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INTERACTION ENERGY AND FORCE BETWEEN ALPHA-ALPHA DIMER

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ABSTRACT

Microtubules (MTs), 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 is conducted to obtain interaction properties of alpha-alpha tubulin in microtubule. For this aim, interaction energy in alpha-alpha dimer was calculated using the molecular dynamic simulation and GROMACS software package. Computational methods, especially protein structure prediction and molecular dynamics (MD) simulations, have been widely used for modeling protein structures and studying their dynamic behaviors. Force-distance diagram for alpha-beta was obtained using the relation between potential energy and force. Each alpha-alpha dimer has nearly 8500 atoms. In a microtubule with 13 protofilaments and 0.1 µm length, there are almost 300 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. Therefore, instead of the alpha-alpha dimer we can consider two spheres with 55 KDa weight that connect with a nonlinear connection such as nonlinear spring. This mechanical model can be used to simulate microtubules using meso and macro-scale simulations such as finite element method for obtaining their mechanical and dynamic properties.

Keywords: Interaction Energy; Alpha tubulin; Microtubule; Nonlinear spring

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INTRODUCTION

Microtubules (MTs), 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 **[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 (the number of protofilaments, the helical pitch, etc.) have been well determined by electron microscopy **[5, 6]**.

Depending on the number of protofilaments, different MT configurations exist. The number of protofilaments in a MT observed in-vivo and in-vitro conditions vary 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 inequivalent tubulin monomers α, β, and staggered arrangement of protofilaments **[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 nanometer scale by radial indentation with a scanning force microscope (SEM) tip **[11]**. Janosi and coworkers **[12]** simulated microtubule as a homogeneous sheet of elastic material with a curved structure.

Computational methods, especially protein structure prediction and molecular dynamics (MD) simulations, have been widely used for modeling protein structures and studying their dynamic behaviors **[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 three decades ago **[14]**. SEPT **[3]** used the package APBS and molecular dynamic to find the lateral and longitudinal bonds along protofilaments. Also, Zeiger 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 dimer are replaced by connection and spring elements, in the structural model, where the interaction will

be obtained using molecular dynamic and GROMACS **[16]** package.

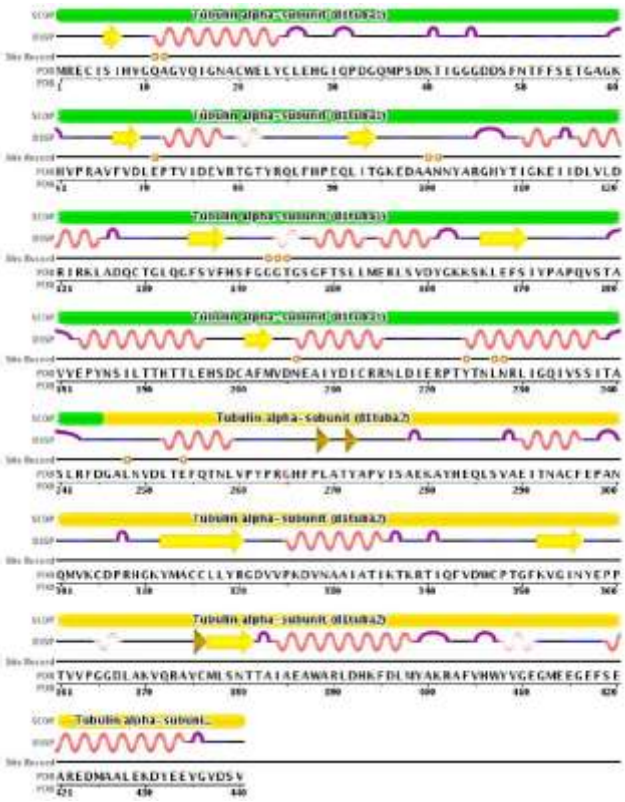

Figure 1: Sequence display of alpha tubulin

MATERIALS AND METHODS

Docking Alpha-alpha tubulins

For a better understanding of the biological function of a protein, knowledge of its three-dimensional structure is crucial. Solving protein structures is mainly achieved by two different 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 solved and 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. Next to these experimental approaches, theoretical methods to study protein complexes at a structural level based on docking are now emerging that have been well developed during the past few years. There are now a number of programs performing "ab initio" protein-protein docking **[17, 18]**. 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 **[19]**.

In this work, the high ambiguity driven docking approach (HADDOCK) **[19]** that 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, has been used. 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.

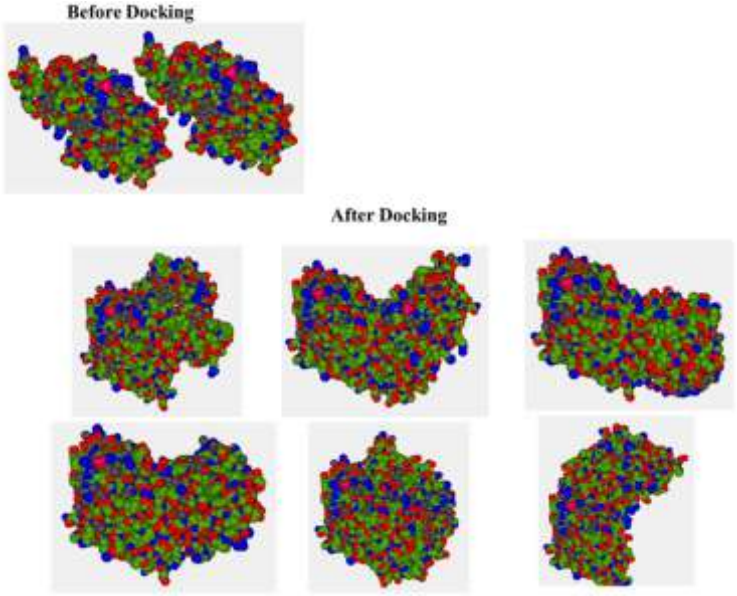

Figure 2: Alpha-alpha tubulins before docking and 6 sample of docked tubulins

Microtubules are biopolymers built from globular proteins (alpha-beta dimer) with 46 ×65 ×80 A˚ dimensions **[20]** bound together in a head-to-tail state to form protofilaments **[21]**, which are, in turn, aligned in parallel mode to generate the microtubule. The atomic structure of alpha-alpha tubulin applied in the present work, generated by HADDOCK program.

GROMACS 4.5.3 software **[22]** 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 **[23]**. 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:

$$V^{phys}(\vec{r}(t);\lambda,\mu)=V^{bon}(\vec{r}(t);\lambda,\mu)+V^{nonb}(\vec{r}(t);\lambda,\mu)=V^{bond}(\vec{r}(t);\lambda,\mu)+V^{angle}(\vec{r}(t);\lambda,\mu)+$$
$$V^{har}(\vec{r}(t);\lambda)+V^{trig}(\vec{r}(t);\lambda,\mu)+V^{nonb}(\vec{r}(t);\lambda,\mu)=\mu[V^{bond}(\vec{r}^{\,4D}(t);\lambda)+V^{angle}(\vec{r}^{\,4D}(t);\lambda)+$$
$$V^{trig}(\vec{r}^{\,4D}(t);\lambda)]+(1-\mu)[V^{bond}(\vec{r}^{\,3D}(t);\lambda)+V^{angle}(\vec{r}^{\,3D}(t);\lambda)+V^{trig}(\vec{r}^{\,3D}(t);\lambda)]+V^{har}(\vec{r}^{\,3D}(t);\lambda)+$$
$$V^{nonb}(\vec{r}(t);\lambda,\mu) \qquad (2)$$

and the nonphysical terms is:

$$V^{special}(\vec{r}(t))=V^{pr}(\vec{r}^{\,3D}(t))+V^{dr}(\vec{r}(t))+V^{dlr}(\vec{r}^{\,3D}(t))+V^{Jr}(\vec{r}^{\,3D}(t))+$$
$$V^{le}(\vec{r}^{\,3D}(t))+V^{fdr}(\vec{r}^{\,4thD}(t)) \qquad (3)$$

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) **[20]**.

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.

The structure was first energy minimized and then placed inside the rectangular box of size 18·9·8 nm. 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, 31 Na+ ions were added to the 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.

RESULT AND DISCUSSION

The applied structure for $\alpha\alpha$ tubulin simulation has a potential energy of $V = -1.88*10^6\ {kJ}/{mol}$ after energy minimization in the environment (after 100 ps), while the original one had potential energy of $V = -1.4*10^6\ {kJ}/{mol}$, which indicates a significant reduction in potential energy. **Figure 3** shows the change in potential energy over the time steps.

Next, two ensembles including NVT and NPT were used to equilibrate the system. In NVT or canonical ensemble, the number of particles (N), system volume (V), and temperature (T) are kept as either constant or conserved. Potential, kinetic, and total energy diagrams are shown in **Figure 4**.

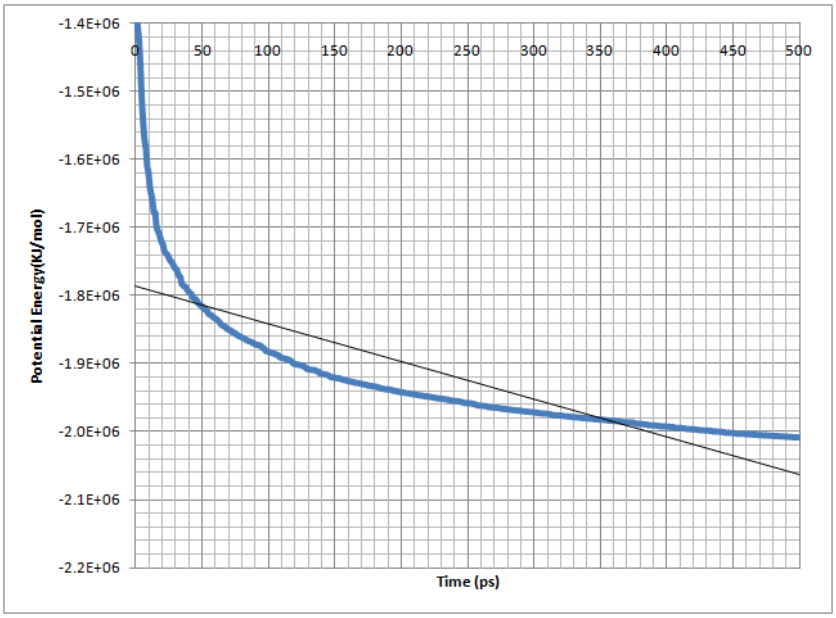

Figure 3: Potential energy minimization in alpha-alpha dimer

To simulate constant temperature, Berendsen algorithm [24] with external heat bath was used. The effect of this algorithm is that a deviation of the system temperature from T_0 which is slowly corrected according to:

$$\frac{dT}{dt} = \frac{T_0 - T}{\tau} \qquad (4)$$

which means that temperature deviation decays exponentially with a time constant τ.

The reference temperature in this step was set to $T_0 = 300\,K$. Fig. 4 shows the temperature changes diagram over the time periods. Here, the varying pressure results in a constant volume. **Figure 4** represents the pressure change over the time periods.

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 5**, the temperature and pressure slightly fluctuate around a constant value, while the reference pressure value is 1 bar.

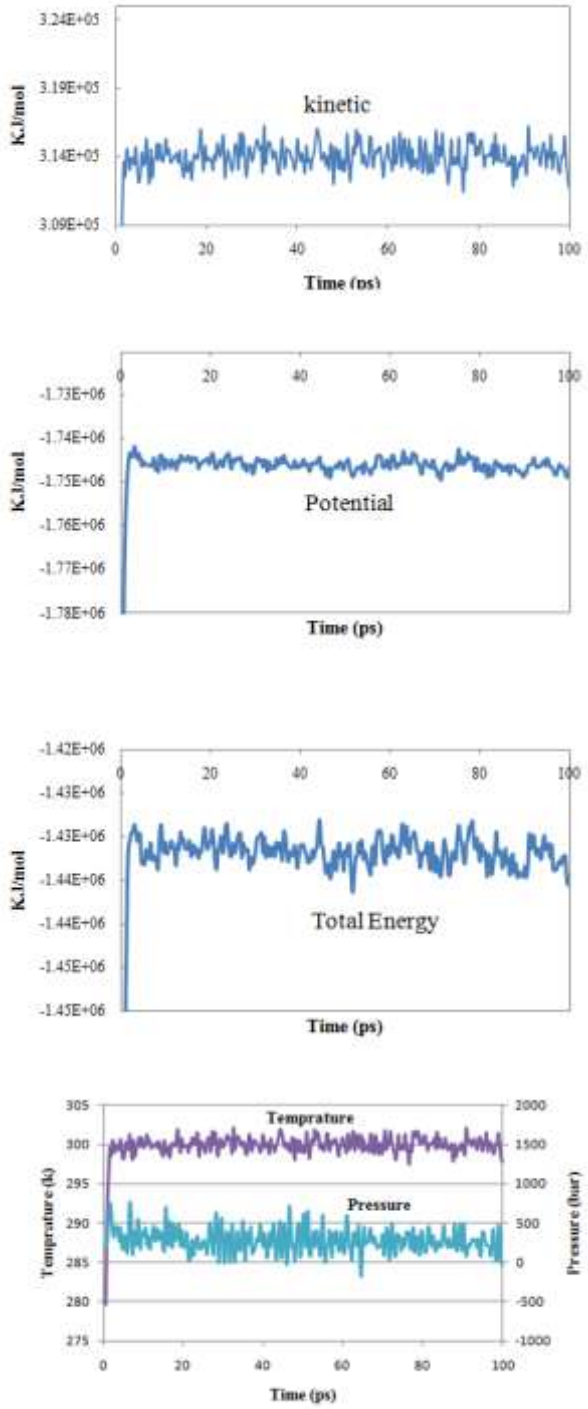

Figure 4: Potential, kinetic and total energy and temperature and Pressure diagram in NVT ensemble.

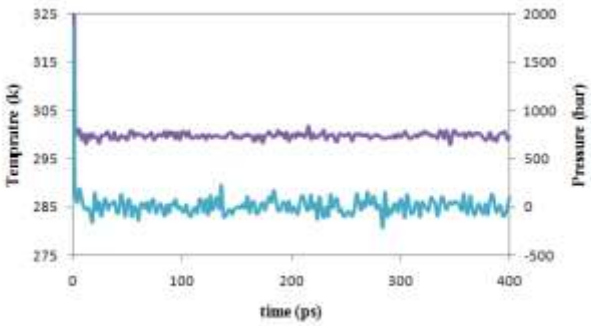

Figure 5: Temperature and pressure fluctuation in NPT ensemble

Furthermore, the energy values are shown in **Figure 6**.

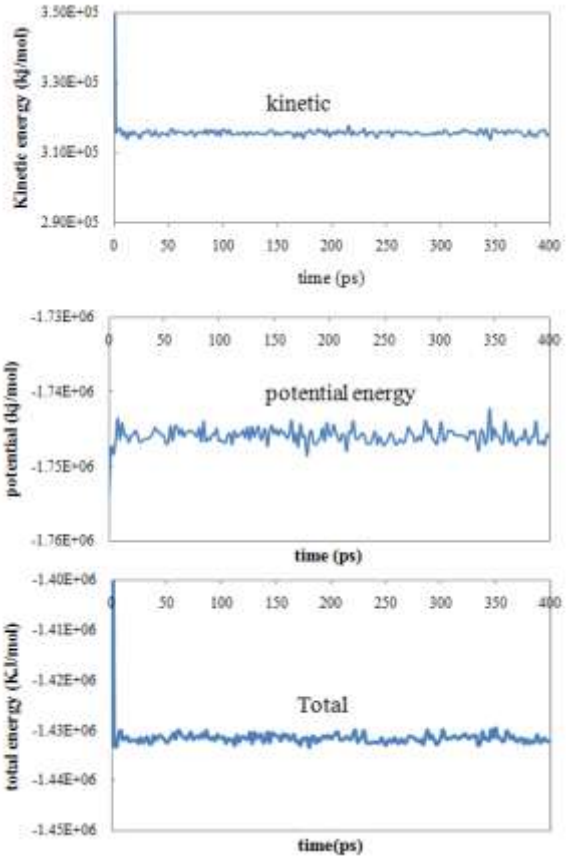

Figure 6: Diagram of energies in NPT ensemble

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, 14 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 $\alpha\alpha$ tubulins versus distance (d) was obtained and the data were plotted in **Figure 7** and fitted with a third order polynomial function that approximates potential energy as a function of distance (d).

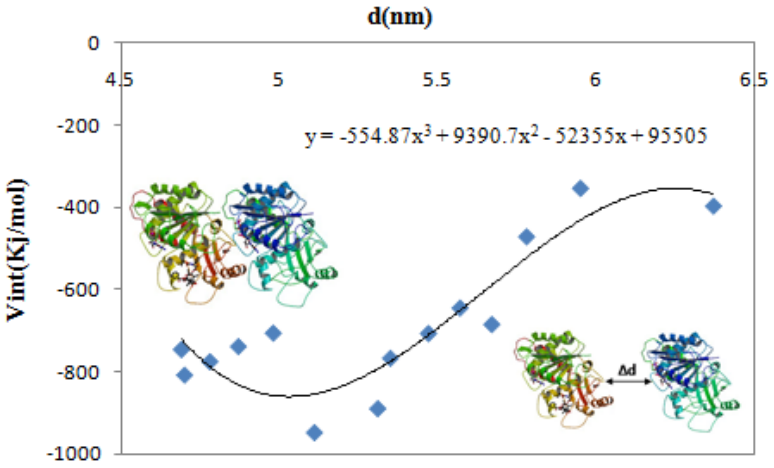

Figure 7: Interaction energy versus distance in alpha-alpha dimer

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

$$V(B)-V(A)=-\int_{A}^{B}F(x).dx \qquad (5)$$

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:

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

So, derivative of the energy function, the force-displacement can be obtained which plotted in **Figure 8**.

Structural Molecular mechanic:

Each alpha-alpha dimer has nearly 8500 atoms. In a microtubule with 13 protofilaments and 0.1 µm length, there are almost 300 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-beta dimer we can consider two spheres with 55 KDa weight that connect with a nonlinear connection such as nonlinear spring (**Figure 9**). The mechanical properties of nonlinear connector are shown in **Figure 8**.

However, alpha-alpha, beta-beta, and beta – alpha interactions are required to be obtained for microtubule simulation. These interactions will be calculated in future works.

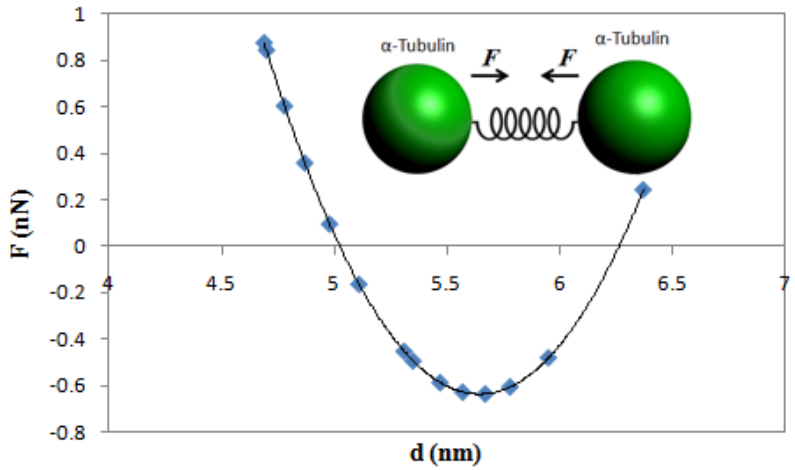

Figure 8: Force versus distance in alpha-alpha dimer

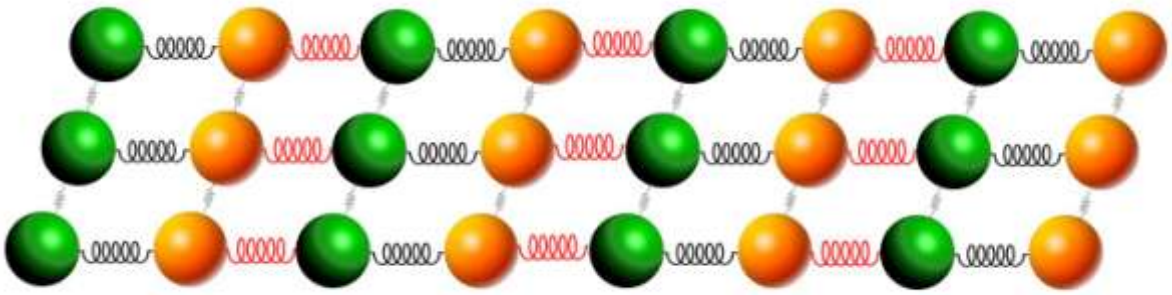

Figure 9: Structural mechanic microtubule with nonlinear spring

CONCLUSION

This work tried to obtain interaction properties of alpha-beta tubulin in microtubule. In this regard, interaction energy between alpha-beta dimer was calculated and interaction energy VS distance diagram was plotted using the molecular dynamic simulation and GROMACS package. Simulation conditions and NPT and NVT ensembles were fully discussed in this work. . Force-distance diagram between alpha-beta was plotted according to relationship between potential energy and force. By using this diagram, we

can consider a mechanical model with two solid spheres which connect with a nonlinear connector instead of the complicated alpha-beta dimer structure with 8500 atoms. This mechanical model can be applied for simulating the microtubules using mesoscale simulation like finite element method for obtaining their mechanical and dynamic properties.

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