

1 **Effect of Amino Acid Mutations on Intra-Dimer Tubulin-Tubulin Binding**
2 **Strength of Microtubules**

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1 **1 The effect of the stiffness of virtual spring in SMD**

2 Additional simulations have been performed to justify the choices for cut off radius, spring
3 stiffness and loading rate. First, three different spring stiffness are used to perform intra-dimer
4 tubulin-tubulin (IDTT) SMD, namely 2.08, 4.86, and 9.72 N/m. Figure S1(a) shows that the force-
5 displacement responses in IDTT SMD are very close to each other despite using different spring
6 stiffness, indicating that the spring stiffness plays a negligible role given it falls into the reasonable
7 range. However, we admit that the spring stiffness will definitely influence the accuracy of the
8 results in SMD if the magnitude of it is too large or too small. For example, if the spring constant
9 is too large, there would be great fluctuations in the force-displacement responses. Actually, in
10 Figure S3, we can clearly see that the fluctuations in the curve with $K = 9.72$ N/m are much greater
11 than those in the curves with $K = 2.08$ and 4.86 N/m. On the other hand, if the spring stiffness is
12 too small, a significant amount of computing resources would be required to perform SMD.

13 **2 The effect of the separation cutoff radius for inter-particle potential**

14 With respect to the cutoff radius, we choose 1.2 nm in the current paper, which is sufficient given
15 that the value of σ , the finite distance at which the inter-particle potential is zero, is smaller than
16 0.48 nm. Typically, in molecular dynamics simulations, a cut off radius no less than 2.5σ would
17 be considered to be enough for an accurate calculation of van der Waals forces. In addition, we
18 perform additional simulations using the cutoff radius greater than 1.2 nm, namely 1.5nm and 2.0
19 nm. Figure S1(b) shows that the three force-displacement curves using different cut off radii are
20 very close to each other, indicating that the cut off radius 1.2 nm we choose is sufficient to
21 guarantee the accuracy of simulations.

22 **3 The effect of loading rate in SMD**

23 Additional simulations are performed to justify our choice in loading rate, in which the magnitude
24 of loading rates ranges from 1 to 20 m/s. In all simulations, the displacement of SMD atom is
25 always 4 nm. As we can see from Figure S2(a), when the loading rate is 20 m/s, the resultant force
26 keeps increasing as the displacement increases. However, as the loading rate decreases, the force-
27 displacement curves show a force-decaying trend as the displacement further increases. Moreover,
28 as we can see from Figure S2(b) and (c), both stiffness and rupture force decrease rapidly at first
29 and then enter a plateau associated with the decrease of loading rates from 20 to 1 m/s. Therefore,
30 the loading rate 1 m/s is reasonable according to our analysis.

1 **4 The effect of coordination (pdb) files**

2 Additional simulations are performed using the pdb file with accession number “4i4t” from a
3 recently published paper.¹ In Figure S3 and S4, the energy contribution of residues on IDTT
4 binding is plotted for α and β tubulin, respectively, in comparison with those from our previous
5 results using the pdb file with accession number “1tub”. The data included are averaged on three
6 independent simulation runs and only these residues with the magnitude of energy contribution
7 greater than 20 kcal/mol are singled out and depicted. As we can see from Figure S3(a) and S4(a),
8 almost all of these residues on α and β tubulins with significant energy contributions are still from
9 (-)charged, (+) charged and polar clusters despite the use of a new pdb file, “4i4t”, which is
10 consistent with our findings in the main context. Moreover, from the comparison between Figure
11 S3(a) and (b), one can tell that the residue on α tubulin with greatest reduction on IDTT binding is
12 always ARG105 regardless of the use of different pdb files in simulations, in which the energy
13 contribution of ARG105 is 52.3 ± 8.3 and 54.7 ± 2.3 kcal/mol for “4i4t” and “1tub”, respectively.
14 Similarly, from the comparison between Figure S4(a) and (b), ASP251 remains the residue on β
15 tubulin with greatest reduction on the IDTT binding, in which the energy contribution of ASP251
16 is 54.3 ± 12.6 and 41.7 ± 0.5 kcal/mol for “4i4t” and “1tub”, respectively. However, results do
17 show that there are some differences between data obtained from simulations using “4i4t” and
18 those from “1tub”. For example, as shown in Figure S3, the number of residues on α tubulin with
19 energy contribution greater than 20 kcal/mol is 12 for simulations using “4i4t” while the
20 counterpart for simulations using “1tub” is 18. Besides, the energy contribution varies for a given
21 residue if different pdb files are used in the simulations. For instance, the energy contribution of
22 GLU71 on α tubulin is -104.9 ± 17.7 and -181.9 ± 0.6 kcal/mol for “4i4t” and “1tub”, respectively.

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2 **Figures**

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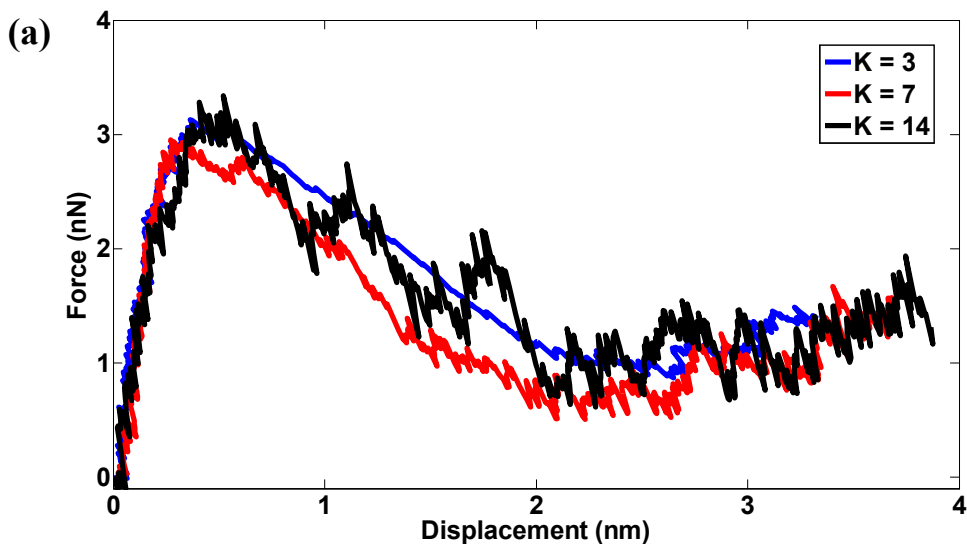
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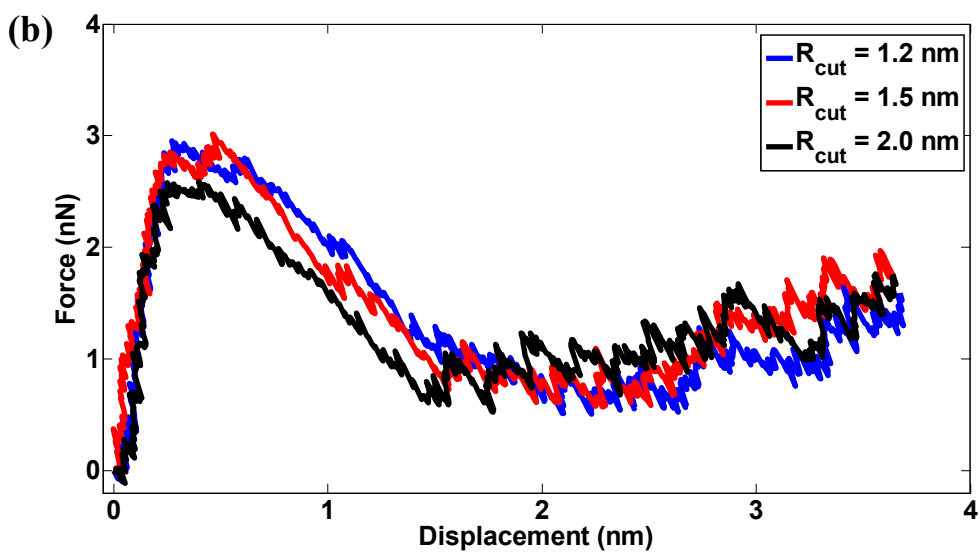
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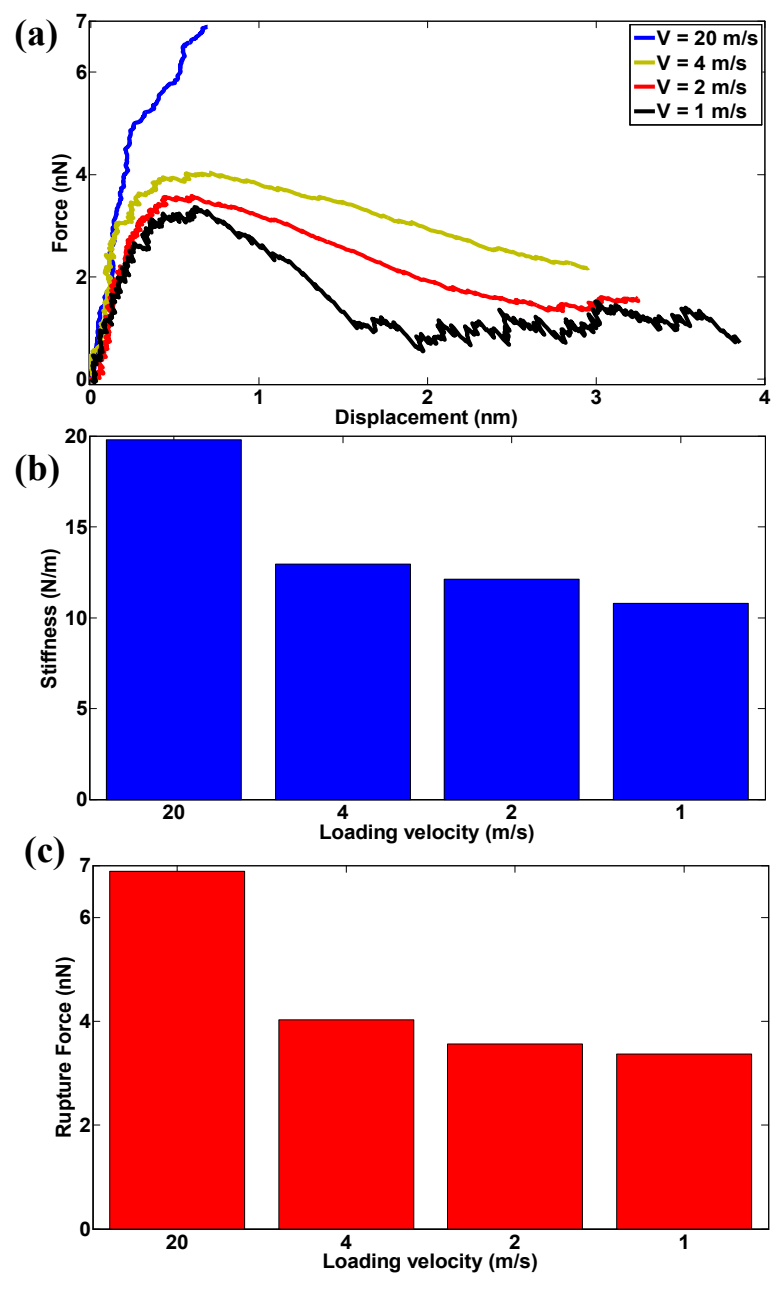
23 **Figure S1.** Force-displacement responses in IDTT SMD (a) using virtual springs with different

24 stiffness; (b) using different cut off radius for calculation of van der Waals forces and electrostatic

25 forces.

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22 **Figure S2.** (a) force-displacement responses using different loading rate; (b) Measured stiffness
23 from curves in subfigure (a); (c) Measured rupture force from curves in subfigure (a).

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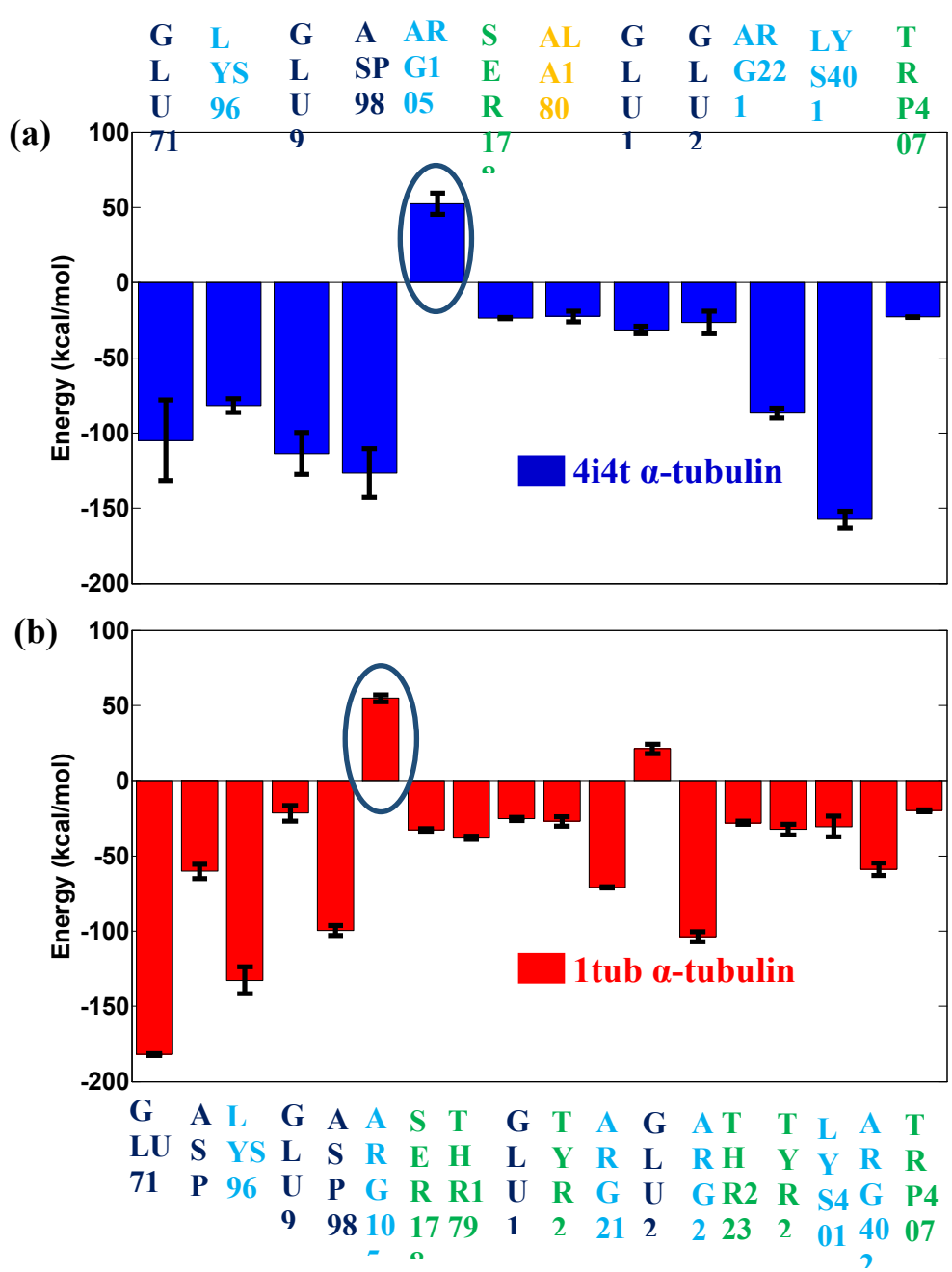


Figure S3. Energy contribution of residues on α tubulin on the IDTT binding from simulations using pdb files with accession number (a) 4i4t; (b) 1tub. (Only these residues with energy contribution bigger than 20 kcal/mol are singled out and shown.)

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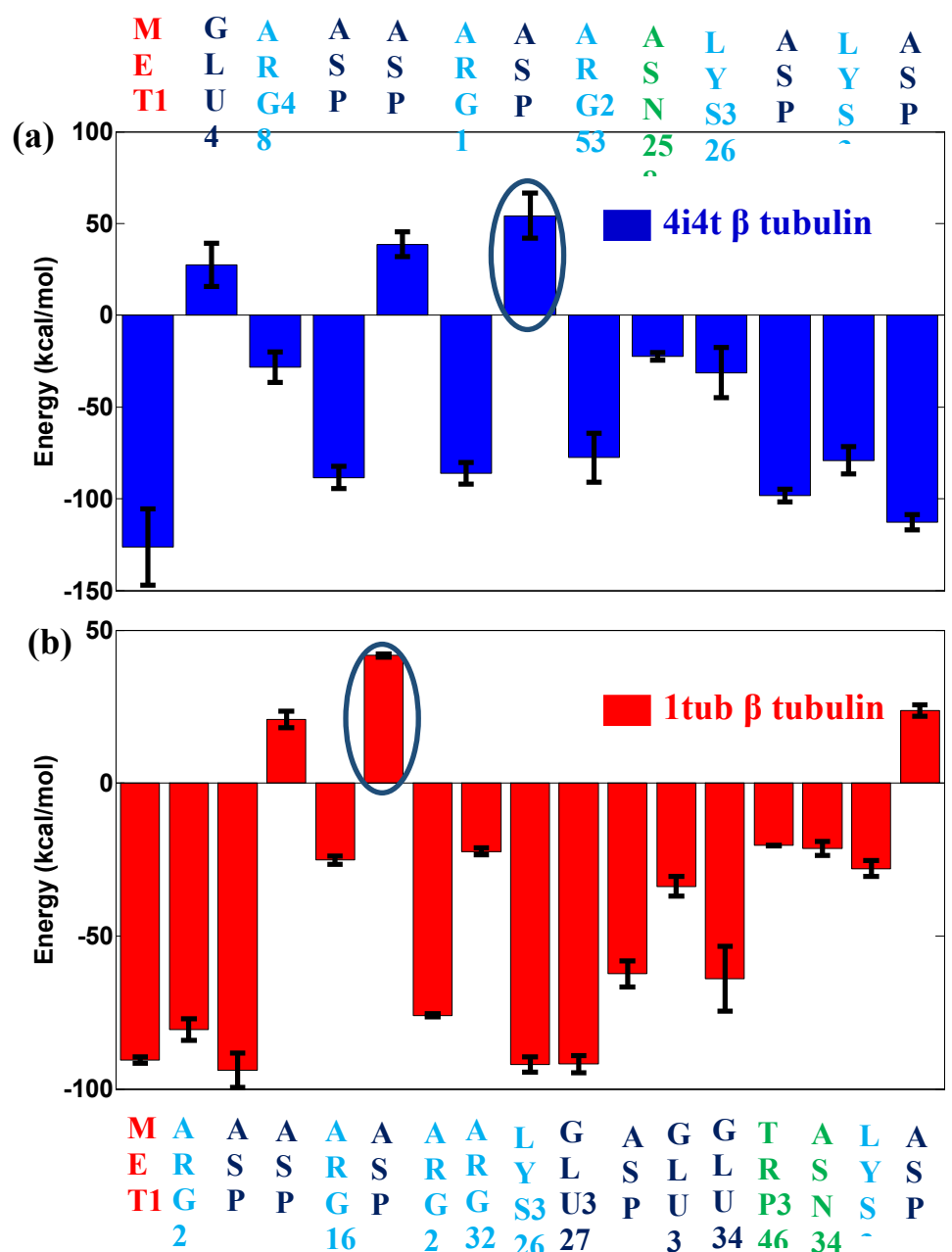


Figure S4. Energy contribution of residues on β tubulin on the IDTT binding from simulations using pdb files with accession number (a) 4i4t; (b) 1tub. (Only these residues with energy contribution bigger than 20 kcal/mol are singled out and shown.)

1 References

- 2 1. A. E. Prota, K. Bargsten, D. Zurwerra, J. J. Field, J. F. Díaz, K.-H. Altmann and M. O. Steinmetz,
3 *Science*, 2013, **339**, 587-590.

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