Molecular Modeling of the Axial and Circumferential Elastic Moduli of Tubulin

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ABSTRACT Microtubules play a number of important mechanical roles in almost all cell types in nearly all major phylogenetic trees. We have used a 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. The axial elastic moduli, in vacuo, were found to be 1.25 GPa and 1.34 GPa for α- and β- bovine tubulin monomers. In the circumferential direction, these moduli were 378 MPa for α- and 460 MPa for β- structures. Using bovine tubulin as a template, 269 homologous tubulin structures were also subjected to simulated tensile loads yielding an average axial elastic modulus of 1.10 ± 0.14 GPa for α-tubulin structures and 1.39 ± 0.68 GPa for β-tubulin. Circumferentially the α- and β-moduli were 936 ± 216 MPa and 658 ± 134 MPa, respectively. Our primary finding is that the axial elastic modulus of tubulin diminishes as the length of the monomer increases. However, in the circumferential direction, no correlation exists. These predicted anisotropies and scale dependencies may assist in interpreting the macroscale behavior of microtubules during mitosis or cell growth. Additionally, an intergenomic approach to investigating the mechanical properties of proteins may provide a way to elucidate the evolutionary mechanical constraints imposed by nature upon individual subcellular components.

INTRODUCTION

Microtubules provide a number of mechanical services in nearly all cell types throughout most of the major branches of the phylogenetic tree including archaea (1). They act as mitotic spindles for cell division (2), maintain transport conduits (3,4), and are used as flagella (5). Recently, they have also been implicated as playing a critical role in consciousness (6). Additionally, microtubules interact with actin filaments and the cellular membrane to provide a foundation that determines cell morphology (7,8). While typically constructed of a heterodimeric lattice, with intermonomeric bond stiffnesses and strengths contributing to cellular-scale behavior, microtubule function and assembly may also be attributed to the mechanical properties of individual tubulin monomers. While tubulin sequences vary significantly across species, the role that specific residues or tertiary-scale interactions contribute to the ultimate behavior of tubulin is difficult to predict (e.g., (9)).

Experimental approaches to determine the mechanical properties of tubulin have included optical tweezers (8), hydrodynamic flow (10), vesicle buckling (11), thermally induced vibrations (12), naturally occurring bending (13), and atomic force microscopy (14). Most of these experiments focus on obtaining buckling stiffness of microtubules and have yielded a wide range of values for axial elastic modulus, 1 MPa to 7 GPa (1 MPa = 1 megapascal = 10^6 N/m^2; 1 GPa = 1 gigapascal = 10^9 N/m^2). These findings have been well reviewed (15).

Modeling approaches for predicting tubulin and microtubule properties include those of Tuszynski et al. (16) and Kerssemakers et al. (17). Often, simulations are run in vacuo, which reduces computational requirements by an exponential factor versus models employing implicit or explicit water. One of the first exhaustive three-dimensional intergenomic homology modeling studies of tubulin focused mainly on geometry, dipole moments, charge distributions, and C-terminus lattice structures, was by Tuszynski et al. (18). Their results offer an exhaustive comparison for the structural properties of homologous tubulin structures in Tuszynski et al. (19), but did not explore mechanical properties.

Here, we establish a framework comparing mechanical properties of members of the same family of proteins. We have performed molecular mechanics simulations on all of the currently sequenced α-, β-, and γ-tubulins. Specifically, we simulated axial and circumferential loading on all structures after mapping them onto a consensus structure (20). Our findings may elucidate the roles that key mutations or conserved regions may have played in driving tubulin toward its mechanically anisotropic state. Additionally, the mechanical effects of directed mutations, or of engineered protein sequences, may be estimated before employing molecular biological techniques.

For special terms and reference data used in this article, see Table 1.

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TABLE 1 Notation

| Symbol | Description |
|--------|-------------|
| D_i   | Inner diameter of tubulin |
| D_o   | Outer diameter of tubulin |
| E_M   | Elastic modulus of tubulin |
| E_memo| Elastic modulus of monomer |
| E     | Elastic (Young’s) modulus |
| F_i   | Force on a MT filament |
| I     | Second moment of inertia |
| K*    | Inverse stiffness of dimer |
| k_a   | Stiffness of α-tubulin |
| k_b   | Stiffness of β-tubulin |
| k_i   | Stiffness of monomer-monomer bond |
| k_d   | Stiffness of monomer-monomer bond |
| k_bond| Stiffness of covalent bond |
| k_stiff| Rotation stiffness of covalent bond |
| L_o   | Unstrained dimer length |
| L_p   | Persistence length |
| L_z   | Axial length of monomer |
| M_i   | Change in length |
| n_i   | Crystal plane number |
| r_i   | Radial direction |
| r_stretch| Stretched bond length |
| r_h  | Atomic separation for Coulomb force |
| r_e   | Equilibrium bond length |
| T     | Temperature |
| U_total| Total simulation energy |
| U_bond| Energy from bond stretching |
| U_angle| Energy from bond bending |
| U_stiff| Energy from bond twisting |
| U_VDW| Energy from van der Waals interactions |
| U_Coulomb| Energy from Coulomb interactions |
| z     | Axial direction |
| γ_i   | Equilibrium value of φ |
| Δ_a   | Deformation of α-tubulin |
| Δ_b   | Deformation of β-tubulin |
| Δ_i   | Deformation of monomer-monomer bond |
| Δ_d   | Deformation of dimer-dimer bond |
| ε     | Strain |
| e_q   | Maximum energy of separation |
| e_j   | Permittivity of free space |
| θ     | Circumferential direction |
| θ_1   | Circumferential position of filament in MT |
| θ_b   | Bent bond angle |
| θ_i   | Equilibrium bond angle |
| κ     | Curvature |
| O_i   | Tetrahedral bond angle |
| ρ     | Radius of curvature |
| σ_i   | Zero energy separation distance |
| φ_i   | Angle between bond planes |

METHODS

Sequences used

We searched for all complete primary tubulin sequences within the Research Collaboratory for Structural Bioinformatics Protein Data Bank (PDB) (21). Utilizing the UniProt protein resource (22), we were able to obtain sequences for 269 tubulin structures. This series includes 96 α-structures, 147 β-structures, and 26 γ-structures. To date, a few hundred tubulin sequences have been identified and sequenced. Even fewer (only two or three) three-dimensional structures of tubulin dimers exist at a significantly high resolution to produce accurate homology models (21).

Structural homology matching

Since the tertiary structures of all nearly all of the presently sequenced tubulins are unknown, a three-dimensional consensus structure template was needed. For this, we selected the highest-resolution structure produced to date. Lowe et al. obtained a 3.5 Å resolution structure of the α-β dimer for bovine tubulin utilizing electron diffraction (PDB Identifier 1JFF) (20). This predicted structure corresponds to that of the tubulin dimer found in zinc-induced tubulin sheets. Although there has been no systematic study to compare the sheet structure with the cylindrical structure, it is reasonable to assume that the individual dimers and monomers within the sheet are more flat in the circumferential direction. Recent simulation and imaging work (24) of a 15-filament structure indicates that the GDP versus GTP state of β-tubulin may be responsible for microtubule stability. Specifically, Krebs et al. (24) suggest that, since the 15-filament structure represents an intermediate form between the ~10-Å radius-of-curvature of a native microtubule and the infinite radius-of-curvature of the zinc-induced sheets, it may serve as a predictor of microtubule stability. Ideally, for microtubule-scale mechanical property prediction, tubulin-straining simulations such as those we have performed would be done on the curved configuration. However, since current experimental techniques preclude this level of detail, we are limited to the sheet configuration.

For γ-tubulin, we used the 2.71 Å resolution structure (PDB Identifier 1Z5V) obtained by Aldaz et al. (25). Utilizing the structure predicted by Lowe et al. (20) as a template for other α-β-tubulin structures, and Aldaz’s structure for γ-tubulin, we created homology models of all tertiary structures. We began by using nanoscale molecular dynamics (NAMD) downloaded from the University of Illinois at Urbana-Champaign’s Theoretical and Computational Biophysics Group (26) and separated the dimers into their monomeric units. From the dimer PDB files, a protein structure file (PSF) was created using NAMD’s psfgen package, the topology file required for this PSF (using Chemistry at Harvard Molecular Mechanics, i.e., CHARMM, Ver. 22, for proteins and lipids). Topology files contain bond connectivity, angle, and charge distribution information. The parameter file, also CHARMM Ver. 22, contains force constants, equilibrium geometries, and various other calculations required to perform energy balances (27,28). Cutoffs were set in the force-field parameters at 12 Å. At 20 steps per cycle, and a 100-step minimization was performed on the monomer to produce a local minimum energy structure for α-, β-, and γ-tubulin (Fig. 1). This approach was necessary because the problem of de novo prediction of three-dimensional structure from a one-dimensional sequence is exceedingly difficult and frequently yields nonunique solutions (29).

To perform energy minimization of the structures to be stretched we used SWISS-MODEL (http://swissmodel.expasy.org/SWISS-MODEL.html). Briefly, SWISS-MODEL follows the following protocol: initially it checks the sequence identity with the target. It then creates a ProModII job by first superimposing three-dimensional structures of the two related proteins and generates multiple alignments with the sequence to be modeled. By using the positions of atoms that are most similar between the template structure and predicted structure, it creates a framework and rebuilds any lacking loops. It then completes and corrects the backbone structure and the side chains, verifies the model structure quality, and finally refines the structure with energy minimization using GROMOS96. Lastly, a PSF file is produced and BLAST analysis is provided. The series of amino-acid sequences produced an average similarity of 85.82% and standard deviation of 9.39% with the template structures. Structures with a similarity at <25% were automatically rejected by the SWISS-MODEL server. Sequences with <50% similarity were usually a result of incomplete or fragmentary structures. However, these structures were still included in the simulation of stretching the tubulin structures. Sequence alignment and similarities were independently verified using CLUSTAL W (30).

To enhance the likelihood of finding the likely global minimal energy structure, in several test cases, we allowed our minimization procedure to run for 10,000 steps rather than the recommended 100 steps of steepest descent, followed by 200–300 steps of conjugate gradient energy minimization. In these extended simulations, no more than 5–10% difference was observed in total energy. Only one structure failed to stabilize (TBA8_CAEEL), regard-
less of the number of time steps (31). While the sequences of all tubulin structures we studied are published, their exact three-dimensional structures have yet to be determined. Once the 269 tubulin homologous models were created, visual molecular dynamics (VMD) was used to visualize the structures to verify that three-dimensional consensus mapping resulted in globular protein structures of densities comparable to the template structure. All structural predictions were performed in vacuo. While this is a limitation of the model, since the force constants developed for NAMD through CHARMM were developed within an explicit water framework, recent work using a ubiquitin model indicates that this approach leads to errors that are statistically insignificant (p < 0.01) (32).

The majority of the structural data for MT(microtubules) has been acquired from highly purified preparations, thus our simulations most likely closely represent the material behavior of tubulin in isolated microtubules. In a manner consistent with Tuszynski’s approach, we worked under the assumption that errors within each model are negligible when compared against a group of models (19). This error can be reduced by using an initial minimization run before the tensile test is performed. Another notable quality of the molecular deformation experiments is that in general, the α-tubulin molecules exhibit multiple moduli as the protein unfolds (see Fig. 5). This type of behavior has been observed in fabric failure (34), but is not observed in solid structures.

Parameters used, boundary conditions, and optimization

Steered molecular dynamics (SMD) offers programmable dynamic simulation utilizing NAMD (35). The NAMD software was loaded with the original PDB files, PSF file, a reference file (1JFF and 1Z5V), and a configuration file to perform the simulation following previously developed methods (21,36). Briefly, NAMD is a parallel molecular dynamics code specifically designed for the simulation of large biomolecular systems. The software is open-source and available free of charge. It allows the user to perform chemical and conformation free energy calculations with multiple timestep integration. For our application, the ability to create scriptable code in Tool Command Language integrated with SMD allowed us to perform repeatable dynamic simulations of all structures we considered with the exception of one incomplete sequence: TBA8_CAEEL.

While there are no standards for simulated molecular mechanical property characterization, standard macroscale mechanical tensile tests utilize dog-bone-shaped specimens to ensure a concentration of loading on a narrow portion of the sample with a precisely known cross-sectional area. In general, these tests result in a scale-invariant elastic modulus until smaller dimensions are reached, where moduli tend to increase and become more variable (37,38). While single molecule experiments have been performed on single proteins as they unfold (e.g., (39)), the opportunity to interrogate a single tubulin monomer in its naturally occurring state has not been realized. Thus, the Cartesian coordinates for every atom in the PDB structure were tabulated to determine a suitable region to act as a grasping area. This is shown in Fig. 2, which depicts a histogram of the distribution for a human tubulin species, similar to that of 1JFFB, in the axial direction. A histogram of the z-axis positions of each atom as provided in the PDB files was plotted in 3.3 Å increments using MS Excel. The C-termini tails of tubulin monomers, because of the extensive number of possible interactions that are still undetermined, were cut off before performing the simulations. Thus, an entire line of residues was removed—preventing the possibility that this relatively flexible region would dictate the simulation behavior. To facilitate our virtual tensile testing, we labeled 10% of the most distal N-terminus atoms as fixed atoms and 20% of the remaining most distal C-terminus atoms as steered atoms. These atoms were labeled appropriately in each PDB file with a value of 1.00 in the appropriate Fixed or Steered column.

We used SMD to pull the 6377-atom α-monomer and 6574-atom β-monomer in tension. Fixed atoms were held rigid, while steered atoms were directed by an SMD atom, pulled axially at 0.005 Å per time step. This translates to 2.5 Å/ps with a time step of 2 fs. The SMD “dummy” atom pulls the steered atom with a spring constant of 7 kcal mol⁻¹ Å⁻² ≅ 500 pN Å⁻¹ = 5 Nm⁻¹, (1 kcal/mol⁻¹ = 69.5 pN Å⁻¹).

These values were selected based upon a series of optimization simulations. We performed an initial set of simulations on the 1JFF β-monomer at a series of velocities ranging from 0.5 to 0.005 Å/ps. A velocity of 0.05 Å/ns was found to be asymptotic in that it achieved an elastic modulus that was within 2% of the modulus measured at the slower velocities. At velocities slower than this, computational time became unreasonable and produced errors in energy minimization cascades over long time-periods. Simulations run faster than 0.05 Å/ns resulted in inaccuracies caused by overstretched bond angles (Fig. 3). The velocity of pulling also reflects the effect of hy-
To optimize computational resources, we performed our simulations at a series of velocities ranging from 0.5 to 0.005 Å/ns. At rates <0.05 Å/ns, modulus results were unaffected.

In calculating the iterative energies, the presence of hydrogen adds an extra force component to the system. In reality, the monomer may be more plastic as a consequence of hydrating the structure, resulting in lower moduli. Faster pulling rates also result in more brittle behavior.

Total simulation energy, $U_{\text{total}}$, is calculated as a sum of contributions from three primary deformation modes (35,42), as well as van der Waals forces and Coulomb forces, as

$$U_{\text{total}} = U_{\text{bond}} + U_{\text{angle}} + U_{\text{dihedral}} + U_{\text{vdW}} + U_{\text{Coulomb}}.$$  

Each of these individual energies are found from

$$U_{\text{bond}} = \sum_{\text{bonds}} k_{\text{bond}} (r_i - r_{\text{eq}})^2,$$

$$U_{\text{angle}} = \sum_{\text{angles}} k_{\text{angle}} (\theta_i - \theta_{\text{eq}})^2,$$

$$U_{\text{dihedral}} = \sum_{\text{dihedrals}} \left( k_{\text{dihedral}} \left[ 1 + \cos(n_i \phi_i - \gamma_i) \right] \right),$$

$$U_{\text{vdW}} = \sum_{i,j} 4\epsilon_{ij} \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^6,$$

$$U_{\text{Coulomb}} = \sum_{i,j} \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}}.$$  

The variable, $k_{\text{bond}}$, represents the axial bond stiffness; $r_i$ is the stretched bond length; $r_{\text{eq}}$ is the equilibrium bond length; $k_{\text{angle}}$ is the torsional bond stiffness; $\theta_i$ is the bond forming angle; $\theta_{\text{eq}}$ is the equilibrium bond angle; $k_{\text{dihedral}}$ is the torsional bond stiffness; $n_i$ is the periodicity of the crystal structure or the number of instances of a plane of a given orientation; $\phi$ is the angle between adjacent planes; $\gamma$ is the equilibrium value of $\phi$ defined on a per-atom basis; “O” (omicron) is the angle between the first three atoms in a tetrahedral structure where there is no crystal periodicity, i.e., ($n = 0$); $\epsilon_i$, the maximum depth of the energy potential well for atomic separation; $\sigma_{ij}$ is the distance between atom $i$ and atom $j$ at which the energy is zero; $r_{ij}$ is the atomic separation distance; $q_i$ and $q_j$ are the charges of the respective atoms; $\epsilon_0$ is the permittivity of free space; and $r_{ij}$ is the distance separating atom $i$ and atom $j$.

### Axial modulus

Data output from the NAMD software in the form of energy and displacement was converted to force/displacement. Energy was determined by utilizing the equations in Li and Wu (1), which govern the bonding interactions between atomic groups. These equations utilize the CHARMM parameter sets as well as atomic position at each interval of the testing procedure. As the procedure is displacement-controlled, the resulting energy was converted to axial force by dividing the resulting energy by the given axial displacement at each increment, $f = U_{\text{total}}/\Delta L$. Strain was obtained by dividing the increment displacement by the total length of each monomer ($\varepsilon = \Delta L/L_0$). The axial lengths of the template $\alpha$- and $\beta$-monomers were determined to be $5.789$ nm and $6.042$ nm, respectively. Note that these dimensions are greater than the value of $4$ nm typically reported in the literature. This discrepancy is caused by the overlap of $\sim 2$ nm between the monomers in their lattice configuration. The axial period of the center-to-center locations of individual monomers is $\sim 4$ nm, while their overall length is closer to $6$ nm. Stress was calculated by determining the force per unit cross-sectional area, $\sigma = F/A_0$. For $\alpha$- and $\beta$-tubulin, cross-sectional area was determined by averaging the area of three least-squares ellipses drawn about the surface in the transverse direction at the center of the structure, at $40$ and $60\%$ of the distance between the bottommost and topmost of the steered and fixed atoms. The resulting in-average transverse cross-sectional areas of $\alpha$ and $\beta$ were $25.43$ nm$^2$ and $27.88$ nm$^2$, respectively. This algorithm was applied to all structures to estimate the molecular cross-sectional area. All simulations were run at a constant temperature of $300$ K.

Stress/strain curves for the simulated tensile tests were then produced for all simulations. The qualitative behavior of each of the simulations indicate that the individual molecules respond in a manner similar to that of macro-scale material sample responds under tensile load, with the exception that slope variations associated with discrete binding events at the molecular scale are undetectable in a macroscale tensile test.

### Circumferential modulus

When a microtubule is stretched, monomers interact both axially and circumferentially. While the precise response to multiaxial loading has yet to be determined, it is assumed that tubulin monomers will exhibit anisotropic behavior based on both their antisymmetric structure and their assembly modes (18). Thus, to determine the degree of anisotropy, the tensile tests described above were repeated on all structures in the circumferential direction. The axis of applied displacement we used was chosen to simulate the forces imposed by the binding with conjoining dimers within the helical structure of the microtubule.

With a total of 538 stress/strain curves produced (269 curves for axial tensile models and 269 curves for circumferential tensile models), we plotted our predicted elastic modulus values against the following physical parameters as determined by Tuszynski et al. (19); net dipole moment; net charge; volume; and surface area. Further characteristics such as number of residues, cross-sectional area, number of atoms, homology similarity, and percent distribution of each individual amino acid were also plotted as a function of the axial elastic modulus. Linear regression statistics demonstrated that, while none of these characteristics produced any observable trends, one prominent trend was an inverse correlation between axial stiffness and axial length.

### Polyglycine simulations

To test the effects of simulation size on elastic modulus results, we also performed identical simulations on both linear and helical oligomeric glycine chains of lengths ranging from $10$ Å to $500$ Å. The first and last group of residues in the structure was deemed as fixed and steered atoms. The simulation directed a linear displacement along the axial direction of the glycine chain. These simulations were used to determine whether long-range electrostatic interactions contributed significantly to the simulation energy. Specifically, as the chains are stretched, covalent interactions dominate electrostatic interactions. Additionally, increasing the chain length of an oligomeric structure in vacuo was expected to artificially stiffen the structure as more residues are added, since additional residues added to either end may still interact with interior residues. This trend is expected to continue until a length is reached at which these boundary conditions become less prevalent.
RESULTS

Axial modulus

As seen in the stress/strain curves in Figs. 4b and 5, our simulations demonstrate a failure curve reminiscent of polymerlike failure curves. There is an elastic region from 0 to 0.350 strain, followed by plastic deformation from 0.350 to 0.475 strain, and ultimately failure above 0.475 strain. These particular values are unique to the bovine β-tubulin structure. However, this overall shape was demonstrated by both the α- and β-template 1JFF monomers. In nature, a strain of 0.3 or greater is highly unlikely to ever occur. However, as microtubules have recently been used as potential components for nanomachinery (e.g., (43,44)), this may become a critical design parameter.

The axial modulus for each monomer was calculated in a manner similar to those outlined by Shah (45). For α-tubulin, the modulus was 12.51 pN/A˚² (1.25 GPa). For β-tubulin, the modulus was 13.35 pN/A˚² (1.34 GPa). These values agree well with other recent AFM and finite element analysis results that predict the modulus to be ~1.4 GPa (46). To evaluate whether our predicted elastic moduli agree with recently measured mechanical properties of single microtubules, we developed a beam-mechanics model wherein each monomer was given a spring constant, $k$, based on its predicted modulus, $E$, its area, $A$, and its length, $L$, via $k = EA/L$ (see Appendix). We also assigned spring constants to the α-β binding site and the β-α binding sites, giving them values 0.1, 1.0, and 10 times that of the monomer stiffness. For these values, we found persistence lengths of 0.4, 2.3, and 4.1 mm, respectively. This agrees remarkably well with the recent empirical results of Pampaloni et al. (47), who found MT persistence lengths to range between 0.2 and 5 mm for MTs ranging in length from 2 to 40 μm.

To quantify correlation between monomer geometry and elastic modulus, we plotted all moduli as a function of monomer length (Fig. 6). These data are summarized in Table 2. Our primary finding was that as monomer axial length increased, axial stiffness decreased. The regression lines for the α- and

FIGURE 4  (a) Incrementally stretched structure of 1JFFB ($\sigma$, stress; $\varepsilon$, strain). (A) $\varepsilon = 0.00$, $\sigma = 0.00$ MPa; (B) $\varepsilon = 0.041$, $\sigma = 210$; (C) $\varepsilon = 0.083$, $\sigma = 323$; (D) $\varepsilon = 0.124$, $\sigma = 522$; (E) $\varepsilon = 0.166$, $\sigma = 735$; (F) $\varepsilon = 0.207$, $\sigma = 1005$; (G) $\varepsilon = 0.248$, $\sigma = 1107$; (H) $\varepsilon = 0.290$, $\sigma = 1326$; (I) $\varepsilon = 0.331$, $\sigma = 1528$; and (J) $\varepsilon = 0.372$, $\sigma = 1567$. (b) Stress/strain plot for 1JFFB.
Typical bond energies are $-9.1 \times 10^{-21}$ to $-2.4 \times 10^{-20}$ J laterally and $-2.8 \times 10^{-20}$ to $-3.9 \times 10^{-20}$ J longitudinally (48). The typical work-to-failure of most our model systems were $-5.1 \times 10^{-18}$ J for $\beta$ and $-3.0 \times 10^{-18}$ J for $\alpha$. This is consistent with the observation that microtubule failure occurs between, rather than within, monomers.

**Circumferential modulus**

Elastic moduli in the circumferential direction were approximately one-third of those in the axial direction. To our knowledge, this is the first report of tubulin anisotropy at the tertiary level. We found an average circumferential elastic modulus of 935.6 MPa for $\alpha$ and 658.4 MPa for $\beta$ across all structures. The circumferential elastic moduli of the $\alpha$, $\beta$, and $\gamma$ yielded no discernible trends as a function of axial length, circumferential length, cross-sectional area, volume, net charge, net dipole moment, residue fraction, number of atoms or number of residues—i.e., regression statistics demonstrated no significant correlation between the properties predicted by Tuszynski and monomer length. The results for circumferential modulus as a function of circumferential length are shown in Fig. 7 and summarized in Table 3.

Since we performed simulated stretching on the flat rather than the curved form of tubulin, the question remains open as to whether our results would be similar if the curved form found in MTs were to have been used. Paramount in this consideration is whether the superposition principle of mechanics (49) may be applied to MD. The superposition principle, as it applies to beam equations, states that the stress or strain state resulting from the three primary modes of loading (tension/compression, bending, or torsion) may be calculated separately and summed to find the overall state of the system. For example, if a beam is loaded in pure tension and subsequently in bending, the resulting stress state is the sum of the two. To our knowledge, such an investigation has not been undertaken in the MD literature, but deserves investigation.

**Polyglycine simulations**

The polyglycine control simulations resulted in an inverse trend: longer structures were stiffer than shorter structures and approached an asymptote near a length of 75 Å. This effect is attributable to long-range interactions among individual atoms in the simulation, i.e., central atoms are affected by a greater number of boundary atoms, but as the fraction of...
boundary atoms diminishes, so does this effect. This correlation stood in direct contrast to the inverse correlation between axial stiffness and axial length, thus bolstering the validity of our approach.

**DISCUSSION**

We have used a molecular mechanics approach to perform tensile tests on individual tubulin monomers and determined values for elastic moduli for all currently known complete sequences. The results obtained from the simulations for each species were tabulated for cross-species comparisons. Sequences were chosen by Keeling and Doolittle, who demonstrated the divergent evolution of tubulin structures (50). Carpenter et al. (51) built upon Keeling and Doolittle’s homology models, calculating structural and physical properties for >300 sequences, noting that a large fraction of these monomeric structures were incomplete. We have found that the axial modulus of elasticity decreases as a function of monomer length, whereas the circumferential modulus showed no such trend.
One potential limitation of our approach is that since we used bovine tubulin as our template structure, the possibility exists that our predicted structures likely had conformations similar to that of the template, and that this may have resulted in our predicted structures being confined to a local energy minimum rather than the global energy minimum. Restated, the method we chose for energy minimization is likely to have found the energy minimum closest to that of the bovine tubulin. The possibility exists that we did not find the global minimum. Other methods, such as the conformational space annealing genetic algorithms, have been shown to more efficiently and effectively find global minimums (53,54). However, what has not yet been determined is whether the predicted global minimum represents the in vivo state of the protein. Thus, finding a global minimum, while certainly providing an unequivocal standard for protein structure prediction, to our knowledge, has yet to be systematically compared to in vivo protein structure.

We also found reasonable agreement between the predicted moduli of the monomers simulated and the global behavior of individual MTs (47). One limitation of our beam analysis is that we did not include a separate stiffness for the axial monomer-monomer bonds versus the dimer-dimer bonds. Since the native form of tubulin in the cell is dimeric rather than monomeric, it is likely that the monomer-monomer bond is stiffer than the dimer-dimer bond. However, in our order-of-magnitude approximation (Figs. 8 and 9), varying this stiffness from 0.1 to 10 times that of the predicted stiffness of individual monomers resulted in persistence length predictions all within the recent experimental results of Pampaloni et al. (47). Additionally, since the binding stiffness at the seam of the microtubule may have an energy different from that between the other filaments, this may have an effect on the MT-scale mechanical behavior. This is likely to manifest itself if shear interactions are accounted for. In our first-order analysis, we only considered axial interactions. An analysis that does include shear interactions (e.g., (47)) may benefit by assigning a separate shear modulus to this portion of the structure.

Unfortunately, no other empirical three-dimensional atomistic models of tubulin species exist. Previous studies, such as Tuszyński et al. (19), used software such as MODELLER to create the homologous structures to the template protein. However, because of the large number of structures under investigation in our study, we decided to use protocol SWISS-MODEL because of its known speed and accuracy. An additional limitation of our study is that most of the high-resolution structures have been determined from crystalline preparations and are likely different from the native tubular form. However, since it is likely that tubulin oscillates about some minimal energy tertiary conformation in vivo, it seems reasonable to use the models generated by SWISS-MODEL (55) as approximations to demonstrate trends in stiffness behavior.

Presumably, as tubulin evolves, it performs a balancing act by maintaining a sequence that allows it to not only attain a structure that is mechanically the most efficient for sustaining compressive loads (i.e., a hollow cylinder) but also allows for rapid assembly and disassembly. Through evolution, the sequences within each species it serves change in a combination of ways that nature deems as either beneficial or detrimental, as it meets, or fails to meet, demands from external pressures (e.g., (19)). Through an intergenomic mechanical analysis such as ours, a demonstration of how evolution has affected the structure and strength of this protein may become possible. For example, by further analyzing the positions within the phylogenetic tree of tubulin sequences and the tubulin’s mechanical characteristics, a clearer picture emerges of what specific key mutations may have occurred to meet new demands. These techniques may also enable engineering of the tubulin sequence and thus the monomer structure to modify microtubule polymerization and mechanical loading characteristics.

It is important to note that the accuracy of the results depend greatly on the original PDB structure. With this in mind, these simulations do offer an approximate model to in situ behavior while offering insight into mechanical properties as well as overall trends. For example, we anticipate that, once
more-complete data is reported on the complete sequences of all tubulin-expressing organisms, mechanical characteristics may help explain why a microtubule primarily used for mitosis in one organism, may have different mechanical properties than one used primarily for locomotion in another. We hope that, eventually, an approach such as ours, augmented by more advanced knowledge of additional structures as well as the inclusion of explicit water and a more effective energy minimization technique such as conformational space annealing, may begin to elucidate how tubulin’s ancestor, FtsZ (56), evolved through various species to obtain its present form. We also hope that an analysis such as ours may be used to engineer novel tubulin structures for advanced nanotechnological devices (e.g., (43,57)). We are optimistic that this intergenomic approach may open the door to bulk modeling of multiple protein systems and homologs, across other structural proteins such as collagen, or other organellar structures or DNA-binding proteins, etc.

### Table 3: Tabular data of all circumferential moduli

| Alpha       | Beta       | Gamma     |
|-------------|------------|-----------|
| IJFFA       | 1038       | 460       |
| TBA_AVESA   | 1126       | 503       |
| TBA_BOMO    | 1111       | 524       |
| TBA_CANAL   | 1007       | 729       |
| TBA_CHLVU   | 591        | 574       |
| TBADICDI    | 380        | 553       |
| TBA_EUGR    | 920        | 799       |
| TBA_EUPOC   | 755        | 456       |
| TBA_EUPVA   | 829        | 695       |
| TBA_HAECO   | 817        | 682       |
| TBA_MYCGR   | 715        | 557       |
| TBA_NOTVI   | 831        | 633       |
| TBA_OCTDO   | 936        | 717       |
| TBA_OCTVU   | 919        | 657       |
| TBA_OnCke   | 872        | 612       |
| TBA_Oxygr   | 988        | 617       |
| TBA_Pig     | 812        | 704       |
| TBA_Plafk   | 851        | 591       |
| TBA_Playo   | 897        | 694       |
| TBA_Produ   | 928        | 786       |
| TBA_Sorma   | 840        | 712       |
| TBA_TetPy   | 654        | 686       |
| TBA_TetTh   | 898        | 622       |
| TBA_Torma   | 838        | 671       |
| TBA_Toxgo   | 692        | 209       |
| TBA_TryB    | 738        | 590       |
| TBA_Trycr   | 943        | 464       |
| TBA_Wheat   | 1075       | 913       |
| TBA_Xenla   | 897        | 508       |
| TBA_AneP    | 988        | 452       |
| TBA_Arath   | 765        | 726       |
| TBA_Chick   | 336        | 722       |
| TBA_Chllre  | 1012       | 561       |
| TBA_Drome   | 1322       | 658       |
| TBA_EleIn   | 1252       | 565       |
| TBA_Emen    | 895        | 688       |
| TBA_EntH   | 1021       | 517       |
| TBA_Homam   | 1121       | 715       |
| TBA_HorV    | 806        | 761       |
| TBA_Human   | 841        | 814       |
| TBA_Mauze   | 847        | 602       |
| TBA_Mouse   | 1258       | 1155      |
| TBA_Neucr   | 893        | 752       |
| TBA_Orysa   | 872        | 494       |
| TBA_ParlI   | 1094       | 543       |
| TBA_PeA     | 1171       | 488       |
| TBA_PeelA   | 1148       | 718       |
| TBA_Penca   | 1389       | 776       |
| TBA_Schoo   | 950        | 551       |

| Avg. 936 ± 216 | TBB_TetTh | Avg. 658 ± 134 | Avg. 741 ± 293 |
|----------------|-----------|----------------|-----------------|
| 551            | 860       | 800            | 1159            |
| 752            | 850       | 800            | 1159            |
| 775            | 850       | 800            | 1159            |
| 800            | 850       | 800            | 1159            |
| 825            | 850       | 800            | 1159            |
| 850            | 850       | 800            | 1159            |
| 875            | 850       | 800            | 1159            |
| 900            | 850       | 800            | 1159            |
| 925            | 850       | 800            | 1159            |
| 950            | 850       | 800            | 1159            |
| 975            | 850       | 800            | 1159            |
| 1000           | 850       | 800            | 1159            |
| 1025           | 850       | 800            | 1159            |
| 1050           | 850       | 800            | 1159            |
| 1075           | 850       | 800            | 1159            |
| 1100           | 850       | 800            | 1159            |
| 1125           | 850       | 800            | 1159            |
| 1150           | 850       | 800            | 1159            |
| 1175           | 850       | 800            | 1159            |
| 1200           | 850       | 800            | 1159            |

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mens typically are weaker than smaller ones (58). This may be explained through a weakest-link analogy, whereby the more molecular bonds that are added to a structure, the more likely it becomes that a weaker bond will be added. In this work, this statistical explanation may also explain why a more compliant structure is created as additional binding sites are added. Of particular interest may be the investigation of evolutionary trends that drove tubulin to its current state as it evolved to support its myriad of mechanical roles (59,60).

Future work will include using values obtained for the elastic moduli and incorporating them into a finite element model to perform bending and buckling tests (e.g., (61)). We will assume the microtubule to be a fully stable polymerized chain. We will use the commonly accepted 13:3 lattice structure; 13 dimers with a helical pitch of 3 per complete revolution; and assemble the dimers assuming the central axis of the microtubule to be straight (62). The radius of the tube will be set to 11.2 nm (63). While the data shown in this work are for tension only, we realize that compression and torsion are also important loading modes and will be modeled in future simulations. As the mechanical properties of the different types of microtubules are determined, additional microtubules will be incorporated into the simulation. In addition, these simulations were performed in vacuo. In vivo fluid interactions may have a small but significant impact on results (e.g., (64,65)). Dimer-dimer interactions are also an important consideration (shear, multiaxial loading, etc.). Future work will include simulation of dimer structures, and ultimately the superquaternary structure of microtubules themselves.

**APPENDIX: RELATION OF MONOMER MODULUS**

**$E_{\text{MONO}}$ TO MICROTUBULE MODULUS $E_{\text{MT}}$**

Typically in composite or multiscale structures, the smaller subunits tend to be stronger and stiffer than the macroscale structure (e.g., (66)). If the predicted moduli determined by our method are to inform the tubulin-scale behavior, a multiscale approach is warranted. Beginning with the length-dependent persistence length measurements recently completed by Pampaloni et al. (47), we may make an estimate of the axial elastic modulus (Young’s modulus) of a microtubule and compare it to our results. The persistence length, $l_p$, of a molecule is defined as

$$l_p = \frac{EI}{k_B T},$$

where $E$ is Young’s modulus of elasticity, $I$ is the second moment of inertia, $k_B$ is Boltzmann’s constant, and $T$ is temperature in Kelvins. An intuitive way to interpret this relationship is that $l_p$ represents the ratio between the order-preserving $EI$ of the numerator and the disorder-maintaining $k_B T$ of the denominator. The numerator has dimensions of energy × length, while the denominator has dimensions of energy, resulting in a characteristic length that predicts how closely correlated the position of one end of a molecule (or supermolecular structure in the case of a microtubule) is with the other end. The persistence length of individual microtubules has been reported to be

![Microtubule persistence length](image)
5 mm for microtubules with contour lengths of 40 μm, and close to 100 nm for microtubules with contour lengths <3 μm. Solving Eq. 3 for $E$ and using $D_0 = 25$ nm, $D_3 = 10$ nm, $k_B = 1.38 \times 10^{-23}$, $T = 310 \text{K}$, and $l_p = 100$ nm to 5 mm, results in a predicted $E_{MT}$ of 22.9 kPa to 1.14 MPa, or 3–5 orders-of-magnitude less than the $E_{nano}$ found in our study. Thus it is likely that the binding both between and within dimers govern the microtubule’s behavior. A discrete model that models spring constants of individual monomers and the spring constants of their binding follows.

The beam-bending moment equation is

$$M_i = E l \kappa,$$  \hspace{1cm} (4)

where $M_i$ is the bending moment about the radial axis, and $\kappa$ is the beam curvature, with dimensions of length $^{-1}$. I.e., $\kappa = 1/\rho$, where $\rho$ is the radius of curvature at the center of the microtubule. Eliminating $EI$ between Eqs. 3 and 4 results in

$$l_p = \frac{M_i}{k_B \rho T}.$$  \hspace{1cm} (5)

The next challenge is to relate the bending moment, $M_i$, acting upon the microtubule to its curvature. The moment may be taken as the sum of all of the individual forces acting within each filament as

$$l_p = \frac{\sum_{i=1}^{13} \kappa_i}{k_B T},$$  \hspace{1cm} (6)

where $\kappa_i$ takes on the values of $R \sin \theta_i$, where $R$ is the effective radius of the microtubule ~10.5 nm and $\theta$ is the circumferential position of the individual filaments, i.e., $\theta = 0, 2\pi/13, 4\pi/13, \ldots, 24\pi/13$. The value $\kappa$ has become discretized, since each filament’s curvature differs, those being in compression having a greater curvature than those in tension. The force in each filament is shared by each α-subunit and each β-subunit as well as by the $\alpha$-β bond and $\beta$-α bonds. Expressing $P$ as a function of total bending-displacement of each of these, $\Delta = \Delta_k + \Delta_p + \Delta_{\alpha\beta} + \Delta_{\beta\alpha}$ and the spring constant of each $k_{\alpha\beta}, k_{\beta\alpha}, k_{\kappa\alpha},$ and $k_{\kappa\beta}$, results in

$$l_p = \frac{\sum_{i=1}^{13} \kappa_i}{k_B T},$$  \hspace{1cm} (7)

where $K' = 1/k_{\alpha\beta} + 1/k_{\beta\alpha} + 1/k_{\kappa\alpha} + 1/k_{\kappa\beta}$. Assuming a consistent curvature, $\kappa$, throughout the MT, the individual displacement, $\Delta_k$, of each monomer reduces to $k_B R \Delta_k$, where $L_0$ is a dimer length, $R$ is the average radial distance of a monomer from the center of the MT, and $k_i$ is the curvature of the $i$th filament ($i = 1 \ldots 13$). The spring constants, $k_{\alpha\beta}$ and $k_{\beta\alpha}$, in units of N/m, may be taken directly from the simulation data and were $-5$ N/m. Since the spring constants for the $\alpha$-β bonds and $\beta$-α bonds are not known, we may use these as the independent variables to help determine the contribution individual monomer stiffness makes to MT stiffness. The most straightforward way to do this is through the persistence length,

$$l_p = \frac{\sum_{i=1}^{13} R^2 k_i \sin \theta_i}{k_B T}.$$  \hspace{1cm} (8)

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