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# Microtubule assembly dynamics: new insights at the nanoscale

Melissa K Gardner[1], Alan J Hunt[2], Holly V Goodson[3] and David J Odde[1]

Although the dynamic self-assembly behavior of microtubule ends has been well characterized at the spatial resolution of light microscopy (~200 nm), the single-molecule events that lead to these dynamics are less clear. Recently, a number of *in vitro* studies used novel approaches combining laser tweezers, microfabricated chambers, and high-resolution tracking of microtubule-bound beads to characterize mechanochemical aspects of MT dynamics at nanometer scale resolution. In addition, computational modeling is providing a framework for integrating these experimental results into physically plausible models of molecular scale microtubule dynamics. These nanoscale studies are providing new fundamental insights about microtubule assembly, and will be important for advancing our understanding of how microtubule dynamic instability is regulated *in vivo* via microtubule-associated proteins, therapeutic agents, and mechanical forces.

**Addresses**
[1] Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN 55455, United States
[2] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, United States
[3] Department of Chemistry and Biochemistry and Interdisciplinary Center for the Study of Biocomplexity, University of Notre Dame, Notre Dame, IN 46556, United States

Corresponding author: Odde, David J (oddex002@umn.edu)

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## Introduction

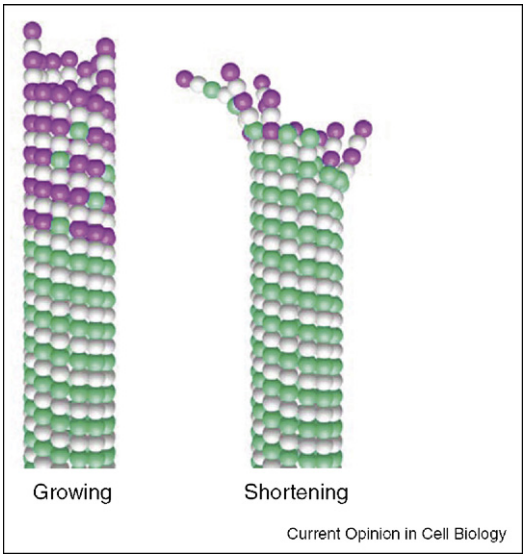
Microtubules (MTs) are dynamic, growing and shortening at their ends via αβ-tubulin dimer addition and loss. These dynamics are important for rapid cellular restructuring of the cytoskeleton, as well as for mediating the delivery of cellular cargos. For example, during mitosis, dynamic MTs mediate the alignment of sister chromatids at the spindle equator during metaphase, and ultimately segregate the sister chromatids into the nascent daughter cells during anaphase [1].

Unlike most self-assembled polymers, single MTs undergo an unusual assembly process, called dynamic instability, where MTs switch back and forth stochastically between extended periods (seconds–minutes) of growth and shortening. To quantitatively characterize dynamic instability, four parameters have been defined: the growing and shortening rates of MT ends, and the frequencies of switching between these two states. The frequency at which a growing MT tip switches to a shortening state is called the 'catastrophe' frequency, while 'rescue' frequency is the frequency at which a shortening MT tip switches into a growing state [2]. Numerous studies using light microscopy have quantitatively characterized this behavior both *in vitro* and *in vivo* [3–5].

Although dynamic instability has been well characterized at the spatial resolution of light microscopy (~200 nm), dynamic characterization at single-molecule resolution is just now emerging. Until recently, our understanding of MTs at single-molecule resolution has rested largely on electron microscopy studies. These studies have shown that MTs are typically composed of 13 individual protofilaments consisting of αβ-tubulin heterodimers lined up end-to-end (Figure 1). The electron microscopy images of growing and shortening MT tips reveal that shortening MT ends can have outwardly curling individual protofilaments, while growing ends are relatively straight [6–8,9•] (Figure 1). Tubulin heterodimers that are added to MT tips are GTP-bound. The difference in structure between growing and shortening MT tips has been attributed to a difference in preferred orientation of GTP versus GDP nucleotide-containing αβ-tubulin heterodimers [10,11] because the GTP nucleotide is subsequently hydrolyzed to GDP after incorporation into the MT lattice. Specifically, the GDP-bound tubulin heterodimer is believed to curl outwardly when exposed at MT ends, resulting in destabilization of the lateral bonds between the tubulin subunits. This destabilization then leads to depolymerization of the MT lattice. Conversely, a 'cap' of GTP-bound tubulin heterodimers at the MT tip leads to relatively straight growing ends that stabilize the MT from rapid depolymerization (Figure 1, magenta). However, the size and structure of the stabilizing GTP-tubulin cap remains unclear.

Although electron microscopy data has provided important nanoscale information, it has not supplied direct information about MT dynamics because samples are fixed before imaging. Recent studies, highlighted below, are now directly collecting information about MT dynamics at the nanoscale. Here, novel approaches combining laser tweezers, microfabricated chambers, and high-resolution tracking of MT-attached beads were used to characterize various aspects of MT dynamics at nan-

**Figure 1**




Microtubule structure at the nanoscale. Microtubules *in vivo* are typically composed of 13 individual protofilaments organized into a tube configuration. The individual protofilaments consist of stacked αβ-tubulin heterodimers with an exchangeable GDP/GTP binding site on the β subunit (GDP-tubulin αβ subunits are shown in white and green; GTP-tubulin αβ subunits shown in white and magenta). A GTP-tubulin 'cap' stabilizes MT growth (left, magenta), possibly by keeping individual protofilament subunits in close proximity to each other. By contrast, depolymerizing MT tips tend to have curled protofilaments because of the preferential outward kinking of exposed GDP-tubulin subunits (right). Cartoons depict output from the mechanochemical model of VanBuren *et al.* [25].

ometer scale resolution. These studies have led to new insights into the following: first, the mechanisms of dynamic instability behavior at the single-molecule level; second, the effect of compressive loading on these behaviors; third, the forces generated by a single depolymerizing MT protofilament; and fourth, the effect of tensile force coupling on MT plus-end dynamic instability. These studies, combined with computational modeling, provide a new framework for understanding MT assembly dynamics.

## Microtubule dynamics at the nanoscale

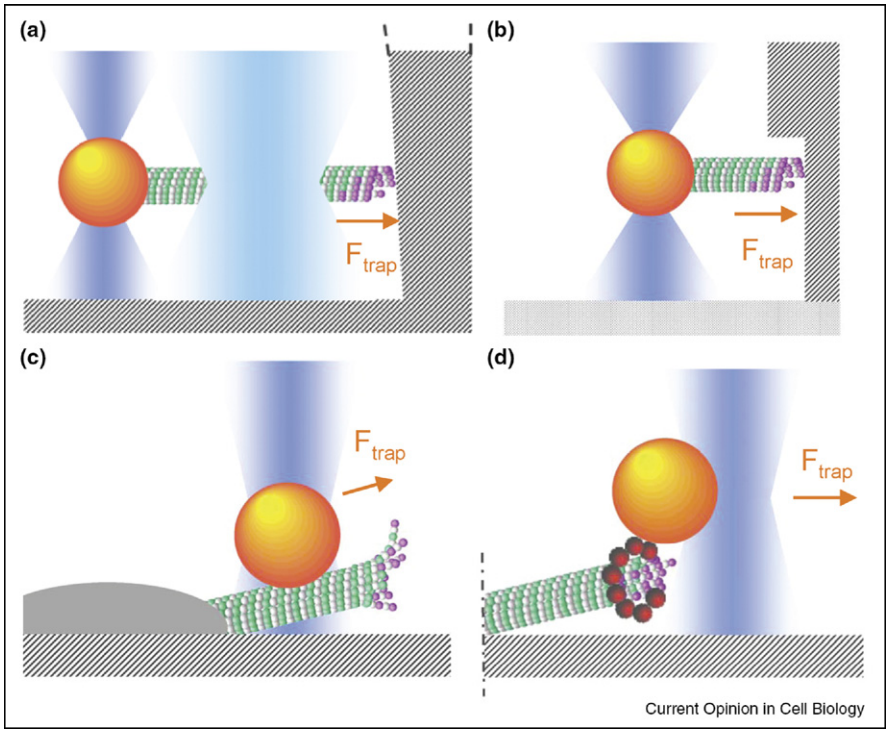
New technologies have recently been developed for characterizing *in vitro* MT dynamics at molecular scale resolution. Recent work by Kerssemakers *et al.* [12••] first reported the dynamic growth and shortening behavior of MT plus-ends at the resolution of a single tubulin dimer (8 nm). Here, the authors held a bead/axoneme construct in an optical trap, and then allowed growth of a single MT into a rigid microfabricated barrier (Figure 2a) [13,14]. As the MT minus-end moved away from the barrier because of growth of the leading protofilament against the barrier, the bead position was displaced. By measuring the displacement of the bead in the trap over time, the authors were able to measure MT plus-end assembly dynamics of the leading protofilament at a sampling rate of 25 Hz and at a spatial resolution of 5–10 nm. The results reported by Kerssemakers and co-workers provided an unprecedented look into MT plus-end dynamics at near molecular-level resolution.

Interestingly, the relatively constant MT plus-end growth and shortening rates observed with light microscopy that have traditionally characterized 'dynamic instability' behavior turn out to be highly variable at molecular scale resolution. Periods of rapid growth were observed that were clearly interspersed with periods of slower growth and even occasional shortening. Kerssemakers *et al.* [12••] interpreted this rapid growth behavior as being 'step-like', in which rapid length changes were regularly interrupted with periods of slower changes in length. The addition of XMAP215, a microtubule-associated protein (MAP) known to increase the MT growth rate [15,16], increased the frequency and duration of rapid MT plus-end length changes at the nanoscale. In addition, the step-like events appeared to be larger, on the order of tens of nanometers, which approaches the experimentally measured length of XMAP215 [17]. It was suggested that small oligomers, composed of two or more subunits, can add to growing MT plus-ends, and that addition of these oligomers is further promoted by XMAP215 (step size 40–60 nm) because the size of the steps in the absence of XMAP215 (20–30 nm) exceeded the size of a single subunit length (8 nm). This study is highly significant because it demonstrated the feasibility of measuring MT assembly dynamics at near molecular-level resolution. This represented a resolution improvement of more than an order of magnitude in going from the light microscope (~200 nm), which has been the gold standard for observing MT assembly for the past 20 years, to the laser-tweezers-tracking method (5–10 nm).

More recently, Schek *et al.* [18••] reported nanoscale MT dynamics using a similar experimental geometry to that of Kerssemakers *et al.* (Figure 2b). By collecting unfiltered data at higher temporal resolution (5 kHz), the authors were able to temporally average out thermal noise to report the molecular scale behavior of MT plus-end assembly at ~3.5 nm spatial resolution. In addition, the force generated from bead deflections in the laser trap because of polymerization at the MT plus-end was clamped to specific values by regular (10 Hz) adjustments to the laser trap position. Thus, leading protofilament MT plus-end growth could be characterized at higher temporal and spatial resolution and under conditions of relatively constant, low compressive load.

Similar to results reported by Kerssemakers *et al.* [12••], Schek *et al.* [18••] reported highly variable MT plus-end growth behavior. Here, rapid advances in plus-end polymerization were balanced by periods of slow growth

**Figure 2**




Depiction of experimental assays for measuring various aspects of nanoscale MT dynamics. **(a)** The experimental assay designed by Kerssemakers *et al.* [12] for measuring MT assembly dynamics at the nanoscale. A microtubule-attached bead (orange, bead not to scale) is centered via a two-component 'keyhole' optical trap (dark blue/light blue). The dark blue 'point' trap serves to keep the MT plus-end (magenta) in contact with a microfabricated barrier (hatched vertical section, direction of force shown by orange arrow), and the light blue 'line' trap serves to orient the MT perpendicular to the barrier wall. Deflection of the bead away from the center of the 'point' trap (dark blue) reflects leading protofilament length fluctuations at the MT plus-end (magenta). **(b)** An assay designed by Schek *et al.* [18] similarly measured MT plus-end leading protofilament length fluctuations (magenta) via deflection of a bead (orange, bead not to scale) away from the center point of a laser trap (dark blue). In contrast to the Kerrsemakers *et al.* assay, the position of the laser trap itself was updated at 10 Hz frequency to maintain a constant force at the MT tip as the MT increased in length during polymerization. **(c)** In an assay designed by Grishchuk *et al.* [26], brief deflections in the position of a bead (orange, bead not to scale) conjugated to the microtubule lattice (green/magenta) are recorded as the MT tip depolymerizes. GDP-tubulin subunits should displace the bead as the MT tip depolymerization proceeds past the site of bead attachment because of their preference to curl radially outward when exposed at the MT tip. Thus, forces produced by the depolymerizing plus-end are inferred from the magnitude of bead deflections from the center of the trap (laser trap — dark blue; resisting force provided by trapped bead shown as orange arrow). **(d)** In an assay designed by Franck *et al.* [30] tension is applied at MT plus-ends by pulling a MT away from a laser-trapped bead (laser trap — blue, bead — orange, not to scale), while maintaining the bead at a constant position relative to the center of the trap. Mechanochemical coupling is achieved between the MT plus-end (magenta) and the bead (orange) via the Dam1p protein complex (red), which forms a key kinetochore component in yeast. MT length fluctuations are recorded by measuring the distance between the MT minus-end and the bead position.

and, surprisingly, shortening excursions of ~40 nm or more that did not result in plus-end catastrophe. In this work, the improved temporal resolution of the experiment showed that periods of rapid growth, that could appear step-like at lower resolution, were in fact made up of a series of small (nm) increments. In over 16 000 observations, there were not any events that corresponded to the 20–30 nm steps suggested by Kerssemakers *et al.* [12••], demonstrating that oligomer-mediated addition was extremely rare under the conditions of the Schek *et al.* [18••] experiment.

While Schek *et al.*'s experiments allowed direct measurement of characteristics such as nanoscale changes in leading protofilament length, they also provided more indirect but equally important insight into the size and structure of the stabilizing GTP cap. A long-standing view in the literature is that the GTP-cap could be as thin as a single layer [19–23]. However, frequent nanoscale shortening events of 40 nm or more (~5 tubulin subunit layers) without MT catastrophe suggested a more substantial stabilizing GTP-cap depth. To interpret these fluctuations, Schek *et al.* compared the observed dynamics to those predicted by a GTP-cap model that assumes first-order hydrolysis once a GTP-tubulin is buried in the lattice [24,25•]. Here, an approximately exponentially distributed GTP-cap, typically composed of ~40–60 GTP-tubulins, was sufficient to explain the substantial nanoscale shortening events observed during MT plus-end growth.

Finally, by allowing for regular adjustments to the bead laser trap position such that nearly constant compressive loads were maintained at the growing MT tip, Schek *et al.* reported the effect of increased compressive load on nanoscale MT growth behavior. Interestingly, the variability in nanoscale growth behavior was suppressed at higher compressive forces as compared to low force behavior, an effect that was predicted by computational modeling [24]. Using the model as a guide, it was hypothesized that leading protofilaments have greater difficulty remaining ahead of lagging protofilaments when the compressive force is relatively large and the probability of subunit addition to leading protofilaments is therefore relatively small. The shortening that occurs when the leading protofilament depolymerizes is decreased at higher compressive forces because lagging protofilaments stay closer to the leading protofilament. As a result, the amplitude of both nanoscale growth and shortening excursions during assembly under load is suppressed at higher force. The overall consequence is that the net growth rate is only weakly reduced by load (up to 2.5 pN). In summary, the study by Schek *et al.* [18••] showed that a single-layer GTP-cap model is very unlikely, that MT growth by oligomers is very unlikely, and that net growth is only weakly slowed by compressive load. Together, the studies of Kerssemakers *et al.* and Schek *et al.* open new doors for MT research, where MAPs and drugs can be added and the effects observed at near molecular resolution. An important aspect of these efforts will be the integration of computational models for MT assembly in the presence of MAPs and drugs, whose predictions can be directly compared to these experiments.

## Mechanochemical coupling at the microtubule plus-end

Recently, methods developed both for indirectly measuring force at depolymerizing MT plus-ends and for directly applying force at dynamic MT plus-ends provided insight into how mechanochemical coupling at MT plus-ends could mediate cellular movement of cargos attached at plus-ends, and how attachment of these cargos could in turn regulate the dynamics of MT plus-ends.

In particular, recent work by Grishchuk *et al.* [26••] examined force production by disassembling MTs. Here, the authors conjugated glass beads onto the sides of depolymerizing MTs, and then used laser tweezers to measure the forces exerted by depolymerizing MT tips (Figure 2c). It was predicted that curling protofilaments at the depolymerizing MT tip would briefly pull the lattice-attached bead away from the center of the trap during MT depolymerization past the bead. Thus, by monitoring bead movements away from the center of the laser trap, a force spike could be recorded as the depolymerizing MT tip contacted the bead. The authors measured raw force magnitudes of ~0.2 pN from an estimated one to two depolymerizing MT protofilaments. By taking into account the size of the bead and the likely protofilament length in contact with the bead, the calculated force produced by a single depolymerizing protofilament was estimated to be ~5 pN, which would translate to 30–65 pN if 13 protofilaments were acting in concert. The extent of force generation is consistent with a computational model for protofilament peeling [27•]. Thus, the forces produced by depolymerizing MT plus-ends are estimated to be on the order of multiple molecular motors [28,29] suggesting that MT depolymerization forces may be crucial in intracellular motility, such as in chromosome movements during mitosis.

Interestingly, Grishchuk *et al.* [26] also noted that the duration of bead attachment during disassembly was increased by tension at the MT tip acting to oppose the outward peeling of disassembling protofilaments. Thus, although outwardly peeling MT tips could exert significant forces during depolymerization, tensional loads exerted through a coupling mechanism at the MT tip could in turn act to alter tip structure and regulate MT plus-end assembly dynamics. Therefore, dynamic instability at the MT tip could result in a 'give-and-take' between both force-generating outward peeling of disassembling protofilaments, and, as tensional loads increase through a coupling mechanism at the MT tip, a switch to net polymerization and growth of individual protofilaments. This balance could contribute to the 'directional instability' of chromosomes during mitosis, in which chromosomes oscillate between poleward and anti-poleward movement. During anaphase, this balance is ultimately tipped in favor of net depolymerization at the MT tip, as chromosomes separate and move to opposite poles.

To further study the effect of tensional loads exerted through a coupling mechanism at the MT tip, Franck *et al.* [30••] considered the effect of tension at the MT tip though the use of a biologically relevant force coupler. Here, the authors used the yeast Dam1 complex, which has been shown to form rings around MTs *in vitro* [31,32], to exert continuous tension at the plus-ends of MTs. *In vivo*, the Dam1 complex is a key component of the yeast kinetochore that links MT plus-ends to chromosomes ([33], reviewed by [34]). Specifically, beads coated with the Dam1p complex were attached to the tips of MTs, and then tension was applied via optical trapping of the tip-attached bead (Figure 2d). In contrast to the reduced MT plus-end growth rates that are observed under compressive loading of the tip [18••,35–37], tensile loading via a biological coupler had little effect on the growth rate of the MT plus-end. However, the other three parameters of dynamic instability were significantly affected by tensile loading. In particular, the MT plus-end catastrophe frequency was reduced under tension, while the rescue frequency was increased. In addition, the MT shortening rate was greatly reduced, similar to results reported by Grishchuk *et al.* [26••].

Overall, Franck *et al.* [30••] found that tension applied to the MT plus-end via the Dam1p yeast kinetochore coupler promoted net MT assembly relative to a MT tip with no load on it. Promotion of MT plus-end assembly via tension is consistent with previous *in vivo* studies showing that increased tension slowed or even reversed kinetochore poleward movement during mitosis [38–41]. However, *in vivo* depolymerizing kinetochore MTs could possibly just detach from the kinetochore coupler when under load, rather than slowing their shortening or rescuing. As the kinetochores in these *in vivo* studies were attached to multiple MTs, this tension effect could then be explained by an alternate model in which increased MT release and capture at the kinetochore was dominant, rather than increased net MT assembly because of the tensional load [42]. In this alternate model, increased MT release under tension would lead to selective enrichment of MTs in a polymerization state, favoring kinetochore movement in the direction of the tension [43]. More recently, it was shown that tension at the kinetochore promotes net kinetochore MT assembly in budding yeast, which has only a single MT per kinetochore, similar to the *in vitro* system designed by Franck *et al.* [44•]. Thus, the *in vitro* study by Franck *et al.* [30••] nicely corroborates the *in vivo* findings of Gardner *et al.* [44•] where MT release from the yeast kinetochore is minimal [45]. Future work in molecular modeling of tensional forces exerted via biological coupling at the MT plus-end will be useful for dissecting how forces affect tubulin addition and loss events. These studies will also be helpful in determining how poleward chromosome movement is driven by microtubule dynamics, which has been hypothesized to be driven by a sliding sleeve mechanism [46], or by a peeling protofilament mechanism [27].

## Implications for *in vivo* regulation of MT dynamics

Taken together, these studies suggest that mechanical and physical influences likely make fundamental contributions to MT behavior and function *in vivo*. Indeed, it is clear that mechanical processes could have strong influence over much of biochemistry [47,48]. As described above, tensile forces coupled to the MT tip through the yeast Dam1p protein complex reduce MT plus-end catastrophe, enhance rescue, and decrease the depolymerization rate.

By contrast, these recent experiments and earlier studies show that compressive forces slow MT growth and promote catastrophe [18••,36,37]. Suppression of MT assembly by compressive force probably occurs by sterically suppressing the addition of fresh GTP-tubulin, and thereby reducing the size of the GTP cap [36,37]. This effect may partially account for the observation that MTs in cells frequently undergo catastrophe when they reach the cell edge [49]. This 'premature' induction of catastrophe by the cell edge may in turn increase the amount of unpolymerized tubulin and contribute to the observed persistent growth of MTs in the cell interior [50]. These examples of how physical considerations contribute in a fundamental way to cytoskeletal behavior *in vitro* are but a few of those that will facilitate our understanding of *in vivo* dynamics in the future.

## Conclusions

In summary, observations of MT plus-end dynamics at the nanoscale coupled with computational modeling of these dynamics argue that the GTP-tubulin 'cap' is larger than a single layer, and that this cap configuration can be accounted for by stochastic hydrolysis of lattice-incorporated GTP-tubulin subunits. These *in vitro* studies have demonstrated that forces at the MT plus-end can have a significant effect on MT dynamics, though effects on growth rate appear to be weak, and in turn, forces induced by depolymerizing MTs can be harnessed to do work such as that of moving chromosomes.

The surprising extent of variability in MT growth dynamics at the nanoscale revealed through these studies potentially opens the door to a wealth of new molecular-level information. Further experiments in perturbing these dynamics either mechanically or chemically will be informative for improving the understanding of MT assembly, and in shedding light on the function of various drugs and MAPs.

These *in vitro* studies suggest that mechanochemical coupling at the MT tip is likely to be important for regulation of MT plus-end dynamics *in vivo*. Thus, the data gathered from *in vitro* studies will be useful in ultimately integrating the general principles discovered for the role of force in the regulation of MT plus-end dynamics into a broader understanding of MT length control at the cellular level.

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