Thermal fluctuations of grafted microtubules provide evidence of a length-dependent persistence length

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Communicated by Robert H. Austin, Princeton University, Princeton, NJ, May 13, 2006 (received for review June 25, 2005)

Microtubules are hollow cylindrical structures that constitute one of the three major classes of cytoskeletal filaments. On the mesoscopic length scale of a cell, their material properties are characterized by a single stiffness parameter, the persistence length $\ell_p$. Its value, in general, depends on the microscopic interactions between the constituent tubulin dimers and the architecture of the microtubule. Here, we use single-particle tracking methods combined with a fluctuation analysis to systematically study the dependence of $\ell_p$ on the total filament length $L$. Microtubules are grafted to a substrate with one end free to fluctuate in three dimensions. A fluorescent bead is attached proximally to the free tip and is used to record the thermal fluctuations of the microtubule’s end. The position distribution functions obtained with this assay allow the precise measurement of $\ell_p$ for microtubules of different contour length $L$. Upon varying $L$ between 2.6 and 47.5 $\mu$m, we find a systematic increase of $\ell_p$ from 110 to 5,035 $\mu$m. At the same time we verify that, for a given filament length, the persistence length is constant over the filament within the experimental accuracy. We interpret this length dependence as a consequence of a nonnegligible shear deflection determined by subnanometer relative displacement of adjacent protofilaments. Our results may shine new light on the function of microtubules as sophisticated nanometer-sized molecular machines and give a unified explanation of seemingly uncorrelated spreading of microtubules’ stiffness previously reported in literature.

nanomechanics | protofilaments | single-particle tracking | thermal fluctuation analysis

The mechanics of living cells is largely determined by the cytoskeleton, a self-organizing and highly dynamic network of filamentous proteins of different lengths and stiffnesses (1). Understanding the elastic response of purified cytoskeletal filaments is fundamental for the elucidation of the rheological behavior of the cytoskeleton. Microtubules (MTs) are hollow cylindrical filaments formed by, on average, 13 tubulin protofilaments (PFs) assembled in parallel. The MT outer and inner diameters are ~25 and 15 nm, respectively. In cells, MTs are generally 1–10 $\mu$m long, whereas in axons their length can be 50–100 $\mu$m (2).

The tubular structure of MTs implies a minimal cross-sectional area, hence a high strength and stiffness combined with low density. In recent years, the mechanical properties of MTs have been investigated by several experimental approaches, such as thermal fluctuations (3–6), atomic force microscopy (AFM) (7–10), and optical tweezers (11–13).

The standard reference model to describe the mechanical properties of a biopolymer on length scales much larger than any microscopic scale (the tube diameter for MTs) is the worm-like chain model (14, 15). It is characterized in terms of a flexural rigidity $\kappa$ (neglecting torsional rigidity). The combined effect of flexural rigidity and thermal fluctuations on the conformation of the filament is given by the ratio $\kappa/k_BT$, which defines a characteristic length scale, the persistence length $\ell_p$. In a fluctuation analysis, $\ell_p$ determines the width of the probability distribution functions of the filament contour, e.g., the probability distribution function of the end-to-end distance of the filament $P(R)$ (16). The relationship between the flexural rigidity, or equivalently $\ell_p$, with the architecture of the MT and the molecular interactions between the constituent dimeric tubulin proteins is, in general, non trivial. Trivial relationships will hold only in the case of simple architectures. For example, in the case of a homogeneous elastic rod with a fixed cross section and a Young modulus $E, \kappa = EI$, where $I$ is the second moment of area of the cross section.

Recently, there has been clear experimental evidence that a model that relates $\kappa$ to a single modulus $E$ is too naive: for taxol-stabilized MTs the measured flexural rigidities vary in a range between $1.9 \times 10^{-24}$ $\text{nm}^2$ (11) and $2.15 \times 10^{-23}$ $\text{nm}^2$ (3). The rigidity values of MTs range between $4.7 \times 10^{-24}$ $\text{nm}^2$ (11) and $3.4 \times 10^{-23}$ $\text{nm}^2$ (4). The origin of this scattering is still a matter of debate. Recently, it was shown that increasing the growth velocity in vitro may decrease the flexural rigidity by a factor of 2 (4). However, this is not sufficient to explain the observed broad range of MT stiffness values reported in literature.

We suggest that the solution of this puzzle might be looked for rather in the complex mechanical behavior due to strongly anisotropic material properties of MTs. Although usually modeled as isotropic cylinders in literature, MTs have strongly anisotropic elastic properties determined by their discrete PF structure: inter-PF bonds are weaker than the head–tail $\alpha\beta\alpha\beta$ tubulin bonds along the PF (7, 8, 17, 18), and adjacent PFs can slightly shift relative to each other (19). Hence, it is expected that, in the language of continuum mechanics, the shear modulus $G$ of a MT (determined by the deformability of lateral bonds) differs significantly from its Young’s modulus $E$ (9, 10, 20) (determined by longitudinal tubulin interactions along the PFs). Indeed, recent experiments with AFM yielded a shear modulus that was 2–3 orders of magnitude smaller than the Young’s modulus (9, 10, 20). Moreover, an extremely low transverse buckling stress of $P_t = 600$ Pa has been measured on MTs (21) subject to osmotic pressure. This is four orders of magnitude smaller than the buckling stress calculated by modeling a MT as an isotropic shell with $E \sim 1.6$ GPa.

From engineering literature on fiber reinforced materials, it is known that a key parameter is the total filament length $L$. The main goal of this work is a systematic study of the dependence of $\ell_p$ on $L$, by performing a thermal fluctuation analysis that exploits the fact that the random fluctuations of the polymer...
contour reveal its intrinsic stiffness. Although the persistence length $\ell_p$ has been measured with great accuracy by means of studying $P(R)$ (22), a detailed study of the mechanics of MTs based on probability distributions is complicated by their high stiffness, requiring an assay with very high spatial resolution. Here, we apply single-particle tracking for measuring the probability distribution function $P(x, y)$ of grafted MTs. From the measured probability distributions we obtain the MTs persistence length and systematically investigate its dependence on the total contour length $L$ over a broad range (from 2.6 to 47.5 $\mu$m). Thermal fluctuations, as originally suggested by Einstein, represent the most natural way to “probe the intangible” (23), in our case the mechanics of biological nanometer-sized specimens nondestructively and over extended times. Thermal fluctuations already have been taken into account to model the structural and elastic properties of MT tips as evidenced by cryoelectron microscopy (24).

**Results**

The experimental situation is depicted in Fig. 1 a and b. In the assay, we select single grafted MTs with one bead attached proximally to the tip. A MT with two free ends would allow only the measurement of $P(R)$: grafting one end of a MT allows for a direct precise measurement of $P(x, y)$ and of the reduced distribution functions $P(x)$ and $P(y)$, which are more sensitive to the elastic parameters and, in our case, allow a precise measurement of $\ell_p$. Because the beads and the MTs emit fluorescence at two different wavelengths, they can be observed independently by simply exchanging the filter set in the microscope. The attached bead acts as a neutral tracer. Fig. 2a shows an example of the distribution resulting from the projection of the three-dimensional fluctuations of the MT’s tip on the $x, y$ plane. The distribution is obtained by measuring the position of the fluorescent bead attached at the MT’s end in at least $10^4$ frames. Measuring the tip’s deflection by tracking a strongly fluorescent bead allows one to evaluate with great precision the rigidity of very short MTs, whereas measuring fluctuations in shape on short MTs (3–5) might be problematic, given the much smaller amplitude of the fluctuations. The bead’s position is automatically tracked frame-by-frame with a custom particle-tracking routine. The coordinates of the bead’s centroid can be determined with an accuracy of 1 nm and better under optimal conditions (25). Here, the accuracy was reduced to $\approx$10 nm. A stack containing the fluorescent MTs is recorded separately for the determination of $L$.

The recorded particle position is used to obtain the probability distribution function $P(x, y)$ of the free end in the $x, y$ plane and the corresponding reduced distribution functions $P(x)$ and $P(y)$ (Fig. 2 b and c), obtained by integrating $P(x, y)$ over the coordinates $y$ and $x$, respectively. These can be directly compared with theoretical predictions based on the worm-like chain model, as reported in ref. 26. Given the high persistence length of a MT ($\ell_p/L$ can be as high as several hundred), the longitudinal distribution function $P(x)$ is peaked toward full stretching, as in Fig. 2b, and varies over a typical length scale $L_\perp = L^2/\ell_p$. A non-Gaussian and asymmetric shape of $P(x)$ is clearly visible in Fig. 2b. In contrast, $P(y)$ is a Gaussian the width of which is given by the transverse length scale $L_\parallel = \sqrt{L^2/3\ell_p}$, as represented in Fig. 2c (27, 28). By measuring the contour length $L$, $\ell_p$ can be extracted from both $P(x)$ and $P(y)$. The measured $P(x)$ and $P(y)$ correspond to convolution products between the theoretical functions $P_{\text{th}}(x)$ and $P_{\text{th}}(y)$ predicted by the worm-like chain model (27, 28) and the function that represents the experimental precision in the measurement of the distance between the tip of the filament and the attachment point on the substrate. Because longitudinal and transverse fluctuations scale differently, the convolution has a negligible effect on $P_{\text{th}}(y)$, in contrast to $P_{\text{th}}(x)$, the shape of which is much more sensitive to small variations of the experimental precision. For this reason, we focused on $P(y)$ for the determination of $\ell_p$ in this work. The persistence lengths obtained from $P(y)$ for 48 grafted MTs of different contour
lengths are shown in Fig. 3. For a given length \( L \), we have attached beads at different locations along the MT. Fig. 4 shows a typical realization of the experiment: we found that the persistence lengths independently obtained from the fluctuations of the two beads were consistent with \( \ell_p \) being independent on the arc-length position along the contour of the MT.** On the contrary, upon variation of \( L \) between 2.6 and 47.5 \( \mu \)m, we observe a systematic increase of \( \ell_p \) from a minimum value of 110 \( \mu \)m to a maximum of 5,035 \( \mu \)m.

As shown in Fig. 3, the values of \( \ell_p \) measured on MTs spread with increasing \( L \). This spreading might be partially due to differences in the structures of single MTs. In fact, in MTs polymerized in vitro the number of PFs may vary from one MT to another and even along a given MT, and several lattice types are possible (29). The probability of defects in the MT lattice may also increase with the contour length. Because there is also evidence that the stiffness of MTs can vary with the polymerization velocity (4), we carefully monitored the MT polymerization temperature. The diameter of the bead is much larger than the MT’s diameter; thus, the viscous drag acting on the bead is large and will influence the motion of the MT. However, at the time scale of video microscopy-based single-particle tracking (in our case 16 frames per s, corresponding to a time resolution of \( \approx 60 \) ms) the influence of the bead size is negligible and the fluctuations of the bead reflect accurately the thermal motion of the bare MT’s tip.11 The tip of the MT in our assay is at a distance of tens of micrometers from the coverslips; hence, we can neglect hydrodynamic coupling and direct non-spe-

cific interaction with the surface. A potential experimental source of error might be a loose binding of the bead to the MT, which could lead to an underestimation of \( \ell_p \). This issue has been tested by pulling the bead with optical tweezers at high laser power, ensuring that it attaches firmly to the MT and does not roll or twist on it. In addition, by strongly bending the MT with optical tweezers, we ensured that the segment of the MT anchored to the substrate does not change its orientation during thermal fluctuations, a further possible source of uncertainty in the measurement of \( \ell_p \). Theoretical calculations show also that the fluctuations of the angle of torsion along the whole MT are below 7° and can be safely neglected.

**Linear Elasticity Theory for Composite Structures**

It is known that linear elasticity theory works well to model the mechanical properties of nanometer-sized materials, like biofilms and carbon nanotubes (31, 32). As shown in ref. 24, the various shapes observed at the MT’s tip and its mechanical properties can be explained by applying an elastic sheet model in the framework of linear elasticity theory. Because a MT is a tubular assembly of discrete PFs assembled in parallel, its mechanical response can be understood by analogy with anisotropic fiber reinforced materials (33) rather than by a direct application of the theory of elasticity for homogeneous structures (34, 35).

The description of the stress–strain relationship in a generally anisotropic structure requires the introduction of a \( 6 \times 6 \) stiffness matrix containing the elastic moduli along the different orientations and the couplings between different strains. In an isotropic structure, off-diagonal elements (coupling terms) become zero, and the independent elastic parameters reduce to 3: the Young modulus \( E \), the shear modulus \( G \), and the Poisson ratio \( \nu \). Given their cylindrical symmetry and the PF structure, MTs are transversely isotropic, meaning that they are anisotropic along the longitudinal direction, whereas their cross section has an isotropic response to stresses (Fig. 5). For transversely isotropic materials, the tensile/compressive stress–strain relationship is expressed by (33)

\[
\begin{pmatrix}
\sigma_x \\
\sigma_y \\
\sigma_z
\end{pmatrix} =
\begin{pmatrix}
E_1 & -E_2/v_{12} & -E_2/v_{23} \\
-E_1/v_{12} & E_2 & -E_2/v_{23} \\
-E_1/v_{13} & -E_2/v_{23} & E_2
\end{pmatrix}
\begin{pmatrix}
e_x \\
e_y \\
e_z
\end{pmatrix},
\]

where \( \sigma_x, \sigma_y, \) and \( \sigma_z \) are the stresses along the \( x, y, \) and \( z \) axes and \( e_x, e_y, \) and \( e_z \) are the corresponding strains; \( E_1 \) and \( E_2 \) are the longitudinal and transverse Young moduli, respectively, and \( v_{12}, v_{23}, \) and \( v_{13} \) are the Poisson ratios.13 The shear stress–strain relationships are described by the following matrix:

\[
\begin{pmatrix}
\tau_{xy} \\
\tau_{xz} \\
\tau_{yz}
\end{pmatrix} =
\begin{pmatrix}
G & -G/v_{12} & 0 \\
-G/v_{12} & G & 0 \\
0 & 0 & G
\end{pmatrix}
\begin{pmatrix}
e_{xy} \\
e_{xz} \\
e_{yz}
\end{pmatrix},
\]

12Symmetry considerations impose that \( E_1/v_{23} = E_1/v_{12} \), so that there are four independent elastic parameters.
fluctuations in the tip’s position can be interpreted as deflections due to thermal forces. Given the boundary conditions and the geometry of the system, only the elastic moduli $E_1$ and $G_{13}$ are involved in bending on the $x$, $y$ plane. By assuming the deflection as due to a point load $P$ applied at the free end of the grafted filament, the total deflection of the MT ($\delta$) is the sum of both bending ($\delta_B$) and shear deformations ($\delta_S$) (10, 33):

$$\delta = \delta_B + \delta_S = \frac{PL^3}{\alpha E_1 I_z} + \frac{PL}{G_{13} k A} = \frac{PL^3}{\alpha e k_B T},$$

where $P$ is the lateral load at the MT’s tip, $I_z = 1/4\pi(R_{in}^4 - R_{out}^4)$ is the second moment of the tube’s cross-sectional area $A$, and $k$ is a geometrical correction factor the value of which is 0.72 for a hollow cylindrical beam (36) and $\alpha = 3$ for a grafted beam. The last equality, with $k_B T$ the thermal energy, represents the linear response of a polymer with persistence length $\ell_p$ in the same loading configuration and with the same boundary conditions (37). Through the relationship $E_1 I_z = \ell_p^3 k_B T$, Eq. 3 can be used to obtain the length dependence of $\ell_p$:

$$\ell_p = \ell_p^3 \left( 1 + \frac{3E_1 I_z}{G_{13} k A L^2} \right)^{-1},$$

where $\ell_p^3$ is the persistence length for “long” MTs ($L \gg \sqrt{3E_1 I_z/G_{13} k A} = 21 \mu m$). This relationship does not depend on the nature of the applied force; hence, fluctuations due to thermal forces are perfectly suitable for its direct test. Eq. 4 provides a good fit to the experimental points, giving a value of $\ell_p^3 = 0.3 \pm 0.8 mm$ in agreement with previous measurements on taxol-stabilized MTs (4). This value corresponds to $E_1 = 1.51 \pm 0.19$ GPa and a ratio of $\delta_S/\delta_B = (21/L)^2$ when $L$ is expressed in micrometers, corresponding to an extremely large ratio $E_1/G_{13} \sim 10^6$. This result is surprising and must be interpreted carefully. First, it is important to remark that $E_1$ and $G_{13}$ are not linked in this case by the Poisson relationship $G = E/2(1 + \nu)$, because of the mechanical anisotropy on the plane $x_3y_3$. Hence, a large $E_1/G_{13}$ ratio does not imply an exceedingly high $\nu$, which would lead to a diagonal collapse (complete flattening) of the cross section upon bending. Second, at microscopic level a very small shear modulus $G_{13}$ implies a high compliancy of the lateral inter-PF bonds.

From electron microscopy studies, it is known that the compliancy of inter-PF bonds allows one to accommodate a broad range of structures of the MT lattice, which can differ in PFs and helix-start numbers (19, 38–40). Variability within a range of ±0.2 nm in the relative position of tubulin dimers on adjacent PFs was also observed in these studies and interpreted as the result of thermally driven deformations of the lateral contacts between PFs (19, 38). Electron crystallography maps obtained at 8- and 20-A resolution give insight about the molecular origin of longitudinal and lateral interactions in MTs (41, 42). One key structural element in determining lateral interactions between dimers is a long loop (the S7–H9 loop, also called “M-loop”; Fig. 6c) that protrudes from the PF side, interacting with the loop H1–S2 and the α-helix H3 on the surface of the neighboring PF (41, 42). Such interaction involves an important electrostatic contribution, at variance with the αf-αf tubulin bonds along the PF, which are linked by prevalently hydrophobic and polar interactions on a very large interface (≈3,000 Å$^2$) and with extremely high complementarity in shape, thus suggesting that short-range Van der Waals forces are important in intra-PF contacts between tubulins (41, 42). It is known that head–tail intra-PF contacts between tubulin dimers are stronger than lateral inter-PF contacts. Sept et al. (18) have calculated that longitudinal bonds along the PF are 7 kcal/mol stronger than lateral ones. Estimating the elastic constant of a single longitudinal bond from the potential profile calculated by Sept et al.

**Discussion**

In the following discussion, we will model MTs as transversely isotropic hollow cylindrical beams and assume that the small

$$\begin{pmatrix} \tau_x \\ \tau_y \end{pmatrix} = \begin{pmatrix} G_{13} & 0 & 0 \\ 0 & G_{13} & 0 \\ 0 & 0 & G_{23} \end{pmatrix} \begin{pmatrix} \gamma_{xy} \\ \gamma_{yx} \end{pmatrix}. \tag{2}$$

Here, $\tau_i$ is the shear stress along the $i$ direction, and $\gamma_{ij}$ represents the strain angle in the $ij$ plane. In transversely isotropic materials there is no coupling between transverse ($G_{23}$) and longitudinal ($G_{13}$) shear moduli. Conversely, the transverse shear and Young moduli are linked by the relationship $G_{23} = E_2/2(1 + \nu_{23})$, given the transverse isotropy.18

18The total number of independent elastic parameters is thus 5.

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**Fig. 5.** Mechanical anisotropy of a MT. (a) $x$, $y$, and $z$ geometrical axes; 1, 2, and 3, material axes of the MT. (b) Bending generates a tensile/compressive stress $\sigma_x = E_1 \epsilon_x$ and a shear stress $\tau_{zx} = G_{13} \gamma_{zx}$ between adjacent PFs ($E_1$ and $G_{13}$ are longitudinal Young’s and shear modulus, respectively; $P$ is the load, $\epsilon_x$ is the tensile strain along $x$, and $\gamma_{zx}$ is the longitudinal shear strain). Given the anisotropy in the tubulin interaction across and along PFs, $E_1$ and $G_{13}$ may differ by several orders of magnitude. (c) Similarly to fiber-reinforced materials, MTs are transversely isotropic, implying that on the plane of the cross section $E_2 = E_3$ and that the Poisson’s relationship between the Young’s modulus and the transverse shear modulus $G_{23}$ still holds.

$$\begin{pmatrix} \tau_x \\ \tau_y \end{pmatrix} = \begin{pmatrix} G_{13} & 0 & 0 \\ 0 & G_{13} & 0 \\ 0 & 0 & G_{23} \end{pmatrix} \begin{pmatrix} \gamma_{xy} \\ \gamma_{yx} \end{pmatrix}. \tag{2}$$

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results in $k_{\text{long}} = 4 \text{ N/m}$ (43), which is 40 times larger than the elastic constant measured in a transverse compression by an AFM tip, $k_{\text{trans}} = 0.1 \text{ N/m}$ (7). Thus, a large amount of experimental data, including the assembly/disassembly dynamics of MTs, electron crystallography maps (41, 42), cryoelectron microscopy (19, 38, 44), and small-angle x-ray diffraction (21, 45), suggest a pronounced mechanical anisotropy of MTs.

Microscopically, the longitudinal elastic modulus $E_1$ is expected to arise from the strong $\alpha\beta$-tubulin bonds along single PFs, whereas the M-loop might be the prevalent contribution to the longitudinal shear modulus $G_{13}$. The energy barrier adjacent PFs must overcome to produce the tiny relative displacement observed by electron microscopy is likely very small [on the order of thermal fluctuations (19)], at least for small strains within 0.2 nm (19, 38, 44). Therefore, MTs could easily absorb shear stresses associated with bending by slightly shifting the relative position of neighboring PFs. Indeed, our measurement of a very large $E_1/G_{13}$ ratio confirms such a hypothesis. As shown in Fig. 6, we estimate the amount of PF shear strain produced by the observed MT deflection to be on the subnanometer scale and hence consistent with the measurements of PF relative displacement provided by cryoelectron microscopy (19, 38, 44). For forces larger than the thermal force, the compliance of the lateral connection between PFs might be eventually exceeded, leading to deformation of tubulin subunits. Higher forces can be obtained, for instance, by further increasing the osmotic pressure in the medium (45). Tubulin subunits have a high stiffness, typical of proteins, of $\approx 2 \text{ GPa}$ (46). Deformation of the stiff tubulin dimers might be the explanation of the postbuckling elastic response measured in ref. 45. The shear modulus $G_{13}$ may be considered as the result of two possible contributions: a vertical shear modulus ($G_{13}$) arising from stretching of intra-PF tubulin, and a horizontal shear modulus ($G_{13}$), determined by lateral bonds between PFs (Fig. 6). The above-mentioned structural considerations imply that $G_{13} \ll G_{13}$. Consequently, the shear deflection is given by

$$\delta_s = \frac{PL}{kA} \left( \frac{1}{G_{13}} + \frac{1}{G_{13}} \right)$$

and, in the case of a thermally fluctuating MT, would be largely dominated by the horizontal shear modulus, determined by the compliance of inter-PF bonds. In case a cross-linker like glutaraldehyde is present to stabilize MTs, the relative displacement between PFs would be hindered and the magnitude of $G_{13}$ and $G_{13}$ would be comparable, thus implying a higher overall value of $G_{13}$ and a lower $E_1/G_{13}$ ratio. This stabilization partly explains the difference between our results and the $E/G$ ratio previously reported in AFM experiments on glutaraldehyde-stabilized MTs (9, 10). In contrast, to stabilize MTs we used the drug taxol, which inserts in the MT lattice and may relieve the MT internal tension inducing a further decrease of the shear modulus (11). In addition, AFM experiments (7, 9, 10) are likely to probe the structure of MTs by involving the transversal modulus $G_{23}$, whereas the main contribution to shear stress in our thermal bending experiments is given by $G_{13}$. Consequently, the $E/G$ ratio measured in AFM experiments is likely different from the $E_1/G_{13}$ ratio measured in this work.

Conclusions

We have developed an assay based on single-particle tracking to measure the reduced distribution functions $P(x)$ and $P(y)$ for the tip’s position of grafted MTs with high spatial resolution. Through our fluctuation analysis, we have found a systematic and strong increase of MT persistence length with the total contour length $L$. For short MTs ($L = 2–3 \mu m$), we observe an $\ell_p$ on the order of a few hundred micrometers, at least one order of magnitude smaller than for long ones ($L \approx 30–40 \mu m$). This property can be explained by the anisotropic structure of MTs and described by linear elasticity theory. Our results together with those in refs. 9, 10, and 21 suggest that the inter-PF bonds are highly compliant (at least for small strains), leading to a very low longitudinal shear modulus $G_{13}$ in MTs. The dependence of $\ell_p$ on $L$ in MTs might also contribute to explaining the broad range of measured MT persistence lengths found in the literature for taxol-stabilized and bare MTs (0.5–8 mm) (3, 4, 11). Noticeably, the maximum variability of $\ell_p$ occurs at contour lengths close to the typical length scale of a cell ($L \approx 12 \mu m$). It is interesting to speculate about the biological significance of this property. MTs are able to accomplish various tasks in differently sized cells and during different stages of a cell’s life cycle. On one hand, the flexibility of a MT allows it to search space laterally for binding partners or to bend and continue growing in a modified direction when encountering obstacles, for example when growing MTs hit the side of a small fission yeast cell. On the other hand, sufficient rigidity is important in situations in which MTs need to resist pushing forces such as in large vertebrate cells during anaphase of mitosis, when the spindle elongates. The length dependence of MT rigidity might have allowed cells...
during evolution to use MTs in an optimal way both for lateral searching and for exerting pushing forces in different contexts and especially on different length scales.

Materials and Methods

Biotinylated tetramethylrhodamine-labeled MTs (25 μM pig brain tubulin dimers of which 10% was labeled and 10% was biotinylated) were polymerized for 20 min at 37°C with 2 mM GTP in BRB80 buffer (80 mM Pipes, pH 6.8/2 mM MgCl₂/1 mM EGTA). After polymerization, MTs were centrifuged at 215,000 × g and washed once with BRB80. Pellets were resuspended in 20 μM paclitaxel (Sigma-Aldrich) solved in BRB80 buffer.

A gold grid for transmission electron microscopy with linear mesh (Agar) was glued onto a round glass coverslip (170 μm thick and 15 mm in diameter; Menzel-Gläser, Braunschweig, Germany) by a tiny amount of a fluid silicon rubber (Elastosil E43; Wacker Chemie) deposited carefully onto the grid's rim. After drying of the adhesive, the grid surface was treated with a basic/oxidizing cleaning solution (H₂O₂/NaH₂O₂; 1:1.5 volumes) for 1 h. The mounted grid was then rinsed with deionized water and ethanol and incubated overnight with a 5 mM solution of mercaptoethanesulfonic acid (Sigma-Aldrich) to obtain an ordered self-assembled monolayer with the reactive carboxylic groups exposed toward the outside on the gold surface. After overnight incubation the grid was washed with ethanol and dried with a nitrogen flow. A flow chamber was then realized by mounting the coverslip with the grid onto a rectangular one (30 × 22 mm) with double-sided adhesive tape as a spacer. The distance between the grid and the bottom coverslip was 30–40 μm, and the total volume of the flow cell was ~20 μl. The exposed carboxylic groups were activated by flowing into the chamber 20 μl of an aqueous solution of 100 mM N-hydroxysuccinimide and 100 mM EDC (both from Sigma-Aldrich).

The MT suspension was diluted 1:100 (vol/vol) in 20 μM paclitaxel/BRB80. About 20 μl of the MTs' suspension was made to flow into the chamber and incubated for 1/2 h. Subsequently, ~20 μl of an oxygen scavenger solution (hemoglobin diluted 1:22 from a saturated solution, 0.5 mg/ml glucose oxidase/0.14 mg/ml catalase/46 mM glucose) in 20 μM BRB80/paclitaxel, containing a diluted suspension of beads, was exchanged in the flow chamber. The beads were neutral-red coated and yellow-green fluorescent with a diameter of 250 or 500 nm (F8811 and F8813; Molecular Probes; excitation 505 nm, emission 515 nm). The chamber was finally sealed with a small quantity of silicon grease at both ends to avoid evaporation of the medium.

The single-particle tracking set-up was based on a conventional epifluorescence microscope (Axiovert; Zeiss) (25). The MTs and the beads, fluorescing, respectively, at 585 and 515 nm, were imaged by using a peltri-cooled sensitive camera (Sensicam; PCO). MTs with a single bead attached at the tip were selected, for the measurement. Alternatively, optical tweezers were used for attaching the bead. A stack of 10⁵ frames of the bead's thermal fluctuations were recorded for each MT, at a rate of 16 frames per s, setting an integration time of 25–50 ms. Each stack was processed by tracking automatically the bead's position in each frame by a custom particle-tracking routine implemented with the data analysis software IGOR PRO (WaveMetrics) (25).

We acknowledge helpful discussions with J. Howard and C. F. Schmidt. We thank A. Rohrbach, E. H. K. Stelzer, and J. Swoger for substantial support and many interesting discussions. G.L. was supported by European Commission HPMF-CT-2001-01423 and MERG-CT-2004-513598. F.P. was supported by German Research Foundation Grant FL351/2-1.