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Nanobiomechanical Properties of Microtubules

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Abstract
Microtubules, the active filaments with tubular shapes, play important roles in a wide range of cellular functions, including structural supports, mitosis, cytokinesis, and vesicular transport, which are essential for the growth and division of eukaryotic cells. Finding properties of microtubules is one of the main concerns of scientists. This work helps 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 and GROMACS software package. Force-distance diagrams for these dimers were obtained using the relation between potential energy and force. Each dimer has nearly 8500 atoms. There are more than 100 tubulins in a microtubule with 13 protofilaments and 0.1 μm length. 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 to obtain the interaction force between tubulins. Therefore, instead of the each tubulin we can consider one sphere with 55 kDa weights that connect to another tubulin with a nonlinear connection such as nonlinear spring. The mechanical model of microtubule was used to calculate Young's modulus based on finite element method. The Young's modulus has good agreement with previous works.
Keywords*: Finite element, Microtubules, Nanoproperties, Young's modulus.*

1. INRODUCTION

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 of α and β tubulins [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 nanometres scale by radial indentation with a scanning force microscope (SEM) tip [11]. Janosi and co-workers (2002) simulated microtubule as a

homogeneous sheet of elastic material with a curved structure [12].

Modelling protein structures and studying their dynamic behaviours using computational methods and molecular dynamics simulation (MD) have been used in recent years [13]. MD simulations have become an important tool in studying the physical basis of the structure and function of biomolecules since the first simulation work was published about 1985 [14]. Sept (2003) used the package APBS and molecular dynamic to find the lateral and longitudinal bonds along protofilaments [3]. Also, Zeiger (2008) 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].

In this work, the bond-related inter-atomic interactions of alpha-beta, beta-alpha, beta-beta and alpha-alpha dimers have been replaced by connection and spring elements, in the structural model, where the interaction has been obtained using molecular dynamic and GROMACS package [16]. The structural model was modelled using finite element software and mechanical properties of microtubule were obtained. This method helps to find mechanical properties of bio-nanomaterials with more accuracy and fast calculation. Also, it's the first time that a modeling of microtubule has been done from molecular dynamic to mechanical structure simulation.

2. MATERIALS AND METHODS

Microtubules are biopolymers built from globular proteins (alpha and beta monomers) with 46 ×65 ×40 A˚ dimensions [16] bound together to form protofilaments, which are aligned in parallel mode to generate the microtubule [17].

Protein structures are usually achieved by two methods: X-ray crystallography and nuclear magnetic resonance (NMR) [13]. From the statistics of the protein data bank (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 [16, 17]. 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 [18].
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) [18]. HADDOCK makes use of biochemical and/or biophysical interaction data such as 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 [18]. This work found the best solutions generated by HADDOCK, that is, the structures with the lowest intermolecular energy term (Figure 1).

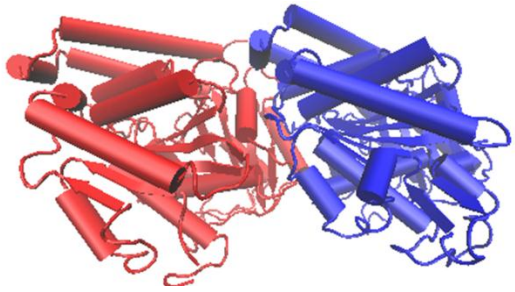

Figure1. *Alpha-beta dimer.*

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 with the GROMOS96 43a1 force field was used to perform the simulation [19]. GROMOS96 has been developed for the dynamic modelling of biomolecules using the methods of molecular dynamics, stochastic dynamics, and energy minimization [20]. Cut-offs of 1 nm was used for non-bond interactions (van der Waals and electrostatic) [19]. Moreover, the time step was set to 2 fs for all MD simulations [19]. The structure was first energy minimized and then placed inside the rectangular box with $18\times9\times8\ nm$ size. The rest of the box was filled with water molecules to explicitly model water in the system. To balance the negative charge of the dimer, Na^{+} ions were added to each solution. The entire system was then energy minimized again and after that heated up to 300K by coupling it to an external heat bath for 50 ps. Figure 2 shows energy minimization for alpha-beta and alpha -alpha dimers.

To simulate constant temperature, Berenson algorithm with external heat bath was used [21]. The effect of this algorithm is that a deviation of the system temperature from T_0 slowly corrected according to:

$$\frac{dT}{dt}=\frac{T_0-T}{\tau} \qquad (1)$$

It means that temperature deviation decays exponentially with a time constant τ. The reference temperature in this step was set to $T_0=300K$.

Similar to the temperature coupling, the system can also be coupled to a "pressure bath" in NPT ensemble. Berendsen algorithm applied in this work rescales the coordinates and box vectors every step. As shown in Figure 3, the temperature and pressure slightly fluctuate around a constant value, while the reference pressure value is 1 bar.

2.1. Interaction energy between monomers:

As the next step, pulling molecular dynamic simulation was performed for 200 ps duration.

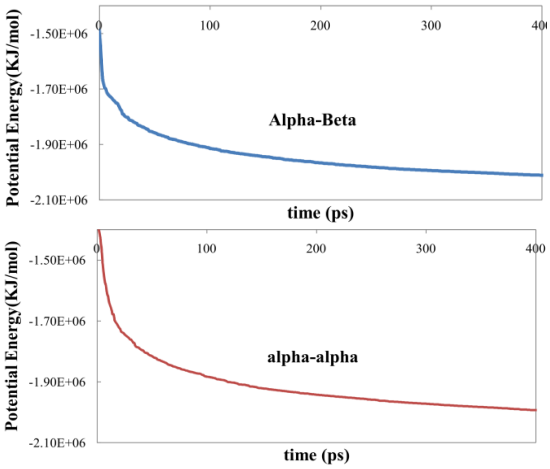

Figure2. *Energy minimization for alpha-beta and alpha -alpha dimers.*

The distance between two monomers changed 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 Figure 4 and fitted with a third order polynomial function that approximates potential energy as a function of distance (d).

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

$$V(B)-V(A)=-\int_{A}^{B}F(x).dx \qquad (2)$$

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

$$-\frac{V(B)-V(A)}{X(B)-X(A)}=Force\ \ or\ \ -\frac{\partial V}{\partial x}=F \qquad (3)$$

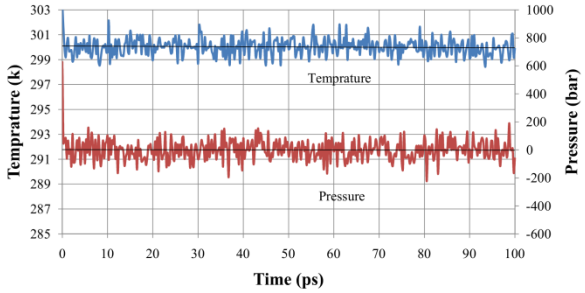

Figure3. *The temperature and pressure slightly fluctuate around a constant value*

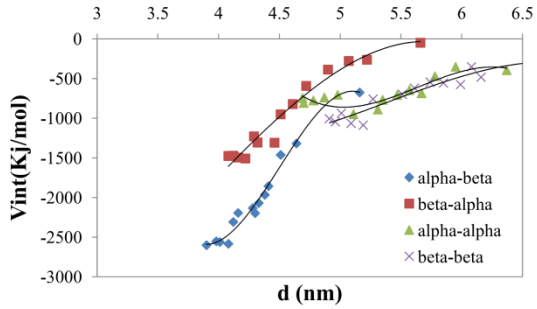

Figure4. *Interaction energy between $\alpha\beta$, $\beta\alpha$, $\alpha\alpha$ and $\beta\beta$ tubulins versus distance (d)*

So, using derivation of the energy function, the force-displacement can be obtained and plotted in Figure 5.

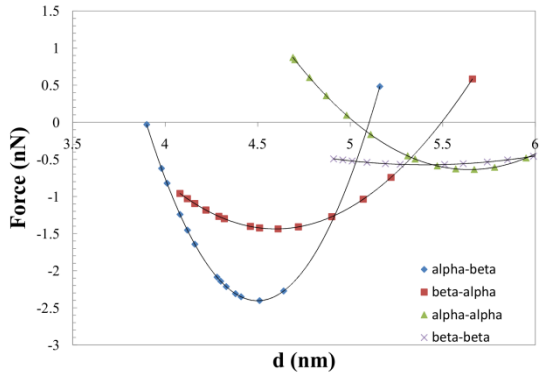

Figure5. *Force-displacement diagram between $\alpha\beta$, $\beta\alpha$, $\alpha\alpha$ and $\beta\beta$ tubulins.*

2.2. Structural molecular mechanic:

Each alpha or beta tubulin has nearly 4300 atoms. In a microtubule with 13 protofilaments and 0.1 µm 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 to obtain 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 (Figure 6). The mechanical properties of nonlinear connector are shown in Figure 5.

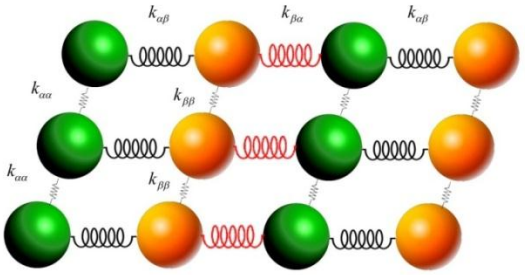

Figure6. *Structural model of tubulins connected by springs*

2.3. Finite element analysis and mechanical properties:

Microtubule can be treated as a frame-like structure (Figure 7) 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 (as Figure 5.). A microtubule with 13 protofilaments and 3 start- helix was considered. All necessary dimensions for simulation can be found in Figure 8.

For calculating Young modulus, an axial load pulled MT from one side while the other side was constrained (Figure 9).

Using Hook's law, Young's modulus of MT can be calculated as:

$$\sigma = E\,\varepsilon \tag{4}$$

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

$$\frac{F}{A} = E\frac{\Delta L}{L} \qquad (5)$$

Which F is pulled force, A is section area, and L is length of MT.

After calculating, Young's modulus of MT has been obtained about 1GPa. The value has good agreement with previous works. Frederick and co-workers (1993) found Young's modulus of MT about 1.2 GPa [22]. They measured it from thermal fluctuation in shape. Also, Brian (1995) found it 1.4 GPa [23].

The Differences between this work and other works comes from the differences between simulation and experimental works.

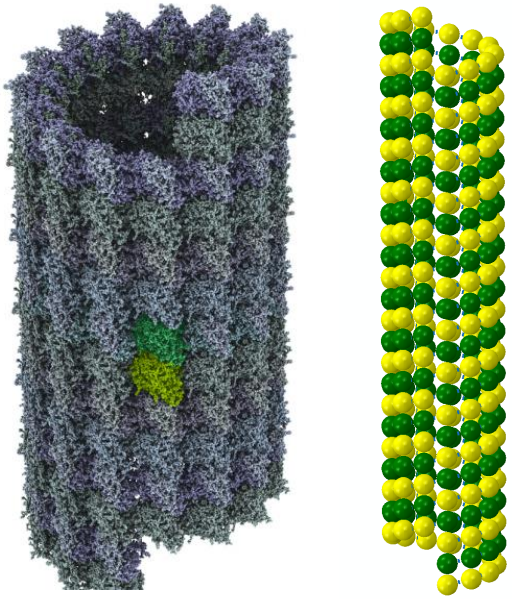

Figure7*. Microtubule and its structural mechanic model*

3. CONCLUSION

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 each tubulin we can consider one sphere with 55 KDa weights that connect to another tubulin with a nonlinear connection such as nonlinear spring.

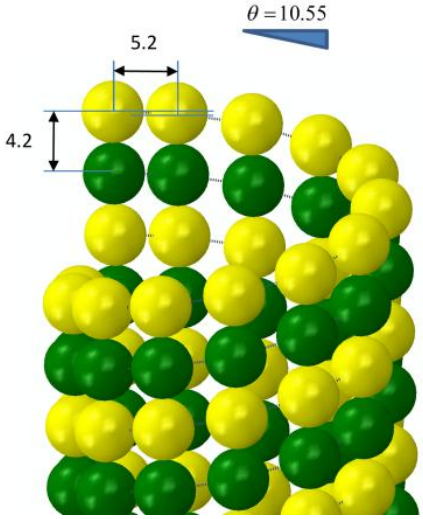

Figure8*. Simulation dimension for modeling MT*

The mechanical model of microtubule has been used to calculate Young's modulus based on finite element method. Young's modulus of MT was obtained about 1GPa. The Young's modulus has good agreement with previous works.

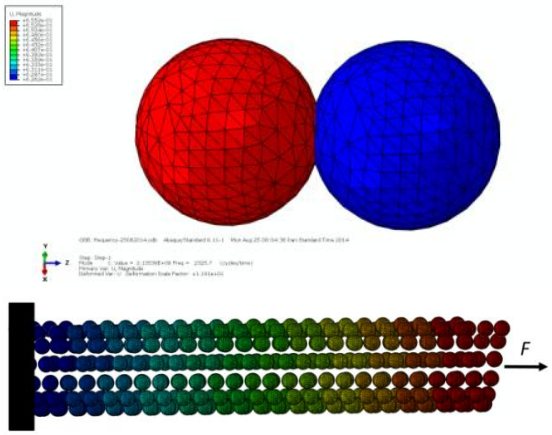

Figure9*. Finite element model of a dimer and a microtubule*

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