# Non-equilibrium effects of 'hydrolysis': consequences on kinetics and size regulation of microtubules

**Dipjyoti Das**

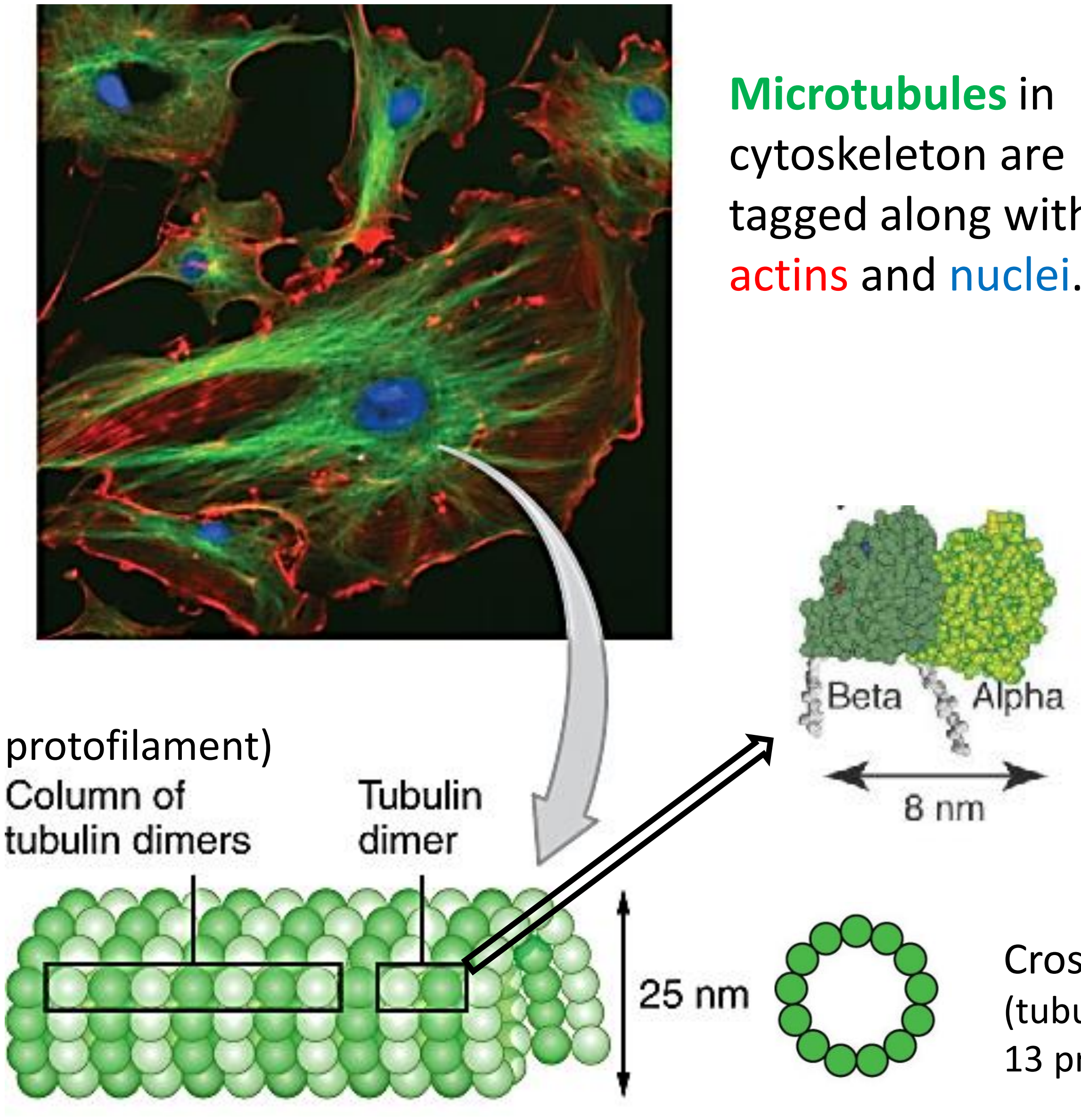
**Department of Biological Sciences**

**Indian Institute of Science Education and Research (IISER) Kolkata**

(Dec 11, 2020)

IISER KOLKATA

# Microtubules: Structure




**Microtubules** in cytoskeleton are tagged along with actins and nuclei.

(a protofilament)

The building-block of a MT

Cross-section (tubulin-ring from 13 protofilaments)

Source: http://rsb.info.nih.gov/ij/images/

# Microtubules: Some functions

- Structural rigidity

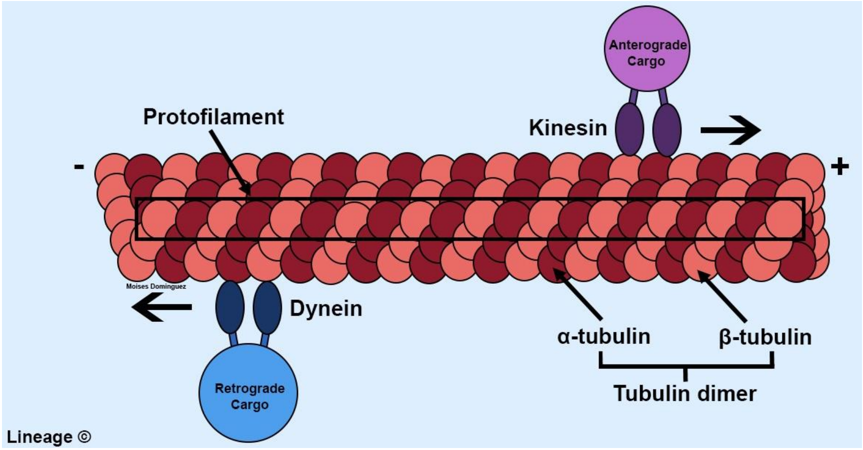
Persistence length ~ 1-5 mm ;  rigid over a cell dimension.
(Frederick Gittes et al., JCB, 1993; Howard, J. “Mechanics of motor proteins and the cytoskeleton”.)

# Microtubules: Some functions

- Structural rigidity

Persistence length ~ 1-5 mm ; rigid over a cell dimension.
(Frederick Gittes et al., JCB, 1993; Howard, J. “Mechanics of motor proteins and the cytoskeleton”.)

- Act as tracks for intracellular transport

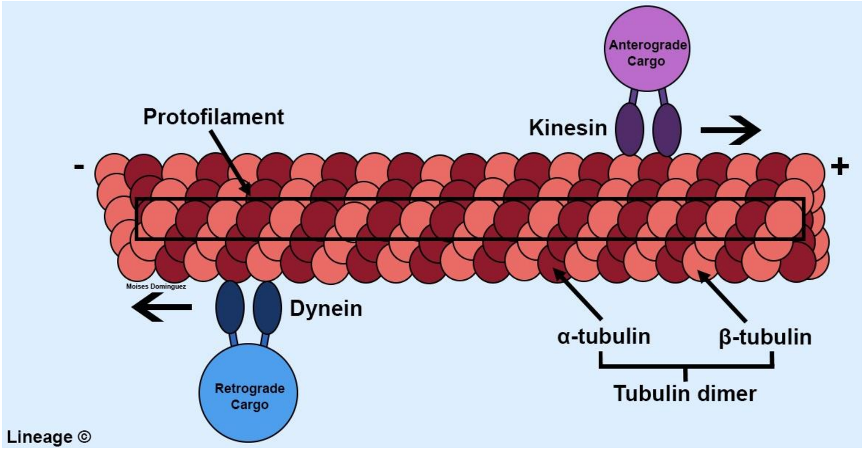


# Microtubules: Some functions

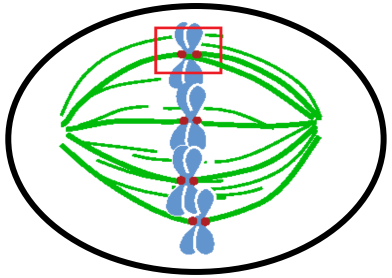
- Structural rigidity

Persistence length ~ 1-5 mm ;  rigid over a cell dimension.
(Frederick Gittes et al., JCB, 1993; Howard, J. “Mechanics of motor proteins and the cytoskeleton”.)

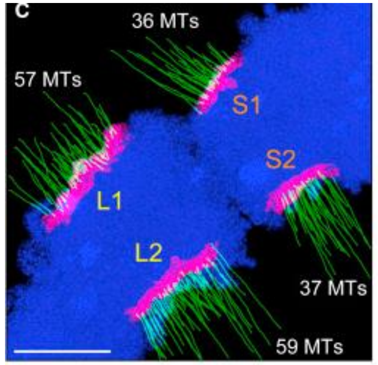
- Act as tracks for intracellular transport




- Help in chromosome segregation during cell division



During mitosis microtubules are attached to kinetochores on chromosomes.




(Danica Drpic et al., Curr Bio, 2018.)

# Stochastic kinetics of a microtubule (dynamic instability)

Length


Time


TIRF image at 12 μM tubulin concentration
(Gardner et al., Cell, 2011)

# Stochastic kinetics of a microtubule (dynamic instability)

Length


Time

Seed Dynamic MT

TIRF image at 12 μM tubulin concentration
(Gardner et al., Cell, 2011)

WHY 'dynamic instability' ? → **"Hydrolysis"**

GTP-tubulin → GDP-tubulin
hydrolysis

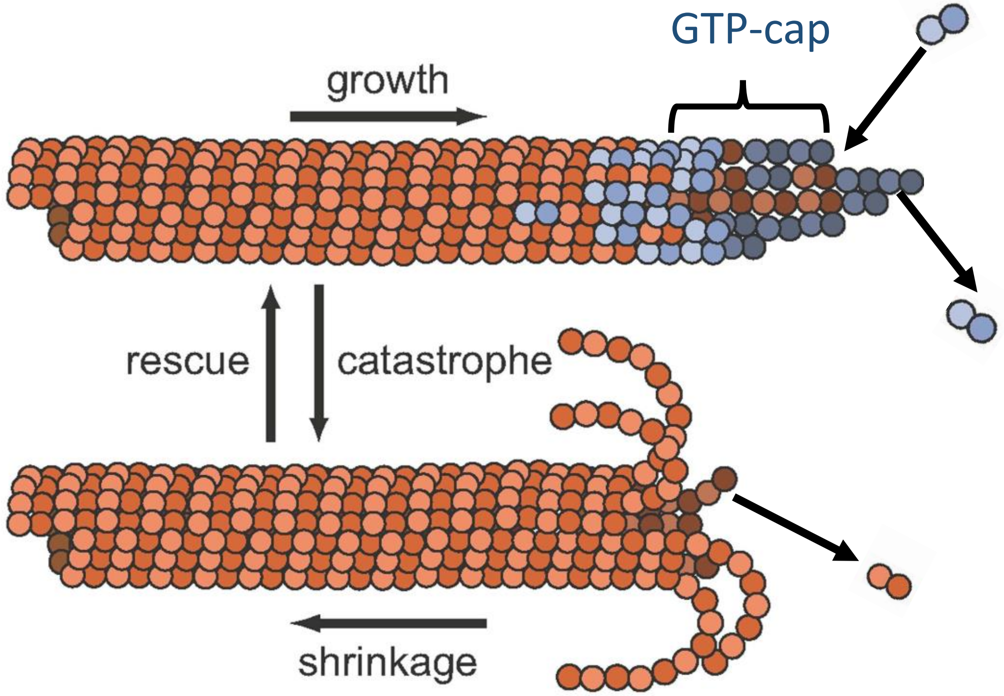
(A **mostly irreversible 'chemical switch'** on the MT lattice)

# Stochastic kinetics of a microtubule (dynamic instability)

Length


Time


TIRF image at 12 µM tubulin concentration (Gardner et al., Cell, 2011)




WHY 'dynamic instability' ? → **"Hydrolysis"**

GTP-tubulin → GDP-tubulin
hydrolysis

(A **mostly irreversible 'chemical switch'** on the MT lattice)

Depolymerization rate of GDP-tubulin >> Depolymerization of GTP-tubulin
**(290-700 /s)** **(~24 /s)**

# More on ‘hydrolysis’

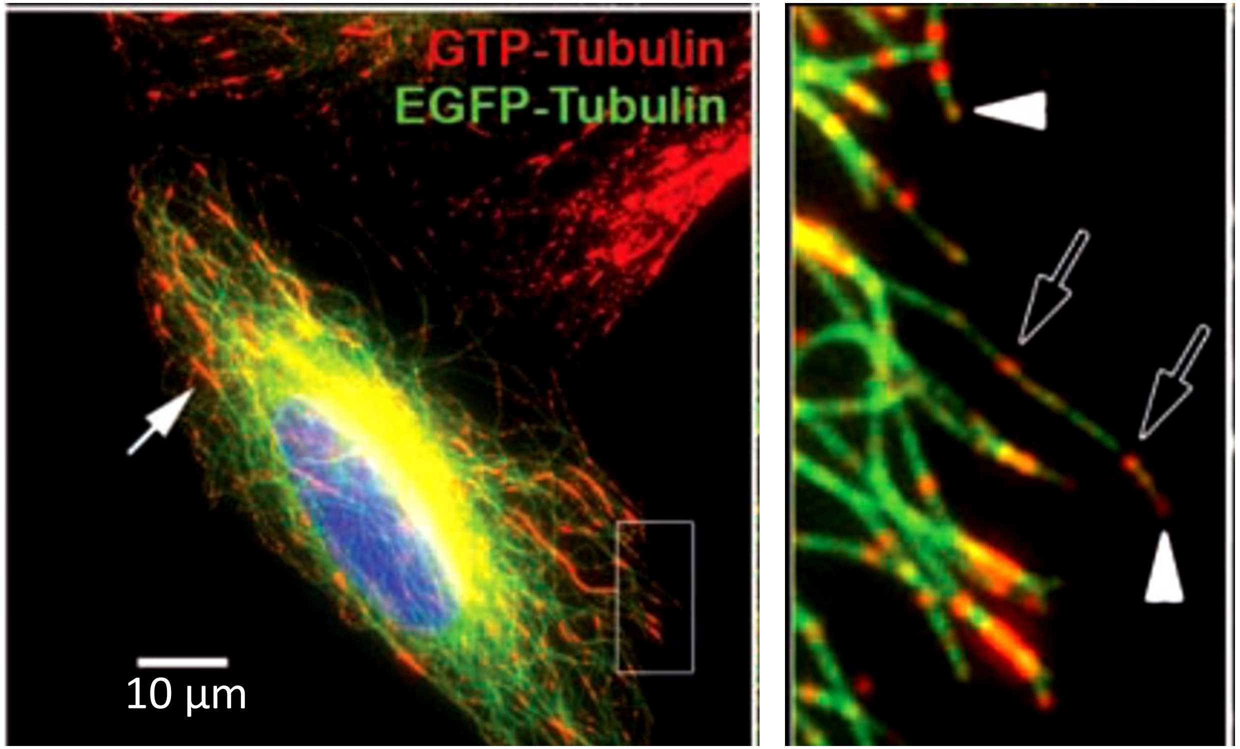
- Almost **non-hydrolysable tubulins (GMPCPP-tubulin) DO NOT show dynamic instability** (Mitchison, MBoC, 1992)

# More on 'hydrolysis'

- Almost **non-hydrolysable tubulins (GMPCPP-tubulin) DO NOT show dynamic instability** (Mitchison, MBoC, 1992)
- Hydrolysis takes place **randomly and irreversibly (nonequilibrium dynamics)**.

**GTP-tubulin 'islands' are seen in experiments.**
(A Dimitrov et al., Science, 2008)

Theory: Sumedha et al., PRE, 2011



# A nonequilibrium statistical physics perspective

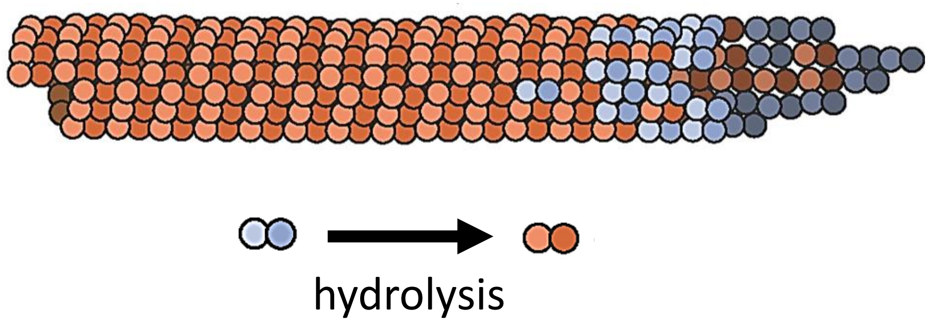
Random & irreversible hydrolysis can lead to nonequilibrium dynamics of a microtubule.

A single microtubule → Multiple microtubules → Emergence of collective phenomena (?)

# Models of microtubules

**1. Highly 'detailed' models:**

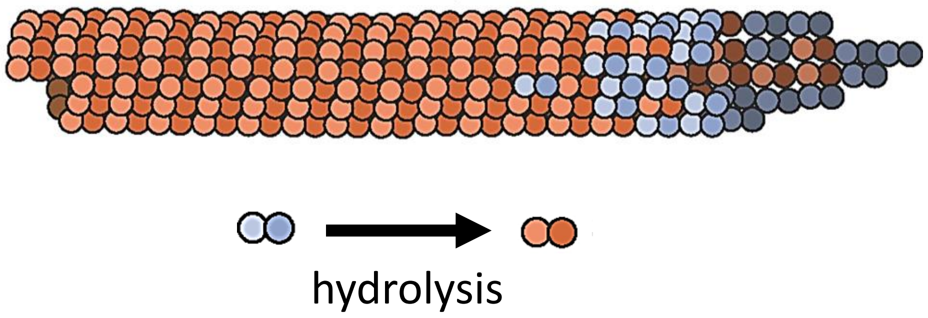
VanBuren et al, PNAS, 2002;
Margolin et al, MBoC, 2012;
Molodtsov et al., Biophys J, 2005;
Jemseena & Manoj, PRE, 2019;
Aparna et al., Soft matter, 2019.



# Models of microtubules

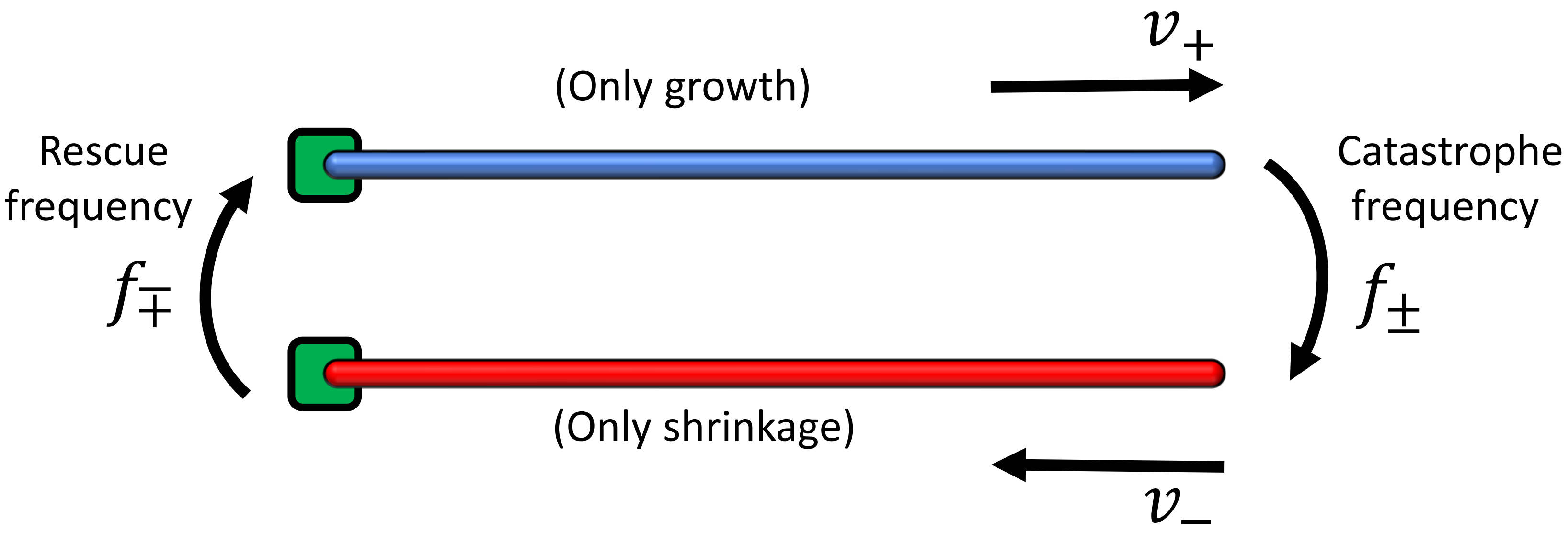
## 1. Highly 'detailed' models:

VanBuren et al, PNAS, 2002;
Margolin et al, MBoC, 2012;
Molodtsov et al., Biophys J, 2005;
Jemseena & Manoj, PRE, 2019;
Aparna et al., Soft matter, 2019.




## 2. Simple 'coarse-grained' models:

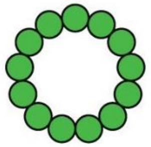
Dogterom & Leibler,
PRL, 1993



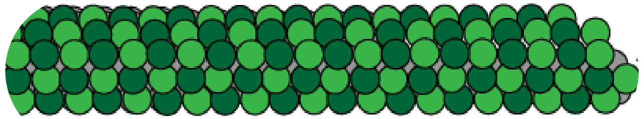
# Models of microtubules: Our approach

**An intermediate level of 'coarse-graining' :**

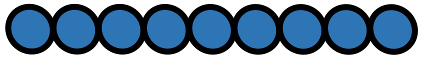
Ranjith & Kolomeisky et al., BPJ , 2009 & 2010; Aparna et al, Sci Rep., 2019; J. Howard, BioEssays (review), 2013.



$\stackrel{\text{def}}{=}$



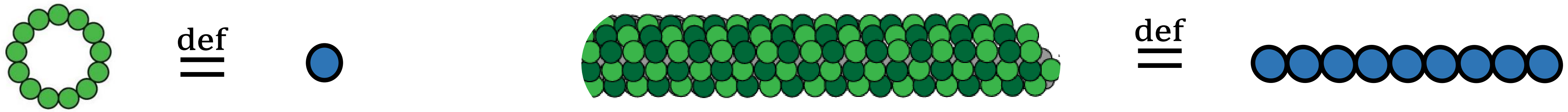
$\stackrel{\text{def}}{=}$



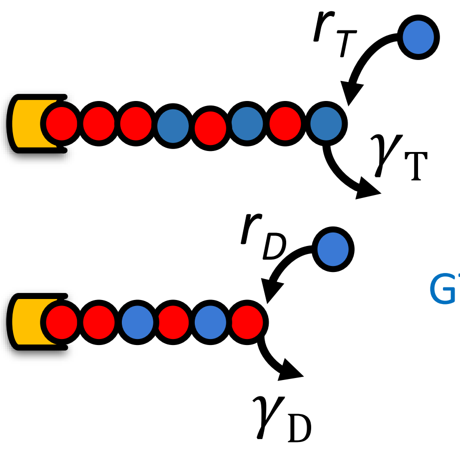
(tubulin ring) (a subunit)

# Models of microtubules: Our approach

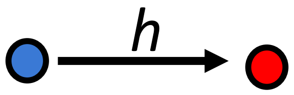
**An intermediate level of 'coarse-graining' :**




(tubulin ring) (a subunit)




(Aparna et al, Sci Rep., 2019)



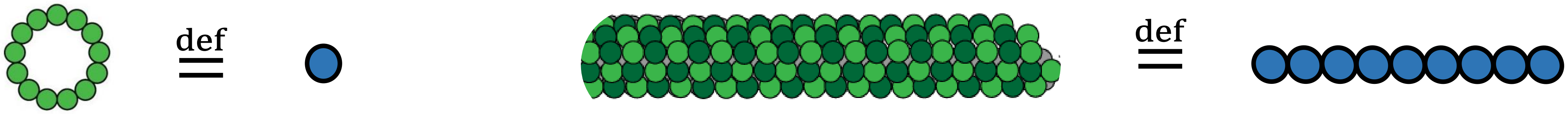

GTP-subunit GDP-subunit

| Processes | Rate |
|---|---|
| Subunit assembly rate when the tip is GTP-bound | $r_T$ |
| Subunit assembly rate when the tip is GDP-bound | $r_D$ |
| Subunit disassembly rate when the tip is GTP-bound | $\gamma_T$ |
| Subunit disassembly rate when the tip is GDP-bound | $\gamma_D$ |
| Hydrolysis | $h$ |

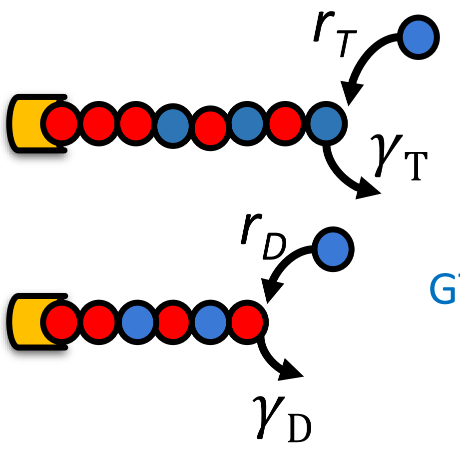
**Rates must be supplied from *in vitro* experiments.**

# Models of microtubules: Our approach

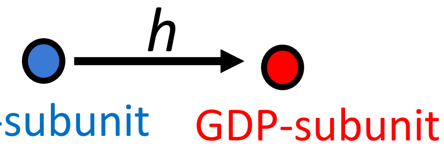
**An intermediate level of 'coarse-graining' :**




(tubulin ring) (a subunit)




(Aparna et al, Sci Rep., 2019)



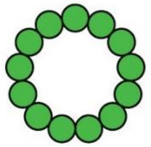

Recall: $\gamma_D >> \gamma_T$

| Processes | Rate |
|---|---|
| Subunit assembly rate when the tip is GTP-bound | $r_T$ |
| Subunit assembly rate when the tip is GDP-bound | $r_D$ |
| Subunit disassembly rate when the tip is GTP-bound | $\gamma_T$ |
| Subunit disassembly rate when the tip is GDP-bound | $\gamma_D$ |
| Hydrolysis | $h$ |

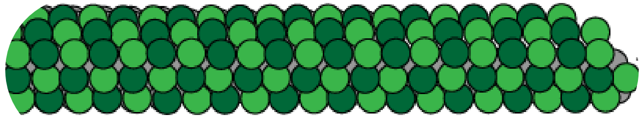
**Rates must be supplied from *in vitro* experiments.**

# Models of microtubules: Our approach

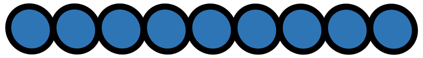
**An intermediate level of ‘coarse-graining’ :**



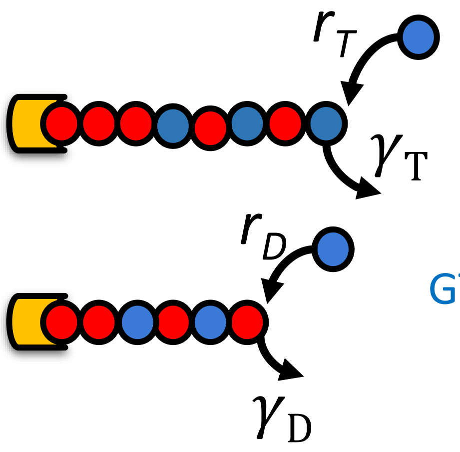
$\stackrel{\text{def}}{=}$



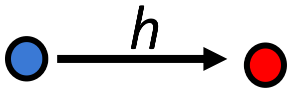
$\stackrel{\text{def}}{=}$



(tubulin ring) (a subunit)



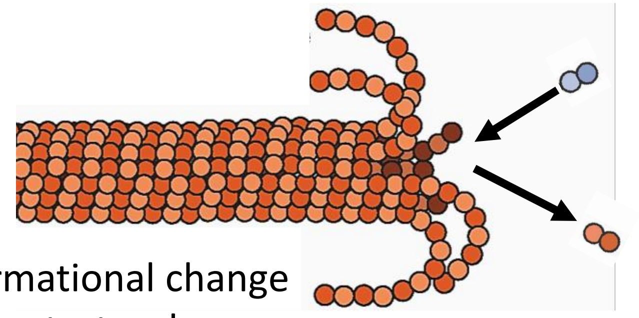

(Aparna et al, Sci Rep., 2019)




GTP-subunit GDP-subunit

Recall: $\gamma_D >> \gamma_T$

Also, $r_D < r_T$



Conformational change during catastrophe

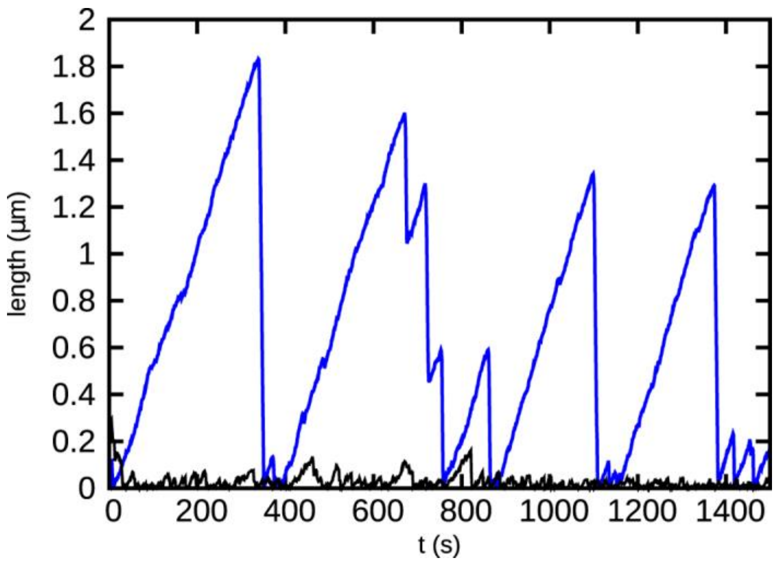
| Processes | Rate |
|---|---|
| Subunit assembly rate when the tip is GTP-bound | $r_T$ |
| Subunit assembly rate when the tip is GDP-bound | $r_D$ |
| Subunit disassembly rate when the tip is GTP-bound | $\gamma_T$ |
| Subunit disassembly rate when the tip is GDP-bound | $\gamma_D$ |
| Hydrolysis | $h$ |

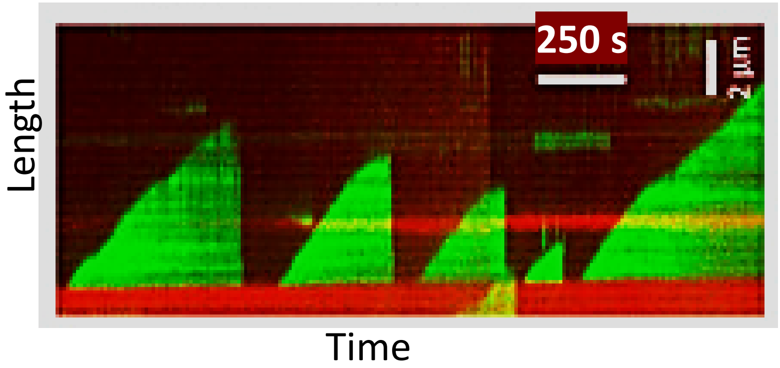
**Rates must be supplied from *in vitro* experiments.**

# Experimental correspondence for the ‘coarse-grained’ model

- Captures the length-vs-time traces

(Aparna et al, Sci Rep., 2019)

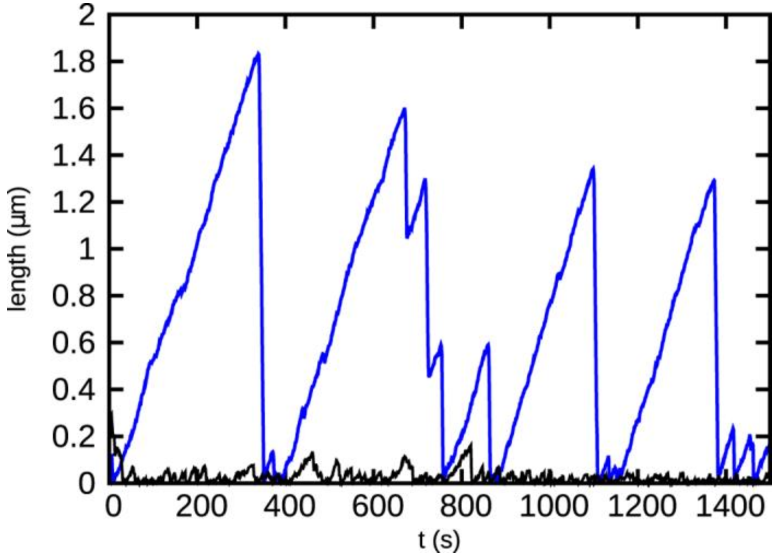


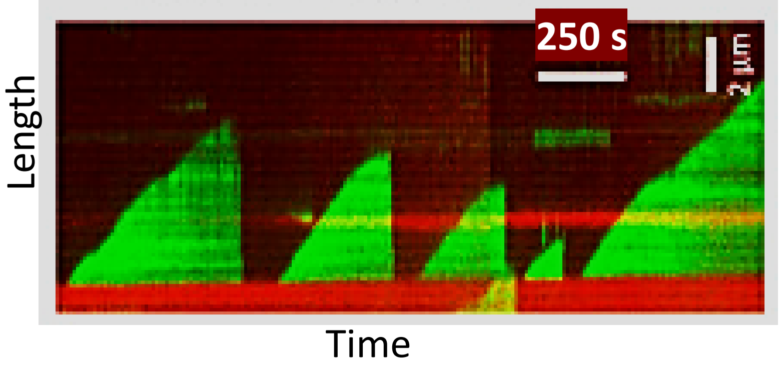



# Experimental correspondence for the 'coarse-grained' model

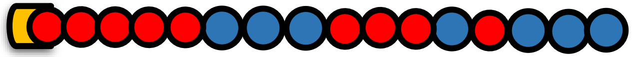
- Captures the length-vs-time traces

(Aparna et al, Sci Rep., 2019)



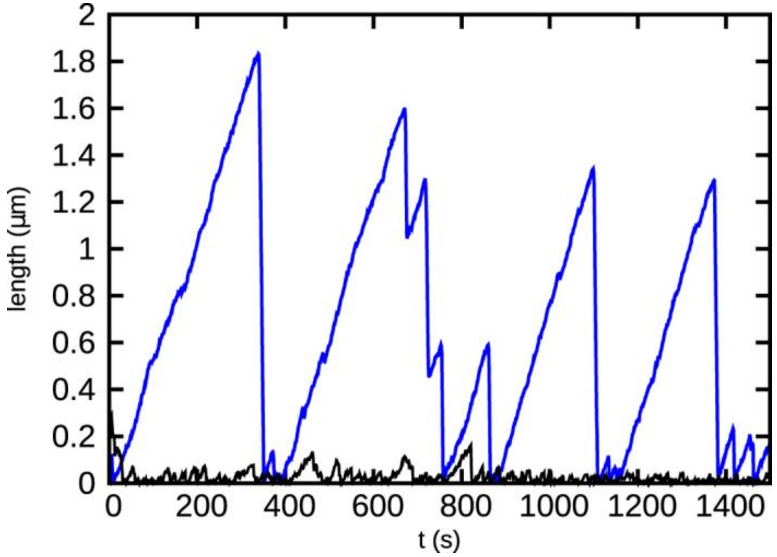

- Produce 'GTP-islands' in simulations

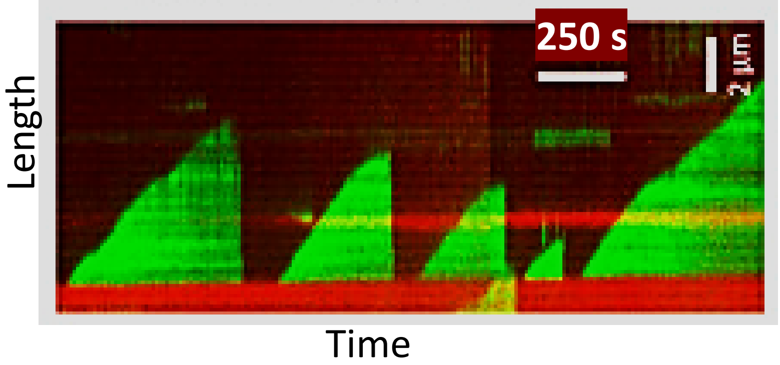


# Experimental correspondence for the ‘coarse-grained’ model

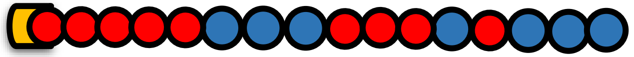
- Captures the length-vs-time traces

(Aparna et al, Sci Rep., 2019)

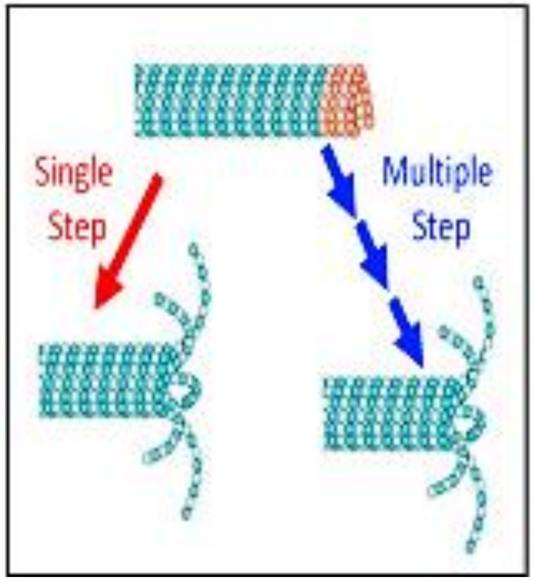



- Produce ‘GTP-islands’ in simulations



- Multi-step catastrophe



# Are there 'out-of-equilibrium' collective effects?

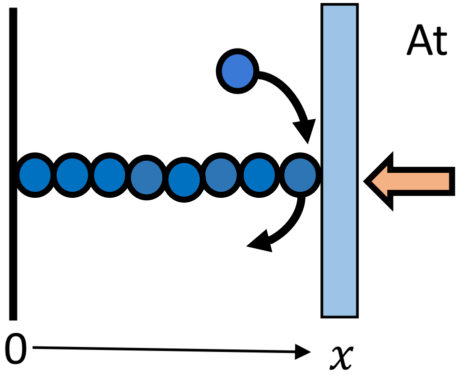
- Collective force generation by microtubules

D. Das et al., New J Phys & PloS One, 2014; T. Bameta & D. Das et al., PRE, 2017 (editor's choice)

- Length regulation of microtubules

S. Satheesan & D. Das , 2020 (under review)

# Equilibrium ensures additivity of stall forces




At "stall", $f = f_1^s$, average velocity is ZERO.

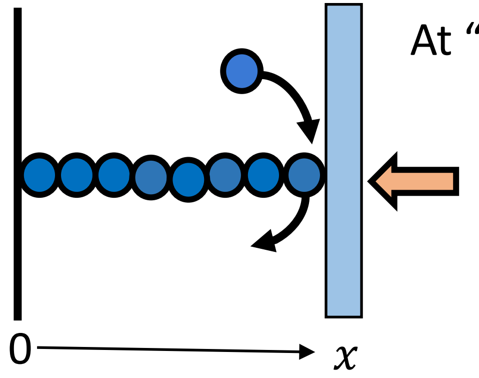
Force (f)

$$P_1(x) = \frac{1}{Z}\, e^{\beta \epsilon x}\, e^{-\beta f_1^s x} = \frac{1}{Z}\, e^{\beta x (\epsilon - f_1^s)}$$

→ $f_1^s = \epsilon$

($\epsilon$ is energy per subunit)

# Equilibrium ensures additivity of stall forces

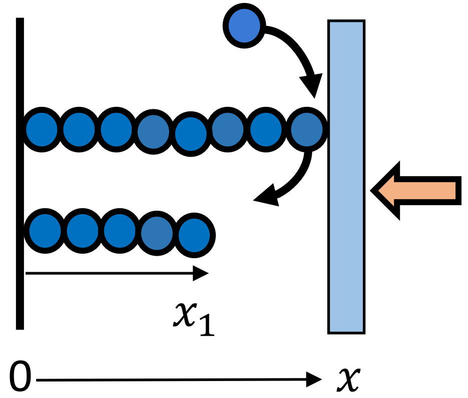



At "stall", $f = f_1^S$, average velocity is ZERO.

$$P_1(x) = \frac{1}{Z}\, e^{\beta \epsilon x}\, e^{-\beta f_1^S x} = \frac{1}{Z}\, e^{\beta x(\epsilon - f_1^S)}$$

→ $f_1^S = \epsilon$

($\epsilon$ is energy per subunit)



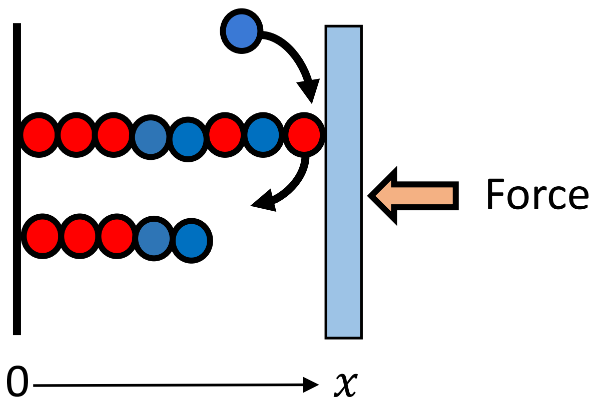

$$P_2(x) = \frac{1}{Z_2} e^{\beta \epsilon x}\, e^{-\beta f_2^S x}\left(2 \sum_{x_1=0}^{x} e^{\beta \epsilon\, x_1}\right)$$

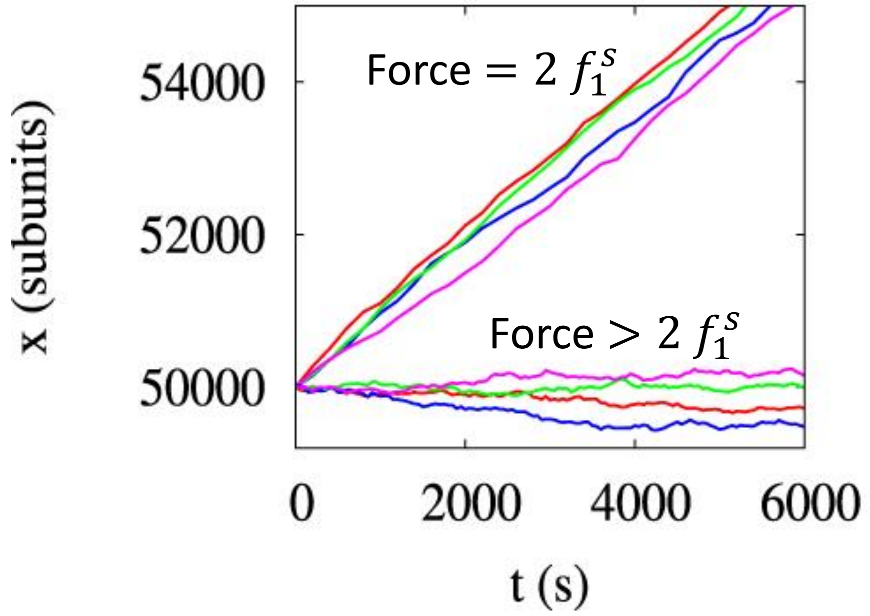
$\sim e^{\beta x(2\epsilon - f_2^S)}$

→ $f_2^S = 2\epsilon = 2\, f_1^S$

*Without hydrolysis,* $f_N^S = \mathrm{N}\, f_1^S$

# Nonequilibrium random hydrolysis makes stall forces nonadditive




*With hydrolysis,* $f_N^s > N\ f_1^s$

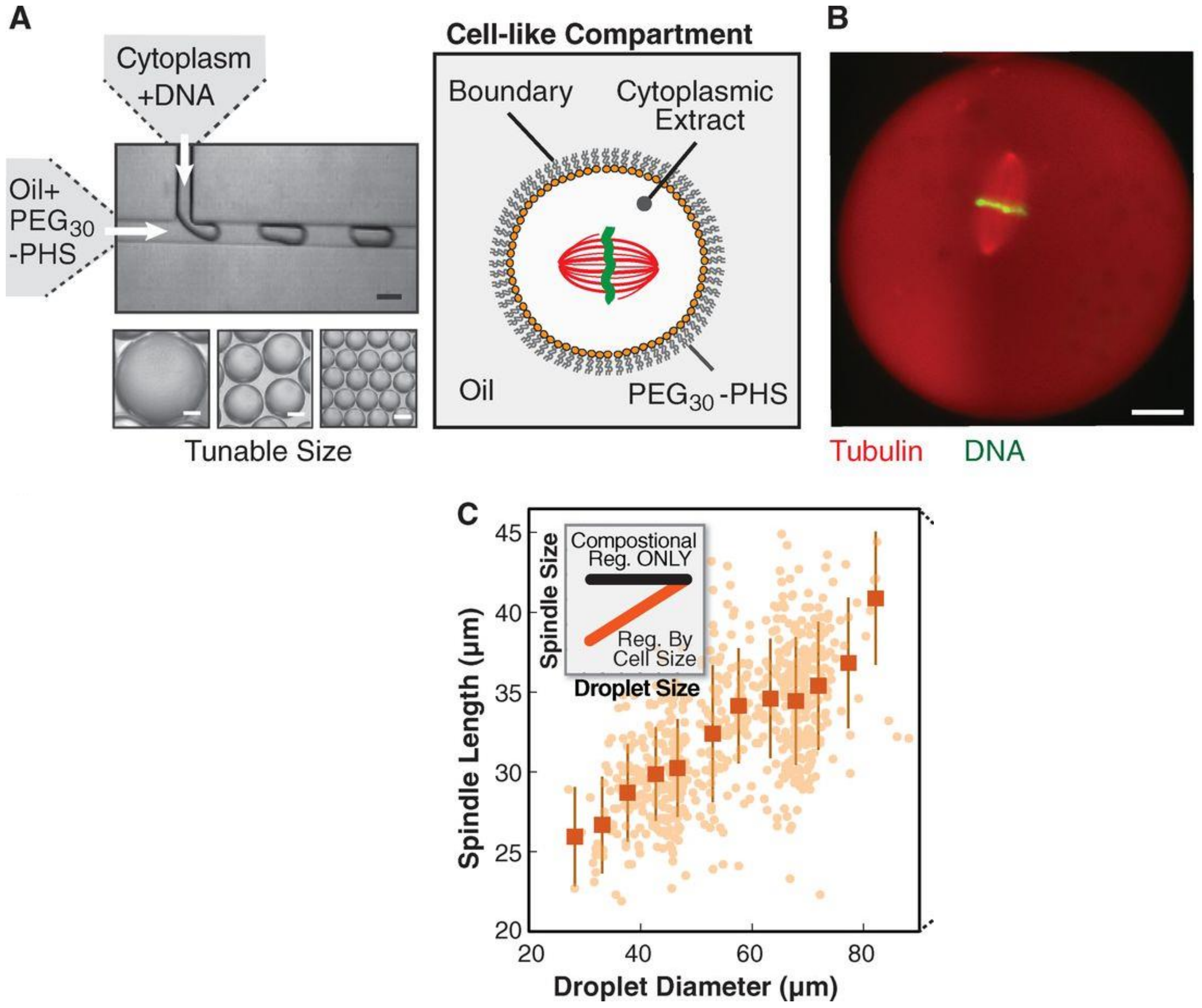
# How cells control sizes of subcellular structures?

## Size regulation of microtubules in a limiting subunit pool

S. Satheesan & D. Das , 2020 (under review)

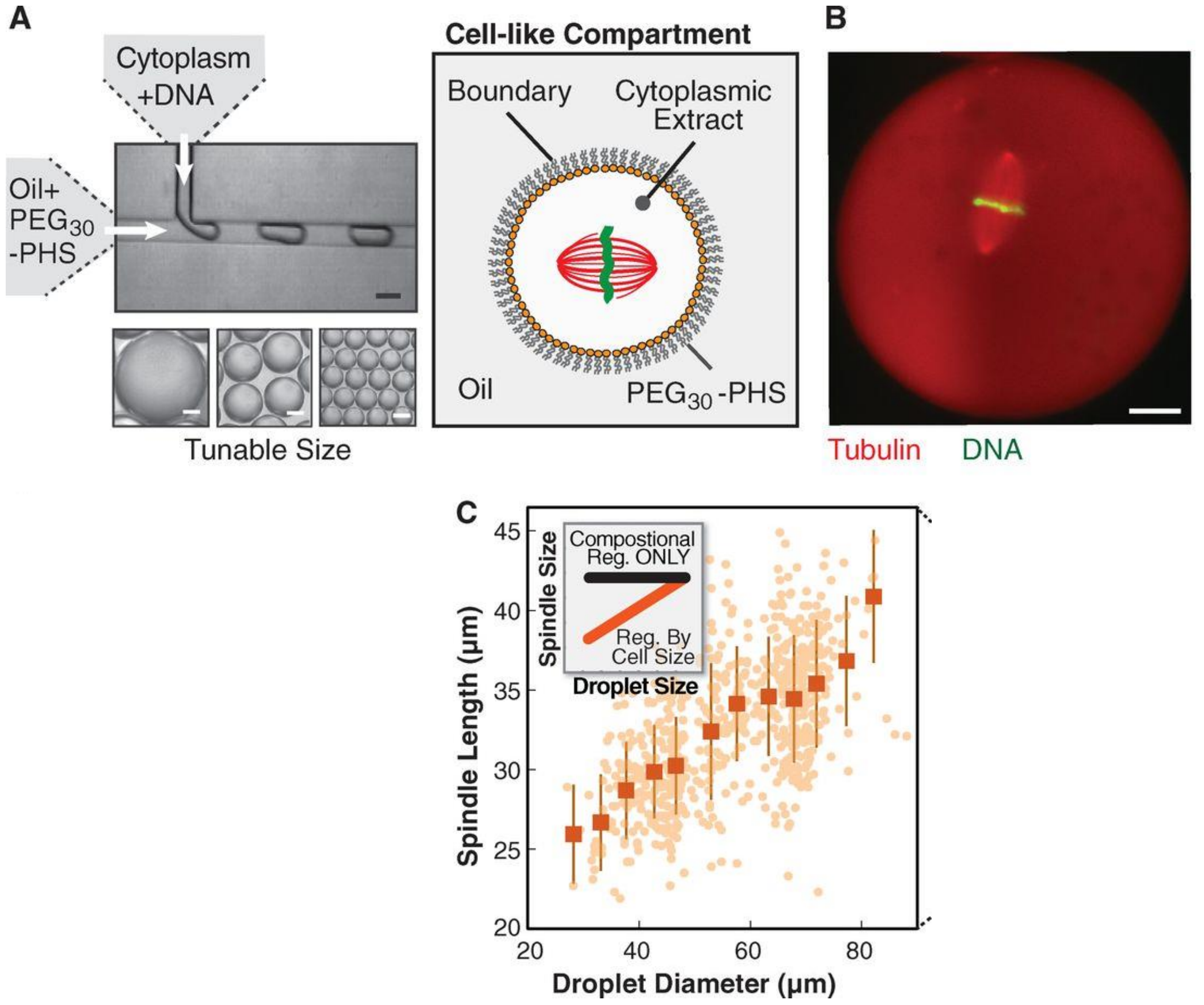
# Limiting pool of building blocks can control sizes of subcellular structures

Sizes of mitotic spindles and also its constituent MTs scale with cytoplasmic volume.
(Good et al., Science, 2013; Hazel et al., Science, 2013; Winey et al., JCB, 1995)



# Limiting pool of building blocks can control sizes of subcellular structures

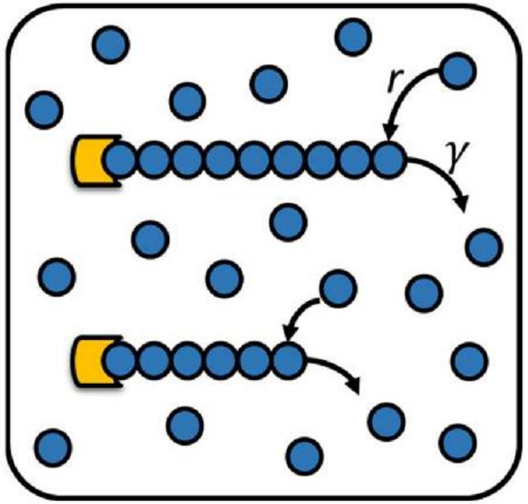
Sizes of mitotic spindles and also its constituent MTs scale with cytoplasmic volume.
(Good et al., Science, 2013; Hazel et al., Science, 2013; Winey et al., JCB, 1995)




**An intuitive idea of size control:**
Assembly and disassembly of balances each other in **a limiting pool of building-blocks.**

# Length distributions of filaments in a limiting subunit pool (without hydrolysis)

Consider **N subunits** and **F nucleation sites**.



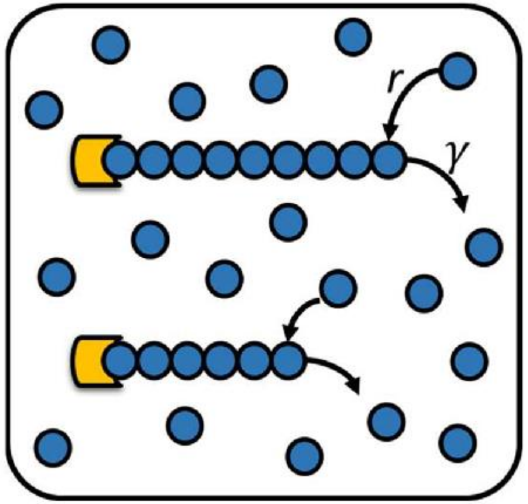

L Mohapatra et al., Cell systems, 2017.

# Length distributions of filaments in a limiting subunit pool (without hydrolysis)

Consider **N subunits** and **F nucleation sites**.

**m(t):** number of free subunits. At steady state: $rP(m) = \gamma P(m-1)$

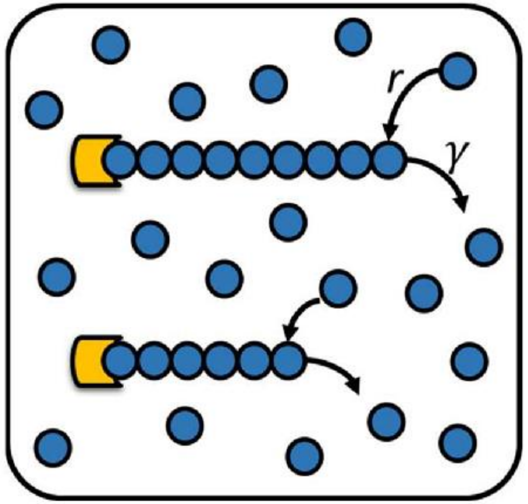
$P(m) \rightarrow$ ***Poisson*** with mean K and std. dev $\sqrt{K}$ (dissociation const. $\mathrm{K} = \frac{\gamma}{r}$)




L Mohapatra et al., Cell systems, 2017.

# Length distributions of filaments in a limiting subunit pool (without hydrolysis)

Consider **N subunits** and **F nucleation sites**.




**m(t):** number of free subunits. At steady state: $rP(m) = \gamma P(m-1)$

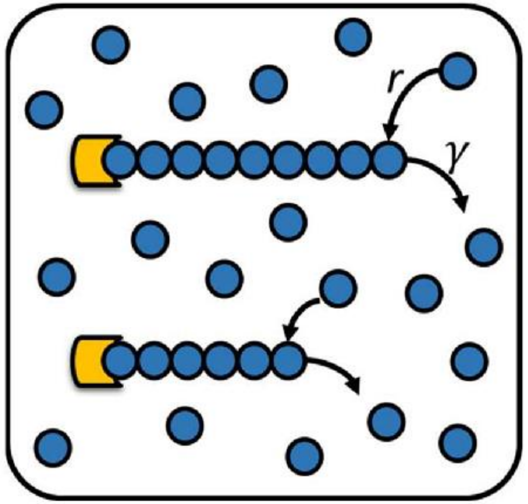
$P(m) \rightarrow \boldsymbol{Poisson}$ with mean K and std. dev $\sqrt{K}$ (dissociation const. $\mathrm{K} = \frac{\gamma}{r}$)

- **Length distribution of a single filament (F=1):**

Also $\boldsymbol{Poisson}$ with mean $= (N - \langle m \rangle) = (\mathrm{N} - \mathrm{K})$, and std. dev ~$\sqrt{K}$

L Mohapatra et al., Cell systems, 2017.

# Length distributions of filaments in a limiting subunit pool (without hydrolysis)




Consider **N subunits** and **F nucleation sites**.

**m(t):** number of free subunits. At steady state: $rP(m) = \gamma P(m-1)$

$P(m) \rightarrow$ ***Poisson*** with mean K and std. dev $\sqrt{K}$ (dissociation const. $\mathrm{K} = \frac{\gamma}{r}$)

- **Length distribution of a single filament (F=1):**

Also ***Poisson*** with mean $= (N - \langle m \rangle) = (\mathrm{N} - \mathrm{K})$, and std. dev ~$\sqrt{K}$

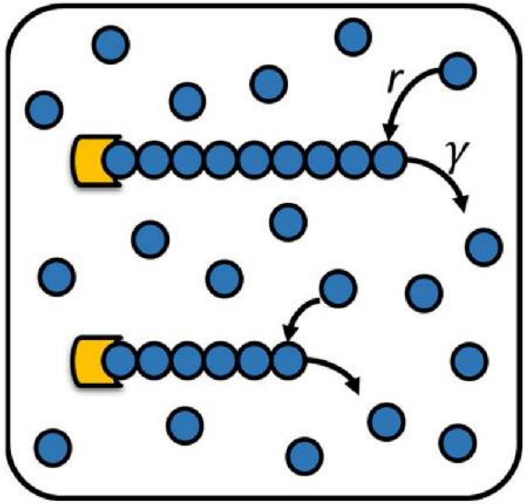
- **Individual length distribution in a collection of many filaments (F>1):**

Since distribution of free subunits is narrow with negligible fluctuation, $l_1 + l_2 \ldots + l_F \approx (N - K)$

L Mohapatra et al., Cell systems, 2017.

# Length distributions of filaments in a limiting subunit pool (without hydrolysis)

Consider **N subunits** and **F nucleation sites**.




**m(t):** number of free subunits. At steady state: $rP(m) = \gamma P(m-1)$

$P(m) \rightarrow \boldsymbol{Poisson}$ with mean K and std. dev $\sqrt{K}$ (dissociation const. $\mathrm{K} = \frac{\gamma}{r}$)

- **Length distribution of a single filament (F=1):**

Also $\boldsymbol{Poisson}$ with mean $= (N - \langle m \rangle) = (\mathrm{N} - \mathrm{K})$, and std. dev ~$\sqrt{K}$

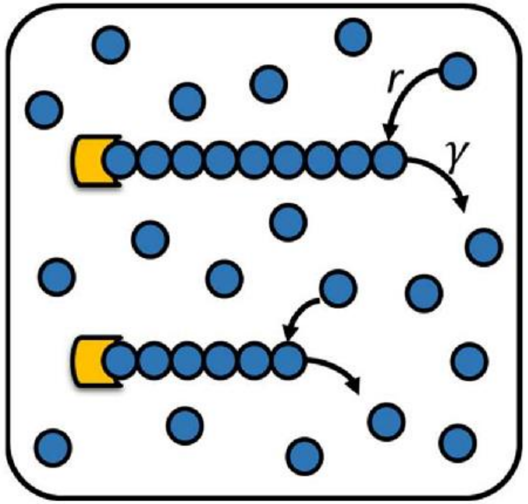
- **Individual length distribution in a collection of many filaments (F>1):**

Since distribution of free subunits is narrow with negligible fluctuation, $l_1 + l_2 \ldots + l_F \approx (N - K)$

Number of configurations $\Rightarrow$ arranging $(\mathrm{N}-\mathrm{K})$ objects in F boxes without constrints: $\binom{N-K+F-1}{F-1}$

L Mohapatra et al., Cell systems, 2017.

# Length distributions of filaments in a limiting subunit pool (without hydrolysis)




Consider **N subunits** and **F nucleation sites**.

**m(t):** number of free subunits. At steady state: $rP(m) = \gamma P(m-1)$

$P(m) \rightarrow \boldsymbol{Poisson}$ with mean K and std. dev $\sqrt{K}$ (dissociation const. $\mathrm{K} = \frac{\gamma}{r}$)

- **Length distribution of a single filament (F=1):**

Also $\boldsymbol{Poisson}$ with mean $= (N - \langle m \rangle) = (\mathrm{N} - \mathrm{K})$, and std. dev ~$\sqrt{K}$

- **Individual length distribution in a collection of many filaments (F>1):**

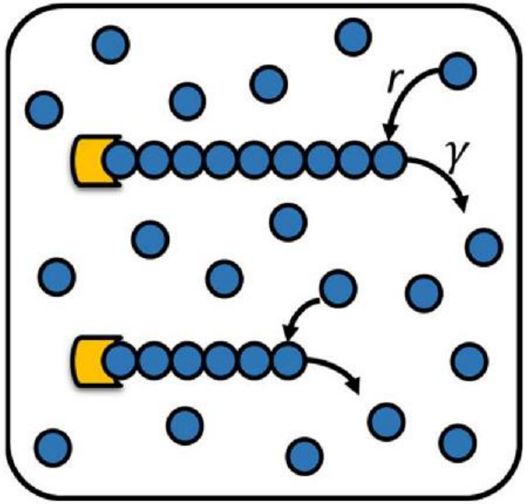
Since distribution of free subunits is narrow with negligible fluctuation, $l_1 + l_2 \ldots + l_F \approx (N - K)$

Number of configurations $\Rightarrow$ arranging $(\mathrm{N} - \mathrm{K})$ objects in F boxes without constrints: $\binom{N-K+F-1}{F-1}$

Now, consider filament 1 in the collection, $l_2 \ldots + l_F \approx (N - K - l_1)$. Number of configurations: $\binom{N - l_1 - K + F - 2}{F-2}$

L Mohapatra et al., Cell systems, 2017.

# Length distributions of filaments in a limiting subunit pool (without hydrolysis)




Consider **N subunits** and **F nucleation sites**.

**m(t):** number of free subunits. At steady state: $rP(m) = \gamma P(m-1)$

$P(m) \rightarrow \boldsymbol{Poisson}$ with mean K and std. dev $\sqrt{K}$ (dissociation const. $\mathrm{K} = \frac{\gamma}{r}$)

- **Length distribution of a single filament (F=1):**

Also $\boldsymbol{Poisson}$ with mean $= (N - \langle m \rangle) = (\mathrm{N} - \mathrm{K})$, and std. dev ~$\sqrt{K}$

- **Individual length distribution in a collection of many filaments (F>1):**

Since distribution of free subunits is narrow with negligible fluctuation, $l_1 + l_2 \ldots + l_F \approx (N - K)$

Number of configurations $\Rightarrow$ arranging $(\mathrm{N} - \mathrm{K})$ objects in F boxes without constrints: $\binom{N - K + F - 1}{F - 1}$

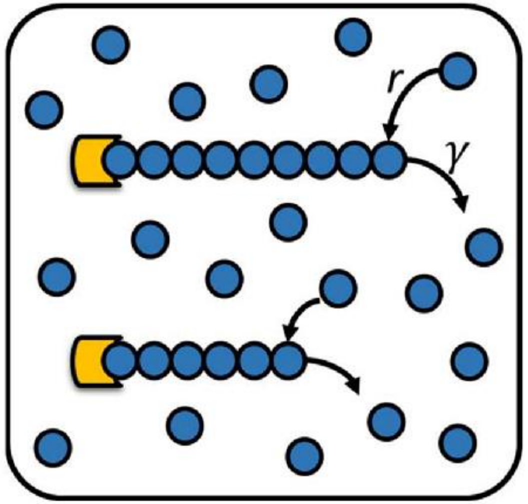
Now, consider filament 1 in the collection, $l_2 \ldots + l_F \approx (N - K - l_1)$. Number of configurations: $\binom{N - l_1 - K + F - 2}{F - 2}$

Therefore, $P(l_1) = \binom{N - l_1 - K + F - 2}{F - 2} / \binom{N - K + F - 1}{F - 1} \approx \frac{F-1}{N-K}\left(1 - \frac{l_1}{N-K}\right)^{F-2}$

L Mohapatra et al., Cell systems, 2017.

# Length distributions of filaments in a limiting subunit pool (without hydrolysis)

Consider **N subunits** and **F nucleation sites**.




**m(t):** number of free subunits. At steady state: $rP(m) = \gamma P(m-1)$

$P(m) \rightarrow \boldsymbol{Poisson}$ with mean K and std. dev $\sqrt{K}$ (dissociation const. $\mathrm{K} = \frac{\gamma}{r}$)

- **Length distribution of a single filament (F=1):**

Also $\boldsymbol{Poisson}$ with mean $= (N - \langle m \rangle) = (\mathrm{N} - \mathrm{K})$, and std. dev ~$\sqrt{K}$

- **Individual length distribution in a collection of many filaments (F>1):**

Since distribution of free subunits is narrow with negligible fluctuation, $l_1 + l_2 \ldots + l_F \approx (N - K)$

Number of configurations ⇒ arranging (N − K) objects in F boxes without constrints: $\binom{N-K+F-1}{F-1}$

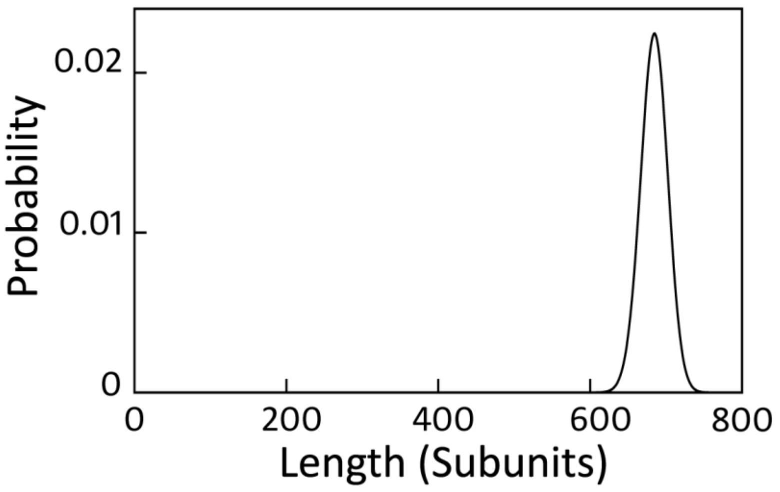
Now, consider filament 1 in the collection, $l_2 \ldots + l_F \approx (N - K - l_1)$. Number of configurations: $\binom{N - l_1 - K + F - 2}{F-2}$

Therefore, $P(l_1)$= $\binom{N - l_1 - K + F - 2}{F-2} / \binom{N-K+F-1}{F-1} \approx \frac{F-1}{N-K} \left(1 - \frac{l_1}{N-K}\right)^{F-2}$

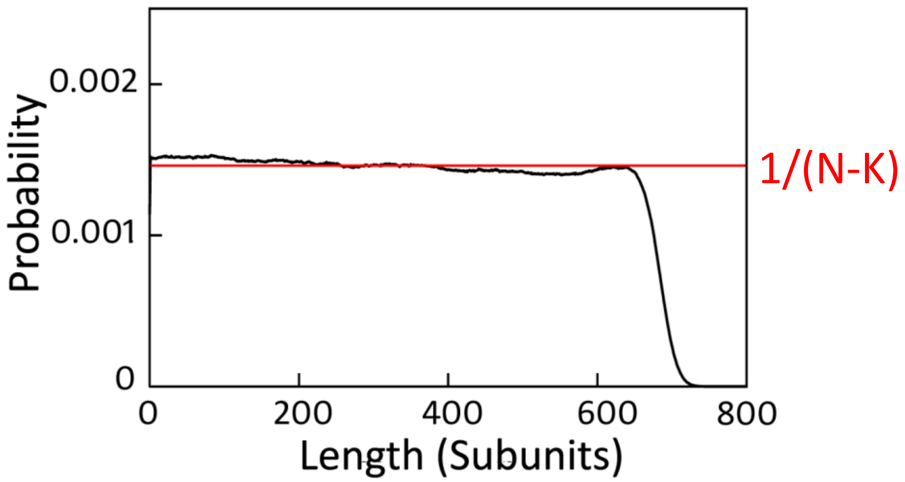
**For 2 filaments (F=2):** $P(l_1) = \frac{1}{N-K}$ in the interval [0, N-K] → **Uniform Dist.**

L Mohapatra et al., Cell systems, 2017.

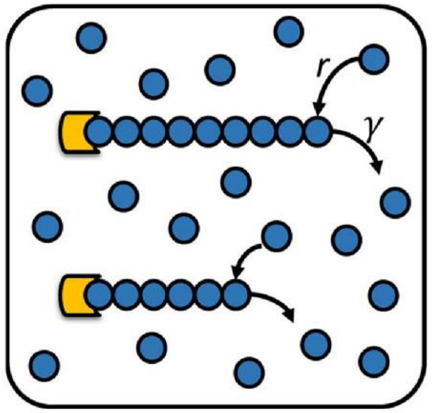
# So…a limiting subunit pool by itself cannot control filament lengths



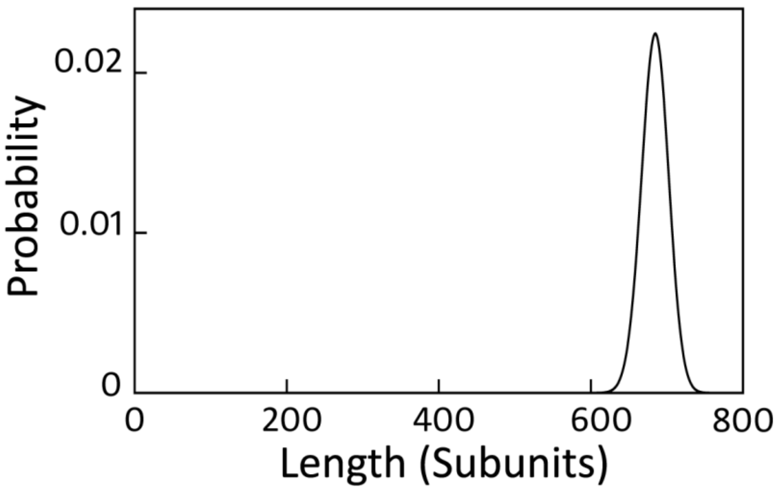

Single filament length distribution:
Poisson



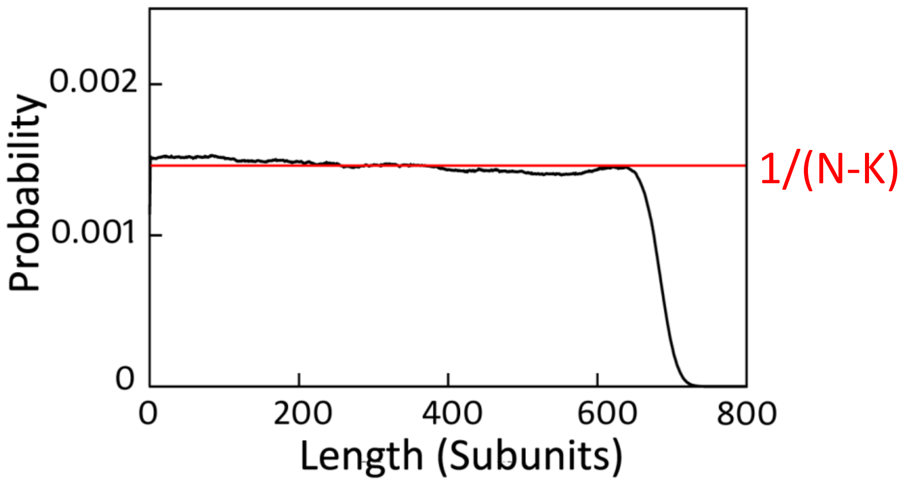

individual length distribution
in a collection of 2-filaments:
Uniform



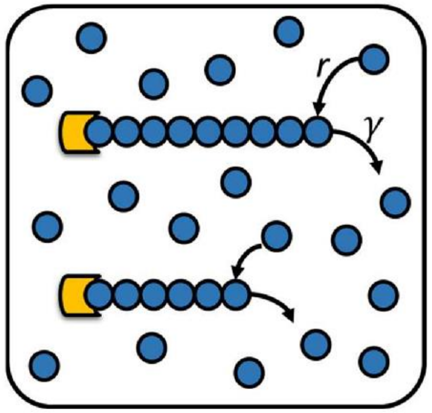
# So…a limiting subunit pool by itself cannot control filament lengths




Single filament length distribution:
Poisson

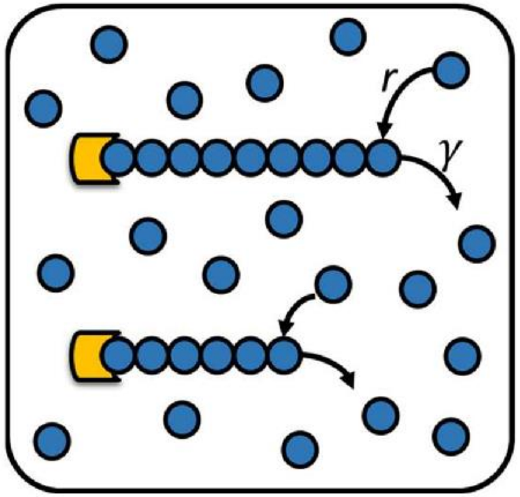



individual length distribution
in a collection of 2-filaments:
Uniform

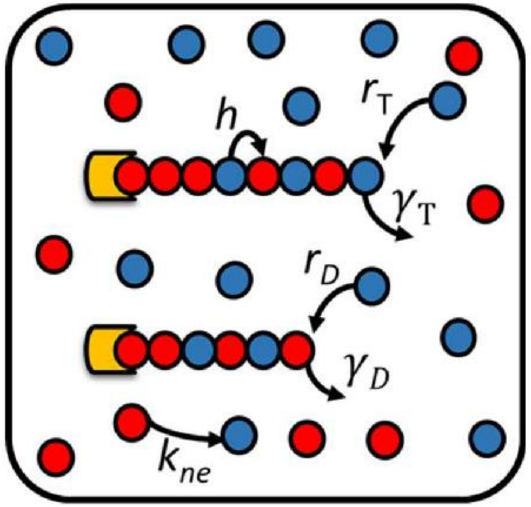



We ask: How hydrolysis affects filament length distributions in a limiting subunit pool?

# Homogenous (without hydrolysis) vs. heterogeneous (with hydrolysis) pool



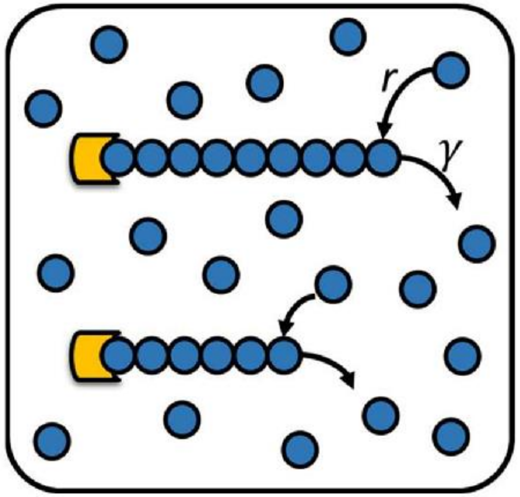

No hydrolysis



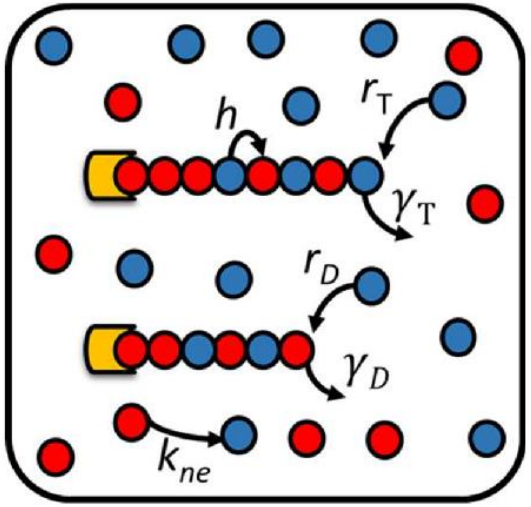

with hydrolysis

| Processes | Rate |
|---|---|
| Subunit assembly rate when the tip is GTP-bound | $r_T$ |
| Subunit assembly rate when the tip is GDP-bound | $r_D$ |
| Subunit disassembly rate when the tip is GTP-bound | $\gamma_T$ |
| Subunit disassembly rate when the tip is GDP-bound | $\gamma_D$ |
| Hydrolysis | $h$ |
| Nucleotide exchange (in the solution) | $k_{ne}$ |

# Homogenous (without hydrolysis) vs. heterogeneous (with hydrolysis) pool




No hydrolysis



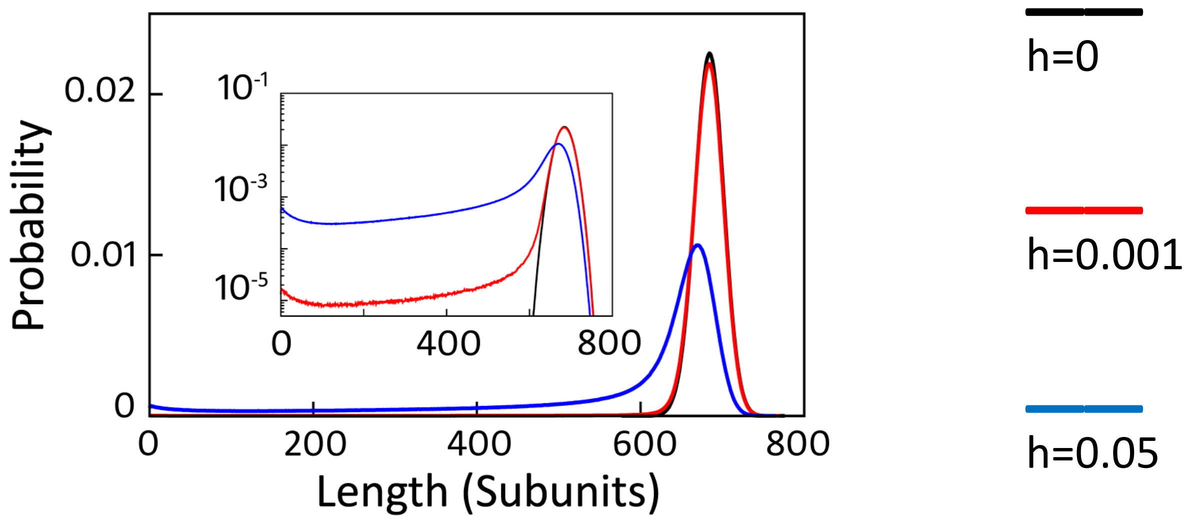

with hydrolysis

| Processes | Rate |
|---|---|
| Subunit assembly rate when the tip is GTP-bound | $r_T$ |
| Subunit assembly rate when the tip is GDP-bound | $r_D$ |
| Subunit disassembly rate when the tip is GTP-bound | $\gamma_T$ |
| Subunit disassembly rate when the tip is GDP-bound | $\gamma_D$ |
| Hydrolysis | $h$ |
| Nucleotide exchange (in the solution) | $k_{ne}$ |

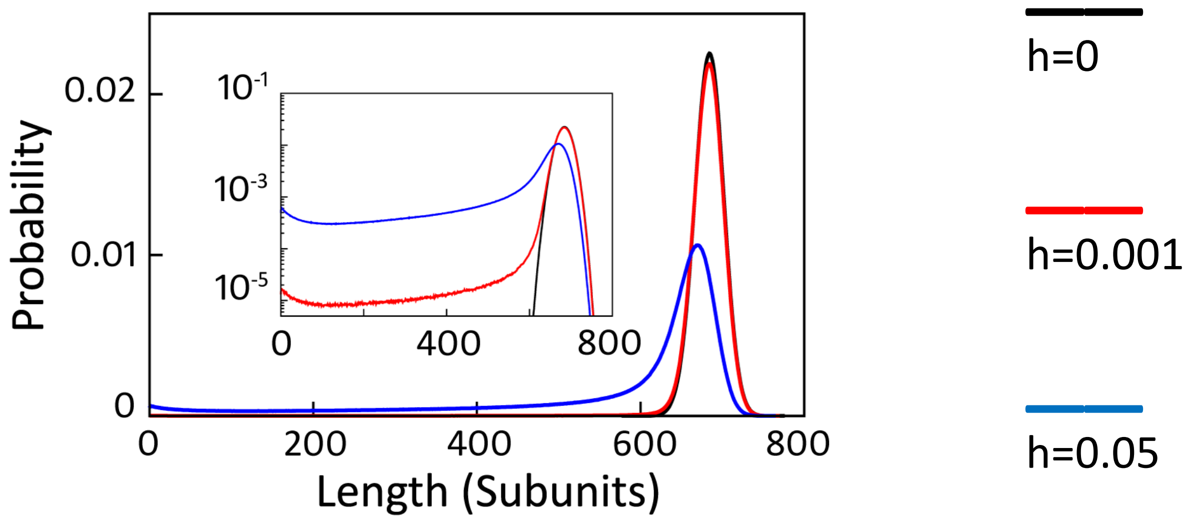
**We take *in vitro* parameters for microtubules and simulate.**

# Results

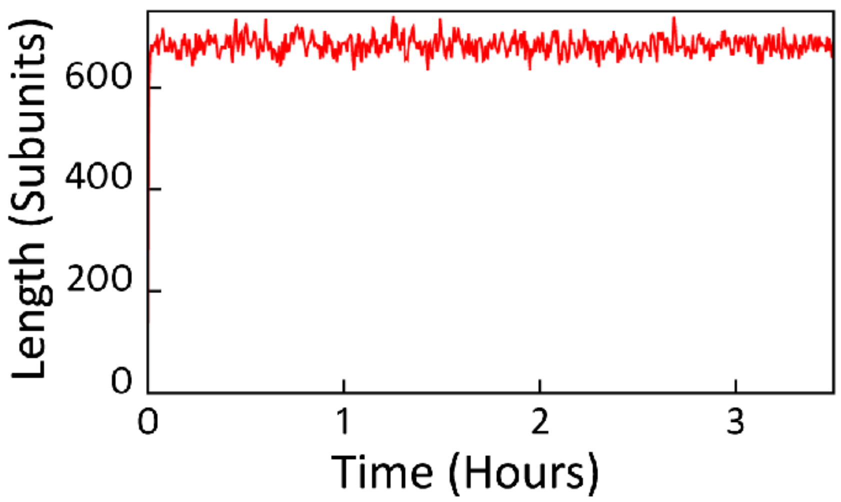
# A single microtubule in limiting subunit pool



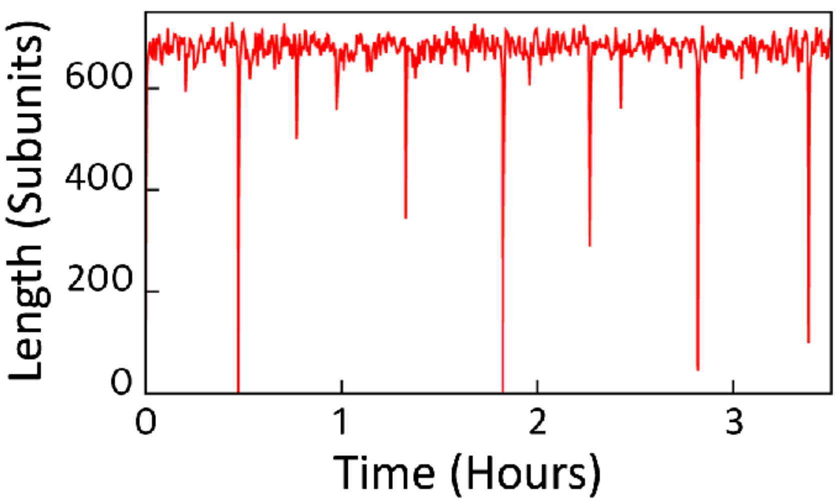
# A single microtubule in limiting subunit pool



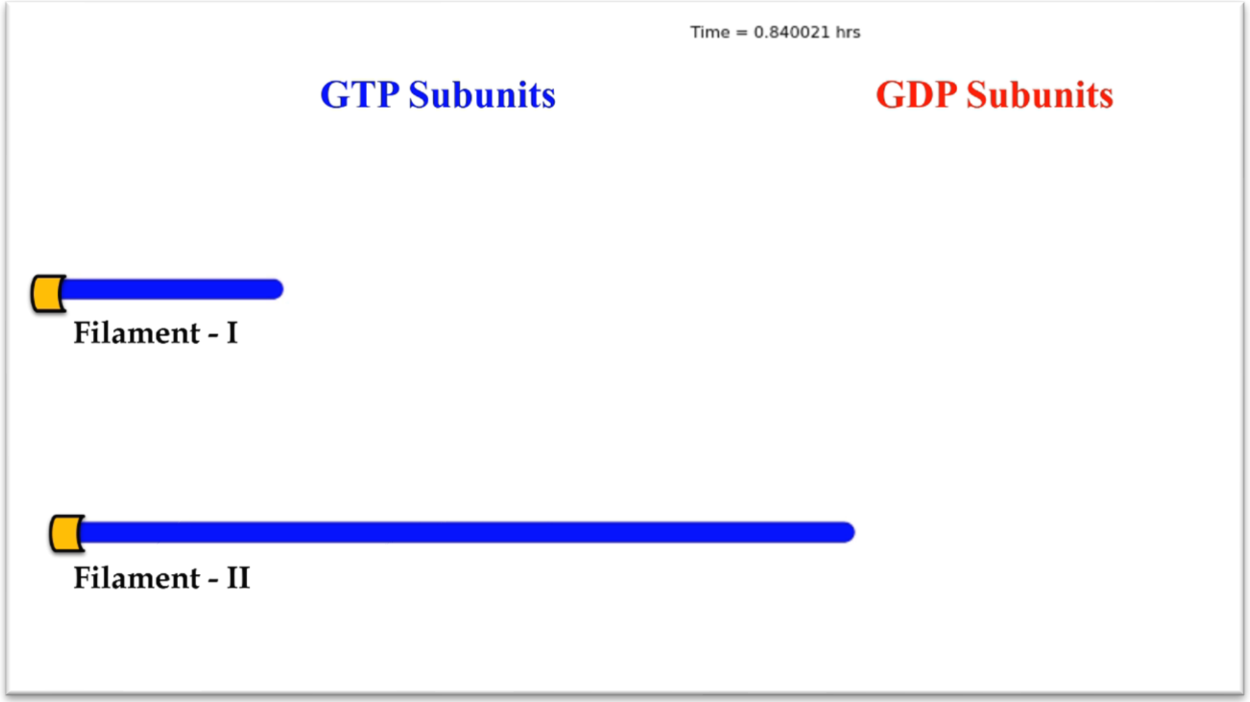

No hydrolysis



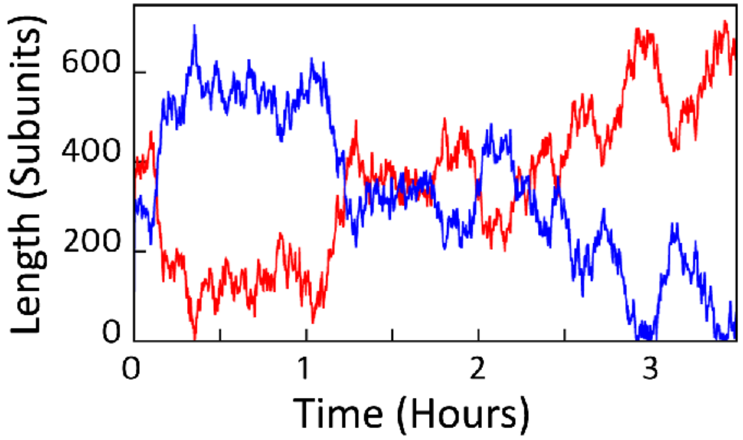

with hydrolysis

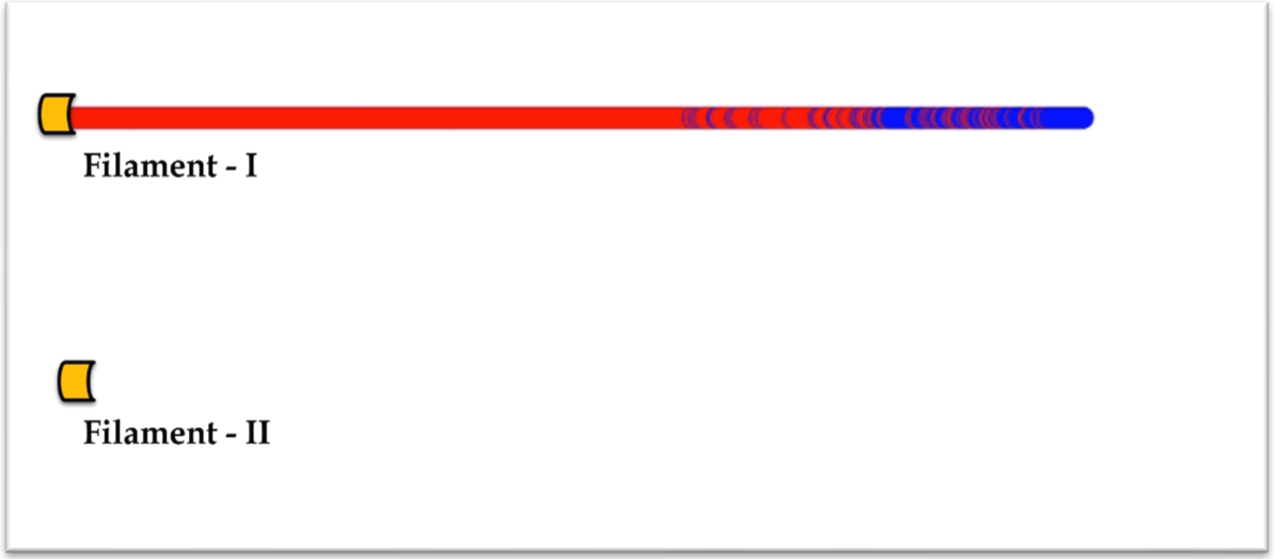


# Two microtubules in limiting subunit pool

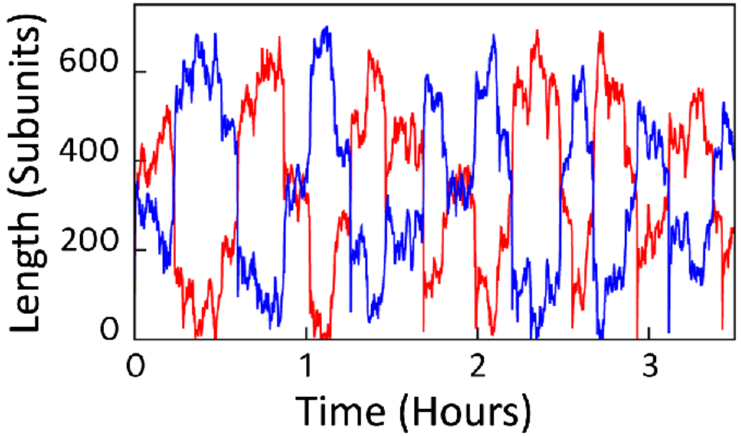



No
hydrolysis

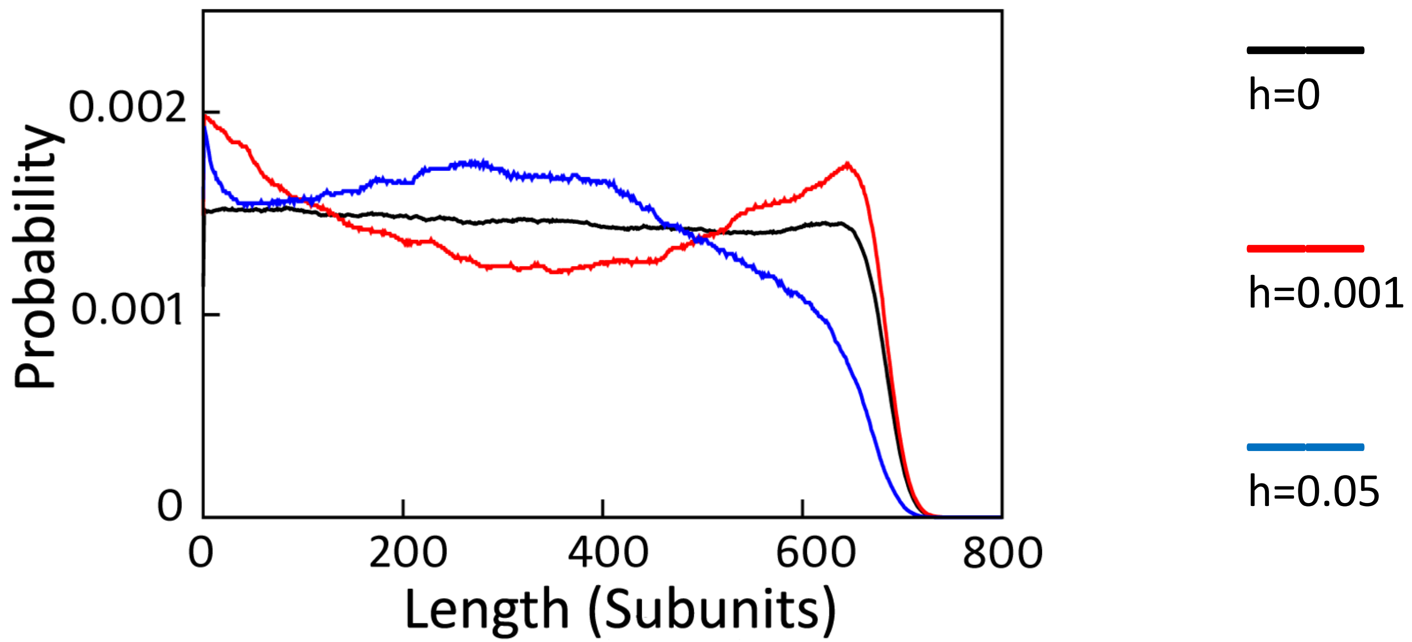


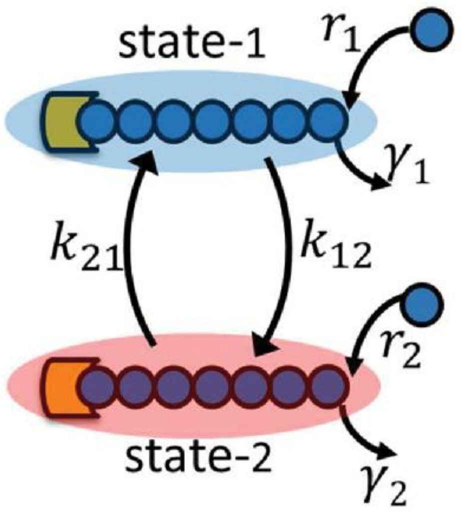

With
hydrolysis



# Bimodal distribution of individual lengths for two microtubules



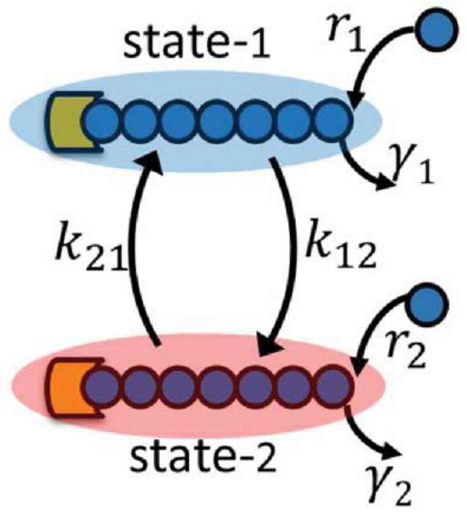
# Similar results for a simple ‘two-state’ model



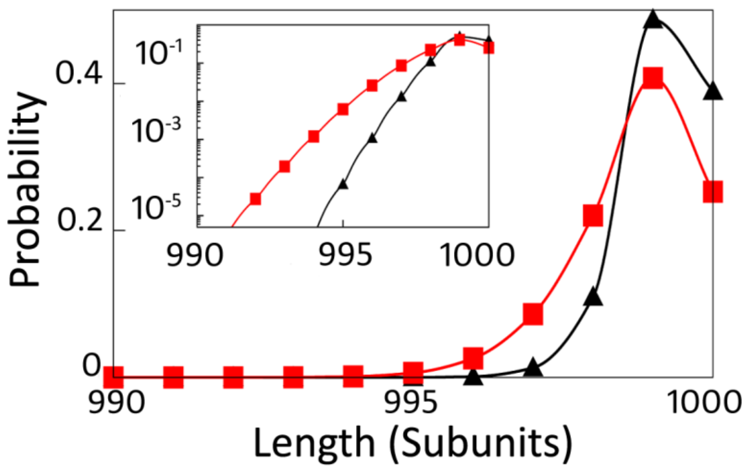

Single filament **Hill model**
(Hill T L et al., PNAS, 1984)

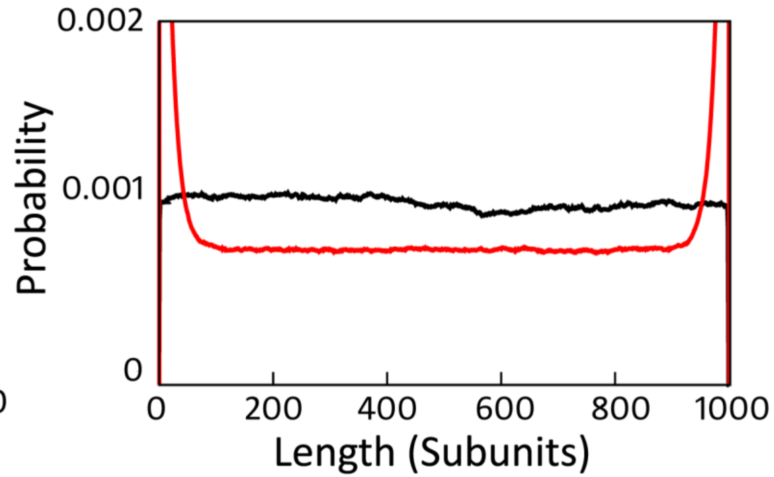
| Processes | Rate |
|---|---|
| assembly in state 1 | $r_1$ |
| assembly in state 2 | $\gamma_1$ |
| disassembly in state 1 | $r_2$ |
| disassembly in state 2 | $\gamma_2$ |
| State switching (1→ 2 & 2→ 1) | $k_{12}$ , $k_{21}$ |

# Similar results for a simple ‘two-state’ model




Single filament **Hill model**
(Hill T L et al., PNAS, 1984)



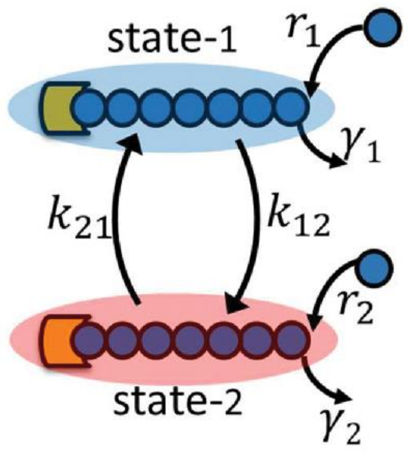




$k_{12} = k_{21}$=0

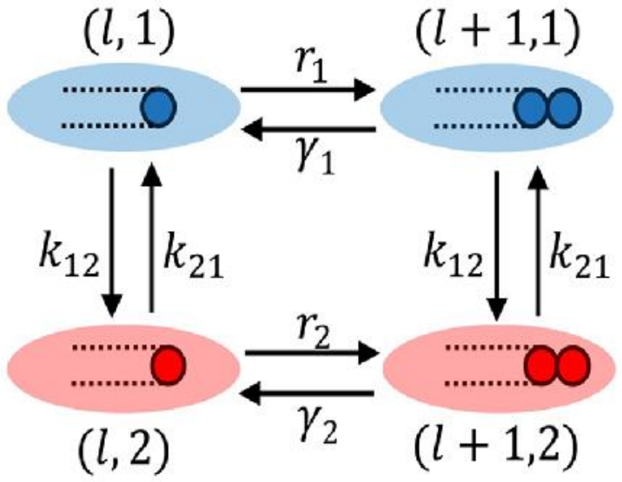
$k_{12} = k_{21}$= 0.001

Emergence of bimodality is linked with the deviation from reversible/equilibrium dynamics

# Departure from ‘equilibrium’ in Hill model




Single filament **Hill model**
(Hill T L et al., PNAS, 1984)

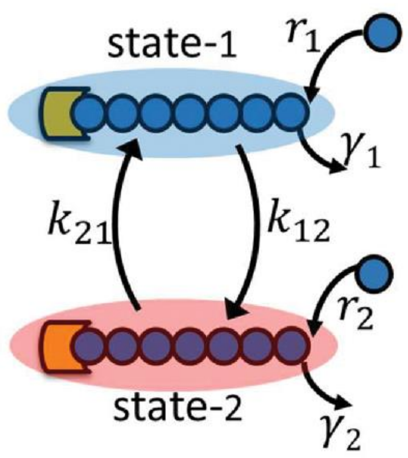



**Kolmogorov’s criterion:**

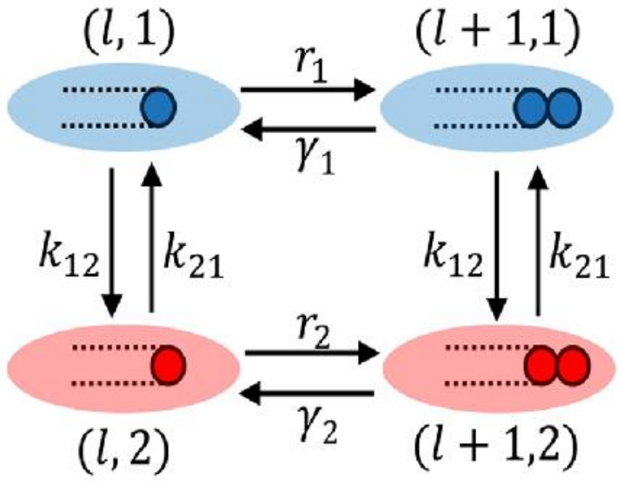
$r_1\, k_{12}\, \gamma_2\, k_{21} = \gamma_1\, k_{12}\, r_2\, k_{21}$

$$\Rightarrow \frac{r_1}{\gamma_1} = \frac{r_2}{\gamma_2}$$

# Departure from 'equilibrium' in Hill model



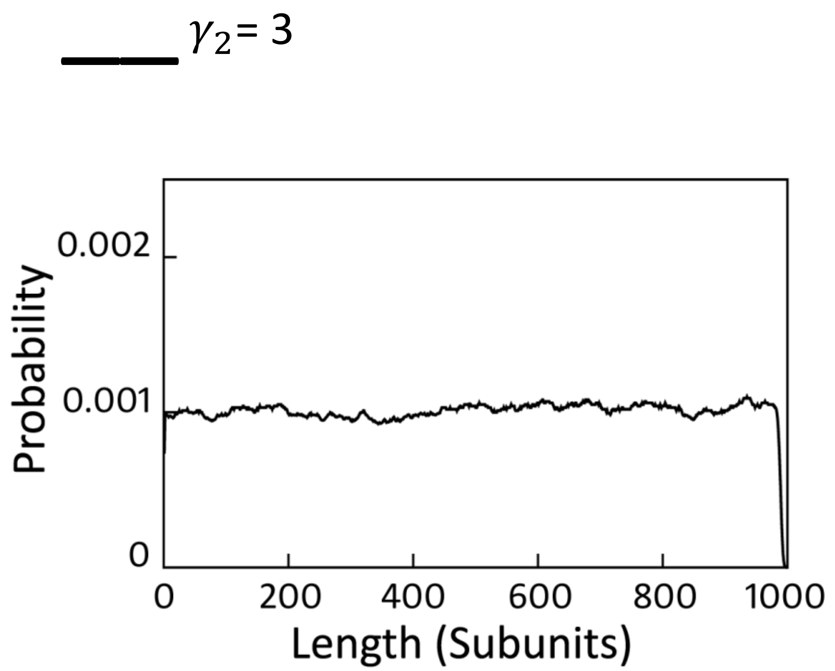

Single filament **Hill model**
(Hill T L et al., PNAS, 1984)




**Kolmogorov's criterion:**

$r_1\ k_{12}\ \gamma_2\ k_{21} = \gamma_1\ k_{12}\ r_2\ k_{21}$

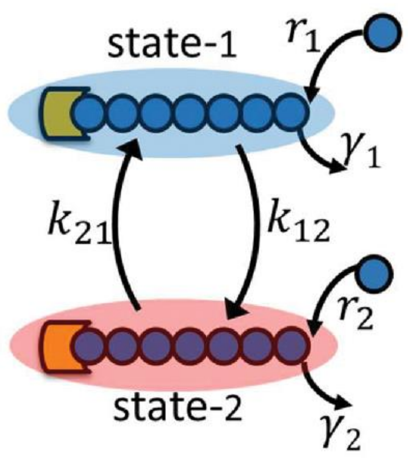
$$\Rightarrow \frac{r_1}{\gamma_1} = \frac{r_2}{\gamma_2}$$



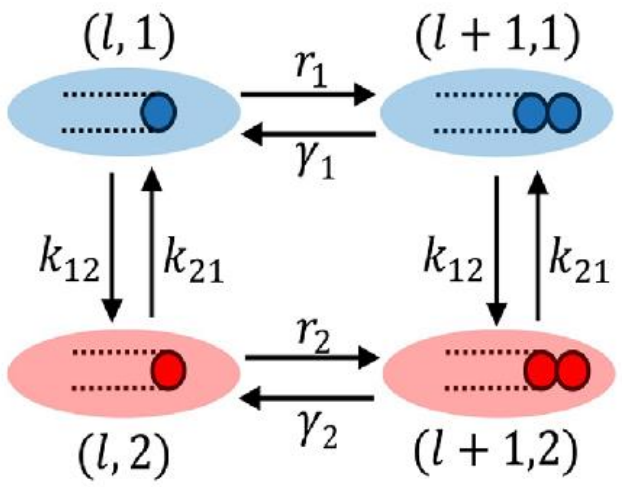

We set: $k_{12} = k_{21} = 0.005\ s^{-1}$, $r_1$=0.5 $s^{-1}$, $\gamma_1 = 5\ s^{-1}$, $r_2$= 0.3 $s^{-1}$.

$\boldsymbol{\gamma_2 = 3\ s^{-1}}$ **corresponds to equilibrium**

# Departure from ‘equilibrium’ in Hill model



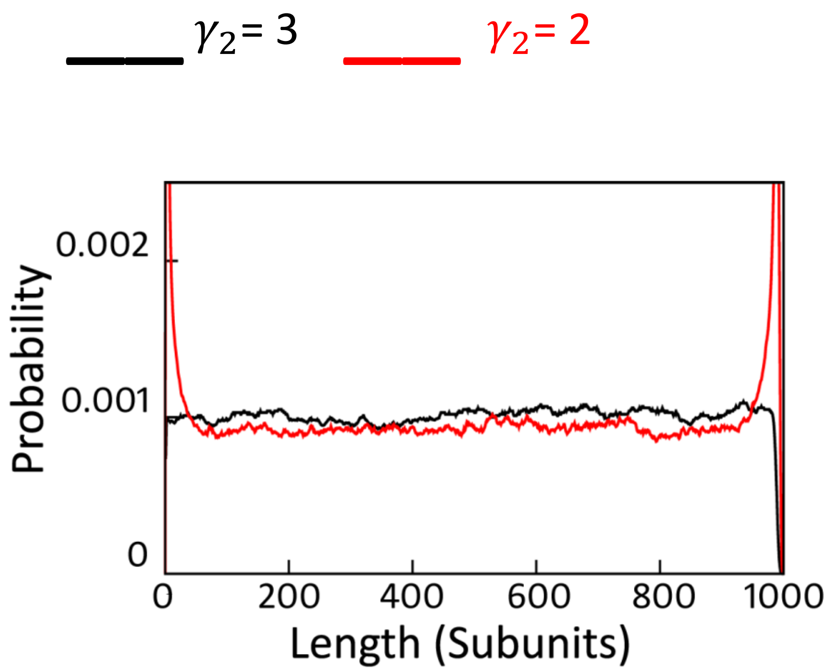

Single filament **Hill model**
(Hill T L et al., PNAS, 1984)




**Kolmogorov’s criterion:**

$r_1\, k_{12}\, \gamma_2\, k_{21} = \gamma_1\, k_{12}\, r_2\, k_{21}$

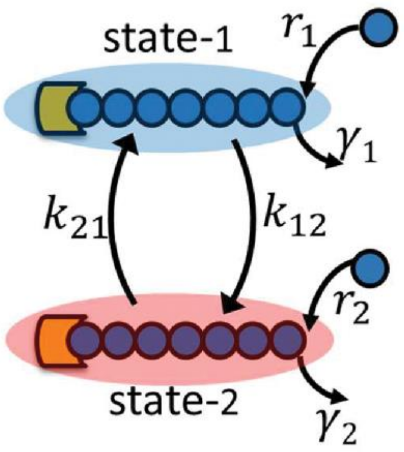
$$\Rightarrow \frac{r_1}{\gamma_1} = \frac{r_2}{\gamma_2}$$




We set: $k_{12} = k_{21} = 0.005\ s^{-1}$, $r_1 = 0.5\ s^{-1}, \gamma_1 = 5\ s^{-1}$, $r_2 = 0.3\ s^{-1}$.

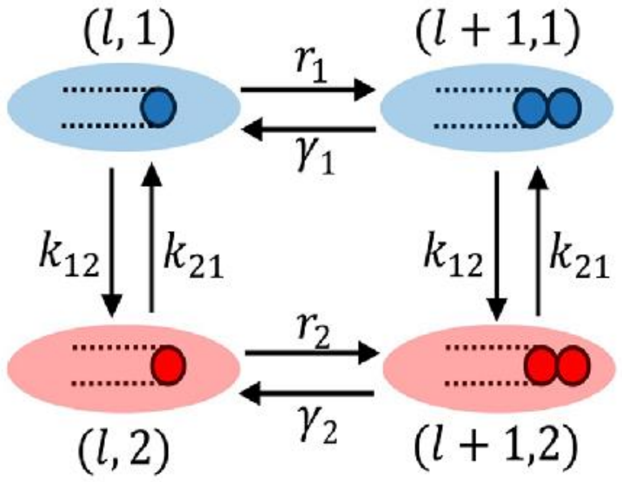
$\gamma_2 = 3\ s^{-1}$ **corresponds to equilibrium**

# Departure from ‘equilibrium’ in Hill model




Single filament **Hill model**

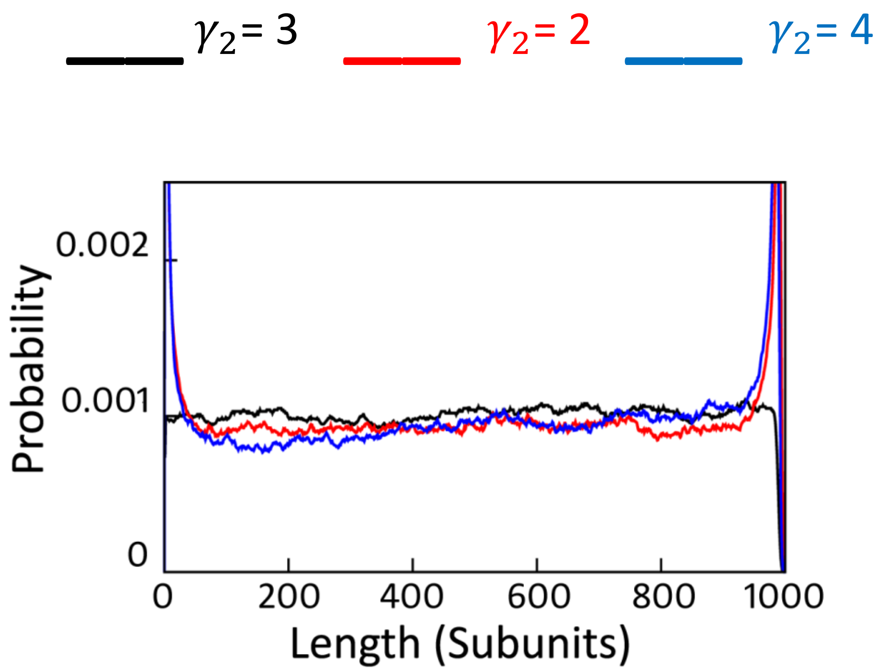
(Hill T L et al., PNAS, 1984)




**Kolmogorov’s criterion:**

$$r_1\, k_{12}\, \gamma_2\, k_{21} = \gamma_1\, k_{12}\, r_2\, k_{21}$$

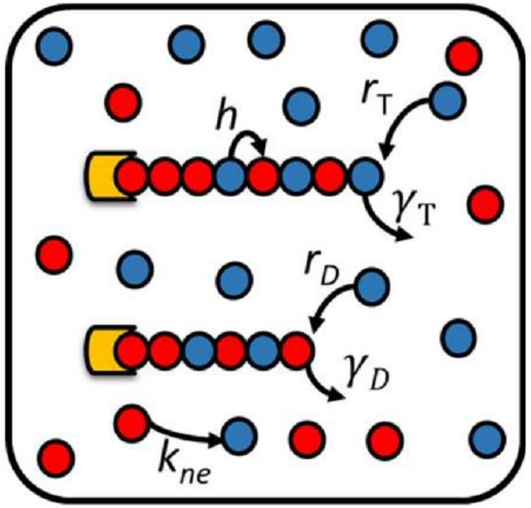
$$\Rightarrow \frac{r_1}{\gamma_1} = \frac{r_2}{\gamma_2}$$




We set: $k_{12} = k_{21} = 0.005\ s^{-1}$, $r_1$=0.5 $s^{-1}$, $\gamma_1 = 5\ s^{-1}$, $r_2$= 0.3 $s^{-1}$.

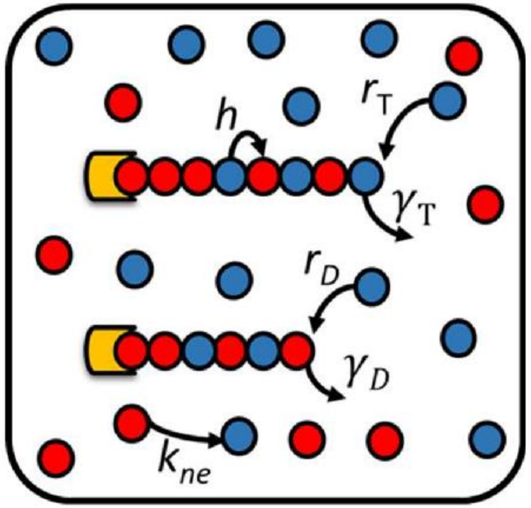
$\boldsymbol{\gamma_2 = 3\ s^{-1}}$ **corresponds to equilibrium**

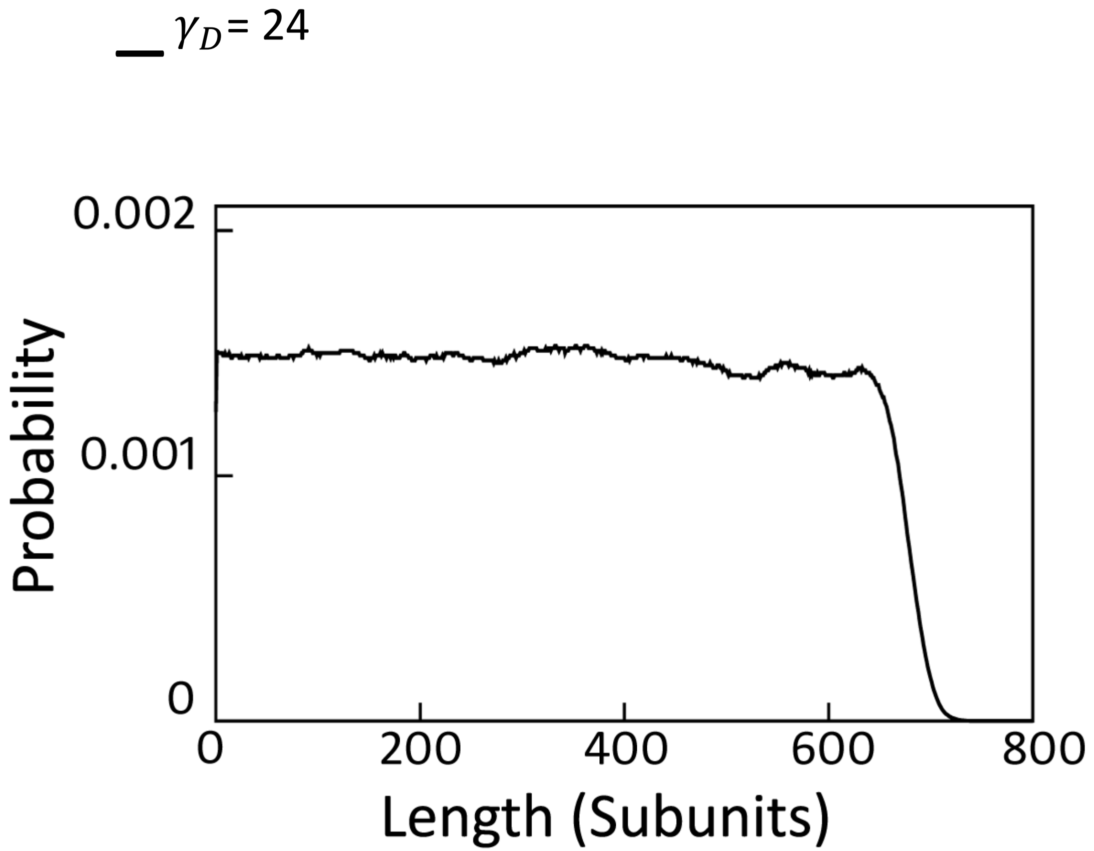
# Departure from 'equilibrium' in hydrolysis model




If we set $r_T = r_D$
then,
**$\gamma_T$ = $\gamma_D$ effectively corresponds to reversibility/equilibrium**

# Departure from 'equilibrium' in hydrolysis model

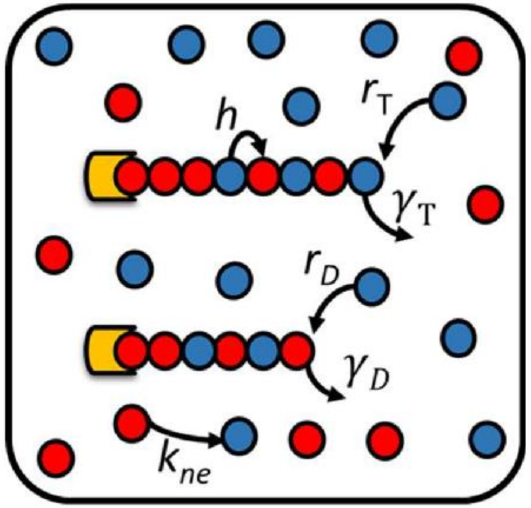


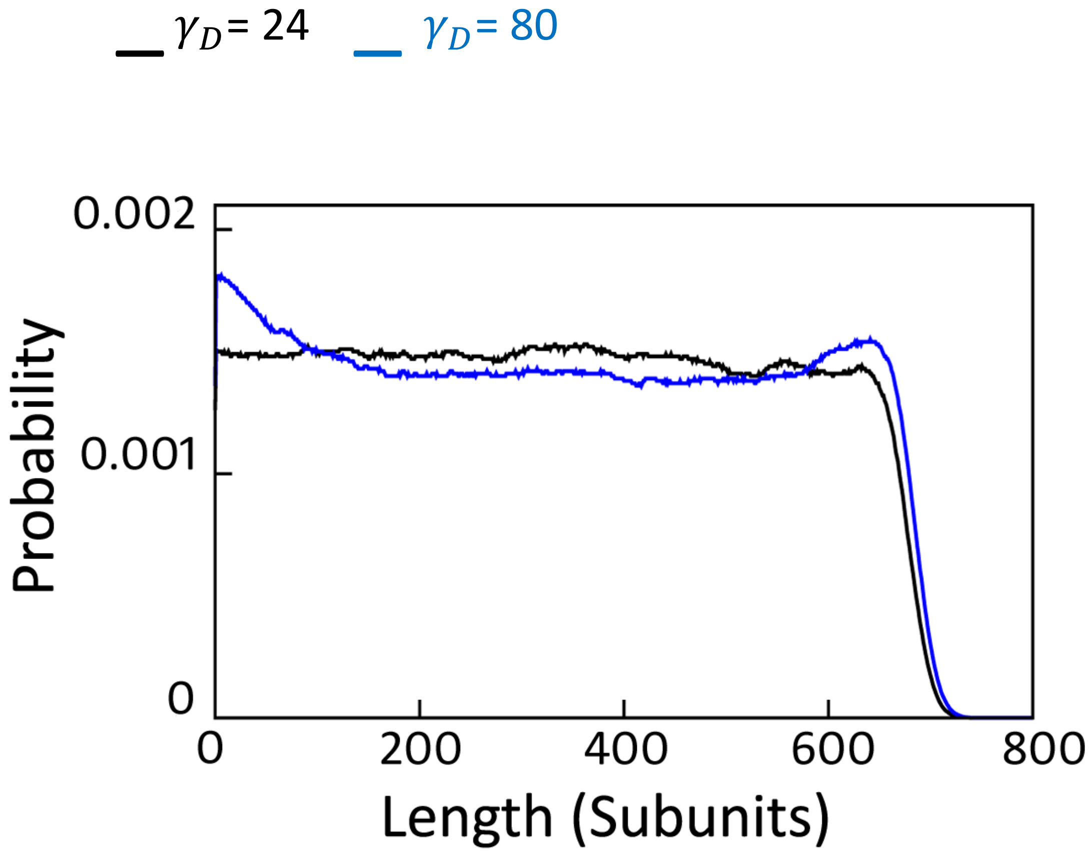




If we set $r_T = r_D$
then,
**$\gamma_T$ = $\gamma_D$ effectively corresponds to reversibility/equilibrium**

We set: $\gamma_T = 24$

# Departure from 'equilibrium' in hydrolysis model

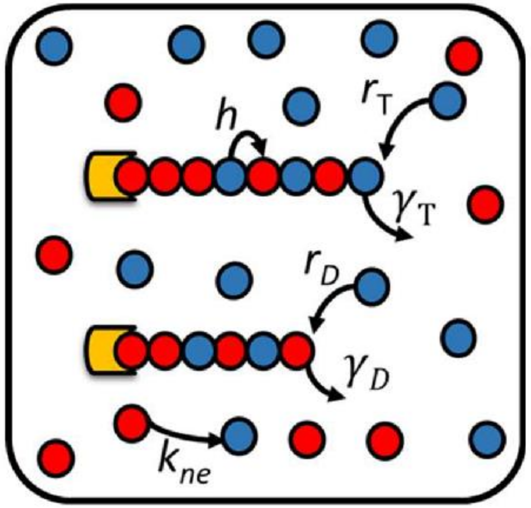


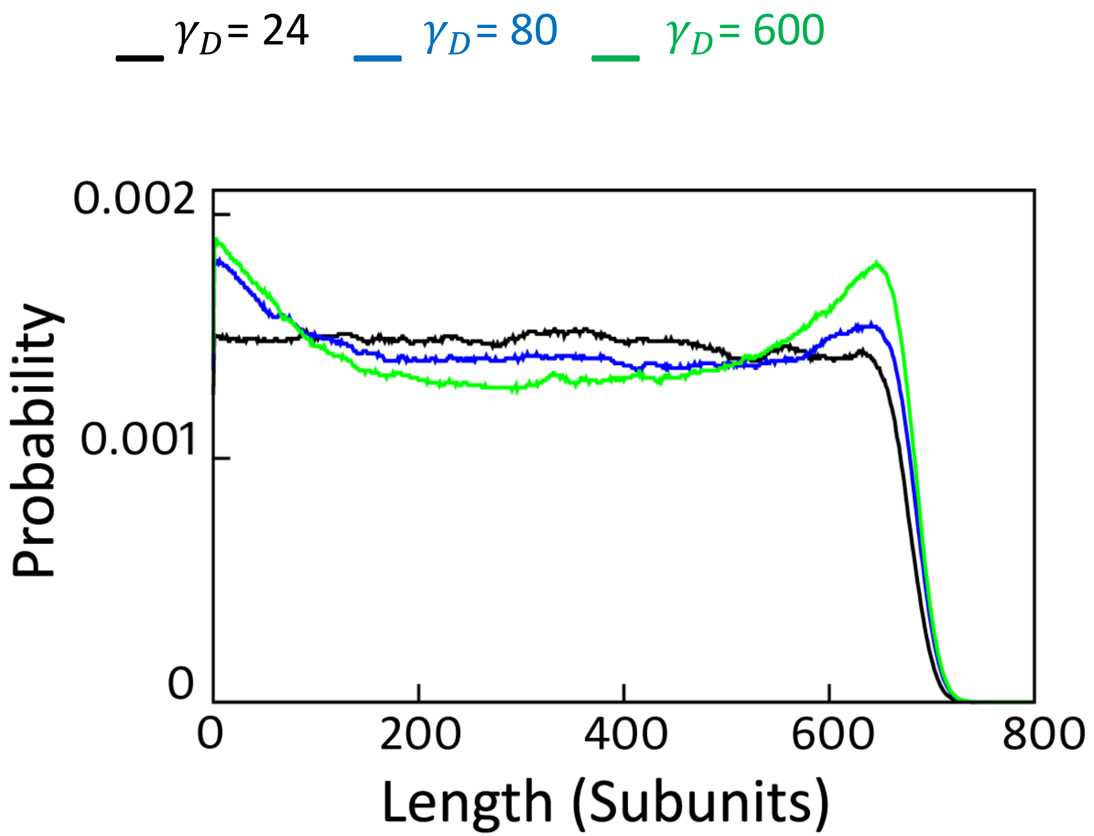

If we set $r_T = r_D$
then,
**$\gamma_T$ = $\gamma_D$ effectively corresponds to reversibility/equilibrium**

We set: $\gamma_T = 24$

# Departure from ‘equilibrium’ in hydrolysis model

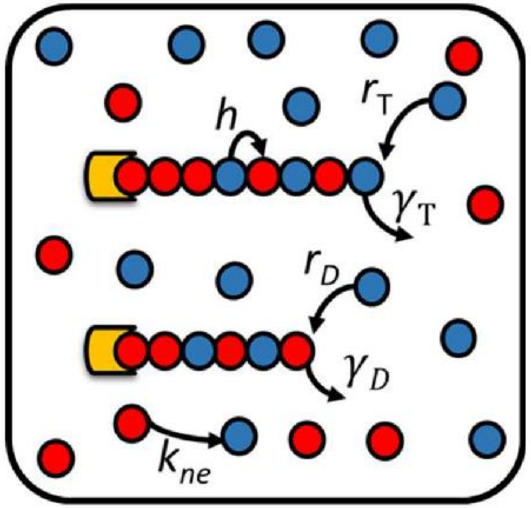


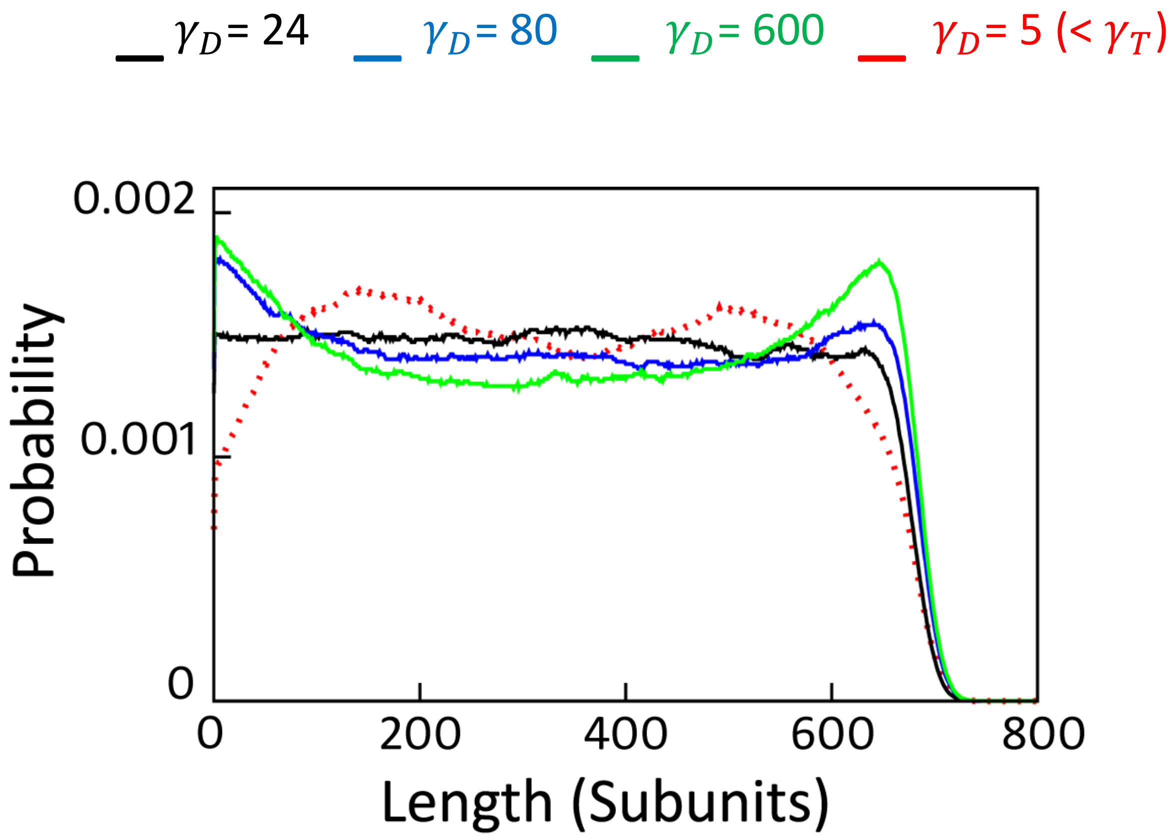

If we set $r_T = r_D$
then,
**$\gamma_T$ = $\gamma_D$ effectively corresponds to reversibility/equilibrium**




We set: $\gamma_T = 24$

# Departure from 'equilibrium' in hydrolysis model

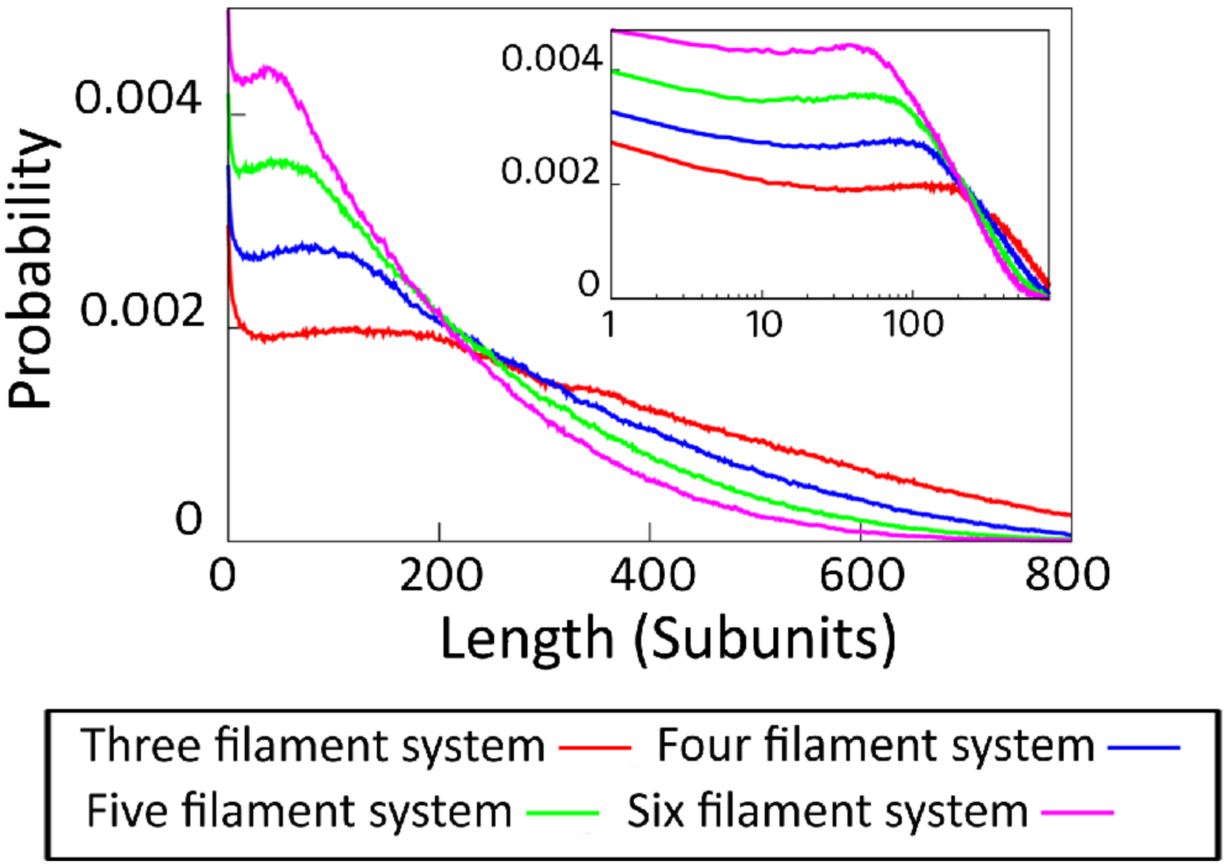



If we set $r_T = r_D$
then,
**$\gamma_T = \gamma_D$ effectively corresponds to reversibility/equilibrium**

We set: $\gamma_T = 24$

# Bimodality in multiple microtubules



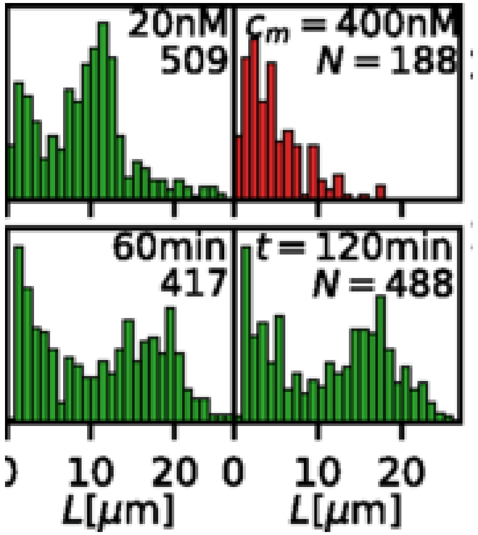
# How can we test the predictions?

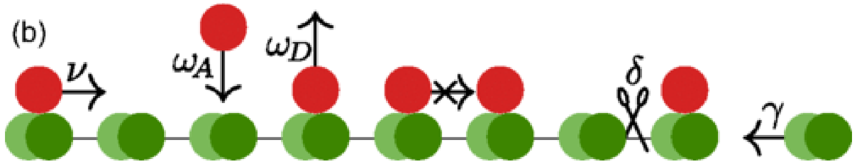
- *In vitro* experiments can be designed with GMPCPP-tubulins (nonhydrolyzable) as a control
- Solutions’ pH level is known to affect GTP hydrolysis, by which hydrolysis rate may be tuned.

# How can we test the predictions?

- *In vitro* experiments can be designed with GMPCPP-tubulins (nonhydrolyzable) as a control
- Solutions' pH level is known to affect GTP hydrolysis, by which hydrolysis rate may be tuned.

➢ A recent *in vitro* experiment with finite pools of kip3 motors and GMPCPP tubulins showed bimodality. (Rank et al., PRL, 2018)



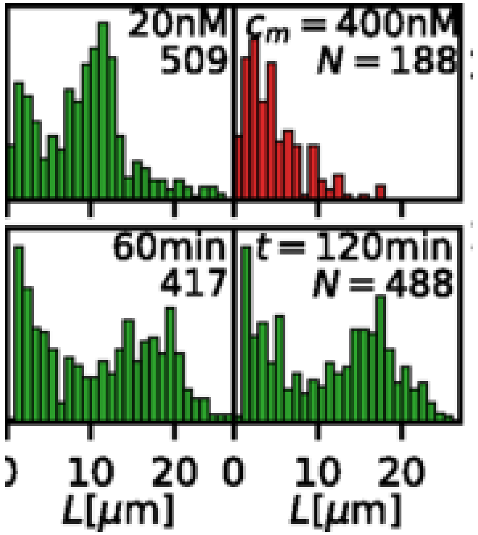



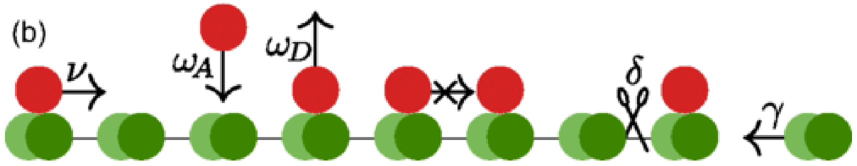

Similar to a 'two-state' model in essence.

# How can we test the predictions?

- *In vitro* experiments can be designed with GMPCPP-tubulins (nonhydrolyzable) as a control
- Solutions' pH level is known to affect GTP hydrolysis, by which hydrolysis rate may be tuned.

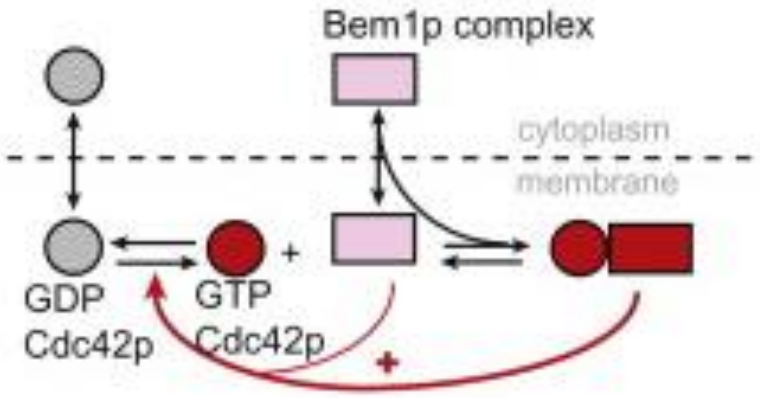
➢ A recent *in vitro* experiment with finite pools of kip3 motors and GMPCPP tubulins showed bimodality. (Rank et al., PRL, 2018)




Similar to a 'two-state' model in essence.

➢ Yeast polarity protein Cdc42 oscillate in sizes *in vivo*.
(Howell et al., Cell, 2012)



# Summary

- Hydrolysis acts like a irreversible 'chemical switch' that makes microtubule dynamics nonequilibrium in nature.

- Hydrolysis leads to a number of collective effects in multiple filaments.

  - Collective stall force of multiple filaments is not just the sum of individual forces.

  - In a limiting pool of subunits, individual filaments toggle stochastically between 'higher length' and 'lower length' → Bimodal length distribution

- The larger the difference of kinetic rates between GTP-bound & GDP-bound states, the more prominent collective effects are expected

- Actin and ParM filaments also exhibit hydrolysis: our results can carry forward.

# Acknowledgements

- Old works at IITB:  Dibyendu , Ranjith, Mandar, Tripti
- **Recent work at our IISERK lab:  Sankeert (BS-MS)**
- Computational facility: DBT Ramalingaswami fellowship & SERB start-up grant

That's all for today

Thank you