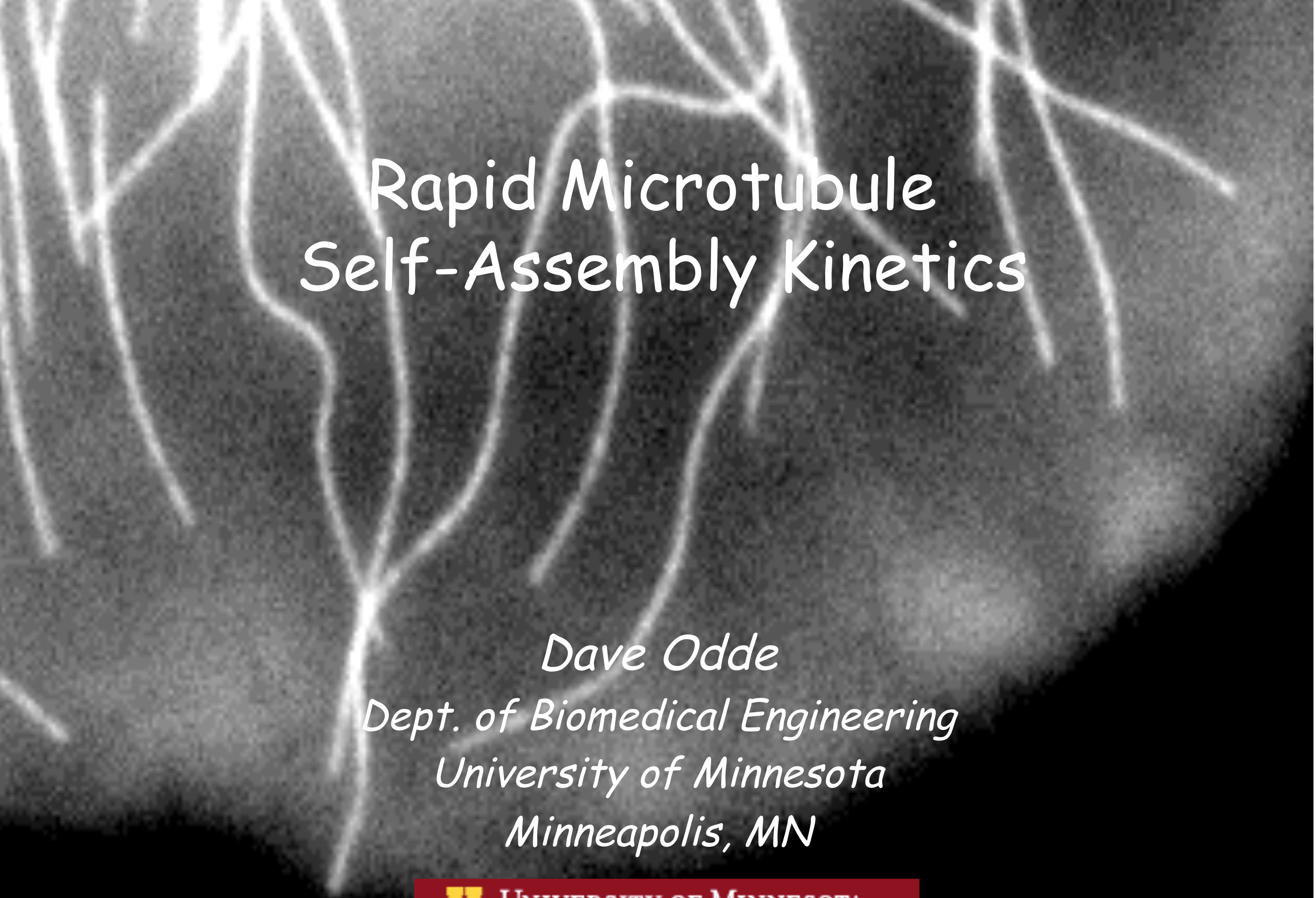

Rapid Microtubule Self-Assembly Kinetics

Dave Odde

Dept. of Biomedical Engineering

University of Minnesota

Minneapolis, MN

UNIVERSITY OF MINNESOTA

Acknowledgements

Melissa Gardner

- U of Minnesota
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  - Blake Charlebois
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- Lehigh U
  - Vince VanBuren (Texas A&M U)
  - Lynne Cassimeris
- Eötvös Loránd U
  - Imre Janosi
- Max Planck Inst-MCBG, Dresden
  - Joe Howard

<u>Funding</u>: NIGMS, NIBIB, NSF, Whitaker Fdn, U of Minnesota IMA

Microtubules

Molecular
"Nanoscale"

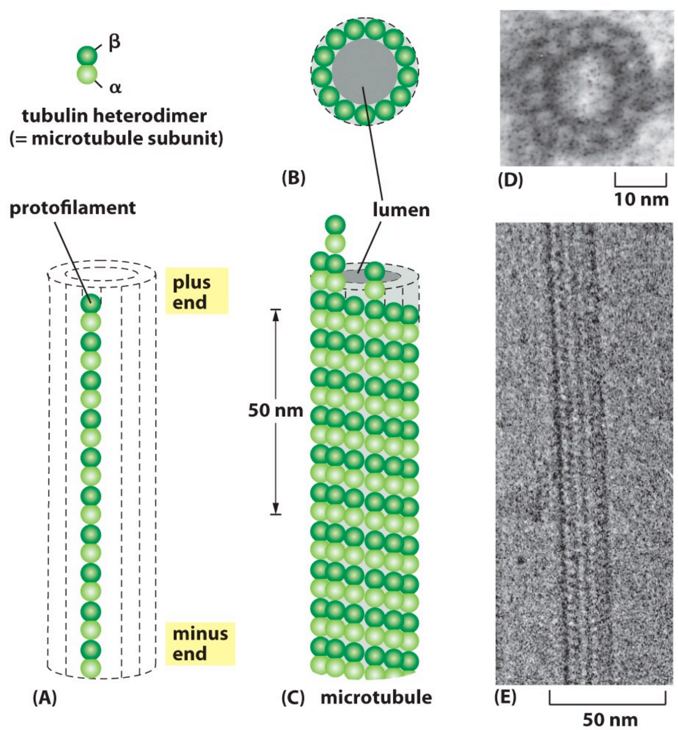


Figure 17-9 Essential Cell Biology 3/e (© Garland Science 2010)

Cellular
"Microscale"

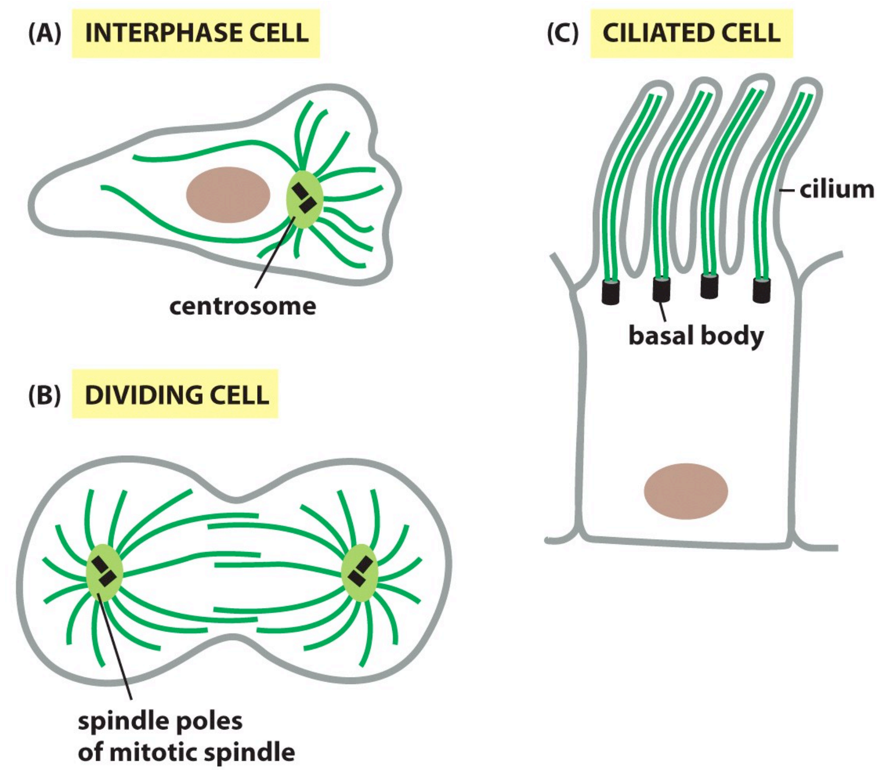
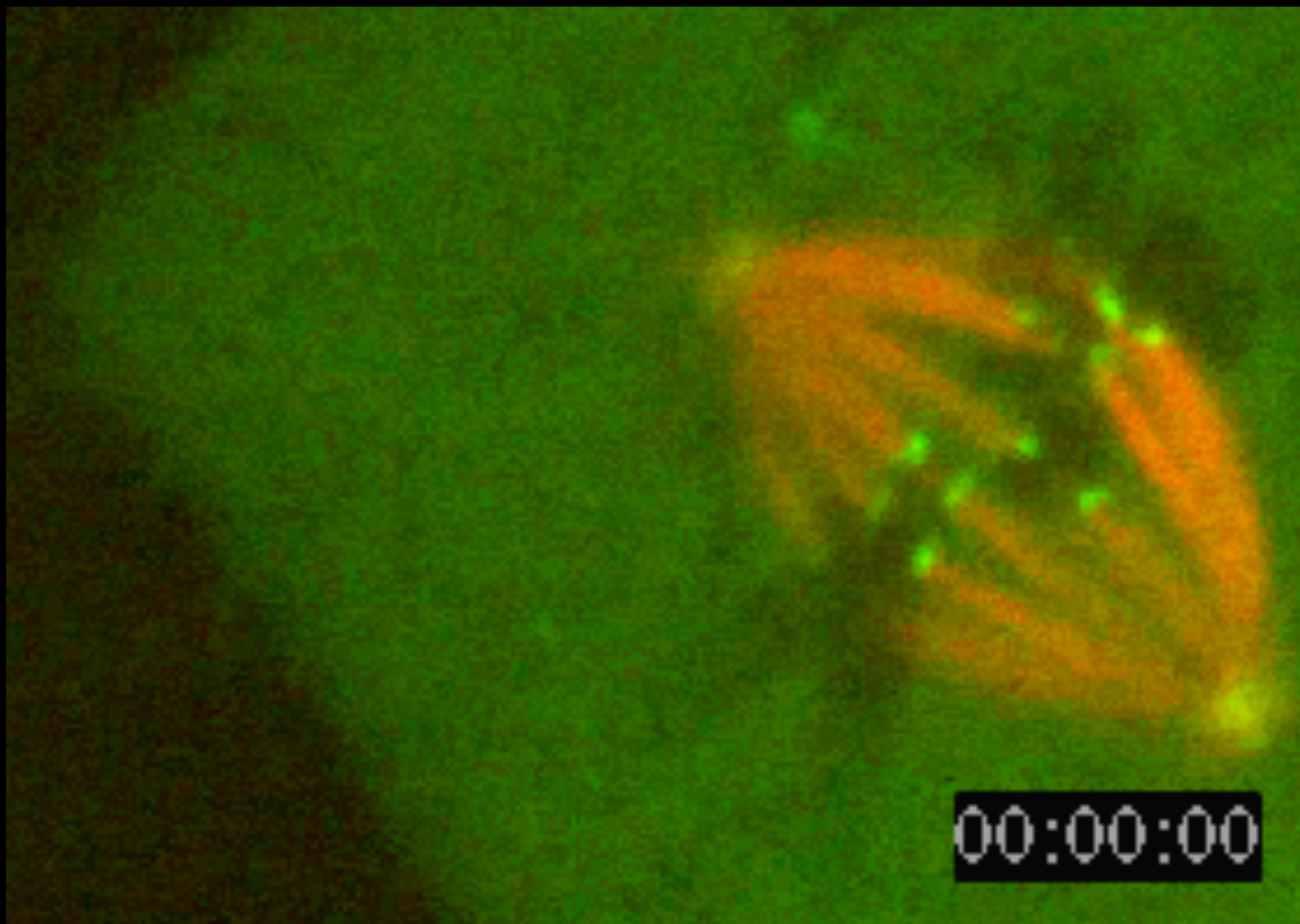


Figure 17-8 Essential Cell Biology 3/e (© Garland Science 2010)

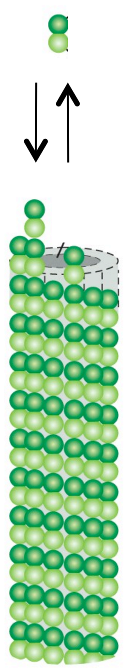
Microtubule Dynamics in the Mitotic Spindle



Cimini and Salmon, Current Biology, 2004

Oosawa 1D Model

J. theor. Biol. (1970) **27**, 69–86



Size Distribution of Protein Polymers

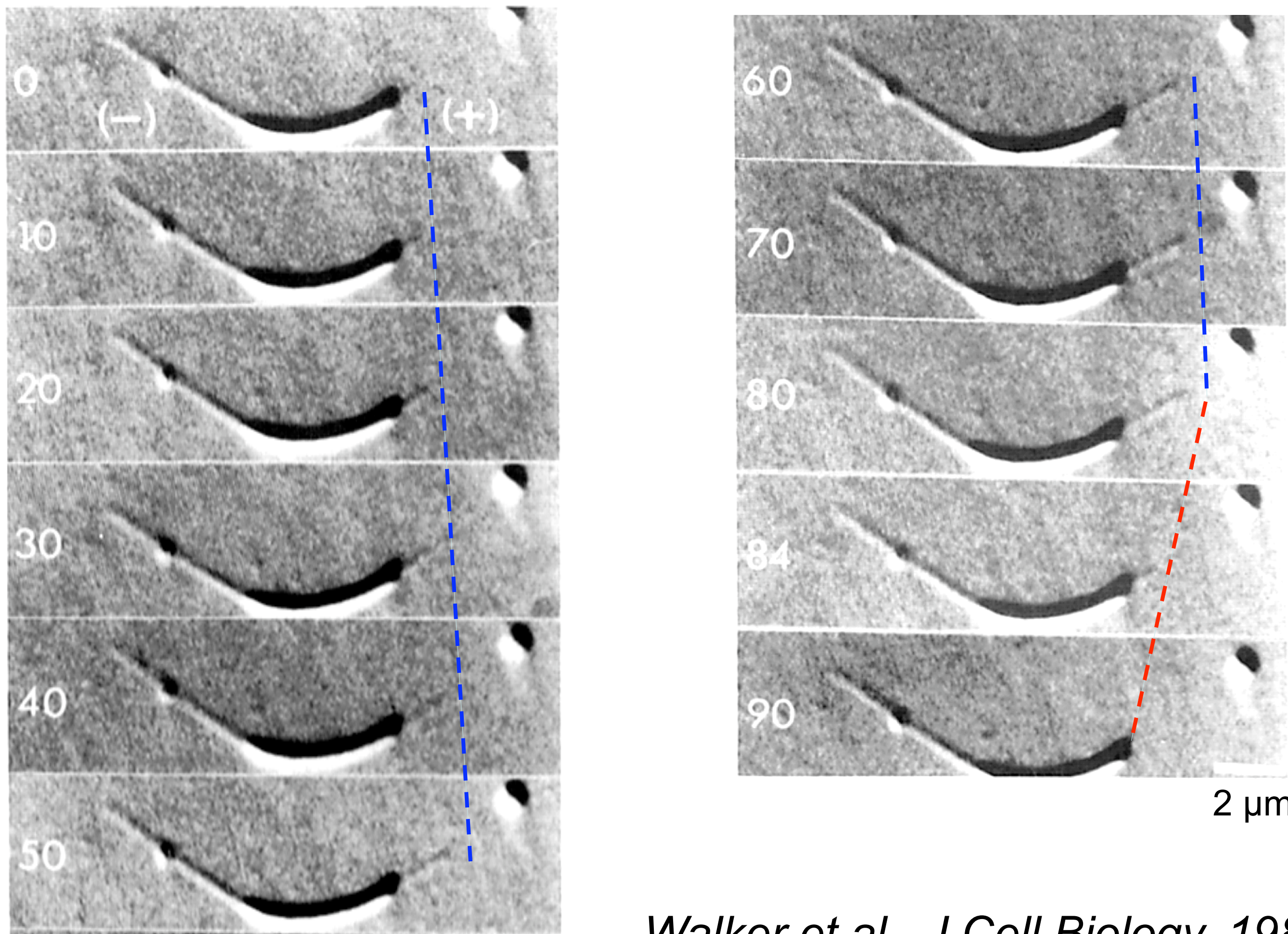
FUMIO OOSAWA

Institute of Molecular Biology, Faculty of Science, Nagoya University, Nagoya, Japan

$$dc_p/dt = (k_+ c_1 - k_-)m$$

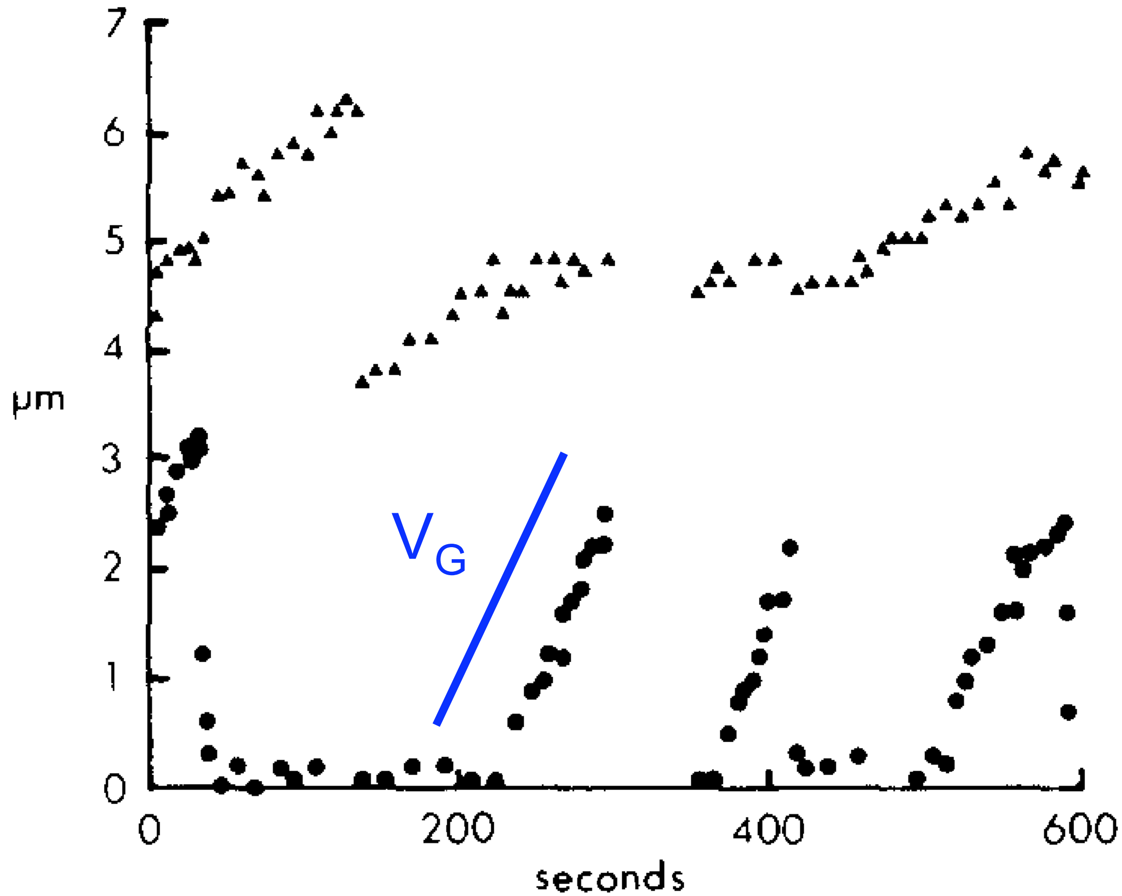
$$V_G = k_{on}[tubulin] - k_{off}$$

Direct Observation of MT Dynamic Instability In Vitro by Light Microscopy



Walker et al., J Cell Biology, 1988
(see also Horio and Hotani, Nature, 1986)

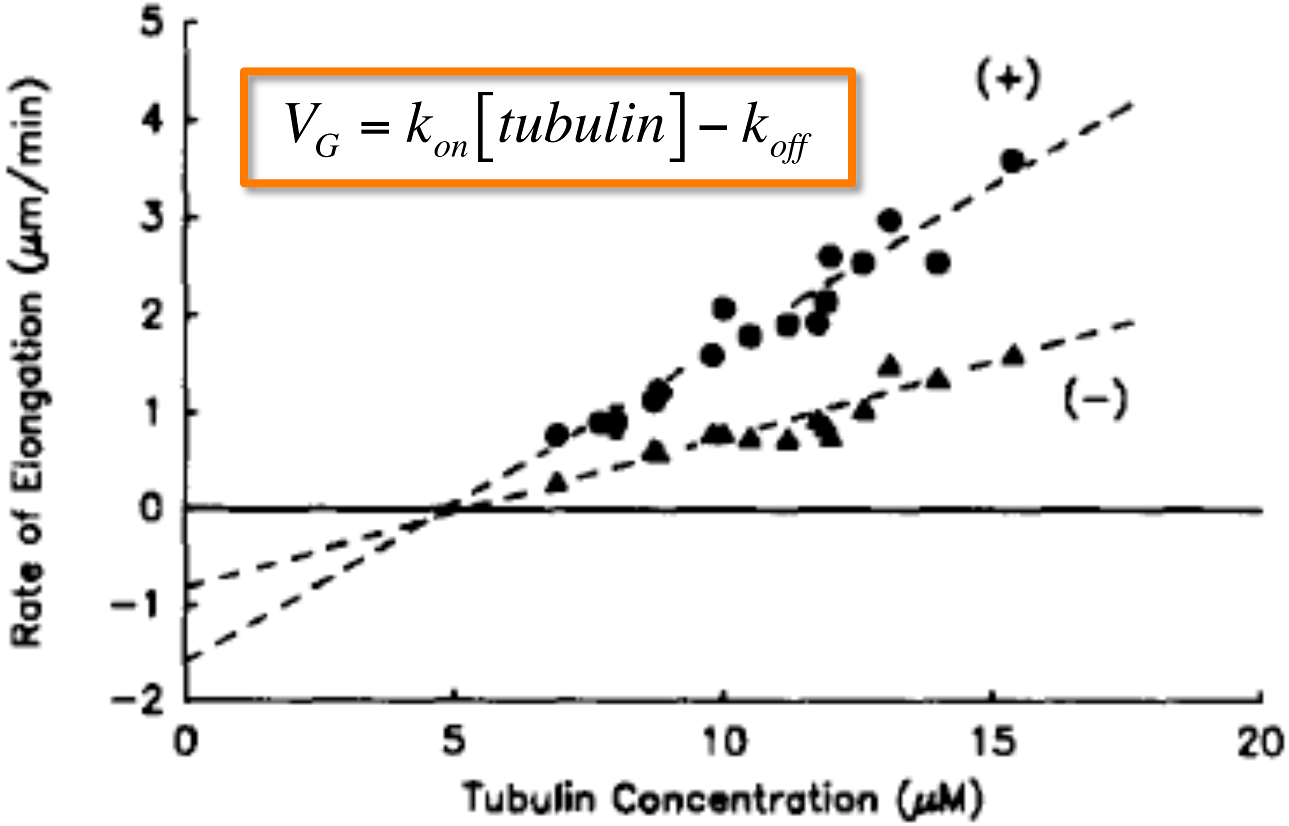
Direct Observation of MT Assembly In Vitro by Light Microscopy



Walker et al., JCB, 1988

Microtubule Growth Rate:
1D Model

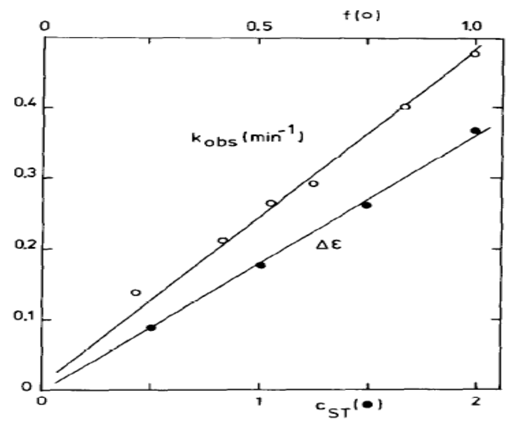
$$V_G = k_{on}\left[tubulin\right] - k_{off}$$



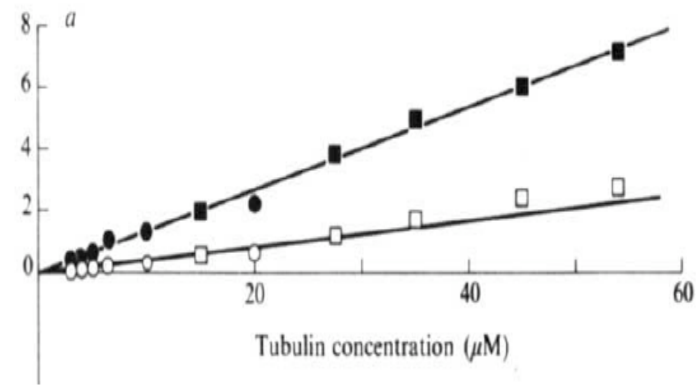
Walker et al., JCB, 1988

Success of the 1D Model

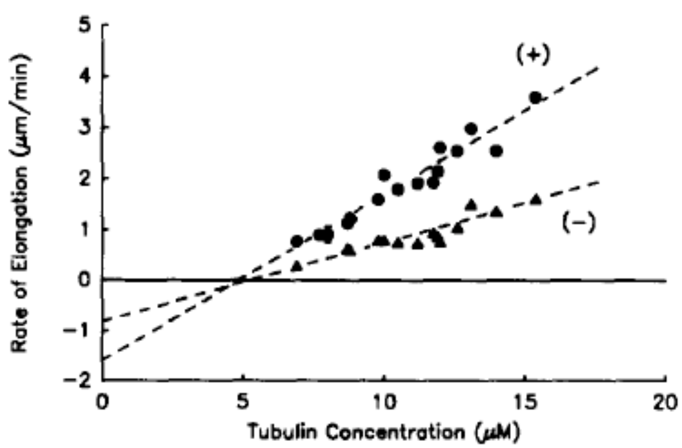
Engelborghs et al. (1977)



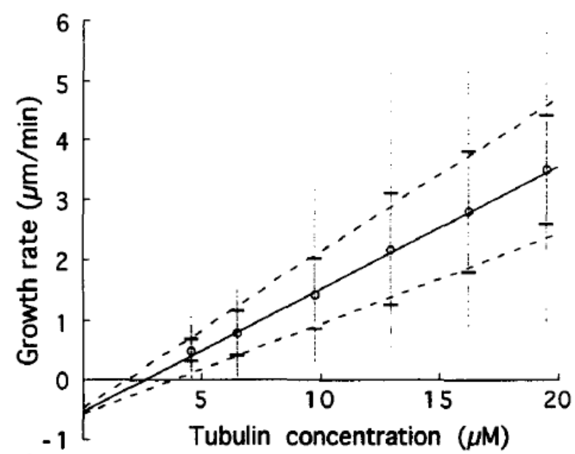
Mitchison and Kirschner (1984)



Walker et al., J Cell Biol (1988)



Chretien et al., J Cell Biol (1995)



Kinetics of Microtubule Assembly: 1D model

Source	On-Rate Constant $\mu M^{-1} s^{-1} MT^{-1}$	Off-Rate Constant $s^{-1} MT^{-1}$
Engelborghs et al. (1977)	3.9±1.8	25.7±7.1
Bergan & Borisy (1980)	7.2	17
Farrell & Jordan (1982)	1.00±0.36	2.00±0.18
Carlier, Hill, and Chen (1984)	4.2	2.3
Mitchison & Kirschner (1984)	3.82	0.37
Gard & Kirschner (1987)	1.4	--
Walker et al. (1988)	8.9±0.3	44±14
O'Brien et al. (1990)	6.8	25-30
Drechsel et al. (1992)	3	0.1
Trinczek et al. (1993)	6.9±0.5	32±6.0
Hyman et al. (GMPCPP) (1992)	5	0.1
Chretien et al. (1995)	5.7	14.1
Brouhard et al. (GMPCPP) (2008)	4.9±2.3	0.49±0.12
Mean	**4.8**	**15.0**

$k_{on} =\sim 5\ \mu M^{-1}\ s^{-1}\ MT^{-1}$

1D model

At 10 μM tubulin:

$k_{on}^{*} = k_{on}[tub] = +50\ s^{-1}$

$k_{off} = -10\ s^{-1}$

$net = +40\ s^{-1}$

Consequences of the 1D Model

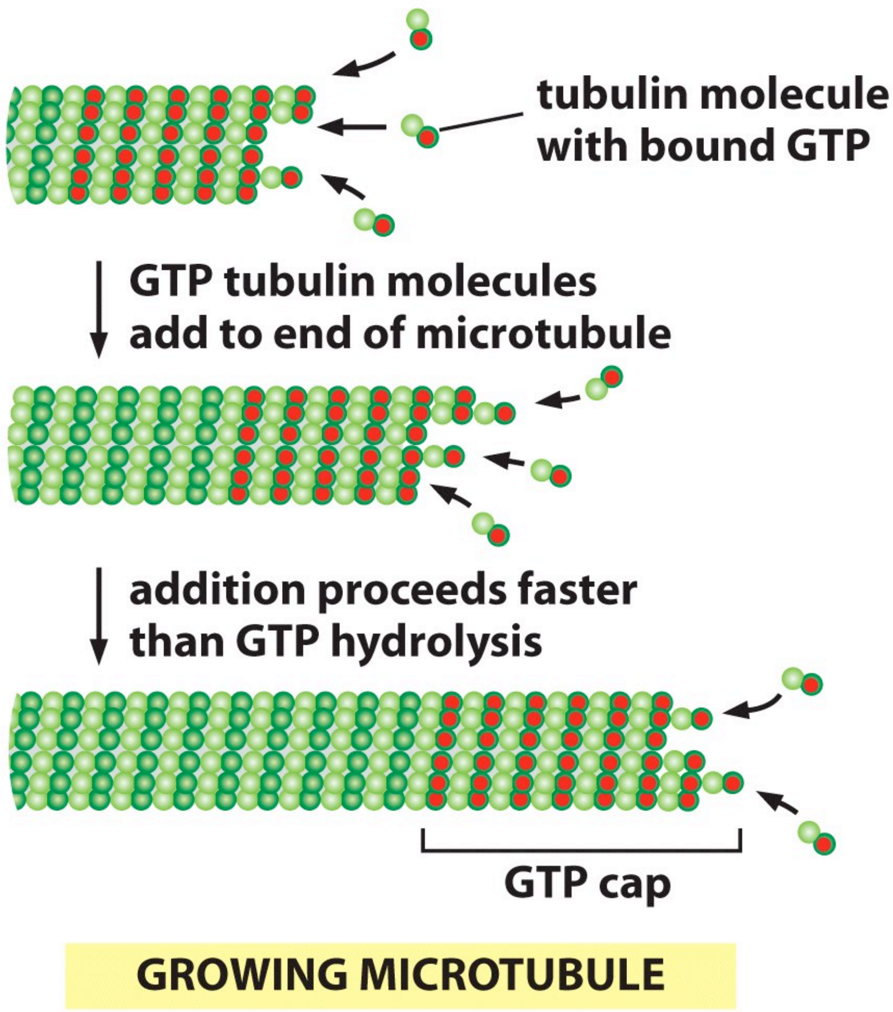


Figure 17-12a Essential Cell Biology 3/e (© Garland Science 2010)

At 10 μM tubulin:

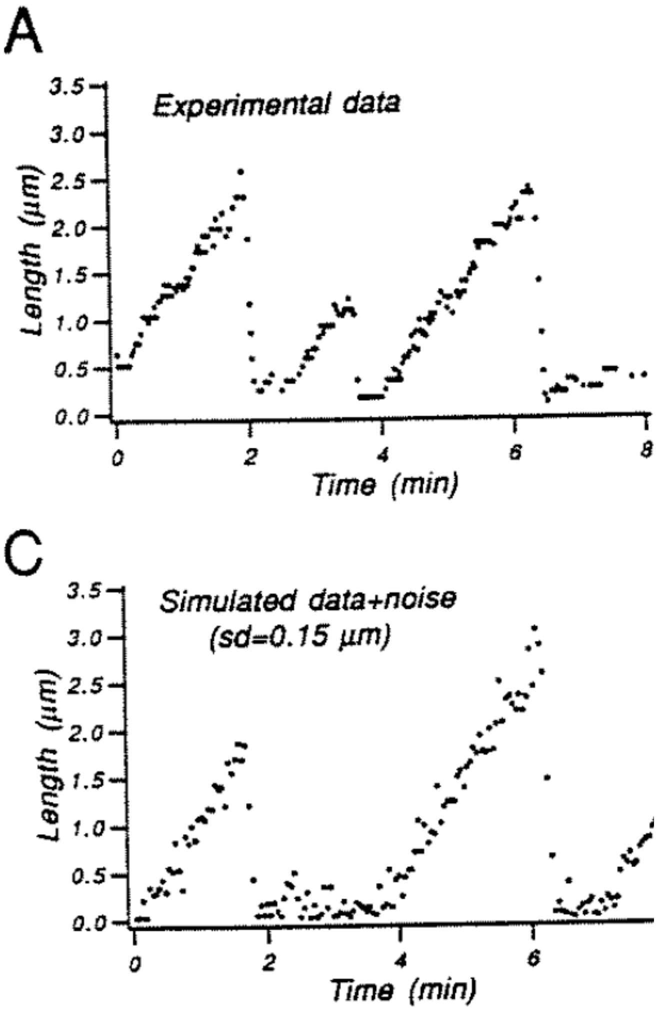
$k_{on}^* = k_{on}[tub] = \quad +50\ s^{-1}$

$k_{off} = \quad -10\ s^{-1}$

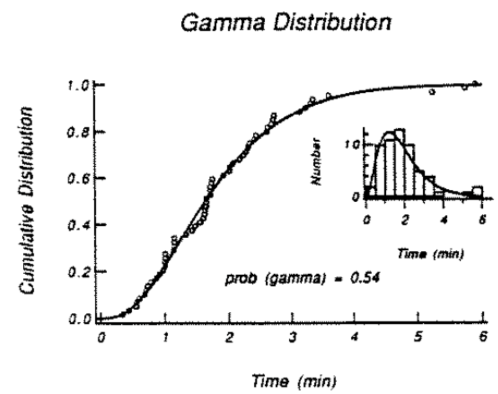
net = $+40\ s^{-1}$

How is assembly regulated by MAPs and drugs?

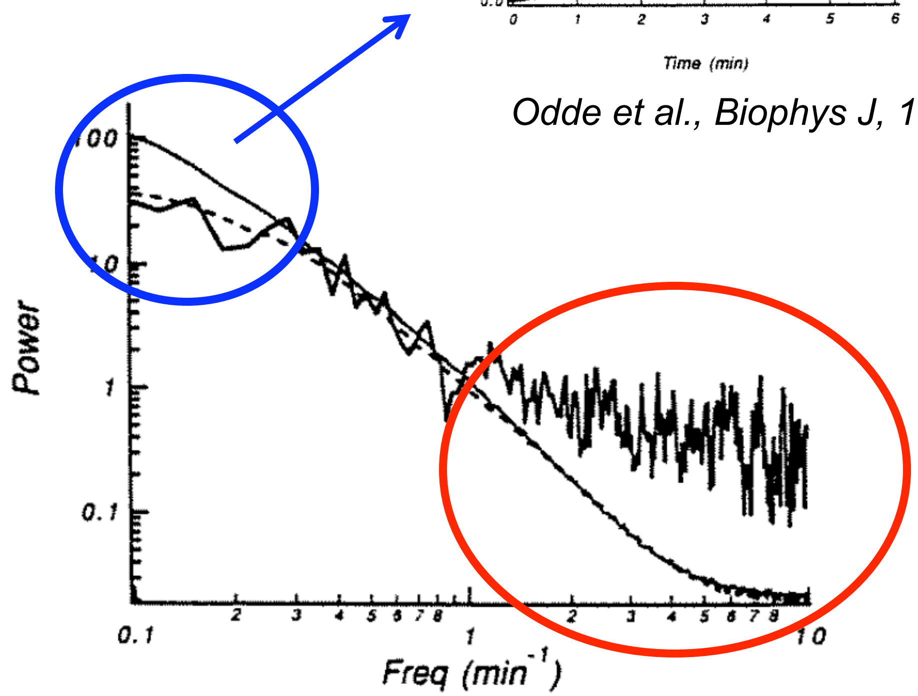
Problems with 1D model?



Odde et al., AIChE J, 1996



Odde et al., Biophys J, 1995

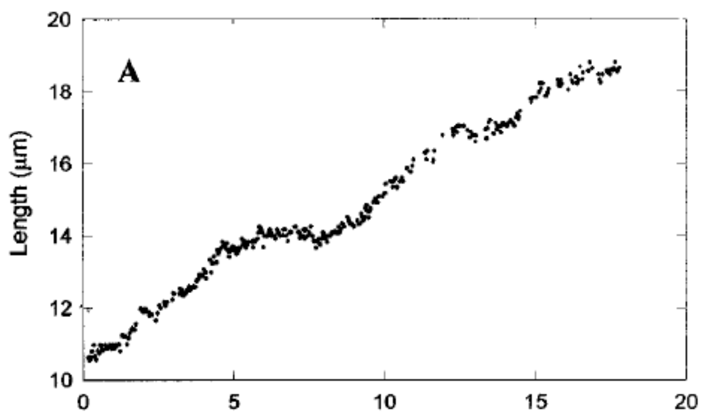


Long times

Short times

Questions raised by 1D model

- Why is the assembly rate so variable within a growth phase?



Dye and Williams, Biochemistry, 1996

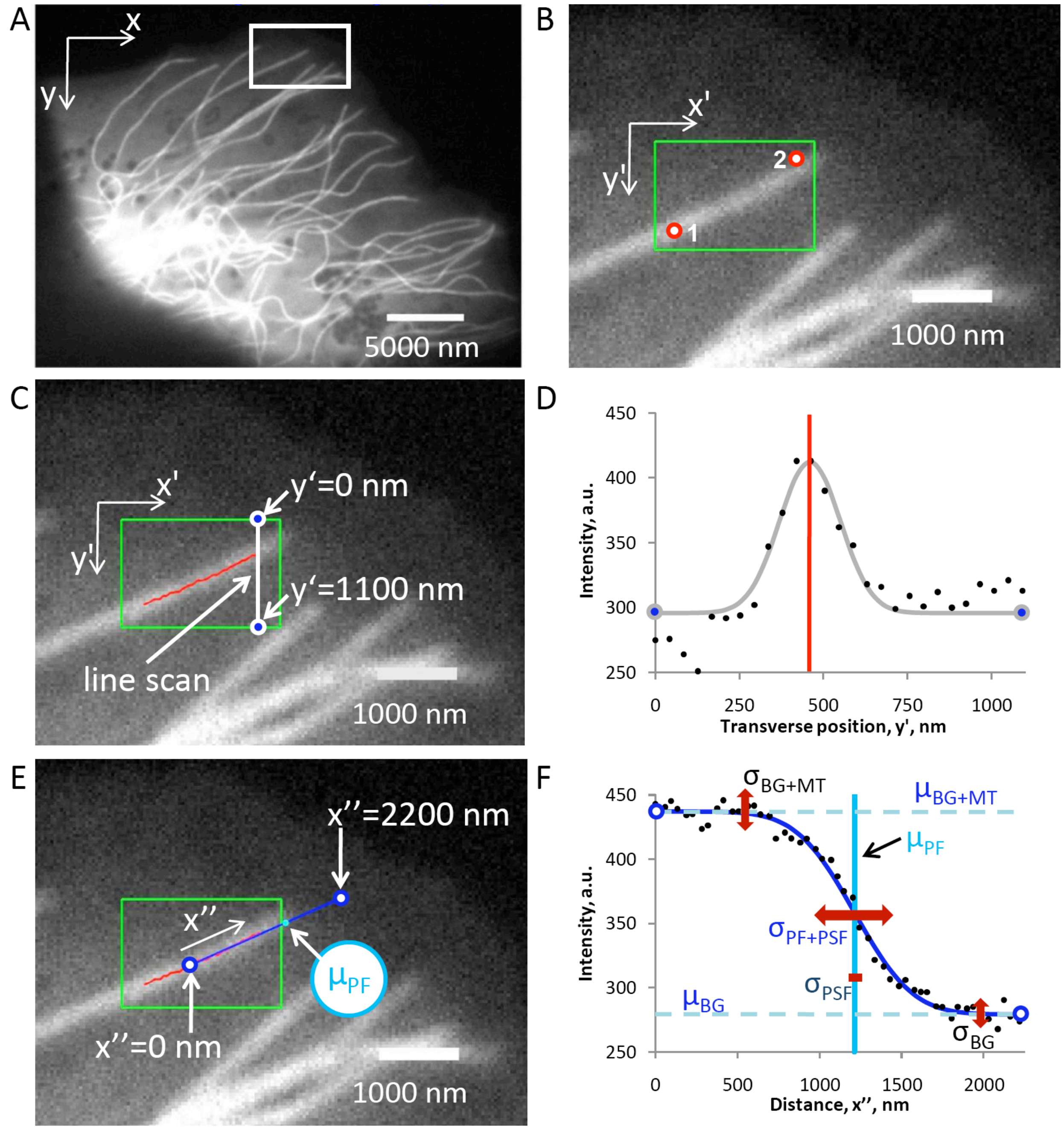
See also:
- *Gildersleeve et al., JBC,1992*
- *Odde et al., AIChE J, 1996*
- *Pedigo et al., Biophys J, 2002*
- *Schek, Gardner et al., Curr Biol, 2007*

Source	Resolution	Nucleotide
Dye and Williams, 1996	115 nm	GMPCPP
Schek, Gardner et al., 2007	4 nm	GTP
Not yet done (?)	*4 nm*	*GMPCPP*

Microtubule Tip Tracking

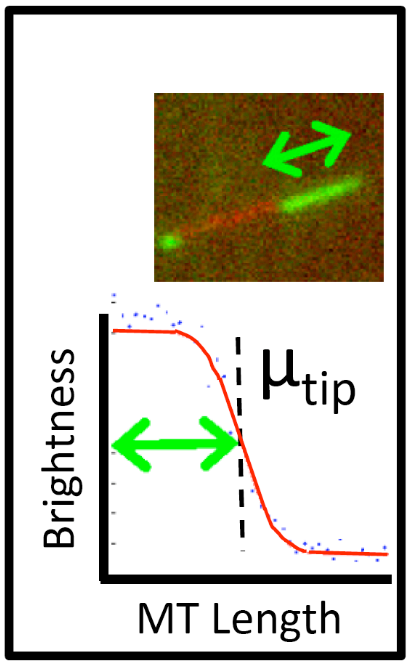
Accuracy: ~36 nm

Demchouk et al., *Cellular and Molecular Bioengineering*, 2011



Microtubule Assembly at the Nanoscale

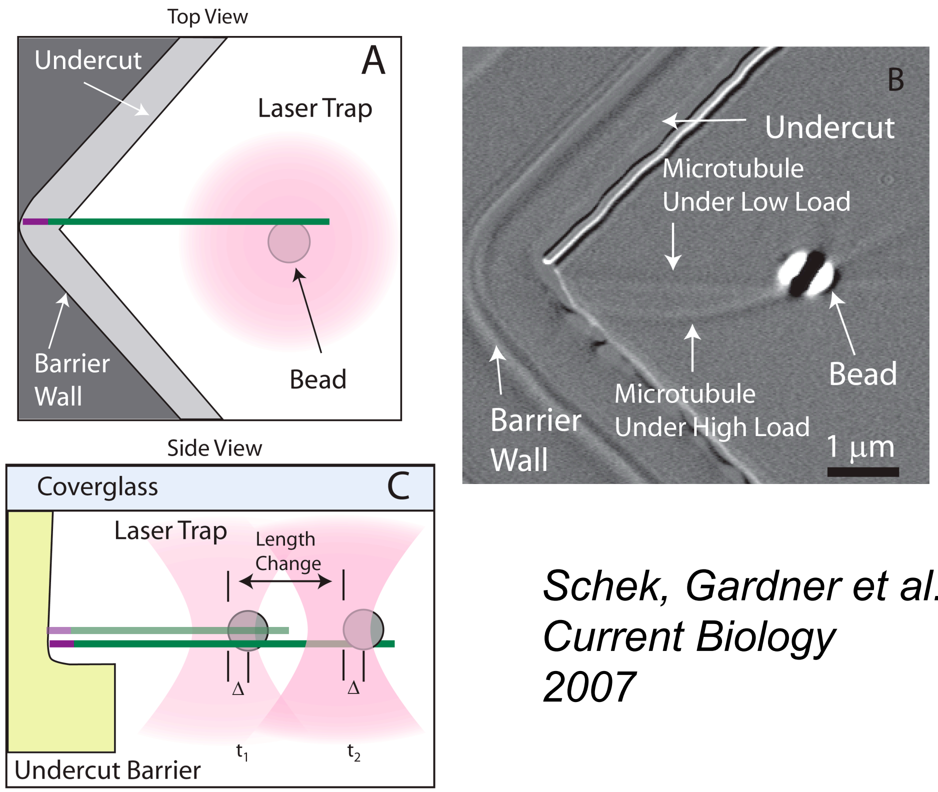
TIRF Microscopy



Demtchouk et al., Cellular and Molecular Bioengineering, 2011

Accuracy: ~11 nm

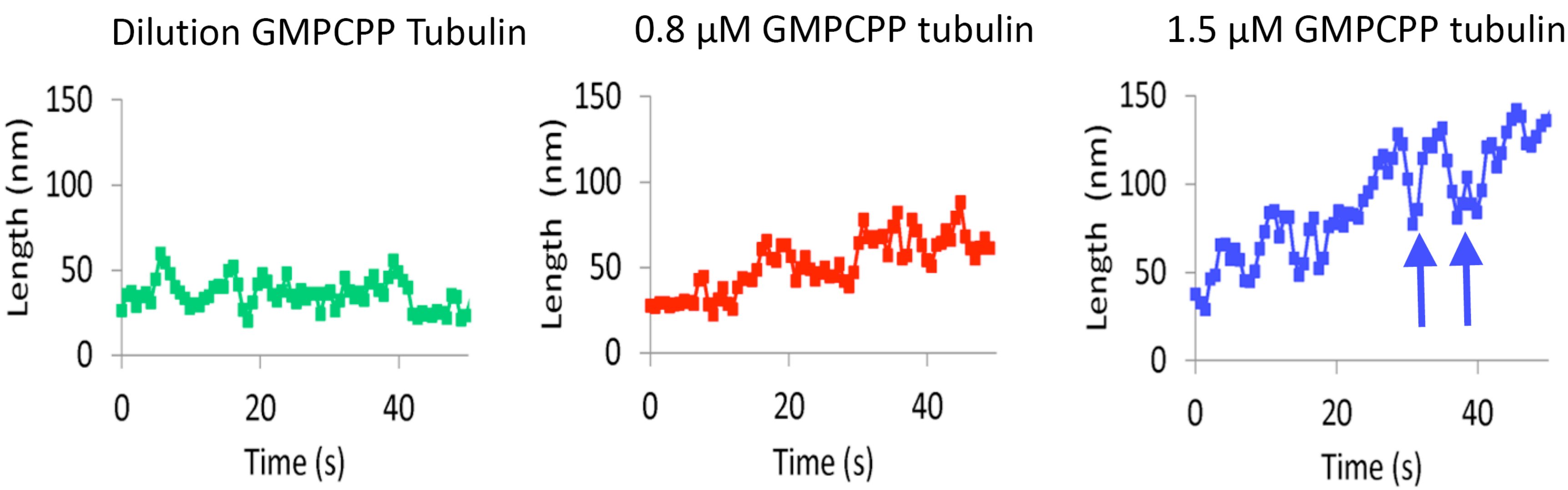
Laser Tweezers



Schek, Gardner et al., Current Biology 2007

Accuracy: ~4 nm

TIRF: GMPCPP-Microtubule Assembly at the Nanoscale



Gardner et al., Cell, in press

Predictions of the 1D model

For a given time interval τ:

Mean # subunits on: $$\lambda_+ = k_{on}\left[tubulin\right]\tau$$

Mean # subunits off: $$\lambda_- = k_{off}\tau$$

Increment mean: $$\mu = \lambda_+ - \lambda_-$$

Increment variance: $$\sigma^2 = \lambda_+ + \lambda_-$$ σ= ~2-4 nm

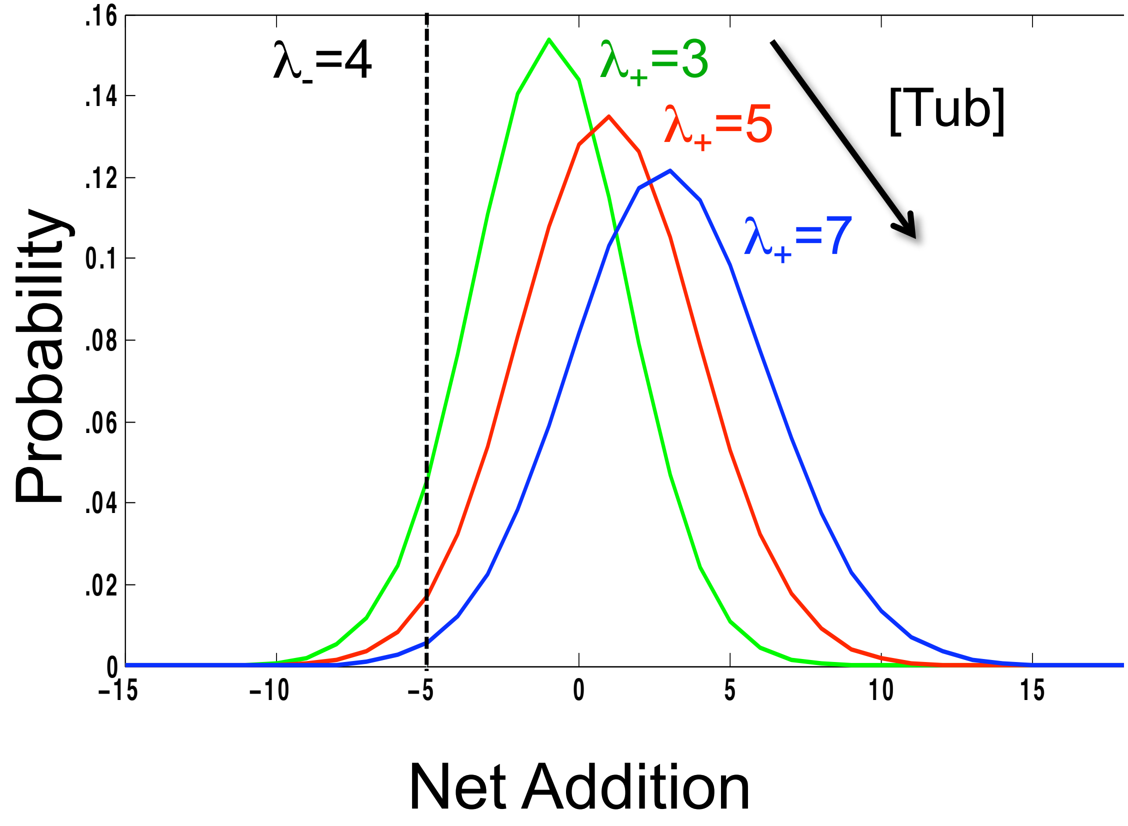
1D model

As [tubulin] increases:

Probability of large negative increment should decrease

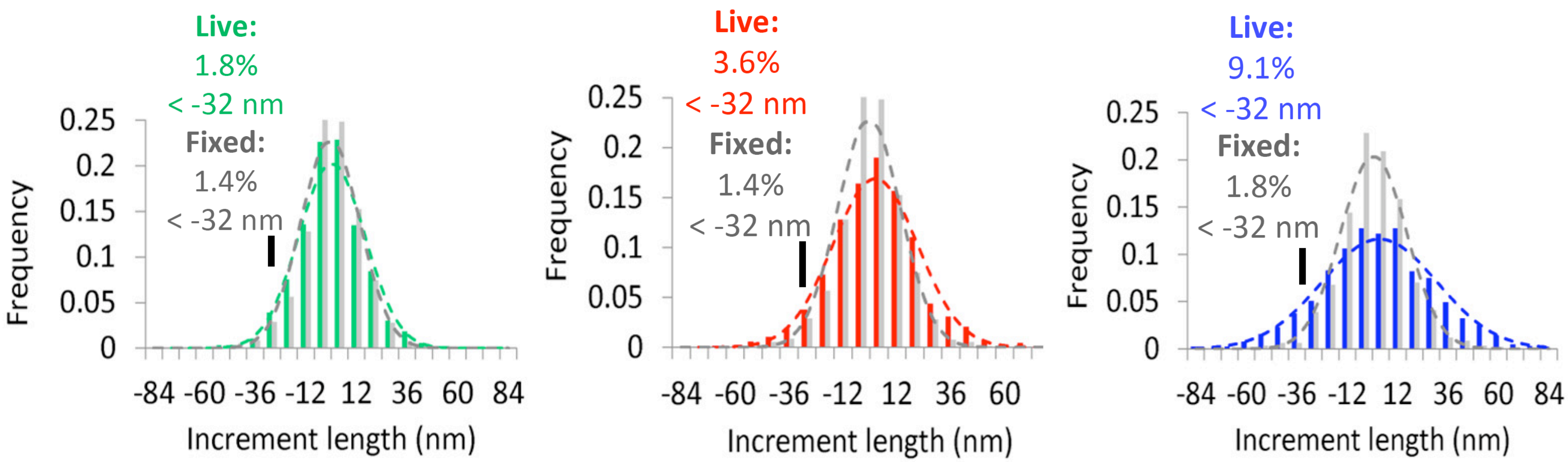
Gardner et al., Cell, in press

Predictions of the 1D model



Skellam, 1946

TIRF: Growing GMPCPP-Microtubule Increment Length Distribution

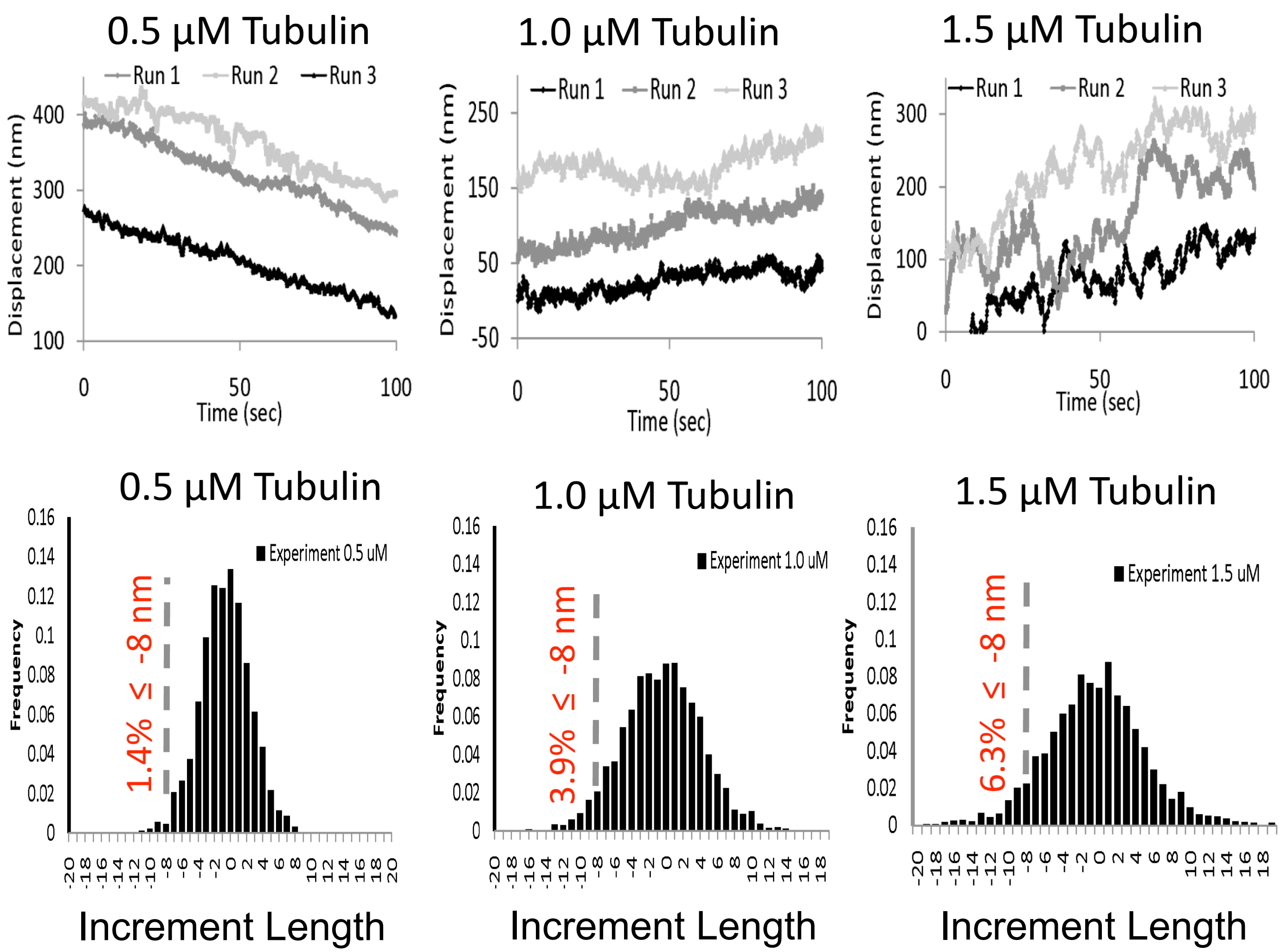


Large *negative* increments are *more* common at higher tubulin concentrations, opposite of 1D model prediction

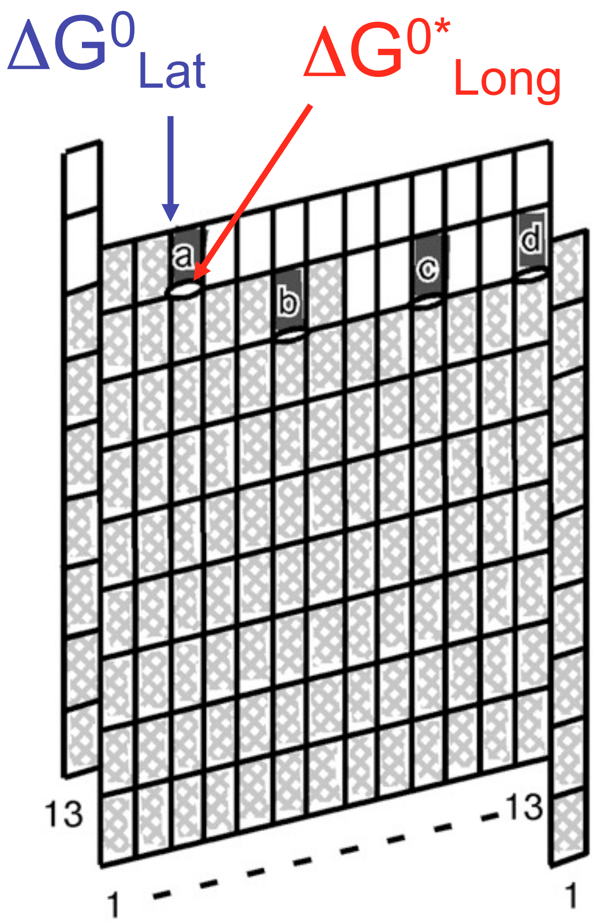
Off-rate is increasing with free concentration

Gardner et al., Cell, in press

Tweezers: GMPCPP-Microtubule Assembly at the Nanoscale



2D Model of Microtubule Assembly



For every PF:

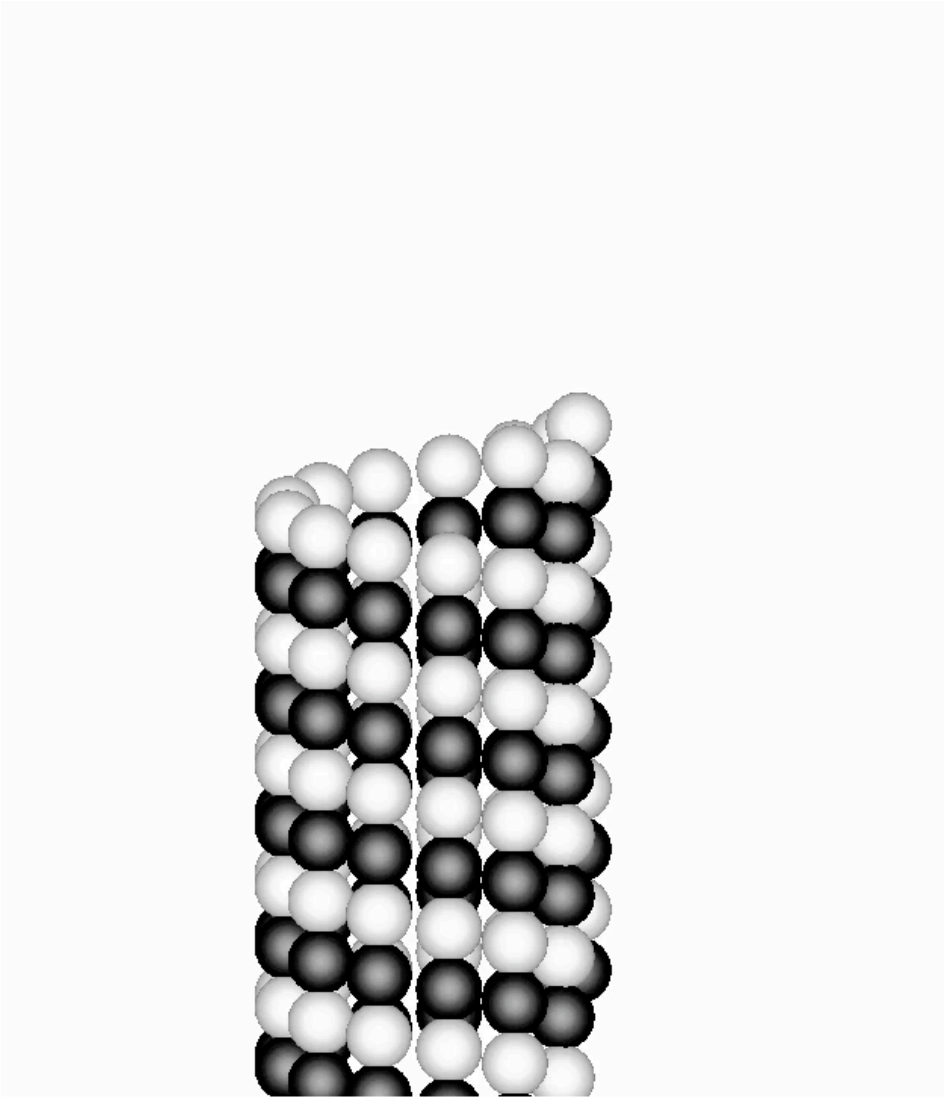
$$k_{on}^* = k_{on}[Tub - GTP]$$

$$k_{off} = k_{on}\, e^{\sum \Delta G^0 / k_B T}$$

VanBuren et al., PNAS 2002

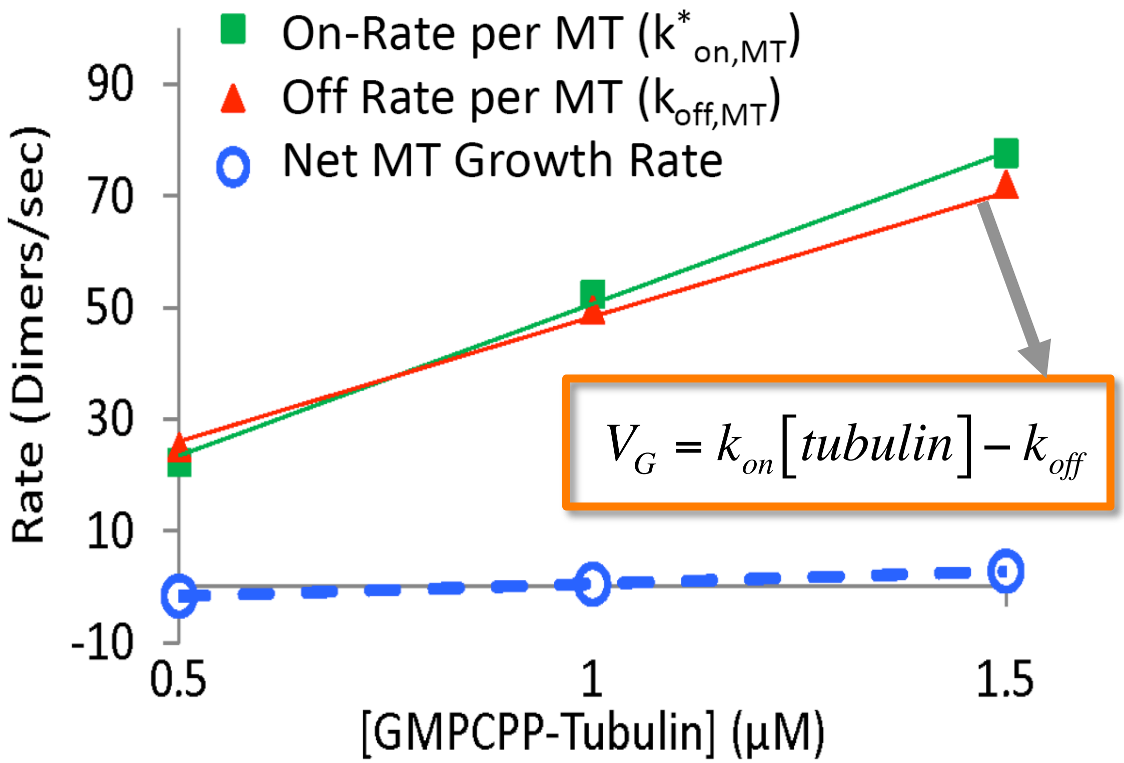
2D Model of Microtubule Assembly

10x slower than real-time,
1 sec total



VanBuren et al., PNAS 2002; Animation by M. Gardner

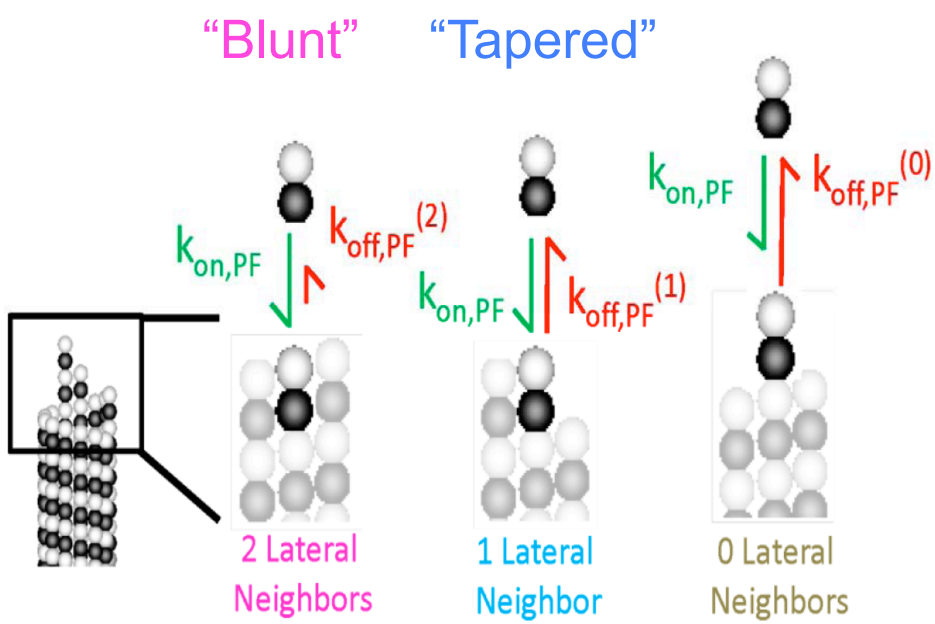
2D Model: k_{off} is *not* constant



- *On and off rates are nearly equal at all concentrations*
- *2D model requires a higher on-rate constant than 1D model*

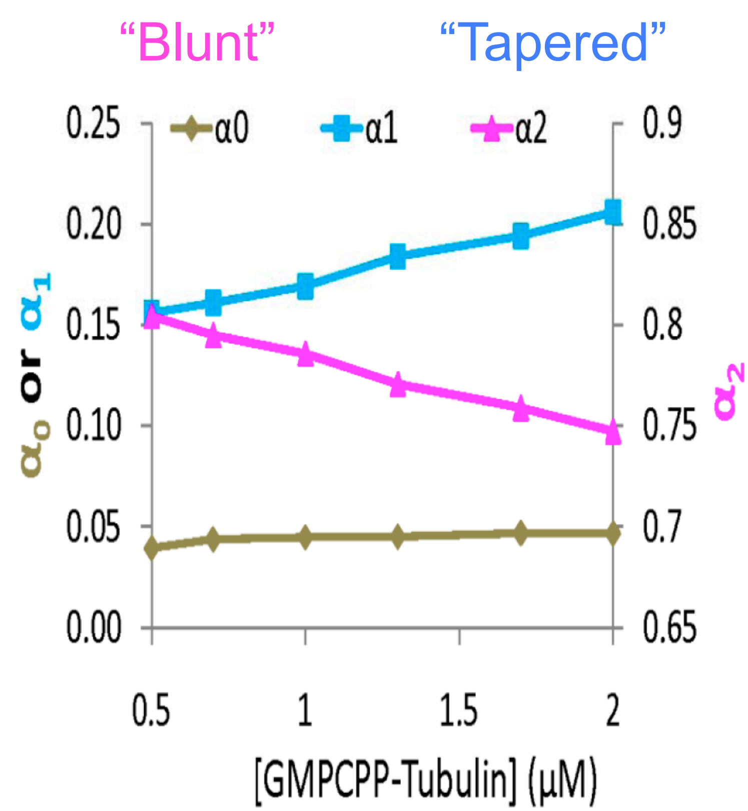
Gardner et al., Cell, in press

2D Model tip structures: From 2 lateral neighbors to 1 lateral neighbor



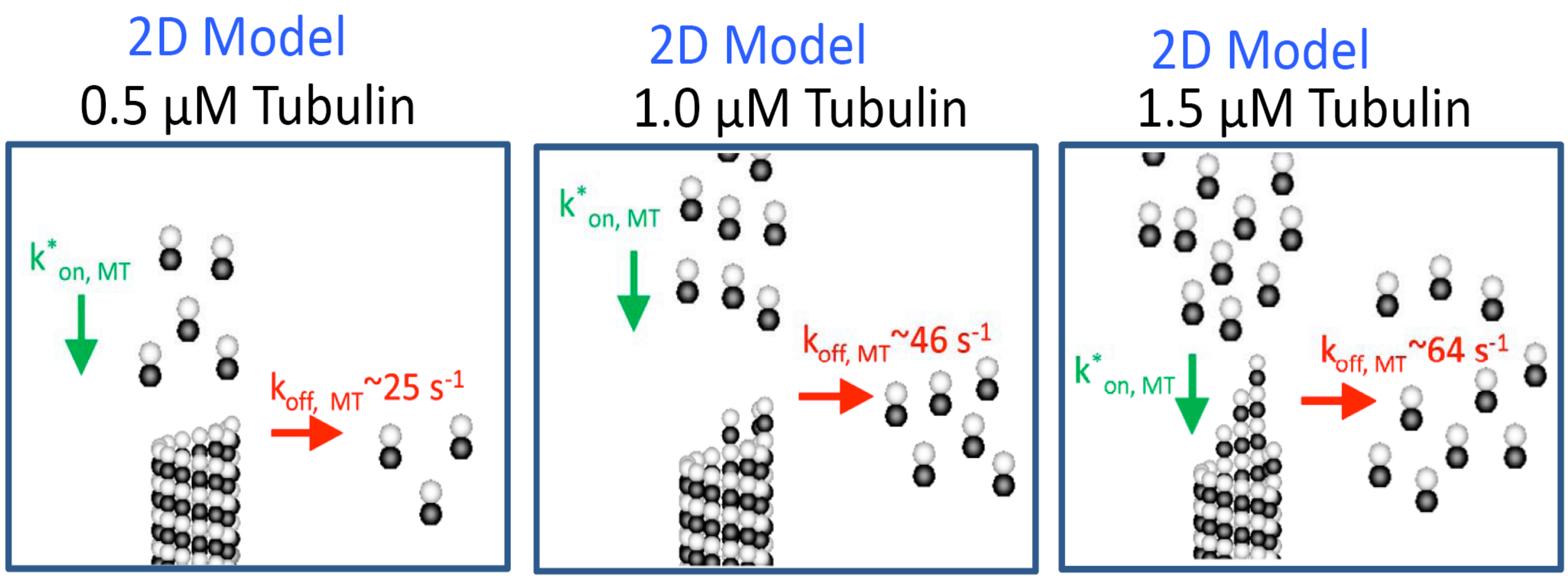
$$\langle k_{off,\ PF}\rangle = \alpha_2 k_{off,\ PF}^{(2)} + \alpha_1 k_{off,\ PF}^{(1)} + \alpha_0 k_{off,\ PF}^{(0)}$$

$$k_{off,\ MT} = N_{PF}\langle k_{off,\ PF}\rangle$$



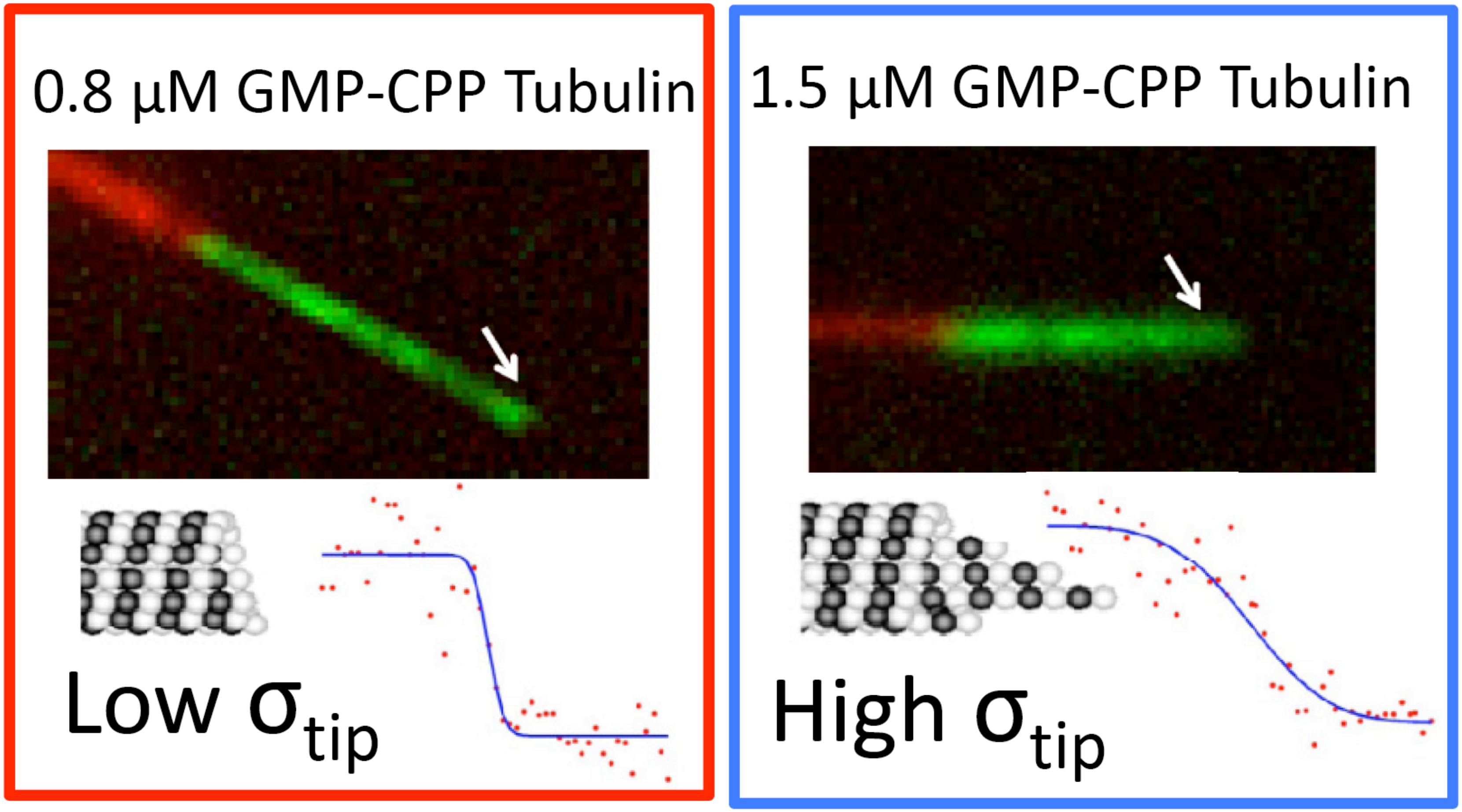
Gardner et al., Cell, in press

2D Model: MT tips are more tapered at higher tubulin concentrations



Gardner et al., Cell, in press

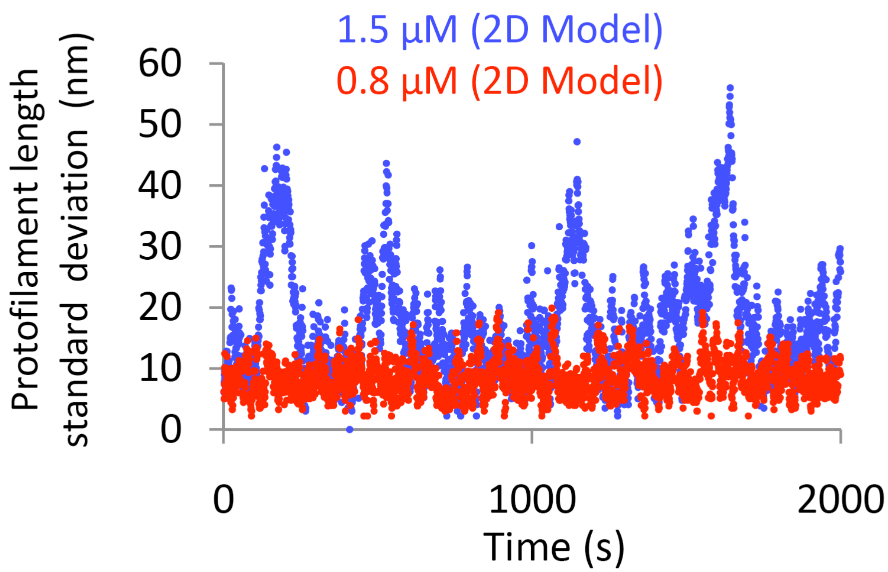
TIRF: Measuring Tip Structure Variance

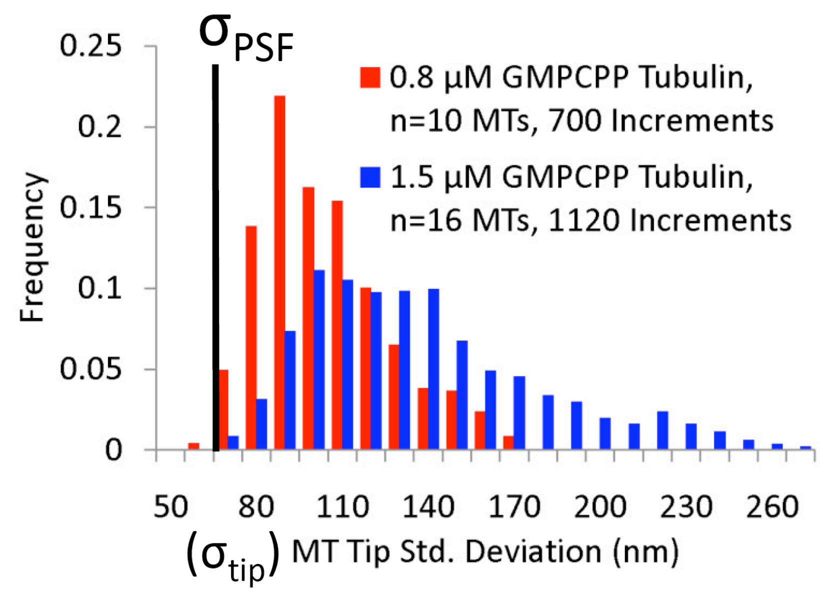


Demchouk et al., Cellular and Molecular Bioengineering, 2011

Gardner et al., Cell, in press

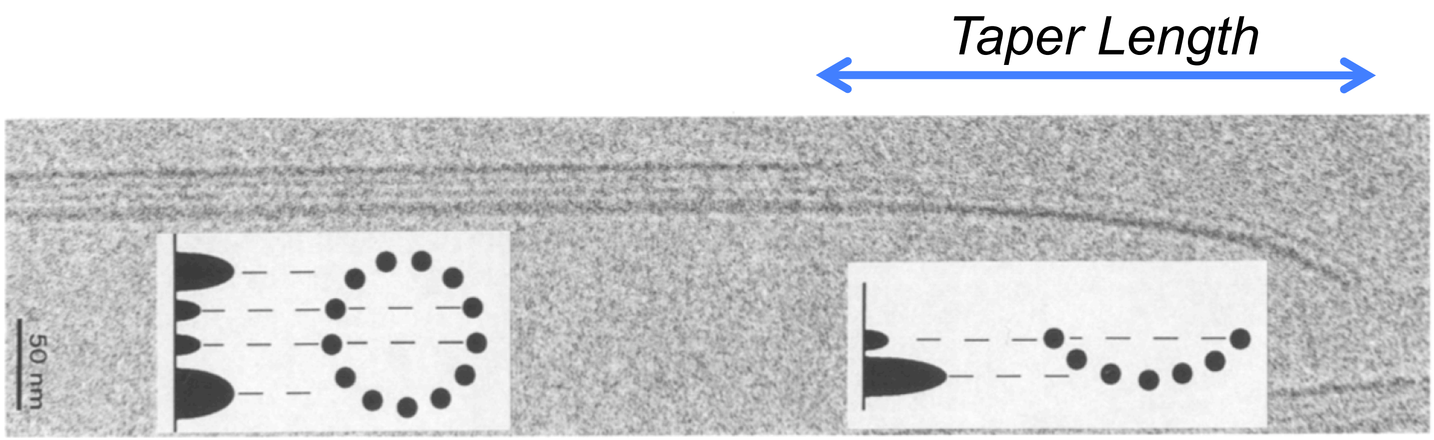
TIRF: Measuring Tip Structure Variance

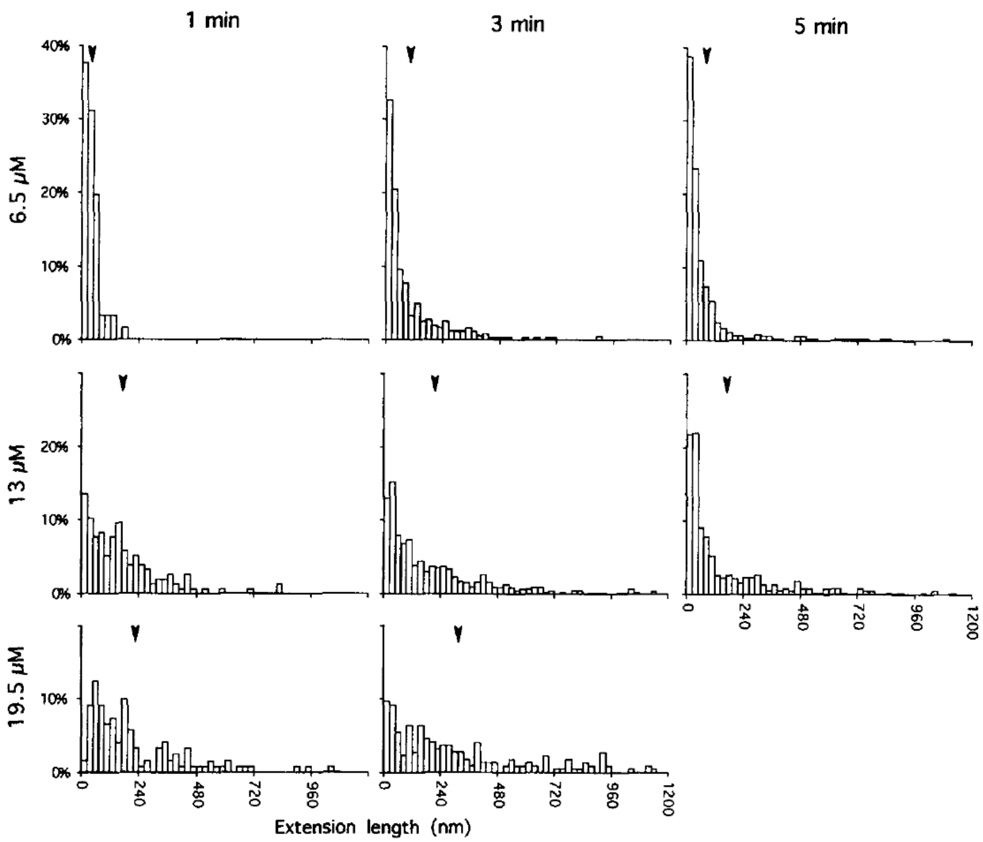




Protofilament lengths are <u>more variable</u> at higher tubulin concentrations, consistent with 2D model

Gardner et al., Cell, in press

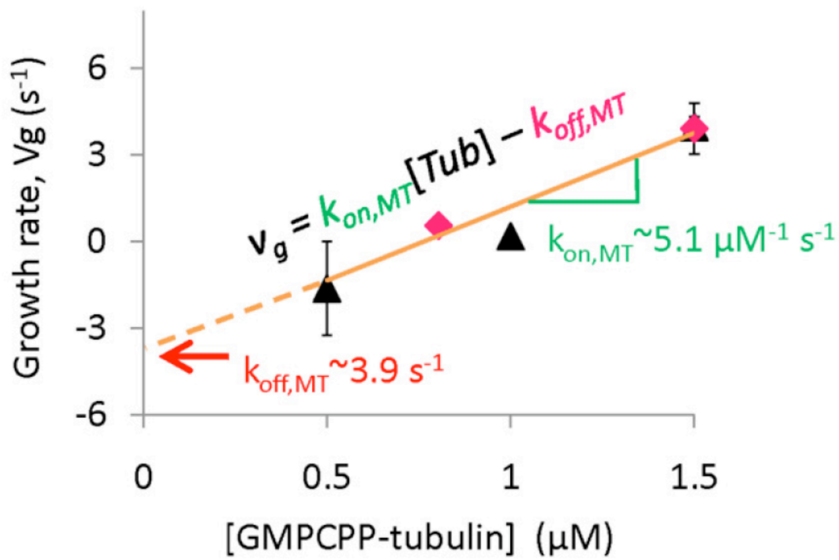


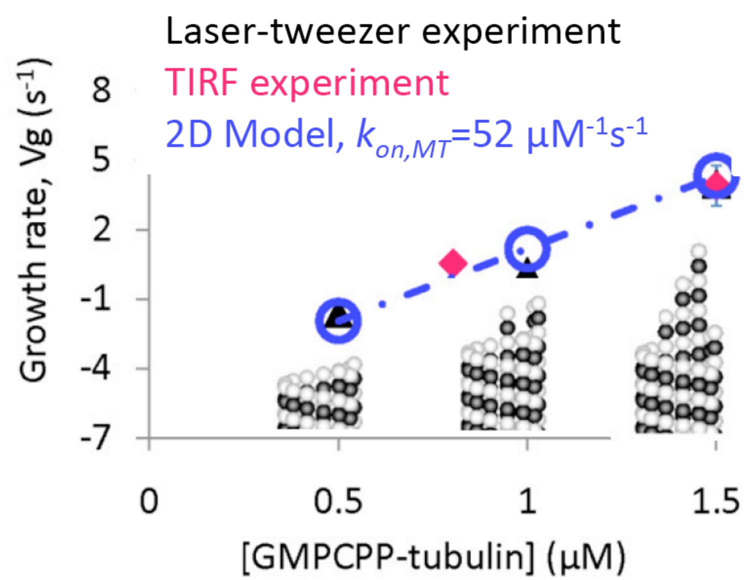


Taper length increases with increasing [tubulin]

Chretien et al., J Cell Biology, 1995

Net growth rate:
1D model v. 2D model





<u>At 1 μM GMPCPP-tubulin:</u> 1D model

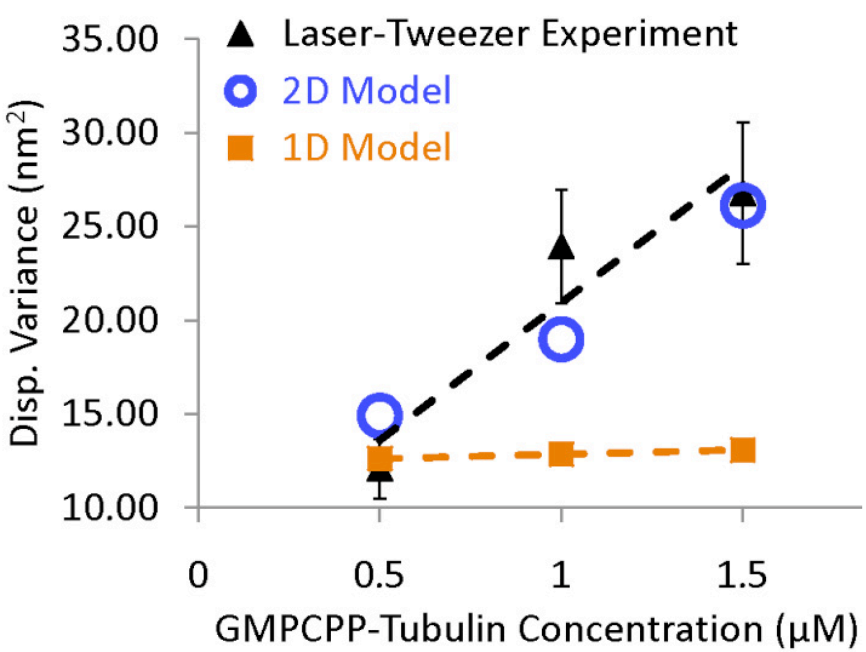
k_{on}^*	$= k_{on}$[tub] =	+5 s^{-1}
k_{off}	=	- 4 s^{-1}
net	=	+1 s^{-1}

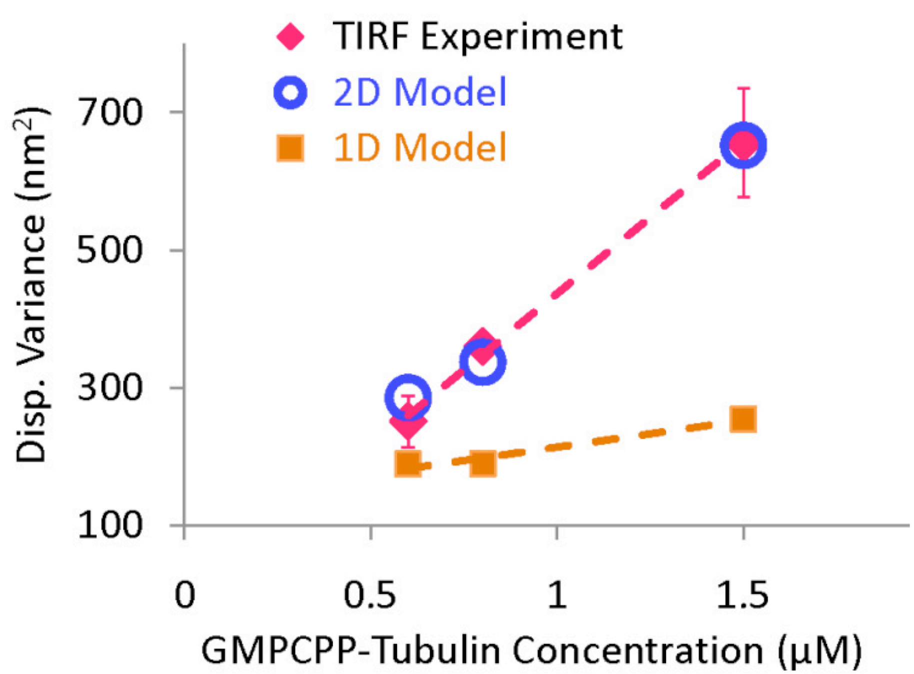
<u>At 1 μM GMPCPP-tubulin:</u> 2D model

k_{on}^*	$= k_{on}$[tub] =	+52 s^{-1}
k_{off}	=	- 51 s^{-1}
net	=	+1 s^{-1}

Gardner et al., Cell, in press

Growth rate variance:
1D model v. 2D model





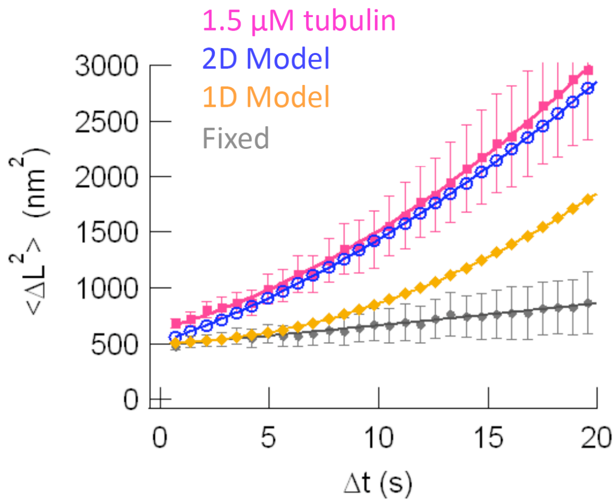
Gardner et al., Cell, in press

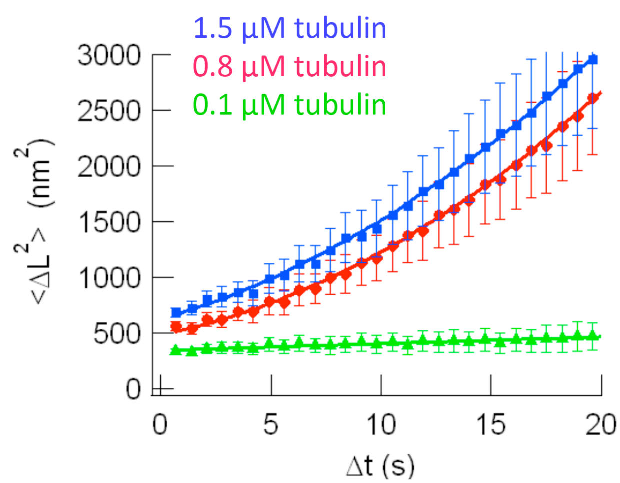
Estimation of GMPCPP-tubulin on and off rates

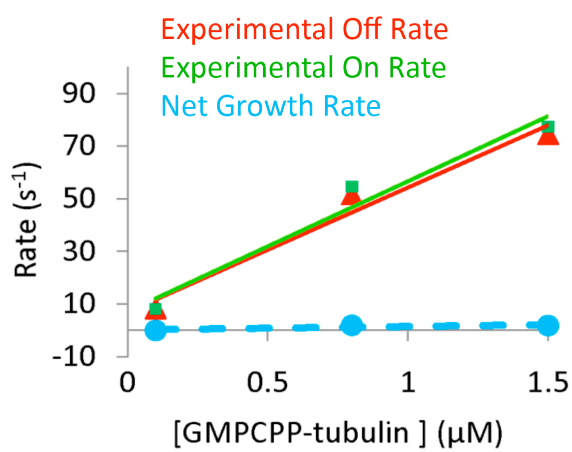
$$\langle \Delta L^2 \rangle = \sigma_0 + 2Dt + v^2 t^2$$

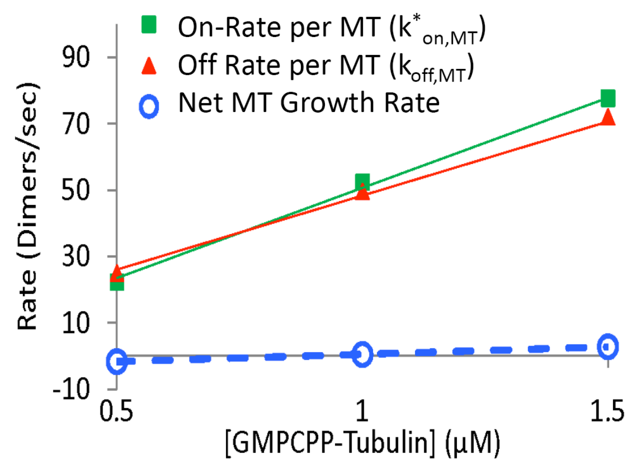
$$D = a^2 (k_{on}^* + k_{off})/2$$

$$v = k_{on}^* - k_{off}$$

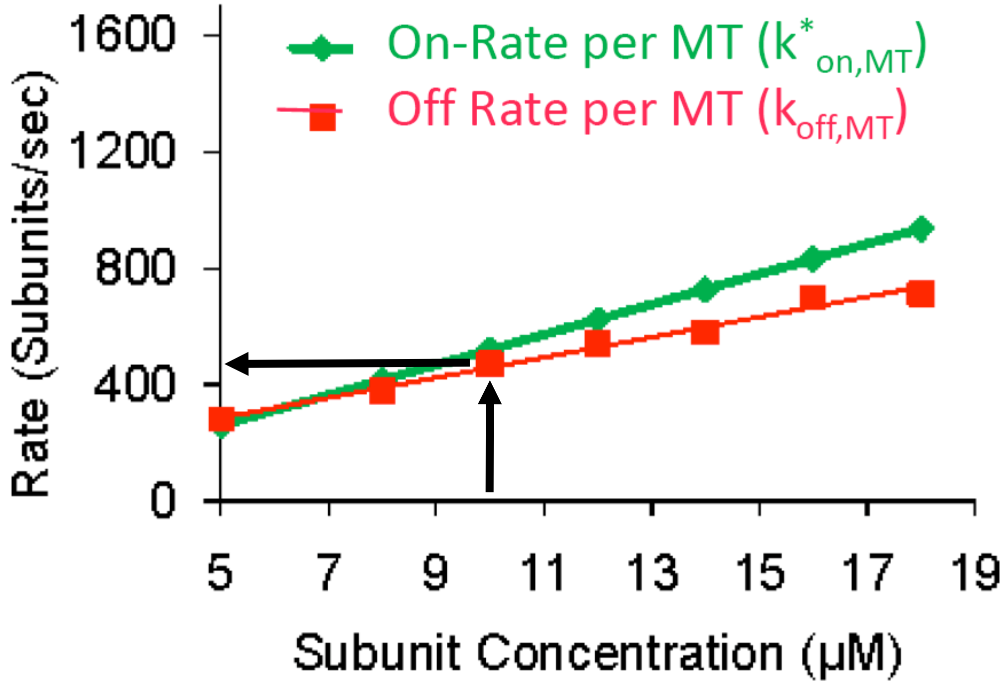








Predicted near 1 kHz kinetics of GTP-tubulin MT assembly in vitro



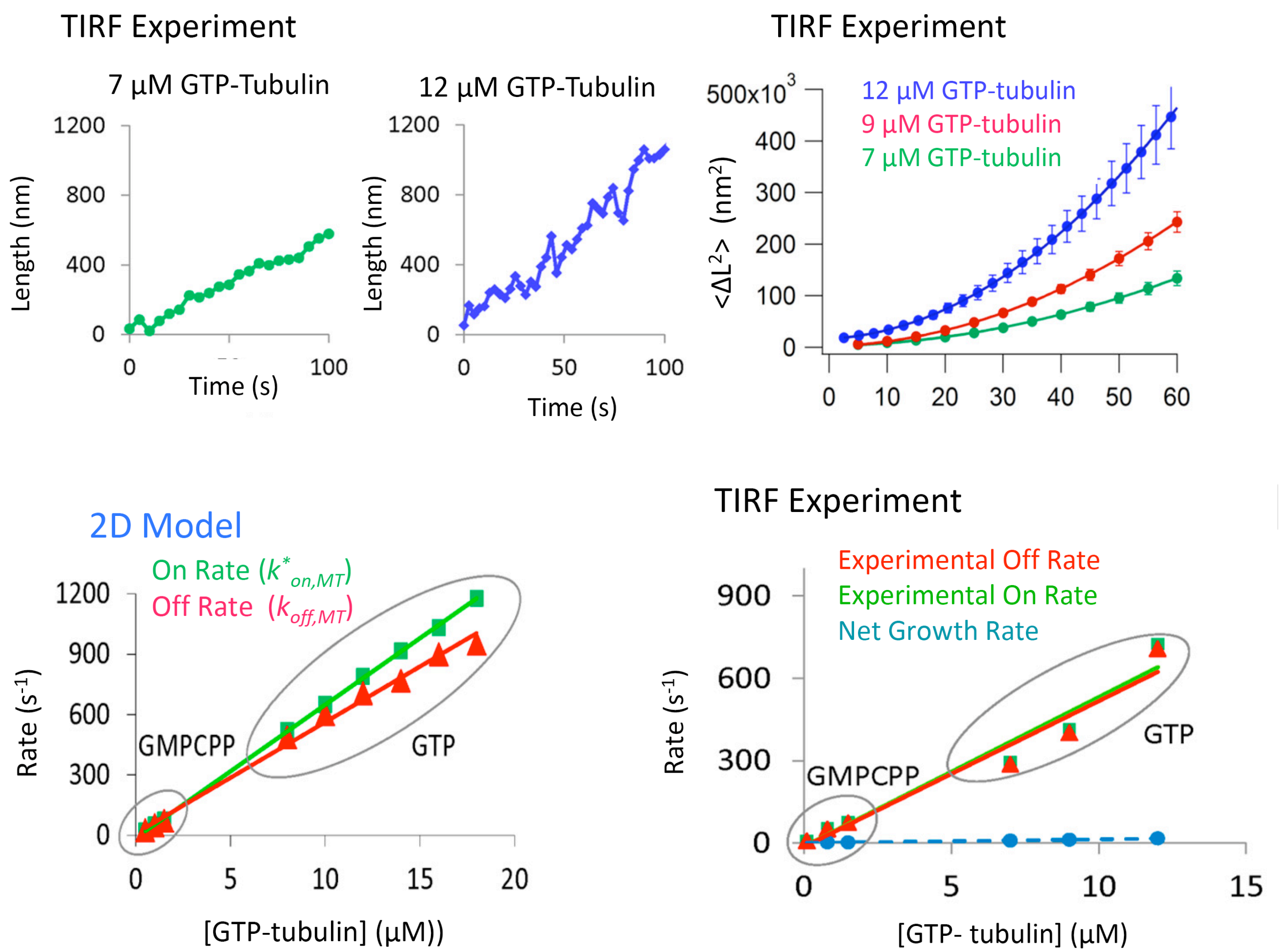
At 10 μM GTP-tubulin: 2D model

k_{on}^*	$= k_{on}[tub] =$	$+520\ s^{-1}$
k_{off}	=	$-\ 480\ s^{-1}$
net	=	$+40\ s^{-1}$

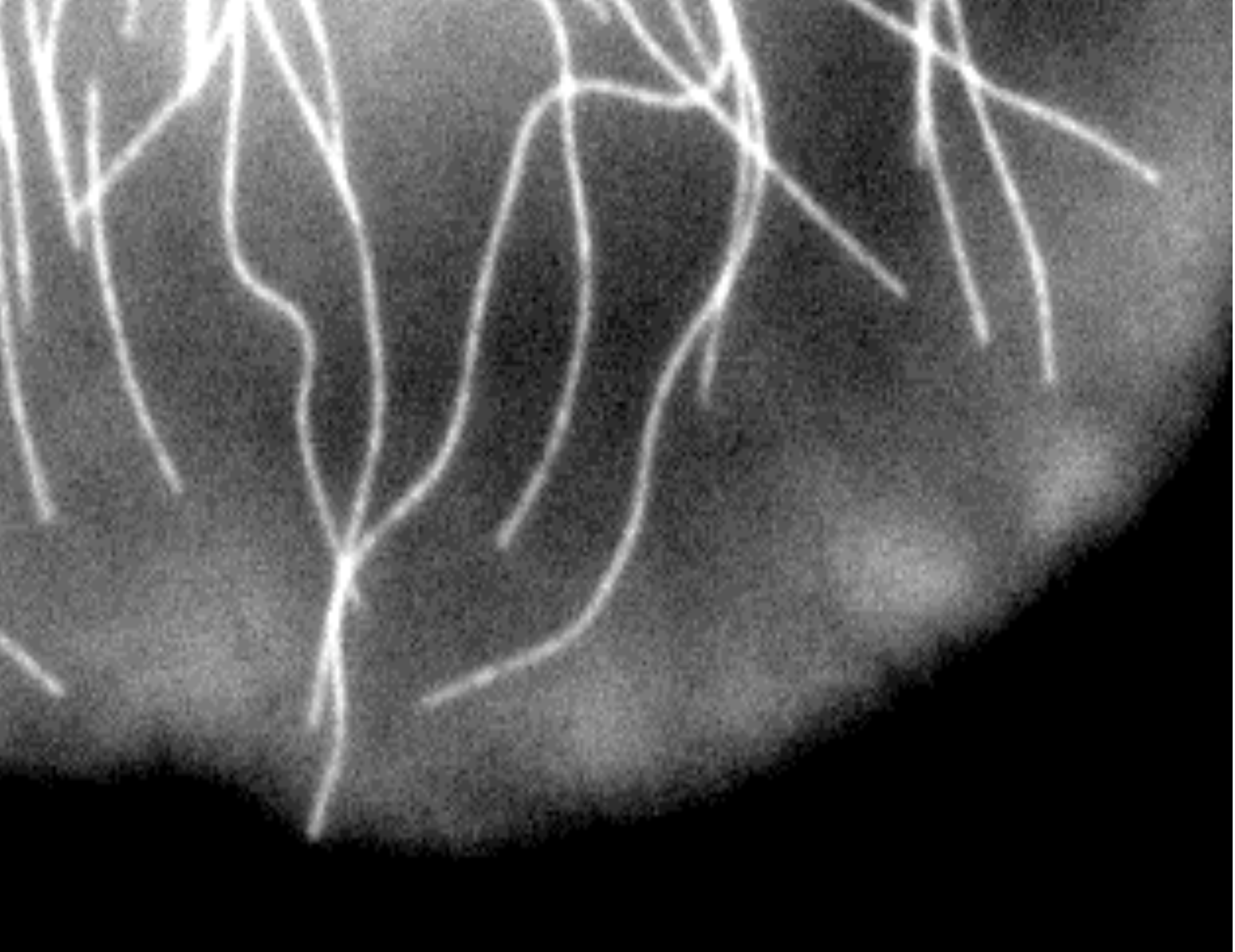
$520\ s^{-1} + 480\ s^{-1} = 1000$ events/s = 1 kHz

Gardner et al., Cell, in press

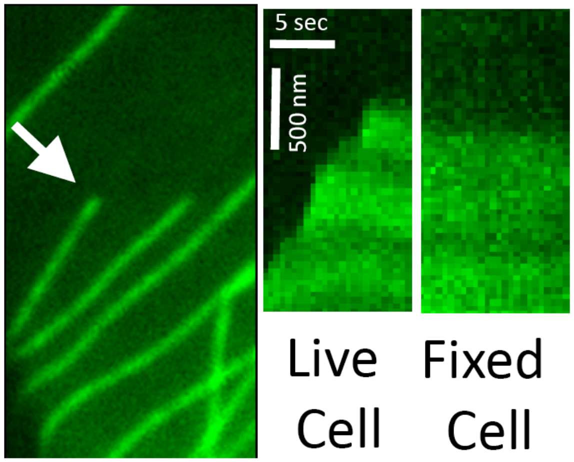
Kinetics of GTP-tubulin in vitro

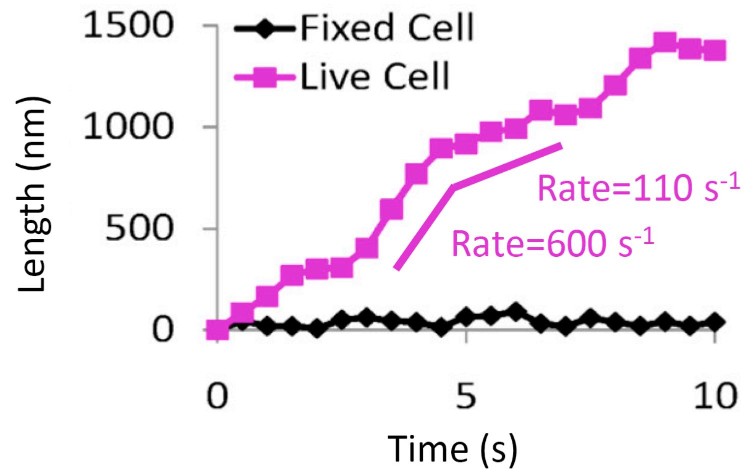


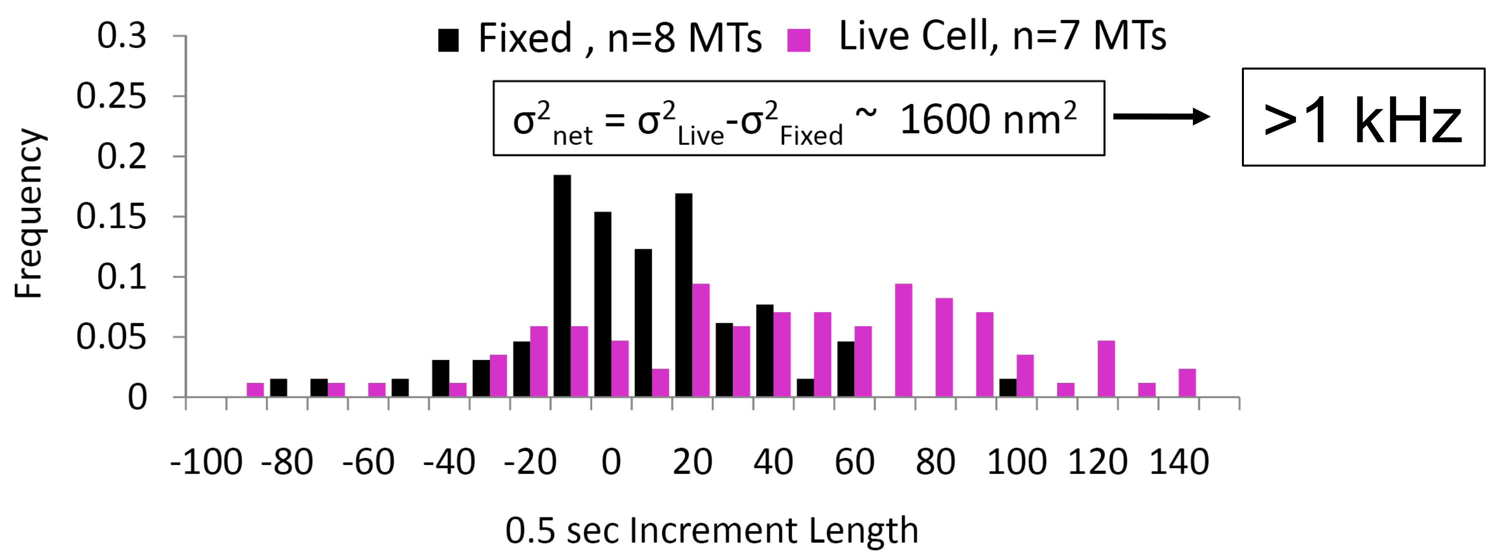
Gardner et al., Cell, in press



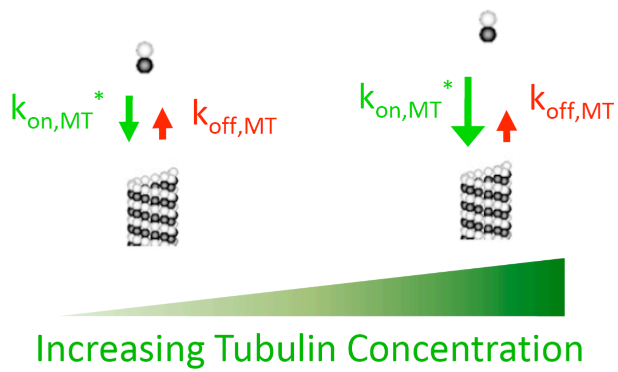
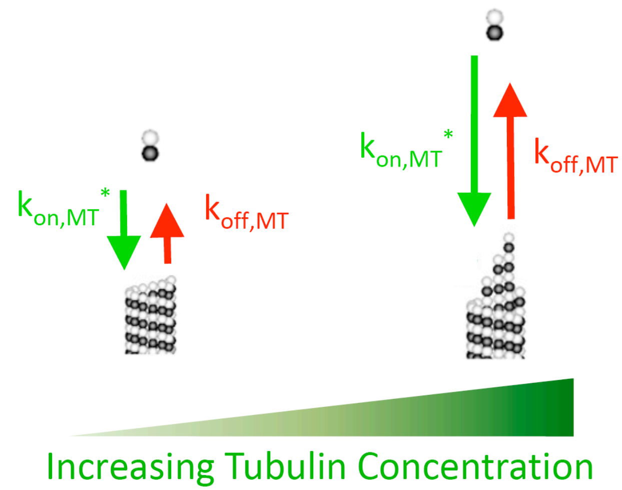
In Vivo Kinetics of MT Assembly







The Kinetics of Microtubule Assembly

<table>
<tr><th></th><th>Previously</th><th>This Study</th></tr>
<tr><td>$k_{on,MT}$</td><td>~5 $\mu M^{-1}s^{-1}$</td><td>~58 ± 4 $\mu M^{-1}s^{-1}$</td></tr>
<tr><td>$k_{off,MT}$</td><td>Constant</td><td>Concentration Dependent</td></tr>
<tr><td>Kinetics</td><td>Slow Kinetics

</td><td>Rapid Tip-State Dependent Kinetics

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Gardner et al., Cell, in press

Conclusions

- 1D model (Oosawa) is problematic when applied to *multiprotofilament* polymers: *k_{off} is not constant*
- 1D model parameters are not physically meaningful
- 2D model captures mean and variance with consistent parameters, and predicts tip structures
- Tubulin on and off rates are 10-fold higher than previously estimated
- Tubulin on and off rates are *nearly equal* at all tubulin concentrations
- Rates of addition-loss at 10 μM GTP-tubulin are estimated to be ~1 kHz
- In vivo kinetics are at least as rapid as in vitro kinetics (>1 kHz)
- **Regulation via MAPs and drugs requires only weak or infrequent alteration of off-rate via alteration of lateral or longitudinal bonds**