Catastrophic Depolymerization of Microtubules Driven by Subunit Shape Change

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DYNAMICS OF MICROTUBULE DEPOLYMERIZATION

In Fig. S1, we provide calculations of microtubule (MT) size and dimer dissociation over time, which complement the snapshots of behavior in Fig. 2 and the movies in Supplemental Material (SM). Here, we focus on uncapped GDP-MTs, which are populated with compressed α -subunits, undergoing catastrophic depolymerization or exhibiting stable leaves. Simulation protocols are as described in the main text.

Fig. S1(a) demonstrates that during catastrophic depolymerization, unpeeling occurs at an overall constant rate. This is established by calculating the net change $\Delta n_{\text{rows}}(\Delta t)$ in number of intact dimer rows over elapsed time Δt . Rows are categorized as intact if all 13 dimer participants (seam to seam) remain a part of the MT and of their subunits are bonded laterally. (The change $\Delta n_{\text{rows}}(\Delta t)$ directly correlates with the change in MT length, as typified in the time-lapse snapshots of main text Fig. 2(e-g).) On the other hand, for MTs exhibiting stable leaves, we initially observe loss of intact rows (often at comparable rates to the depolymerizing cases), but ultimately the net change in rows levels off as the leaves reach a quasi-equilibrium. As shown in Fig. S1(a), these stable leaves can be quite long — up to 20 dimer rows or more in length — given very strong vertical attractions (and lateral attractions strong enough to prevent mid-tubule instability).

Fig. S1(b) shows that GDP-MTs undergoing catastrophic depolymerization exhibit constant



FIG. S1. MT sizes and dimer dissociation over time for uncapped GDP-MTs with attraction strengths $A_{\rm L} = 2.2k_{\rm B}T$ and various $A_{\rm V} = 3.0, 3.2, \text{ and } 3.4k_{\rm B}T$ (exhibit *catastrophic depolymerization*, i.e., *catastrophe*, squares) and $4.4k_{\rm B}T$ (stable leaves, triangles). As in Fig. 2 in the main text, MTs are prebuilt with 40 dimer rows (520 dimers) with bottom two rows tethered. (a) Net change in number of *intact* dimer rows $\Delta n_{\rm rows}(\Delta t)$ over elapsed time Δt . (b) Net change in the number of dimers $\Delta n_{\rm dimers}(\Delta t)$ due to dissociation from the plus end. (c) Ratio of number of dimers released over the number of dimers that are unpeeled, which can be calculated as $f(\Delta t) = \Delta n_{\rm dimers}(\Delta t)/(13\Delta n_{\rm rows}(\Delta t))$. In (a-b), dashed lines denote complete depolymerization.

rates of net dimer losses (i.e., numbers of dimers released), as quantified by the net change in the number of dimers $\Delta n_{\text{dimers}}(\Delta t)$ — eventually hundreds of dimers are released from the propagating ram's horns (see main text Fig. 2). In contrast, GDP-MTs exhibiting stable leaves exhibit very little dimer dissociation despite considerable unpeeling. Generally, we observe that any lost dimers are usually released from one filament (not necessarily the seam). This apparently occurs because systems prone to forming stable leaves tend to form *evenly-sized* leaves. Given 13 protofilaments, this means four leaves that are three protofilaments wide, with the remaining filament left relatively unstable and frequently "passed" back and forth between adjacent leaves (during which the terminal dimer is weakly bound and likely to dissociate).

We combine the data from Figs. S1(a-b) to calculate the fraction $f(\Delta t)$ of unpeeled dimers that are actually released over time, shown in Fig. S1(c). Excepting initial startup, GDP-MTs undergoing catastrophic depolymerization exhibit constant rates of net dimer losses roughly commensurate with the rate of unpeeling, resulting in ratios close to 1.0 (though with smaller ratios for higher A_V , corresponding to longer leaves). For GDP-MTs with stable leaves, this ratio is understandably low.

Finally, we note that for fixed lateral attraction strength $A_{\rm L}$, the rate of unpeeling negatively correlates with vertical attraction strength $A_{\rm V}$. This corresponds with our observation that the continued propagation of unpeeling directly coincides with (arguably, depends upon) the successful dissociation of dimers, which in turn exposes new rows of dimers that can be unpeeled, etc. In turn, the lower limit of unpeeling rate essentially corresponds with stable leaves at strong attraction strengths, where the tendency of unpeel is ultimately frustrated by lack of dimer dissociation (at long times, the unpeeling rate obviously approaches zero).

DISSOCIATION TIMES OF MICROTUBULE FRAGMENTS

As briefly discussed in the main text, we calculate the dissociation times of small GTP-MT fragments built with uncompressed α -subunits (Fig. S2) to find the ranges in attraction strength where GTP-MTs are highly stable. Here, fragment stability acts as a proxy for whole-MT stability because GTP-MTs at attraction strengths of interest (e.g., overlapping the region where uncapped GDP-MTs depolymerize) only slowly depolymerize from their plus ends, each starting with the weakest-bound top dimer. This dimer is bound to only two neighboring dimers, one vertical and one lateral, due to the pitch-3 architecture of the MTs (see Fig. 2) — hence the focus on 2x2 (LxV) fragments where each dimer begins with this bonding motif. We choose the small fragments — as opposed to, e.g., simulating short MTs — because we require many independent runs.

Fig. S2 shows the setup of a single simulation run, where we initialize a 2x2 fragment in isolation and propagate its trajectory following the protocols in the main text. We do not fix the fragment center-of-mass, allowing the fragment to translate in a cubic box with $L = 30\sigma$ as it vibrates and rotates. We run thousands of realizations at each combination of $A_{\rm L}$ and $A_{\rm V}$ and record when the primary dissociation event occurs — i.e., the instant where the initial fragment breaks into two or more non-bonded groups of dimers. For weaker attraction strength combinations, e.g., $A_{\rm L} = 1.8k_{\rm B}T$ and $A_{\rm V} = 2.0k_{\rm B}T$, we perform upwards of $2x10^4$ simulations runs out to timescales of $5x10^3\tau$. For the strongest attraction strengths, e.g., $A_{\rm L} = 2.2k_{\rm B}T$ and $A_{\rm V} = 3.4k_{\rm B}T$, we perform upwards of $4x10^4$ out to timescales of $2x10^5\tau$. Once we have collected individual dissociation times at a given combination of $A_{\rm L}$ and $A_{\rm V}$, we calculate the *characteristic dissociation time* $t_{\rm d}$ by fitting the normalized histogram h(t) of dissociation times with $h(t) = A \exp(-t/t_{\rm d})$, where A is



FIG. S2. Snapshots of a GTP-MT fragment simulation, one of many analyzed to obtain characteristic dissociation times t_d for dimers. (a) Initial configuration: a 2x2 fragment of dimers bonded in perfect chiral register. (b-e) Time-lapse of fragment dissociation (labeled by elapsed time Δt), where we collect the time corresponding to the *primary dissociation event*, i.e., the first instant where the initial fragment breaks into two or more sets of dimers.



FIG. S3. Diagram of dissociation times t_d for GTP-MT fragments as function of vertical (A_V) and lateral (A_L) attraction strengths. Alternative axes show the maximum (absolute) bonding energies possible between vertical $(A_{V,S})$ or lateral $(A_{L,S})$ subunit surfaces of two dimers. Solid symbols denote the combinations of attractive strengths for which simulations were performed. Solid contour lines denote constant t_d values interpolated from the simulation results; the dotted portion of the $t_d = 20000\tau$ contour is an extrapolation from leftward simulated points at high A_V .

an arbitrary constant.

Fig. S3 compiles the characteristic dissociation times t_d as a function of attraction strengths, where by comparison with Fig. 3, we observe that GTP-MT fragment stability increases rapidly within the parameter ranges where uncapped GDP-MTs exhibit catastrophic depolymerization, stable leaves, or long-term stability. Note that these calculations provide a *conservative* proxy for GTP-MT stability as the fragments lack the collective structural support of complete rings.



FIG. S4. Snapshots from a simulation used to calculate free-energy landscape F for dissociation of exposed dimers (i.e., dimers with only one lateral and one vertical neighbor, which occur because of MT pitch) from short a GTP-MT. (a-d) Umbrella sampling is used to gradually move top (bottom) exposed dimer from (a) the bound state where orientation is fixed due to neighboring wedges and attractive beads are in perfect register to (d) distances away from the MT where the dimer experiences no attractive interactions and has translational and orientational freedom. The umbrella sampling coordinate for each exposed dimer, denoted ξ , is the root mean square deviation (RMSD) of its bead positions relative to its pocket position. For each panel, we provide the reference ξ_0 of the umbrella sampling window at the elapsed time Δt of the snapshot. All other dimers in the MT are tethered. (d-e) Alternative close-up views of the short MT just after initialization.

BARRIERS TO DIMER DISSOCIATION FROM MICROTUBULE ENDS

To complement the above calculations of dissociation times, we also calculate approximate freeenergy profiles for dissociation of dimers from the ends of short GTP-MTs, which allows us to estimate as a function of attraction strengths the free-energy barriers separating the bound and bulk states. This provides a quantitative basis for our claim, also based on visual observations, that whole GTP-MTs are stable across the parameter space where uncapped GDP-MTs exhibit catastrophic depolymerization.

As shown in Fig. S4, we perform umbrella-sampling simulations of dimers initially in the bound state (where they are bonded to two neighboring dimers, one lateral and one vertical) and then are pulled away such that they have almost complete transitional and orientational freedom, excepting occasional impacts with the tethered MT. In our setup, the short GTP-MTs (tethered in-place) and the two dissociating dimers are simulated at overall packing fraction $\phi = 0.0085$, which is consistent with the whole-MT simulations and approximately corresponds to an average tubulin concentration $C = 30\mu$ M. The latter lies slightly above the concentrations typical *in vitro* and under physiological conditions, which range from 0.1-10 μ M [4].

The sampling coordinate is the root mean square deviation (RMSD), denoted ξ , of all bead positions in the dimer relative to the average bead positions when in the bound state where $\xi = 0$ (i.e., oriented correctly at the tubule end and overlapping bonding sites with neighbors). This is a reasonable choice of collective variable as it gradually increases the translational and orientational freedom of the dimer as it leaves the binding site. We calculate profiles out to RMSD values where the free-energies start to decrease as expected for dilute bulk conditions, with the dimer entropically favored to translate away from the bound state and access larger volumes for a given RMSD (i.e., where $p(\text{RMSD}) \propto 4\pi(\text{RMSD})^2$, with RMSD increasingly dominated by the average distance of



FIG. S5. Free energies F as a function of dimer RMSD relative to the bound state, denoted ξ , for select combinations of $A_{\rm L}$ and $A_{\rm V}$. Profiles are normalized relative to their values at $\xi = 5.0\sigma$, which aligns the regions of the profiles above $\xi \approx 4.0\sigma$ that approach the dilute bulk conditions away from the MT ends (see text). The free energy barriers for dissociation $\Delta F^{\rm MAX}$ are the *barrier maxima* separating the bound states and the large RMSD decay regions minus the *bound state* minimums. The bounds of $\Delta F^{\rm MAX}$ are shown with arrows for the $A_{\rm L} = 1.4k_{\rm B}T$ and $A_{\rm V} = 4.0k_{\rm B}T$ case. Excepting the purely entropic reference case with $A_{\rm L} = 0.0k_{\rm B}T$ and $A_{\rm V} = 0.0k_{\rm B}T$, profiles for all systems considered have the same qualitative shape as the profiles shown here, with one local maximum between the bound state and large-RMSD values.

the dimer beads from the bound state.) Note that our free-energy calculations resemble, but are distinct from, some approaches [1–3] for calculating standard binding free energies $\Delta G_{\text{bind}}^{\circ}$ of, e.g., ligand-protein associations.

For each simulation (at a given combination of $A_{\rm V}$ and $A_{\rm L}$), we use the COLVARS package for LAMMPS [5, 6] to constrain the dimer in a series of overlapping RMSD windows. The biasing potential of each window is harmonic and given by $U(\xi) = (1/2)k(\xi - \xi_i^2)$, where the spring constant is $k = 50.0k_{\rm B}T/\sigma^2$ and ξ_i is the reference RMSD of window *i*. In each simulation, the first and last windows have $\xi_i = 0.0\sigma$ and $\xi_i = 5.0\sigma$, respectively, where the interval between windows is $\Delta\xi_i = 0.25\sigma$. We move sequentially between windows (i.e., gradually increasing RMSD), using the last configuration of a current window as the first for the new window and allowing the new stage to relax for $5x10^4\tau$ prior to collecting statistics. (This protocol ends up indistinguishable from independently initializing each window.) We then collect $5x10^3$ RMSD measurements over $2.0x10^4\tau$ in simulation time.

From the simulation, we then measure the histograms $\rho_i(\xi)$ of instantaneous RMSDs exhibiting by the dimer in each umbrella-sampling window *i* and self-consistently combine them into a single histogram $\rho(\xi)$ spanning the entire range of RMSD values via the weighted histogram analysis method (WHAM) [7]. During the re-weighting of our window histograms, we require that window weights all converge within error tolerance 1×10^{-3} . This single combined histogram $\rho(\xi)$ is then inverted to obtain the potential of mean force (PMF), i.e., free-energy landscape



FIG. S6. Diagram of free-energy barrier ΔF^{MAX} for dimer dissociation from the ends of GTP-MTs as function of vertical (A_{V}) and lateral (A_{L}) attraction strengths. Alternative axes show the maximum (absolute) bonding energies possible between vertical $(A_{\text{V,S}})$ or lateral $(A_{\text{L,S}})$ subunit surfaces of two dimers. Solid lines trace free energy barriers ΔF^{MAX} in increments of $2k_{\text{B}}T$. Simulations were performed at A_{L} increments of $0.2k_{\text{B}}T$ and A_{V} increments of $0.4k_{\text{B}}T$ (not marked for visual clarity).

 $F(\xi) = -k_{\rm B}T \ln\{\rho(\xi)\} + C$ where C is an arbitrary constant. Free-energy profiles for several combinations of attraction strengths are given in Fig. S5, which for attraction strengths greater than zero all exhibit similar shapes.

As shown in Fig. S5, from each free-energy profile, we calculate the approximate free-energy barrier to dissociation ΔF^{MAX} relative to the bound state. These are compiled in Fig. S6, which demonstrates that over the parameter ranges where uncapped GDP-MTs exhibit catastrophic depolymerization, stable leaves, or long-term stability, the dissociation of exposed dimers from GTP-MTs (or, e.g., GTP caps) is frustrated by free-energy barriers of at least $12k_{\text{B}}T$ (see Fig. 3).

MECHANICAL PROPERTIES

We calculate the mechanical properties of GTP-MTs and capped GDP-MTs to contextualize the model relative to real MTs, focusing on $A_{\rm L} = 2.2k_{\rm B}T$ and $A_{\rm V} = 3.2k_{\rm B}T$ as this combination lies in the center of the catastrophic depolymerization region. We find that the model MTs in this region are highly stiff, similar to recent calculations for tubules built from wedge monomers [9].

To obtain Young's modulus E and shear modulus G we apply the methods of Sept and MacKintosh [10] that were developed for equilibrium simulations of MT fragments. We perform simulations of prebuilt MTs that are 40 dimer rows long (i.e., 320 nm) using the protocols in the main text. Following Ref. [10], we extract the centers of mass of all dimer subunits for each simulation configuration, and collect necessary statistics (described below) over each possible subset of 6 dimer subunits. Each subset comprises 2x3 (LxV) adjacent *subunits* and we combine statistics from all possible groupings in the tubules having subunits positioned at least 7 dimer rows away from the



FIG. S7. Normalized probability distribution functions (pdfs) of equilibrium (a) interdimer vertical distance d, (b) inter-filament vertical offset p, and (c) end deflection x. Symbols correspond to distributions measured from GTP-MTs (blue squares) and capped GDP-MTs (gold circles) with 40 dimer rows and attraction strengths $A_{\rm L} = 2.2k_{\rm B}T$ and $A_{\rm V} = 3.2k_{\rm B}T$. Deflection distributions are averaged over the x- and y-directions relative to tethered ends of the MTs (on average, MTs are directed along the z-axis; see main text Fig. 2). Solid black lines are Gaussian fits to the data. In (b) and (c), results for the GDP-MTs are multiplied by a factor of three for visual clarity.

MT ends. Thus, all fragment subunits are at least 5 dimer rows away from any tethered or capped regions, and we capture only mid-tubule lattice properties. Note that the bottom subunits of a fragment can be α - or β -subunits, or one of each if the fragment portion crosses the MT seam. Each vertical half of a fragment spans the α (or β) subunit of a given dimer to the same subunit in the dimer above it (i.e., one dimer length).

To obtain E, we begin by applying the equipartition relation

$$E^{(2D)} = \frac{1}{2} \frac{d}{w} \frac{k_{\rm B}T}{\langle \Delta d^2 \rangle} \tag{1}$$

where $E^{(2D)}$ is the average local modulus of the 2D fragment, d is the average vertical dimer spacing, $w = 2.65\sigma$ is observed average filament width, and $\langle \Delta d^2 \rangle$ is the (Gaussian) variance associated with average vertical dimer spacing d (see Fig. S7(a)). Given the approximation $E = E^{(2D)}h$, where $h = 3\sigma$ is tubule thickness, we find that GTP- and GDP-MTs respectively have $E = 158.4k_{\rm B}T/\sigma^3$ and $77.1k_{\rm B}T/\sigma^3$. In the main text, we convert these values to units of MPa given the conversions $k_{\rm B}T = 4.1$ pN-nm and $\sigma \approx 1.5$ nm (also used below).

We obtain G by beginning with the analogous relation

$$G^{(2D)} = \frac{2}{3} \frac{w}{d} \frac{k_{\rm B} T}{\langle \Delta p^2 \rangle} \tag{2}$$

where $\langle \Delta p^2 \rangle$ is the variance in the vertical offset p between the vertical halves of the fragment (adjacent protofilaments) (see Fig. S7(b)). Given the approximation $G = G^{(2D)}h$, we find that GTP- and GDP-MTs respectively have $G = 26.0k_{\rm B}T/\sigma^3$ and $21.3k_{\rm B}T/\sigma^3$.

To measure persistence length $\ell_{\rm p}$, we mimic the equilibrium MT deflection experiments of Pampaloni et. al. [11]. Here, we perform simulations of MTs that are 40 dimers long and follow the protocols in the main text, except that we increase the Langevin damping constant to $1000\tau^{-1}$ to lower the effective viscosity of the implicit solvent and improve sampling of whole-tubule motions. The quantity of interest here is the (lateral) deflection x between the centers of mass of the top



FIG. S8. Uncapped GDP-MT behavior as a function of compression (i.e., tilt) angle θ and attractive bead cutoff distance r_c (results in the main text are for $\theta = 15^{\circ}$ and $r_c = 0.5\sigma$. Attraction strengths are fixed at $A_L = 2.2k_BT$ and $A_V = 3.2k_BT$. GDP-MTs that are *stable* (diamonds) exhibit no unpeeling or breakage over time. GDP-MTs exhibiting *fraying* (circles) frequently have broken lateral bonds in the top dimer row, but never unpeel. GDP-MTs undergoing *catastrophic depolymerization* (i.e., *catastrophe*, squares) unpeel from their uncapped ends and release dimers. At all other states, GDP-MTs exhibit one or more spontaneous *mid-tubule break(s)* (plus signs). Note that similar behaviors are observed for attraction strength combinations $A_L = 2.2k_BT$, $A_V = 3.0k_BT$ and $A_L = 2.2k_BT$, $A_V = 3.4k_BT$.

and bottom rows of dimers (where the bottom is tethered), which are collected in Fig. S7(c). We then apply the relation

$$\ell_{\rm p} = \frac{L^3}{3\langle \Delta x^2 \rangle} \tag{3}$$

where $\langle \Delta x^2 \rangle$ is the (Gaussian) variance of the MT deflection x and L is the average tubule contour length, which for GTP- and GDP-MTs is respectively $L = 240\sigma$ and 234σ . In turn, we find that GTP- and GDP-MTs respectively have $\ell_p = 3.54 \times 10^5 \sigma$ and $1.94 \times 10^5 \sigma$.

EFFECTS OF ATTRACTION CUTOFF AND α -SUBUNIT TILT ANGLE

Finally, we briefly survey how the choices of compression (i.e., tilt) angle θ and attractive bead cutoff distance r_c impact the behavior of uncapped GDP-MTs. Here, we focus on attraction strengths ($A_L = 2.2k_BT$ and $A_L = 3.2k_BT$) that fall squarely in the catastrophic depolymerization region given the choices $\theta = 15^{\circ}$ and $r_c = 0.5\sigma$ used in the main text. MTs are initially 40 dimer rows long, and we follow the simulation protocols as described in the main text.

One notable finding is that for a given r_c value, catastrophic depolymerization occurs over a *narrow* range of θ -values — spanning only about 2° — which reflects the delicate balance of geometric frustration required for inducing unpeeling only at an uncapped MT end (similar to the discussion of main text Fig. 3): too small of a θ value does not induce bond frustration sufficient to destabilize the tubule, but too large of a θ value favors curved filament configurations very strongly, producing many breaks over the MT lattice instead of a steadily propagating unpeeling only from the plus end.

It is also apparent that for a given combination of attractions strengths, the choice of attractive bead cutoff r_c has no qualitative impact on the way GDP-MT behavior changes as a function of θ ; rather, increasing r_c simply requires a corresponding increase in θ to induce the bond frustration necessary for catastrophic depolymerization. For the attraction strengths here, one can observe catastrophic depolymerization for a given r_c by choosing $\theta = 25.0r_c + 2.5$. Of course, in the main text we focus on the combination of $\theta = 15^{\circ}$ and $r_c = 0.5\sigma$, which demonstrates that α -subunit compression can induce catastrophic depolymerization given attractive bead sizes comparable in scale to tubulin binding structures and a θ -value comparable to that associated with the "bent" tubulin conformation in experiments [12, 13]. However, the results here indicate that catastrophic depolymerization can be *predictably* induced for other reasonable combinations of parameters.

- [1] T. Simonson, G. Archontis, and M. Karplus, Acc. Chem. Res. **35**, 430 (2002).
- [2] H.-J. Woo and B. Roux, Proc. Natl. Acad. Sci. U. S. A. 102, 6825 (2005).
- [3] Y. Deng and B. Roux, J. Phys. Chem. B **113**, 2234 (2009).
- [4] M. Gardner, B. Charlebois, I. Jánosi, J. Howard, A. Hunt, and D. Odde, Cell 146, 582 (2011).
- [5] G. Fiorin, M. L. Klein, and J. Hénin, Mol. Phys. **111**, 3345 (2013).
- [6] S. Plimpton, J. Comput. Phys. **117**, 1 (1995).
- [7] S. Kumar, J. M. Rosenberg, D. Bouzida, R. H. Swendsen, and P. A. Kollman, J. Comput. Chem. 13, 1011 (1992).
- [8] H. P. Erickson, J. Mol. Biol. **206**, 465 (1989).
- [9] M. J. Stevens, J. Chem. Phys. 147, 044902 (2017).
- [10] D. Sept and F. C. MacKintosh, Phys. Rev. Lett. **104**, 018101 (2010).
- [11] F. Pampaloni, G. Lattanzi, A. Jonáš, T. Surrey, E. Frey, and E.-L. Florin, Proc. Natl. Acad. Sci. U. S. A. 103, 10248 (2006).
- [12] R. B. Ravelli, B. Gigant, P. A. Curmi, I. Jourdain, S. Lachkar, A. Sobel, and M. Knossow, Nature 428, 198 (2004).
- [13] G. M. Alushin, G. C. Lander, E. H. Kellogg, R. Zhang, D. Baker, and E. Nogales, Cell 157, 1117 (2014).