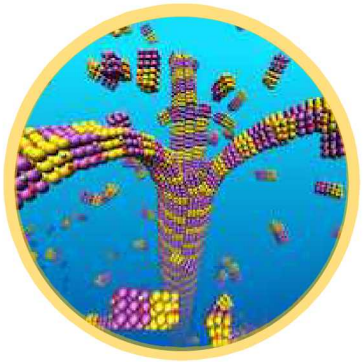
SAND2019-14536C

Catastrophic depolymerization of microtubules driven by tubulin shape change



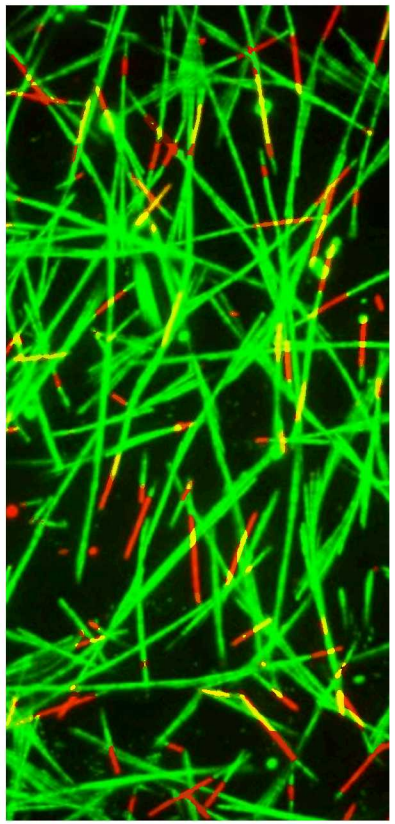
Jonathan A. Bollinger and Mark J. Stevens

Center for Integrated Nanotechnologies
Sandia National Laboratories

March 8, 2018

This work was performed, in part, at the Center for Integrated Nanotechnologies, an Office of Science User Facility operated for the U.S. Department of Energy (DOE) Office of Science. Sandia National Laboratories is a multi-mission laboratory managed and operated by National Technology and Engineering Solutions of Sandia, LLC., a wholly owned subsidiary of Honeywell International, Inc., for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-NA0003525.

Microtubules are uniquely responsive biopolymers



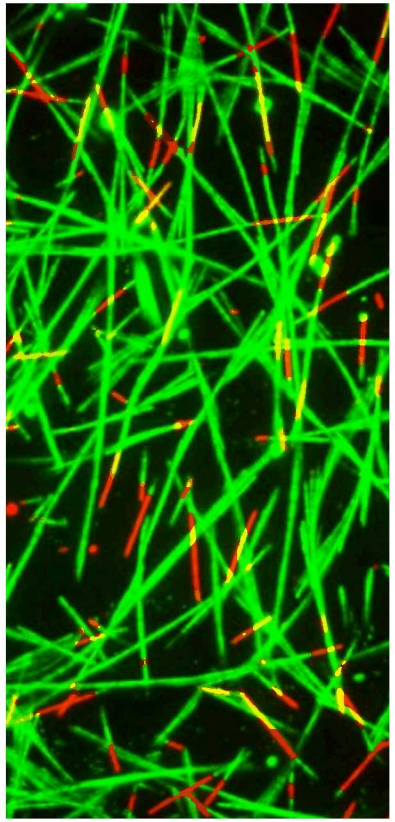
Dyed microtubules in epithelial cells. Zanic group, Vanderbilt School of Medicine

High aspect-ratio fibers (d~25nm, L~10um) that self-assemble from tubulin in eukaryotic cells

Critical for cell function: cytoskeletal structure, mitosis, tracks for motor-proteins

Duality: microtubules are highly stiff, yet can catastrophically unpeel

Microtubules are uniquely responsive biopolymers



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Critical for cell function: cytoskeletal structure, mitosis, tracks for motor-proteins

Duality: microtubules are highly stiff, yet can catastrophically unpeel

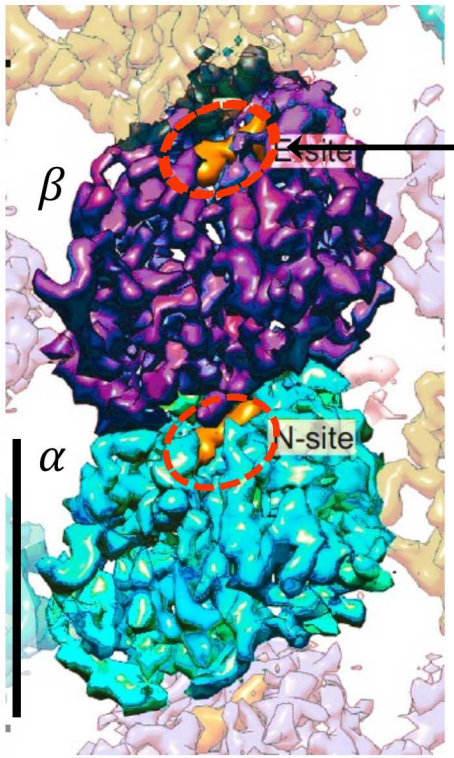
Goals

Develop **minimal model** for microtubule behavior (capture duality)

Validate **"design rules"** for building blocks with active (dis)assembly

Toward **synthetic** reconfigurable fiber/gel/film systems

$\alpha\beta$-tubulin and microtubule ***dynamic instability***

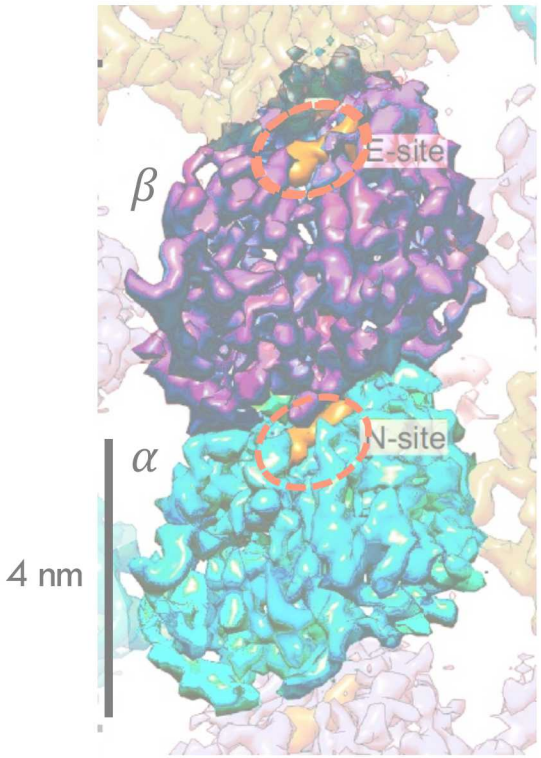


High-res (~8 A) Cryo-EM reconstruction (Alushin)

Active binding site for **GTP or GDP**, which are **exchangeable**

Alushin, et al. Cell, 157, 2014

$\alpha\beta$-tubulin and microtubule ***dynamic instability***



Polymerization of GTP-tubulin

GDP

GTP

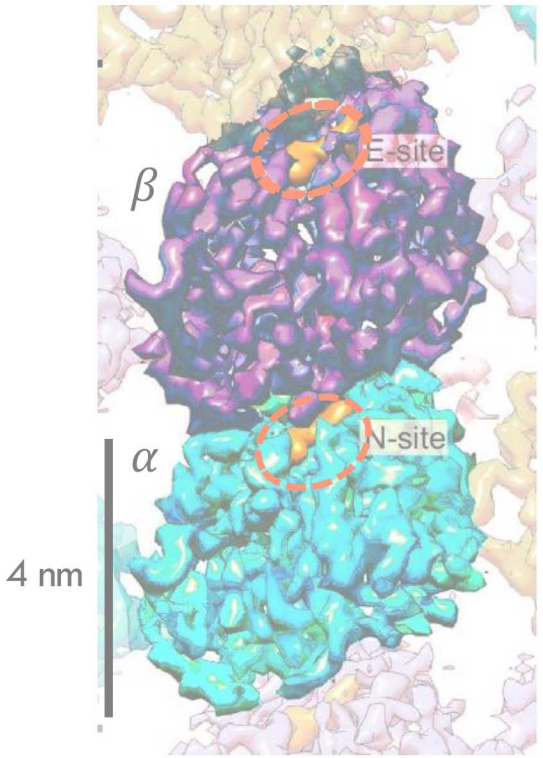
GTP-tubulin "cap" at leading plus end

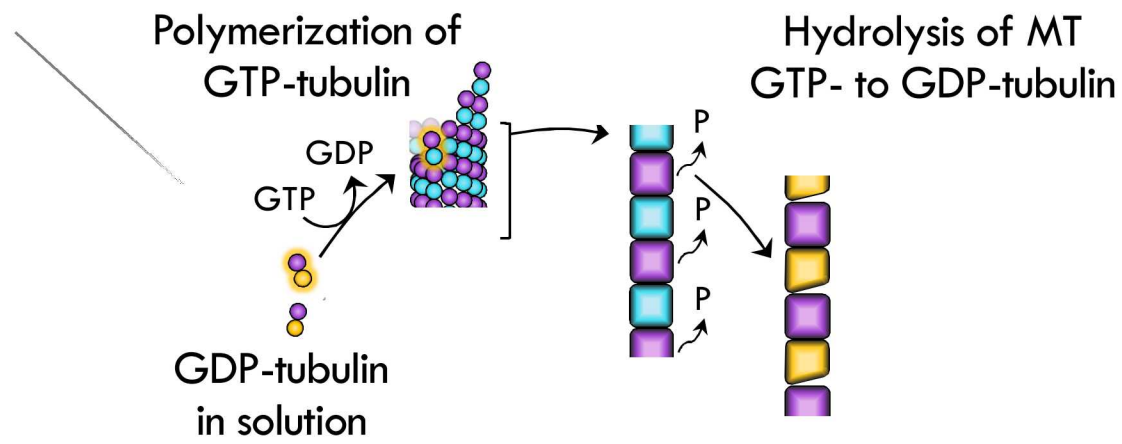
GDP-tubulin in solution

Alushin, et al. Cell, 157, 2014

Bollinger and Stevens, Soft Matter, 2018.

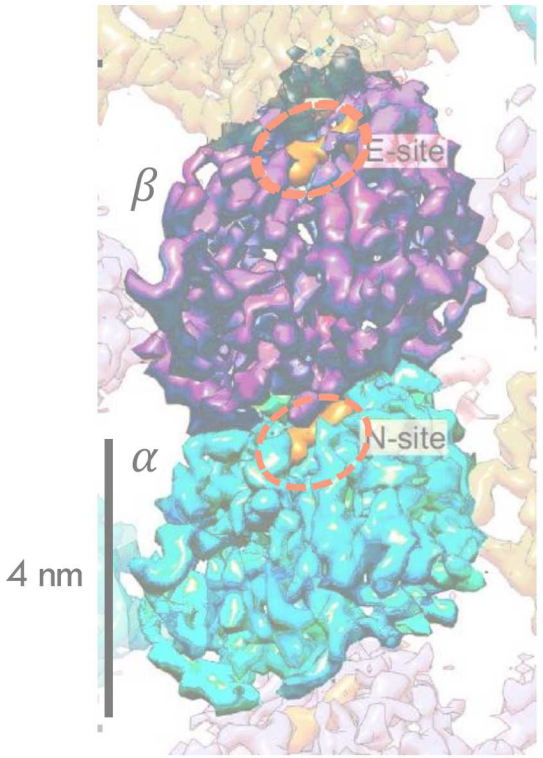
$\alpha\beta$-tubulin and microtubule ***dynamic instability***

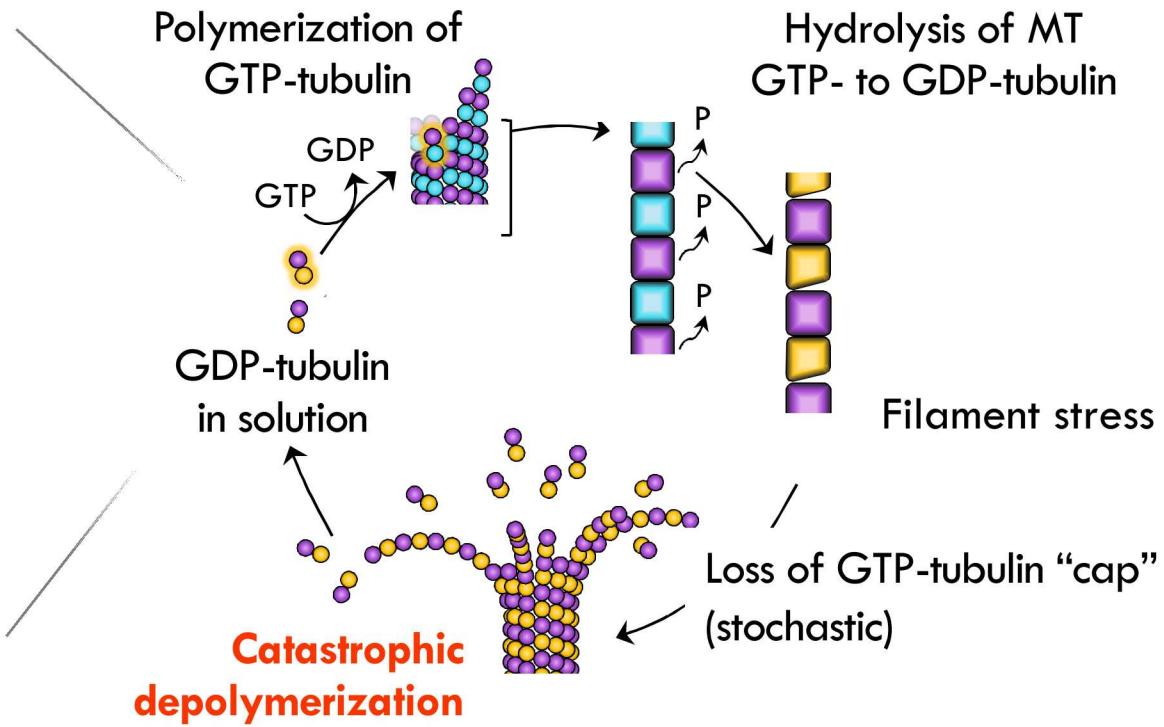




Alushin, et al. Cell, 157, 2014
Bollinger and Stevens, Soft Matter, 2018.

$\alpha\beta$-tubulin and microtubule ***dynamic instability***



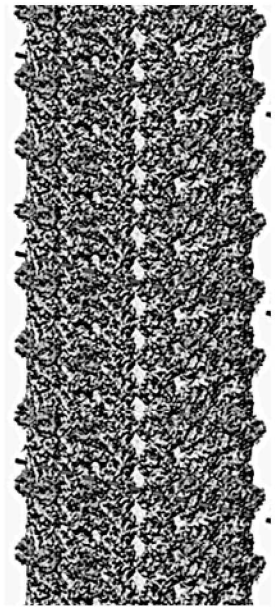


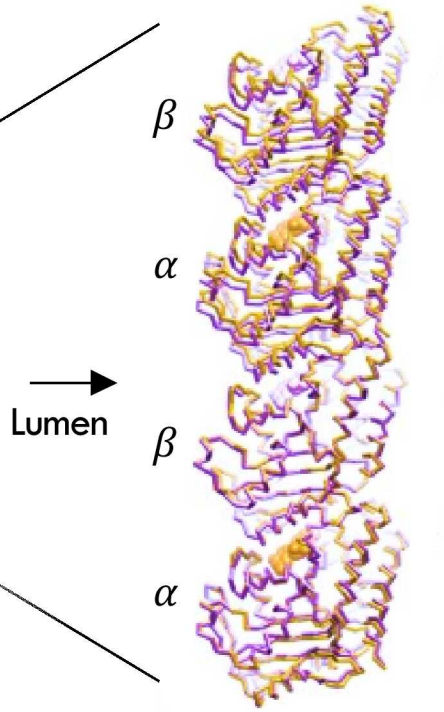
Phosphorylation/hydrolysis-driven **cycle** of microtubules

Alushin, et al. Cell, 157, 2014

Bollinger and Stevens, Soft Matter, 2018.

Is depolymerization driven by tubulin ***shape change?***



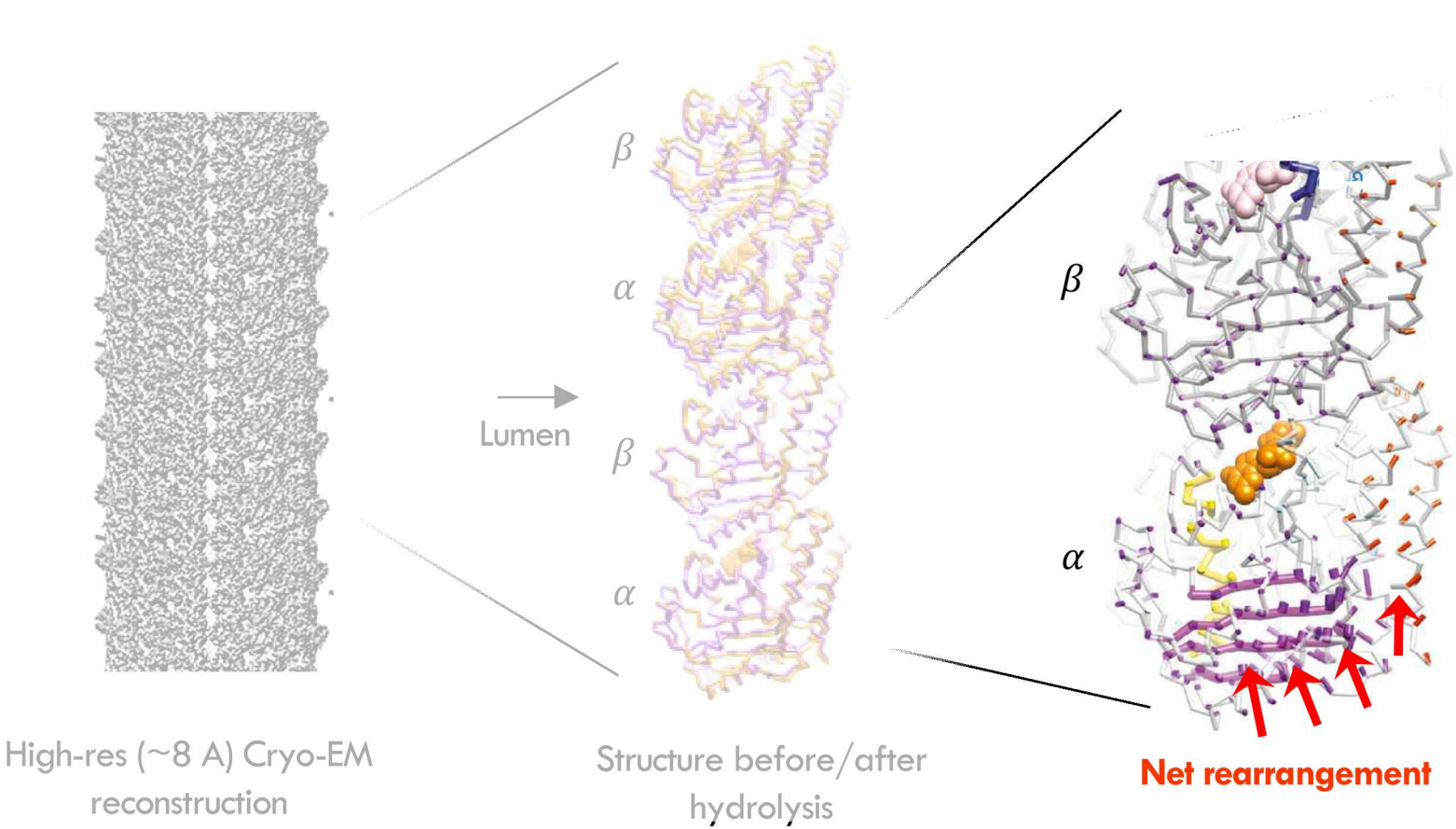


High-res (~8 A) Cryo-EM reconstruction

Structure before/after hydrolysis

Alushin, et al. Cell, 157, 2014
Zhang, et al. Cell,162, 2015

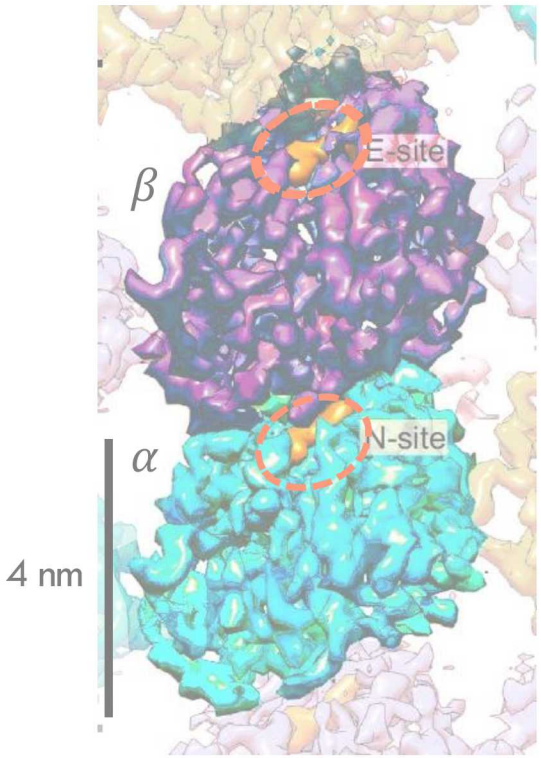
Is depolymerization driven by tubulin *shape change?*

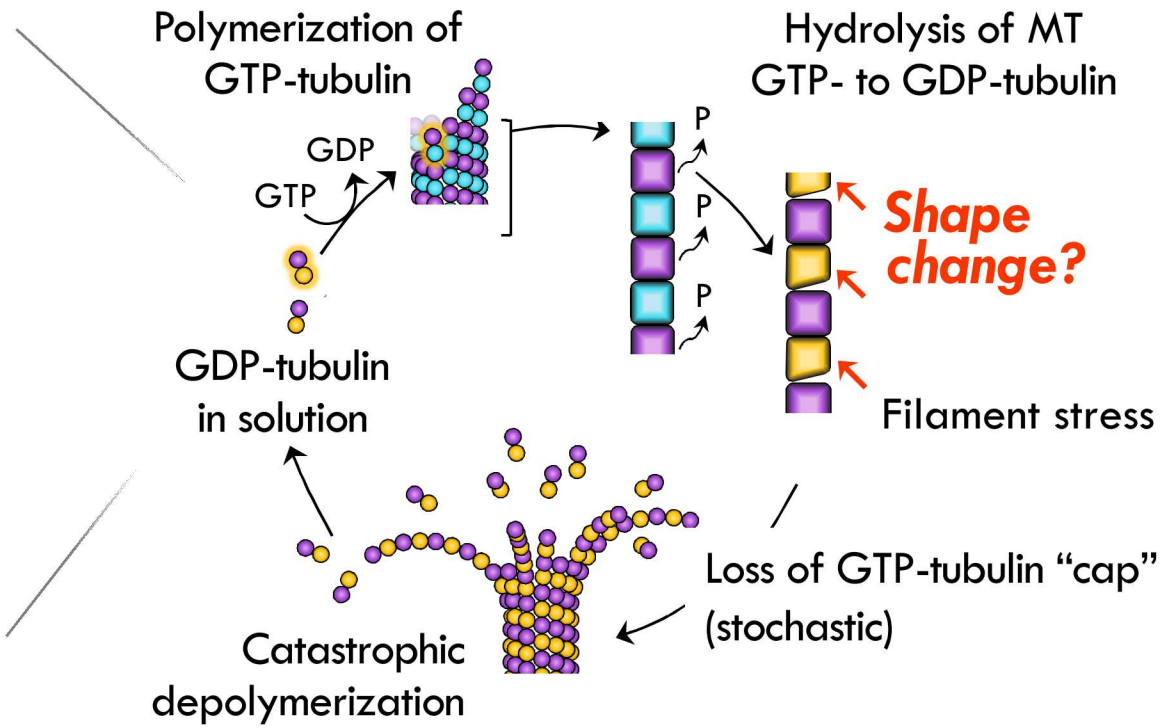


Lattice hydrolysis associated with **α-subunit compression**

Alushin, et al. Cell, 157, 2014
Zhang, et al. Cell,162, 2015

Is depolymerization driven by tubulin *shape change?*

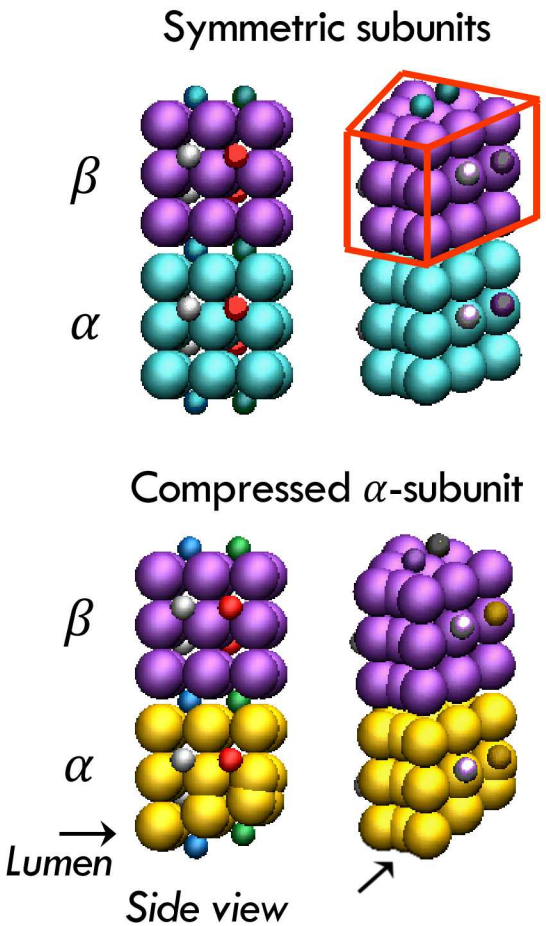




Hypothesis: modest **shape change** drives depolymerization

Alushin, et al. Cell, 157, 2014
Bollinger and Stevens, Soft Matter, 2018.

Minimal model for tubulin dimer

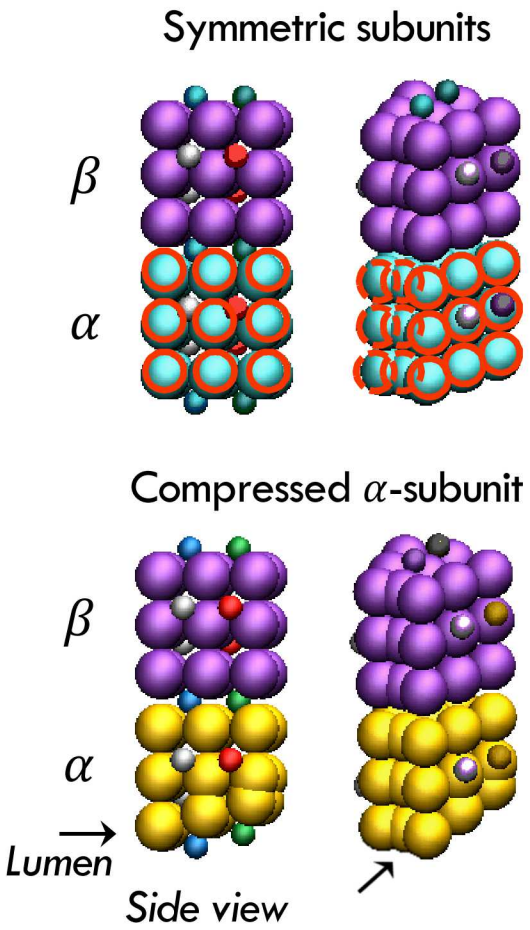


Rigid wedge-shaped subunits

Simplest hollow-tube building block; angled for ring of 13 protofilaments

Bollinger and Stevens, Soft Matter, 2018.

Minimal model for tubulin dimer



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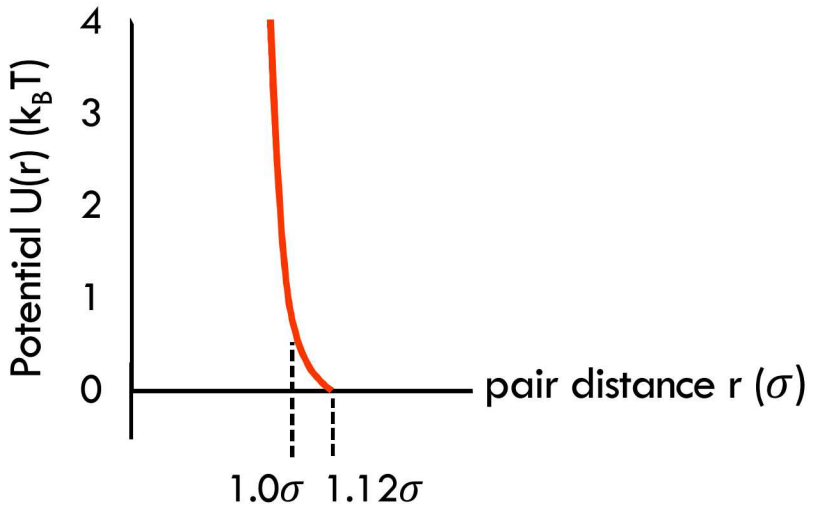
3x3x3 repulsive beads (12-6 LJ cut/shifted)

Subunit excluded volume, all size $\equiv 1\sigma$, equal mass



Bollinger and Stevens, Soft Matter, 2018.

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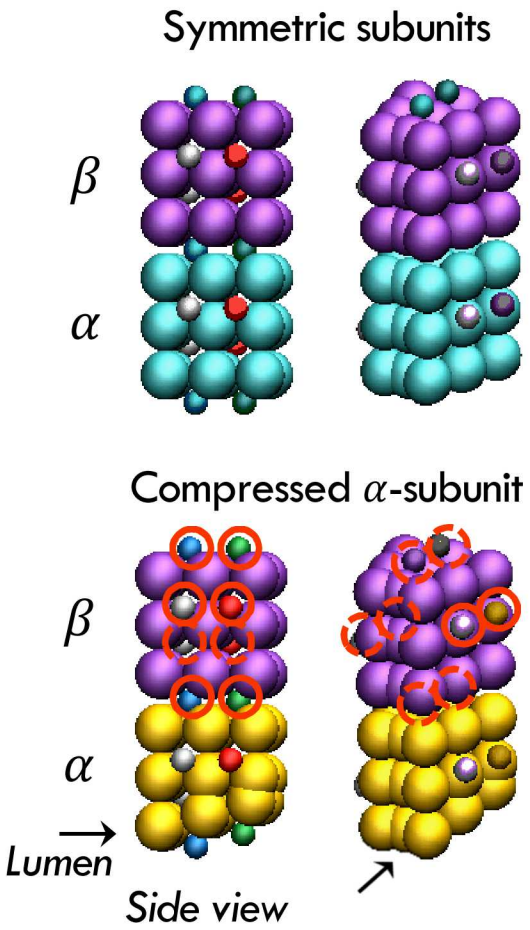

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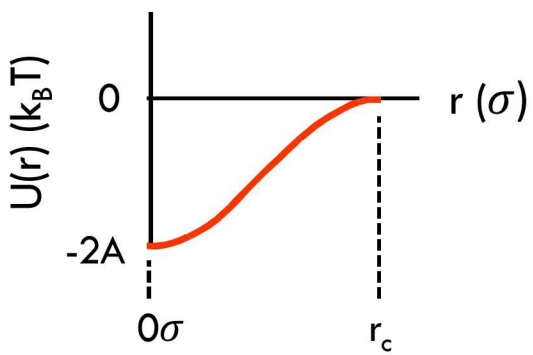
4 pairs attractive-well beads (cosine form)

Side-specific, vertically offset across subunit, enforces orientation/chirality



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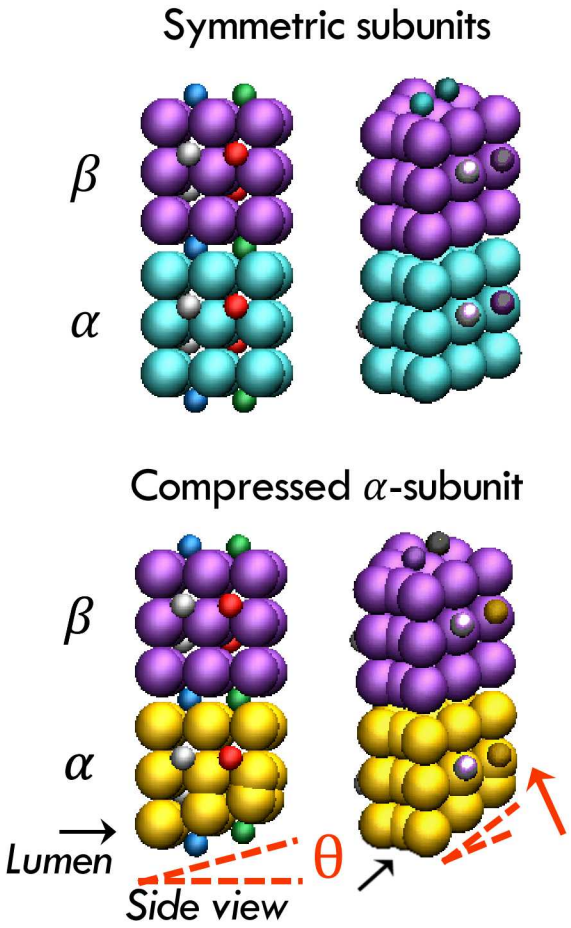

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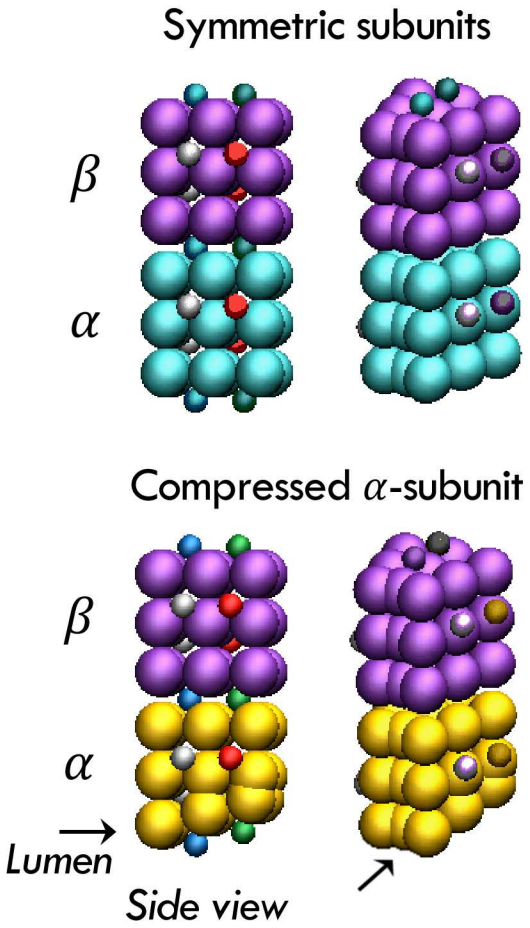
Compression of a-subunit (angle θ)

Rearrangement due to MT dephosphorylation

Bollinger and Stevens, Soft Matter, 2018.

Minimal model for tubulin dimer

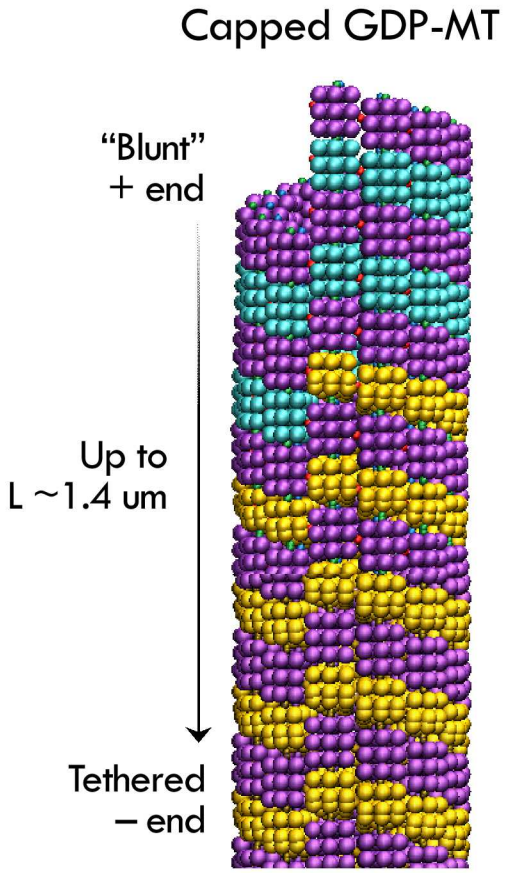


Relevant parameter space:

- A_L Lateral attraction strength (2x per bead)
- A_V Vertical attraction strength (2x per bead)
- θ Compression angle (**fixed** at 15°, reflects deformation in cyro-EM)
- r_{cut} Attraction lengthscale (**fixed** at 0.5σ, reflects binding region size)

Bollinger and Stevens, Soft Matter, 2018.

Simulations of coarse-grained microtubules



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Simulation protocols:

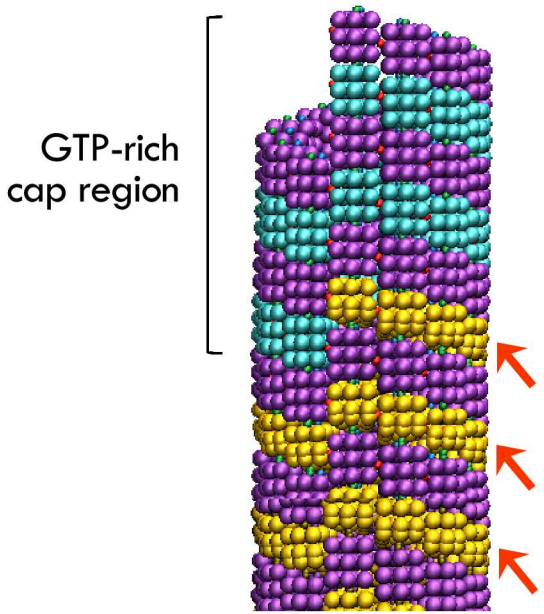
Observe dynamics of **single MTs** (C_{eff}~100 uM)

Molecular dynamics via LAMMPS with Langevin thermostat

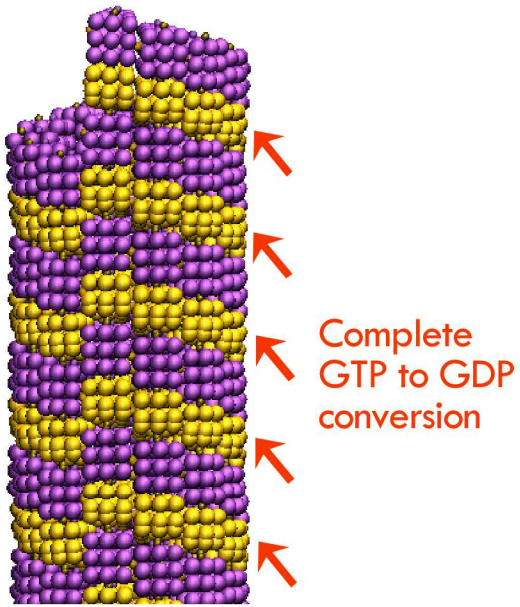
Bollinger and Stevens, Soft Matter, 2018.

1. Can shape frustration drive catastrophic depolymerization?

Capped GDP-MT



Uncapped GDP-MT



Observe respective model microtubule behaviors as function of { **cap, A_L , A_V** }

Bollinger and Stevens, Soft Matter, 2018.

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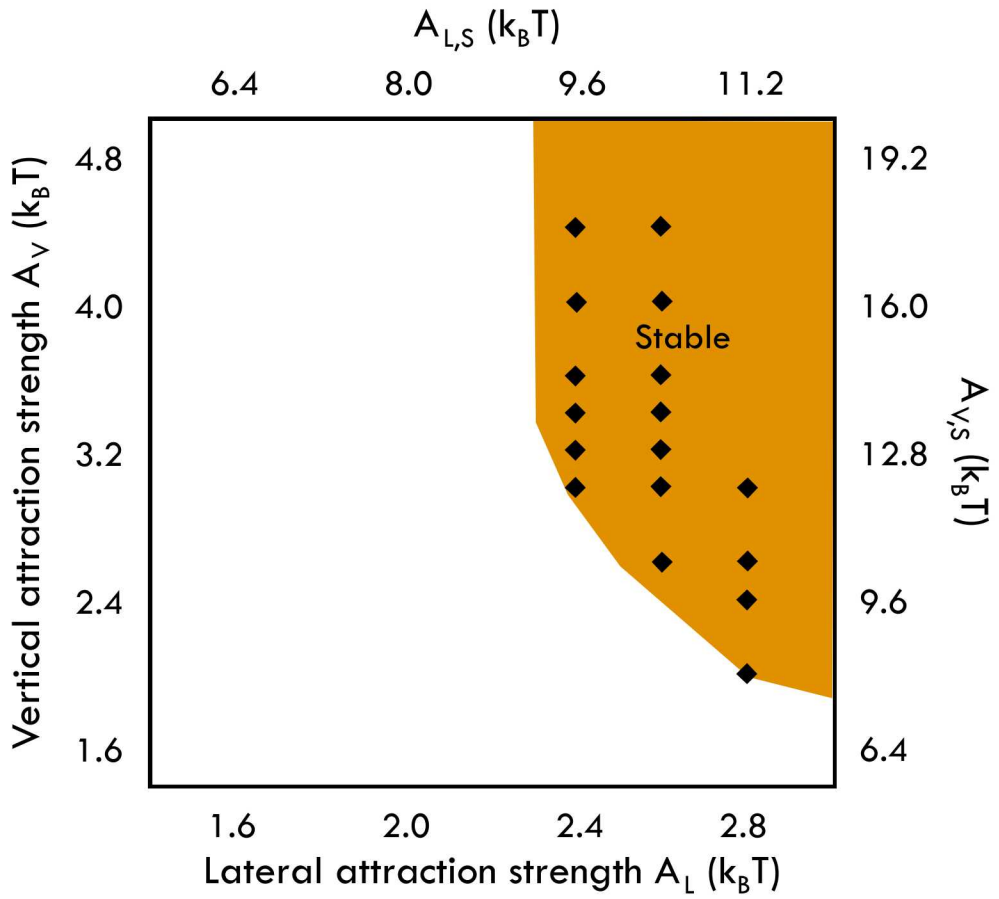
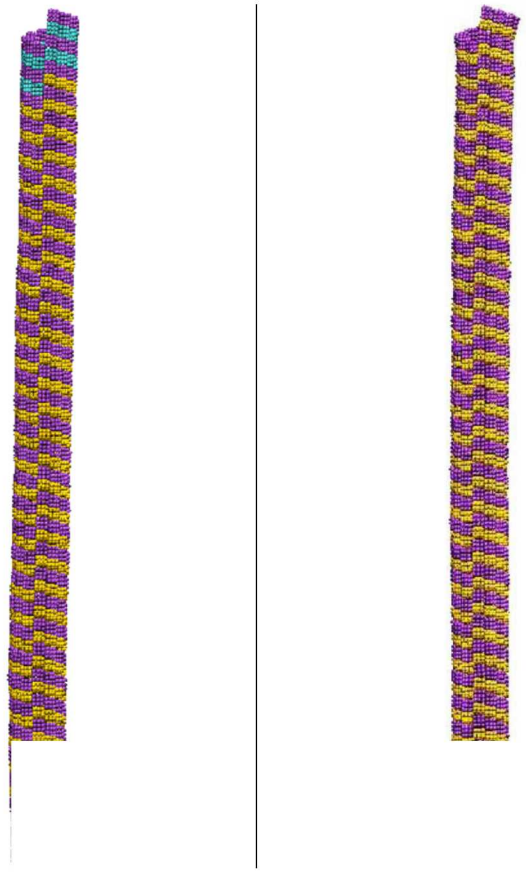


Observed behavior:
Stable



Bollinger and Stevens, Soft Matter, 2018.

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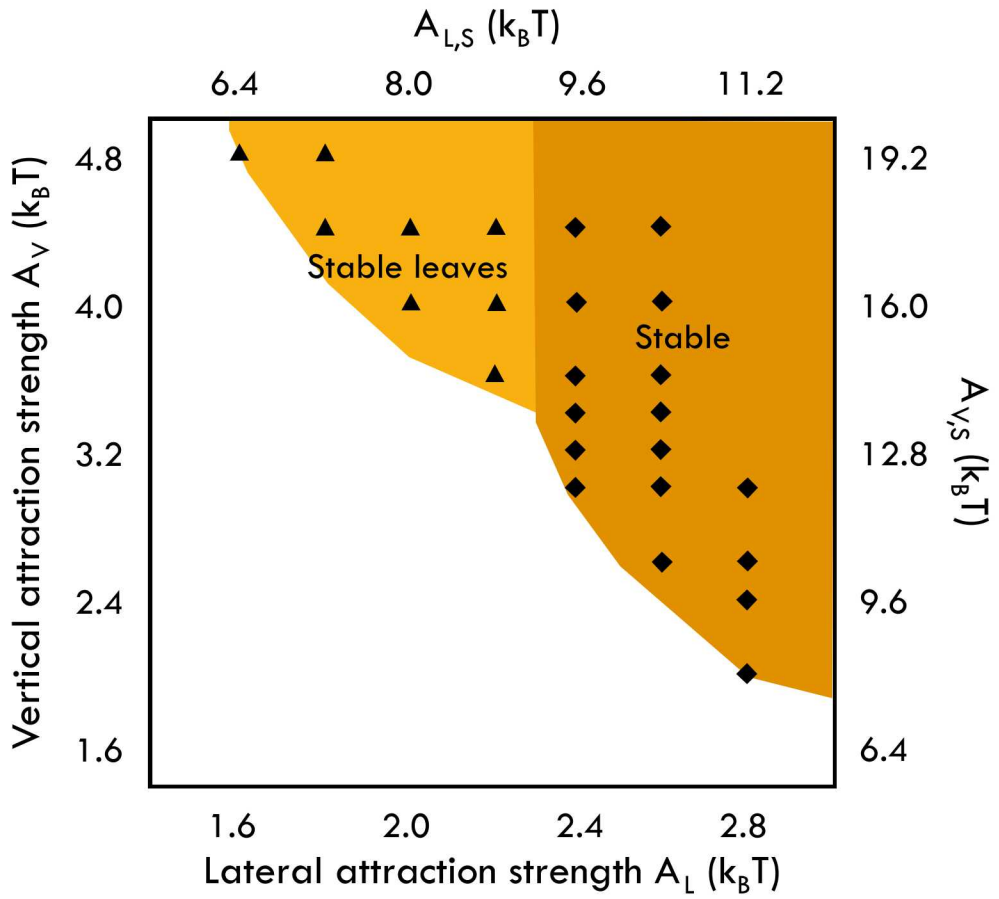
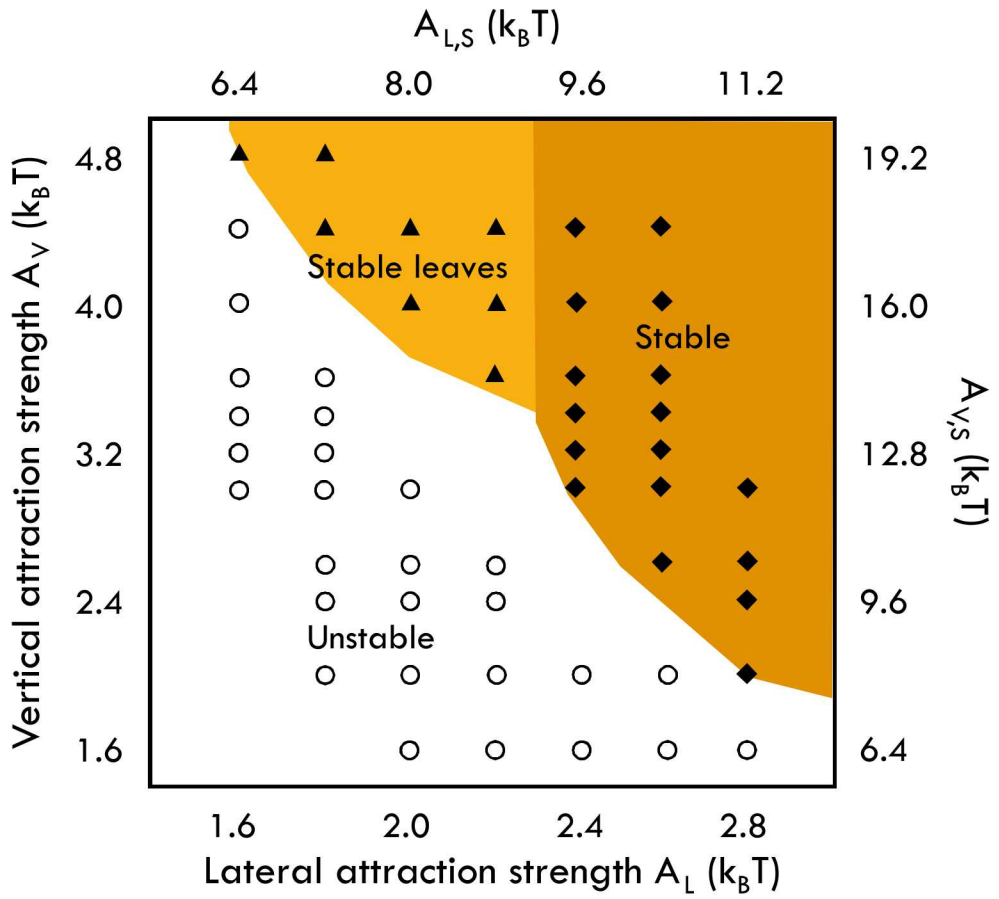


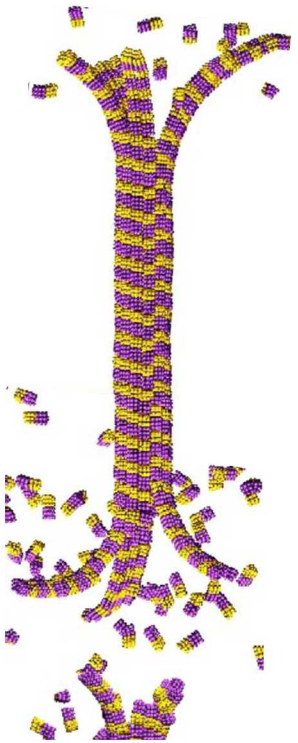
Observed behavior:
"Stable leaves"

Bollinger and Stevens, Soft Matter, 2018.

1. Can shape frustration drive catastrophic depolymerization?



Observed behavior:
Unstable



Bollinger and Stevens, Soft Matter, 2018.

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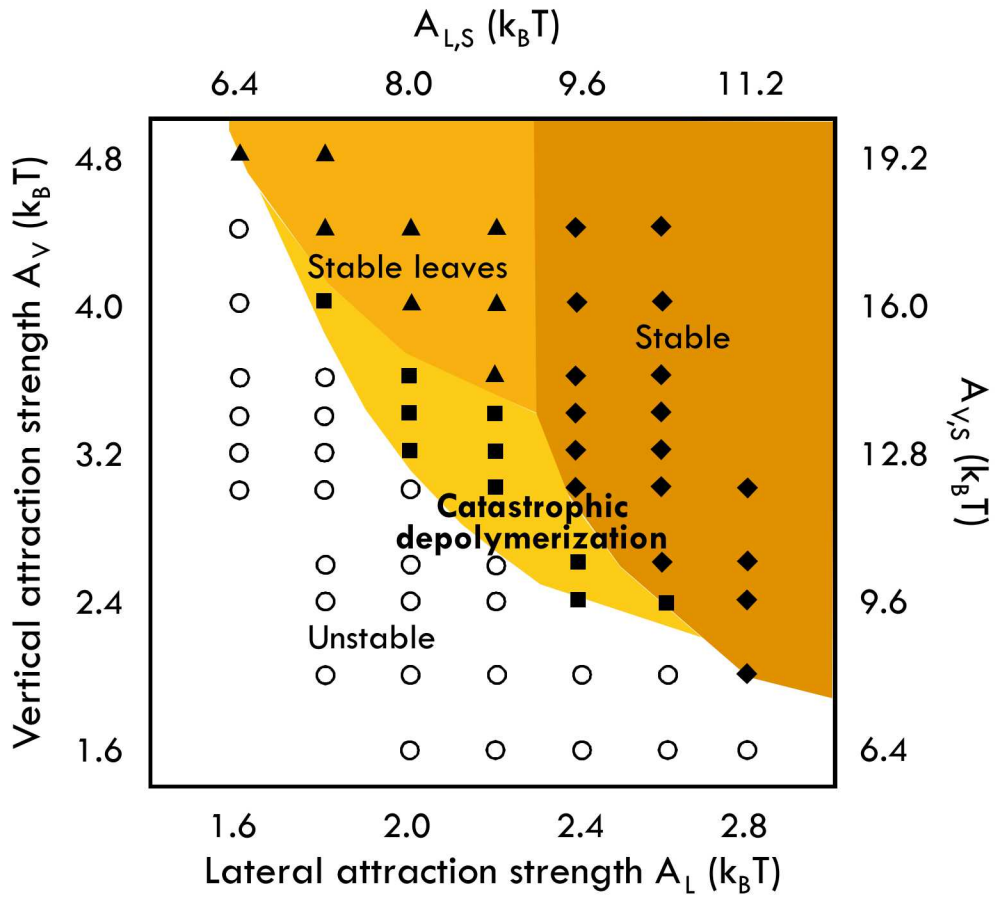
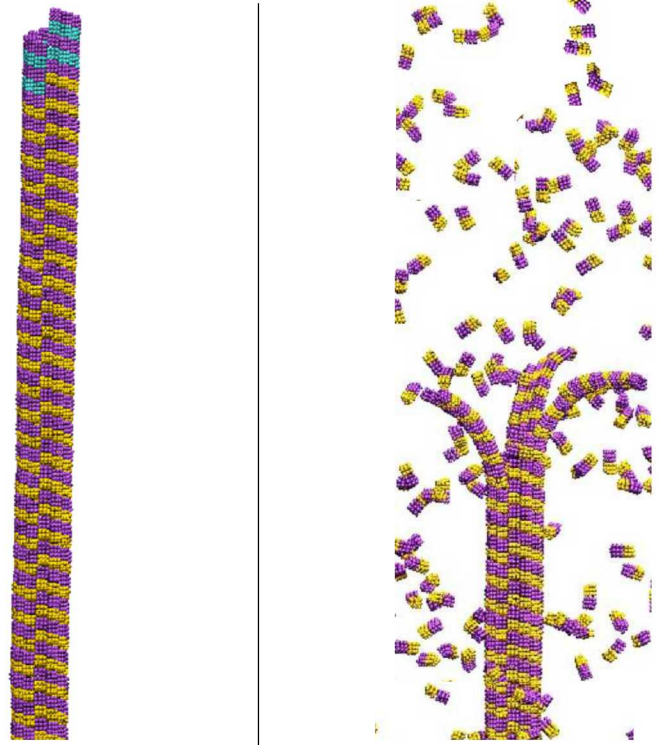


Observed behavior:

Catastrophic depolymerization



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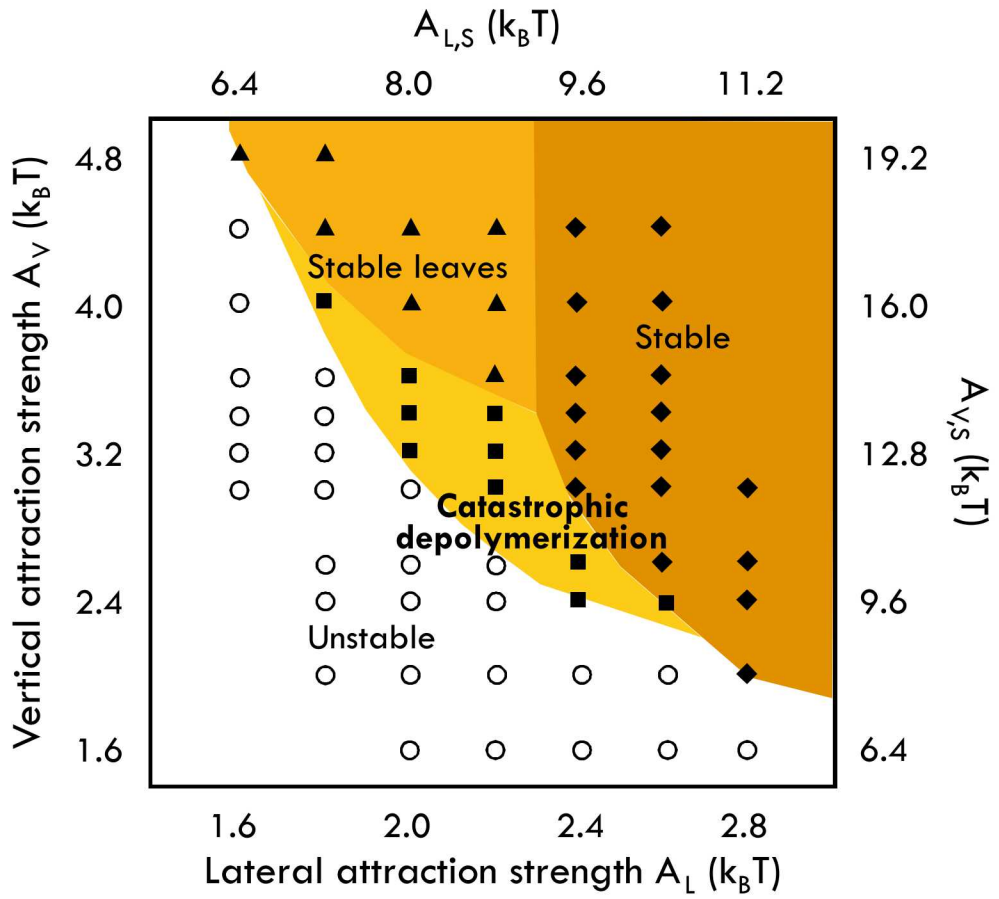

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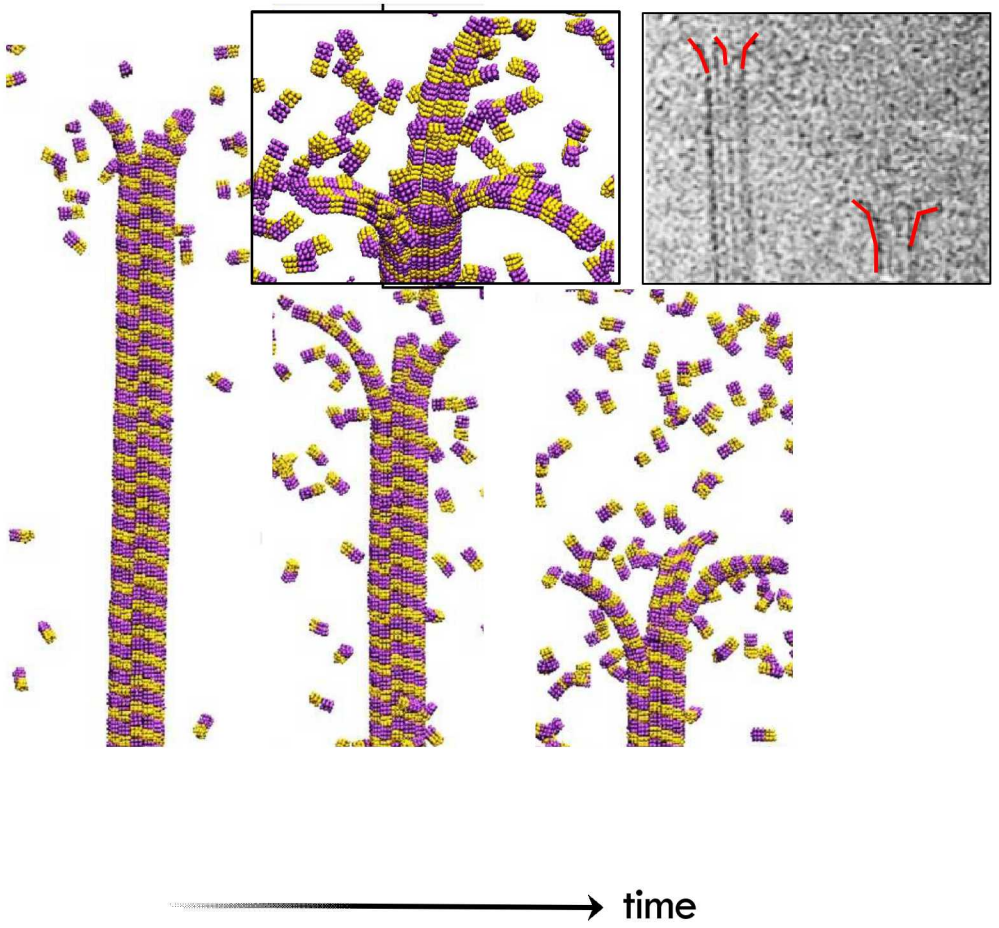
Catastrophic depolymerization



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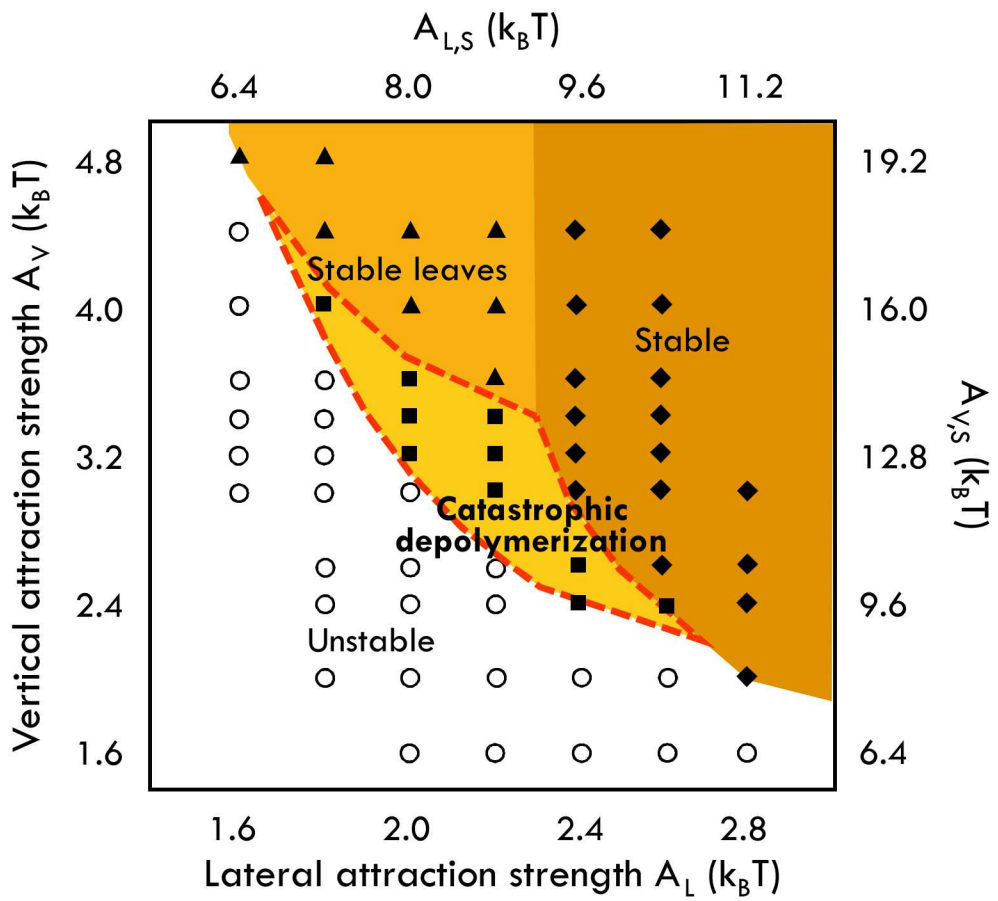
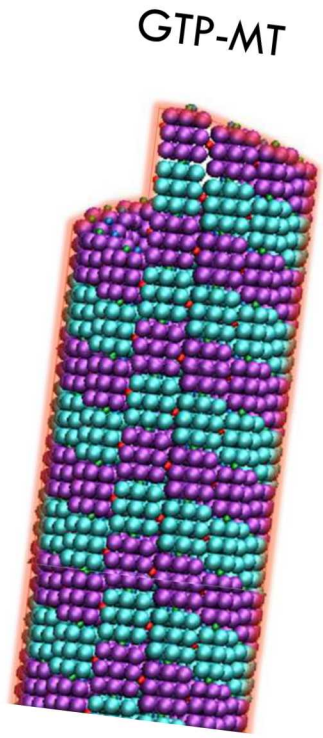
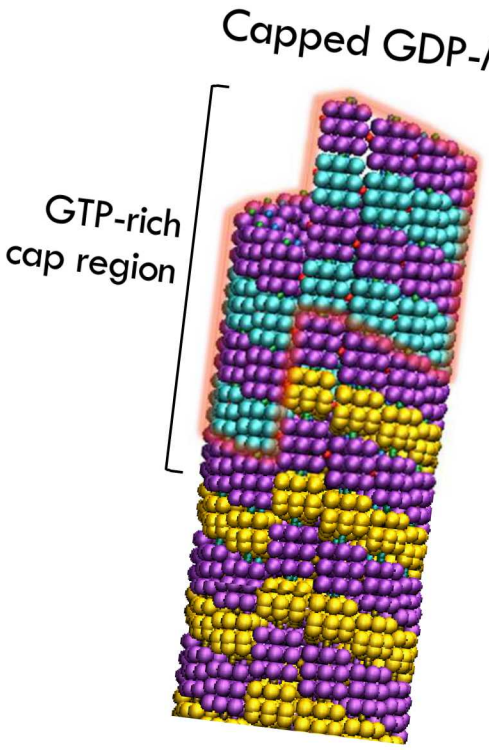


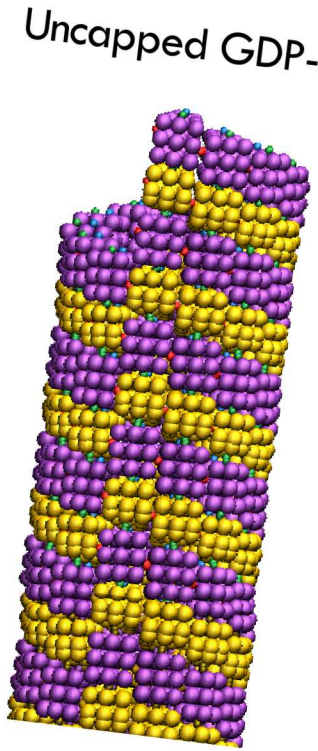
Subtle balance of attractions underlies depolymerization region

Bollinger and Stevens, Soft Matter, 2018.

2. Consistent with stability for non-hydrolyzed caps and lattice regions?



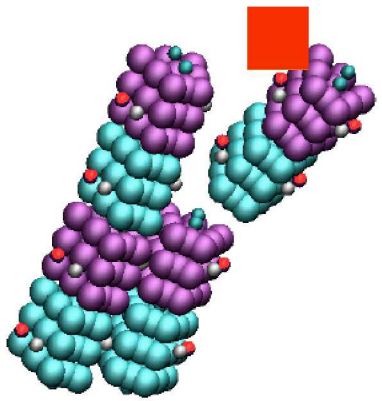


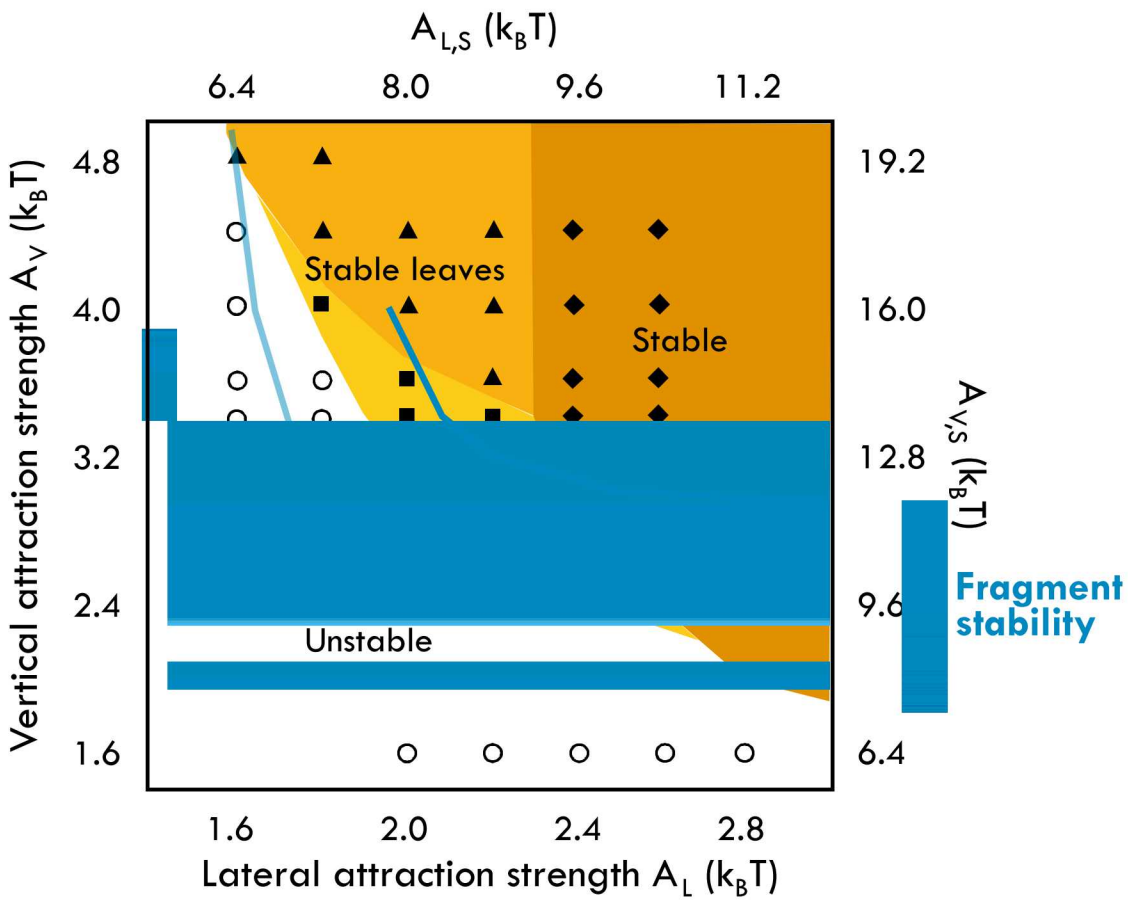


GTP-rich MTs and GTP-tubulin caps (i.e., **non-hydrolyzed** regions) should be stable

Bollinger and Stevens, Soft Matter, 2018.

2. Consistent with stability for non-hydrolyzed caps and lattice regions?

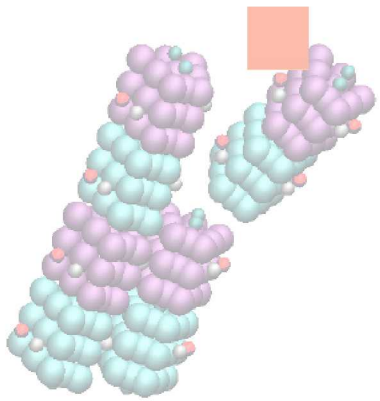


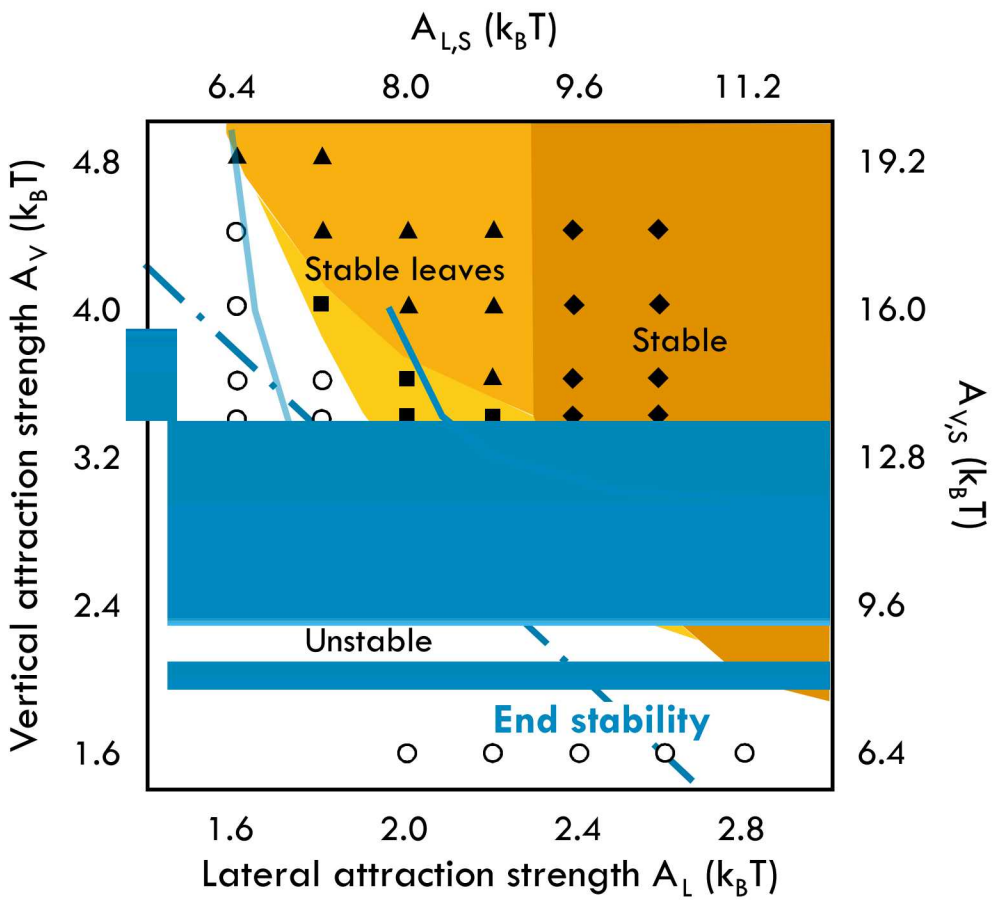


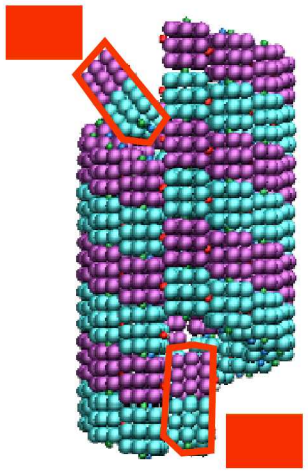
Dynamic stability of GTP-MT fragments **exponentially increases** over region of interest

Bollinger and Stevens, Soft Matter, 2018.

2. Consistent with stability for non-hydrolyzed caps and lattice regions?







$12k_BT$ free-energy barriers to dimer dissociation from GTP-MT regions (e.g., caps)

Bollinger and Stevens, Soft Matter, 2018.

3. Physically-plausible model tubule properties, e.g., mechanics?

Comparison of experiments to model MTs at A_L=2.2,A_V=3.2 k_BT, L~400um

Property	**Experiments (w/ Taxol)**	**Model GTP-MT**
Persistence length **L_p (um)**	~600 (L < 400um)	530
Young's modulus **E (MPa)**	100 to 2000	270
Shear modulus **G (MPa)**	1.4 to 48.0	44

Model GTP-MT persistence length and axial/shear stiffness **in line with experimental estimates** for stabilized MTs

Kis, et. al., PRL, 89, 2002; Taute, et. al. PRL, 100, 2008; Sept and MacKintosh, PRL, 104, 2010
Bollinger and Stevens, Soft Matter, 2018.

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Comparison of experiments to model MTs at A_L=2.2,A_V=3.2 k_BT, L~400um

Property	**Experiments (w/ Taxol)**	**Model GTP-MT**	**Capped GDP-MT**
Persistence length **L_p (um)**	~600 (L < 400um)	530	290
Young's modulus **E (MPa)**	100 to 2000	270	131
Shear modulus **G (MPa)**	1.4 to 48.0	44	36

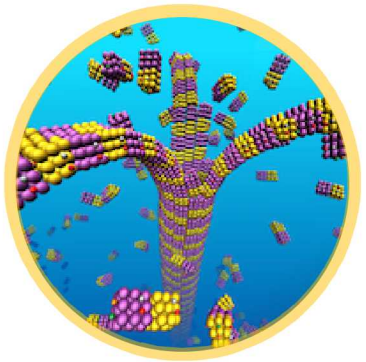
Shape change increases model MT flexibility—

collective result of subunit rattling due to bond frustration

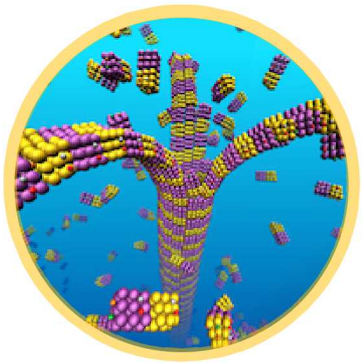
Kis, et. al., PRL, 89, 2002; Taute, et. al. PRL, 100, 2008; Sept and MacKintosh, PRL, 104, 2010
Bollinger and Stevens, Soft Matter, 2018.

Concluding remarks

- New **minimal model** for tubulin/microtubules demonstrates how modest shape frustration can drive microtubule depolymerization
- Model microtubules exhibit mechanical responses **in line with experimental estimates**
- Coarse-grained model suitable for ongoing simulation investigations of microtubule **nucleation and growth pathways**



Catastrophic depolymerization of microtubules driven by tubulin shape change



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Sandia National Laboratories

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