Mechanical Heterogeneity of Microtubules

Oane Gros

Layman's summary

Cells contain an organizational network of stiff tubes called microtubules. These tubes are able to grow and shrink and act as a roadmap that organizes transport in the cell. Through these properties, microtubules contribute to the shape and functioning of cells. A key feature of the microtubule that allows its cellular function is that a microtubule is very stiff. Thus, it has been a relevant experimental question how stiff microtubules are and how they behave when put under force. This review tries to summarize the experimental work quantifying and characterizing the behavior of microtubules under force.

The measurement of microtubule mechanics mostly focuses on the fitting of the 'flexural rigidity' parameter, which is a product of material properties when we assume that microtubules are elastic. This comes from similar models that are used for designing columns for buildings in structural, applied on a microscopic scale. To measure this value, it is necessary to put a known force on the microtubule and measure how much it deflects or bends. In the past 40 years many methods have been tried to put this force on the microtubule. These methods of force application are, among others, a constant flow of fluid around the microtubule, pulling or pushing the microtubule with optical tweezers (lasers that can precisely exert force), active probing with a mechanical arm in atomic force microscopy, or watching the shape fluctuations under thermal forces. Each of these methods have their own drawbacks and advantages.

The summarized results of all major studies into microtubule flexural rigidity are shown in Table 1. The combination of the different techniques and almost 40 years of research have revealed some of the major mechanical features of microtubules but have also shown that these experiments are hard to execute and relate to the biological system. The current view on microtubule flexural rigidity is that this falls within a range of values, where the polymerization (growth) speed of the microtubule is one of the important dependencies. How much the rigidity depends on the microtubule length is still not completely clear. Additionally, many other factors can change how the microtubule is chemically structured which in turn affects the biophysical properties of the microtubule. Here it is relevant that some non-cellular compounds, such as the drug Taxol, are used in many experiments, and many of the altering factors that are present in cells are not reconstituted in the experiments, partially because not all interacting factors are known for microtubule subtypes. For the future it may thus be necessary to first identify what other compounds are interacting with a specific cellular microtubule, and what the resulting microtubule structure is to accurately say anything about its rigidity and function in the cell.

Abstract

The study of microtubule deflection under force lies exactly on the interface of biology and physics. Microtubules are polyproteins that assemble into rigid beams, that carry biologically relevant forces. To quantify this rigidity, much research has been done in the past 40 years. First measurements matched to Euler-Bernoulli beam theory to get the material properties of microtubules and found varying results. In the subsequent years *in vitro* work with various techniques of applying force on microtubules have given better estimates of rigidity, found that rigidity can depend on length and polymerization speed, and have shown that in many conditions more complicated beam models may be necessary. However, variation in sample preparation that yield different lattices or dynamics stabilization are still often present. This review serves to assess the state of the field, provide an overview, and propose a direction to attempt further microtubule rigidity studies in tandem with novel microscopy methods to try to link biophysical properties to the microtubule structure.

Introduction

Microtubules (MTs) are rigid and dynamic polyprotein fibers that are part of the eukaryotic cytoskeleton. MTs are assembled of α- and β-tubulin dimers forming protofilaments in a densely packed filament lattice (Manka & Moores, 2018; Nogales, 2001). Across biology, the MT lattice conformations can be markedly different in the number of protofilaments (Chaaban & Brouhard, 2017) and lattice conformation (Debs et al., 2020), which can be dynamically changed (Cross, 2019). One of the features of the MT is the very high axial rigidity of the assembled fiber, which is used in multiple biological processes, such as mechanotransduction (Porshneva & Montagnac,

2021) and cell motility (Garcin & Straube, 2019; Ishikawa, 2017). MTs also play a large role as regulatory elements as they can serve as transport network for motor proteins (Burute & Kapitein, 2019).

To understand the function of the MT, a biophysical model of its deflection under force is relevant. The measurements necessary to make this model have been attempted numerous times in different fashions over the past 40 years by reconstituting MTs *in vitro*. These reconstituted MTs can then be tested without too many confounding factors, by putting the fiber under force and measuring its deflection. The way to exert force on the MT can be through thermal forces, optical traps, atomic force microscopy and other techniques. These measurements can then subsequently be fit to a model for column buckling as it is known for large-scale structural engineering, such as Euler-Bernoulli beam theory. Euler-Bernoulli beam theory models the properties of a stiff 1-dimensional rod, where the material properties are captured in the flexural rigidity, a product of the elastic (Young's) modulus and the area moment of inertia (defined by the area cross section of the rod).

All the *in vitro* experimental techniques used to measure MT stiffness bring along their own issues. Many of these problems have since been partially resolved, such as MT localization precision and sample purification. The progress in technical ability has given the current field a good analysis of the range of magnitude and major dependencies of MT rigidity. Various data also shows that Euler-Bernoulli beam models are not sufficient to model MTs, and more detailed models have since been developed, based on the *in vitro* data. This includes relatively simple Timoshenko beam models including shear deformation, orthotropic shell models modeling the 2D cross-section of the MT and models that take the atomistic reality into account. With the current state of the field, we can say that there is some understanding of how pure MTs behave although some experimental differentiators remain. One such issue is the necessity of chemical stabilization of the MT dynamics for many active force-exerting assays, where it is also known that the MT is structurally and biophysically changed by this stabilization, and another is the remaining heterogeneity within and between studies.

This report attempts to review the past 40 years of MT rigidity research and consolidate this into a current view on MT rigidity *in vitro*, and thus give an update to this part of the review by (Hawkins et al., 2010). Care is also taken to see how this *in vitro* view compares with MTs *in vivo*, where MTs are part of a much more complicated network of interaction and are both actively regulated by and active regulators of cellular processes.

MT structure and dynamics

MTs assemble from dimer subunits of α- and β-tubulin. The dimers assemble end-to-end into protofilaments that combine laterally to form a tubular MT lattice with circular cross-section (Nogales, 2001; Nogales et al., 1998). Tubulin can spontaneously assemble into a MT 'seed', from which further polymerization is possible, but is in biological circumstances often nucleated by a specific nucleating protein (Roostalu & Surrey, 2017). Nucleating proteins can force MTs to contain a specific number and conformation of protofilaments. Multiple conformations of MT exist across eukaryotes, containing between 11 and 16 protofilaments in a single ring (Chaaban & Brouhard, 2017). Doublet and triplet semi-interlocking rings also appear, and are characteristic for but not exclusive to axonemes and centrioles, respectively (Chaaban & Brouhard, 2017; Ishikawa, 2017; LeGuennec et al., 2021). The 13-protofilament MT, nucleated by the γ-TuRC complex is the predominant canonical form (Chaaban & Brouhard, 2017; Zupa et al., 2021). With 13 protofilaments, two lattices are supported, termed A- and B-lattice, both conformations have a straight alignment of protofilaments without hypertwisting. The common B-lattice forms a left-handed staggered 3 monomer-start helix, which leaves a 'seam' of A-lattice contacts, with the major lateral contacts between α and β tubulin (Kikkawa et al., 1994; McIntosh et al., 2009), B-lattice MTs with multiple A-lattice seams are also common, but less stable (Debs et al., 2020; Katsuki et al., 2014).

Both spontaneously seeded and nucleated MTs polymerize by adding GTP-bound tubulin dimers to a MT end. The incorporated GTP-tubulin is subsequently hydrolyzed, through which most of the MT-lattice is in a GDP-bound state. The growing GTP-bound tip is thought to protect the fiber from depolymerization (Cleary & Hancock, 2021), leading to the characteristic dynamic instability of MTs, where a MT tip can be in a GTP-bound growing state or, once the tip is also hydrolyzed goes into a shrinking depolymerization phase (Hill & Chen, 1984; Hyman et al., 1992; Mitchison & Kirschner, 1984). Free MTs have two distinct ends, the 'plus' and 'minus' end, and dynamics can happen on either end (Walker et al., 1988). However, most plus

end dynamics are faster, and minus ends are often stabilized in cells, so the most assessed dynamics are at the plus end (Cleary & Hancock, 2021; van Haren & Wittmann, 2019).

The MT structure *in vivo* is thus already not a clear-cut phenomenon. This is further complicated during the lifetime of a fiber, as the chemical properties of MTs can be changed by numerous factors (Cross, 2019). This is most clear in the form of factors that alter the tip dynamics (Jiang & Akhmanova, 2011), where the chemical structure is sufficiently altered by accessory proteins to change the dynamic state of the tip. However, many factors also exist that change the MT along its length. Structural microtubule-associated proteins (MAPs) bind alongside the MT and change the organization and dynamics of MTs, these are often innately disorganized proteins that bind between the protofilaments of MTs (Bodakuntla et al., 2019). Molecular motors, MAPs that walk on the MT, have been shown to locally compact the lattice (Peet et al., 2018; Shima et al., 2018), or even rip out tubulin subunits from the filament (Triclin et al., 2021). On top of this, there are also tubulin post-translational modifications (PTMs), most of which change the C-terminal tail that hangs outside the MT column (Magiera & Janke, 2014). K40 lysine acetylation is notable as this modification is done in the lumen of the MT, changing the tubulin lattice packing (Eshun-Wilson et al., 2019). Acetylation is one of the ways a MT can be 'stabilized', where MTs are no longer growing or shrinking and resistant to physical stress (Janke & Montagnac, 2017).

The chemical packing of tubulin into a rod gives the MT filament emergent physical properties, as it gains a lot of rigidity along its lengthwise axis. MTs can thus withstand and exert a lot of force within the cell. A notable example of how this is used biologically are the doublet MTs in the axoneme of ciliated cells, where the rigidity of the MTs give the axoneme the rigidity to exert force (Ishikawa, 2017). To be able to understand how the rigidity of the MT contributes to cellular biophysics, we need to know how the MT deforms under force. For this, we can liken the MT fiber to a beam (or column/stiff rod) as they are modeled in structural engineering.

Beam Theory

To understand the values being researched, it is relevant to first introduce the modeling of macroscopic beams as it is used in structural engineering. The simplest physical model of a beam under force comes from Euler-Bernoulli beam theory (Connor & Faraji, 2016). Euler-Bernoulli beam theory was defined in the eighteenth century and assumes that a beam behaves as a spring-like elastic material and will return to its undeformed state when a load is removed. Thus, a beam has internal elastic forces that counteract an applied load. The general equation for the deflection $w(x)$ of a uniform rod in z as it is under force in x is as follows: $EI\frac{d^4w(x)}{dx^4} = q(x)$, where q($x$) is the distributed load along the rod, and EI is the flexural rigidity of the column. The flexural rigidity itself is a product of the elastic modulus of the material (E or Young's modulus, the material resistance to bending following Hooke's law) and the column's second moment of area of the beam cross-section (I). This equation also gives a means to calculate both the bending moment $M = -EI\frac{d^2w(x)}{dx^2}$ (rotational bending force) and shear force $Q = \frac{dM}{dx} = -\ EI\frac{d^3w(x)}{dx^3}$ (forces along the beam axis) inside the beam. These internal forces can then be used to calculate stress. Another relevant feature that is now computable, is the critical buckling force $P_{cr} = \frac{\pi^2 EI}{(KL)^2}$ defining the critical force under which a column will deform or buckle, dependent on the EI, end conditions (K) and column length (L) (Connor & Faraji, 2016). The discovery of this set of relatively simple equations to physically model columns have been invaluable for structural engineering, as structural soundness of constructions can be modeled more easily and accurately.

Euler-Bernoulli beam theory models a very simple beam. A major factor subsequent structural engineering-beam models added was the shear deformation of a beam, termed (Bresse-Ehrenfest-)Timoshenko beam theory (Challamel & Elishakoff, 2019). Modeling shear deformation is especially relevant to model sandwich composite beams, where lateral contacts between beam fibers are not as strong and the resistance to shear force (shear modulus) is low, this also allows the modeling of very thick beams.

These forms of beam theory are models for simplified macroscopic beams. However, MTs are polymers at a micrometer scale. This scale difference makes the physics more complicated, as measurements get harder and thermal forces can have a strong effect on the column. The general model that MT studies use is the assumption of a Worm-Like Chain polymer, as an isotropic and homogeneous continuously elastic material (Marantan & Mahadevan, 2018). With

short stiff polymers, far under their persistence length (persistence length $L_p = \frac{EI}{k_bT}$), these chains can be modeled as rigid beams under macroscopic beam. Because MTs are observed to be very stiff, they fit under this assumption. However, more precise measurements have since shown that more complicated models are necessary to model the deflection of MTs accurately. To understand how these models were developed it is good to first assess the *in vitro* r force measurements on MTs.

Initial Measurements

Flexural rigidity measurements on MTs *in vitro* are very hard, as the both the scale and chemical nature of the MT make it hard to control the unknown parameters. The first measurements of MT rigidity were done by assessing the end-to-end distance of MTs affixed to a coverslip, where the measured end-to-end distance is a product of persistence length L_p and MT contour length L (Mizushima-Sugano et al., 1983). These measured values are on the very low end of subsequent measurements with 0.45 pN・μm^2 for GDP-assembled singlet MTs. This has been attributed to measurement error either from intrinsically bent MTs (Gittes et al., 1993) or due to the fixing to the coverslip (Hawkins et al., 2010). It may also have been that the observed dynamics in their MTs can be attributed to possible active MAPs in the 60% pure tubulin mixture. Similar measurements based on end-to-end distance were done by (Dye et al., 1993) to assess the stiffness effect of the drug paclitaxel (here termed under the more commonly used brand name Taxol). Taxol is a small molecule that is used as chemotherapeutic drug due to its function of mitotic arrest, and binds on the luminal side to β-tubulin, changing MT conformation (Kellogg et al., 2017). The (Dye et al., 1993) study did not fix the MTs to the coverslip but had MTs grow from sea urchin axoneme nucleation in a controlled flow chamber. However, the added flow force in this system was not considered in the analysis.

The two previous methods represent the difference in *active* and *passive* measurements of MT flexural rigidity. Passive measurements rely on thermal forces to cause deflection in the MT and active measurements rely on an explicitly applied force to bend the MT. The problem with applying a load is that the load needs to be controlled, constant and known, which is hard on the scale of the MT. Thermal forces are known and constant if temperature is controlled, however, due to the high stiffness of the MT, long measurement times and long MTs are necessary to measure thermal fluctuations in a single fiber.

Passive measurements were improved in (Gittes et al., 1993): here spontaneously seeded MTs were taken and assessed through their thermal fluctuations when floating freely. Because thermal forces at specific temperatures are known, the thermal fluctuations of the fiber can be measured, and rigidity can be found through decomposing the fluctuations into Fourier cosine modes. Because this form of measurements takes much more time (over 15 minutes of imaging at 5-20 s intervals) and MTs were not affixed to the coverslip, the Taxol was used to stabilize the tip dynamics and protect the MT from depolymerization. Using Taxol-stabilized free MT thermal fluctuations, a value of EI of 21.5 pN・μm^2 was found. It is noted that the MTs used were mostly (69%) assembled with 14 protofilaments, and 13, 15 and 16-pf MTs were also present. Subsequent work by the same group (Mickey & Howard, 1995) narrows this down to only 14-pf MTs with various stabilization methods aside from Taxol. This includes GMPCPP (a nonhydrolyzable GTP-analogue) incorporation in the lattice, GMPCPP caps on GDP-tubulin lattices, and purified Tau (a common MAP). Notable is here that the pure GDP-tubulin lattice with GTP caps was measured at 26 pN・μm^2 and were less stiff than Taxol-stabilized GDP-MTs.

Active measurements simultaneously developed. The principle of the flow chamber of (Dye et al., 1993) was expanded into calibrated flow measurements under specific flow conditions by (Venier et al., 1994). Here the deflection of MTs under a known flow rate were measured. This is difficult as flow rates are not the same in the entire chamber due to fluid mechanics. To correct for this, thermal fluctuations without flow were also measured and agreed with the active measurements. Stiffnesses were measured for GDP lattices, Taxol and Taxotere (Taxol homologue) stabilization, and non-hydrolysable GDP analogues GDP・ BeF_3^- and GDP ・ AlF_4^-. GDP-lattice MTs were found to have a rigidity of 9.2 pN・μm^2, and stiffness decreased with Taxol-stabilization. Calibrated flow was subsequently also used by (Kurz & Williams, 1995), measuring MAP-stabilization of MTs, which found much more stiff MTs of 35.8 pN・μm^2, with no major difference between MAP-stabilized and pure tubulin MTs, here growth was

inhibited in unstabilized MTs by slightly lowering tubulin concentration, but the remaining growth was not accounted for in analysis.

The results of the first measurements under calibrated flow and thermal fluctuations vary more than three-fold for minimally stabilized GDP-lattices. The effect of the attempted stabilizations with Taxol, MAPs and GMPCPP also give qualitatively different results between studies. Controlling experimental parameters and analysis artefacts is incredibly difficult in this work and retrieving where differences between results come from can be hard. Thus, the question of what the exact flexural rigidity of MTs is stayed open, and many other techniques were used to attempt to find this value. Because of the breadth of the work, further values will be listed in Table 1, and not named in the text.

Optical Trapping

Active measurements subsequently developed into multiple forms. The use of optical traps to exert force on MTs was used to be able to control the exerted force better than calibrated flow models did (Felgner et al., 1996, 1997; Kurachi et al., 1995). Multiple ways to measure rigidity exist with optical traps. One way is to measure the buckling force of the MT, first done by pulling on MT-attached polystyrene beads (Kurachi et al., 1995; Takasone et al., 2002). Similar measurements with dual optical traps were done later (Kikumoto et al., 2006; van Mameren et al., 2009). Where (Kikumoto et al., 2006) were able to assess MT buckling with optical traps without Taxol, by adding the MTs to around 70% deuterium oxide to prevent disassembly.

Work done in (Felgner et al., 1996) utilizes an optical trap both by following the relaxation after the MT tip was displaced (RELAX method) and moving the axoneme anchor while trapping the middle of the MT and assessing the lag time between anchor and MT end fluctuations (WIGGLE). Subsequent work by the same group only utilizes the RELAX method (Felgner et al., 1997). The RELAX measurements include pure GDP MTs, decreasing rigidity with Taxol and increasing with MAPs. (Felgner et al., 1997) found a linear relationship between the binding strength of MAPs with MT rigidity.

However, one of the major problems of active force measurements is that the applied forces bring the MT into different deformation regimes, not included in the Euler or Timoshenko beam models that were used to analyze the data. Using dual optical traps to continuously bend GMPCPP-stabilized MTs, (Memet et al., 2018) found that MT cross-sectional flattening affects rigidity measurements already at relatively low strain, which may affect most analyses where MTs are put under force. Under this constrant strain, lower stiffnesses were found, implying a 'softening' under strain. Work by (Koch et al., 2017) also used dual optical traps with MTs under high forces to assess higher deformation modes. However, these used a continuously oscillating bead to exert force on Taxol- and GMPCPP-stabilized MTs. This showed an increase in MT stiffness with increasing frequencies (within biological ranges such as heartbeat).

Thermal Fluctuations

Passive measurements of MT stiffness were done without explicit stabilization in (Cassimeris et al., 2001; Dogterom & Yurke, 1997; Janson & Dogterom, 2004a, 2004b). The use of spontaneously nucleated short anchored GMPCPP-MTs allows a consistent and biologically reasonable seed for MT nucleation (Gittes et al., 1996). Flexibility of the unstabilized MTs grown from GMPCPP seed was best quantified in (Janson & Dogterom, 2004a): here a new decomposition of Fourier modes was used to allow for MTs that grow during measurement. This analysis uses the solution to the cantilever beam problem to model the clamped end and is only dependent on the currently analyzed MT length. This analysis showed a polymerization speed-dependent MT rigidity, where fast-growing MTs are 2-fold less stiff than slow-growing MTs.

Work with stabilized MTs was still done after this, such as the measurement of Taxol-stabilized MTs in thermal fluctuations by (Pampaloni et al., 2006) finding a length-dependence of MT stiffness, where MT rigidity increases with MT contour length. Here a fluorescent bead on the end of the stabilized MT was tracked and around 3-fold increase of stiffness was found between MTs of ~3 µm and ~20 µm length. This was attributed to a very low shear modulus (material resistance to shear deformation) of the measured MTs from very weak lateral bonds between protofilaments. Subsequent work (Taute et al., 2008) showed that the length dependence based on Timoshenko beam theory fails for MTs shorter than 5 µm in length. Computational image analysis of fluctuating MTs developed later on, which allowed for less biased assessment, higher throughput, and also subpixel precision of filament tracking with Taxol-stabilized MTs (Brangwynne et al., 2007; Valdman et al., 2012). The results shows that

even with subpixel precision the measurements need long (>15 μm) contour length MTs to have measurements that overcome the measurement error with thermal fluctuations (Brangwynne et al., 2007). Taking a large range (18-66 μm (Brangwynne et al., 2007)) of MT contour lengths does show a slight correlation between length and persistence length. taking a smaller window (10-20 μm (Valdman et al., 2012)) did not reproduce this, and either study also shows variation that seems intrinsic to the preparation of the MTs. The spectral analysis for subpixel localization of (Valdman et al., 2012) was subsequently used to assess MT stiffness with GMPCPP, GTP-γ-S and Taxol stabilizations in varying combinations to test the rigidity effect of EB1 in varying concentrations (Lopez & Valentine, 2014).

Temperature-dependence of Taxol-stabilized MTs (Kawaguchi et al., 2008) and GMPCPP-stabilized and pure GDP-lattice MTs (Kawaguchi & Yamaguchi, 2010) were measured. Here, pure GDP-lattices were created by having many MTs in the chamber and only measuring those that were stochastically neither growing nor shrinking. These studies reproduce a length-dependency only for the MTs without Taxol, and only find strong temperature dependence (decreasing rigidity with increasing temperature) for these MTs without Taxol. Other dependencies investigated with Taxol-stabilization include the method of tubulin purification (Hawkins et al., 2012), GMPCPP/GTP-γ-S and MAPs in combination with Taxol (Hawkins et al., 2013), and salt (NaCl) concentration at polymerization (Harris et al., 2018), notably, these studies do not find a length-dependent MT rigidity. By controlling salt concentration at polymerization, the number of protofilaments and relative commonality of A-lattice (lateral bonds between α- and β-tubulin) saturation can be controlled. Interestingly, the high-salt/high-A-lattice MTs were only slightly less stiff, which was likely due to the lower protofilament number (Harris et al., 2018). Researching the double stabilization also reveals that Tau only affects Taxol-stabilized MTs if Tau is present during MT polymerization and double stabilizations do not have additive effects, but one is usually dominant (Hawkins et al., 2013). Another interesting feature in these studies is that the spread of persistence length measurements can very clearly fit a gaussian distribution when transformed with natural log. This gives some clearer indication on how the scattered data deviates than most studies were able to do. A notable point, however, is that (Hawkins et al., 2012, 2013) underestimated MT rigidities by a factor two due to incorrect shape digitization, as noted and quantified by (Harris et al., 2018). Subsequent work studied GMPCPP-seeded MTs with thermal fluctuations by Taxol-stabilizing after measuring polymerization speed under different tubulin concentrations (Isozaki et al., 2017; Zhou et al., 2020, 2021), and reproduces the dependence of MT stiffness on polymerization speed found by (Janson & Dogterom, 2004a). This work also reassesses the digitization error and confirms the point that short MTs need much better localization to measure (Zhou et al., 2021).

Atomic Force Microscopy

To measure the MT force response, multiple studies have utilized atomic force microscopy (AFM), where imaging is done by physically probing the sample. Because AFM measurements exert force on the MT, samples are usually fixed with glutaraldehyde. The first AFM studies measure MT elasticity by taking the force response when indenting MTs that were immobilized against a flat surface, taking a range of glutaraldehyde concentrations and extrapolating the point at 0% glutaraldehyde (Vinckier et al., 1996). This extrapolation assumes that the initial addition of any glutaraldehyde does not make changes to the MT lattice packing. Under these conditions, the addition of Taxol or Taxotere no longer affects MT elasticity (Vinckier et al., 1996). To not only retrieve indentation data, but also bending elasticity, (Kis et al., 2002, 2008) did AFM on MTs attached to a surface with pores of around 0.2 μm the MTs were suspended over. These MTs were also stabilized with glutaraldehyde, although this method does allow for some extra features, such as separate direct measurement of Young's and Shear moduli.

AFM measurement of MTs without glutaraldehyde were done by (de Pablo et al., 2003; Schaap et al., 2006). Here immobilized Taxol-stabilized MTs were indented against a flat surface. This showed that these MTs deformed linearly up to 3 nN of applied force (~3.6 nm), but indented irreversibly after this threshold force. However, the indentation elasticity does not give complete information on the bending elasticity. Measurements of unstabilized MTs were also measured with AFM, but only in air-dried conditions, where all fluids are removed from the sample (Hamon et al., 2010). This results in collapsed MT tubules, and force curves are not retrievable. Recent work on triplet MTs of centrioles uses a new form of AFM, actuating the cantilever probe through photothermal means, which achieves much higher imaging speed and

much gentler sample probing while still retrieving force curves (Nievergelt et al., 2018). This has not yet been used on singlet MTs.

Other Techniques

Multiple other forms of active force application on MTs have been used to measure MT rigidity. Measurements of buckling and collapse under vesicle tension were done (Elbaum et al., 1996), where vesicles with MTs were put under increasing pressure through microaspiration to measure buckling force of the MT. While the pressure at the moment the MT collapses can be clearly measured, this does assume that physical failure of the column rigidity happens before chemical depolymerization or breakage.

A way to analyze the rigidity of extremely short MTs (<1 μm) was attempted using gliding assays, where the trajectories of Taxol-stabilized MTs on a kinesin-coated surface are tracked. Here the tangent angle trajectory can be tracked, which is a random walk that is affected by rigidity. The variance in trajectory can thus give clear measurements for MT persistence length (van den Heuvel et al., 2007). Another method uses a similar gliding assay, put under an electrical field that consistently applies a homogeneous force on the MTs (van den Heuvel et al., 2008). Gliding assays were also used for longer MTs in combination with thermal fluctuation experiments (Isozaki et al., 2017; Zhou et al., 2021), supporting the rigidity findings by showing different emergent MT organizations and sorting in the gliding assay for MTs with different rigidities. MT rigidity measurement with gliding assays was also done by (Kawaguchi et al., 2008), however, these did not track the position of gliding fibers, but used a temperature pulse to create a gradient of kinesin activity that could buckle the MT.

Microrheology of Taxol-stabilized MT networks, where highly crosslinked networks of MTs are probed with magnetic tweezers, was also done. However, force responses were here mostly defined by the reversible binding of crosslinkers, and not as much from column mechanics of MTs (Yang et al., 2013).

Another interesting way to apply force to the MT was done by (Needleman et al., 2005), by adding osmotic pressure to the MT. Here the fact that MTs are hollow was used, as polymers were added that could not enter the MT lumen and thus created an osmotic pressure on the MT. From this pressure, MTs buckle into 'rectangular' cross sections analyzed with small-angle x-ray diffraction. With sufficient pressure, elastic bending was achieved, but the authors do not have a full analysis for this, which was later done in (Wang et al., 2006). The buckling pressure for the interprotofilament walls into the rectangular conformation is quantified for pure GDP-MTs, with MAP mixture, Taxol and KCl.

MTs as Beams

From the *in vitro* data we can understand that the model of MTs as Euler-Bernoulli beams is probably an oversimplified understanding of MT physical behavior. Modeling MTs as an Euler-Bernoulli beam assumes that the MT is a linearly elastic, homogeneous isotropic material without shear deformation. Many of the *in vitro* results contradicts some of these assumptions, such as the length-dependencies (Pampaloni et al., 2006) and reversible softening under continual strain (Memet et al., 2018). Thus, the modeling of MTs evolved alongside the newly gathered data. To give an overview on the understanding of MT rigidity following from the data, a quick overview of *in silico* modeling of MTs is given here, a more thorough review can be found in (Liew et al., 2015).

A relevant expansion to the MT model as a beam is the orthotropic shell model (Wang et al., 2006), made to explain the found anisotropy in the data through MT structure. Here the buckling data of (Needleman et al., 2005) is used to fit to, and combining earlier theoretical work of (Sirenko et al., 1996; Tuszyński, Luchko, et al., 2005) and the measurements of (de Pablo et al., 2003) to construct an MT model that includes a longitudinal, shear and circumferential modulus, a defined Poisson's ratio, mass density per unit volume and equivalent and effective thicknesses. A similar orthotropic shell model was proposed by (Gu et al., 2009), with included transverse shearing. The model proposed in (Pampaloni et al., 2006) can also be counted as an orthotropic shell model. These models give much lower buckling forces for short (< 4-5 μm) MTs and support an inherent length-dependent MT rigidity. With these models, the MT length-dependence is mostly mapped to the shear deformation of the protofilaments sliding in relation to one another.

Computational analysis of the bulk behavior of the MT that goes more detailed than the orthotropic shell model needs to account for the way in which strain is transferred between

atoms. This has been done by modeling the MT as spheroid tubulins with fictitious-bond vectors connecting protofilaments with higher orders of the Cauchy-Born rule to curve the lattice model (Liew et al., 2011), this model shows a circumferential and longitudinal value that falls in the expected range, although these results are also limited by the experimental parameters put in. Another way to handle the atomistic scale of the MT is to use Eringen's non-local elasticity, which serves to bridge the gap between atomic lattice theory and classical elasticity theory by taking the stress at a single point as a function of strain at all points (Povstenko, 1999). Models specifically for MT deflection with nonlocal theory have been made, either in isolation (Civalek & Demir, 2011; Heireche et al., 2010) or in more complicated scenarios using Finite Element Method (Civalek & Demir, 2016). The results here show that these nonlocal effects may have relevance for the bending moment of MTs, but this depends on a nonlocal parameter that is hard to fit. Fitting this parameter has been attempted using molecular dynamics (MD) models (Motamedi & Sohail, 2018).

MD simulations of MTs have generally been a relevant tool alongside the 'top-down' studies discussed up to now. The MD approach consists of modeling the Newtonian forces on each atom in the system and assessing thermal dynamics and response to forces within the 'complete' atomistic model. The benefits of this include that many forces can exactly be quantified and the entire system is described. However, the all-atom description quickly makes models very large, especially due to the short modeled timestep that is limited by the timescale of atom movement (Hollingsworth & Dror, 2018). Full-atom MD models have been made for separate tubulin dimers (Tuszynski et al., 2004; Tuszyński, Brown, et al., 2005), up to 78 dimers in a 6-turn MT (Nasedkin et al., 2021), and can reveal some insights on the stability of the lattice for different tubulin states and thermal fluctuations of small lattice elements. Methods to coarse-grain the molecular dynamics, by abstracting regions of known interactions into single nodes have been done and have reached µm-length MTs, but lose the accuracy of all-atom MD (Deriu et al., 2012; Kmiecik et al., 2016; Zha et al., 2021).

State of the Art

The study into flexural rigidity of MTs has thus spanned almost 40 years now from the first quantification by (Mizushima-Sugano et al., 1983). The *in vitro* measurement of MT flexural rigidity has, in essence, mostly had a single goal: what are the biophysical deformation features of a MT under force, when we remove the complexity of the cell. Supplementary to this question is whether and how this is changed by other biological components. Through this time some of the major problems of the experimental technique have been solved. Where early active measurements struggle with analyzing the elastic deformation of MTs because of the low thresholds for non-elastic deformation, the recent optical trapping assays specifically target these higher modes of deformation and can quantify these features (Koch et al., 2017; Memet et al., 2018). Similarly, while earlier thermal fluctuation measurements struggle with image digitization to capture enough of the motion of a short stiff fiber to get the rigidity, recent work has explicitly improved on subpixel precision analysis of microscopic images of filaments (Brangwynne et al., 2007; Valdman et al., 2012).

The problem of MT dynamics stabilization has proven more difficult to remove from all measurements. While some of the earliest works on MT rigidity already show that Taxol stabilization has strong effects on MT rigidity and were unclear on its effects, almost all other works still use Taxol-stabilized MTs. Taxol-stabilization solves some of the difficult problems of MT rigidity measurements, as it allows MTs to go to grow very long, survive at this length for a long time and sustain more force. Many active measurements are nigh-impossible with unstabilized MTs, and thermal fluctuations are much harder to analyze with fewer frames and shorter MTs that are actively growing. Notable is here that (Janson & Dogterom, 2004a) did manage to analyze actively growing GDP-lattice MTs that were around 30 µm during imaging, well above the lower thresholds of measurement error. However, subsequent works have still opted to stabilize MTs.

Another major feature that still causes wide variations within single studies and is handled very differently between studies is the matter of MT preparation and sample heterogeneity. The tubulin used has varied between different animal sources (bovine, porcine and ovine), and nucleation of MTs is usually done via spontaneous aggregation into MTs. While controlled nucleation does not guarantee specific packing of tubulin or a protofilament number, spontaneous nucleation of MTs can yield varying populations under different polymerization conditions (Reid et al., 2017). Some of the first measurements already calculated how relative

changes of protofilament number can affect MT stiffness by power 3 (equating to $1-(13/14)^3=20\%$ change between 14 and 13 pf) purely through area moment of inertia I (Gittes et al., 1993). However, rigidity changes can be expected to be even more as changes in chemical structure will change material properties such as Young's modulus. Some of the early calibrated flow used sea urchin sperm axoneme nucleation, which may have lessened this issue. Studies that did try to address the heterogeneity of samples include (Harris et al., 2018; Hawkins et al., 2012), where the effects of tubulin purification method and salt concentration in polymerization were tested, among others. Arguably, the control of polymerization rate that allowed (Janson & Dogterom, 2004a; Zhou et al., 2021) to find a velocity-dependent rigidity can be included as analysis of polymerization conditions.

Another open issue is related more to results than methods: while strong evidence has been supplied for both a polymerization-speed dependent rigidity (Janson & Dogterom, 2004a; Zhou et al., 2021), the contour length-dependent rigidity that was found (Brangwynne et al., 2007; Kawaguchi & Yamaguchi, 2010; Koch et al., 2017; Pampaloni et al., 2006; Takasone et al., 2002; Taute et al., 2008) was not found in other studies (Hawkins et al., 2012, 2013; Isozaki et al., 2017; Valdman et al., 2012). The question of length dependence is thus still open. This especially hard to answer as MT shear deformation may give a good reason for a true length-dependence, but experimentally many other factors may correlate with length. This is for example that short MTs are harder precisely localize to the accuracy that is necessary, or that longer MTs may have lived longer or grown faster, implying different lattice quality.

Biological Consequence

To be able to quantify MT rigidity, both the *in vitro* and *in silico* measurements strip away much of the complexity of biological MT environment and modification. The cytoskeletal network, MT PTMs and the wide variation of MAPs can all significantly change MT physical behavior. To assess the *in vivo* rigidity is much harder, as all these confounding factors can no longer be disentangled. This has been attempted (Bicek et al., 2007; Brangwynne et al., 2006), but clear values of rigidity cannot be retrieved. The major points the *in vitro* experiments struggle with, like creating a uniform pool of MTs that are structurally and physically similar, as well as the extremely unstable nature of the MT, are features of the MT that are used by the cell. The heterogeneity of MTs is used in cells through multiple chemically distinct pools of MTs that coexist, with different MAP binding rates which can regulate transport by binding different motors (Janke & Magiera, 2020). The instability is used in many cases, but a clear example is in the formation of the mitotic spindle, where dynamic MTs are used to traverse the 3D search space to find the chromosomes and are stabilized after making contact (Verma & Maresca, 2022). Cells are able to stabilize MTs through means such as K40-acetylation (Janke & Montagnac, 2017), and do not have access to Taxol.

The plasticity of the MT lattice is very broad. MT structure has been shown to be affected by PTMs, MAP binding, nucleation conditions and more (Cross, 2019). Recent work has also shown how lattice defects along the MT can affect dynamics at the tip (Rai et al., 2021) and shown cooperative changing of MT growth rate between MAPs through interaction with the MT (Zanic et al., 2013), it is thus likely that changes in the lattice can propagate along the MT and change its biophysical features at long range. And because tip dynamics can be altered significantly, there is a high likelihood of the lattice rigidity also being variable. One of the open questions to relate the biological context to the measurements of rigidity is thus to find what MT structures are actually inside cells, and how to reconstitute these or measure them *in vivo*.

Another interesting thing to consider is in which situations the exact value of rigidity will matter to cellular behavior. In many cells, MTs have an active regulatory role as a transport network (Burute & Kapitein, 2019), where the high rigidity of MTs can be expected to mostly aid in making straight paths. Similarly for spindle assembly and mitosis, many of the used features of MTs, such as rapid dynamics to search for chromosomes and the depolymerization-based pulling of the kinetochore (Verma & Maresca, 2022) seem to not explicitly exert force through rigidity, outside of ensuring a relative measure of straight orientation. A clear example where MTs do have mechanical function is in providing pushing forces through polymerization or dynein pulling. This is used in processes such as mesenchymal migration (Garcin & Straube, 2019; Zhovmer et al., 2022) and centrosome positioning (Elric & Etienne-Manneville, 2014). Other examples of rigidity-based MT force exertion are long range mechanotransduction (Seetharaman et al., 2021), and as compressive elements in a tensegrity based cell structure (Mehrbod & Mofrad, 2011). The use of MTs as force-exerting elements in these biological

contexts necessarily depends on the rigidity of the MT. Thus, it is also important to consider how the cells control the rigidity of these MTs, and how much this rigidity will matter. For example, the range of mechanotransduction or the effectivity of centrosome positioning may depend on specific MT rigidities and may not work for other subtypes of MTs or within different biological circumstances.

Conclusion and Discussion

In the almost forty years since the first *in vitro* measurement of MT rigidity, the field has come a long way, from a range of magnitude where the studies varied four-fold to current methods where good estimates of pure MT rigidity are known, some major dependencies of rigidity are known, and higher order deformations have been studied in Taxol-stabilized MTs. Some of the larger issues remain with MT sample variation within and between studies. Despite these strides in knowledge, reconciliation with biological MTs is still difficult. The cell biology creates subtypes of MTs that are still poorly understood that carry out different functions. The elucidation of MT rigidity in cell biology will thus probably require targeted identification of force-exerting MTs, to derive what features need to be reconstituted or probed to assess how the rigidity is regulated in the broad cellular context.

The current models and measured values are not fully integrated, but we can distill some general rules of around 13-pf GDP-lattice MTs from the field. Growing GDP-lattices of around 30 μm length have rigidities between 18 and 28 pN・μm^2 (Janson & Dogterom, 2004a), depending on polymerization speeds. Slow-growing MTs are expected to be more stiff than fast-growing MTs. A more disputed finding is the length-dependence. Some find MTs to range three-fold in rigidity between 10 μm length and 70 μm length, when Taxol-stabilized (Brangwynne et al., 2007; Pampaloni et al., 2006), supported by modeling (Wang et al., 2006). These give a range of 1 to 4 mm persistence length between 10 and 70 μm length (Brangwynne et al., 2007). Modeling and experiments have also shown that short (< 5 μm) MTs behave differently (Gu et al., 2009; Taute et al., 2008; Wang et al., 2006) with a measured limit of around 0.2 mm *Lp* (van den Heuvel et al., 2008). However, this finding is contradicted by other research that does not find a length dependence between 5 and 30 μm and smaller ranges for Taxol-stabilized MTs (Hawkins et al., 2013; Isozaki et al., 2017; Valdman et al., 2012).

For the understanding and modeling of the cytoskeleton, the range of magnitude and dependencies of flexural rigidity of pure GDP-lattice MTs that have been found are already very powerful tool. Modeling MTs as beams reproduces much of their physical behavior, while being vastly less computationally expensive than taking precise atomic structure. Modeling has been done within the measured range of rigidity to retrieve its effect under biological boundary conditions (Dmitrieff et al., 2017; Pinot et al., 2009), but modeling can also be done for more complex cytoskeletal systems where a generic MT Euler-Bernoulli column model is deemed sufficiently detailed to account for the variation in the system (most of the research done with the open-source framework *Cytosim* (Nedelec & Foethke, 2007)), which is still a more computationally intense solution than assuming MTs are infinitely stiff rods, as is sometimes done (Lamson et al., 2021). As such the understanding of MTs as columns gives us a lot of power to understand the larger cytoskeletal interplay by allowing easy computation of the general behavior within the range of values that have been measured.

However, some situations are present where a more detailed and specific analysis of MT rigidity may be necessary. This is especially relevant when measuring the forces that MTs exert. An example of this is work where the *in vitro* force exertion of MT polymerization was measured (Dogterom & Yurke, 1997; Janson & Dogterom, 2004b), where the rigidity of the MTs was also measured, as no meaningful statements could be made about the force-exertion without knowing the exact rigidity of these MTs. Similarly, exact rigidities may also be necessary to understand the more complicated force-exerting functions of MTs *in vivo*, such as mechanotransduction. The next step in retrieving the rigidity of force-exerting MTs may not be more rigorous *in vitro* testing, but more so the identification of the MT that is exerting force *in vivo*. As long as we do not know structure of the nucleated MTs that are being assessed, and what other components are altering it, we cannot reconstitute or model it. An interesting direction here is the recent work of (Zha et al., 2021), where the cryo-EM tomography structure of *in vivo* cellular MTs was taken, and was modeled with coarse-grained molecular dynamics simulations at μm length and timescales large enough to measure thermal fluctuations. This technique may directly link *in vivo* structure with flexural rigidity. However, this study did validate their coarse-grained bond strength of their native structure against the glutaraldehyde-stabilized MTs of (Kis et al., 2002).

It would be an interesting endeavor to do similar simulations of cryo-EM structures of MTs side-by-side with an *in vitro* thermal fluctuation assay, to model the known rigidity for validation and use this valid model on native *in vivo* structures as well.

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Table 1.
A table summarizing bending rigidity findings between studies. All Young's/Shear modulus measurements are left out.
Note that only reported values are given (outside of some noted exceptions) and flexural rigidity EI is analogous to persistence length Lp ($L_p = \frac{EI}{k_b T}$). Because not all studies name the temperature during measurement, conversions were not done for all. If values were taken along a linear axis that was not binned, ranges are given and the dependency found is named under Condition.
Rhodamine-labeling is only named when separate results are presented. Variation in polymerization condition such as GTP concentration, glycerol and others are not named. Temperature given is the temperature during measurement, polymerization temperatures are often at 35-37 ℃, but can differ from this.

Reference	Stabili-zation	Nucleotide	Nucleation	Source	Method	Forces	Temp (℃)	Length (μm)	Tubulin (μM)	Condition	*EI* (pN • μm²)	±*EI*	*Lp* (mm)	±*Lp*	Additional meas-urements	*Notes*
(Mizushima-Sugano et al., 1983)	-	GTP	spontaneous	porcine	affixed to coverslip	passive	25	-	-	-	0.45		0.74			*60 % pure GTP tubulin, converted values*
(Dye et al., 1993)	-	GTP	sea urchin sperm axoneme	-	shape under flow	active	37	10-15	11.83	-			0.33	0.09		*Converted values*
	Taxol												0.031	0.008		
	Taxol + MAPs												0.074	0.009		
(Gittes et al., 1993)	Taxol	GTP	spontaneous	-	Thermal fluct-uations	passive	25	24.5-57.2	18.2-54.6		22	1.5				
										Rhodamine-tagged	21	1				
(Venier et al., 1994)	-	GTP	sea urchin sperm axoneme	porcine	Calibrated flow	active	37	9-19	20	-	8.5	2	2	0.43		*10 μM tubulin flow, named concentration under polymerization*
		GDP BeF3									29	5	6.85	1.3		
		GDP AlF4									25	5	5.87	1.3		
		GTP			Thermal fluct-uations	passive					9.2	0.9	2.2	0.22		
	Taxol								12		4.7	0.4	1.17	0.1		
	Taxotere								12		4.8	0.4				
	-	GDP BeF3							20		26	2.7	6.1	0.64		
(Kurachi et al., 1995)	MAPs	GTP	spontaneous	bovine	Optical trap buckling (beads), elastica	active	37	10	2.73	-	34	17				
								30			200	60				
	Taxol							5			1	0.65				
								20			20	6				
(Kurz & Williams, 1995)	-	GTP	sea urchin sperm axoneme	bovine	Calibrated flow	active	37	15-31	11.83	-	35.8	9.5	8.4	2.2		
	MAPs										39.5	12.5	9.4	2.7		
	-					passive							6.2	0.8		

	MAPs				Thermal fluct-uations								6.5	0.8		
(Mickey & Howard, 1995)	Taxol	GTP	spontaneous	bovine	Thermal fluct-uations	passive	37	24-68	20	-	32	2				
							25				21	1				
	Tau	GTP					37				34	3				
	GMPCPP cap	GTP									26	2				
	-	GMPCPP							1		62	9				
(Elbaum et al., 1996)	-	GTP	spontaneous	bovine	Buckling in vesicles	active	27	38 (single instance)	30		26	10				
(Felgner et al., 1996)	-	GTP	Algae (*Chlamydomonas reinhardtii*) axoneme	porcine	RELAX	active	22-25	5-20	9.1	-	3.7	0.8				
	Taxol										1	0.3				
	MAP mixture										16	3				
	-				WIGGLE						4.7	0.4				
	Taxol										1.9	0.1				
	MAP mixture										18	3				
(Dogterom & Yurke, 1997)	OXS	GTP	GMPCPP seed	bovine	Thermal fluct-uations, middle and end point	passive	22	10-20	25	-	34	7				
(Felgner et al., 1997)	-	GTP	Algae (Chlamydomonas reinhardtii) axoneme	porcine	RELAX	active	22-25	5-20	9.1	-	3.8	0.9				
	2 % Tau saturation										4.5	1.5				
	18 % Tau saturation										8.9	1.3				
	48 % Tau saturation										9.4	2.6				
	85 % Tau saturation										10.4	3.1				
	MAP2C										15.1	3.3				
	MAP2D										16.1	2.7				
	MAP2C3										14.5	3.8				
	HT40										10.4	3.1				
	MAP mixture										16	3				
	-	GTP		porcine		passive	-	-	10	-	17.5	2.2				

(Cassimeris et al., 2001)	XMAP215		axoneme (unnamed origin)		Thermal fluct-uations end point						18.5	2				
(Takasone et al., 2002)	Taxol	GTP	spontaneous	porcine	Optical trap (beads), cantilever /bending	active	22	3-20	18.2	EI ∝ L^2	1-20					*Values estimated from graph as table was not shown*
					Optical trap buckling (beads), compressive						0.5-10					
					Optical trap buckling (beads), elastica						0.5-10					
(Janson & Dogterom, 2004a)	-	GTP	GMPCPP seed	bovine	Thermal fluct-uations	passive	23	28.5-34.5	26	-			6.6	0.9		
	OXS							27.6-33.5	28				4.2	0.3		
(Janson & Dogterom, 2004b)	OXS	GTP	GMPCPP seed	-	Thermal fluct-uations end point	passive	-	-	20	-	21.2	1.7				
									28		13.7	1.4				
(Kikumoto et al., 2006)	Taxol	GTP	spontaneous	bovine	Dual optical trap buckling (anti-tub beads)	active	33 ± 1	7-23	27.3	-	2	1.1				*No length dependency*
	Deu-terium oxide										7.9	0.7				
(Pampaloni et al., 2006)	Taxol	GTP	spontaneous	porcine	Thermal fluct-uations single point (attached bead)	passive	-	2.6-47.5	25	$l_p = l_p^{\infty}(1 + \left(\frac{L_{crit}}{L}\right)^2)^{-1}$		0.11 - 5.035			*Lcrit = 21 µm, L_p∞ =6.3±0.8 mm;*	*dual tracked points were also checked for consistency*
(Brangwynne et al., 2007)	Taxol	GTP	spontaneous	bovine	Thermal fluct-uations	passive	room temp-erature	18-66	-	$l_p = l_p^{\infty}(1 + \left(\frac{L_{crit}}{L}\right)^2)^{-1}$			0.8-4.2			
								18-25					2.8	1		
								25-66					1.5	0.7		
van den Heuvel et al., 2007)	Taxol	GTP	spontaneous	bovine	Gliding assay trajectories	active	-	< 1	0.008				0.24	0.03		

Reference	Stabili-zation	Nucleotide	Nucleation	Source	Method	Forces	Temp (℃)	Length (μm)	Tubulin (μM)	Condition	*EI* (pN • μm²)	*±EI*	*Lp* (mm)	*±Lp*	Additional meas-urements	*Notes*
(Kawaguchi et al., 2008)	Taxol	GTP	spontaneous	porcine	Thermal fluct-uations clamped end to bead	passive	20-35	5.6-17.2	20	Dependency on Temperature	2.54	0.52			*Temperature does not affect stiffness*	
					Buckling in gliding assay (Temperature Pulse Micro-scopy)	active	20 (with 1-2 ℃ pulse)	5.3-7.8		-	2.7–7.8					
							35 (with 1-2 ℃ pulse)				2.7–7.8					
(Taute et al., 2008)	Taxol	GTP	spontaneous	-	Thermal fluct-uations single point (attached bead)	passive	-	2.2-5	-	-			0.58	0.1		
								5-27.9		EI ∝ L^2			0.5-3			
(van den Heuvel et al., 2008)	Taxol	GTP	spontaneous	bovine	Gliding assay trajectories with electrical field	active	-	0.1	0.2	-			0.08	0.02		
(van Mameren et al., 2009)	Taxol	GTP	spontaneous	porcine	Dual optical trap buckling (streptavidin beads)	active	unable to infer	-	-	single	6.1	1.3	1.4			*Rigidity and persistence length do not result in a consistent temperature*
(Kawaguchi & Yamaguchi, 2010)	-	GTP	spontaneous	porcine	Thermal fluct-uations clamped end to bead	passive	20	5-20	20	EI ∝ L	8.12	1.52			*Slight increases with MT length, mean values are reported*	
		GMPCPP									12	1.7				
		GTP					25				7.3	1.95				
		GMPCPP									10.6	1.69				
		GTP					30				6.26	1.55				
		GMPCPP									10.3	2.18				
		GTP					35				6.1	1.92				
		GMPCPP									10.1	1.99				
		GTP					20-35	8-12		EI decreases with T	8.12-5.41					

		GMPCPP									11.9-8.9					
(Hawkins et al., 2012)	Taxol + OXS	GTP	spontaneous	porcine	Thermal fluct-uations	passive	-	5-31	45.4	4 hours stored, Commercial tubulin, 16% rhodamine			1.6	0.2	*No length dependence found*	*Under-estimated by exactly factor 2 (Harris,2018)*
										4 hours stored, In-house purified tubulin, 16% rhodamine			0.6	0.1		
										24 hours stored, Commercial tubulin, 16% rhodamine			0.8	0.1		
										24 hours stored, In-house purified tubulin, 16% rhodamine			0.6	0.1		
										24 hours stored, In-house purified tubulin, 7% rhodamine			0.77			
										24 hours stored, In-house purified tubulin, 10% rhodamine			0.57			
										24 hours stored, In-house purified tubulin, 16% rhodamine			0.58			
										24 hours stored, In-house purified tubulin, 25% rhodamine			0.77			

(Valdman et al., 2012)	Taxol	GTP	spontaneous	bovine	Thermal fluct-uations	passive	ambient	10-20	20	-			3.45	0.22	*No length dependence found*	
(Hawkins et al., 2013)	Taxol	GTP	spontaneous	porcine	Thermal fluct-uations	passive	25	5-30	45.5	-	2.5	0.5	0.6	0.1	*No length dependence found*	*Under-estimated by exactly factor 2 (Harris,2018) due to shape digitization*
	-	GMPCPP									8	2	1.8	0.5		
	Taxol	GMPCPP									8	3	1.9	0.7		
	-	GTP-γ-S									2.1	0.6	0.51	0.1		
	Taxol	GTP-γ-S									2.1	0.5	0.51	0.1		
	Taxol, Tau copolymerization	GTP							0.45		15	4	4	1		
	Taxol, Tau post-polymerization										2	0.3	0.49	0.07		
	Taxol, MAP4 post-polymerization										2.2	0.4	0.6	0.1		
(Lopez & Valentine, 2014)	Taxol	GTP	spontaneous	bovine	Thermal fluct-uations	passive	room temp-erature	15-20	50	-			2.4			*non-gaussian wide distributions, GMPCPP seeds are described: nucleation might be this*
	-	GMPCPP							2	with EB1 concen-trations (at 0 EB1 named here)			4.8		*EB1 increases rigidity up to 6.7-10 mm*	
	Taxol	GMPCPP							50				6.8		*EB1 does not affect stiffness*	
		GTP-γ-S											3.2		*EB1 slighty increases stiffness up to mean 5 mm*	
	Taxol (post-polymerization)	GMPCPP							2 or 50	-			4.8			
(Isozaki et al., 2017)	Taxol + Tau + OXS	GMPCPP	GMPCPP seeds	porcine	Thermal fluct-uations	passive	~ 27 (room temp-erature)	2-17	10	-			16		*No length dependency*	*values estimated from graph as table was not shown*
	Taxol + OXS												16			
	Taxol + Tau + OXS	GTP											9			
	Taxol + OXS												8.7			
									30				3.2			
(Koch et al., 2017)	Taxol + OXS	GTP	spontaneous	-	dual optical	active	-	5	-		-	-	0.33	0.05	*Oscillation-frequency*	

	High Taxol + OXS				trap oscillations					lp(*ω*)=*G*′(*ω*)·2.16*L*^4≈lp(0)+lp(*ω*>*ω*)	-	-	0.33	0.05	*dependent stiffness is found, getting stiffer with frequency ω, Lp(0), Lp without oscillation, named here*	
	High Taxol + OXS	GMPCPP									-	-	0.33	0.05		
	Taxol + OXS	GTP						15			-	-	4.06	0.26		
	High Taxol + OXS										-	-	5.8	0.39		
	High Taxol + OXS	GMPCPP									-	-	12.1	0.66		
(Harris et al., 2018)	Taxol	GTP	spontaneous	porcine	Thermal fluct-uations	passive	-	8-35	45.5	(expected 12-13 pf)	4.9	0.2	1.19	0.04		
										NaCl (expected 10 pf)	2.83	0.08	0.69	0.02		
(Memet et al., 2018)	-	GMPCPP	spontaneous	bovine	Dual optical trap continuous bending	active	-	7.1	118.3	-	12				*MT only fits to stiffness with strain ε < -0.1, and undergoes significant reversible softening under higher strain*	
(Zhou et al., 2021)	Taxol	GTP	GMPCPP seeds	porcine	Thermal fluct-uations	passive	37 (presumed from context)	-	20	-	0.71	0.36				
									30		0.8	0.34				
									35		0.75	0.29				
									40		0.7	0.32				
									50		0.63	0.2				
									75		0.43	0.31				
									100		0.27	0.1				
									150		0.31	0.18				
									200		0.32	0.39				