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Tubulin: from atomistic structure to supramolecular mechanical properties

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Abstract Microtubules (MTs) are fundamental structural elements in the cytoskeleton of eukaryotic cells. Their unique mechanical properties depend on the properties of the tubulin dimer, its interactions with surrounding dimers and the geometric organization within the MT. While the geometry has already been well described in experimental works, the mechanical characteristics of the dimer as well as of the individual monomers have up to date not been described. These may therefore provide new, additional insight to the microtubule tensile properties. In this paper we construct a mesoscale model of MT with a bottom-up approach. First, we evaluate the elastic constants of each of the two monomers together with the interaction force between them by means of molecular dynamics (MD) simulations carried out in an explicit water environment. Using the MD results, we model a 1 μm long MT as a cylinder constituted by interacting elastic elements and examine its properties via finite element method (FEM). The obtained results show an elastic constant value for α-tubulin of 11 N/m, while for the β-tubulin the elastic constant was measured to be 15.6 N/m. Concerning interactions between neighbouring monomers, the elastic constants along the protofilament (45 N/m for the intra-dimer interface and 18 N/m for the inter-dimer interface) are more rigid than elastic constants calculated for lateral interfaces (11 and 15 N/m). The mesoscale model provides mechanical properties of the whole MT, thus allowing the comparison with data obtained by other previous experimental and theoretical studies. We report here a Young modulus of 1.66 GPa for the MT under axial tension. In perspective our approach provides a simple tool for the analysis of MT mechanical behaviour under different conditions.

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Introduction

Microtubules (MTs) are long, hollow cylinders made of protofilaments which bind together laterally along the long axis of the cylinder and form the microtubule wall [1]. The elemental base of the protofilaments is the tubulin heterodimer which consists of an α- and a β-tubulin monomer. Both are globular molecules with a relatively high degree of similarity from the primary to the tertiary structure level. Their orientation in the dimer is almost the same, and stacked together on top of each other as they are in the protofilament they seem very alike [2]. However, the structures do also have individual features that separate them. One important difference is that in the MT the α-monomer binds a GTP molecule while the β-monomer binds a GDP molecule.

Different MT configurations exist depending on the number of protofilaments included in the MT wall. The number of protofilaments in a MT observed in-vivo and in-vitro varies widely, from 8 to 19 [3], making the MT structure highly polymorphic. Most cellular microtubules have 13 protofilaments.

In microtubules the protofilaments bind together laterally with a slight shift generating a spiral with a pitch of 2, 3 or 4 monomers' length [3]. For MTs with 3 start-helices, the neighbouring monomers form spirals of all-α- and

all-β-tubulins except at the seam of the microtubule where α-tubulins bind laterally to β-tubulins and vice versa. For MTs with 2 or 4 start-helices, there is no seam and the spirals consist of all-α- and all-β-tubulins.

Microtubules are essential structural elements present in all eukaryotic cells. They form motor protein "tracks" which are used for directed transport within the cell and they are part of the spindle apparatus used to move and segregate the chromosomes during cell division. They also play a purely mechanical role for the cell maintaining its shape.

Despite the many experimental and computational efforts the mechanical properties of the microtubule are still debated. The experimental methods included optical tweezers [4, 5], hydrodynamics flow [6], atomic force microscopy (AFM) [7], and thermal fluctuation and viscosity measurements [8]. The reported values of Young's modulus vary between 0.1 GPa [7] and 2.5 GPa [5]. Gittes et al. [8] estimated a length independent MT flexural rigidity of $2.15 \cdot 10^{-23}$ Nm^2 based on thermal fluctuations in shape. From this value they calculated the Young's modulus for MTs to be 1.2 GPa using the assumption that the MT is both homogeneous and isotropic in structure. The length of the MTs in the experiments ranges between 25 μm and 65 μm. MTs of this length are typically found in cilia.

It should be remarked that both the isotropy assumption and the length independence are now generally questioned. In a more recent experiment by Kis et al. [7] the elastic deformation of MTs bound to a surface with holes of different sizes was directly measured with AFM. The AFM tip was used to apply a force on the MT and the deflection was measured and used to estimate simultaneously both Young's and shear moduli. The results show a significant difference between the Young's modulus (Y) of 100 MPa and the shear modulus (G) of 1.4 MPa, evidencing that the MT is highly anisotropic.

These results indicate that the MT flexural rigidity is not length independent and consequently, it does not seem to be an appropriate parameter to describe the MT mechanical behaviour at least not for sufficiently short MTs (few μm long MTs). For short microtubules the sliding between adjacent protofilaments is relevant during MT bending. On the contrary very long MTs (25–65 μm) are more rigid and the Young's modulus dominates the mechanical behaviour since only a slight sliding occurs between adjacent protofilaments in bending [9]. Thus, the value reported by Gittes et al. [8] might result in a correct evaluation of the Young's modulus of the MT, since neglecting the shearing for sufficiently long MT merely introduces small errors.

However, even at their best, these measurements are not able to identify the origin of the shear and Young's modulus values on the basis of the different forces between and within the monomers. Therefore, they cannot provide the desired resolution at the single monomer level needed for a thorough understanding of the MTs mechanical behaviour.

On the theoretical level several attempts have been made to produce a bottom-up approach corresponding to the one here presented. Such attempts include finite element models, but so far all these models have been based on assumptions on how the interactions vary with distance [9–11]. In particular these works use reported experimental values of Young modulus and shear modulus to define the interactions between the mechanical properties of the building blocks of the MT.

In this work we use molecular dynamics (MD) to obtain mechanical characteristics of the different types of interactions present in the MT both between and within monomers. The characteristics are used to create a simple MT model with finite elements methods (FEM) from which we are able to evaluate the overall MT's mechanical properties. In particular, we demonstrate that it is possible to make a full description of the microtubule using MD combined with the available experimental data on the atomistic structure.

Methods

Molecular models and equilibration of the structures for MD simulations

With the aim to characterize the tubulin monomers and all the monomer-to-monomer interactions involved in the MT structure, we used the atomic structure of $\alpha\beta$-tubulin, 1TUB.pdb [2], available from the RSCB Protein Data Bank [12]. Due to a large variation among monomer isotypes present in the crystallographic assay a smaller part of the C-terminal is missing in both monomers. Other pdb structures exist [13] but were not taking into consideration since a larger number of amino acids are missing despite the fact that some of them have a better resolution.

The chosen structure was modified before use. A missing Mg^{2+} ion present in other structures was added based on data contained in the refined structure 1JFF.pdb [13]. Furthermore, a Taxol™ molecule was removed. Taxol™ is used as a stabilizing agent for crystal formation of the microtubules but is not present under physiological conditions.

Gromacs 3.3.1 software [14] with the GROMOS96 43a1 force field was used to perform all the simulations using cut-offs of 1 nm for non-bond interactions (van der Waals and electrostatic). The time step was set to 2 fs for all the MD simulations.

The structure was first energy minimized in vacuum before it was centred and orientated along the x-axis in a rectangular box of size 18×9×8 nm with periodic boundary conditions. The rest of the box was filled with Single Point

Charge (SPC) [15] water molecules to explicitly model water in the system. To balance the strong negative charge of the dimer and neutralize the total system charge 40 Na^+ ions were added to the solution.

The entire system was then energy minimized again and after that heated to 300 K by coupling it to an external heat bath for 35 ps [16]. Finally the system was left to equilibrate for 800 ps in order to stabilize the structure in terms of temperature and energy oscillations.

The equilibrated structure was the basis for all further dynamics done for the mechanical characterization.

Mechanical characterization of interactions between monomers

The mechanical characterization was done with MD simulations in which the interaction energy between two monomers was measured at different distances. On the basis of the previously mentioned equilibrated structure four different molecular systems were generated, each consisting of two monomers: the intra-dimer ($\alpha\beta$-interaction), inter-dimer ($\beta\alpha$-interaction) and lateral ($\alpha\alpha$- and $\beta\beta$-interaction) complex (Fig. 1).

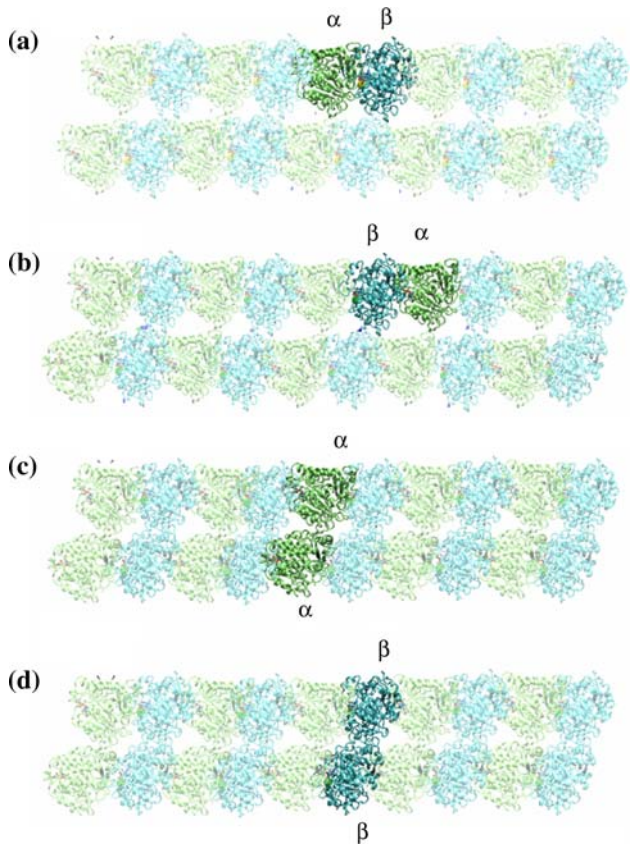

Fig. 1 Different interfaces in MT lattice: longitudinal interactions involving $\alpha\beta$-tubulin (**a**) and $\beta\alpha$-tubulin interfaces (**b**) and lateral interactions involving $\alpha\alpha$-tubulin (**c**) and $\beta\beta$-tubulin interfaces (**d**)

For each system, the interaction interfaces were identified and then created on the basis of the contact surfaces described in literature [17]. After the set up of the relative position of the two monomers, the water molecules and ions were added and each system was heated and equilibrated for 200 ps adding a position restraint in the form of a harmonic potential (V_{pr}) to each C_α-atom:

$$V_{pr}\left(r_{C\alpha,i}\right) = \frac{1}{2}k_{pr}\left|r_{C\alpha,i}(t) - r_{C\alpha,i}(0)\right|^2 \quad (1)$$

where k_{pr} is the elastic constant of the harmonic potential and $r_{C\alpha,i}$ is the position of the C_α atom of the i-th residue.

For each molecular system several configurations were generated with different distances (d) between the centres of mass (CM) of the two monomers. A pulling MD procedure was used to generate different initial configurations moving the monomers ($\Delta d = d - d_0$) along the line connecting the two CMs (Fig. 2) closer and apart with respect to the initial position (d_0).

For each distance the interaction energy term, i.e., the sum of the Coulomb and van der Waals terms, was obtained by running a MD simulation for at least 100 ps while the protein backbone structure was maintained restraining the monomer C_α-atoms. This prevents marked structural changes in the monomer shape, which are considered in the single monomer testing.

During the MD simulation, the monomer-to-monomer interaction energy and CMs distance were sampled every 0.2 ps. The last 50 ps are considered to determine the mean value of interaction energy and CMs distance for each configuration.

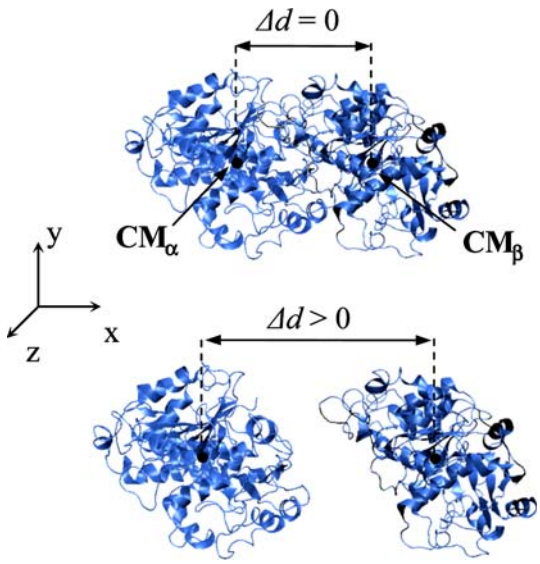

Fig. 2 Set up of the configurations with different monomer-to-monomer distances

The mean value of the interaction potential energy (V_{int}) between the two monomers as a function of the mean value of the CMs distance (d) was fitted with a 3rd order polynomial function. Consequently, the force (F_{int}) can be obtained as first derivative with the opposite sign of the interaction potential energy with respect to the distance. The elastic constant (k_{int}) is calculated as second derivative of the interaction potential energy.

Mechanical characterization of single monomers

The mechanical properties of α- and β-monomers were tested using an AFM-like approach in which springs were applied to deform the monomer (Fig. 3).

First, water and ions were removed from the equilibrated structure and two independent systems consisting of α- or β-tubulin were extracted. Single monomer systems were then solvated by adding SPC water molecules and neutralized with Na^+ ions. Then the system was energy minimized, heated to 300 K and equilibrated for 200 ps while all the C_α-atoms of the monomer were restrained (see Eq. 1).

Two pull groups (P and P') were defined, selecting C_α-atoms of the residues involved in the longitudinal interactions between adjacent monomers along the protofilament. A spring was connected to the CM of each group and characterized by an elastic constant (k_s) equal to 10 nN/nm. The spring elastic constant value was chosen on the basis of a preliminary k_s sensitivity analysis. The value of k_s = 10 nN/nm represents a good compromise between low k_s values that require very time consuming pulling simulations and high k_s values which allow faster simulations but introduce large oscillations in the force values. During MD simulation, the free end of each spring (S and S′) was moved with a constant velocity (v_s) along the x-axis corresponding to the MT longitudinal direction (Fig. 3). Compression and elongation tests were carried out for 2 ns

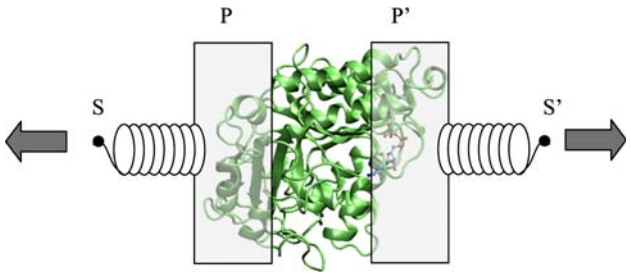

Fig. 3 Scheme of mechanical test for the monomer. The CMs of pull groups (P, P′) are each connected to a spring. Constant rate displacements are imposed to the free end of the springs (S, S′)

MD simulations moving S and S′ with a velocity equal to $5{\cdot}10^{-4}$ nm/ps.

The force $F(t)$ applied to the molecule is given by:

$$F(t) = k_s(|x_S(t) - x_P(t)| + |x_{S'}(t) - x_{P'}(t)|), \tag{2}$$

where $x_S(t)$, $x_{S'}(t)$, $x_P(t)$ and $x_{P'}(t)$ are the CM positions of S, S′, P and P′, respectively. The spring forces at the beginning of the simulation are equal to zero since the free ends of each spring and the CM of its corresponding pull group have the same position.

The deformation $\varepsilon(t)$ in elongation and compression tests was calculated as:

$$\varepsilon(t) = \frac{\Delta L(t)}{L_0} = \frac{L(t) - L_0}{L_0}, \tag{3}$$

where $L(t)$ is the monomer length as function of time calculated as the distance between the CMs of groups P and P'

$$L(t) = |x_P(t) - x_{P'}(t)| \tag{4}$$

and L_0 is the initial monomer length, i.e., for t = 0.

In all tests structure deformations of 10% were carried out.

The elastic constant (k_m) of the monomer then is given by the Hook's law:

$$k_m = \frac{F(t)}{\Delta L(t)} \quad m = \alpha, \beta \tag{5}$$

which corresponds to the slope of the F-Δl curve at the time t.

MT mesoscale model

The mesoscale model of an entire microtubule was built using a finite element (FE) approach. α-tubulin and β-tubulin monomers and all the interactions between monomers in the microtubule lattice were represented as springs. The mechanical properties were set on the basis of the results obtained with MD simulations. In this way the microtubule was simulated as a network of springs.

In order to simplify the microtubule model, thus reducing the number of elements along the protofilament, two different springs $\tilde{k}_{\alpha\beta}$ and $\tilde{k}_{\beta\alpha}$ were defined representing the mechanical behaviour of αβ interface (βα interface) together with the elastic properties of half of the related monomers (Fig. 4a, b):

$$\tilde{k}_{\alpha\beta} = \left(\frac{1}{2k_\alpha} + \frac{1}{2k_\beta} + \frac{1}{k_{\alpha\beta}}\right)^{-1} \tag{6}$$

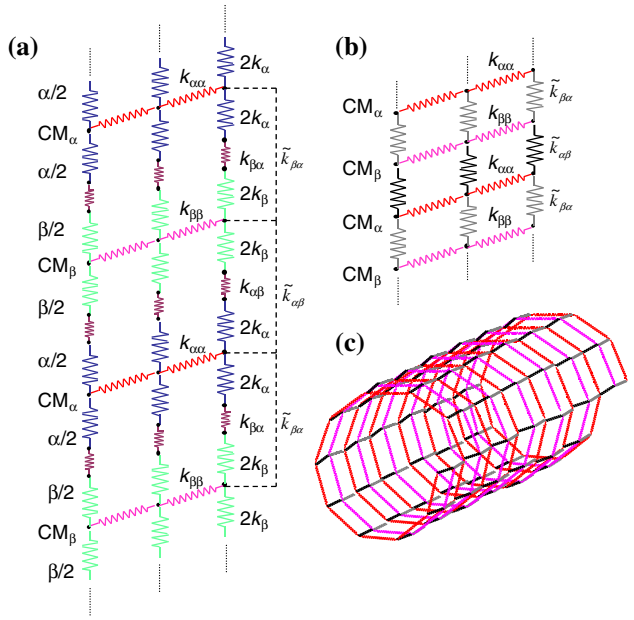

Fig. 4 Scheme of the mesoscale models. (**a**) Extended model of three protofilaments, where α-tubulin and β-tubulin monomers and all the interactions between monomers in the microtubule lattice were represented as springs. (**b**) Simplified model where the interactions along the protofilament together with related half-monomers are condensed in a spring element. (**c**) Lattice model of MT modeled as a cylinder, where tubulin monomers and their interactions are modeled as springs based on the simplified model (**b**)

$$\tilde{k}_{\beta\alpha} = \left(\frac{1}{2k_\alpha} + \frac{1}{2k_\beta} + \frac{1}{k_{\beta\alpha}}\right)^{-1} \tag{7}$$

where k_α and k_β are the elastic properties of tubulin monomers, while $k_{\alpha\beta}$ and $k_{\beta\alpha}$ are the intra- and inter-dimer interaction, respectively.

The microtubule geometry was reconstructed starting from literature data [3] concerning the permitted structures of microtubules. A 1 μm long microtubule constituted by 10 straight protofilaments with 2 start-helices (corresponding to a mismatch of 2 monomers) was built (Fig. 4c). In turn, a displacement of 0.8 nm was set between adjacent filaments. In this case, adjacent protofilaments face a sequence of $\alpha\alpha$ and $\beta\beta$ lateral contacts. Concerning lateral interactions, the behaviour of the lateral contacts was simulated by springs with elastic properties described by $k_{\alpha\alpha}$ and $k_{\beta\beta}$.

The MT, created as a cylinder, had a cross section diameter of about 14 nm, based on data provided by MD simulations. In particular, the position of the nodes of spring elements along and between protofilaments was set on the basis of the distance between the CM of α and β monomers at the minimum of potential energy calculated.

The mesoscale model of microtubules was used to simulate an axial test. The nodes of both extremities of the MT were moved in axial direction symmetrically until a total elongation of 10% was applied. During the simulation the displacement of all nodes are free to move only in the longitudinal direction.

The data obtained by the FE model were used to calculate the MT Young modulus (Y_{MT}) based on force value (F_{MT}) obtained imposing an elongation (ΔL_{MT}) of about 0.1 μm. Thus, Young's modulus was calculated using Hook's law:

$$Y_{MT} = \frac{F_{MT}}{\Delta L_{MT}} \frac{L_{MT}}{A_{MT}}, \tag{8}$$

where L_{MT} is the MT's original length and A_{MT} is the MT's cross sectional area.

To avoid the boundary effects only the central part of the microtubule was considered for data analysis. ABAQUS/Standard version 6.6-1 (ABAQUS/Standard, Hibbitt, Karlsson & Sorensen) was used to perform the numerical analyses at the mesoscale level.

Results

Equilibrated structure for MD simulation

The $\alpha\beta$-tubulin structure refined starting from 1TUB.pdb showed a potential energy of $V = -2.15 \cdot 10^6$ kJ/mol after energy minimization in solvated environment, lower than potential energy calculated for the original structure 1TUB.pdb ($V = +5.28 \cdot 10^{10}$ kJ/mol). A structure analysis with PROCHECK at this point showed that 95.1% of the residues fall in the permitted regions.

A decrease in potential energy from about $-1.76 \cdot 10^6$ kJ/mol to $-1.78 \cdot 10^6$ kJ/mol was observed in the first 200–300 ps of equilibration, then the potential energy is stabilized and the standard deviation of the potential energy is reduced to 0.1%.

Mechanical characterization of interactions between monomers

Four different interactions were investigated, namely the $\alpha\beta$-, $\beta\alpha$-, $\alpha\alpha$-, and the $\beta\beta$-interactions. As explained earlier, the $\alpha\beta$- and the $\beta\alpha$-interactions correspond to the monomer-to-monomer interactions within the dimer and between two dimers in the longitudinal direction, respectively. The $\alpha\alpha$- and the $\beta\beta$-interactions correspond to the two different types of lateral contacts.

Using the method described for interaction force calculations, potential energy (V_{int}) vs. distance (d) relations were obtained for all the considered molecular systems.

In all the considered configurations the V_{int} as a function of time resulted stable after 50 ps as reported in

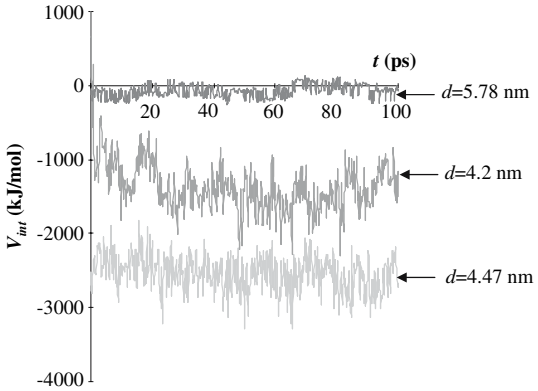

Fig. 5 Interaction energy as function of time as measured at three different distances (5.78, 4.20, 4.47 nm) for the $\beta\beta$-interaction

Fig. 5 for three of the different distances tested for the $\beta\beta$-interaction.

The averaged data points are reported in Fig. 6 and were fitted with a 3rd-order polynomial approximating the expected real energy behaviour as function of the distance (d). Different potential energy minima (V_{min}) together with the corresponding distance (d_{min}) were obtained for each of the four types of interaction as shown in Table 1. From the polynomial expression obtained by the fit forces (F_{int}) as a function of the distance (d) are reported in Fig. 7. The minimum value of the force corresponds to the maximum attractive force acting between the two monomers (F_{max}) occurring when they are a distance d_{max} apart (Table 1). The spring constants (k_{int}) were also evaluated (Table 1).

Mechanical characterization of single monomers

Output data from AFM-like MD simulations consist of the position along the x-axis of the CMs of the P, P′, S and S′ groups at 2 fs intervals. Averages over 1ps non-overlapping

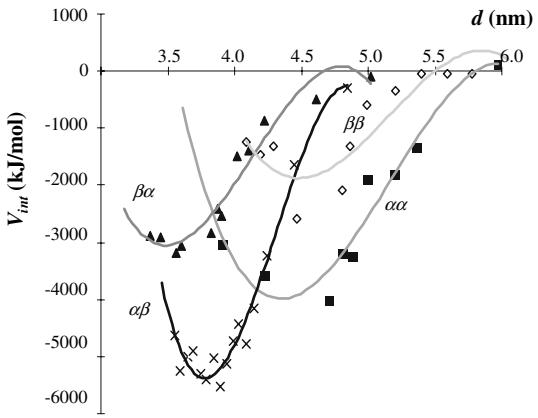

Fig. 6 Interaction energy as function of distance between monomers. Crosses: $\alpha\beta$. Triangles: $\beta\alpha$. Squares: $\alpha\alpha$. Diamonds: $\beta\beta$. The curves in grey tone scale from dark to light show the fitted lines in the same order

Table 1 Minimum interaction energies (V_{min}) together with the distance between the CM of the two monomers, maximum forces of interaction (F_{max}) between the two monomers occurring when they are d_{max} apart, and elastic constants (k_{int}) for the interaction evaluated at the interaction energy minimum distance d_{min}

	$\alpha\beta$	$\beta\alpha$	$\alpha\alpha$	$\beta\beta$
V_{min} (10^3 kJ/mol)	–5.38	–3.05	–4.00	–1.89
d_{min} (nm)	3.78	3.48	4.35	4.48
F_{max} (10^3 pN)	11.9	6.0	6.3	4.1
d_{max} (nm)	4.31	4.14	5.16	5.17
k_{int} (N/m)	44.7	18.3	15.5	11.9

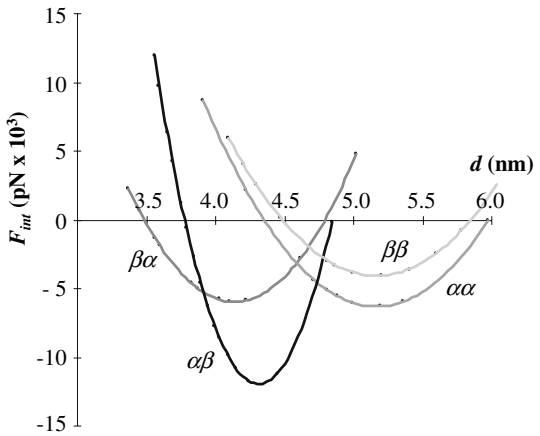

Fig. 7 The curves in grey tone scale from dark to light show $\alpha\beta$, $\beta\alpha$, $\alpha\alpha$, and $\beta\beta$ monomer-to-monomer interaction forces, respectively, as function of distance. Curves are derivative of the fitted 3rd order polynomials (Fig. 6.)

blocks were used to reduce the large number of untreated output data from the simulations.

For the two pull groups together with the springs' free ends, the relative position with respect to the initial position plotted as function of time is shown in Fig. 8. Due to thermal motion the relative positions of P and P′ fluctuate as the groups move apart. The elongation of each spring over time is given by the difference between the position of the free end of the spring and the corresponding pull group.

The monomer contraction and elongation ($\Delta l(t)$; Eq. 3), and the force ($F(t)$; Eq. 2), imposed by the spring were calculated based on the filtered data from the simulation output. The curves showing $F(t)$ versus $\Delta l(t)$ for α-tubulin and β-tubulin in both pulling and compression tests are reported in Fig. 9. Positive $\Delta l(t)$ values imply an elongation of the monomer while negative $\Delta l(t)$ values correspond to a compression.

A straight tendency line is superimposed on the $F(t)$–$\Delta l(t)$ curves for each monomer. Based on Eq. 5, the

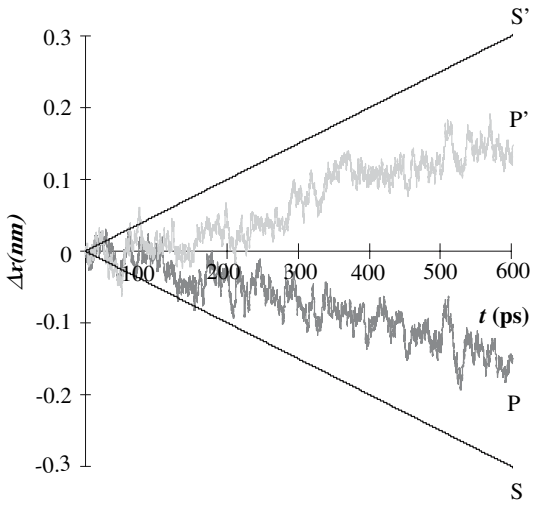

Fig. 8 Relative motion ($x(t)$) of the two pull groups (P, light grey; P′, dark grey) and the one free spring end as a function of time during the α-tubulin elongation test (black)

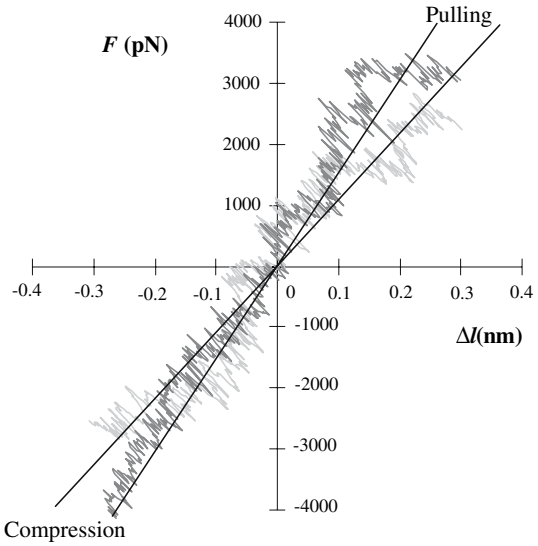

Fig. 9 $F(t)$–$\Delta l(t)$ curve obtained for α-tubulin (light grey) and β-tubulin monomers (dark grey). Tendency lines (black) are superimposed on the curves and their slope represents the elastic behaviour of the monomers

slope of the tendency lines provides the elastic constant values $k_\alpha = 11$ N/m for the α-monomer model and $k_\beta = 15.6$ N/m for the β-monomer model.

The β-tubulin model seems to be slightly more rigid than the α-tubulin model, despite the monomer similarity. However, the value of k_m is affected by the total elongation considered for the analysis. In particular, considering a strain of the monomer up to 5% in elongation and compression the elastic constant value for both α- and β-tubulin is in the range 15–16 N/m.

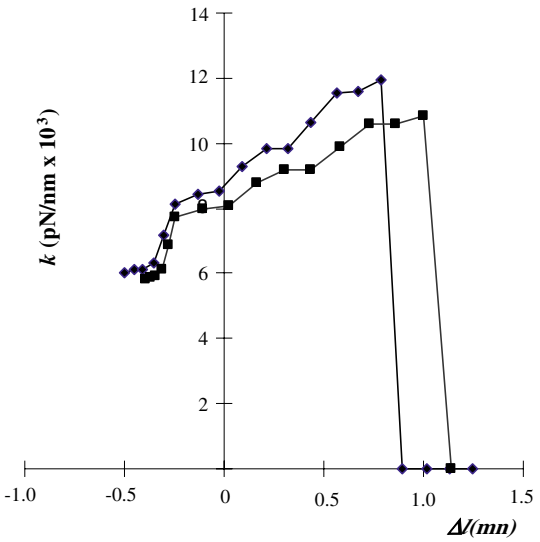

Fig. 10 Mechanical properties of the spring elements along the protofilaments calculated on the basis of MD data ($\tilde{k}_{\alpha\beta}$ (diamonds) for the inter- and $\tilde{k}_{\beta\alpha}$ (squares) for the intra-dimer interactions)

MT mesoscale characterization

Mechanical properties of the spring elements representing the single monomer deformations and all monomer interactions were defined based on the elastic behaviour obtained by MD. Figure 10 shows the curves used as the input parameters, $\tilde{k}_{\alpha\beta}$ and $\tilde{k}_{\beta\alpha}$, in the FE model for the elastic behaviour along the protofilament.

The axial test force-elongation curve for a 1 μm long MT elongated until 10% shows a linear behaviour with slope 335 pN/nm.

The Young modulus (Y) was calculated assuming MT as a hollow cylinder with a cross section of 206.5 nm^2 based on force and displacement data at 10% of MT strain. The cross-section was estimated setting a radial dimension of 4.6 nm for tubulins disposed on a circumference with 14.3 nm of mean diameter. Under these hypotheses Y results to be equal to 1.66 GPa, which is in agreement with data reported in literature [4–8].

Discussion

The aim of the present paper is to increase the understanding of the MT's mechanical behaviour starting from available knowledge about the dimer's molecular structure and the geometry of the MT lattice. A new bottom-up hierarchical approach was developed. The basic building blocks and their interaction interfaces were identified considering the structure of the MT as reported by electron crystallography analyses [2, 13]. The mechanical properties of the building blocks and their interactions were calculated

through MD simulations. The results together with the data on the permitted MT lattice structure obtained from literature [3] were used as input for a FE model. Using advanced FE structural code, a spring elements' model was used to investigate the overall MT behaviour under axial tension. The results obtained are directly comparable to results from MT experiments [4–8]. In contrast, no direct experimental measurements on the dimer are available for comparison. Previously, experimental works have estimated the dimer's mechanical properties starting from the MT structure [7, 8]. Apart from the difficulties in separating the dimer's mechanical properties from the lateral and inter-dimer interactions, these experiments cannot provide detailed knowledge about the individual MT building blocks and their specific interactions. The present work in contrast takes the investigation of the MT to the molecular level.

Also our model has some limitations. The single monomer AFM-like measurements are done at a particular velocity. However, globular protein structures are known to have viscoelastic properties, consequently the strain rate imposed can influence the obtained results. Strain rates imposed in typical AFM pulling/compression experiments on proteins are in the order of 10^{-9} nm/ps [18, 19], while we impose a velocity of $5{\cdot}10^{-4}$ nm/ps.

However a preliminary analysis aimed at evaluating how the velocity influences the obtained elastic constant values (data not published) resulted in no substantial differences between force values (and thus in elastic constant values) calculated at a strain rate of 10^{-3} nm/ps with respect to force values calculated at $5{\cdot}10^{-4}$ nm/ps.

The atoms chosen for the pull groups and the use of the position restraints introduce some arbitrary choices that require further discussion. Different parts of the monomer's surface structure have different mechanical properties, and this is also valid for different parts of the pull group which contains loops and regions weaker than others. Without taking this into account and without a proper pull group definition a pulling approach would only report the mechanical behaviour of the weakest region of the surface. In our work we try to estimate an average behaviour of the whole monomer by maintaining the interface surface fixed. The position restraints used serve precisely this purpose.

Our approach differs from experimental AFM measurements [18] and simple AFM simulations with the spring attached to a single atom [19, 20]. In these studies force values are typically of order 100 pN, while we report values of about 1 nN. Preliminary tests demonstrated that the choice of the C_α-atoms of residues used to set the pull groups (P and P′) influences the output force values. In particular, moving the entire interface instead of a single atom (as in typical AFM simulations) results in higher calculated force values.

Limitations in the method used to obtain the interactions between monomers include the positioning and interaction path used. Different permitted MT configurations like 10:2 or 13:3 should ideally be constructed with different positions and interaction paths of the monomers. Simplifying, we study only the interactions along two perpendicular paths: along the protofilament and laterally. In correspondence, the original structure, 1TUB.pdb, taken from the RCSB Protein Data Bank was obtained from protofilaments in the 2-dimensional planar zinc-sheet configuration. A full investigation in 3 dimensions could be interesting for a later model refinement.

In order to measure the interaction energy a novel approach was used rather than a constrained potential of mean force (PMF). Preliminary docking tests demonstrated that using a distance constraint method or a harmonic umbrella potential acting on the CMs of the monomers has limitations in our case due to the globular shape and size of the investigated molecules. Indeed when arranging the two monomers very close to each other (e.g., with a harmonic umbrella potential acting on each monomer's CM) their structures, squeezed by the high repulsion forces, widely deformed during the simulation. In this way the real distance between the outer surfaces of the two docked monomers cannot be controlled.

Our approach, which restraints each C_α-atom (resulting in a backbone restraint) allowed the structure of each monomer to maintain its shape throughout the whole simulation while lateral groups of each residues on the surface moved and rearranged their positions. The spring constants describing the interactions between the monomers correspond with knowledge about the interactions as described in literature [2, 17] and with experimental evidence concerning the dynamic instability of the MT structure [21]. The general opinion is that during the MT's disassembly, protofilaments break the lateral contacts between the tubulin dimers and peel off from MT's structure. Subsequently, free protofilament segments then break at the inter-dimer interfaces splitting into $\alpha\beta$-tubulin dimers. On the contrary, the $\alpha\beta$-tubulin dimer is known as a very stable structure and can be dissociated only by means of severe chemical treatments [1]. In accordance, our results show that lateral (F_{max} = 6.3 nN for $\alpha\alpha$-interaction and F_{max} = 4.1 nN for $\beta\beta$-interaction) and inter-dimer (F_{max} = 6.0 nN) interactions are much weaker than the interaction between the monomers within the dimer (F_{max} = 11.9 nN). In addition, we note that no substantial difference is found between the values measured for lateral and inter-dimer interactions. Not surprising the energy needed to pull the two monomers within the dimer apart ($\alpha\beta$-interaction) is much greater than the energy needed to pull any of the other dimers apart (Table 1).

It is important to observe that the force-distance relations reported in Fig. 7 were calculated based on interaction

potential energy curves fitted with a 3rd order polynomial function. With this approximation, the force-distance curves are valid around the minimum value and are not applicable far from this minimum value. In particular, it is clear that there is no repulsion force when the monomers are far apart, and the force should be go zero for large distances.

The mesoscale model here developed is based on a simple representation of the MT structure. Indeed, the monomers' deformations and interactions are accounted for by means of mono-dimensional elements. The mesoscale model of the MT does not include thermal fluctuations. However considering the persistence length of the MT about 1–6 mm [1] the bending effect of thermal fluctuations on 1 μm long MT can be neglected.

The FE analysis of a 1 μm long MT under axial tension allowed calculating mechanical characteristics of an entire MT. The Young modulus obtained using our bottom-up approach is 1.66 GPa in agreement with data reported in literature. Up to date, all the experimental works in the past produced a wide range of values for mechanical characteristics of the microtubule (0.1–2.5 GPa) [4–8], and the most of these studies reported a Young modulus of 1–2 GPa.

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References

1. Howard J (2001) In: Mechanics of motor proteins and the cytoskeleton. Sinauer, Sunderland, pp 119–134
2. Nogales E, Wolf SG, Downing KH (1998) Nature 391:199
3. Chrétien D, Wade RH (1991) Biol Cell 71:161
4. Felgner H, Frank R, Schliwa M (1996) J Cell Sci 109:509
5. Kurachi M, Hoshi M, Tashiro H (1997) Cell Mot Cyt 38:201
6. Venier P, Maggs AC, Pantaloni D (1994) J Biol Chem 269:13353
7. Kis A, Kasas S, Babi B, Kulik AJ, Benoît W, Briggs GAD, Schönenberger C, Catsicas S, Forró L (2002) Phys Rev Lett 89:248101
8. Gittes F, Mickey B, Nettleton J, Howard J (1993) J Cell Biol 120:923
9. Kasas S, Kis A, Riederer BM, Forró L, Dietler G, Catsicas S (2004) Chem Phys Chem 5:252
10. Jánosi IM, Chrétien D, Flyvbjerg H (2002) Biophys J 83:1317
11. VanBuren V, Cassimeris L, Odde DJ (2005) Biophys J 89:2911
12. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE (2000) Nucl Acid Res 28:235
13. Löwe J, Li H, Downing KH, Nogales E (2001) J Mol Biol 313:1045
14. Lindahl E, Hess B, van der Spoel D (2001) J Mol Mod 7:306
15. Berendsen HJC, Postma JPM, van Gunstersen WF, Hermans J (1981) In: Interaction model for water in relation to protein hydration. Reidel Publishing Company Dordrecht, Dordrecht, p 331
16. Berendsen HJC, Postma JPM, DiNola A, Haak JR (1984) J Chem Phys 81:3684
17. Nogales E, Whittaker M, Miligan RA, Downing KH (1999) Cell 96:79
18. Lenne PF, Raae AJ, Altmann SM, Saraste M, Hörber JKH (2000) FEBS Lett 476:124
19. Altmann SM, Grünberg RG, Lenne PF, Ylänne J, Raae AJ, Herbert K, Saraste M, Nilges M, Hörber JKH (2002) Struct 10:1085
20. Soncini M, Vesentini S, Ruffoni D, Orsi M, Deriu MA, Redaelli A (2006) Biomech Model Mechanobiol Nov 18;[Epub ahead of print]
21. Erickson HP, Stoffler D (1996) J Cell Biol 135:5