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Authors

Margolin, Gennady
Gregoretti, Ivan V
Cickovski, Trevor M
et al.

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The mechanisms of microtubule catastrophe and rescue: implications from analysis of a dimer-scale computational model

Gennady Margolin[a,b,*,†], Ivan V. Gregoretti[b,c,*,†], Trevor M. Cickovski[d,‡], Chunlei Li[a,b], Wei Shi[a,b,§], Mark S. Alber[a,b,e], and Holly V. Goodson[b,c]

[a]Department of Applied and Computational Mathematics and Statistics, [b]Interdisciplinary Center for the Study of Biocomplexity, [c]Department of Chemistry and Biochemistry, and [d]Department of Computer Science and Engineering, University of Notre Dame, Notre Dame, IN 46556; [e]Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 40202

ABSTRACT Microtubule (MT) dynamic instability is fundamental to many cell functions, but its mechanism remains poorly understood, in part because it is difficult to gain information about the dimer-scale events at the MT tip. To address this issue, we used a dimer-scale computational model of MT assembly that is consistent with tubulin structure and biochemistry, displays dynamic instability, and covers experimentally relevant spans of time. It allows us to correlate macroscopic behaviors (dynamic instability parameters) with microscopic structures (tip conformations) and examine protofilament structure as the tip spontaneously progresses through both catastrophe and rescue. The model's behavior suggests that several commonly held assumptions about MT dynamics should be reconsidered. Moreover, it predicts that short, interprotofilament "cracks" (laterally unbonded regions between protofilaments) exist even at the tips of growing MTs and that rapid fluctuations in the depths of these cracks influence both catastrophe and rescue. We conclude that experimentally observed microtubule behavior can best be explained by a "stochastic cap" model in which tubulin subunits hydrolyze GTP according to a first-order reaction after they are incorporated into the lattice; catastrophe and rescue result from stochastic fluctuations in the size, shape, and extent of lateral bonding of the cap.

INTRODUCTION

Microtubules (MTs) are long, proteinaceous, tubular polymers found in all eukaryotes. MTs act as tracks for vesicle transport, segregate the chromosomes during cell division, and help to establish cell polarity. A key property of MTs necessary for these activities is that

they are highly dynamic: individual MTs transition frequently between phases of elongation and shortening. This behavior is termed dynamic instability, and it is observed both in vivo and in vitro (Mitchison and Kirschner, 1984; Desai and Mitchison, 1997). The resulting length fluctuations allow the MTs to explore space and respond rapidly to both local and global signals (Holy and Leibler, 1994; Wollman et al., 2005). The transitions from growth to shortening and vice versa are known as catastrophe and rescue, respectively. Elongation is achieved by incorporation of new subunits, whereas shortening occurs by subunit detachment. Both processes occur exclusively at the MT tip.

Structurally, MTs are noncovalent polymers of the protein tubulin and typically consist of 13 parallel protofilaments arranged in a hollow tube. Each protofilament is composed of a linear chain of α-β-tubulin heterodimers, resulting in an $(\alpha-\beta)_n$ chain configuration, with the so-called plus end exposing the β monomer (Nogales et al., 1999). The minus end is usually bound to a nucleation site (such as the centrosome) in cells and, so, often is not dynamic. The subunits
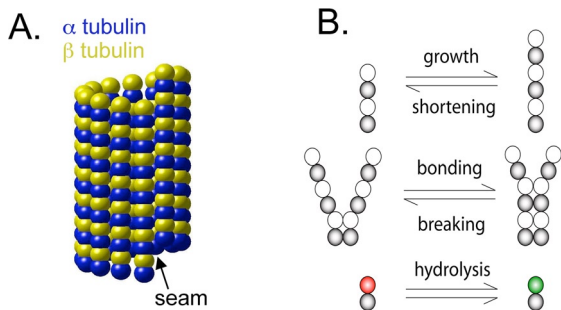
FIGURE 1: (A) Fundamental aspects of microtubule structure. (B) Events incorporated into the simulation. Growth and shortening involve formation or breakage of longitudinal bonds. Bonding and breaking refer to formation or breakage of lateral bonds. Hydrolysis is conversion of GDP tubulin into GDP tubulin. See *Materials and Methods* for more information.

in the protofilaments are generally arranged in a B lattice (α monomers laterally bind α monomers and β monomers bind β monomers), except at the seam, where there is a helical shift of three monomers between the first and last protofilaments, resulting in an A lattice (α monomers bind laterally to β monomers; Figure 1; Song and Mandelkow, 1993; Kikkawa *et al.*, 1994; see also des Georges *et al.*, 2008). The microtubule can be considered as a helix, but structural evidence indicates that the bonds between dimers occur at lateral and longitudinal interfaces (Nogales, 2001). Moreover, de-polymerizing MTs typically have splayed protofilaments or "ram's horns" at their tips (Mandelkow *et al.*, 1991) instead of the blunt tips or other structures that would be expected from helical depolymer-ization. These observations indicate that the functionally significant interactions in microtubules occur along and between protofila-ments instead of along and between helices. Longitudinal bonds along protofilaments appear to be significantly stronger than the lateral bonds between them (VanBuren *et al.*, 2002; Sept *et al.*, 2003). As an additional point, it has been believed that lateral bonds at the seam are weaker than lateral bonds in the rest of the microtu-bule (Simon and Salmon, 1990; Chretien *et al.*, 1995), although re-cent work challenges this idea (Sui and Downing, 2010).

Dynamic instability originates in conformational changes that oc-cur in the tubulin heterodimers after polymerization. Some aspects of this process are clear. These include the facts that tubulin subunits bind the nucleotide GTP, that only GTP-bound tubulin polymerizes into microtubules at physiological tubulin concentrations, that GTP hydrolyzes to GDP rapidly after polymerization, and that replace-ment of GTP by the slowly hydrolysable analogue GMPCPP pro-duces MTs that polymerize normally but depolymerize slowly and experience no catastrophes (reviewed by Desai and Mitchison, 1997; Nogales and Wang, 2006b). In addition, tubulin subunits in microtu-bules have a relatively straight conformation, whereas the GDP sub-units peeling off of depolymerizing MTs are curved (e.g., Mandelkow *et al.*, 1991). These observations have led to typical textbook mod-els of dynamic instability in which a brief delay in GTP hydrolysis af-ter polymerization results in the formation of a GTP tubulin–rich re-gion called a "GTP cap." This GTP cap predisposes the MT to growth because of strong lateral interactions between the GTP sub-units. When this cap is lost (through hydrolysis or other mechanisms), the MT undergoes catastrophe (shifts to depolymerization) because the lateral bonds between the exposed GDP tubulin subunits break as the subunits are allowed to adopt their natural curved conforma-tion (see, e.g., Alberts *et al.*, 2002). In many of these descriptions, GTP hydrolysis is assumed (explicitly or implicitly) to occur in a wave

at the interface between the older, GDP-occupied polymer lattice and the GTP cap (this behavior is known as vectorial hydrolysis), resulting in a microtubule that has a distinct boundary between regions of GDP and GTP tubulin and a solid GTP cap.

Although this view of MT dynamics is widely disseminated, much about it remains controversial, and alternative conceptual models exist. For example, vectorial hydrolysis (which predicts that the faster a microtubule grows, the longer the cap should be) is inconsistent with sudden dilution experiments (which show that MTs undergo catastrophe after approximately the same delay, regardless of how fast they were growing; Voter *et al.*, 1991; Walker *et al.*, 1991). One way to resolve this inconsistency is to postulate that occasional ran-dom GTP hydrolysis events generate new vectorial hydrolysis fronts, thus limiting the cap size (Flyvbjerg *et al.*, 1994). A more serious problem with GTP cap models is that it has been difficult to experi-mentally detect the existence of the GTP cap (Erickson and O'Brien, 1992; Caplow and Fee, 2003), and statistical arguments have im-plied that as little as one GTP dimer per protofilament is sufficient to promote MT growth (Drechsel and Kirschner, 1994; Caplow and Shanks, 1996). These data have led some researchers to propose that the cap consists of as little as one dimer per protofilament. Moreover, cryo–electron microscopy (EM) observation of sheet-like extensions at the ends of growing MTs (Chretien *et al.*, 1995) has led to the increasingly popular idea that the flat sheets, which are often portrayed as blunt, are themselves the functionally significant caps, with tube closure at the seam inducing catastrophe (Chretien *et al.*, 1995; Kueh and Mitchison, 2009; Wade, 2009). The exact connec-tion between tube closure and GTP hydrolysis is unspecified in this model, but it has been proposed that these events are coupled.

The various conceptual models differ significantly, but it has been difficult to distinguish among them experimentally because all seem intuitively to be capable of generating behavior similar to ex-perimentally observed dynamic instability. A major limitation of these conceptual models is that they provide few explicit predic-tions about the dimer-scale events at the MT tip or how these events relate to the processes of catastrophe and rescue.

An alternative way to distinguish between these conceptual models is to build a computational model from the "dimers up" based on experimentally derived knowledge about the biochemi-cal, structural, and mechanical attributes of the tubulin subunits. As-suming that this computational model displays appropriate dynamic behavior, one can then see which if any of the conceptual models is most consistent with the resulting dimer-scale computational model. Moreover, such computational models can provide insight beyond simple confirmation or refutation of particular conceptual models because they are intrinsically more detailed and can potentially gen-erate specific predictions about how particular structures at the tip relate to processes such as catastrophe and rescue.

This approach is intuitively attractive, and, indeed, there is a large body of work on computational models of MT dynamics and energetics (e.g., Chen and Hill, 1985; Hill, 1986; Bayley *et al.*, 1989; Flyvbjerg *et al.*, 1994; VanBuren *et al.*, 2002, 2005; Sept *et al.*, 2003; Sept and MacKintosh, 2010; Molodtsov *et al.*, 2005; Gregoretti *et al.*, 2006; Janulevicius *et al.* 2006; Brun *et al.*, 2009; Ranjith *et al.*, 2009). These models have been built under a variety of simplifying assumptions, and they specify a range of structural detail. However, although the more detailed computational models can better eluci-date the relationships between dimer-scale events, MT behavior, and specific conceptual models, they are also more computation-ally intensive. Thus far, none of the structurally consistent detailed computational models (i.e., those that are protofilament based and explicitly consider formation and breakage of both lateral and

longitudinal bonds between dimers) have reported being able to recapitulate experimentally observed dynamic instability, in part because they have been restricted to simulating only brief spans of time. Although these detailed models (VanBuren *et al.*, 2005) have provided insight into a number of aspects of MT behavior, their impact has been limited by difficulty in comparing their results to those obtained in standard dynamic instability experiments and their inability (thus far) to spontaneously produce both catastrophe and rescue.

To address these issues, we developed a protofilament-based dimer-scale computational model that both explicitly models formation and breakage of lateral and longitudinal bonds and is able to run rapidly enough to simulate experimentally relevant time frames. The structural and biochemical foundations of the model suggest that it is a useful tool for investigating the mechanisms of microtubule dynamics; the similarity of the model's behavior to that of real microtubules across a range of conditions suggests that it reflects physiological processes. This model allows us, for the first time, to examine the processes of catastrophe and rescue as they occur spontaneously in a dimer-scale model that explicitly incorporates lateral interactions between protofilaments.

Our analysis of this model suggests that several commonly held assumptions about microtubules should be reconsidered. The first is the idea that the cap is a distinct structure: the cap that emerges in our simulations is a rapidly fluctuating and discontinuous structure without clear boundaries. We also find no need for vectorial hydrolysis: simple first-order hydrolysis on nonterminal subunits is sufficient to account for MT behavior. These conclusions conflict with standard textbook models but are consistent with the results of some other modeling efforts (VanBuren *et al.*, 2002, 2005). In addition, our work challenges the common assumption that GTP hydrolysis affects primarily lateral bonds. Moreover, we were unable to find parameter sets that produce the commonly portrayed tip structures that consist of sheets open at the seam, arguing against the idea that tube closure plays a dominant role in microtubule dynamics. Instead, we predict that the sheets observed by electron microscopy are extensions off of closed tubes. Finally, our model predicts that short interprotofilament "cracks" (laterally unbonded regions) exist even at the tips of growing microtubules and further predicts that fluctuations in the depths of these cracks play a pivotal role in the mechanisms of both catastrophe and rescue. These observations argue against several of the conceptual models of MT dynamics outlined earlier and support instead a refinement of the "fluctuating cap" model first proposed by Chen and Hill (1985). In this "stochastic cap" model, nonterminal tubulin subunits hydrolyze GTP according to a first-order reaction. Catastrophe and rescue result from stochastic fluctuations in the number and distribution of the GTP subunits and the extent of their lateral bonding.

RESULTS AND DISCUSSION
Overview of the model
Microtubule dynamics is a complex process, but it consists of a series of microscopic chemical events including bond formation, bond breakage, and GTP hydrolysis. Chemical events such as these can generally be described as stochastic (random) processes dependent on chemical rate constants and (when appropriate) the concentrations of the reactants. In addition, since the microtubule is a cooperative structure consisting of many individual subunits (tubulin dimers), the mechanical environment in which a given dimer finds itself will influence these chemical constants and thus the observed rates of bond breakage and formation. For example, a dimer at the tip of a protofilament should be less likely to detach from its lateral neigh-

bors if these neighbors are embedded in the lattice (i.e., laterally bonded to their other neighbors). Moreover, the mechanical strain introduced by GTP hydrolysis of neighboring subunits will also alter the likelihood of bond formation and breakage for a particular subunit.

Given these principles and the established information about the structure of the microtubule, we constructed a model with the following basic attributes:

- We model the MT as a lattice curled on itself, forming a tube with a seam (Figure 1A). Tubulin subunits in the MT have both longitudinal and lateral bonds, meaning that a given dimer in the lattice can participate in as many as four bonds (Figure 1B). Each of these forms and breaks according to defined probabilities; the specific values of the probabilities depend on the state of the surrounding subunits (more on environmental influences later). For the purposes of the studies described here, the MT lattice is composed of 13 protofilaments, but this can be varied by the modeler.

- The MT subunits (tubulin heterodimers) have two conformational states—one prone to polymerization and the other prone to disassembly (in other words, these conformations have different bond formation and breakage probabilities). These states are denoted as GTP-Tu and GDP-Tu, but they could represent other conformational states.

- Conversion from GTP-Tu to GDP-Tu occurs by a simple irreversible first–order process that occurs only on internal subunits, consistent with structural evidence that α monomers act as GTPase-activating proteins (GAPs) for β monomers (Nogales *et al.*, 1998).

- As mentioned earlier, the environment of a dimer influences its behavior: mechanical influences such as constraint in the lattice or GDP-tubulin–induced protofilament bending alter the probabilities of dimer addition and loss. In our model, these mechanical considerations are expressed as environment-dependent changes in probabilities of bond formation and breakage.

- The user-dictated parameters are the rate constants for formation and breakage of the various bonds, the degree of influence of particular environments on these rate constants, the rate constant for GTP hydrolysis, the concentration of tubulin subunits in solution, and the number of protofilaments. In other words, the modeler sets the parameters that would naturally be dictated by the amino acid sequences of the proteins and the environment in which the microtubule is polymerizing. The standard dynamic instability parameters are emergent properties of the system defined by a particular tubulin concentration and set of biochemical rate constants, just as they would be in an experiment.

- The specific parameter values used in this study were chosen by tuning the behavior of the model to match experimental data, including dynamic instability parameters at different tubulin concentrations, depolymerization rate of GTP (GMPCPP) MTs, and measurements of the size of the GTP cap. We also constrained our initial choices for the parameter sets by assumptions drawn from experiment, such as the idea that longitudinal bonds are stronger than lateral bonds (for more detail including the specific values used, see *Materials and Methods* and Supplemental Information). However, it is important to note that the existing experimental data are not sufficient to uniquely determine the user-dictated parameters. Therefore, as will be explained more later, we chose three parameter sets for in-depth analysis and present the results for all three as a way of investigating whether particular

behaviors or characteristics are specific to particular parameter sets or represent more general aspects of the system.

This computational model is conceptually similar to the fluctuating cap model of Chen and Hill (1985), but it is fundamentally different at a structural level because their fluctuating cap model is based on helical interactions between tubulin dimers and also considers "binding" or detachment as single events (Chen and Hill, 1985). In contrast, our model is protofilament based, consistent with present understanding of MT structure (Sept *et al.*, 2003; Nogales and Wang, 2006b), and it separately accounts for both lateral and longitudinal bonds between subunits. The lateral cap model of Bayley and colleagues differs even more significantly because it is based on helical interactions and additionally stipulates that GTP tubulin is restricted to the terminal layer of dimers (i.e., hydrolysis on one subunit is tightly coupled to addition of a new subunit above it; Bayley *et al.*, 1989, 1990; Martin *et al.*, 1993). The model we use has deeper similarity to the two models of Van Buren (VanBuren *et al.*, 2002, 2005), but it has some important distinguishing characteristics. Compared to that of VanBuren *et al.* (2002), our model is more structurally detailed, in that their model does not separately consider lateral and longitudinal bonds and thus cannot consider cracks between the protofilaments such as observed experimentally in shortening MTs (the so-called "ram's horns"). As will be shown later, our model suggests that these cracks play a key role in both catastrophe and rescue. The model of VanBuren *et al.* (2005) does consider both lateral and longitudinal bonds explicitly and calculates for each dimer the probabilities of bond formation and breakage, depending on the local mechanical stress and strain. However, the computational requirements of this model have thus far prohibited simulation of time spans necessary to display the full range of dynamic instability (VanBuren *et al.*, 2005; Schek *et al.*, 2007).

The model we use can be considered to be intermediate between these two Van Buren models in that we do consider explicitly both lateral and longitudinal bonds as in VanBuren *et al.* (2005), but we approximate the mechanical influences by incorporating them into environment-specific kinetic rate constants governing formation and breakage of various types of bonds. The reason for using the kinetic approach instead of the mechanochemical one is that the kinetic approach is orders of magnitude faster, allowing simulation of experimentally relevant time spans (tens of minutes or much longer), which in turn allows both observation of spontaneously occurring catastrophes and rescues and direct comparison to experimental dynamic instability parameters. As will be shown, the ability of our model to produce the full range of dynamic instability behaviors in the course of a single simulation is central to our analysis.

Recapitulation of dynamic instability, parameter adjustment, and initial validation

Typical life history plots for a group of three parameter sets (A–C) is shown in Figure 2. The plots show clear growth and shortening phases, as well as catastrophes and rescues. Set A was our initial parameter set, tuned to approximate the dynamic instability behavior of mammalian brain tubulin in vitro, and sets B and C were iteratively tuned to match additional characteristics of mammalian brain tubulin, including the number of GTP tubulins per tip and the depolymerization rate of GMPCPP tubulin. The dynamic instability measurements for these three sets are given in Table 1. As will be explained later, we focused our efforts on parameter set C because this set best matches the behavior of bovine-brain MTs, but we also examined MTs as assembled under the first two parameter sets to avoid parameter-specific conclusions.
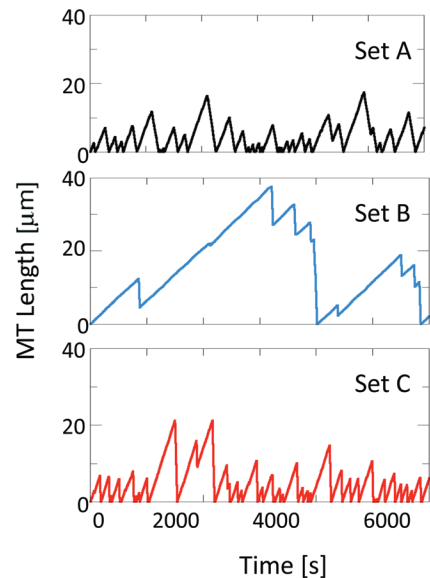

FIGURE 2: Length–history plots of individual MTs simulated with parameter sets A–C as indicated.

The details of parameter tuning are provided in the Supplemental Data, but the simulations conducted to mimic GMPCPP depolymerization experiments were particularly illuminating. GMPCPP is a slowly hydrolysable analogue, and so the rate of depolymerization of GMPCPP MTs in the absence of free tubulin subunits provides a strong constraint for the depolymerization behavior of GTP tubulin. We found that MTs simulated with both parameter sets A and B depolymerized too quickly relative to experimental values (Hyman *et al.*, 1992; Figure 3). However, even after extensive effort, we were unable to find an improved parameter set that both produced dilution-stable GMPCPP microtubules and appropriate dynamic instability values for regular nucleotides.

This observation suggested that we needed to reexamine some of the assumptions used in building the model. We focused on the stipulation that GTP hydrolysis on a subunit alters only its lateral (not longitudinal) bonds because this assumption is explicitly incorporated into most if not all computational models, including the mechanochemical model of VanBuren *et al.* (2005), but it is not structurally dictated. Indeed, the observation that the hydrolysable nucleotide is at the dimer–dimer interface suggests that GTP hydrolysis should alter the longitudinal bond (Nogales and Wang, 2006a; Rice *et al.*, 2008). When we allowed GTP hydrolysis to reduce the strength of the longitudinal bond, we were readily able to identify parameter sets that both produced dilution-stable GMPCPP microtubules and appropriate dynamic instability values for regular nucleotides, leading to set C. An alternative explanation could be that GPMCPP tubulin is an inaccurate mimic of GTP tubulin. It also remains possible that parameter sets exist that both match the range of experiments and have nucleotide-insensitive longitudinal bonds. Nevertheless, we conclude on the basis of our modeling efforts and the existing structural data (e.g., Nogales, 2001) that GTP hydrolysis likely has significant effects on the longitudinal bonds within protofilaments.

To further test the validity of the model, we subjected the simulated MTs polymerized with normal nucleotides to sudden dilution experiments. The logic of this test is as follows: one might expect that faster-growing MTs would have longer GTP caps, and that MTs with longer GTP caps would survive longer after being abruptly deprived of new subunits by sudden dilution. However, experiments

Parameter set	Tubulin concentration (µM)	K_h (s⁻¹)	V_g (dimer lengths/s)	V_s (dimer lengths/s)	F_c (events/s of growth)	F_r (events/s of depolymerization)	Cap (dimer lengths)
A	14	0.2	5.31 ± 0.07	11.7 ± 0.1	0.0079 ± 0.0005	0.0024 ± 0.0007	25.3 ± 3.1
B	10	0.25	1.84 ± 0.01	46 ± 4	0.0018 ± 0.0001	0.04 ± 0.01	5.7 ± 1.2
C	10	0.7	5.64 ± 0.04	61 ± 3	0.0096 ± 0.0002	0.019 ± 0.007	8.8 ± 1.5

F_c, observed catastrophe frequency; F_r, observed frequency of rescue; K_h, hydrolysis rate constant; V_g, observed growth rate; V_s, observed shortening rate. Cap length here is defined for the purposes of comparison as the MT length (mean protofilament length) minus the mean height of all the GTP-Tu dimers in the MT. For measurements of total GTP-Tu number with each parameter set, see Supplemental Figure S1. To convert V_g and V_s into µm min⁻¹, multiply by 0.48. For comparison, Walker et al. (1988) reported plus-end growth and depolymerization velocities at 10 µM tubulin to be ~1.8 and ~25 µm/min, respectively, which correspond to ~3.6 and ~50 dimer lengths per second, respectively. Walker's plus-end transition frequencies at 10 µM tubulin were ~0.004 catastrophe event per second of growth and ~0.02 rescue event per second of depolymerization (we provide these numbers as approximate values because they were extracted by hand from the published graphs; Walker et al., 1988).

TABLE 1: Dynamic instability parameters of simulations performed with the three parameter sets.

show that in real MTs, there is only a weak correlation between initial growth rate and the time to catastrophe (Voter et al., 1991; Walker et al., 1991). These observations have been used to argue against the existence of a GTP cap or its relevance to the catastrophe mechanism (Voter et al., 1991; Walker et al., 1991). We found that when the MTs are simulated with all three parameter sets, they mimic real MTs in displaying little correlation between initial growth rate and the time to catastrophe (Figure 4 and Table 2). These observations confirm the results of VanBuren et al. (2002) in showing that a simple stochastic GTP cap model is compatible with the behavior of MTs observed in sudden dilution experiments.

The similarity of the behavior of the modeled MTs to those observed in experiments with mammalian brain MTs under a variety of conditions suggests that the model is a useful reflection of physiological processes. This in turn suggests that examination of the relationship between the macroscale events of rescue and catastrophe and the microscale events at the MT tip will provide insight into the mechanisms of these transitions. For the work described later, we focused our efforts on parameter set C because it best matches the behavior of experimental mammalian brain microtubules under the range of conditions discussed, but we also examined MTs as assembled under the first two parameter sets to ascertain whether the behaviors observed and principles inferred are parameter specific.

Structure of the MT tip during growth and depolymerization
Before investigating the transitions, it is important to first examine the structure of the tip in the growing and shortening phases. Figure 5 shows representative examples of tip structures for the growing and shrinking phases as found with parameter sets A–C. Examination of these images and the accompanying movies from which they are derived (see Supplemental Data) leads to several predictions about the structure of the MT tip. We stress that some of these predictions are not unique to our work, but it is important to summarize them because they contradict some common assumptions about MT structure. We then use this information as a foundation for our investigations of the mechanisms of the dynamic instability transitions.

The shape of the GTP cap. Examination of Figure 5 and Supplemental Movie S1 suggests that the morphology of the GTP cap during growth is very different from that typically portrayed in textbook descriptions of MT structure: instead of being a continuous (GTP-only) structure with a clear lower boundary, the cap in the model (regardless of parameter set) is a discontinuous, amorphous structure in which the concentration of GTP is generally high at the tip and tapers off toward the MT base. This exponential-like shape

has been predicted by other models (e.g., VanBuren et al., 2002, 2005) and results from the assumption that GTP hydrolysis occurs as a simple first-order process on nonterminal dimers (terminal dimers are not expected to have significant GTPase because the α monomer of one subunit acts as a GAP for a β monomer of the one below it in the lattice; Nogales, 2000). However, the distribution is not precisely exponential because rapid fluctuations in the growth and shrinkage of particular protofilaments and the cooperative nature of interactions between the protofilaments prohibit the existence of an exact relationship between distance from the tip and "age" of the dimer (discussed more later). The prediction that GTP tubulin is sometimes found deeper in the MT (Figure 5 and supplemental movies) is consistent with recent experiments using antibodies directed against
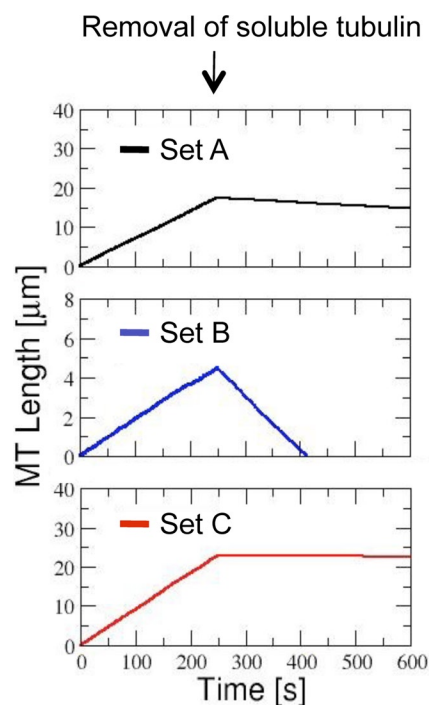

FIGURE 3: Depolymerization of GMPCPP MTs (simulated by setting the hydrolysis rate to zero) after removal of all unpolymerized tubulin, as simulated with parameter sets A (top), B (middle), and C (bottom). These data show that MTs simulated with parameter sets A and B depolymerize too quickly relative to experimental GMPCPP MTs, which have been measured to depolymerize at a rate <1 µm/h (Hyman et al., 1992).
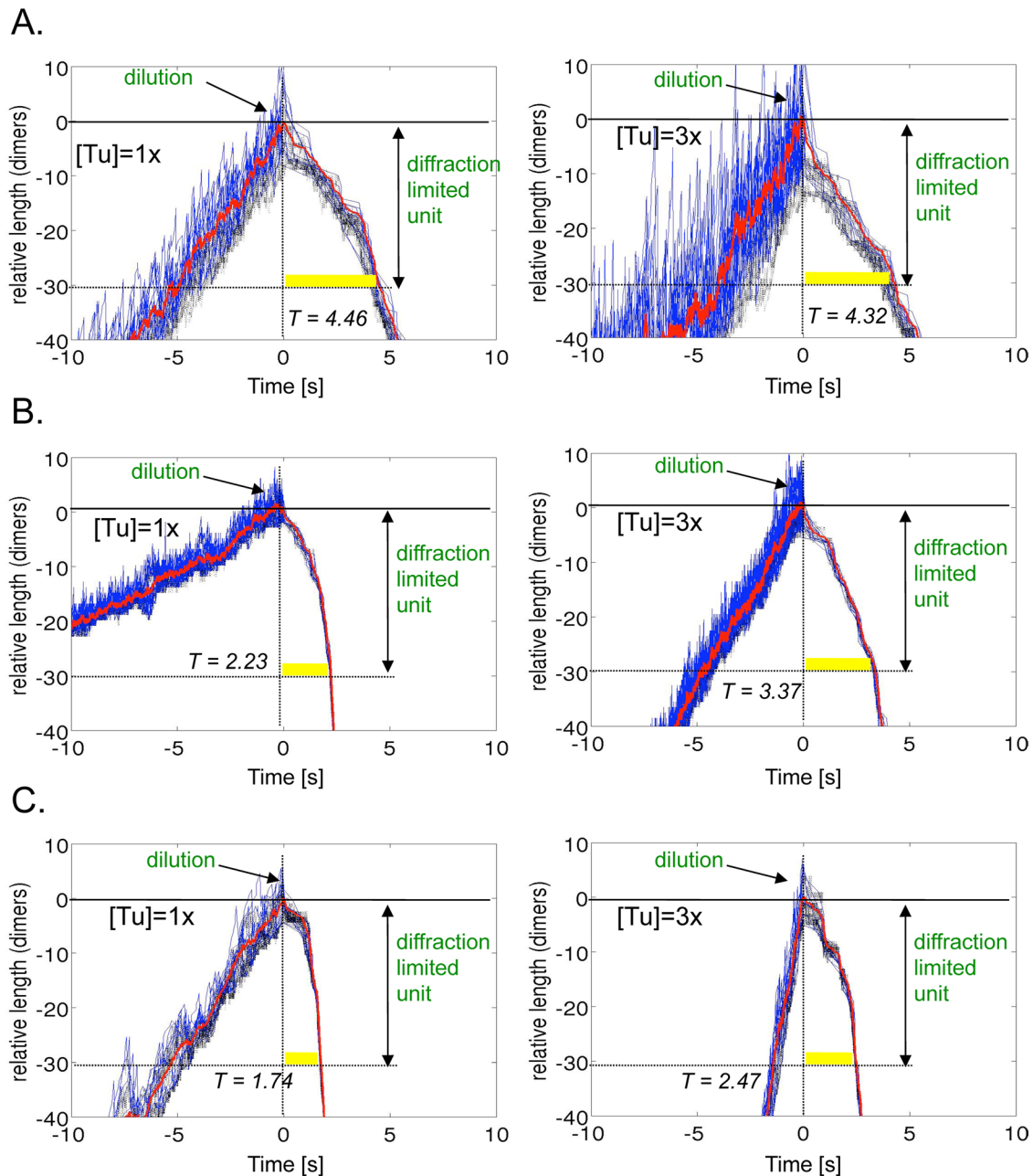
FIGURE 4: Examples of simulations of sudden dilution experiments for parameter sets A (top), B (middle), and C (bottom) at 10 (left) and 30 (right) µM soluble tubulin. The time (in seconds) from dilution to loss of 30 dimer lengths (approximately one diffraction-limited unit) is indicated for each simulation. In each plot, the thin, dark lines indicate the lengths of the 13 protofilaments, and the bold red line represents the average. Statistics for the full set of simulations are provided in Table 2. These data show that the simulated MTs mimic real MTs in displaying little correlation between rate of growth and time to depolymerization after "sudden dilution" (shift into an environment with no soluble tubulin), as observed with experimental MTs (Walker *et al.*, 1991; Voter *et al.*, 1991).

GTP tubulin (Dimitrov *et al.*, 2008), although only with set A did we ever notice GTP subunits occurring >1 µm from the tip (data not shown).

The familiar concept of the GTP cap as a continuous structure with a discrete lower boundary originates in the frequently held idea that GTP hydrolysis occurs vectorially, that is, in a wave progressing from the minus end toward the plus end (Carlier, 1989). The experimental sudden dilution experiments mentioned earlier were the first to cast doubt on the idea that the cap is generated through vectorial hydrolysis (Voter *et al.*, 1991; Walker *et al.*, 1991).

Flyvbjerg and colleagues then demonstrated that vectorial GTP hydrolysis is not sufficient to account for MT dynamic instability, and they proposed a hybrid model in which both vectorial hydrolysis and random hydrolysis occur (Flyvbjerg *et al.*, 1994). However, we concur with VanBuren *et al.* (2002, 2005) in concluding that there is no need to include vectorial hydrolysis at all—we are aware of no structural or biochemical data supporting vectorial hydrolysis, and we find that random GTP hydrolysis on internal (not terminal) dimers is sufficient to account for MT behavior over a range of conditions (Table 1 and supplemental movies). Therefore we suggest

Parameter set	Time to depolymerization (s)	
	10 μM tubulin	30 μM tubulin
A	4.44 ± 0.57	4.29 ± 0.78
B	2.23 ± 0.44	3.39 ± 0.39
C	1.78 ± 0.47	2.50 ± 0.30

Measured from simulations with parameter sets as indicated. Values shown are the average times ± SD (>10 trials) required to lose 30 subunit lengths (approximately equivalent to one diffraction-limited unit, i.e., 240 nm). See Figure 4 for examples of individual dilution simulations.

TABLE 2: Time to depolymerization after sudden dilution to zero soluble tubulin.

that vectorial hydrolysis should be discounted as part of the MT dynamic instability mechanism unless new data are identified to specifically support it.

The shape of the tip during growth. The MT tip that emerges from our model, regardless of parameter set, is a closed tube with dynamic multiprotofilament extensions of varied length (Figure 5 and accompanying supplemental movies). We were initially surprised by this observation because cryo-EM work has been interpreted as showing that MTs grow as flattened, open sheets that close into a tube at a later time (Chretien *et al.*, 1995), and the idea that MTs grow as sheets open at the seam has become widely accepted (e.g., Kueh and Mitchison, 2009). These open sheets are often portrayed as blunt or near blunt (e.g., Chretien *et al.*, 1995; Carvalho *et al.*, 2003; Slep and Vale, 2007). The failure of our model to produce the expected open sheets suggested that the parameters needed further adjustment. However, considerable effort in tuning the strength of the seam and other bonds failed to identify a parameter set that produced open sheets: weaker seams simply produced more frequent catastrophes without open sheets (data not shown).

One explanation of this apparent incompatibility between our model and the existence of open sheets is that it results from over-simplicity in our model—a failure to include an interaction or process. This possibility exists. However, we suggest instead a reconsideration of the idea that MTs grow as sheets. First, reexamination of the cryo-EM evidence suggests that the structure of the MT tip is in fact more closely approximated by the model presented here: the published EM work shows many examples of multiprotofilament extensions such as exist in our model but few clear examples of fully formed open sheets in microtubules assembled either in vitro or in vivo (Chretien *et al.*, 1995; Muller-Reichert *et al.*, 1998; Zovko *et al.*, 2008). Moreover, logic suggests that growth of an extended open sheet would require lateral bonds that are strong relative to the longitudinal bonds—otherwise, the dimers at the edges of the sheet would tend to detach, quickly resolving the "sheet" into an "extension." Instead, a combination of experimental and theoretical work indicates that longitudinal bonds are instead stronger than lateral bonds (VanBuren *et al.*, 2002; Sept *et al.*, 2003). These considerations make the commonly portrayed blunt, open sheets particularly unlikely (see also Hill, 1986). Finally, recent structure work challenges the idea that the seam bond is necessarily weaker than the regular interprotofilament bonds (Sui and Downing, 2010). We conclude that the combination of this evidence suggests that the microtubule tip is most closely approximated by a closed tube with dynamic extensions of various lengths (Figure 5).

VanBuren *et al.* (2005) also reported that sheet-like extensions (not open sheets) are common in their simulations of growing MTs,

but they additionally reported observation of blunt ends. In contrast, we found that blunt ends are rare in growing MTs in all three parameter sets (see supplemental movies). This dissimilarity may be parameter related, but we suggest that it stems at least partially from the fact that the VanBuren simulations started from a defined tip structure that generally consisted of a blunt, four-ringed cap and then ran for a relatively short period of time (seconds). In contrast, our simulations span tens of minutes, so the emergent tip structures are much less likely to be influenced by the starting configuration.

Cracks between protofilaments during growth and the high frequency of GTP tubulin detachment. A final aspect of the tip structure to note is that the model predicts that cracks (regions where neighboring protofilaments are not laterally bonded) often exist even in growing tips. These cracks are particularly prominent in parameter set A, but they are still present in parameter sets B and C, especially immediately after dimers add (Figure 5 and accompanying supplemental movies). Although the existence of such cracks might seem surprising, further reflection indicates that they are expected.
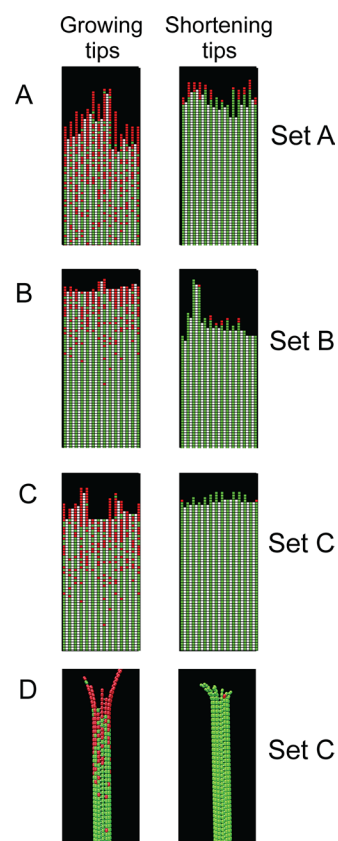

FIGURE 5: Representative tip structures from growing and shortening MTs from each of the three parameter sets. (A–C) Examples of MTs assembled under the indicated parameter sets. They are shown in flat representation, opened at the seam to allow the full tip structure to be viewed. The dimer aspect ratio has been compressed to allow visualization of more of the tip. GTP-bound heterodimers are red, GDP-bound heterodimers are green, and lateral bonds are white. Note that the seam protofilament has been duplicated (leftmost and rightmost) to allow easier assessment of its lateral bonding. (D) The same pair of MTs as in set C, drawn to approximate the three-dimensional structure of the MT to aid interpretation of the flat structures. For additional images of spontaneously occurring tip structures, see Supplemental Movies S1–S11.
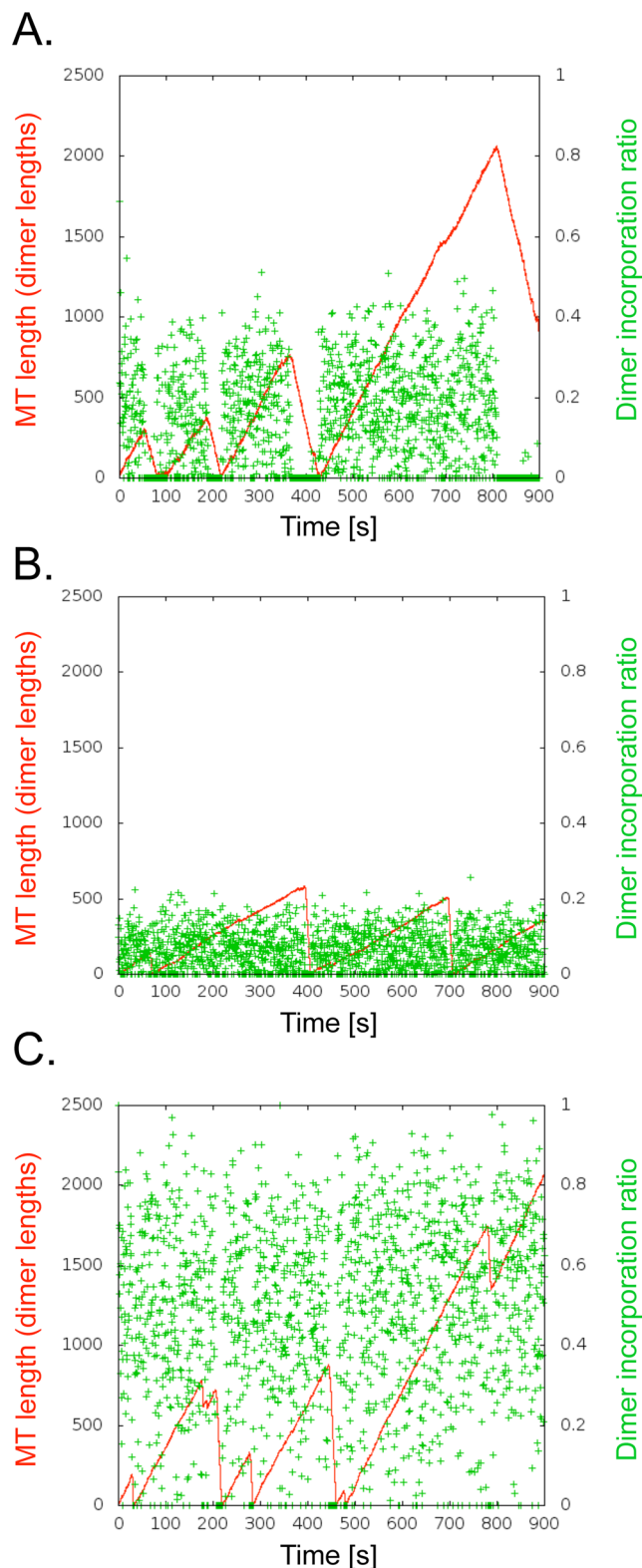
FIGURE 6: Fraction of added dimers that are lost before being incorporated into the MT lattice for parameter sets A (top), B (middle), and C (bottom). The red lines provide a length–history plot with the relevant y-axis at the left, and the green crosses show the corresponding fraction of dimers successfully incorporated into lattice for each second of the simulation, with the relevant y-axis at the right. These data show that with all three parameter sets, a large fraction of subunits detach before being incorporated into the lattice, consistent with recent experimental data (Schek *et al.*, 2007; Gardner *et al.*, 2011).

First, entropic considerations suggest that it is unlikely that both lateral and longitudinal bonds would form simultaneously. Second, if lateral bonds are weaker than longitudinal bonds as indicated by previous work (VanBuren *et al.*, 2002; Sept *et al.*, 2003), and especially if GTP tubulin has an intrinsic bend as suggested by recent structural studies (Rice *et al.*, 2008), it is expected that longitudinal bonds should form before lateral bonds, resulting in cracks between protofilaments even inside the GTP cap. As will be discussed later, we propose that these cracks play a fundamental role in dynamic instability.

These cracks as predicted by our simulations have an additional consequence: when a tubulin dimer binds to a protofilament, it has a significant likelihood of detaching before it can become laterally bonded and incorporated into the lattice (Figure 6). In fact, the fraction of longitudinally bound dimers that become incorporated into the lattice varies greatly from second to second even during periods of what appear to be smooth growth, regardless of parameter set (Figure 6).

Initially this observation might appear to conflict with experiment because the slow depolymerization of GMPCPP tubulin (Hyman *et al.*, 1992) has been interpreted as evidence that the off rate for GTP tubulin is very slow. However, high detachment rates for GTP tubulin during growth have been seen in recent nanoscale experiments (Schek *et al.*, 2007; Gardner *et al.*, 2011) and are also expected from much older experimental analysis of the critical concentration for tubulin elongation (Walker *et al.*, 1988). Moreover, the simulations recapitulate the experimental observation (Gardner *et al.*, 2011) that the rate of subunit detachment per MT (k_{off_MT}) rises as a function of the concentration of soluble tubulin (Figure 7A; also predicted by Hill, 1986). This behavior is seen with all three parameter sets (Figure 7A). In addition, the k_{on_MT} values used in the simulations (16–45 μM^{-1} s^{-1}; see Supplemental Table S1) are faster than the ~5 μM^{-1} s^{-1} expected from earlier work, also consistent with the recent nanoscale experiments (Gardner *et al.*, 2011). Therefore, on the basis of all these data, it seems likely that the rapid GTP tubulin exchange observed in the simulations is a feature of real MTs.

When GTP subunit detachment has been discussed previously, it has been interpreted in terms of the idea that the detached tubulin results from subunits that attached to "unfavorable" sites—for example, protofilaments that lack neighbors (Howard and Hyman, 2009). We instead suggest that *any* subunit has a significant likelihood of detachment before it forms lateral bonds and that the specific likelihood of lateral bond formation/breakage for a particular tubulin dimer depends on its local environment. In fact, the simulations predict that subunits frequently detach as short oligomers, and, as one might expect, this oligomer detachment is most common in the parameter set with the deepest cracks in its growing tips (Figure 7B). These considerations indicate that "growth" is a complex process that reflects both longitudinal and lateral bonding and is dependent on fluctuations in local tip structure.

The cracks in growing tips that are predicted by our model have two additional implications. First, since the rate at which lateral binding follows longitudinal binding is likely to differ between the plus and minus ends, this model provides an explanation for the experimental observation that the two ends differ significantly in their dynamic instability behavior (Walker *et al.*, 1988): the speed of lateral bonding may depend on whether α- or β-tubulin is closest to the tip, especially if the incoming dimer is bent (Rice *et al.*, 2008). Second, suppression of subunit detachment by promoting crack closure provides a mechanism for the activity of MT-binding proteins (see also Gardner *et al.*, 2011).
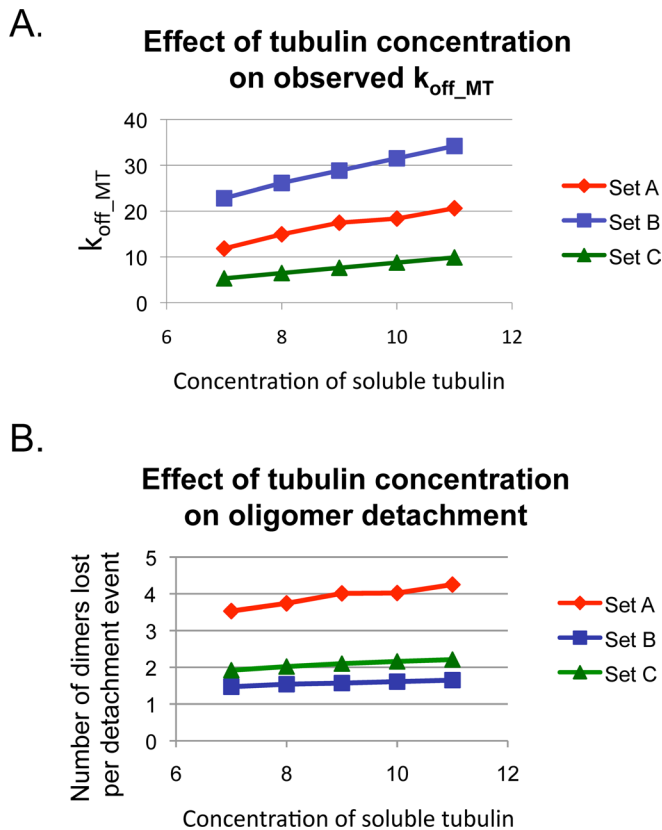
A.

Effect of tubulin concentration on observed k_{off_MT}

B.

Effect of tubulin concentration on oligomer detachment

FIGURE 7: Effect of tubulin concentration on observed k_{off_MT} (A) and oligomer detachment (B) during growth phases. (A) All three parameter sets reproduce the experimentally observed increase of observed k_{off_MT} (measured in units of dimers/second) as the concentration of soluble tubulin increases (Gardner *et al.*, 2011). (B) On average, more than one dimer is lost in each detachment event, indicating that pieces of protofilament are frequently lost; B also shows that the number of dimers lost per detachment event also increases with tubulin concentration, accounting for at least part of the effect in A.

Nature of the GTP cap. The discussion of tip structure during growth raises the question of the nature of the GTP cap. The quasiexponential distribution of GTPs makes it difficult to define the bottom of the cap as it exists in our model, and the ragged, cracked nature of the tip make it similarly difficult to define the top (see Figure 5 and accompanying supplemental movies). On the basis of the structures that emerge from our model, we suggest that an "effective cap" in real MTs consists of a region rich in laterally bonded GTP subunits. What, then, defines the minimal effective cap, that is, the minimal number of GTPs capable of maintaining a microtubule in the growth phase? This question has been the focus of significant experimental effort, but the issue is not yet resolved. We propose that there is no single answer. First, the number of laterally bonded GTP subunits needed for an effective cap will be very parameter dependent. Tubulins from different organisms differ considerably in their biochemical characteristics (Burns, 1991; Davis *et al.*, 1994; Dougherty *et al.*, 2001), so typical cap size should differ from organism to organism. Indeed, yeast MTs contain much more GTP than do mammalian MTs (Davis *et al.*, 1994; Dougherty *et al.*, 2001).

Second, even for a particular type of tubulin, we propose that there is no strictly defined minimum effective cap. This suggestion stems from the dynamic and highly varied structure of the tip: the

minimum cap needed to prevent catastrophe likely depends on the details of the specific tip structure present at a given moment in time. The stochastic (unpredictable) nature of tip growth further complicates the issue of defining the minimal cap: a given tip configuration can only specify the *probability* that a MT will still be growing after a certain amount of elapsed time (discussed more later). For a truly minimal cap (one poised near catastrophe), this probability will necessarily be close to 50%. Note that the effective cap (the set of laterally bound GTP subunits residing in the immediate vicinity of the tip where they constitute majority) is likely smaller than implied by the total number of GTP subunits, providing a potential explanation for the conflict between the relatively large number of GTP tubulins in growing tips as observed with our parameter sets and in some experiments (Melki *et al.*, 1996) and the experimental observation that as few as ~13 GTP (GMPCPP) subunits can be sufficient to stabilize a microtubule (Drechsel and Kirschner, 1994; Caplow and Shanks, 1996).

Structure of the tip during shortening. The most apparent difference between growing and shortening MT tips as predicted by the model is that the tips of depolymerizing MTs have fewer GTP-Tu dimers. Although this conclusion is obvious, it is important to point out that the model predicts that depolymerizing tips do have some GTP-Tu dimers (Figure 5 and the supplemental movies), as is required from the fact that the process of rescue requires that GTP-Tu dimers have some affinity for GDP-Tu polymer. A second predicted difference between growing and shortening MTs concerns the depth of the cracks between protofilaments: the cracks are deeper in depolymerizing MTs, and they terminate in regions of GDP tubulin instead of in regions rich in GTP tubulin (Figure 5 and the supplemental movies). These characteristics result in formation of a frayed end with laterally unbonded, outwardly curled, GDP-rich protofilaments ("ram's horns") that favor continuation of the shortening phase (Figure 5).

Mechanisms of catastrophe and rescue

Attempts to predict transitions from changes in measurable behaviors. Two of the most mysterious aspects of microtubule dynamics are the processes of rescue and catastrophe. Rescue is particularly problematic: given the familiar electron microscopy images of microtubules peeling apart (e.g., Mandelkow *et al.*, 1991), how could the microtubule start growing again? Catastrophe also has puzzling aspects: loss of the cap after sudden dilution seems predictable, but what triggers the transition to catastrophe in a MT growing under conditions of constant tubulin concentration? To address these questions, we examined the structure of the MT tip throughout the course of a simulation and particularly during the transitions, using both movies (Supplemental Movies S1–S8) and more quantitative measures (Figure 8).

As might be expected from the great variability in tip structures observed during both growth and depolymerization (Supplemental Movie S1) and the stochastic nature of subunit addition/loss, we found that there is no single pathway for rescue or catastrophe. Instead, each transition occurs according to its own path (Supplemental Movies S2–S8). Similarly, the significant structural differences between growing and shrinking tips suggest that catastrophe and rescue should be distinguishable processes (i.e., not simply the same process in reverse), and indeed this is what we observed (Supplemental Movies S1–S8).

Even though catastrophe and rescue have variable paths, we expected that some quantifiable aspect of tip structure—raggedness (SD of protofilament lengths), cap size (approximated by the
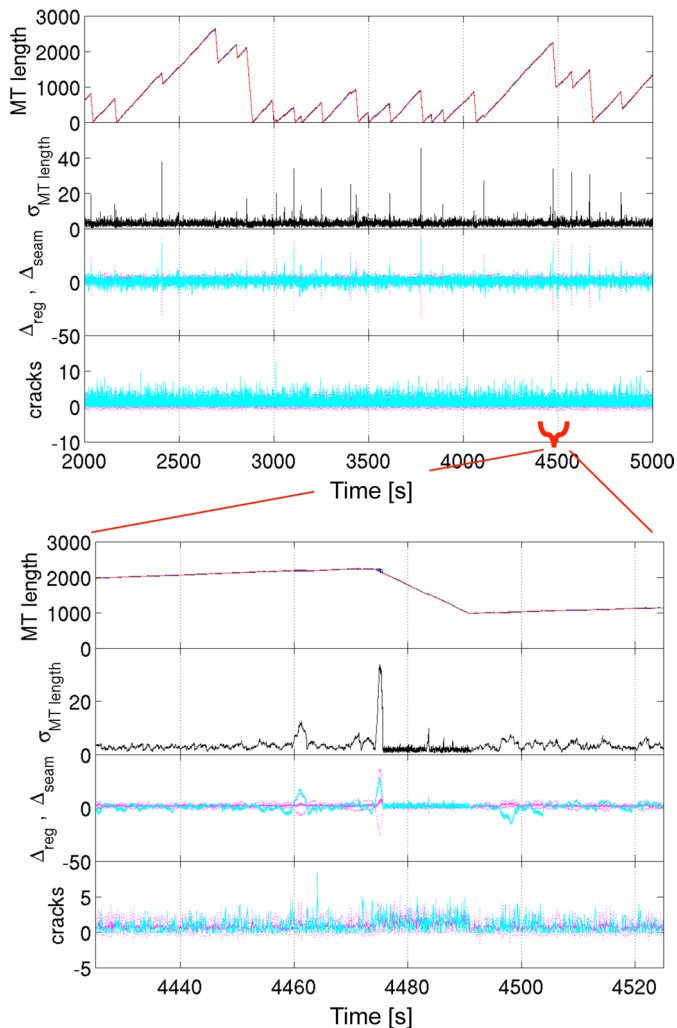
FIGURE 8: Correlation between transitions and characteristics of the MT tip for parameter set C (data for sets A and B can be found in the Supplemental Information). MT length is measured in protofilament lengths, so 1000 corresponds to 8 μm, and time is presented in seconds. Quantities measured are as follows: $\sigma_{MT\,length}$ is the SD of protofilament lengths, and so quantifies the raggedness of the tip; Δ_{reg} (magenta) is the mean protofilament length minus the mean regular bond length and provides a measure of the depth of the cracks between protofilaments; Δ_{seam} (cyan) is the mean protofilament length minus the seam bond length, and so characterizes the crack depth specifically at the seam; "cracks" quantifies the average crack depth d between all pairs of adjacent protofilaments, where d for each pair equals the length of the unbonded part of the shorter of the two protofilaments (regular bonds are indicated in magenta, seam bonds in cyan), thus providing a second characterization of cracks in the MT tip. The top panel shows 3000 s (50 min) of simulated time, while the bottom panel shows at higher temporal resolution the rescue and catastrophe near t = 4480 (indicated in red). Examination of these data shows that although all of these characteristics change with transition, none obviously predicts an incipient rescue or catastrophe. Similar information on the number of GTP tubulins per tip can be found in Supplemental Figure S1.

trophe has occurred. Similar (but opposite) deviations often occur coincident with a rescue. However, we found that none of these parameters could be reliably used to predict when a transition would occur, regardless of parameter set. Simply improving the temporal resolution of these global analyses provided little additional insight: characteristics such as cap length and tip raggedness fluctuate rapidly and significantly throughout the course of a microtubule lifetime, and the deviations that ultimately give rise to a transition are not obviously distinguishable from the vast majority that do not (Figure 8 and Supplemental Figure S2, bottom).
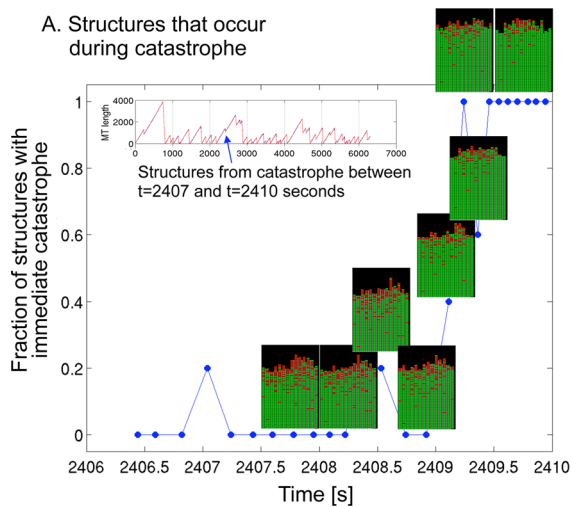
One exception may be tip raggedness, an increase in which precedes the catastrophe in the bottom of Figure 8. Examination of the top of Figure 8 suggests a correlation between increased tip raggedness and the onset of catastrophe for parameter set C. However, the failure of this measure to predict catastrophe in the other parameter sets (Supplemental Figure S2) suggests that although MTs assembled from some types of tubulin might exhibit a connection between tip raggedness and the onset of catastrophe, such a relationship is unlikely to be a universal feature of MTs or dynamic instability.

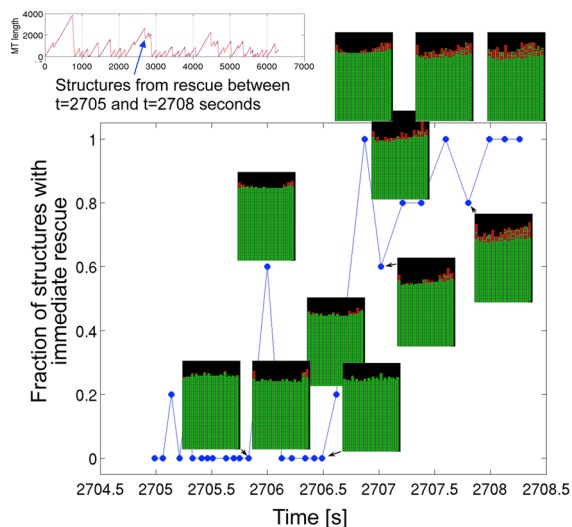***Analysis of transition propensity of spontaneously occurring tip structures***. Empirically, a growing MT is likely to continue growing, and a depolymerizing MT is likely to keep depolymerizing. What event "tips the tip" toward transition? Phrased more precisely, what structure(s) characterize a tip that is more likely to undergo transition? To investigate this question in more detail, we examined the propensity toward transition of particular structures that occurred spontaneously in the course of a transition to see whether we could identify structural features that make transition (as opposed to continued growth or shrinkage) likely. To do this, we extracted the MT tip configurations as they occurred at various time points in the process of an observed catastrophe or rescue and used these configurations to start new simulations. We first performed five new simulations for each of >15 structures occurring spontaneously in the course of six transitions (three rescues, three catastrophes, time points spanning ~3 s each).

Examination of the results (representative examples for set C are shown in Figure 9, A and B) indicates that most spontaneously occurring tip structures are stable in the continued presence of constant tubulin concentration—they are likely to continue either growing or shrinking as appropriate. However, during transition rare structures occur that are unstable—poised to resolve either into growing or shrinking structures, depending on the exact sequence of subsequent bond formation and breakage.

Examination of these transitional structures (Figure 9, A and B, inset, tip structures) suggests that they are characterized by no single attribute. Instead, increased likelihood of rescue correlates with the occurrence of a few lateral bonds between the GTP subunits that typically appear on (and disappear from) otherwise depolymerizing tips (Figure 9, B and C, and Supplemental Movies S3, S5, S7, and S8). Catastrophe-prone tips are harder to categorize but appear to correlate with extension of cracks (laterally unbonded protofilaments) into GDP-rich regions (Figure 9, A and C, and Supplemental Movies S2, S4, S6, and S8). Of course, both catastrophe and rescue correlate with deviations in the number of GTP tubulins at the tip, but we emphasize that our work suggests that it is the termination of the cracks in GTP or GDP-rich regions that is important for determining whether a microtubule grows, shrinks, or transitions, not the number of GTP tubulins itself. The significance of cracks is also supported by our recent analytical (mean-field) study of the role of lateral cracks in microtubule dynamics (Margolin *et al.*, 2011).

number of GTP tubulins), crack depth, and so on—would be predictive of incipient catastrophes or rescues. Examination of Figure 8 and Supplemental Figures S1 and S2 shows that all of these measures do change coincident with the occurrence of a catastrophe, and they deviate significantly from typical growth values after catas-

A. Structures that occur during catastrophe

Structures from catastrophe between t=2407 and t=2410 seconds

B. Structures that occur during a rescue

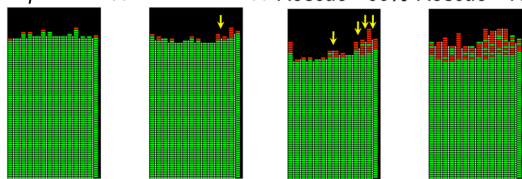Structures from rescue between t=2705 and t=2708 seconds

C. Tip characteristics that correlate with being transition-prone

Rescue:
Becomes more likely when have at least one laterally bonded pair of GTP-Tu subunits
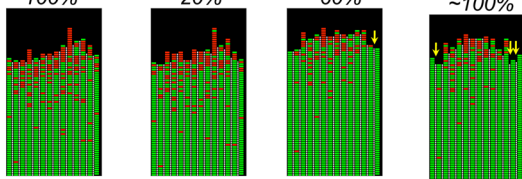
Depol ~100%   Rescue ~20%   Rescue ~60%   Rescue ~100%

Catastrophe:
Correlates with cracks extending into GDP-Tu regions...

Grow ~100%   Catastrophe ~20%   Catastrophe ~60%   Catastrophe ~100%

To investigate the process of transition more thoroughly, we repeated this tip-fate analysis with better statistics (100 simulations for each tip configuration), sampling structures that occurred at regular intervals through a full-length history plot (~1800 simulated seconds), and in parallel sampling the structures that occurred during all the catastrophes and rescues at even higher temporal resolution (~0.1 s; Supplemental Figure S3). The results of this analysis are consistent with the initial investigation of tip fate: transitions are indeed characterized by otherwise rare structures that are poised to "go either way." Consistent with our earlier conclusion that it is difficult to predict transitions, the tip structures in this analysis typically shifted sharply from a growth-prone state to a depolymerization-prone state (or the reverse), with the transition often occurring in 1–2 s of simulated time (Supplemental Figure S3). We were unable to find any features universally characteristic of these transitional structures, other than the observations that rescue is more likely once a tip has at least one lateral bond between GTP subunits; catastrophe becomes more likely when cracks extend into the GDP-rich region (Figure 9C).

Conclusion

We used a molecular-scale computational model of microtubule dynamic instability to investigate the dynamic structure of the microtubule tip and the mechanism of dynamic instability. The model is consistent with known attributes of microtubule structure and biochemistry and differs from previously established models in its unique combination of structural detail, computational speed, and ability to produce the full range of dynamic instability behaviors. More specifically, our model is able to simulate many tens of minutes of experimental time while explicitly accounting for both lateral and longitudinal bonds between tubulin subunits. It allows us to follow both catastrophe and rescue as they occur spontaneously and to do so either macroscopically or at a dimer-by-dimer level, providing a window into the mechanisms of these transitions.

Examination of the tip structures that occur during the course of the simulations suggests that the growing MT tip is best approximated as a closed tube with rapidly fluctuating extensions and that the GTP cap is a discontinuous and rapidly fluctuating

FIGURE 9: To analyze the processes of catastrophe and rescue, tip configurations that occurred spontaneously during a catastrophe and associated rescue were extracted from a simulation at the time points as indicated and used to start new simulations. (A) The fraction of the new simulations that underwent a catastrophe within the first 5 s of the new simulations. (B) The corresponding data for the rescue. Selected tip structures are shown (flat representation), centered above the corresponding data points, except in cases where arrows indicate the relevant data point (structural details are more visible upon magnification). (C) Tip characteristics that correlate with being transition prone. Top, yellow arrows highlight lateral bonding at the tip; bottom, yellow arrows highlight cracks between exposed GDP-capped protofilaments. Taken together with Supplemental Figure S3, these data predict that transition-prone structures occur infrequently during the course of normal MT dynamics and that during transition, the shift from growth-prone to depolymerization-prone structures (or vice versa) is rapid, sometimes <1 s. These data also indicate that transition-prone structures are not easily distinguishable from those that are likely to maintain growth or depolymerization. However, one common feature of catastrophe intermediates is extension of cracks into GDP-rich regions, whereas the appearance of lateral bonds between GTP subunits is a feature of early-rescue intermediates (C).

structure without precise boundaries. These and other conclusions discussed earlier are important because they are contrary to typical assumptions about MT structure, but other researchers have reached at least some of the same conclusions (VanBuren *et al.*, 2002, 2005; see also Howard and Hyman, 2009; Kueh and Mitchison, 2009). The unique aspect of our work is that we predict that cracks (laterally unbonded regions) exist at the tips of both depolymerizing *and* growing microtubules and that these cracks play a significant role in the processes of both rescue and catastrophe. More specifically, increased likelihood of catastrophe correlates with extension of cracks into GDP-rich regions, and rescue correlates with the appearance of a few laterally bonded GTP subunits.

These observations lead us to propose that microtubule dynamics is governed by a "stochastic cap" mechanism in which tubulin subunits hydrolyze GTP according to a first-order reaction after they become nonterminal subunits; catastrophe and rescue result from fluctuations in the size, shape, and extent of lateral bonding of the cap. These fluctuations are chemical in origin and result from the probabilistic nature of single protein–protein interactions and the cooperative nature of the MT tip. Mechanical strains due to subunit bending are obviously involved in these processes and act through their influence on the chemical rate constants (probabilities of subunit addition/bond formation and loss).

This view of MT dynamics can be considered a refinement of the original "fluctuating-cap" model of Chen and Hill (1985) but differs in that their model was helical in nature and so could not consider cracks between protofilaments. It is consistent with the mechanochemical model of VanBuren (2005) and with the related "dynamic cap" conceptual model as described by Howard and Hyman (2009). However, the dynamic cap model suggests that MT behavior can be explained by variation in the number of GTP-Tu subunits at the tip that results from the dynamic nature of subunit addition and loss (Howard and Hyman, 2009; see also Schek *et al.*, 2007; Gardner *et al.*, 2011). The stochastic cap model extends this idea by postulating that MT behavior is governed not only by variations in the number of GTP tubulin subunits, but also by rapid fluctuations in the distribution and lateral bonding of these GTP tubulin subunits. Although we cannot exclude the possibility that structural defects, "tube-closure," or large-scale, thermally induced structural fluctuations influence catastrophe or rescue, there is limited experimental evidence for these conceptual models, and the simulations discussed here show they are not necessary to account for the major aspects of experimentally observed dynamic instability.

In this context, it is important to consider the action of MT-binding proteins. As discussed by Howard and Hyman (2009), the dynamic cap model explains the ability of some MT-binding proteins to increase MT growth rate by suggesting that they suppress subunit loss from growing tips (see also Schek *et al.*, 2007; Gardner *et al.*, 2011). The stochastic cap model extends this idea by providing a mechanism for this suppression of subunit loss: promotion of lateral bond formation (i.e., healing of cracks between protofilaments). The observation that most MT-binding proteins have at least two MT-binding sites (some have many more) linked by relatively unstructured regions suggests that no special activity would be necessary to promote lateral bond formation: proteins could accomplish this simply by binding across multiple protofilaments. In fact, by binding across protofilaments and influencing the strength of lateral bonds, proteins could simultaneously influence MT growth rate, catastrophe, and rescue, as has been experimentally observed (see, e.g., Drechsel *et al.*,

1992). Differential impact on these aspects of dynamic instability could be achieved by the established ability of some proteins to preferentially target the MT lattice or MT tip (Akhmanova and Steinmetz, 2008).

One argument in favor of this stochastic cap model is that it produces behavior very similar to that of experimentally observed dynamic instability. However, although such behavior is consistent with model validity, it is not evidence of validity per se because the ability to produce dynamic instability is neither unique nor limited to closely related models. For example, the lateral cap model has been quite successful in recapitulating and explaining a number of experimental observations, including dynamic instability itself (Bayley *et al.*, 1990; Martin *et al.*, 1993; Vandecandelaere *et al.*, 1994). However, the lateral cap model is based on helical interactions between tubulin dimers, a stipulation that seems incompatible with present understanding of the protofilament nature MT structure. Moreover, both actin and ParM have been observed experimentally to display dynamic instability under certain conditions (Fujiwara *et al.*, 2002; Garner *et al.*, 2004; Kuhn and Pollard, 2005), and computational models for actin dynamic instability have been developed (e.g., Vavylonis *et al.*, 2005; Ranjith *et al.*, 2009). In light of these observations, we justify support for the stochastic cap model of MT dynamic instability on the combination of its biochemical foundation, consistency with MT structure, and ability to produce dynamic instability, and we believe that of the existing conceptual models for dynamic instability, the stochastic cap model provides the most complete explanation for the array of experimental data.

The observation that varied systems can display dynamic instability raises the question of what system characteristics are required for the emergence of dynamic instability (Howard and Hyman, 2009). It is tempting to speculate that the propensity for dynamic instability is intrinsic to any sufficiently cooperative biochemical system that can switch between two or more macroscopically observable quasisteady states. This is likely an overgeneralization, but a key task for the future will be to determine the general principles of systems that display dynamic instability, the conditions under which they display it, and how these principles relate to microtubules, actin, and other systems, both biological and nonbiological.

MATERIALS AND METHODS
The computational model
As noted, we model the microtubule as a lattice composed of 13 protofilaments that is curled on itself, forming a tube with a seam (Figure 1A). The model includes five processes: formation and breakage of longitudinal bonds ("growth" and "shortening"), formation and breakage of lateral bonds ("bonding" and "breaking"), and the transition of each GTP-Tu subunit to GDP-Tu ("hydrolysis"; Figure 1B). At each step the algorithm checks all possible events (excluding hydrolysis) that can occur in any of the protofilaments and bonds and determines the fastest event. This event is then implemented. After that the hydrolysis cycle is run, randomly converting GTP-Tu dimers into GDP-Tu dimers. Then the next step begins. The structure of the algorithm is provided in Supplemental Figure S4.

More specifically, we have the following considerations:

- *Growth* (Figure 1B) is an addition of a single tubulin heterodimer to the end of a protofilament by formation of a longitudinal bond. The probability of growth depends on the rate constant, k_+, and the soluble tubulin concentration, as would be expected

for a typical chemical association event (see Supplemental Table S1 for values used in the various parameter sets).[1]

- *Shortening* is the detachment of a subunit from a protofilament by breakage of a longitudinal bond and can occur only if the subunit lacks lateral bonds. Any part of a laterally unbonded protofilament end can detach, meaning that multiple subunits can fall off simultaneously, which is consistent with experimental observation (Gardner *et al.*, 2011). Shortening is independent of soluble tubulin concentration, as expected for a typical chemical dissociation reaction. Note that, as with k_+, $k_{shorten}$ should not be confused with rate constants for tubulin detachment as given in other work, which typically combine both lateral and longitudinal bond breakage and are sometimes expressed as per MT. In parameter set C (but not A or B), the probability of a shortening event depends on the identity (GTP/GDP) of the subterminal subunit.

- *Bonding* is a formation of a new lateral bond between two dimers of neighboring protofilaments. A new lateral bond can form only if the subunits below it are already laterally bonded.

- *Breaking* is a loss of a lateral bond. The probability of breaking depends on the identity (GTP or GDP) of both subunits, but it also depends on the lateral bonding of the neighboring subunits. Moreover, an existing bond can break only if it is the highest (last) bond between the two protofilaments. These dependences on nucleotide state and lattice environment provide a kinetic approximation of the mechanical aspects of the MT.

- *Hydrolysis* is a first-order process that occurs only in interior subunits (i.e., it does not occur on the terminal subunit of a protofilament), consistent with structural data indicating that binding of α-tubulin is necessary to induce the β-tubulin GTPase (Nogales and Wang, 2006a).

The processes of lateral bond formation and breakage at the seam are governed by different rate constants because the MT structure near the seam differs from that in the rest of the MT (see Supplemental Table S1 for the specifics of each parameter set). In addition, since the lattice is shifted by 1.5 dimers at the seam, the bonds at the seam grow and shorten by half a dimer, in contrast to other bonds that evolve by a full dimer length. This is done to avoid introducing artificial asymmetry at the seam.

The actual rate of each possible event is drawn from the parameters provided in the configuration file or, as appropriate, calculated based on the identities of the neighboring dimers and/or on soluble tubulin concentration. The details of each parameter set are provided in the Supplemental Data. We model the events as Poisson processes with waiting times following an exponential distribution. Then the time for each event is drawn from its respective rate: $t_i = -(\ln r)/k_i$, where r is a random number uniformly distributed between 0 and 1, and k_i is the rate calculated for that event. The event with the shortest time is then implemented, and the process repeats itself (see Supplemental FigureS4 for a flowchart of the simulation process).

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[1]Note that k_+ as used here should not be confused with k_+ as used in other work, which typically is an *effective* rate constant "k_{eff}" that describes incorporation into the lattice and thus includes formation of both longitudinal and lateral bonds. Although it is possible to arrive at an average value for k_{eff}, it is important to point out that k_{eff} is not actually constant at the scale of individual dimer incorporation reactions because the likelihood of incorporation of a particular dimer depends on the specific configuration of the tip and position of the subunit in question (see also Gardner *et al.*, 2011). Moreover, our k_+ is necessarily expressed as "per protofilament," whereas rate constants for growth as derived from experiments or used in models viewing MTs as one-dimensional fibers have sometimes been expressed as "per MT."

The model runs in approximately real time on one CPU (i.e., 10 min of simulated time requires ~10 min of CPU time for a standard Linux machine).

Note: Aspects of this computational model were also presented as part of our mean-field analysis of the role of lateral cracks in microtubule dynamics (Margolin *et al.*, 2011). The resulting theory was successful in describing (predicting and quantifying) the phases of MT dynamics. Specifically, it predicted the existence of stable shortening and growth phases, as well as appearance of an unstable "pause" state, which might play a role in phase transitions. However, the specifics of MT tip structures and their relationships to the phase transitions (catastrophes and rescues) were not addressed by that work. Obtaining insight into these issues and interpreting the computational model in a biological context are goals of the present study.

Parameter tuning

To choose an initial set of user-dictated parameters, we determined the limits of possible values for each parameter as determined by experiment and then arbitrarily varied the parameters within this range to find a set that produced life-like dynamic instability. One of these sets was chosen as set A. We then tuned the behavior of the model against experiment to arrive at sets B and C as described in the Supplemental Data. It is important to emphasize that parameter set C, although tuned against experiments with bovine brain tubulin, can almost certainly be further optimized by tuning against additional constraining experimental data. Thus we have taken care to base our conclusions on behaviors that are not parameter specific (i.e., behaviors that are seen with parameter sets A–C, as well as with many additional parameter sets not described here). In considering the validity of this approach, it is important to remember that real tubulins vary greatly in their specific biochemical properties but display the same dynamic instability behavior (e.g., compare mammalian and yeast tubulin; Dougherty *et al.*, 1998). More specific information about these parameters and their relation to those used in previous models is provided in the Supplemental Information.

Calculation of dynamic instability parameters

To extract the dynamic instability parameters from the simulations, we identified the catastrophes and rescues by applying a thresholding algorithm to the length history of the average protofilament length. More specifically, a transition was detected when the average length changed by more than h dimers in the direction opposite to the current phase. We typically used h of 20 and 50 dimers, which produced similar dynamic instability estimates. Observed growth and shrinkage rates, V_g and V_s, respectively, were extracted from these data by determining the total length change per unit time between transitions. Observed catastrophe frequency and frequency of rescue, F_c and F_r, respectively, were determined by counting the number of catastrophes and rescues per unit time in growth and depolymerization.

Other calculations

For calculation of k_{off_MT} in Figure 7A, we performed one simulation with length 20,000 s for each parameter set. Then, we identified the growth phases and measured the duration of growth phases in seconds (t) and the number of dimers (d) that detached during the growth phases. The quantity of d/t is the effective k_{off_MT}.

For the calculation of average number of dimers lost per detachment event (Figure 7B), we took the growth phases from the same set of three 20,000 s simulations as before and counted the

depolymerization events (e) and all the dimers lost (d) in growth phase. The quantity of d/e is the average number of dimers lost per detachment event.

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