Effect of amino acid mutations on intra-dimer tubulin–tubulin binding strength of microtubules†

Ning Liu, Ramana Pidaparti* and Xianqiao Wang*

Energetic interactions inside αβ-tubulin dimers of a microtubule (MT) with atomic resolutions are of importance in determining the mechanical properties and structural stability of the MT as well as designing self-assembled functional structures from it. Here, we carry out several comprehensive atomistic simulations to investigate the interaction properties within αβ-tubulin dimers and effect of residue mutations on the intra-dimer tubulin–tubulin (IDTT) binding strength. Results indicate that the force-displacement responses of the dimer could be roughly divided into three stages involving increasing, decreasing, and fluctuating forces. Energetic analysis shows that electrostatic interactions dominate the IDTT binding strength. Further per-residue energetic analysis shows that the major part of the interface interaction energy (approximately 72% for α-tubulin and 62% for β-tubulin) comes from amino acid residues with net charges, namely arginine (ARG), lysine (LYS), glutamic acid (GLU), aspartic acid (ASP). Residue mutations are completed for ARG105 on α-tubulin and ASP251 on β-tubulin to study the effect of mutations on the IDTT binding strength. Results indicate that stiffness, rupture force, and interface interaction energy of αβ-tubulin dimer can be improved by up to 28%, 13% and 28%, respectively. Overall, our results provide a thorough atomistic understanding of the IDTT binding strength within αβ-tubulin heterodimers and help pave the way for eventually designing and controlling the self-assembled functional structures from MTs.

1. Introduction

Microtubules (MTs), an essential structural element of cells, are long filamentous hollow cylinders whose surfaces form lattice structures of αβ-tubulin dimers. Due to the high rigidity,1 static microtubules play a very important role in maintaining cell morphology, adjusting placement or transport of subcellular structures, and so on.2 Moreover, their ability to undergo infrequent transitions between polymerization (growth) and de-polymerization (catastrophe or shortening) enables them to play a vital role in cell mitosis, i.e., formation/positioning of the mitotic spindle and search/capture of mitotic chromosomes.3 Therefore, understanding the fundamental mechanisms of different MT functions has drawn a lot of interest experimentally and theoretically. However, a lack of quantitative understanding in the energetic mechanics of αβ-tubulin dimers greatly hinders advances in designing and controlling self-assembled functional structures from MTs.

A variety of experimental techniques have been used to investigate the mechanical properties of MTs, for example, optical tweezers,4 hydrodynamic flow,5 thermally induced vibrations,6 buckling in vesicles,7 atomic force microscopy,8 and indirect tensile tests with a stretch chamber system.9 Despite these remarkable experimental achievements, the underlying mechanism of the mechanics of MTs remains largely unexplored. Because the multi-protofilament structure makes it difficult to establish a direct relation between molecular tubulin
characteristics and experimentally observed MT properties. Therefore, theoretical methods are emerging to establish such a link and unveil the fundamental mechanisms of its mechanical properties. For instance, several coarse-grained models, in which each tubulin is modeled as a point mass, were developed to mimic the self-assembly kinetics of MTs and thus explain the underlying mechanisms. These coarse-grained models have recapitulated some mechanical properties and experimental dependencies of MTs, such as sheet-to-tube transitions during MT growth, conformational change during de-polymerization, pauses in microtubule assembly, etc. A self-organized polymer (SOP) model was adopted to simulate the mechanical behavior of MTs on an experimental time scale using experimental force loads. A key benefit of this method was that it can provide plenty of molecular details about how the αβ-tubulin dimers deform under external loads. In addition, continuum models have also been used to study the static and dynamic properties of MTs. Among these theoretical developments, molecular dynamics simulations has served as a basis for the upper level models, i.e., coarse-grained models and continuum models. Because it offers a straightforward approach to obtain detailed molecular level information, including mechanical interactions between and within αβ-tubulin heterodimers, per-residue energetic analysis, strength of interfacial hydrogen bonds within αβ-tubulin heterodimers, etc. This information helps clarify the underlying mechanism of MT functions, which cannot be obtained through experiments.

Sophisticated transitions between “growth” and “shortening” in MTs play a very important role in multiple cell functions. Minor changes, such as mutations inside αβ-tubulin dimers, can induce a series of cell disorders. For instance, the β-I tubulin R307H single nucleotide polymorphism (SNP) alters the microtubule dynamics and affects the severity of a hereditary thrombocytopenia. The mutations of β-tubulin isotype III (TUBB3) inside human cells can result in a lot of human nervous system disorders, including ocular motility disorder, intellectual and behavioral impairments, facial paralysis, and later-onset axonal sensorimotor polyneuropathy. These aforementioned disorders result from the disturbance of self-assembly dynamics of MTs, which may be partially attributed to the changes in binding strength between or within αβ-tubulin dimers. Therefore, understanding the effect of mutations on the binding strength between tubulins inside MTs is vital to establish the link between molecular level details and the experimentally observed phenomena. In this paper, molecular dynamics simulations are employed to conduct the energetic analysis on the intra-dimer tubulin-tubulin (IDTT) interface of αβ-tubulin dimers and study the effect of mutations on binding strength between α- and β-tubulins within a dimer.

2. Computational models and methods

As shown in Fig. 1(a), a microtubule is a tubular structure inside cells while tubulin dimers, composed of α- and β-tubulins are building blocks of microtubules. The three-dimensional structure of the tubulin dimer was obtained from the pdb file indexed “1tub” from the RCSB protein data bank. It is worth noting that the description of topologies of guanosine diphosphate (GDP) and guanosine triphosphate (GTP) was extracted from the existing topologies of adenosine diphosphate (ADP) and adenosine triphosphate (ATP).

To describe the interactions among atoms in αβ-tubulin dimer, CHARMM36 force field was adopted. The form of the potential energy function is given as follows:

$$E = E_{\text{bond}} + E_{\text{angle}} + E_{\text{dihedrals}} + E_{\text{impropers}} + E_{\text{L-J}} + E_{\text{vdW}} + E_{\text{elec}}$$  \hspace{1cm} (1)$$

where the first five terms account for short-range bonding interactions while the last two terms are associated with long-range van der Waals and electrostatic interactions. The cutoff distance for van der Waals and electrostatic interactions was set to be 12 Å. All molecular dynamics simulations were performed using the Nanoscale Molecular Dynamics (NAMD)28 package. The environmental temperature was set at 310 K and the time step was set at 2 femtosecond. First, the tubulin dimer was immersed into a spherical water droplet with a 6.4 nm radius. The tubulin dimer was placed into water, also called salvation, in order to more closely resemble the cellular environment. It is not necessary to make the shape of water box spherical. However, energy minimization and equilibration will deform the box into the most stable shape with a minimal surface tension, and the most stable shape is a sphere. We need to admit that there are some other aspects of the cellular environment not reflected in our current study, such as pH, concentration of ions, and interactions with other matters inside cellular plasma. These factors do play a very important role in determining the mechanical properties of microtubules, which should be good topics of our future study. Subsequently, a 10 picosecond equilibration was run to obtain a stable configuration. Next, steered molecular dynamics (SMD) simulations were performed to test the IDTT interaction strength. The procedure of steered molecular dynamics can be briefly described as follows. The backbone atoms of α-tubulin were fixed while the backbone atoms of β-tubulin were attached to a SMD atom through a virtual spring as shown in Fig. 1(b). During the simulations, the SMD atom moved at a constant speed and the distance between the SMD atom and the center of the backbone atoms varied, resulting in force changes inside the virtual spring between them.
The resultant force was uniformly distributed to all the backbone atoms of β-tubulin, leading to movement of those atoms and thus movement of the entire β-tubulin. The applied forces by the virtual spring can be expressed as follows,

$$\vec{F} = -\nabla U,$$

$$U = \frac{1}{2} k \left[ |\vec{r} - \vec{r}_0| \cdot \vec{n} \right]^2,$$

where \(\vec{F}\) is the force vector applied on the virtual spring, \(U\) is the potential energy, \(k\) is the stiffness of the spring, \(v\) is the moving velocity of the spring, \(t\) is time, \(\vec{r}\) is the actual position of the SMD atom (see Fig. 1(b)), \(\vec{r}_0\) is the initial position of the SMD atom and \(\vec{n}\) is the pulling direction. The stiffness of the virtual spring was 4.86 N m\(^{-1}\) and the moving speed was set at 1 m s\(^{-1}\). Justifications of choices in spring stiffness and moving speed, as well as the separation distance cutoff radius for the potential force field can be found in Fig. S1 and S2 in ESI. Simulation results are analyzed and visualized through a visual molecular dynamics (VMD) package.

3. Results and discussions

3.1 IDTT stiffness

In this section, tensile tests were performed on the αβ-tubulin dimer to analyze and discuss IDTT interaction strength. In order to eliminate thermal noise, three cases with different initial velocity profiles were performed and analyzed. The differences among these three runs were negligible. Fig. 2 shows the averaged force-displacement responses, which can be roughly divided into 3 stages. In stage I, α- and β-tubulins adhere tightly to each other as shown in the insets A and B from Fig. 2 and thus the force increases intensively until it reaches the first peak. In this stage, the slope of the curve is not strictly constant, indicating the nonlinearity of the IDTT interaction. However, for simplicity, we calculate the IDTT stiffness based on the assumption that it is linear. Results indicate that the IDTT stiffness is 9.93 N m\(^{-1}\), which is smaller than 44.7 N m\(^{-1}\) obtained in a recently published study. It is important to note that in this recent published work, the calculation of the IDTT stiffness did not take the deformation of β-tubulin into account, making the stiffness bigger than that measured in our study. In the current simulation setup, the α carbon atoms of α-tubulin were fixed while the α carbon atoms of β-tubulin were attached to the SMD atom through a virtual spring. Therefore, the deformation of individual tubulins are already included and the resultant stiffness is a combination of the binding stiffness between two tubulins and intrinsic stiffness of individual tubulins. Another important quantity regarding mechanical responses is the rupture force, or the maximum force in stage I, approximately 3.32 nN. After reaching the peak at the end of the stage I, the force starts to decay in the stage II. The α- and β-tubulins start to separate from each other as shown in the inset C of Fig. 2, at the end of the stage II, the relative displacement between α- and β-tubulins is obvious and the force is very small, approximately one third of the rupture force. Subsequently, the force fluctuates and moderately increases due to the unfolding of loops, shown in inset D of Fig. 2, inside β-tubulin in the stage III. In this stage, β-tubulin gradually detaches from α-tubulin except at one binding site. From inset E of Fig. 2, we can see that despite the unfolded loop and one binding site, the separation of β-tubulin from α-tubulin is almost completed. That binding site is attributed to the binding affinity of tubulins to the GTP molecule between them. The dissociation energy of the IDTT binding is 579.9 ± 43.9 kcal mol\(^{-1}\), which was calculated through the force-displacement responses in the first two stages based on three independent cases. Note that this calculated dissociation energy for IDTT binding is much bigger than that for inter-dimer longitudinal (25.7 ± 2.2 kcal mol\(^{-1}\)) or inter-protofilament lateral binding (9.3 ± 0.8 kcal mol\(^{-1}\)) from a recent study, indicating that the IDTT binding is much stronger than the other bindings inside MTs. Our finding is also consistent with the results in a recent study that the fracture of MTs mostly occurs on the inter-dimer or inter-protofilament interface rather
than the IDTT interface. However, the significant differences in loading rates between our study and the previous study may exaggerate the difference between IDTT dissociation energy and inter-dimer longitudinal or inter-protofilament lateral binding energy. In the previous study, the loading rate is in the range from 0.2 to 1 μm s⁻¹, 6 orders of magnitude smaller than the one (1 m s⁻¹) used in our study. The fast loading rate in our study may make the IDTT binding behave stronger, overestimating the difference between IDTT binding and inter-dimer/inter-protofilament binding.

To better illustrate the underlying mechanism of the force-displacement responses of αβ-tubulin dimers, the evolution of averaged displacement and IDTT non-bonded potential energy were shown in Fig. 3 and 4, respectively. As we can see from Fig. 3, the time-displacement relationship is not linear, and can also be divided into three stages as previously shown for the force-displacement responses. In stage I, the displacement increases slowly due to the tight binding between α- and β-tubulins. Subsequently, in stage II, after the force reaches the peak value the displacement increases much faster than that in stage I, indicating the quick separation of β-tubulin from α-tubulin. In stage III, the displacement of β-tubulin increases linearly in terms of time, during which the velocity (1 m s⁻¹) is very close to that of the virtual spring. In other words, the β-tubulin is in force balance. In this stage, due to the large separation between α- and β-tubulins, the IDTT interaction is very weak. Therefore, the friction force from the thermal bath is comparable with the force from the virtual spring, neutralizing the net forces on the β-tubulin to make it move at a constant speed. Fig. 4 shows the evolution of the IDTT non-bonded interaction energy. As shown in the figure, at the starting point, the electrostatic energy is approximately four times larger than the van der Waals energy, indicating that the electrostatic interactions dominate the IDTT binding strength. In stage I, both electrostatic and van der Waals interactions increase intensively, resulting from the tight binding between the two tubulins. After entering stage II, the increase in potential energy becomes slower and slower as the displacement increases, indicating the weakening interaction between the two tubulins, which is in good agreement with results shown in Fig. 2. In stage III, both van der Waals and electrostatic energy fluctuate around zero. In other words, the force from IDTT non-bonded interactions can be negligible in stage III. The force from the virtual spring is compensated by the friction force from the thermal bath. From the preceding analysis, we can conclude that the force-displacement responses are dominated by the electrostatic interactions between α- and β-tubulins. However, the contributions of each specific residue type to the IDTT interaction remain unclear, and are crucial to a thorough understanding of the IDTT binding strength between α- and β-tubulins.

3.2 Amino acid-level energy analysis

In this section, per-residue energetics are performed to examine the effect of types of amino acids on the IDTT binding strength between α- and β-tubulins. As we know, natural proteins are made from 20 types of amino acids. As discussed in a previous study, these 20 types of amino acids can be classified into six clusters: small, hydrophobic, (−)charged, (+)charged, polar and aromatic. Note that there are some overlaps between small and hydrophobic, as well as between polar and aromatic. The classification is based on the features of the side groups. Fig. 5 shows the energy contribution of different amino acid types to the IDTT non-bonded interaction energy. These data sets included in the figure were obtained from the initial configuration right before the tensile tests start. Note that all the results are averaged from three independent runs with different initial velocity profiles in order to eliminate thermal noise. The interaction energy between two residues from different tubulins is calculated as long as the atoms from different residues are in the separation distance cutoff of 12 Å. As shown in Fig. 5, in both α- and β-tubulins, the energy contribution of (−)charged and (+)charged clusters is much larger than those from the other clusters. This finding is in agreement with our previous finding: the force-displacement responses are dominated by the electrostatic interactions between α- and β-tubulins. The domination of amino acids with net charges in terms of IDTT interaction energy contribution indicates the possibility of tuning the IDTT binding strength through mutations of charged amino acids.

Despite the importance of the above key finding, further investigations are needed because the separation distance between two residues also influences the interaction energy in addition to the type of amino acids. To clarify the relation...
between separation distance and the IDTT interaction energy, amino acid residues of α-tubulin with more than 3000 contact pairs with the other tubulin are selected. Fig. 6(a) and (b) show the initial IDTT non-bonded energy and contact pairs as shown in Fig. 2. No clear relationship between contact pairs and the IDTT non-bonded energy is observed as shown in Fig. 6(a) and (b). However, it can be seen that the IDTT non-bonded energy depends heavily on the type of residues. As we can see from Fig. 6(a), residues with net charges have much higher interaction energy with β-tubulin than residues with polar side groups while the latter has higher interaction energy than residues with hydrophobic side groups. Note that, there is a residue, arginine 105 (ARG105) on α-tubulin, with high positive non-bonded energy, indicating that this residue is strongly repulsed by another residue with positive charge, arginine 253 (ARG253) on β-tubulin. Fig. 6(c) shows the relative position between

---

**Fig. 6** (a) Initial IDTT non-bonded interaction energy distribution; (b) initial contact pair distribution of residues on α-tubulin close to β-tubulin; (c) schematic view of the relative position between ARG 105 on α- and ARG253 on β-tubulin.
ARG105 on α-tubulin and ARG253 on β-tubulin, demonstrating that they are in close contact. Similar analysis is performed for β-tubulin. Rather than 3000 contact pairs with the other tubulin, 2000 are chosen as the threshold for residues on β-tubulin. Corresponding results are shown in Fig. 7, which show a similar pattern to the results presented in Fig. 6. However, there are several key findings of interest. The first one is that methionine 1 (MET1) on β-tubulin has exceptionally high interaction energy with the other tubulin as shown in Fig. 7(a). Although MET1 is in the small cluster, it is also the Nitrogen terminus with positive net charges, resulting in high interaction energy with negatively charged residues on α-tubulin. The second one is that aspartic acid 251 (ASP251) has positive interaction energy with α-tubulin due to the strong repulsion from glutamic acid 71 (GLU71) and aspartic acid 98 (ASP98) on α-tubulin. Fig. 7(c) shows the relative position between ASP251 on β and GLU71 and ASP98 on α, indicating that they are in close contact. Additional simulation runs were performed using the pdb file with accession number “4i4t” from a recently published paper and results do not alter above findings. Detailed information, as well as corresponding discussions, can be found in Fig. S3 and S4 in ESI. Based on the above analysis above, a mutation plan can be proposed to tune the IDTT binding strength of the tubulin dimer in order to design functional microtubular structures that target particular properties.

3.3 Effect of point mutation on IDTT stiffness

Results from our previous section reveal that the charged residues dominate the non-bonded interactions between α- and β-tubulins. In addition, we discovered that one residue on each tubulin negatively contributes to the IDTT non-bonded energy, providing us some hints on how to tune the IDTT binding strength through point mutations. For example, ARG105 on α-tubulin has high positive non-bonded interaction energy due to the strong repulsion by ARG253 on β-tubulin. Since ARG253 on β-tubulin contributes positively to the IDTT binding strength as shown in Fig. 7(b), our plan is to mutate ARG105 on α-tubulin to a residue that has net negative charges in order to turn the repulsion into attraction. Similarly, our plan for ASP 251 on β-tubulin is to mutate it to a residue with net positive charges.

Our detailed mutation plan is summarized in Table 1.

Table 1  Mutation plan

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Original state</th>
<th>Mutation state</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation 1</td>
<td>ARG 105(+) on α-tubulin</td>
<td>ASP 105(−) on α-tubulin</td>
</tr>
<tr>
<td></td>
<td>ASP 251(−) on β-tubulin</td>
<td>ARG 251(+) on β-tubulin</td>
</tr>
<tr>
<td>Mutation 2</td>
<td>ARG 105(+) on α-tubulin</td>
<td>ASP 105(−) on α-tubulin</td>
</tr>
<tr>
<td></td>
<td>ASP 251(−) on β-tubulin</td>
<td>ARG 251(+) on β-tubulin</td>
</tr>
<tr>
<td>Mutation 3</td>
<td>ARG 105(+) on α-tubulin</td>
<td>ASP 105(−) on α-tubulin</td>
</tr>
<tr>
<td></td>
<td>ASP 251(−) on β-tubulin</td>
<td>ARG 251(+) on β-tubulin</td>
</tr>
<tr>
<td>Mutation 4</td>
<td>ARG 105(+) on α-tubulin</td>
<td>ASP 105(−) on α-tubulin</td>
</tr>
<tr>
<td></td>
<td>ASP 251(−) on β-tubulin</td>
<td>ARG 251(+) on β-tubulin</td>
</tr>
</tbody>
</table>

To test the effectiveness of point mutations on tuning IDTT binding strength, four different cases were performed as presented in Table 1. For each case, three independent runs were carried out in order to eliminate thermal noise. Fig. 8 shows the initial IDTT non-bonded energy contribution from different
clusters in a control case and four mutation cases, in which the top and bottom panels show the results for α- and β-tubulins, respectively. Results indicate that for all mutation cases, the contribution from charged residue clusters was improved, especially for mutation 2 and 3. However, for other residue clusters, the varying trend of energy contribution is not clear and due to their minor roles, their changes have a relatively small impact on the overall non-bonded energy between α- and β-tubulins. Subsequently, tensile tests were performed and corresponding results in terms of IDTT non-bonded energy, stiffness, rupture force and separation work are shown in Fig. 9. Firstly, IDTT non-bonded energy denotes non-covalent energy contribution between two tubulins inside a αβ-tubulin dimer from both van der Waals forces and electrostatic forces, calculated with a cutoff radius 1.2 nm based on the CHARMM36 force field. Note that energy in hydrophobic interactions is specifically the energy in van der Waals forces between non-polar residues. Secondly, stiffness was determined from the slope of the force-displacement curve analogous to that in Fig. 2, linearly fitting the force and displacement when the displacement is smaller than 0.3 nm. Thirdly, the rupture force was determined from the peak force in force-displacement curves analogous to that in Fig. 2. Lastly, separation work is defined as the work done by the forces from the virtual spring to separate α- and β-tubulins during the tensile test, which is calculated from the force-displacement curves analogous to that in Fig. 2. Note that all the data points were normalized by those from the control case for visualization purposes. The first column in Fig. 9 shows the initial interaction energy between α- and β-tubulins, in which all the mutated cases have better performance than the control case, especially mutations 2 and 3. Due to the improvement of interaction energy between α- and β-tubulins, other mechanical properties, namely stiffness, rupture force and separation work, were improved to a certain extent except mutation 1 in comparison to those in the control case.

4. Conclusions

In this study, molecular dynamics simulations were performed to study the IDTT binding strength inside the αβ-tubulin dimer of the microtubule. Results indicate that the force-displacement responses of IDTT tensile tests can be roughly divided into three stages: force-increasing stage (stage I), force-decreasing stage
(stage II) and force fluctuating stage (stage III). The separation of β-tubulin from α-tubulin is almost complete at the end of stage II according to the energetic analysis. Energetic analysis also indicates that rather than van der Waals energy, electrostatic energy plays a very important role in binding α- and β-tubulins together. Additionally, per-residue energetic analysis was performed to further explore the underlying mechanisms of IDTT binding strength. Results indicate that amino acids with net charges, namely aspartic acid (ASP), glutamic acid (GLU), arginine (ARG) and lysine (LYS), play a major role in the non-bonded energy between α- and β-tubulins. Specifically, two amino acid residues with net charges, ARG 105 on α-tubulin and ASP 251 on β-tubulin, were found to contribute negatively to the IDTT binding, providing us some hints on how to increase the IDTT binding strength through point mutations. Subsequently, four mutation scenarios were implemented on the two sites of α- and β-tubulins and IDTT tensile tests were performed to test the binding strength of the resultant mutated species. Results indicate that the mutations significantly increased the energy contributions of residues with net charges. Consequently, the stiffness, rupture force and separation work improved to a certain extent. In summary, our results help explain the underlying nanoscale mechanisms of the IDTT binding strength of α- and β-tubulin dimer and provide a potential means for controlling binding strength between α- and β-tubulins in MTs through point mutations.

Conflicts of interest
The authors declare no competing financial interests.

Acknowledgements
N. L. and R. P. acknowledge the support from the National Science Foundation (Grant No. CMMI-1610812). X. W. acknowledges supports from National Science Foundation (Grant No. CMMI-1306065). Computational simulations are performed at the UGA Advanced Computing Resource Center.

References


