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Molecular Simulations Show Catastrophic Depolymerization of Microtubules Driven by Subunit Shape Change

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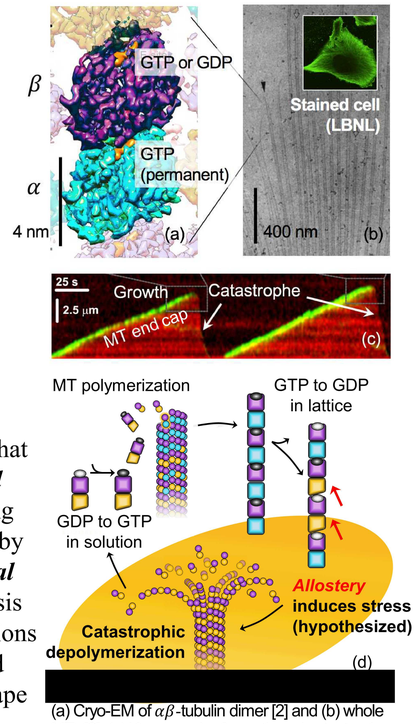
Exploring the origins of microtubule instability

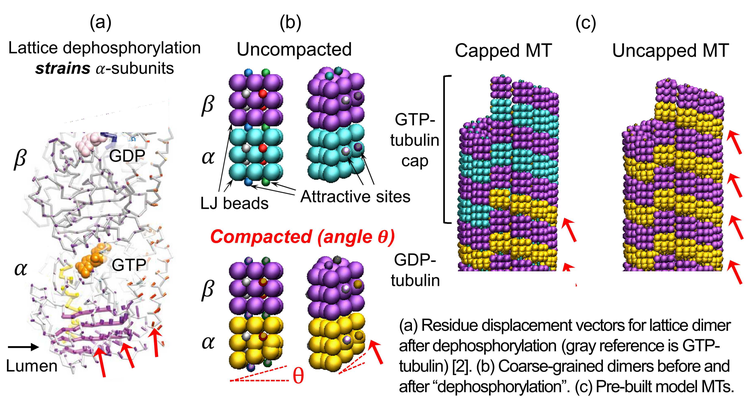
Microtubules (MTs) are stiff biopolymers critical for many cellular processes including mitosis. MTs exhibit a dynamic cycle between growth and ***catastrophic depolymerization***: GTP-tubulin ($\alpha\beta$-dimer with GTP at β-site) self-assembles, but the dephosphorylation of GTP- to GDP-tubulin within the MT causes destabilization [1].

The mechanistic origins of MT depolymerization are not fully understood—one hypothesis is that dephosphorylation induces ***bond frustration between dimers*** along protofilaments, which is caused by ***allosteric responses of individual dimers*** [2]. We test this hypothesis via molecular dynamics simulations of MTs built with coarse-grained dimers, where we can mimic shape transitions known for tubulin.



(a) Cryo-EM of $\alpha\beta$-tubulin dimer [2] and (b) whole MTs [3]. (c) Kymograph of growth and catastrophe cycle (x2) [4]. (d) GTP/GDP-mediated stability.

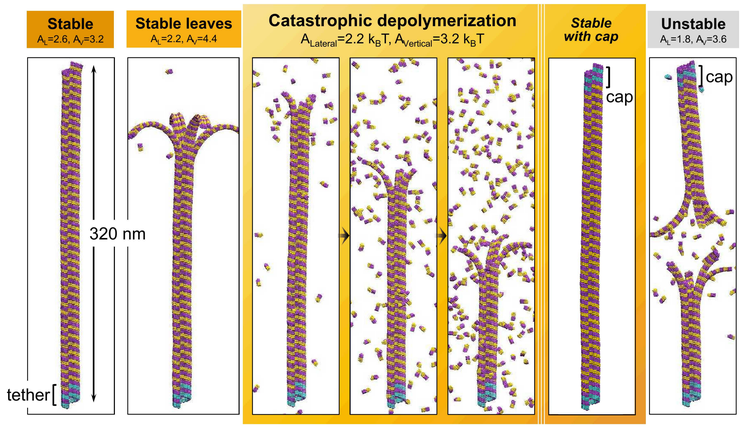
Coarse-grained model of $\alpha\beta$-tubulin



(a) Residue displacement vectors for lattice dimer after dephosphorylation (gray reference is GTP-tubulin) [2]. (b) Coarse-grained dimers before and after "dephosphorylation". (c) Pre-built model MTs.

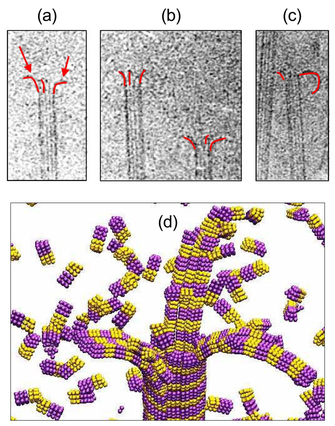
- Implicit-solvent simulations of dimers (bound α- and β-wedges, 27 repulsive beads ea.) w/ attractive site pairs for chiral dimer bonding
- Mimic observed ***strain*** of α-subunit caused by dephosphorylation– ***compacted*** dimer resembles well-known "bent" tubulin [2,5]

Model depolymerization resembles experiments

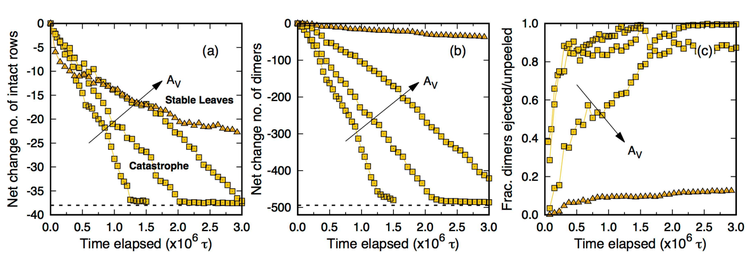


Simulation snapshots of MTs given various interaction strengths. MTs that are stable exhibit no unpeeling or breakage. MTs that exhibit stable leaves unpeel to a quasi-equilibrium length with minimal dimer ejection. MTs undergoing catastrophic depolymerization unpeel and eject dimers (shown as time-lapse) unless stabilized by an uncompacted cap. MTs are considered unstable if they exhibit spontaneous breakage even when capped.

- For uncapped MTs built with ***compacted*** dimers, we observe catastrophic depolymerization at select attraction strengths
- Exposed dimers are released from ***"ram's horns", closely resembling cryo-EM images*** of depolymerizing MTs [4]
- Depolymerization can be ***averted by cap of uncompacted dimers***, as in experiments [1]. (*Required condition for labeling catastrophic depolymerization– otherwise unstable*)
- Sufficiently strong interactions prevent dimer dissociation and even unpeeling

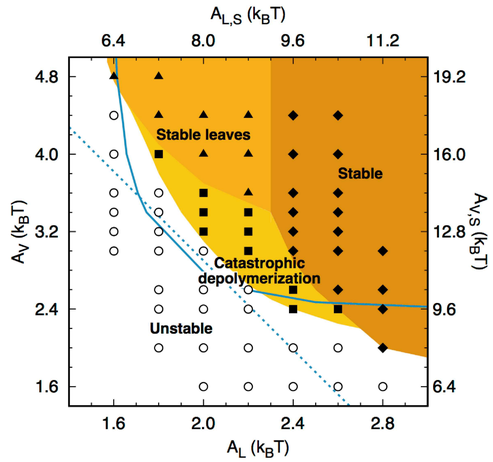


(a-c) Cryo-EM images of real depolymerizing MTs, with traces highlighting characteristic unpeeling "ram's horns" [3]. (d) Close-up view of unpeeling event for depolymerization time-lapse case shown in top figure.



Depolymerization dynamics of uncapped compacted MTs over time for A_L=2.2 k_BT and various A_V = 3.0, 3.2, 3.4 k_BT (all catastrophe) and 4.4 k_BT (stable leaves). MTs are prebuilt with 40 dimer rows (520 dimers). Horizontal dashed lines denote complete depolymerization. Dimers rows are intact if all 13 participants and all lateral bonds remain. Net change in number of dimers reflects dissociation from unpeeling MT end.

Depolymerization conditions & mechanics



Behaviors of compacted MTs as function of vertical A_V and lateral A_L attraction strengths. Secondary axes show the total absolute attraction energies possible between bonded lateral or vertical surfaces of subunits on two adjacent dimers. Solid symbols denote where uncapped MTs exhibit catastrophe, stable leaves, or stability, but capped MTs are perpetually stable. Open circles denote where compacted MTs are unstable with or without caps. Blue lines show boundaries of rapidly increasing stability for uncompacted MTs.

- Depolymerization occurs over narrow region of interaction strengths where vertical interactions are (mostly) dominant
- Analogous to experiments, MTs built with ***uncompacted*** dimers are stable for interaction strengths in catastrophe region [1]
- MTs have high stiffnesses and persistence lengths, comparable to experiments for short MTs [6]
- Shape change shortens MTs and decreases stiffness

Property (@A_L=2.2,A_V=3.2)	**Uncompact (capped)**	**Compact (capped)**
Young's modulus **E (MPa)**	270	131
Shear modulus **G (MPa)**	44	36
Persistence length $\mathbf{L_p}$ **(um)**	530	290

Summary & Conclusions

- Simple α-subunit shape change is sufficient to drive otherwise stable and stiff model MTs to undergo catastrophic depolymerization
- Validates hypothesis that depolymerization-driven allostery, as revealed in recent cryo-EM experiments, explains MT behavior
- Depolymerization occurs for attractions at limit of stability– reflects subtle balance of inducing stress that ***only*** unpeels uncapped end

[1] Mitchison T. and Kirschner M. *Nature*, 312 (1984).
[2] Alushin G.M., et. al. *Cell*, 157 (2014); Zhang R., et. al. *Cell*, 162 (2015).
[3] Chrétien D., Fuller S.D., and Karsenti E. *J. Cell. Biol.*, 129 (1995).
[4] Maurer, S.P., et. al. *Cell* 149 (2012).
[5] Ravelli R.G.B., et. al. *Nature*, 428 (2004).
[6] Taute K.M., et. al. *Phys. Rev. Lett.*, 100 (2008).

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