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Molecular Simulations Show Catastrophic Depolymerization of Microtubules Driven by Subunit Allostery

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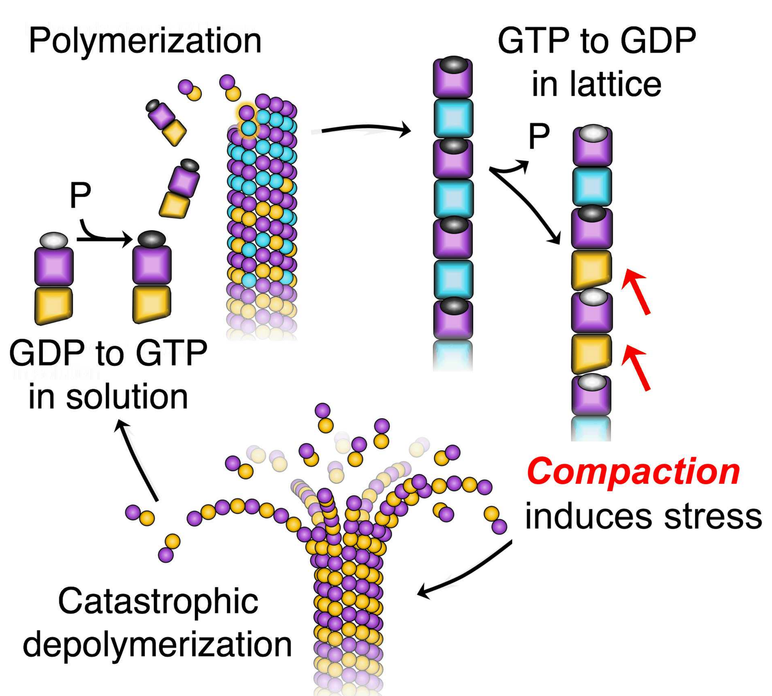
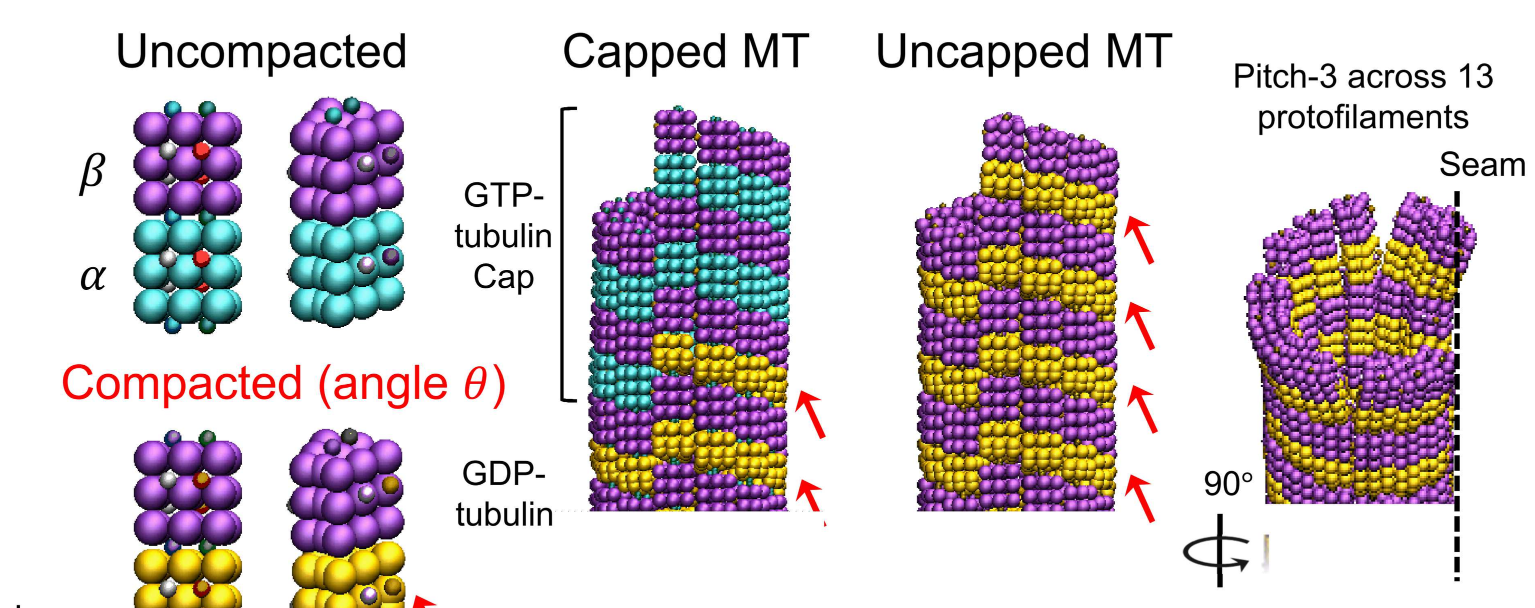
Exploring the origins of microtubule instability

Microtubules (MTs) are rigid biopolymers critical for many cellular processes including mitosis. They exhibit a dynamic instability between growth and ***catastrophic depolymerization***: GTP-tubulin (an $\alpha\beta$-dimer bound with GTP) self-assembles, but the gradual dephosphorylation of GTP- to GDP-tubulin within the tubule causes destabilization [1].



Understanding this MT destabilization mechanism is fundamental for controlling cell division (e.g., interrupting cancerous growth) and designing synthetic particles with similar functionality. It has been hypothesized that dephosphorylation drives destabilization by causing ***allosteric compaction of α-tubulin subunits***, generating mechanical stress [2]— but this has thus far remained untested. We validate this hypothesis by examining molecular dynamics (MD) simulations of MTs built from a coarse-grained model of tubulin where we can selectively incorporate such a shape change.

Coarse-grained model of $\alpha\beta$-tubulin and allosteric compaction



(Left) Close-up of dimer models: each subunit wedge includes 27 repulsive beads and 8 attractive beads (pair on each wedge side). (Right) Tops of pre-built MTs.

- Model $\alpha\beta$-tubulin via wedge dimers with patchy attractions
- Angle θ=15° mimics compaction of outer intermediate domain of α-subunit observed via cryo-EM (also resembles "bent" tubulin conformation in bulk solution) [2,3]
- Prebuild MTs with uncompacted/compacted dimers (optional cap)
- Observe responses via MD simulations with implicit solvent

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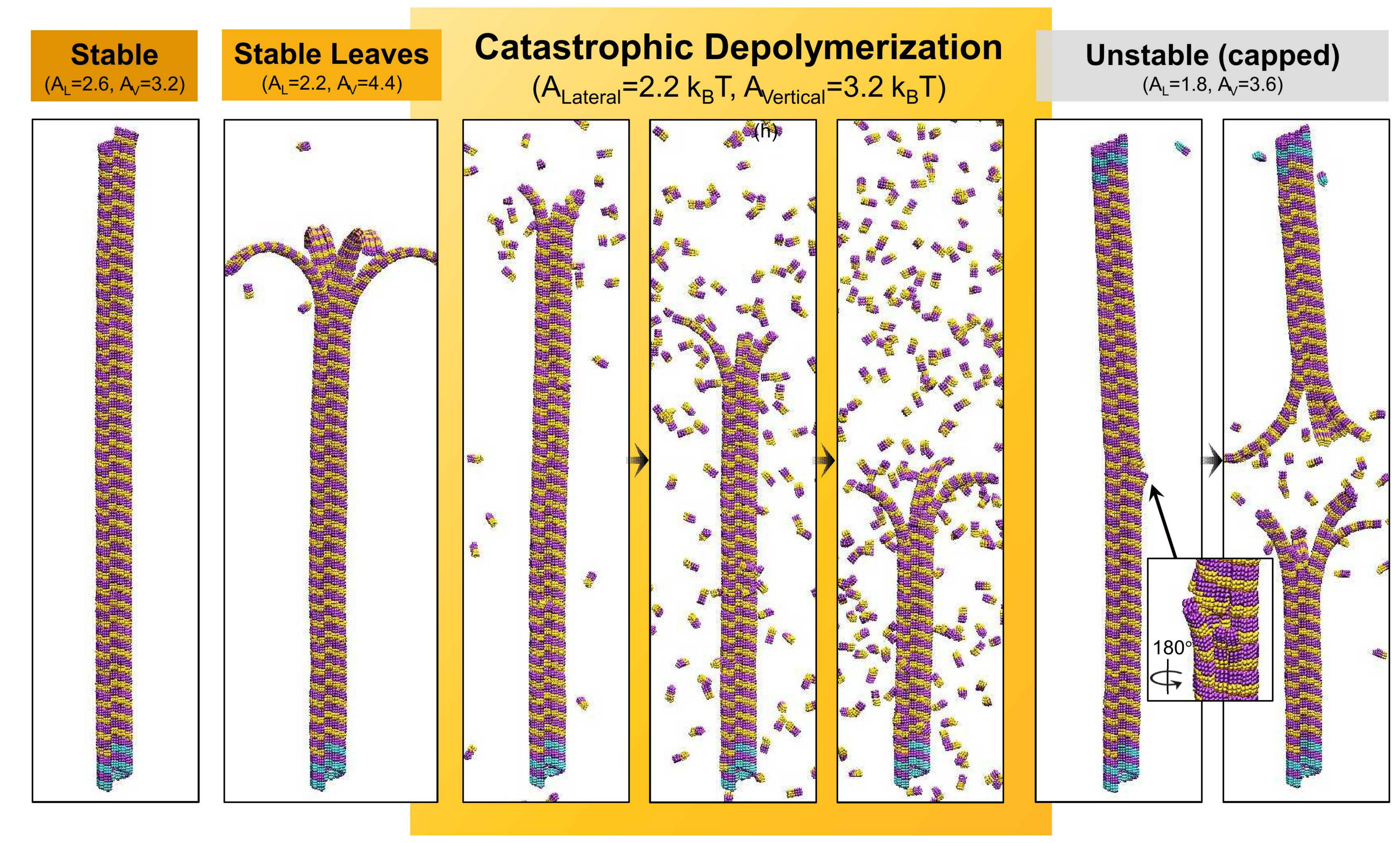
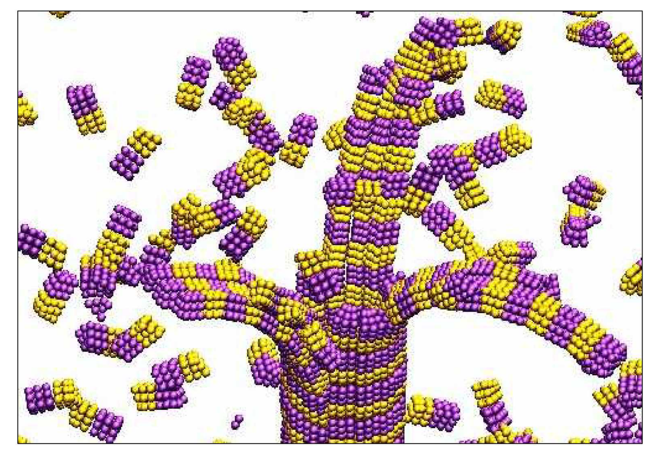
Family of microtubule responses to α-subunit shape change



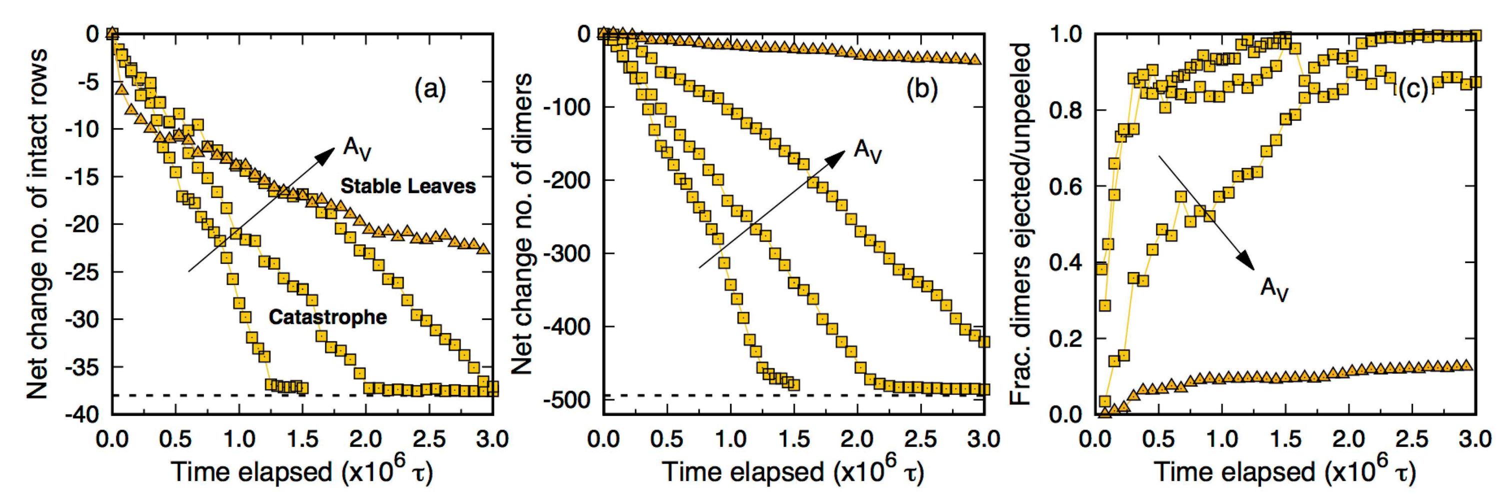
Simulation snapshots of MTs given various interaction strengths. MTs that are stable exhibit no unpeeling or breakage over time. MTs that exhibit stable leaves unpeel to a quasi-equilibrium length with minimal dimer ejection. MTs undergoing catastrophic depolymerization unpeel and eject dimers (shown as time-lapse). MTs are considered unstable if they exhibit spontaneous breakage in the compacted lattice leading to disassembly.

- With compaction, observe catastrophic depolymerization that propagates from uncapped end until exhaustion
- Exposed dimers are ejected from ***"ram's horns", which closely resemble cryo-EM images*** of real depolymerizing MTs [4]



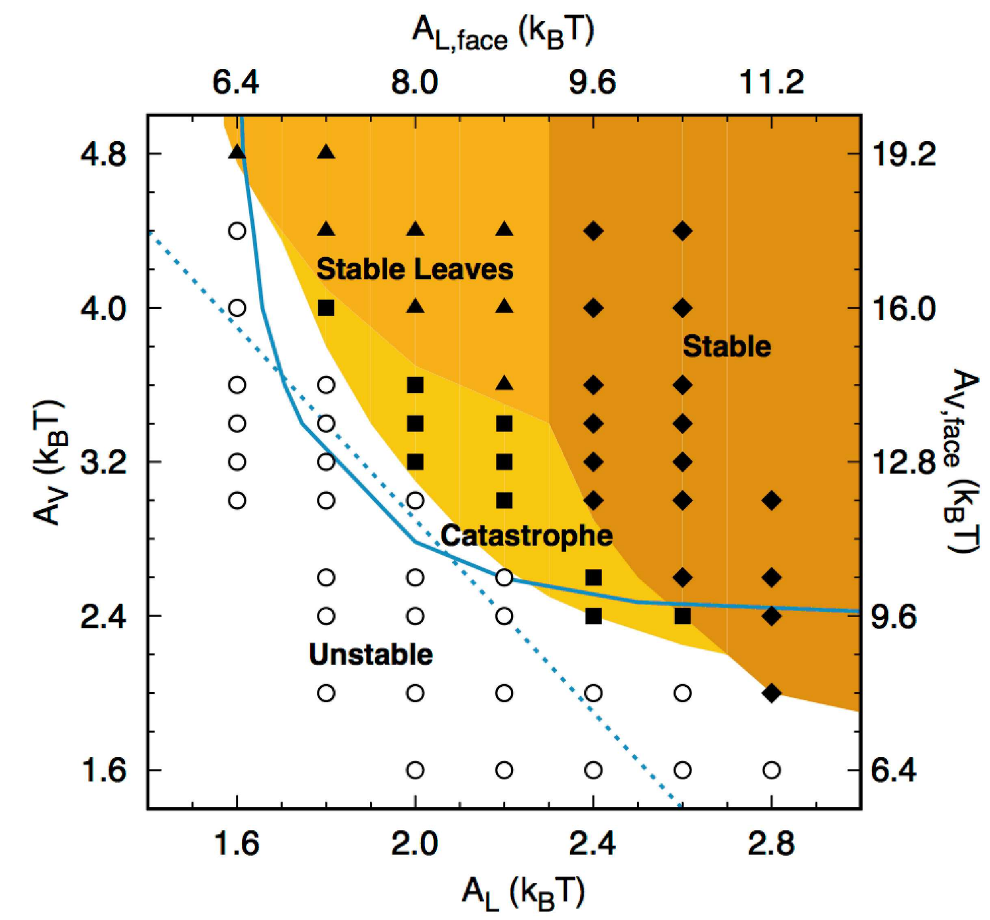
Close-up view of "ram's horns" during unpeeling.

- Confirmed that catastrophic depolymerization can be averted by cap of uncompacted dimers (i.e., GTP-tubulin cap)
- Depolymerization propagates at constant rate, presenting with 3- to 5- prominent "leaves" at each instant
- Sufficiently strong interactions prevent dimer ejection and even unpeeling itself



Sizes 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). As above, MTs are prebuilt with 40 dimer rows (or 520 dimers) with bottom two rows tethered. Horizontal dashed lines denote exhaustion of non-tethered lattice. Dimers rows are intact (seam to seam) if all 13 participants and all lateral bonds remain. Dimers are considered ejected only when they share no cluster connectivity with the MT tether region.

Conditions for depolymerization & impacts on 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 sides 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 slim region of attraction strengths where vertical interactions are (mostly) dominant
- MTs in the catastrophe region that are capped—or built from uncompacted dimers—are stable given same interactions
- Capped MTs have high stiffness & persistence length similar to experiments for short (L < 5μm) MTs [5]
- Compaction 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 **L_p (um)**	530	290

Summary & Future Work

- Simple α-subunit shape change is sufficient to drive otherwise stable and rigid MTs to undergo catastrophic depolymerization
- Depolymerization occurs for interaction ranges at edge of stability–reflecting subtlety of inducing stress that ***only*** unpeels MT end
- Currently exploring how MTs with catastrophe-compatible interactions may homogeneously nucleate and grow

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[2] Alushin G.M., et. al. *Cell*, 157 (2014).
[3] Ravelli R.G.B., et. al. *Nature*, 428 (2004).
[4] Chrétien D., Fuller S.D., and Karsenti E. *J. Cell. Biol.*, 129 (1995).
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