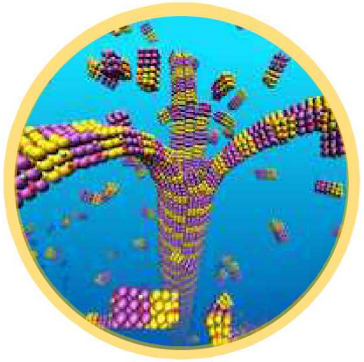
SAND2019-14530C

Onset and arrest of catastrophic depolymerization in microtubules controlled by tubulin subunit shape



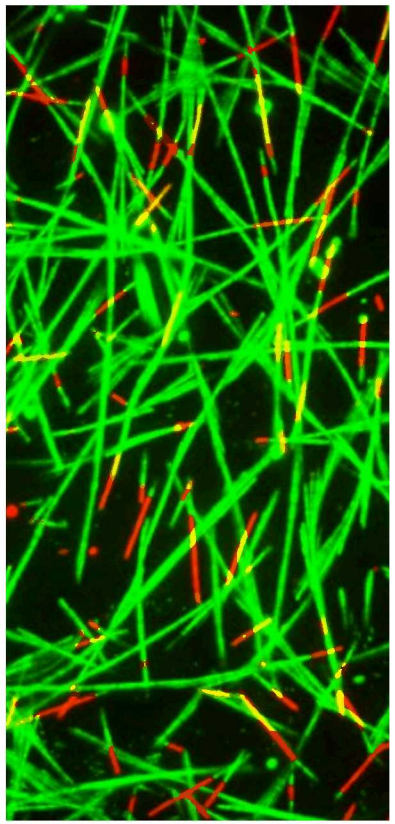
Jonathan A. Bollinger and Mark J. Stevens

Center for Integrated Nanotechnologies
Sandia National Laboratories

March 5, 2019

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



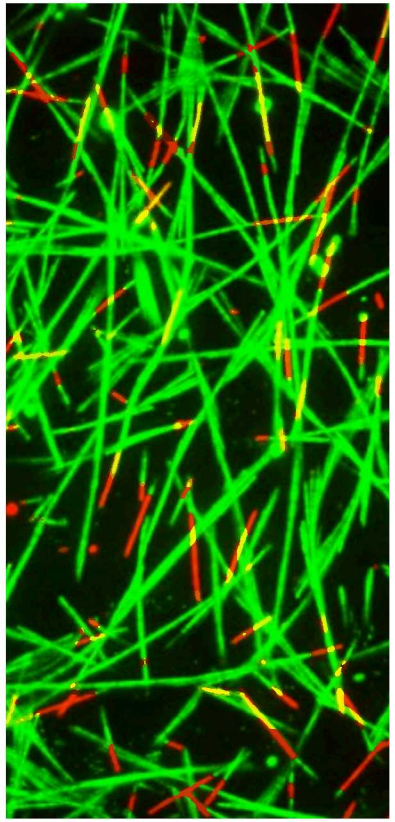
Dyed microtubules in epithelial cells. Zanic group, Vanderbilt School of Medicine

High aspect-ratio, highly-stiff fibers that self-assemble in eukaryotic cells and display **remarkable growth–depolymerization behaviors**

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

Inspiration for synthetic and hybrid **reconfigurable/active fiber materials**

Microtubules are uniquely responsive biopolymers



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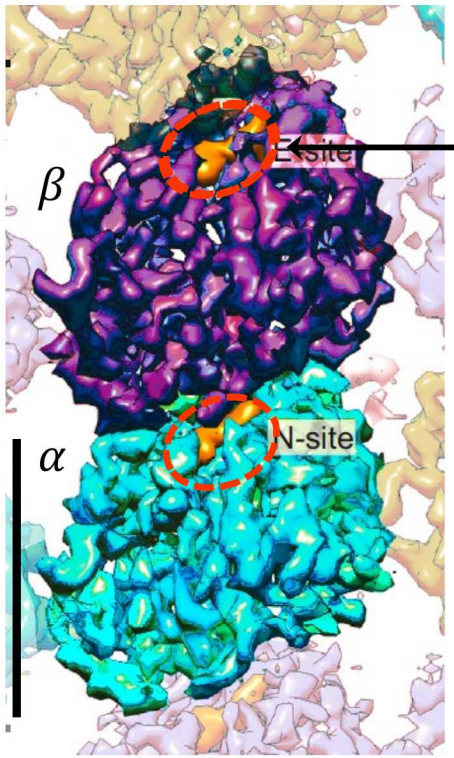
Critical for cell function: cytoskeletal structure, mitosis, tracks for motor-proteins

Inspiration for synthetic and hybrid **reconfigurable/active fiber materials**

Goal: Large-scale molecular simulations of validated tubulin/MT model to reveal how **tubulin shape modulates depolymerization / "rescue"**

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

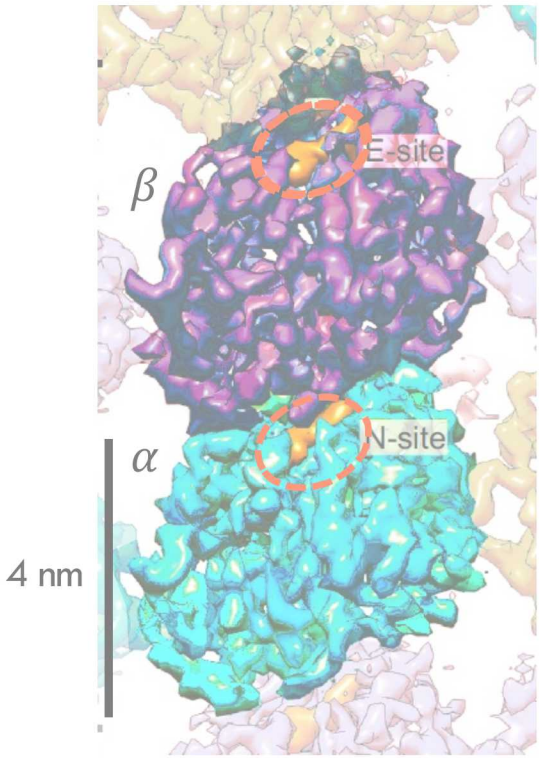


High-res (~8 A) Cryo-EM reconstruction (Alushin)

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

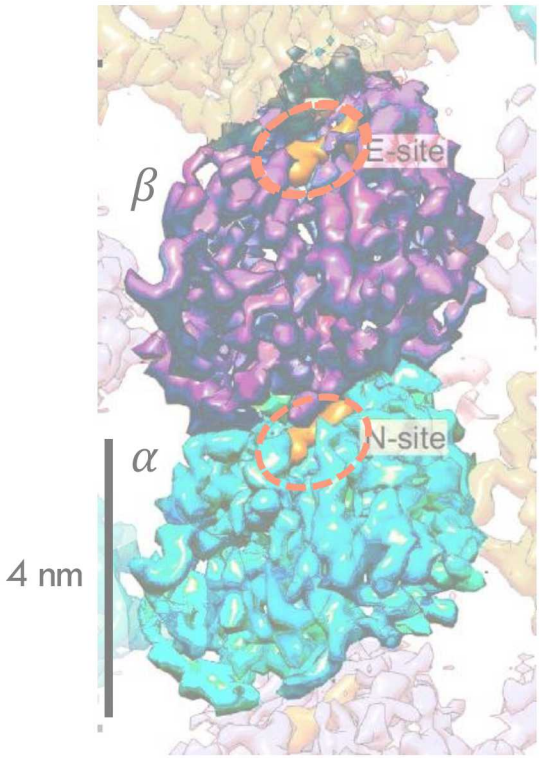
GDP
GTP

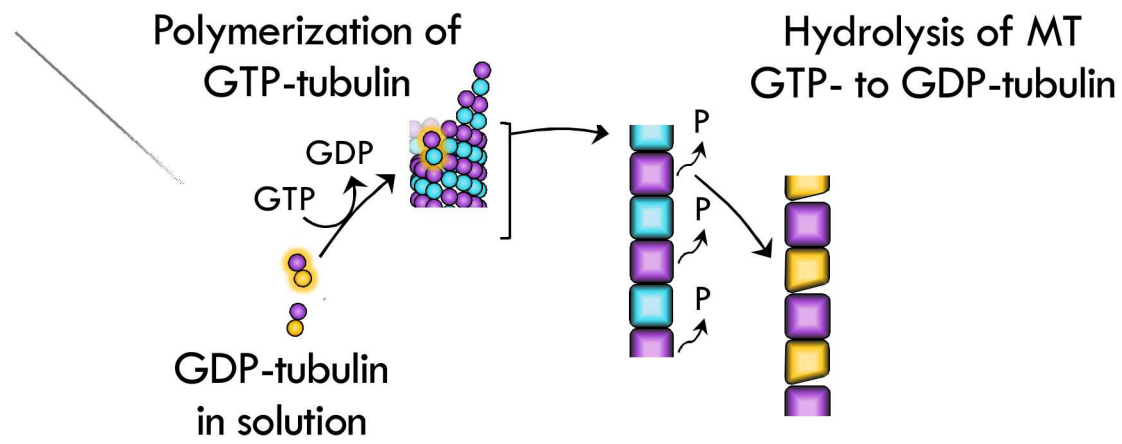
GTP-tubulin "cap" at
leading plus end

GDP-tubulin
in solution

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

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





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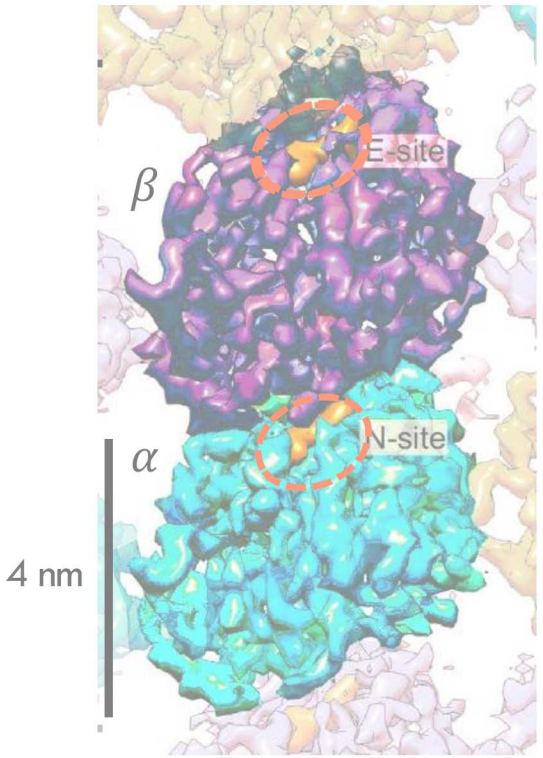
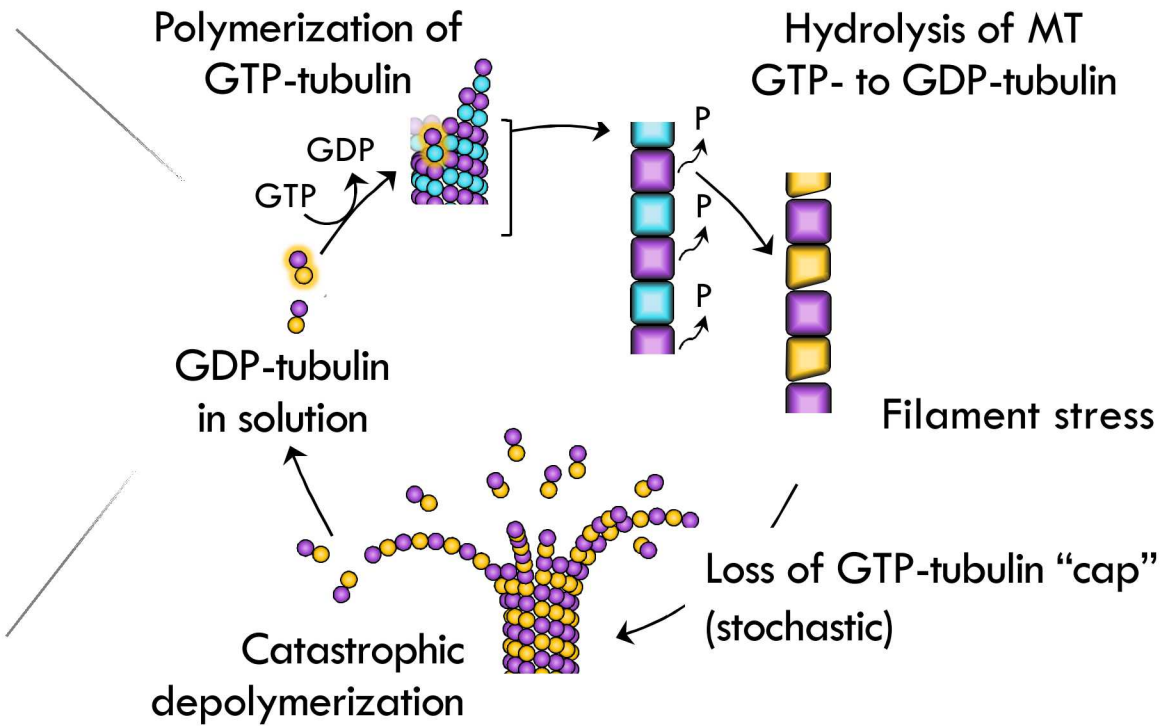
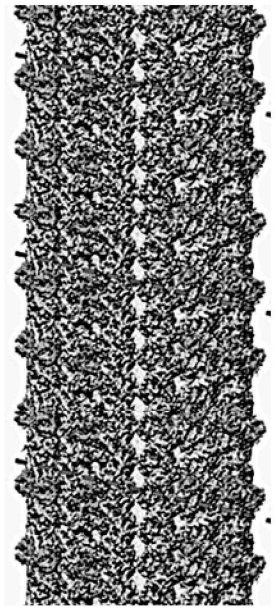




Phosphorylation/hydrolysis-driven **cycle** of growth/depolymerization

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

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



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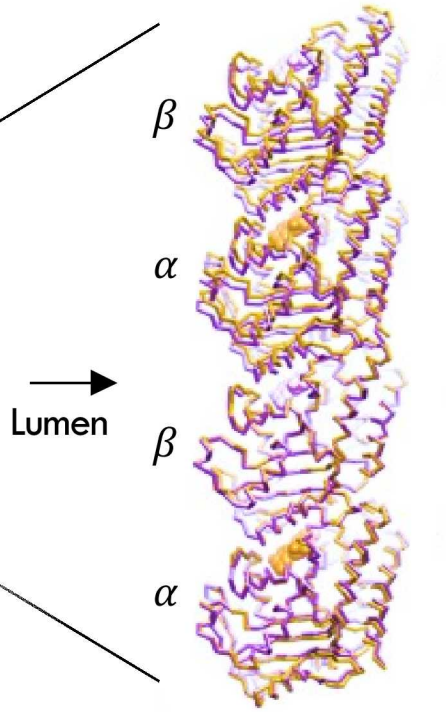
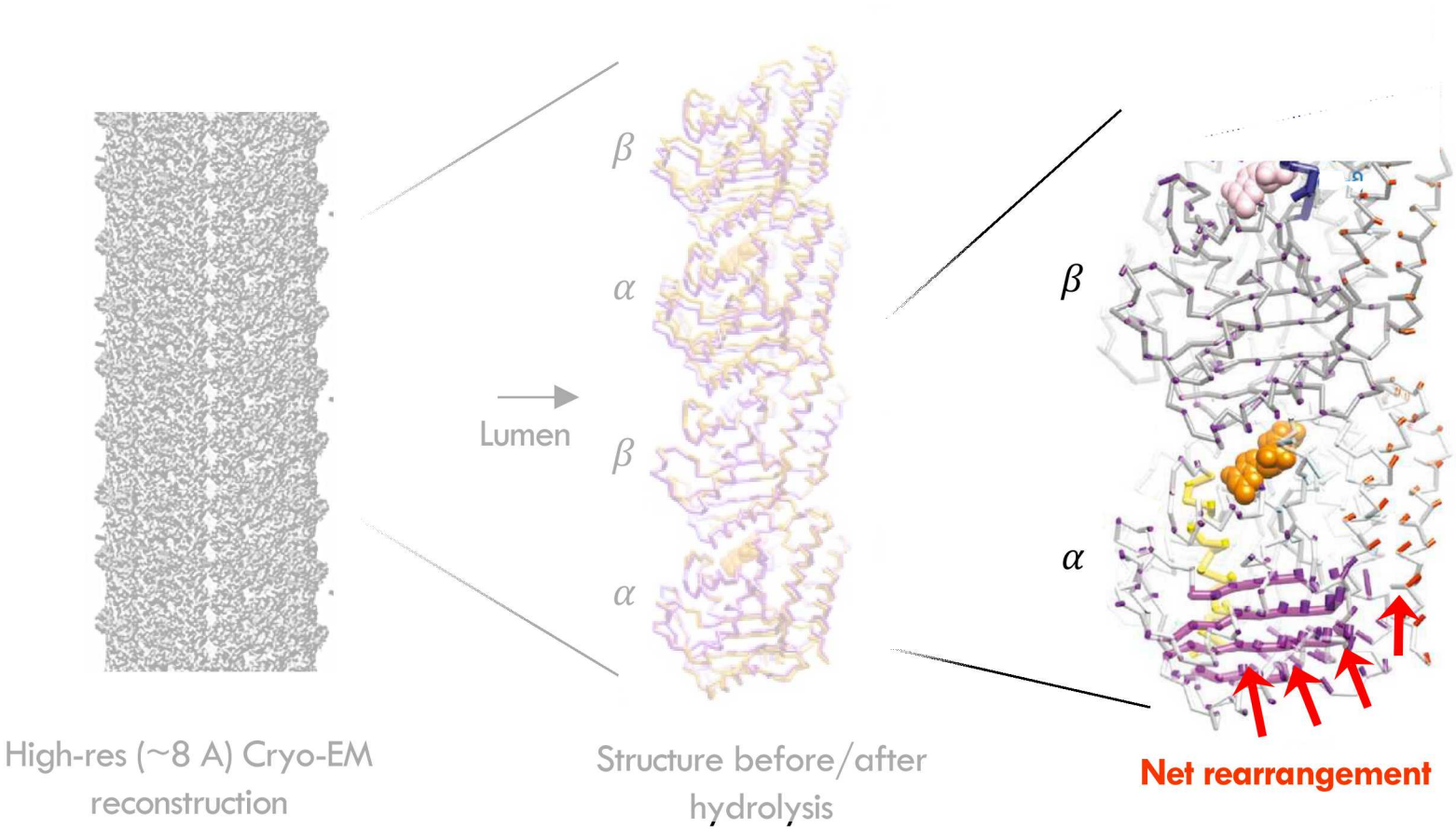


Structure before/after hydrolysis

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

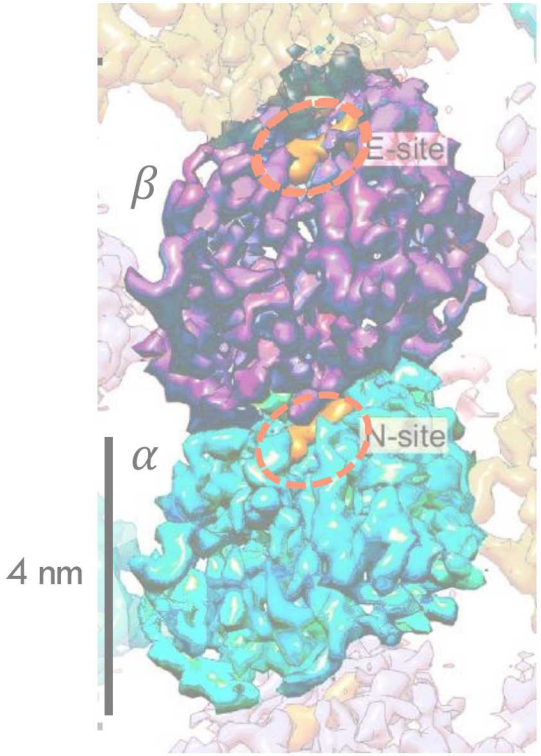
Is depolymerization driven by tubulin *shape change?*

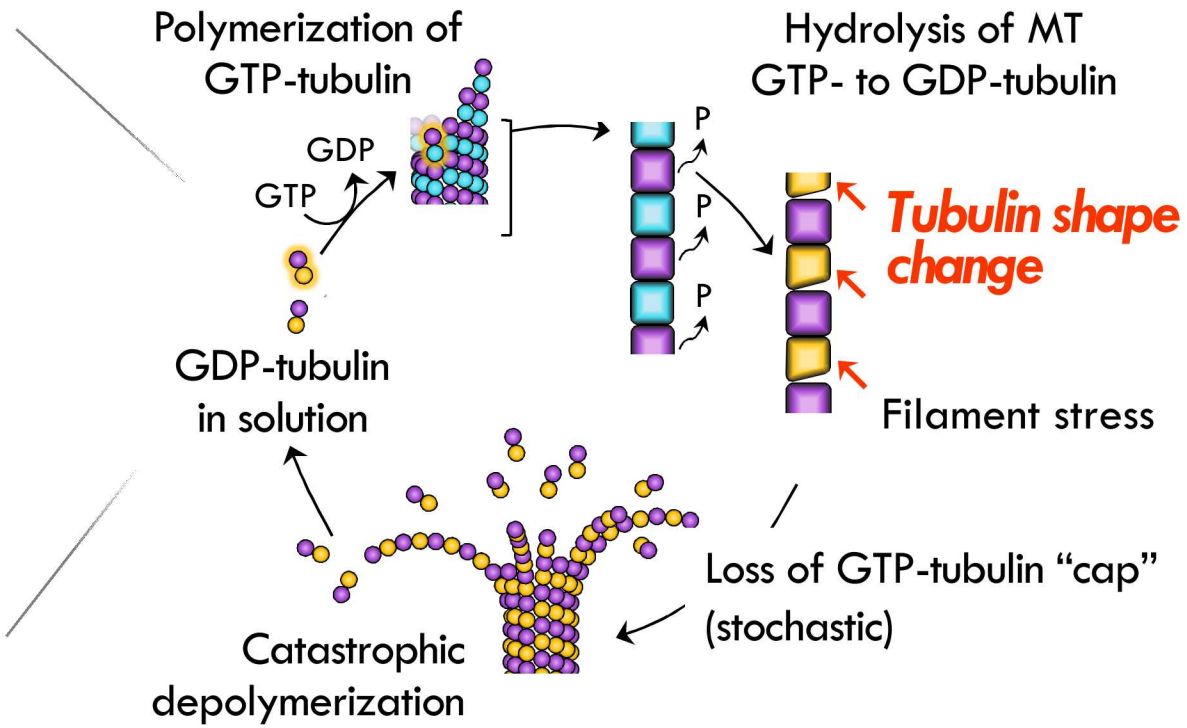


Lattice hydrolysis associated with **α-subunit compression**

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

Is depolymerization driven by tubulin *shape change*?

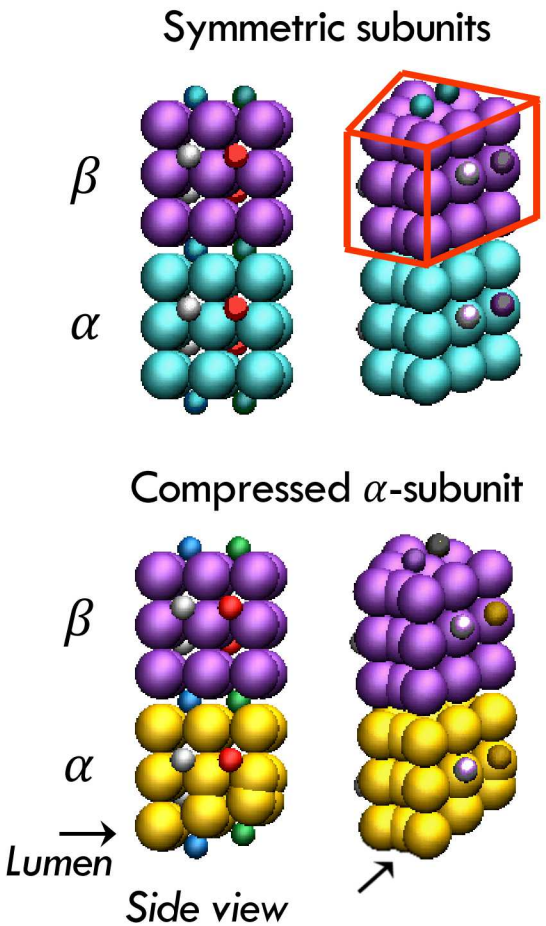




Tubulin **shape-driven cycle** of of growth/depolymerization

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

Minimal model for tubulin dimer

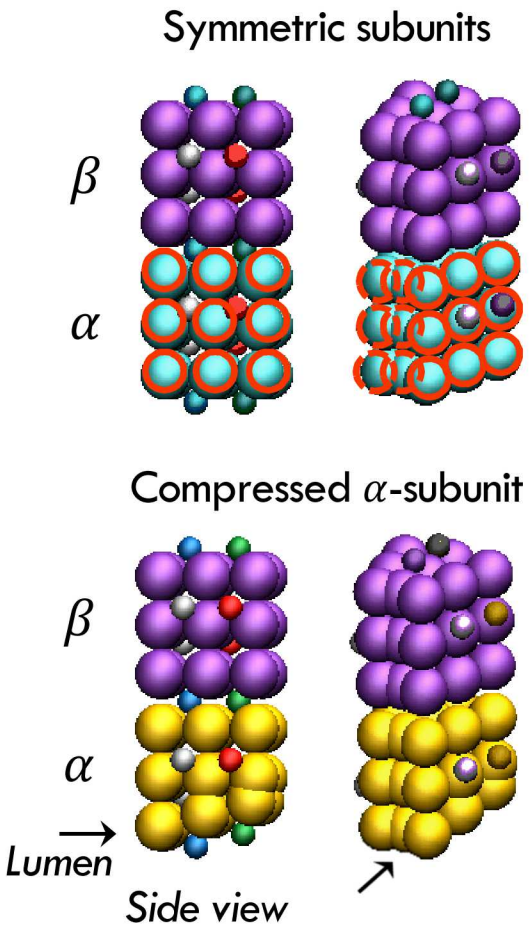


Rigid wedge-shaped subunits

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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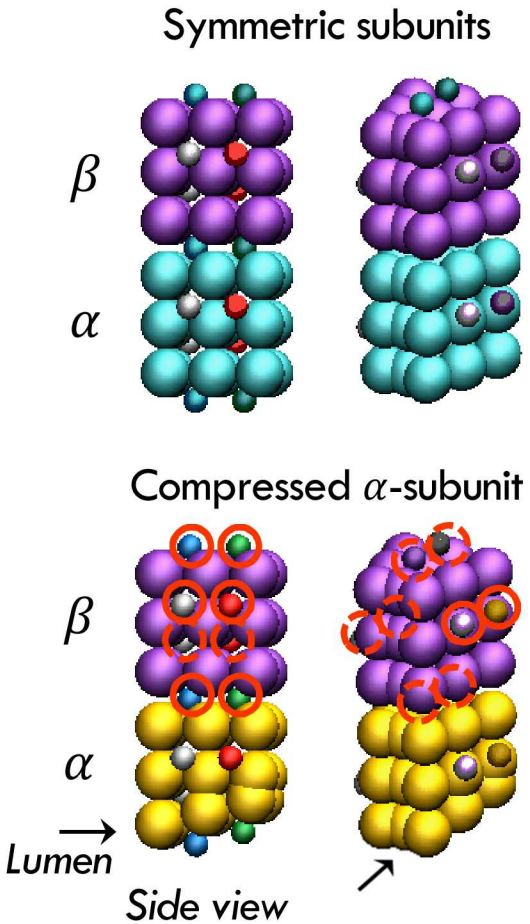

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4 pairs attractive-well beads (vertical vs. lateral)

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

Bollinger and Stevens, Soft Matter, 2018.

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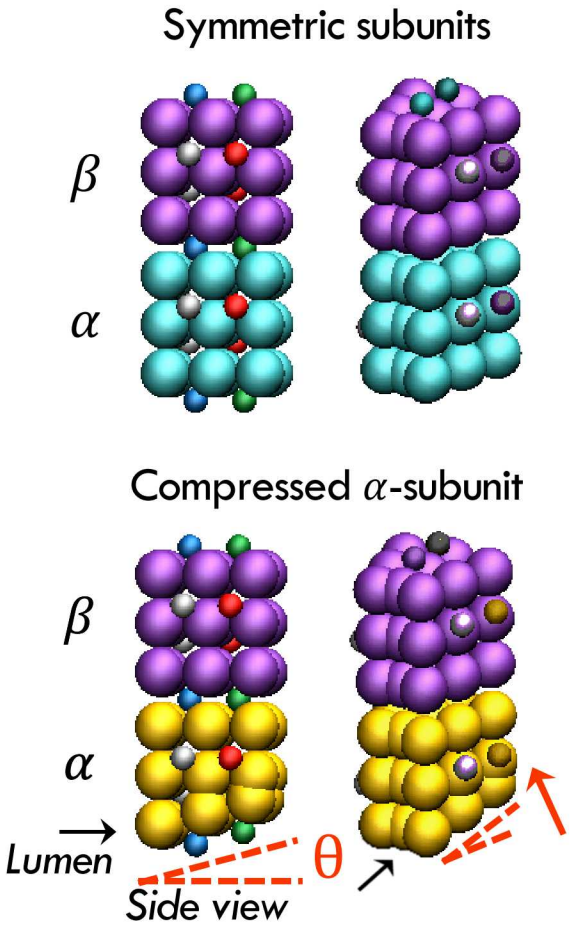

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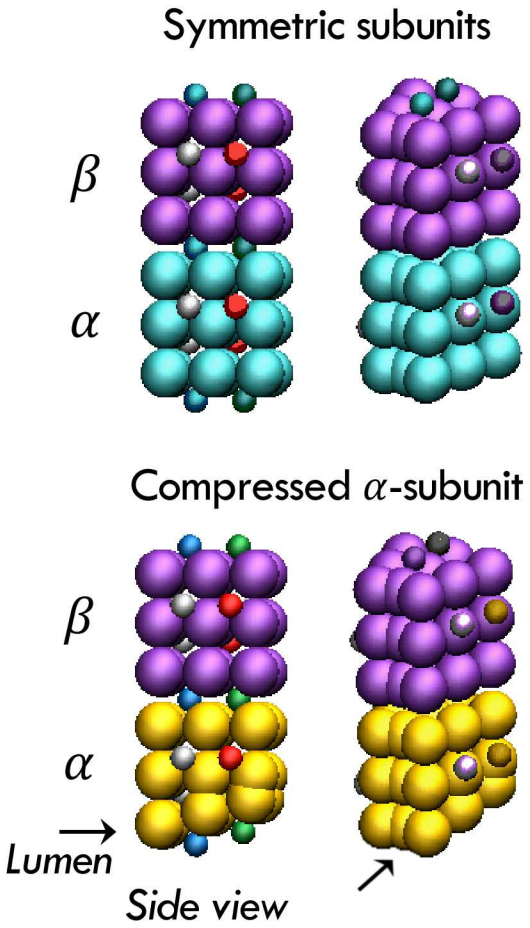
Compression of a-subunit (angle θ)

Rearrangement due to MT dephosphorylation

Bollinger and Stevens, Soft Matter, 2018.

Minimal model for tubulin dimer

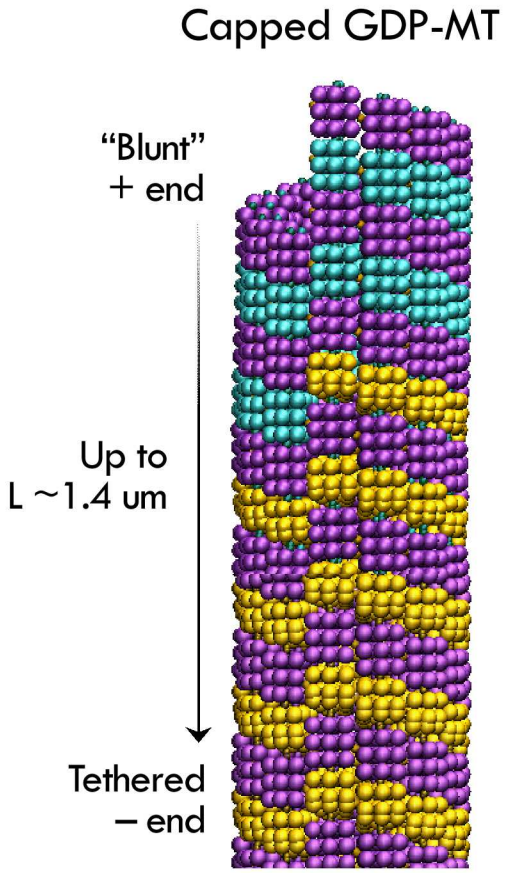


Relevant parameter space:

- 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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- r_{cut} Attraction lengthscale (**fixed** at 0.5σ, reflects binding region size)

Simulation protocols:

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

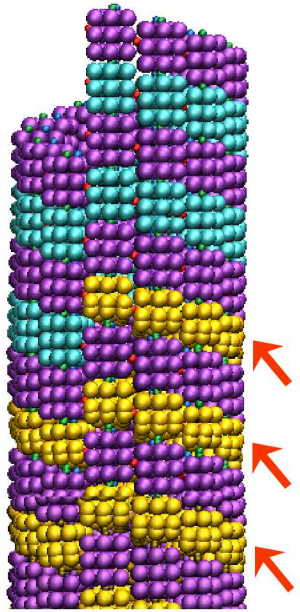
Molecular dynamics via LAMMPS with Langevin thermostat

Bollinger and Stevens, Soft Matter, 2018.

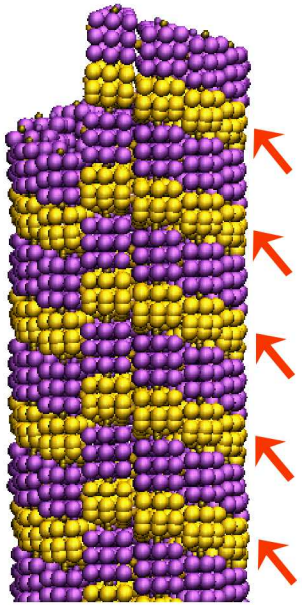
Shape frustration as a driver for stiff yet depolymerizing microtubules

Capped GDP-MT

GTP-rich
cap region



Uncapped GDP-MT

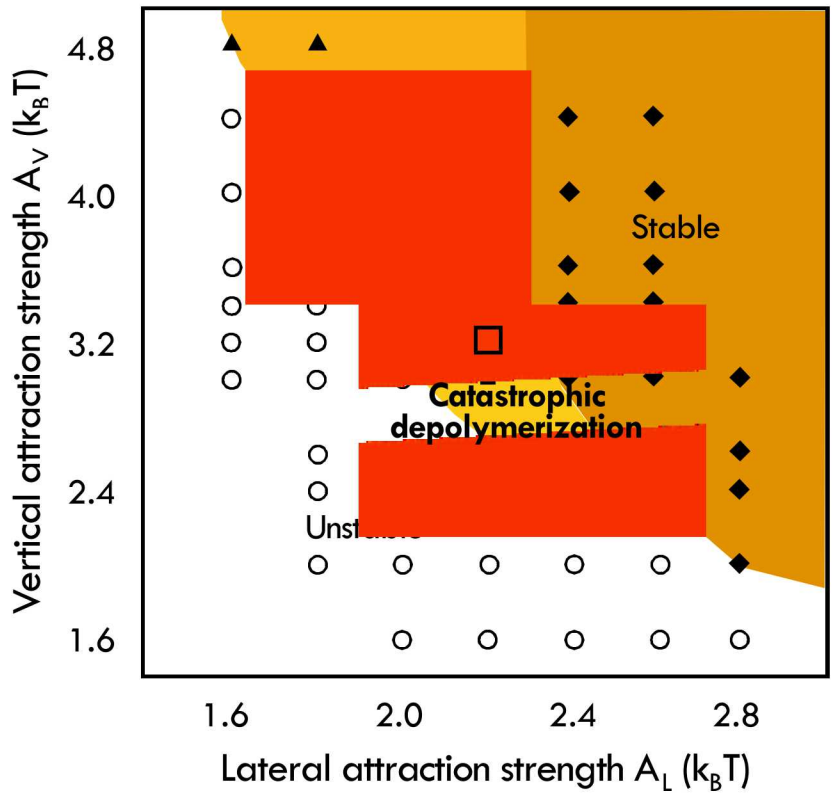


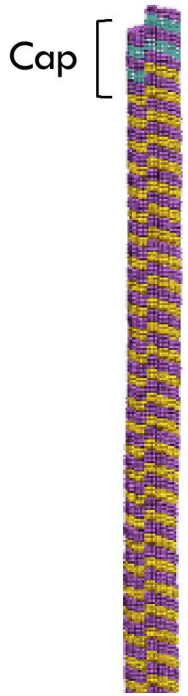
Complete
GTP to GDP
conversion

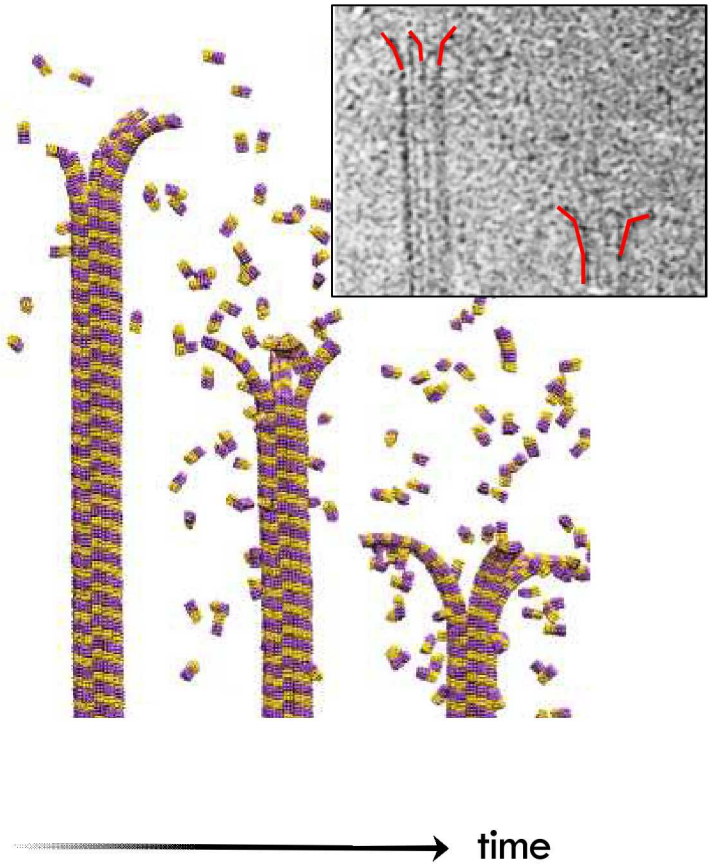
Observe respective model microtubule behaviors as function of { cap, A_L , A_V }

Bollinger and Stevens, Soft Matter, 2018.

Shape frustration as a driver for stiff yet depolymerizing microtubules



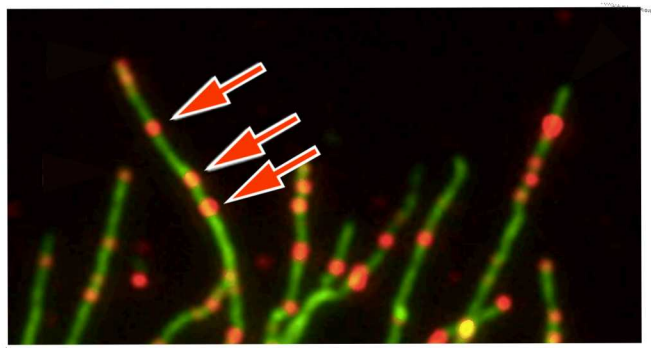




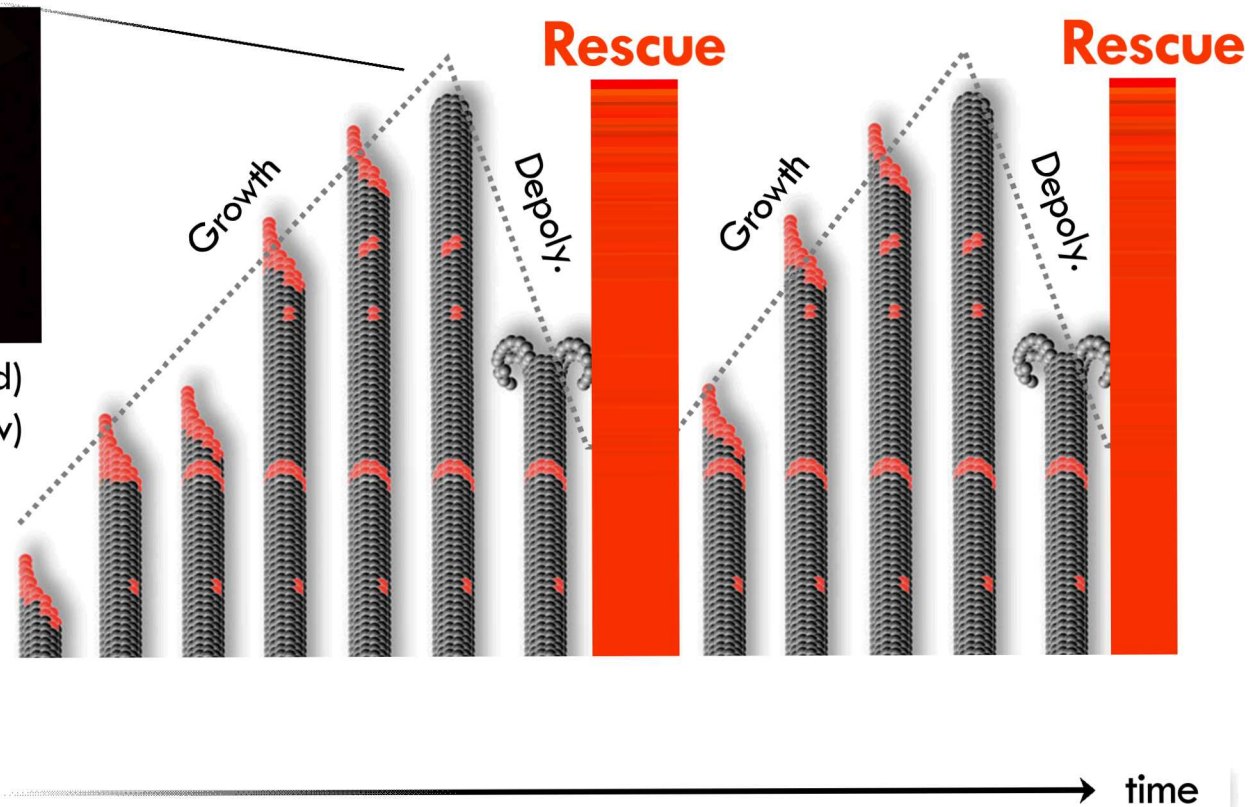
Identified **balance** of shape frustration and binding energy underlying MT behaviors

Chretien, et al. J. Cell Bio., 129, 1995; Bollinger and Stevens, Soft Matter, 2018.

Can regions of non-hydrolyzed tubulin ***interrupt*** depolymerization?



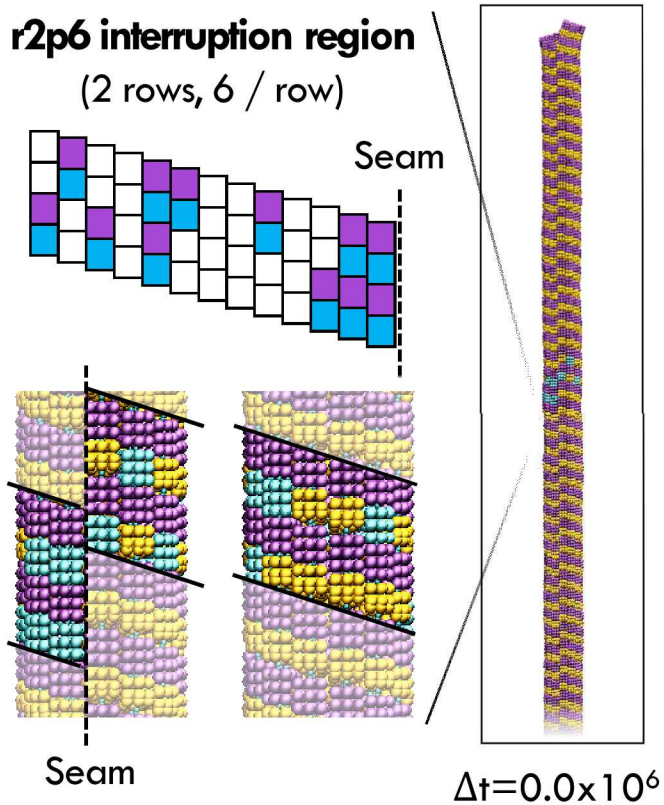
GTP-tubulin "remnants" (red)
in MT lattices (Dimitrov)



Can GTP-tubulin "remnants" facilitate rescue by **interrupting depolymerization?**

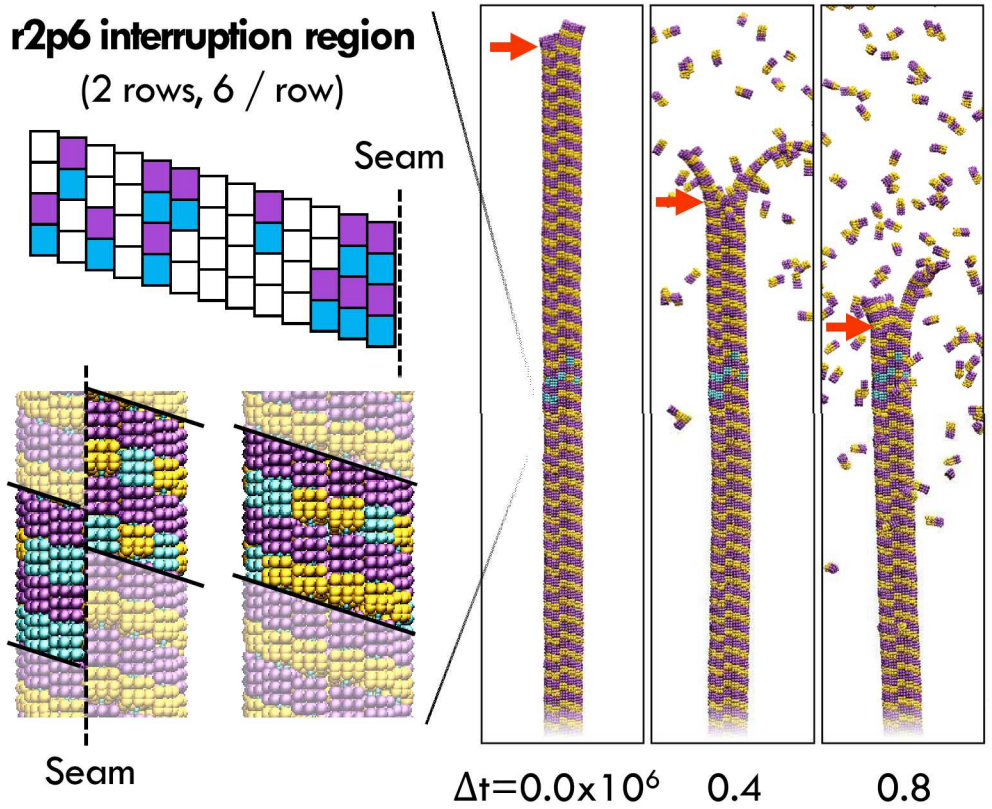
Dimitrov, et. al., Science, 2008.

Can regions of unfrustrated dimers ***interrupt*** depolymerization?



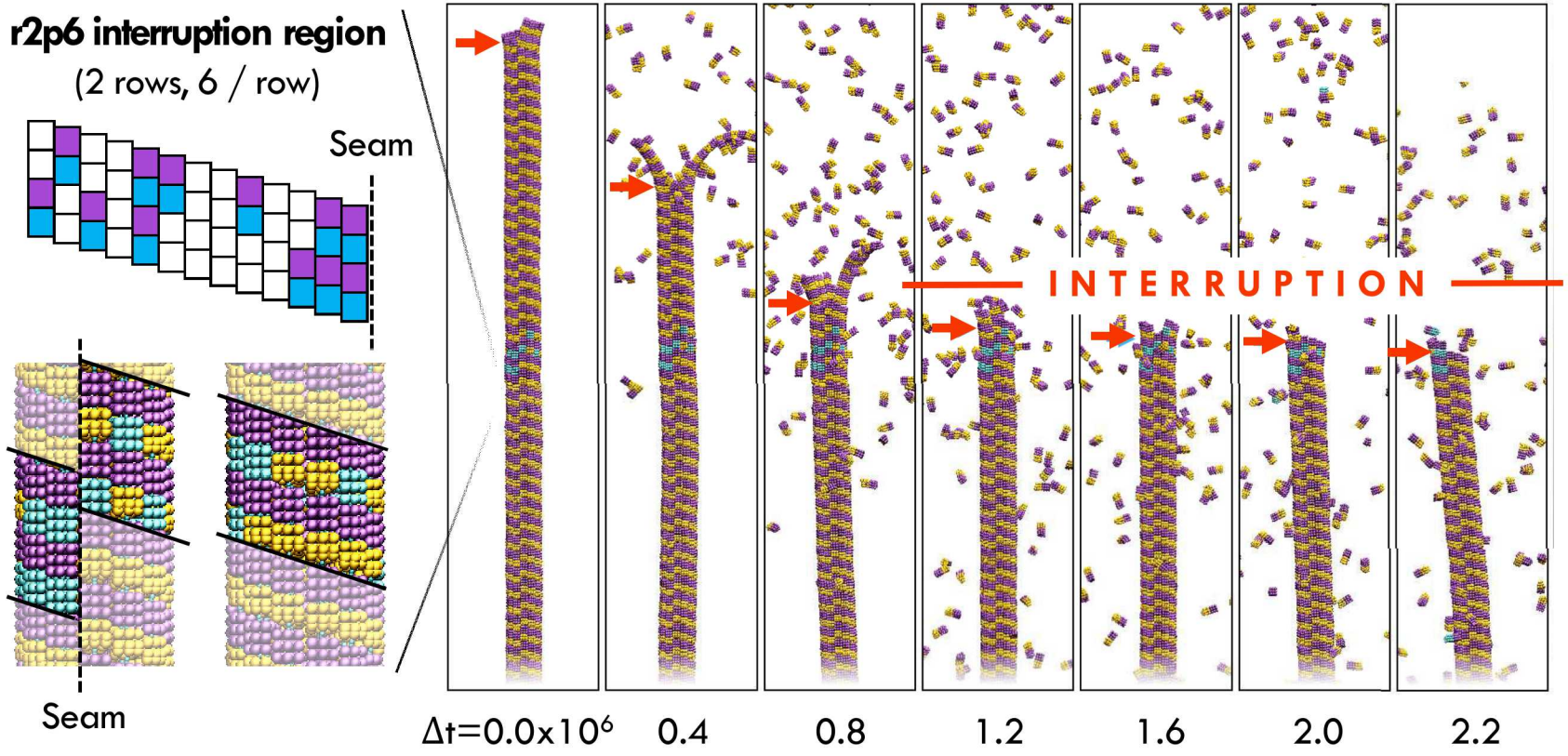
Uncapped GDP-MT with mid-lattice **"interruption region"** containing unfrustrated dimers

Can regions of unfrustrated dimers ***interrupt*** depolymerization?



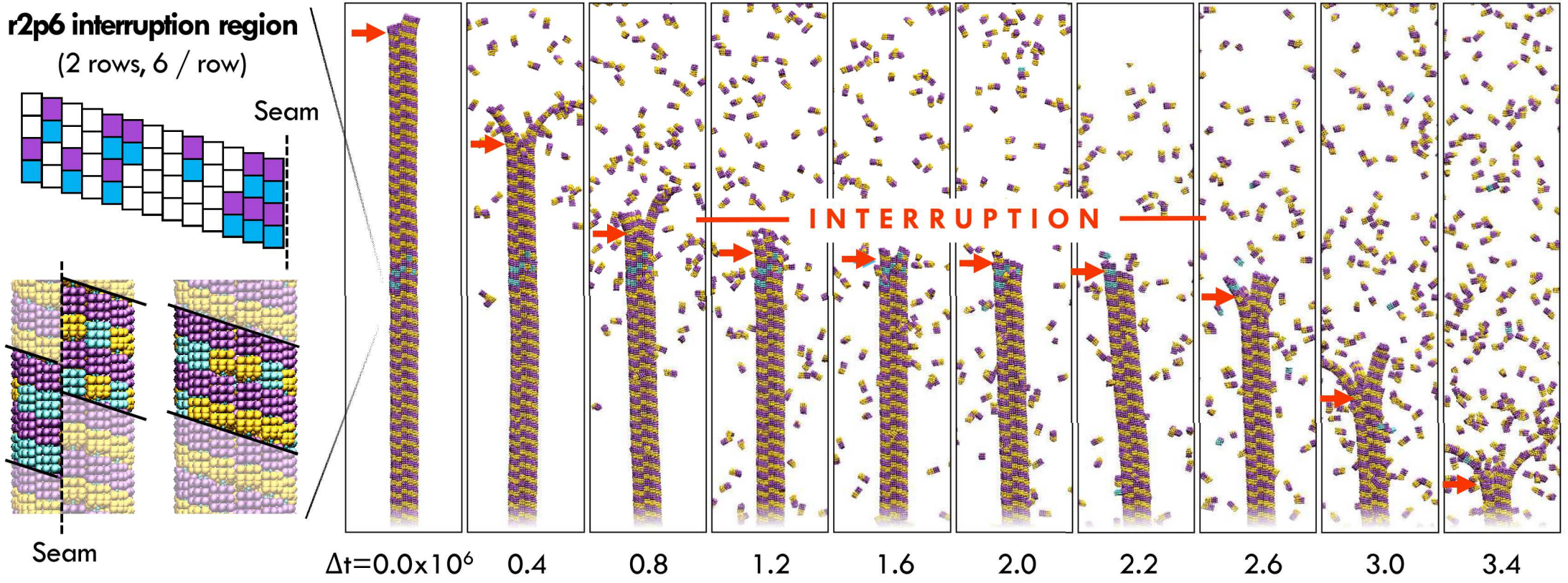
(1) Depolymerization of uncapped GDP-MT **reaches steady-state** rate

Can regions of unfrustrated dimers ***interrupt*** depolymerization?



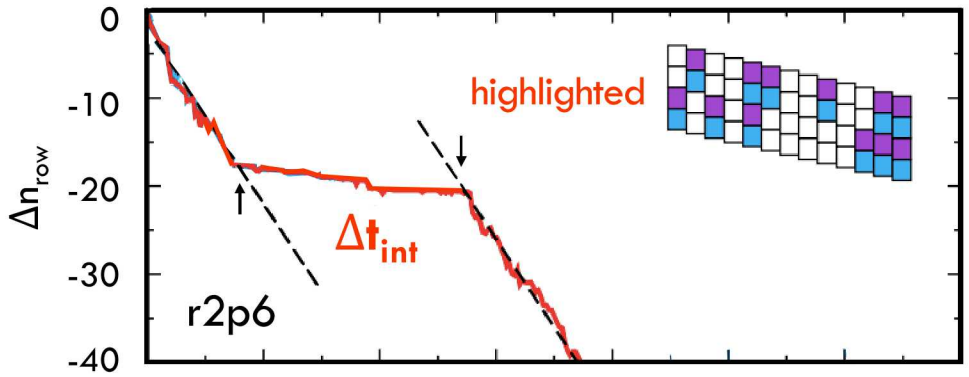
(2) Depolymerization is **interrupted by region** containing unfrustrated dimers

Can regions of unfrustrated dimers ***interrupt*** depolymerization?



(3) Depolymerization **eventually continues** in absence of additional "GTP" refuel

Quantifying interruption time scales from MT length versus time



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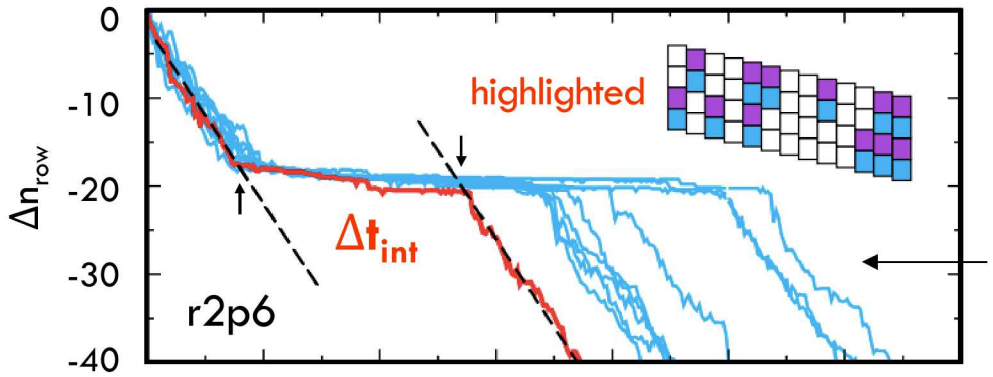
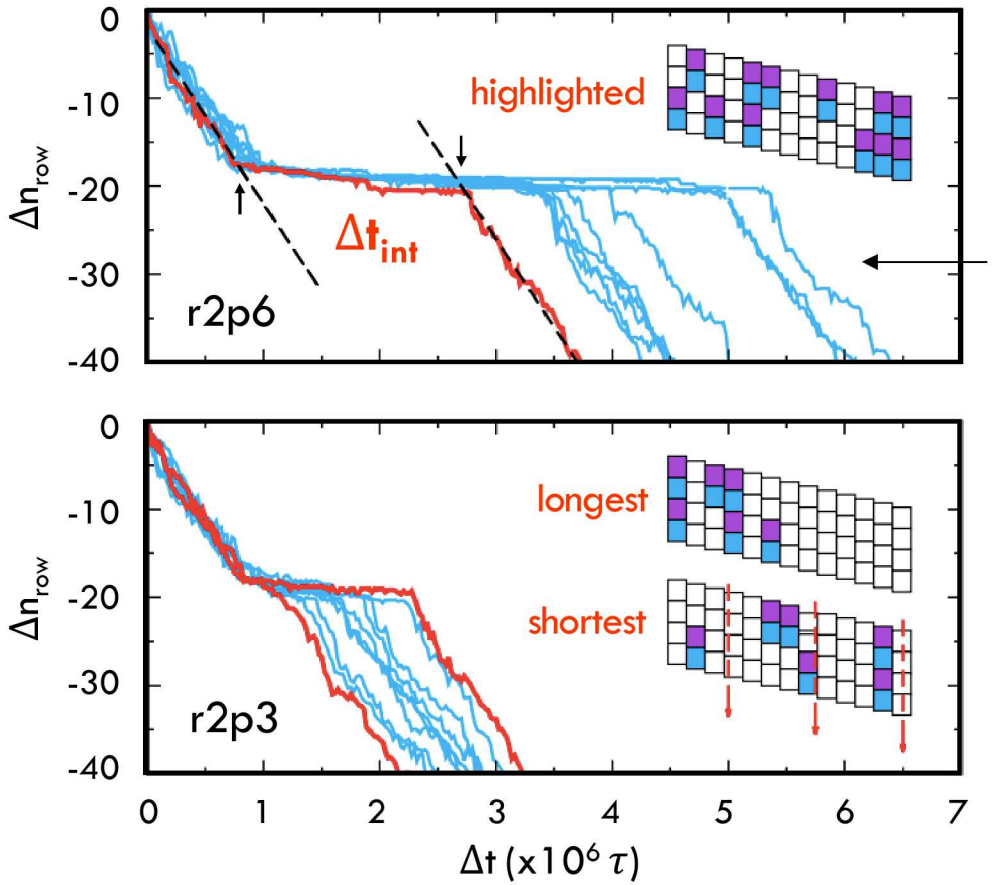


10 independent GDP-MT simulations
(randomly-generated regions)

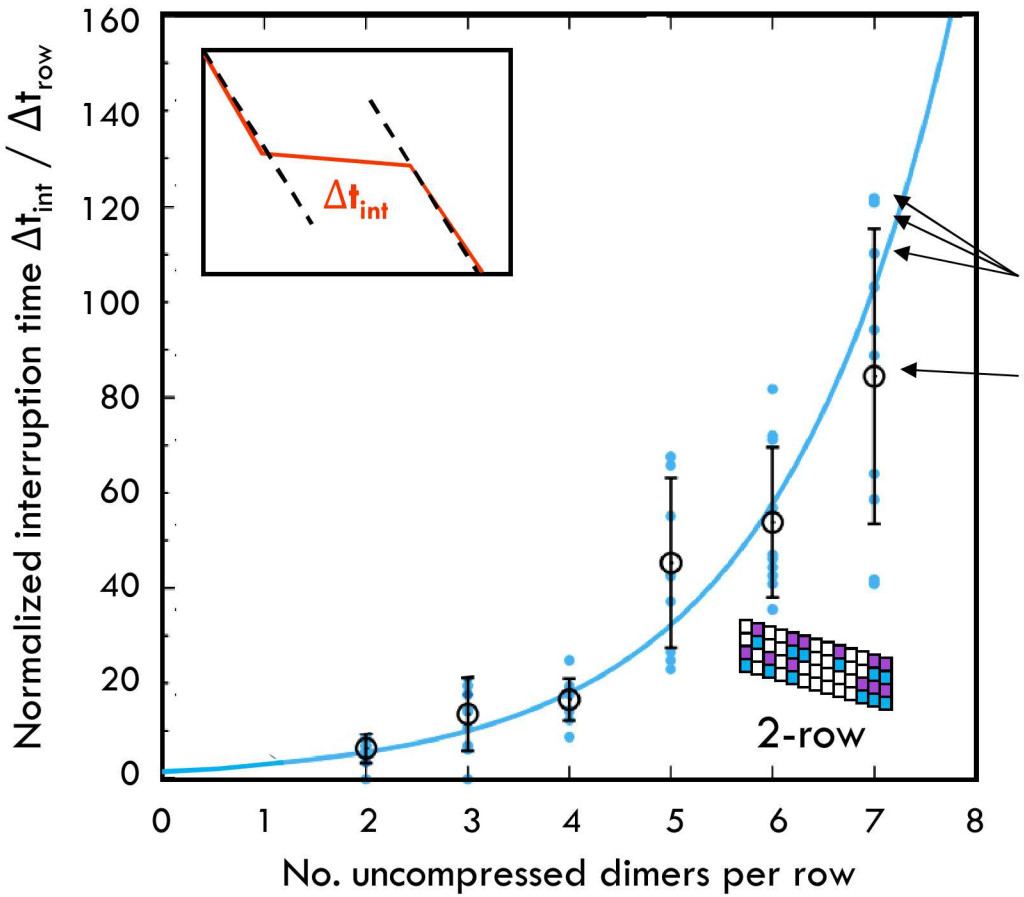
Quantifying interruption time scales from MT length versus time



10 independent GDP-MT simulations
(randomly-generated regions)

Measure interruption w.r.t. **region size** (no. rows) and **composition** (no. dimers / row)

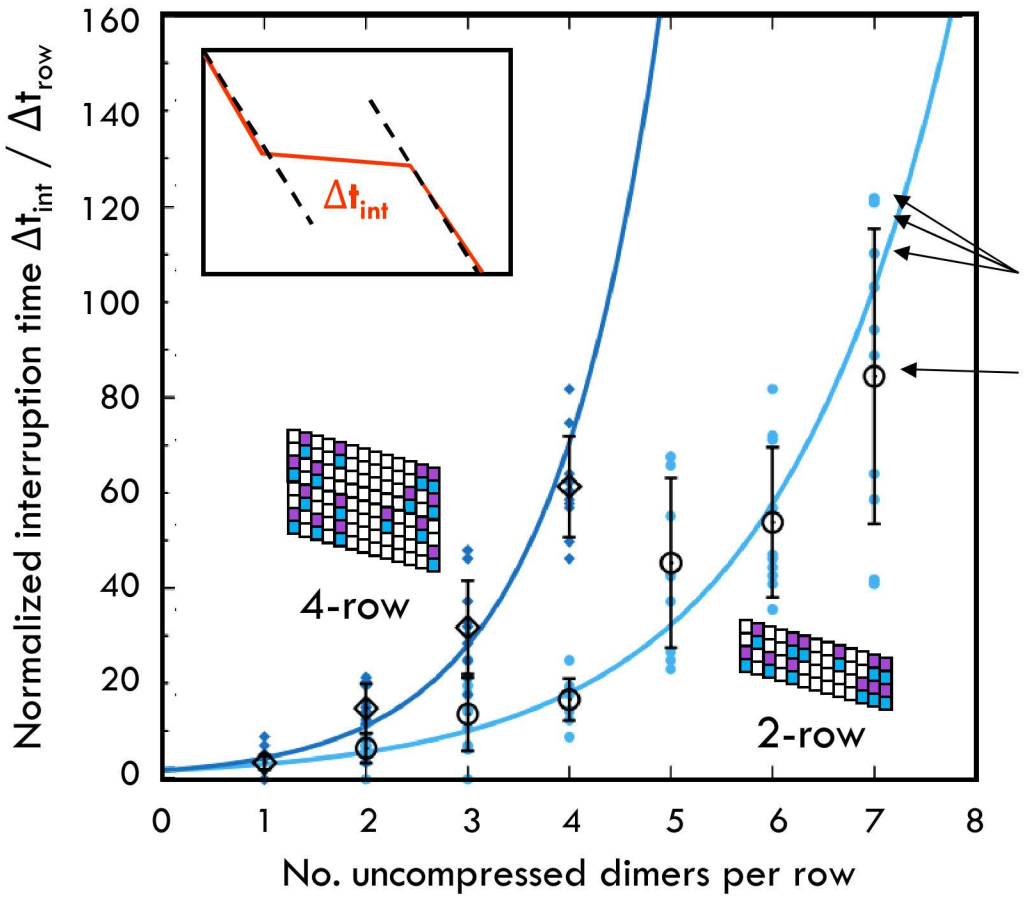
Interruption times (predictably) depend on remnant size & composition



Individual interruption times

Average interruption time (with error)

Interruption times (predictably) depend on remnant size & composition



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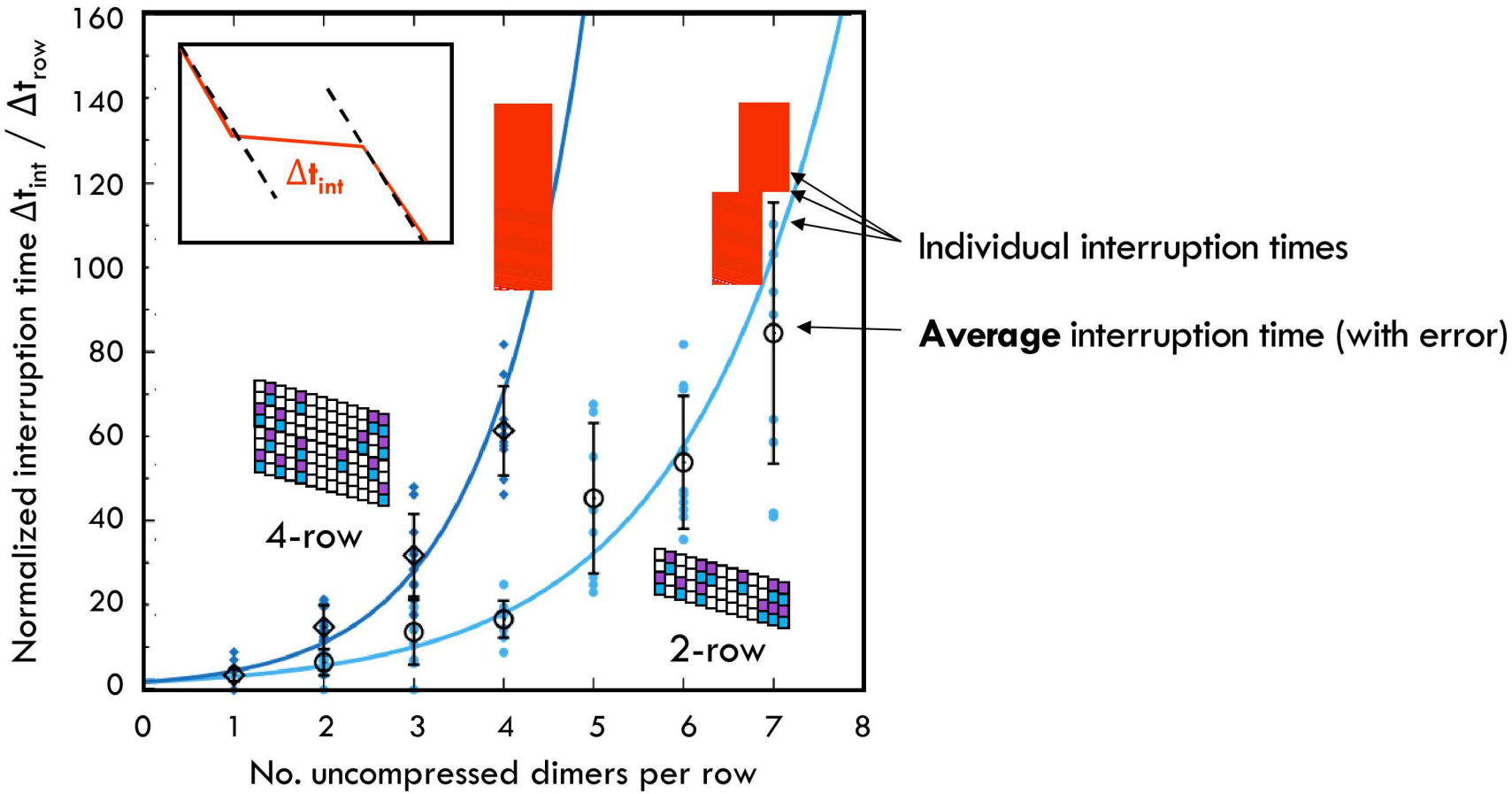
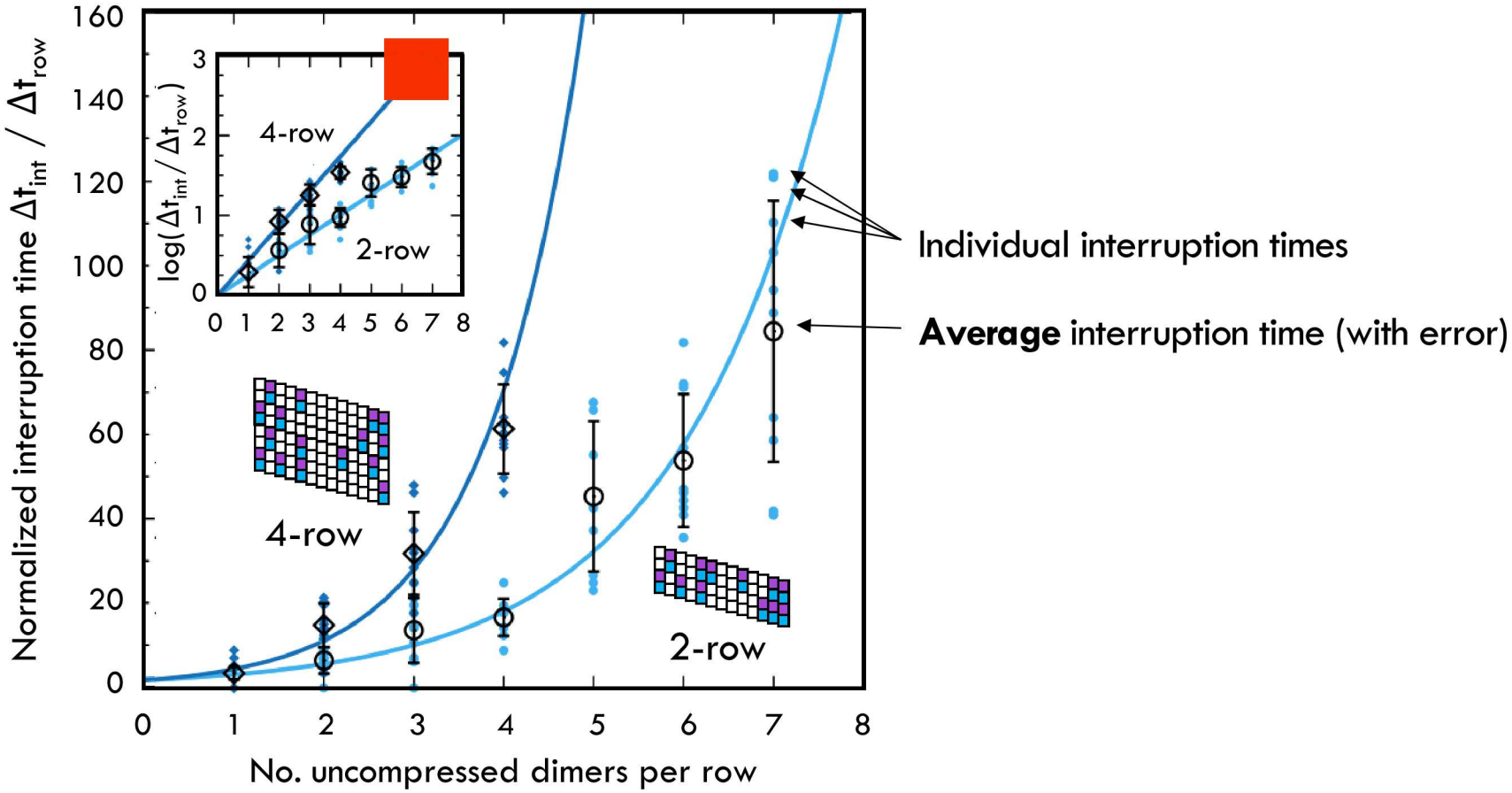


Interruption times **grow exponentially** for 2- or 4-row regions above 30% unfrustrated

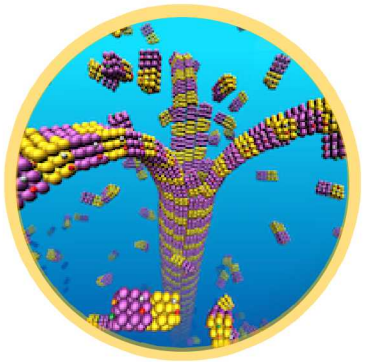
Interruption times (predictably) depend on remnant size & composition



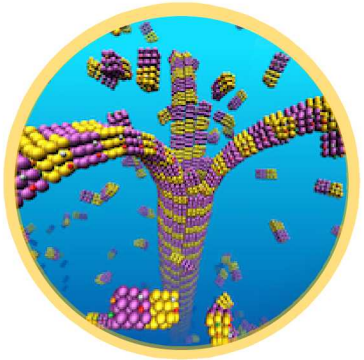
Small patchy regions (e.g., < 30 dimers) stabilize comparable to **unperturbed end cap**

Concluding remarks

- Described recent **minimal model for tubulin/microtubules** that demonstrates how modest shape frustration can drive microtubule depolymerization
- **Consistent with experimental picture** of ”GTP-remnants” as rescue locations, small randomly-initialized regions containing **unfrustrated model dimers can interrupt depolymerization**
- Notably, significant interruption can result from as few as 20 unfrustrated dimers, suggesting that MT depolymerization is **very sensitive to incomplete hydrolysis**



Onset and arrest of catastrophic depolymerization in microtubules controlled by tubulin subunit shape



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