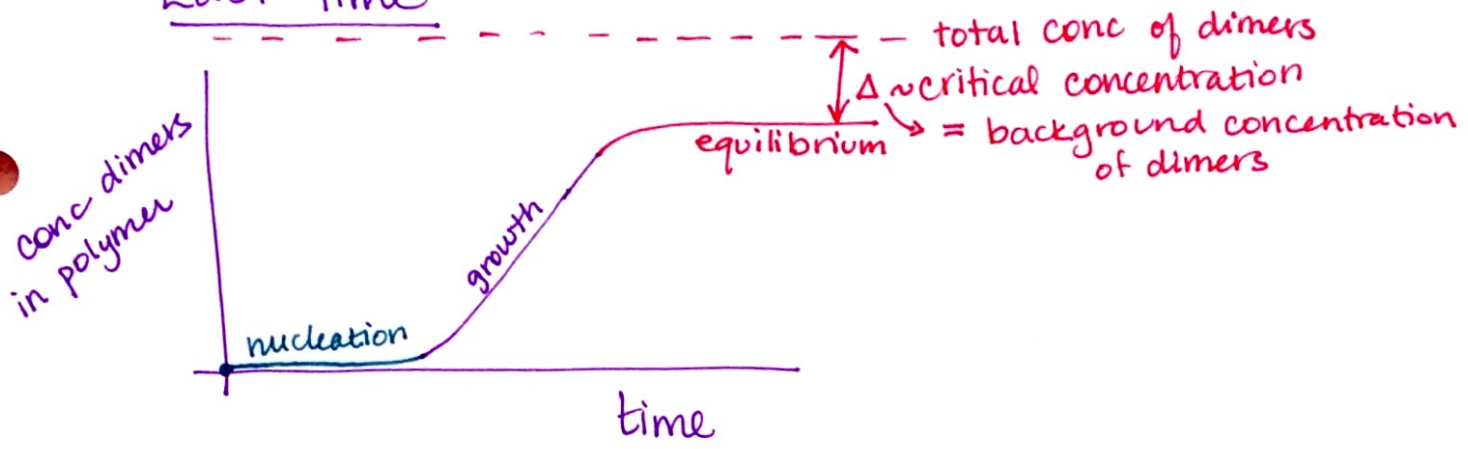
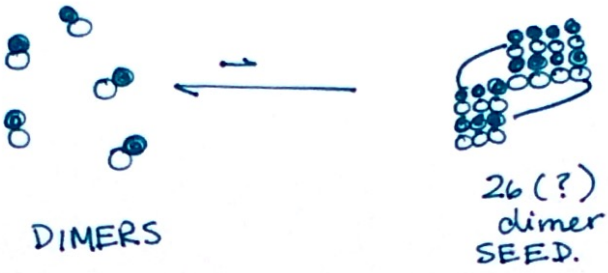
Last Time

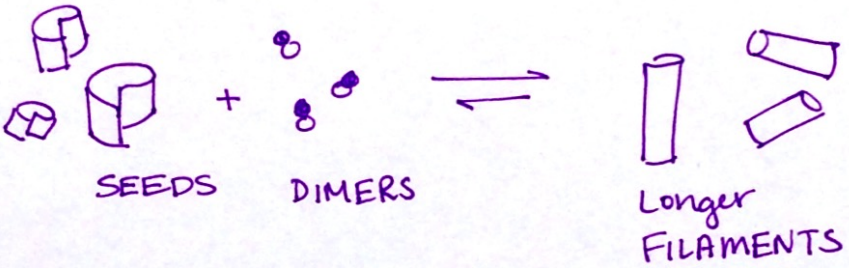


Draw pictures:

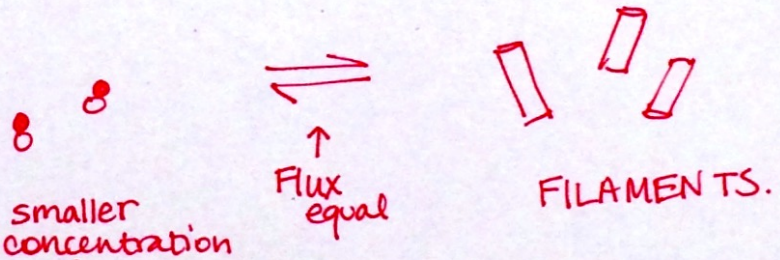
NUCLEATION:



GROWTH:



EQUILIBRIUM



In equilibrium, flux of $\frac{\#\text{ dimers polymering}}{\#\text{ dimers depolymerizing}} = 1$

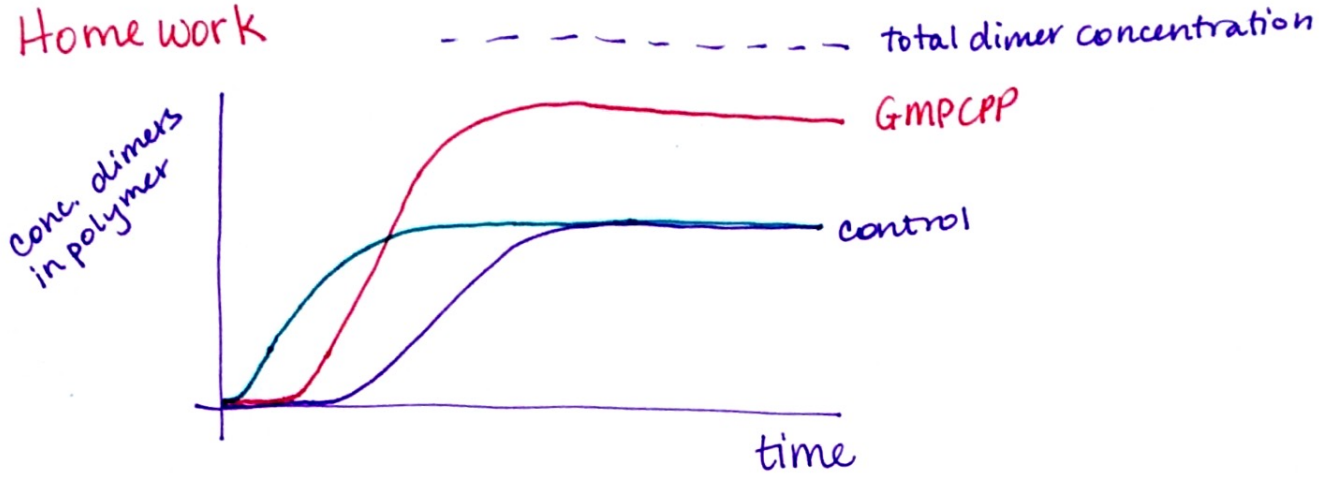
Dimers cannot come from Middle, they must come from ends.

+ dimers are not covalently bound

⇒ Dimers lost / added from ends

↳ dynamic instability

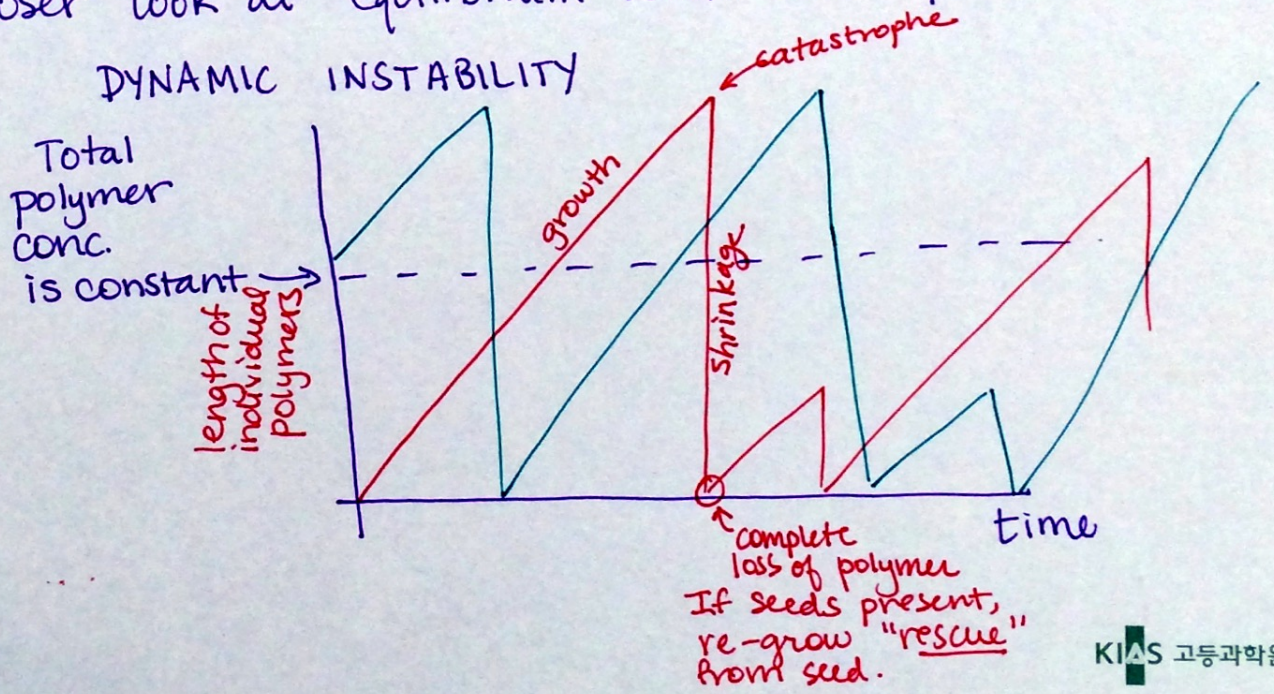
Home work



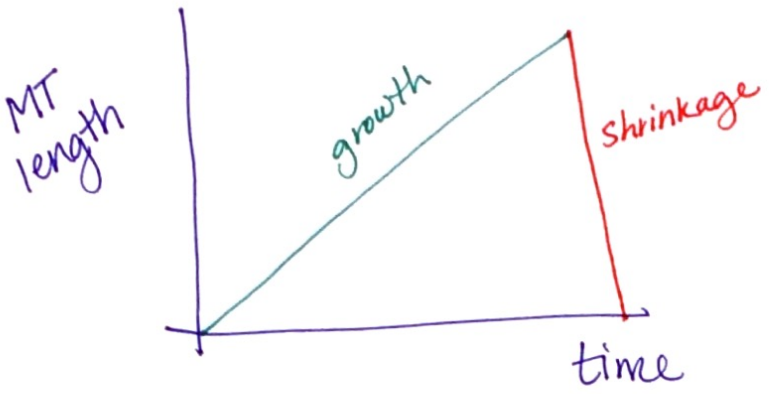
1. Use GMPCPP, no hydrolysis ⇒ polymer stability.
   ⇒ Seed stability ⇒ reduces nucleation time
   ⇒ filament stability → increases conc of dimers in filaments in equilibrium
   ⇒ decreases background concentration of dimers.
   $\Delta_{GMPCPP} < \Delta_{control}$

2. Add seeds
   ⇒ reduces nucleation requirement
   Does not alter the total polymer amt b/c filaments not extra stable.

Closer look at equilibrium when GTP present



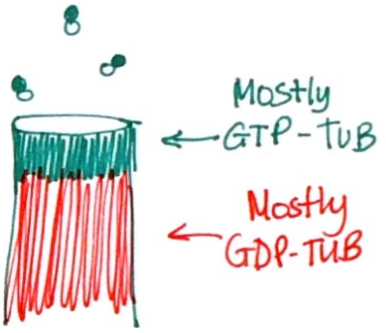
Dynamic Instability - snap shots



Growing Ends

~~Fast Growth~~

Slow Growth : blunt ends



Mostly GTP at top, called GTP-CAP

Fast Growth : assymmetric ends

some curling in growth and shrinkage

sheets

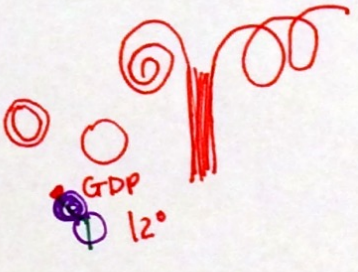
← tapering ends

Shrinking Ends :

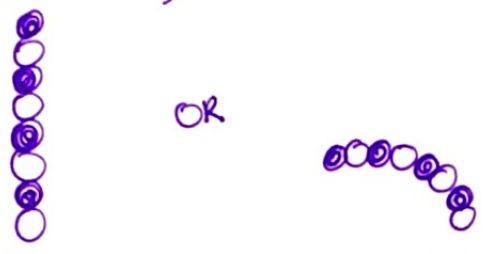
Slow shrink :

← protofilaments peel back

Fast shrink :



If you had a single protofilament, as a precursor, what would it look like?



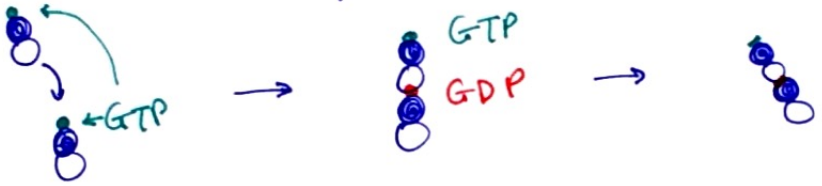
(1) (2)

Depends on nucleotide:

If you use GMPCPP → (1)

GTP → (2)

Remember, GTP → GDP + P_i hydrolysis is catalyzed by next dimer binding



Rate of hydrolysis is probalistic.

Although, binding of next dimer catalyzes rxn, it cannot be instantaneous.

Models with stochastic hydrolysis with rates of ~~0.3/s~~ 0.95 per molecule per second mimicked dynamics

{VanBuren et al PNAS 2002}

The rate of hydrolysis affects the size of the GTP-cap

slow GTP hydrolysis ⇒ large cap.

fast GTP hydrolysis ⇒ small, no cap.

Since each filament is growing and shrinking, how can we predict or model the kinetics?

1970 Fumio Oosawa J. Theoretical Biology
"Size Distribution of Protein Polymers"

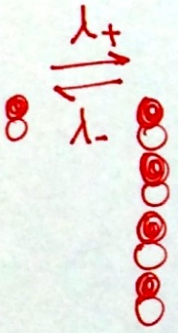
Model is the gold standard to explain actin and microtubule polymerization for 40 years.

We will discuss it and then debunk it.

* Still works for actin, but assumptions wrong for microtubules.

Oosawa model

Effectively a 1D filament model



$$\lambda_+ \equiv \underbrace{k_{on,MT}}_{\text{on rate in } \frac{1}{Ms}} \underbrace{[TUB]}_{\text{tubulin concentration}} \Delta t$$

← time of association

Flux of dimers associating

$$\lambda_- \equiv \underbrace{k_{off,MT}}_{\text{off rate in } \frac{1}{s}} \Delta t$$

Flux of dimers dissociating

$$K_{eq} \equiv \frac{k_{on}}{k_{off}} \quad \text{units } \frac{1}{M}$$

λ_+ = # dimers arriving

λ_- = # dimers leaving

$\{\lambda_+ - \lambda_-\}$ is the distribution of incremental length changes

Assumptions and measureables

$$\frac{dN}{dt} = k_{on}[TUB] - k_{off}$$

- $\frac{dN}{dt}$: rate of dimer adding
- $k_{on}[TUB]$: # dimers adding flux
- k_{off}: # dimers leaving flux

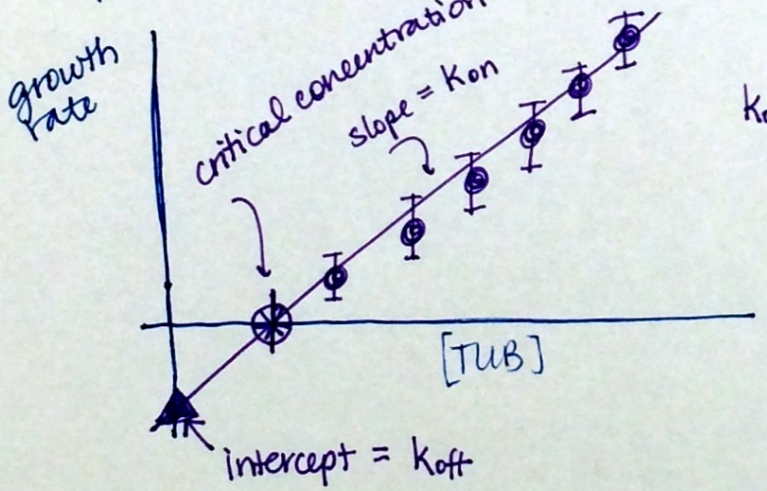
measureable: growth velocity V_g

$V_g \propto \frac{dN}{dt}$ in fact $V_g = a\frac{dN}{dt}$

a: characteristic length scale helical structure

$a \cong 0.6$ nm because of (on average) Because $8\text{nm} \times 1/13 = 0.615\text{nm}$

Measure growth velocity as a function of [TUB], fit to a line, and find slope and intercept:



$k_{on}[TUB] - k_{off} = V_g$ ← in $\frac{1}{s}$

Activity: Use the data at your desk and the Oosawa model to estimate k_{on}, k_{off}, and the critical concentration for the experiments of MT dynamics

	DATA L	DATA M	DATA N	DATA O
Obrien 1990				
Tubulin Concentration (uM)	Tubulin Plus End + 6mM Mg Growth Rate (um/min)	Tubulin Plus End + 0.25mM Mg Growth Rate (um/min)	Tubulin Minus End + 6mM Mg Growth Rate (um/min)	Tubulin Minus End + 0.25 mM Mg Growth Rate (um/min)
7		1.2		0.8
10	2.8	1.2	1.2	0.8
13	3.6	2	1.4	1
14.5	3.2	2.2	1.5	0.95
16	4.2		1.8	
19	5.2	3	2.1	1.8
22	6.8	4.5	4	2.8
24	7.1	5.1	4	3.1

	DATA P	DATA Q
Brouhard, Cell 2008		
Tubulin Concentration (uM)	Tubulin alone Growth Rate (um/min)	Tubulin + XMAP215 Growth Rate (um/min)
4		3
5		4
6		4.5
7	1	5
8		6.25
10	1.5	
13	2	

Hyman 1992 GMPCPP	
Tubulin Concentration (uM)	Tubulin GMPCPP Growth Rate (um/min)
0.25	0.06
0.25	0.045
0.25	0.04
0.5	0.11
0.5	0.12
0.5	0.125
0.75	0.15
0.75	0.16
0.75	0.1
1	0.225
1	0.2
1	0.15

DATA R

	DATA S	DATA T
Mitchison 1984		
Tubulin Concentration (uM)	Tubulin Alone PlusEnd Growth Rate (um/min)	Tubulin Alone MinusEnd Growth Rate (um/min)
3	0.5	0
4	0.7	0.2
5	0.8	0.3
7	1	0.4
10	1.1	0.5
15	2	0.6
20	2.2	0.6
27	4	1
34	5	1.7
45	6	2.1
55	7	2.8

	DATA A	DATA B
Bergen 1980		
Tubulin Concentration (uM)	MT PlusEnd Growth Rate (um/min)	MT Minus End Growth Rate (um/min)
4.545454545	0.5	0.2
6.181818182	1.2	0.4
7.272727273	1.7	0.5
9.090909091	2	0.7

	DATA C	DATA D
Walker 1988		
Tubulin Concentration (uM)	MT PlusEnd Growth Rate (um/min)	MT Minus End Growth Rate (um/min)
7	0.8	0.3
8	0.9	
8.5	0.9	
8.75	1.1	0.5
9	1.2	0.5
9.5	1.5	0.8
10	2	0.8
10.5	1.8	0.75
11	1.9	0.7
11.5	1.9	0.9
12	2.1	0.85
12	2.6	0.8
12.5	2.5	1
13	3	1.4
14	2.5	1.3
15.5	3.5	1.5

	DATA E	DATA F	DATA G
Trinczek 1995			
Tubulin Concentration (uM)	Tubulin alone Growth Rate (um/min)	Tubulin:Tau 5:1	Tubulin:Tau 1:1
4	1.5		
4.5	2.2		
5	2.5		
5.5	3		
7	3.5		
8	4	2.1	
9	4.25	2.25	
10	5	2.5	1.4
11		3	1.5
12		3.5	1.9
13		3.75	2.1
14		4	2.15
15		4.1	3.5

DATA H

Table III. Variations of the Rates in the Growing and Shrinking Phases

	Tubulin concentration	Mean rate	Standard deviation	Coefficient of variation	Time window	Number of microtubules	Number of rates
	μM	$\mu m/min^{-1}$	$\mu m/min^{-1}$		s		
Growth	4.5	0.50	0.20	0.40	7.6	8	142
	6.5	0.78	0.37	0.48	9.3	10	192
	9.7	1.43	0.58	0.41	9.5	8	132
	13.0	2.18	0.92	0.42	7.6	7	97
	16.2	2.79	1.01	0.36	10.6	6	57
	19.5	3.51	0.90	0.26	13.1	11	110
Shrinkage	4.5	−28.0	5.8	0.21	1.2	7	39
	6.5	−34.9	6.1	0.17	1.9	6	36

Individual rate measurements were performed as indicated in Fig. 1 *b*. The mean rates and their associated standard deviations were calculated from the data given in the histograms of Fig. 2 (*b* and *c*). The coefficient of variation is expressed as the ratio of the standard deviation to the mean rate. The time window corresponds to the average time between two length measurements. The number of microtubules analyzed and the number of rate measurements performed are indicated in the two last columns.

DATA I

DATA J

Drechsel 1992		
Tubulin Concentration (uM)	Tubulin alone Growth Rate (um/min)	w/1uM Tau
0.5		0.2
1		0.4
2	0.2	0.8
3	0.4	1.2
5	0.6	
7	0.8	
9	1	

DATA K

Gard 1987	
Tubulin Concentration (uM)	Tubulin from Eggs Growth Rate (um/min)
0.25	0
0.5	0.5
0.75	1
1	1.5
1	2.25
1.25	2
1.5	4.5
1.5	4
1.5	3
2	5
2.5	3.5
2.75	6.5
3.5	4
4	7

Now! Let's tear Oosawa's theory apart and rebuild a better one!

Oosawa's theory made several assumptions

① All monomers are created equal.

⊛ This is the heart of the 1D nature.

We will see that this assumption leads to incorrect results/interpretations

We will need to treat different dimers differently, in particular, depending on where they bind.

② k_{off} is a constant with concentration of TUBULIN.

⊛ We will need $k_{off} \propto k_{on}$ for each protofilament. This follows from biochemistry:

$$K_{eq} = \frac{k_{on}}{k_{off}} = e^{-\Delta G^\circ_{total}/k_B T}$$

↖ Boltzman weighting of free energy of binding

ΔG°_{total} = total free energy of bonds

$k_B T$ = thermal energy

$$\Rightarrow k_{off} = k_{on} e^{\Delta G^\circ_{total}/k_B T}$$

so it ~~cannot~~ is not necessarily constant if k_{on} isn't

and k_{on} will depend on specific location of binding

Assumption of Oosawa Model:

③ All dimers at end have same free energy

$\Rightarrow$ prediction: Likelihood of large MT shortening events will <u>decrease</u> as the subunit arrival rate, λ_+, becomes larger

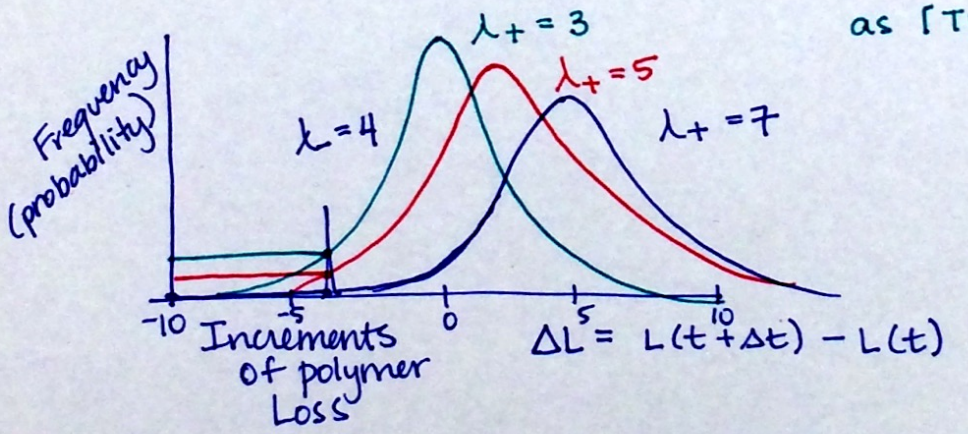
$\lambda_+ = k_{on,MT}\,[TUB]\,\Delta t$

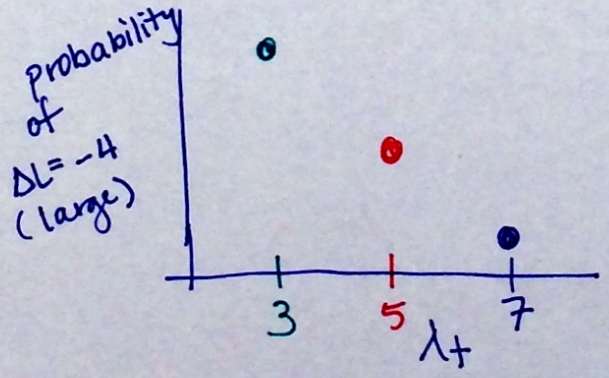
$\Rightarrow$ # large shortening events $\downarrow$ as $[TUB]\uparrow$

Why? This is because MT growth is favored when rate of subunit departure $k_{off,MT}$ remains constant

$\Rightarrow$ Probability of large loss of polymer decreases as [TUB] increases.

as $[TUB]\uparrow$, $\lambda_+\uparrow$

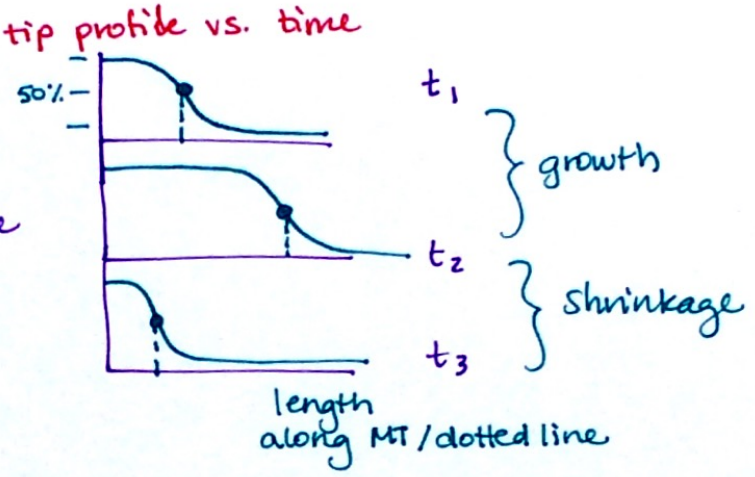




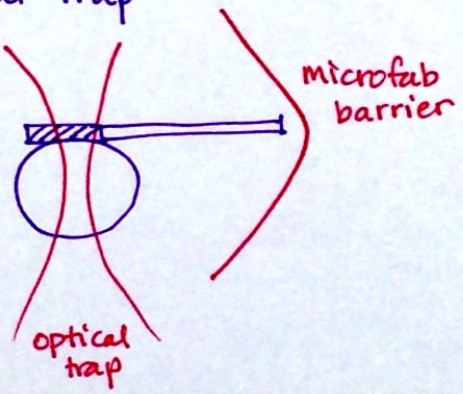
Test Model Using
① GMPCPP MT growth
② TIRF Microscopy w/ high resolution tip tracking
③ Optical trapping with force feedback.

TIRF

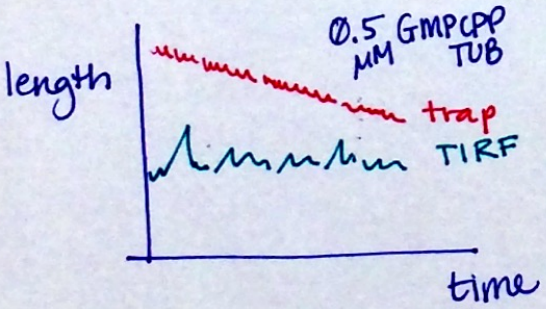
Image in TIRF vs. time

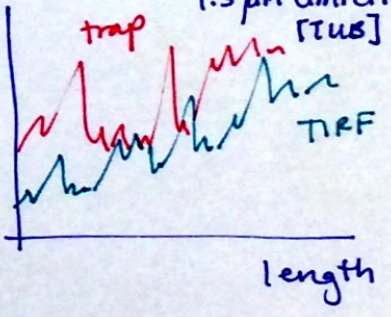


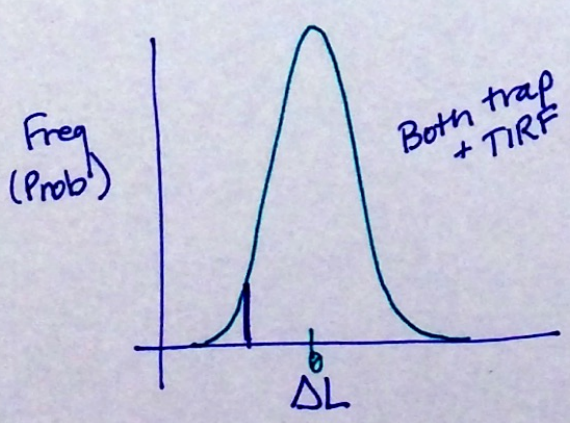
Optical Trap

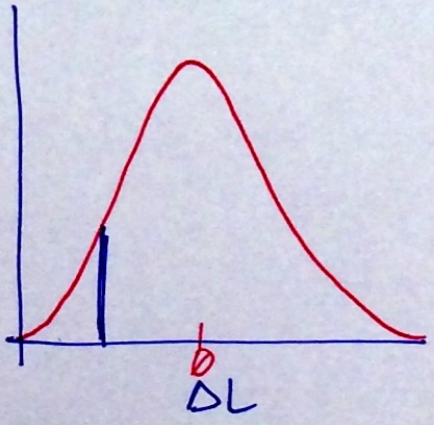


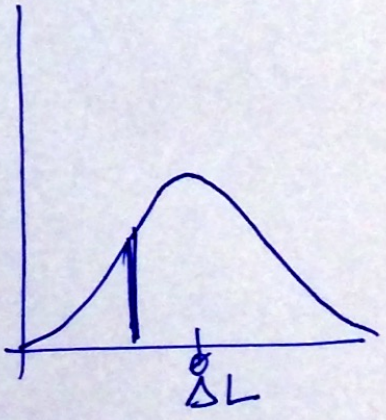
grow into barrier, measure
ΔF, fix constant ε by adjusting
Δx
measure x trap vs. time



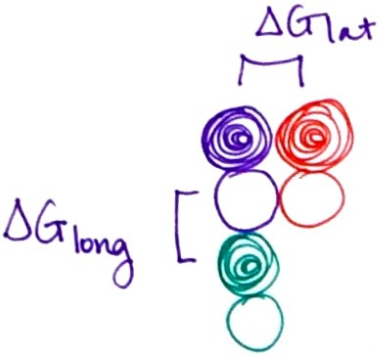








vanBuren 2002 predicted w/ 2D model.



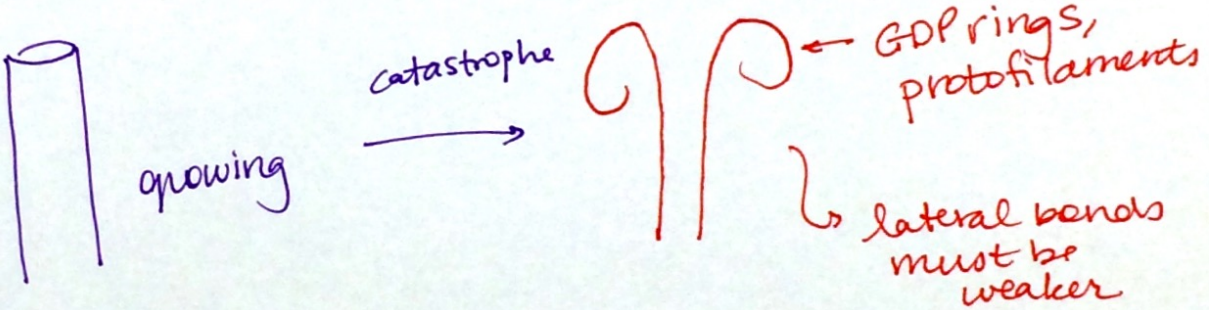
$\Delta G_{long} \sim -18.5 \text{ to } -27.8\ k_BT$

$\Delta G_{lat} \sim -3.2 \text{ to } -5.7\ k_BT$

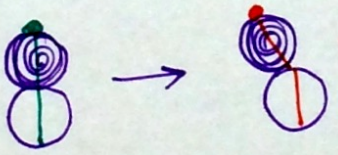
5-FOLD difference in strength

ΔG_{long} stronger than ΔG_{lat}

agrees with structural studies



Also estimated...
mechanical energy stored in dimer



$\sim 2.1 - 2.5\ k_BT$

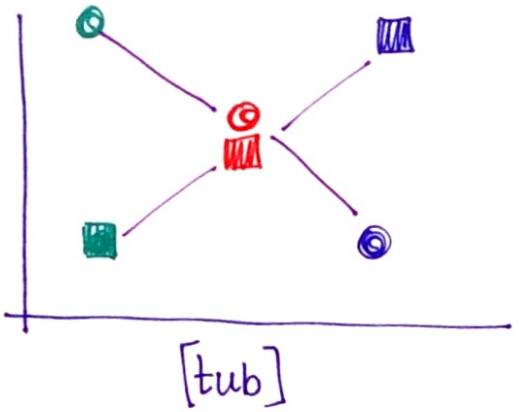
size of GTP cap at end depends on GTP-hydrolysis rate.
when use GTP hydrolysis rate that mimics exp't results,

GTP cap ~ 55 dimers ± 12 dimers (↑ standard deviation)

Theory prediction
Exp't

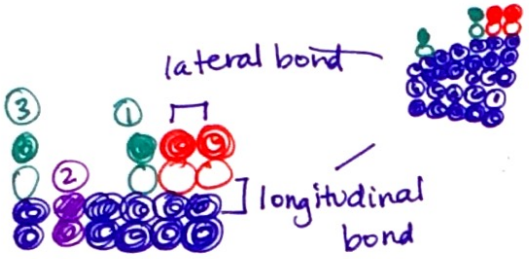
Exp't:

$P(\lambda_- = 4)$



[tub]

2-D Model:



Assumption: free energy of binding depends on lateral and longitudinal bonds.

① Binding of position (1) has 1 longitudinal bond + 1 lateral bond

② Bind of position (2) has 2 ~~longitudinal~~ lateral bonds + 1 longitudinal bond.

③ Binding @ position (3) has 1 longitudinal bond + ∅ lateral bonds.

⇒ These have different bond energies.

$$K_{eq} = \frac{k_{on}}{k_{off}} = \exp\left(-\Delta G^\circ_{total}/kT\right)$$

$$\Rightarrow k_{off} = \frac{k_{on}}{\exp\left(-\Delta G^\circ_{total}/kT\right)}$$

van Buren 2002 estimated where $\Delta G^\circ_{total} = \sum_i \Delta G^\circ_{longitudinal\ bonds} + \sum_i \Delta G^\circ_{lateral\ bonds}$

$\Delta G_{long} \sim -18.5 \to -27.8\ k_BT$

$\Delta G_{lat} \sim -3.2 \to -5.7\ k_BT$

$$\Rightarrow k_{off} \cong f_2 k_{off}^{(2)} + f_1 k_{off}^{(1)} + f_\emptyset k_{off}^{(\emptyset)}$$

$f_2, f_1, f_\emptyset$ = probabilities of dimers at tip w/ 2, 1, ∅ neighbors laterally

$k_{off}^{(2),(1),(\emptyset)}$ = off rates for same types of locations.

If assumptions of 1D Oosawa model are incorrect, than our calculated k_{on}, k_{off} are incorrect. $k_{on} \sim 5\ 1/\mu M \cdot s$, $k_{off} \sim 15\ 1/s$

Use 2D model to estimate k_{on}, k_{off}

$k_{on} \sim 52\ 1/\mu M \cdot s$

$k_{off} \sim 75\ 1/s$ @ $1.5\ \mu M$

$V_g \sim k_{on}[TUB] - k_{off}$ still

$5[TUB] - 15 = 52[TUB] - k_{off}$

$k_{off} - 15 = (52-5)[TUB]$

$k_{off} = (52-5)[TUB] + 15$

$[TUB] = 1.5\ \mu M$

$V_g \cong 3$

$3 = 52(1.5) - k_{off}$ $\quad k_{off} = 75\ 1/s$

Why? Because tip structure changes as V_g changes!

Same as what we saw at ~~beginning~~! beginning. Same / known since 1995.

What about GTP tubulin?

growth variability ↑

⇒ variance of ΔL ↑

on rate / off rate ↑ to ~ 600 1/s

Since $k_{off} \propto k_{on}$ and $k_{off} \sim k_{on}$

BOTH The on and off rates almost kHz

Implications

Prior work with MAPs (like Tau)
had to assume if V_g ↑, then k_{on} ↑

But this doesn't make physical sense

k_{on} is limited by diffusion.

Some MAPs bind multiple dimers
to incorporate oligomers.

TOG/XMAP215

But even this type of mechanism cannot
explain large ↑ of k_{on}...

2D model shows MAPs that ↑ V_g can be affected k_{off} only.

↓ k_{off} will shift to more dimer addion V_g ↑