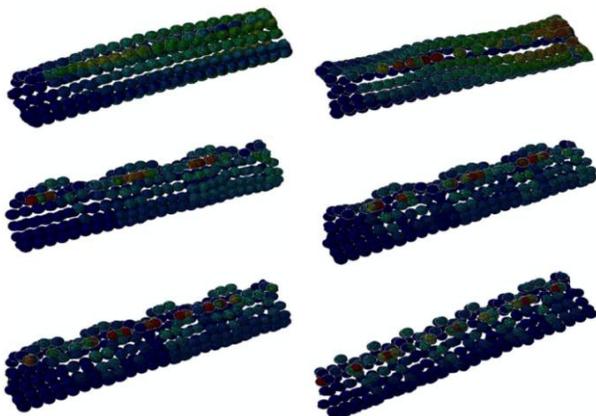


Fig.6
Mode shapes of MTs.

3 CONCLUSIONS

This work is conducted to obtain mechanical properties of microtubule. For this aim, interaction energy in alpha-beta, beta-alpha, alpha-alpha and beta-beta dimers was calculated using the molecular dynamic simulation. Force-distance diagrams for these dimers were obtained using the relation between potential energy and force. After that, instead of the each tubulin we can consider one sphere with 55 KDa weight that connect to another tubulin with a nonlinear connection such as nonlinear spring. The mechanical model of microtubule used to calculate Young's modulus based on finite element method. Young's modulus of MT was obtained about 1Gpa. Obtaining Young's modulus has good agreement with previous researches. Natural frequencies of MTs was also obtained and some mode shapes showed in figures.

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