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Assembly, Forces, and Organization of the Cytoskeleton

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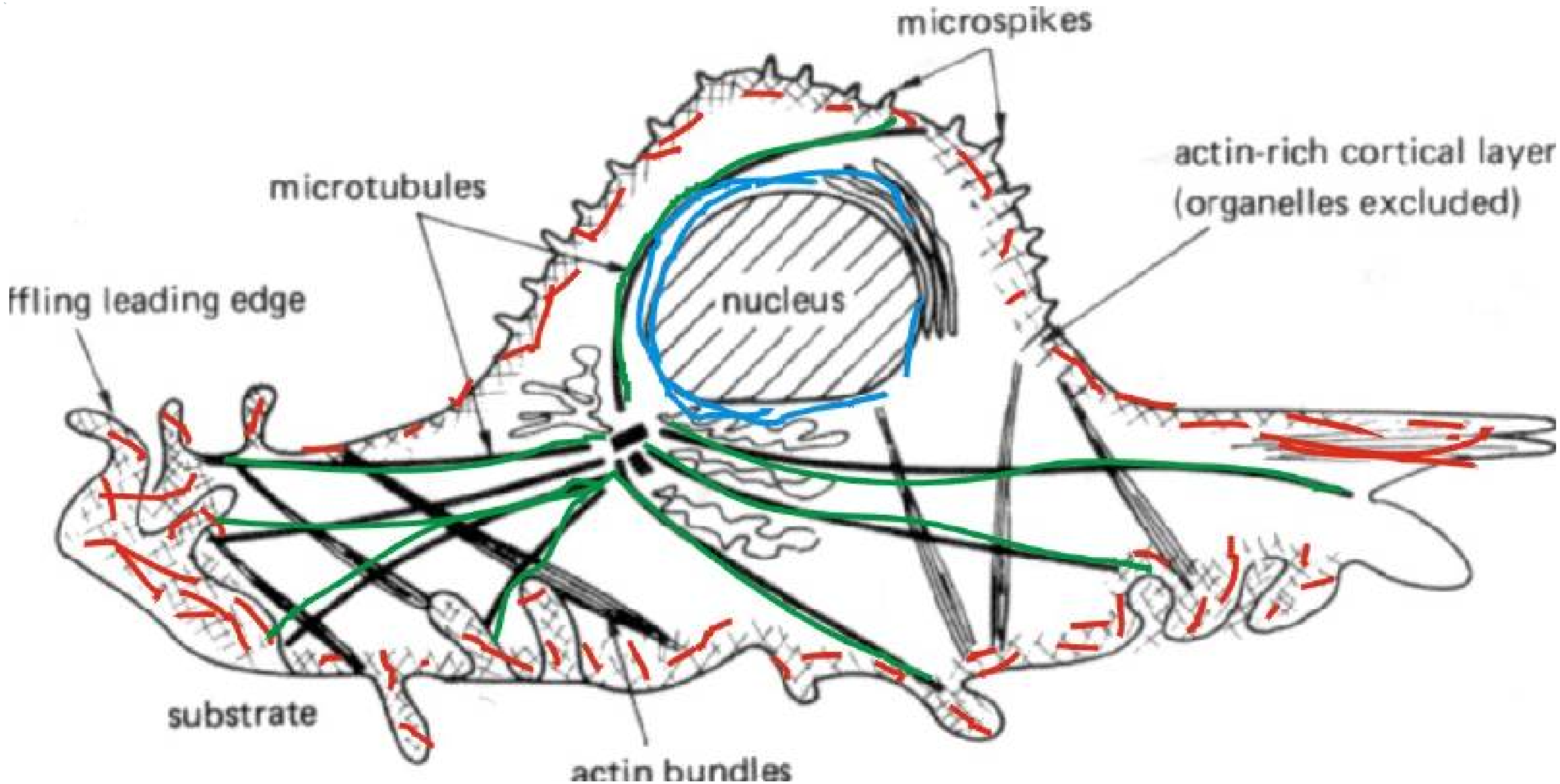
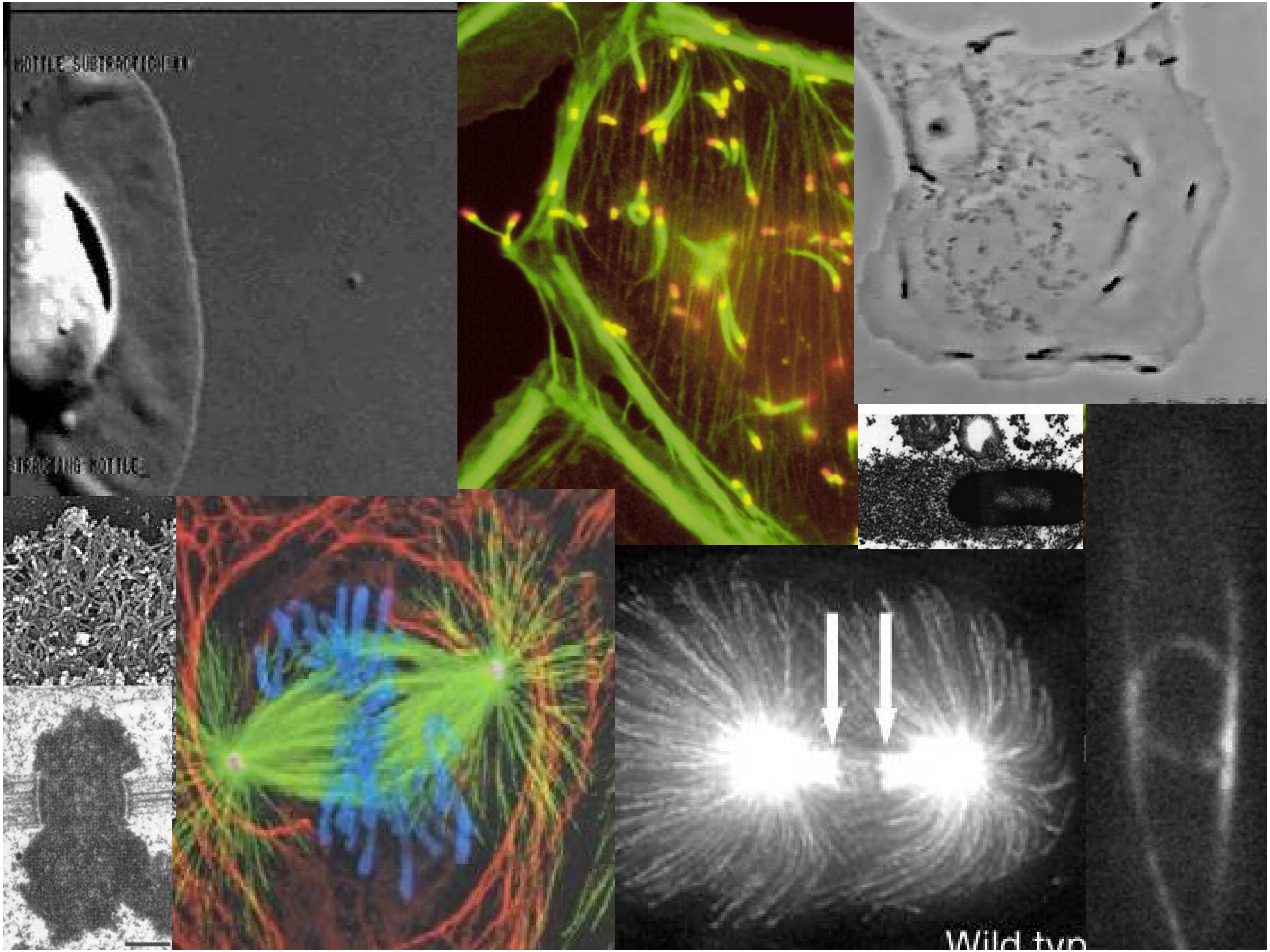
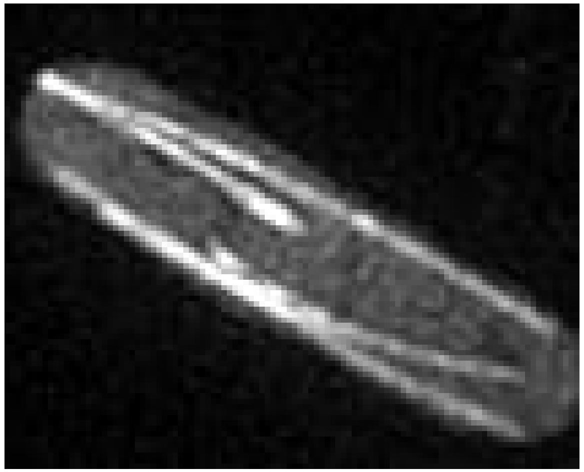
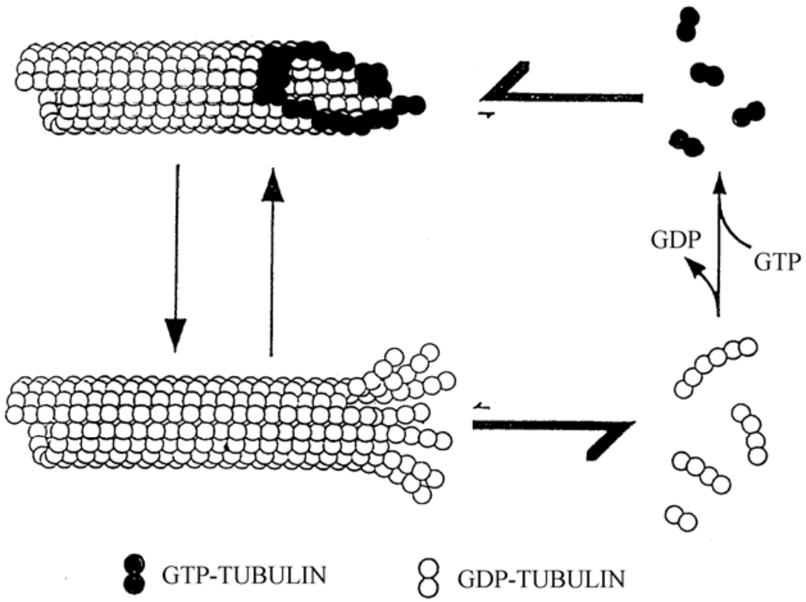

microspikes
actin-rich cortical layer
(organelles excluded)
microtubules
ffling leading edge
nucleus
substrate
actin bundles



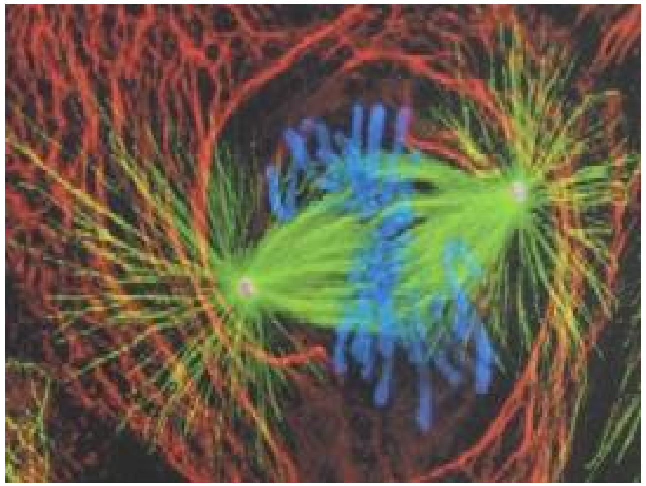
DYNAMIC MICROTUBULES GENERATE FORCES IN CELLS

Nuclear positioning in interphase fission yeast cells



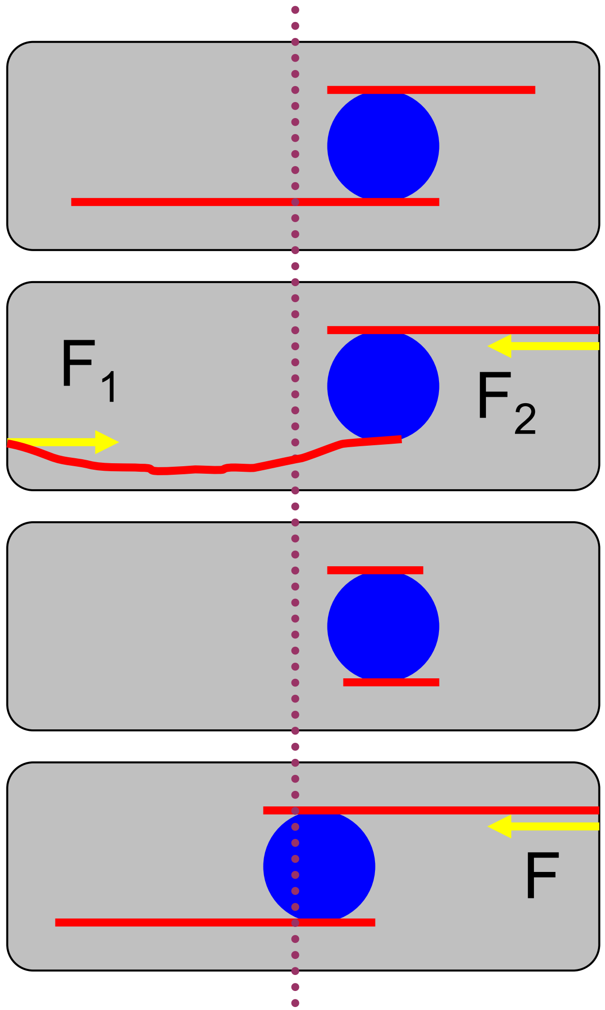


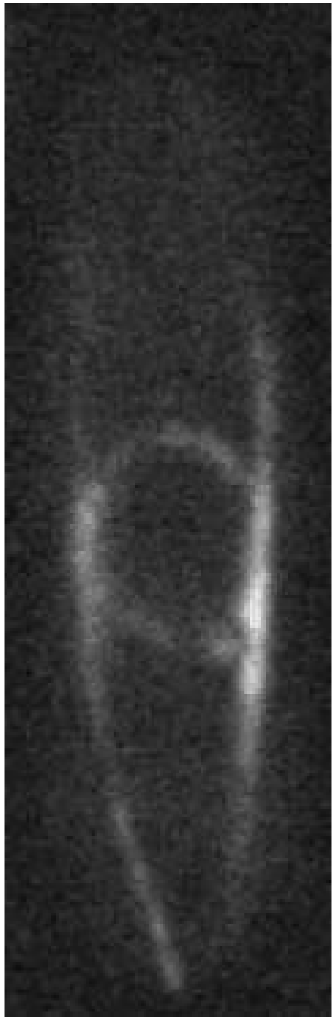
Pushing and pulling at microtubule – kinetochore interface



Rieder et al.

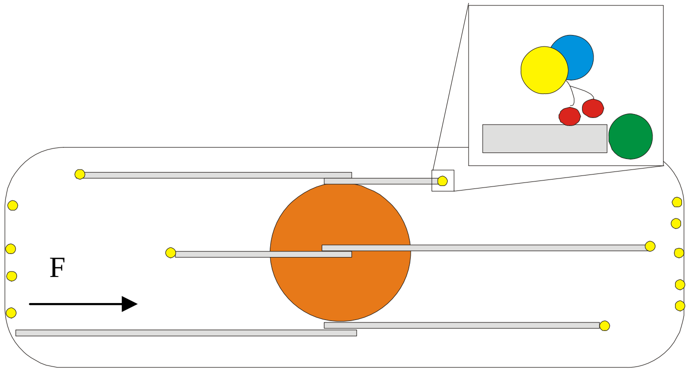
NUCLEAR POSITIONING IN INTERPHASE FISSON YEAST



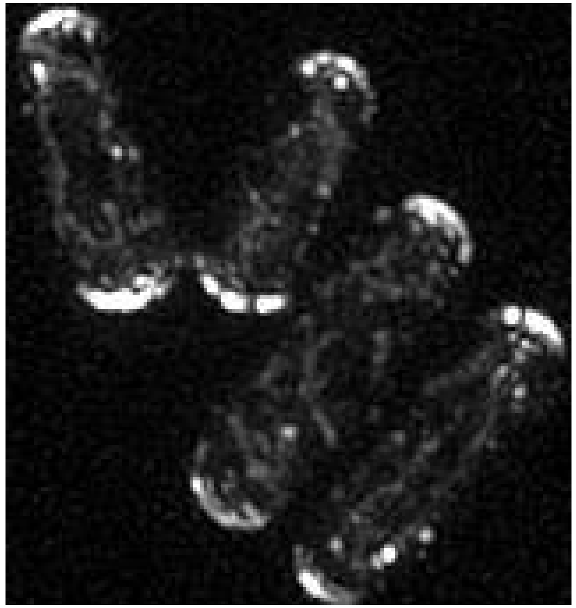


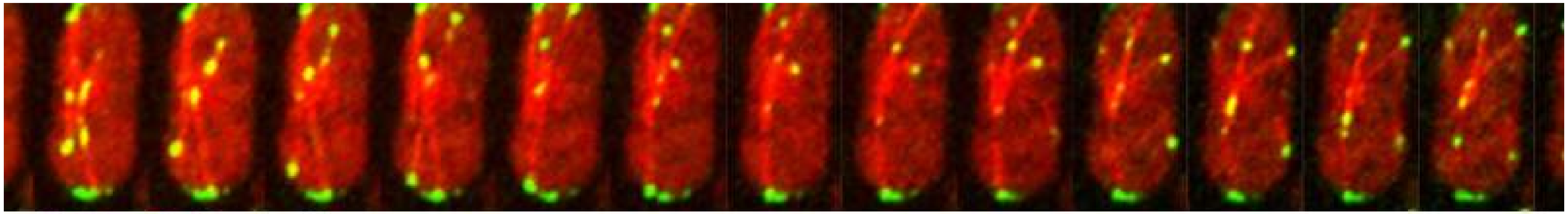
Tran et al (2001), JCB **153**, 397

MT end binding proteins and protein delivery

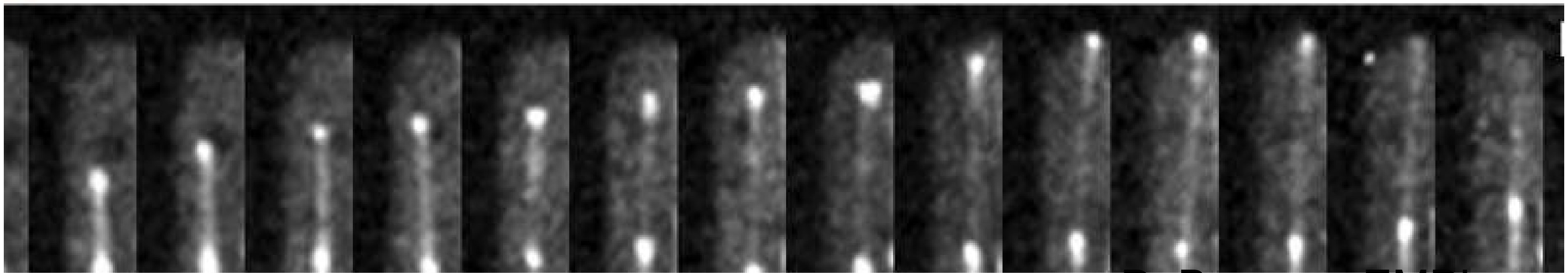


Tea1



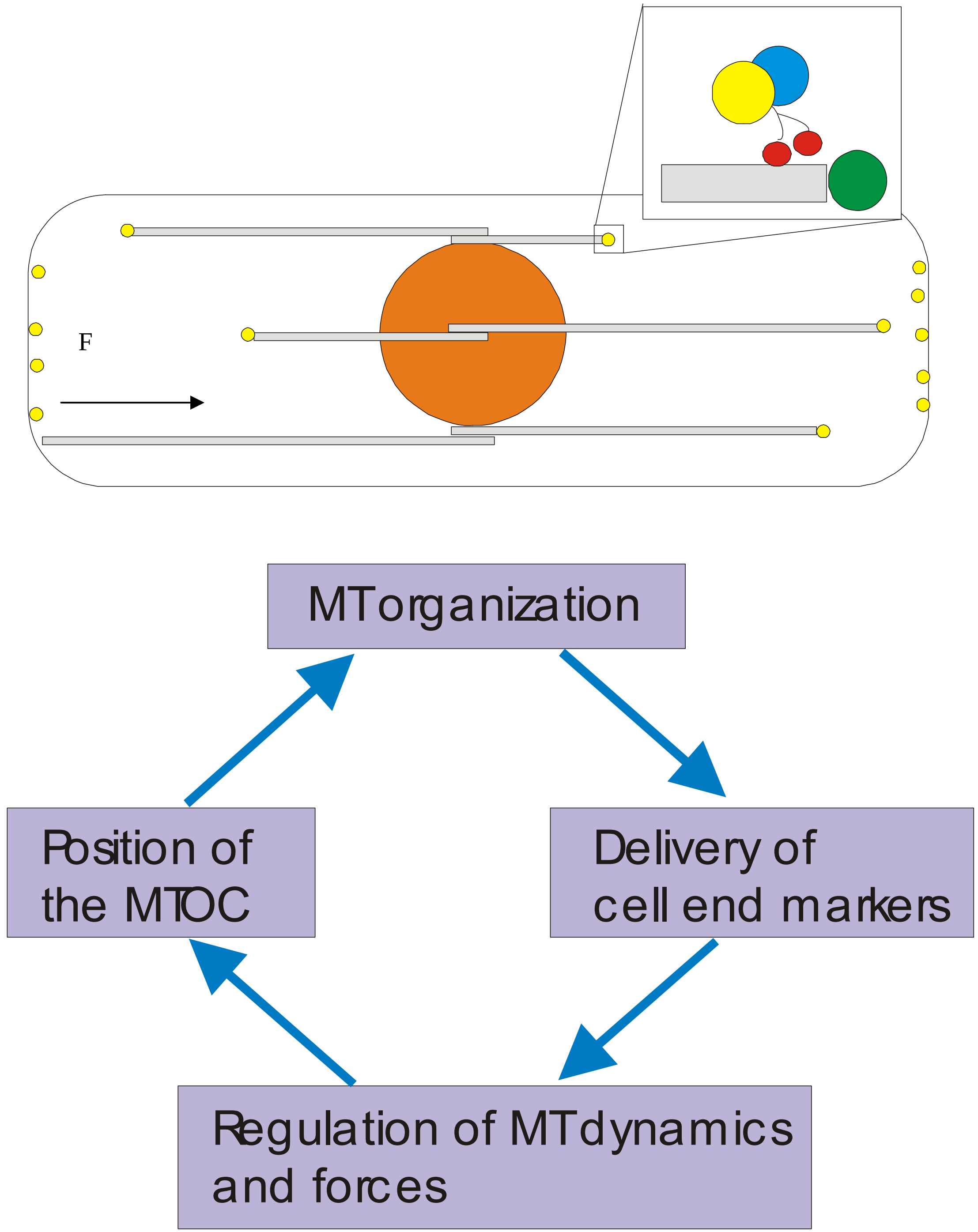


pYFP CFPtubulin



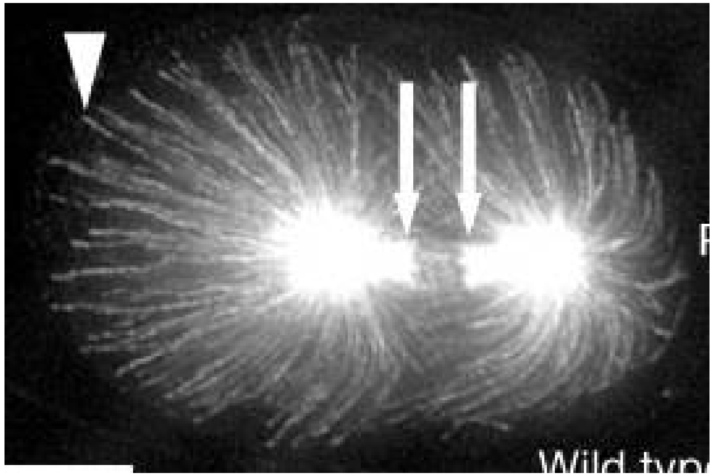
Pmal3p

D. Brunner, EMBL

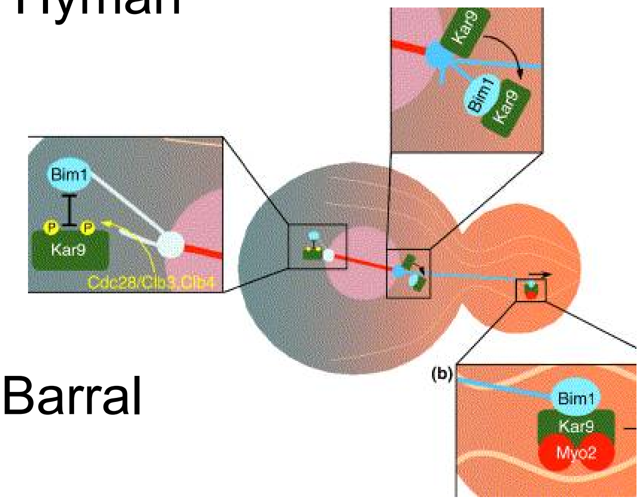

F
MT organization
Delivery of cell end markers
Regulation of MT dynamics and forces
Position of the MTOC

Cell polarity,
Protein patterns, and
MT-cortex interactions

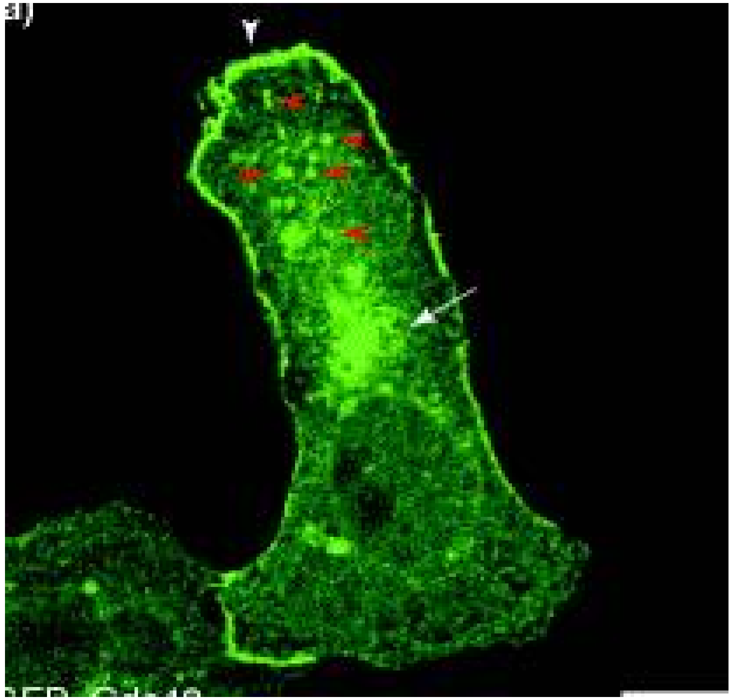
Asymmetric spindle positioning



T. Hyman



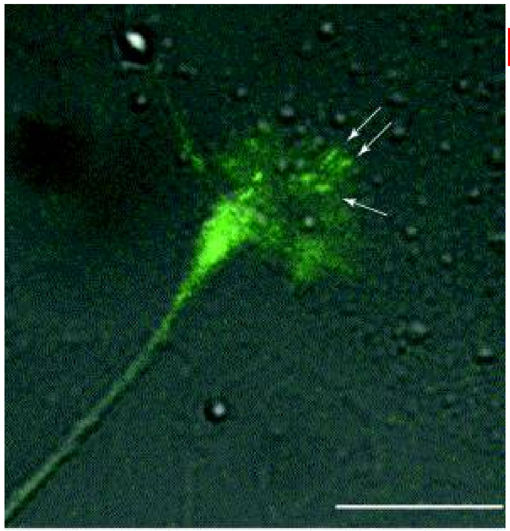
Y. Barral

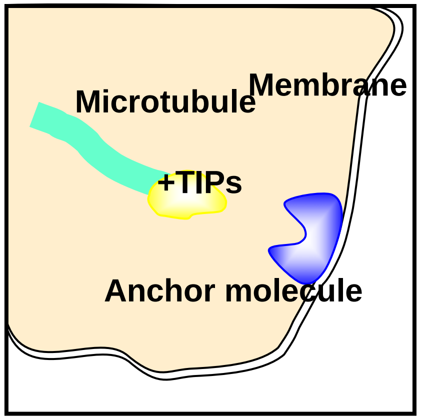


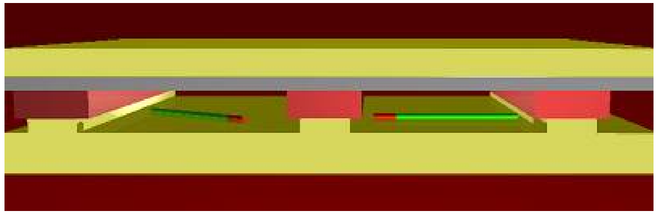
Cell locomotion

K. Kaibuchi

Neuronal growth cone



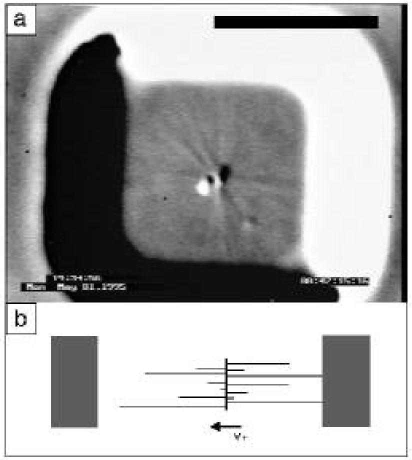




PROGRAM

1) Force generation capabilities on single MT level

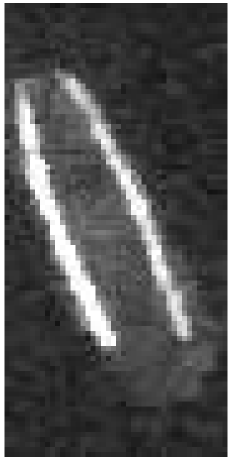
Force-regulation of dynamics



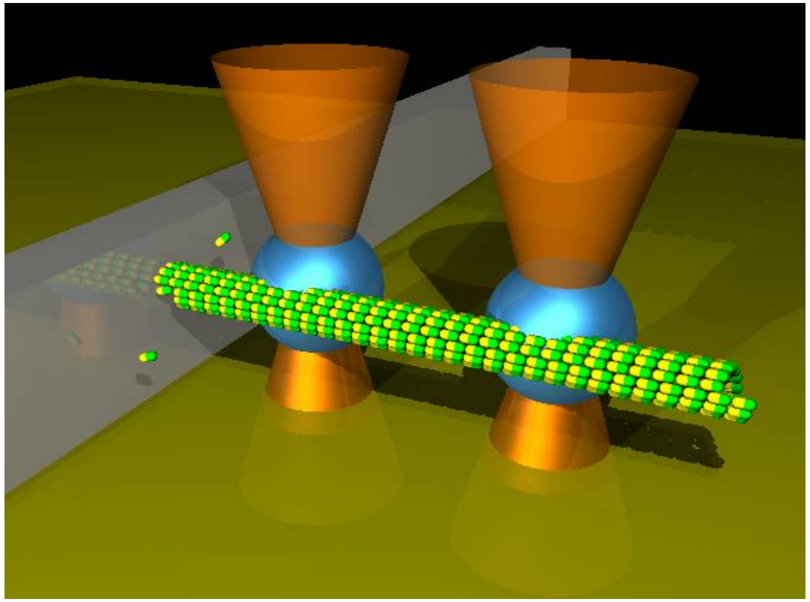
2) Positioning in simple model(s) (experiments)

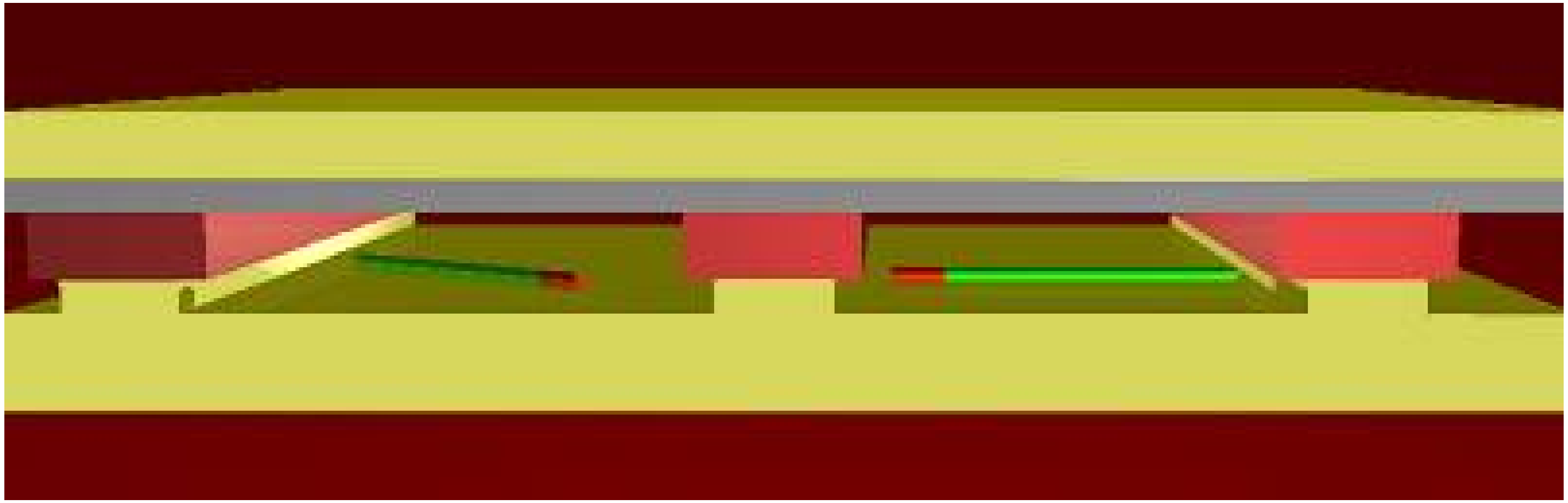
3) Force generation and positioning in vivo

- Regulation ?
- Fine tuning ?



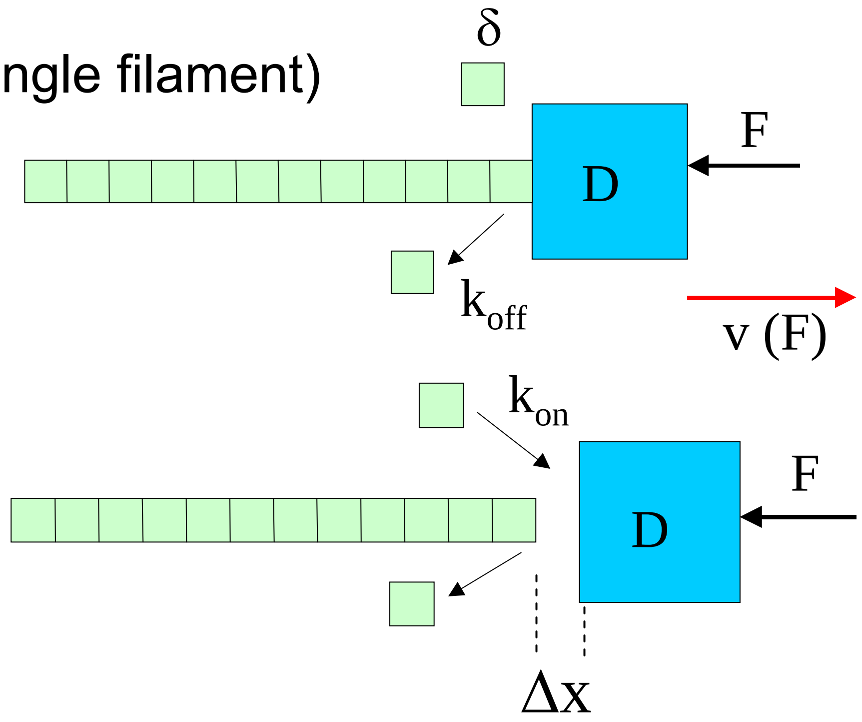
Single MT force generation in vitro

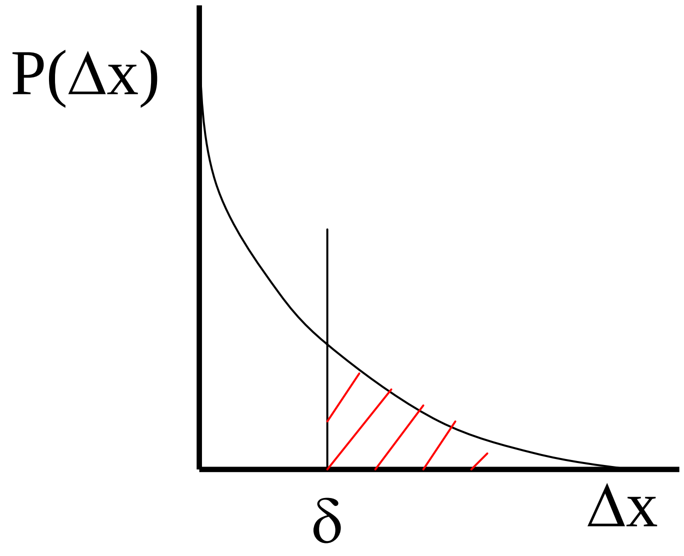




Pushing force: “Brownian ratchet”

(single filament)





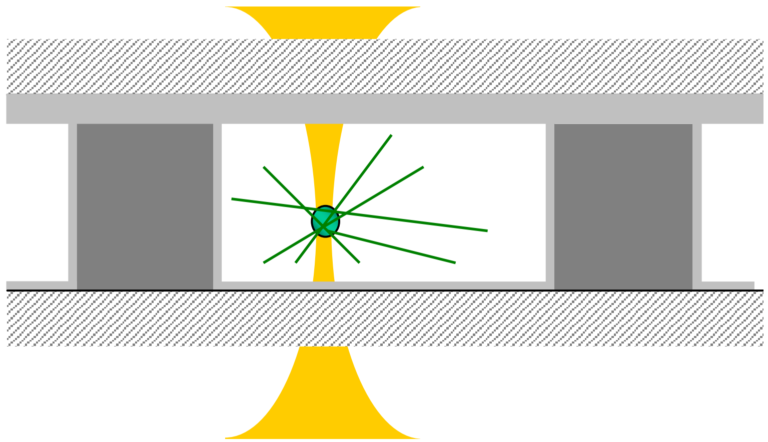
$$P\left(\Delta x \geq \delta\right) = \exp\left(-F\delta / k_B T\right)$$

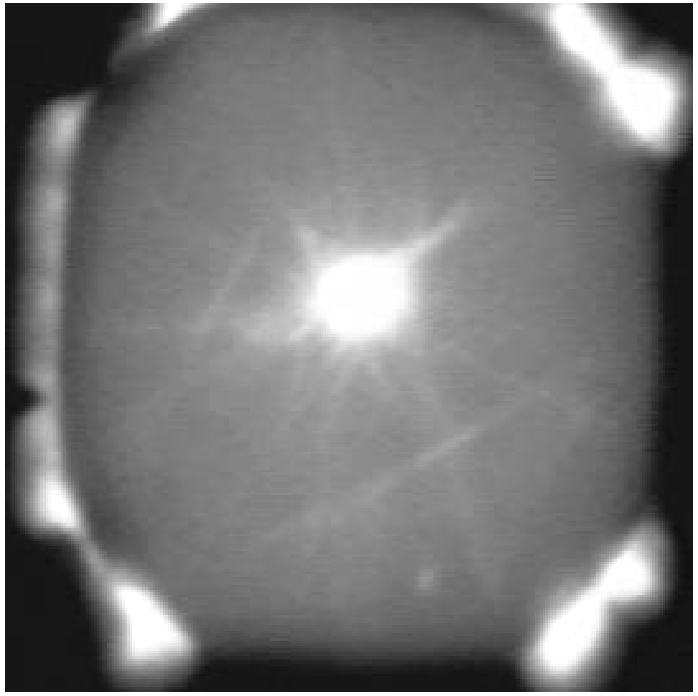
$$\boxed{V\left(F\right) = \delta\left(k_{on} c e^{-F\delta / k_B T} - k_{off}\right)}$$

$$\boxed{F_{\max} = \frac{k_B T}{\delta} \ln\left(\frac{k_{on} c}{k_{off}}\right)}$$

Peskin et al. 93

Aster positioning
in vitro



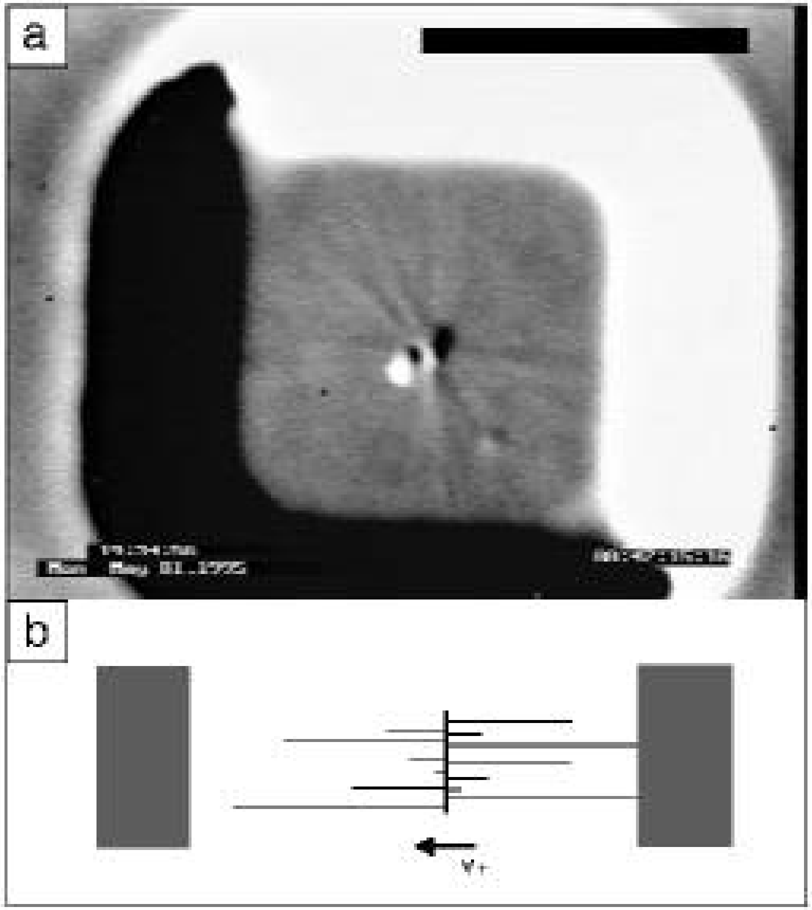


Polymerization forces
enough for aster positioning

1D model

$$\partial_t p_+ = -f_{+-} p_+ + f_{-+} p_- - v_+ \partial_x p_+$$

$$\partial_t p_- = +f_{+-} p_+ - f_{-+} p_- + v_- \partial_x p_-$$



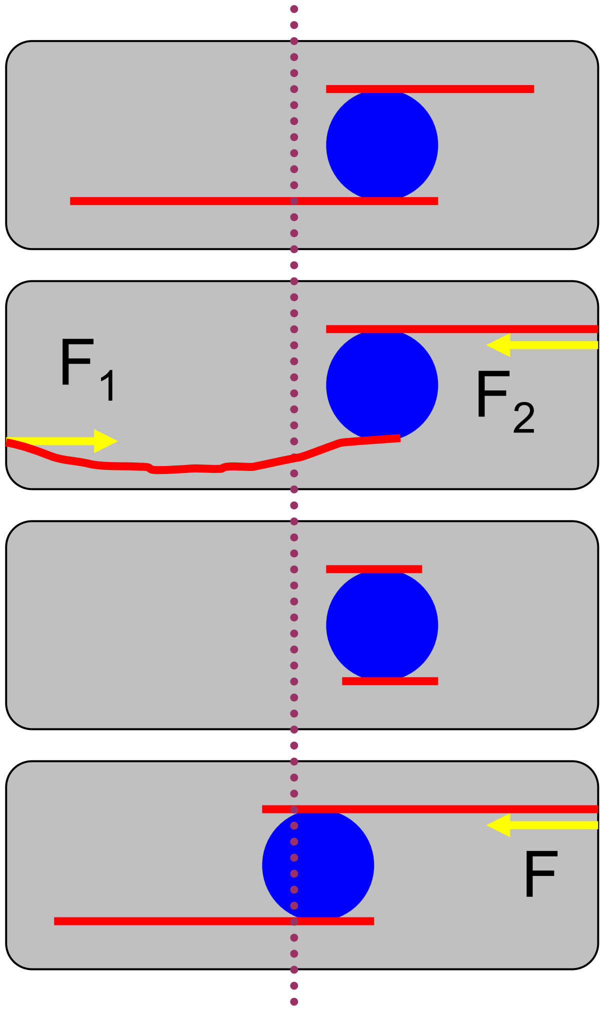
$$f_0 P_0 = v_+ \, p_+ \big|_{x=0}$$

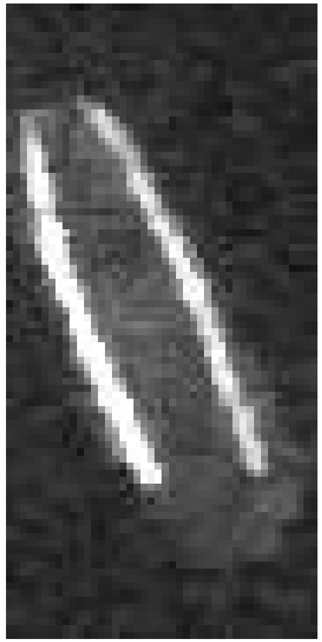
$$f_b P_L = v_- \, p_- \big|_{x=L}$$

$$\partial_t P_0 = v_- \, p_- \big|_{x=0} - f_0 P_0$$

$$\partial_t P_L = v_+ \, p_+ \big|_{x=L} - f_b P_L$$

NUCLEAR POSITIONING IN INTERPHASE FISSON YEAST

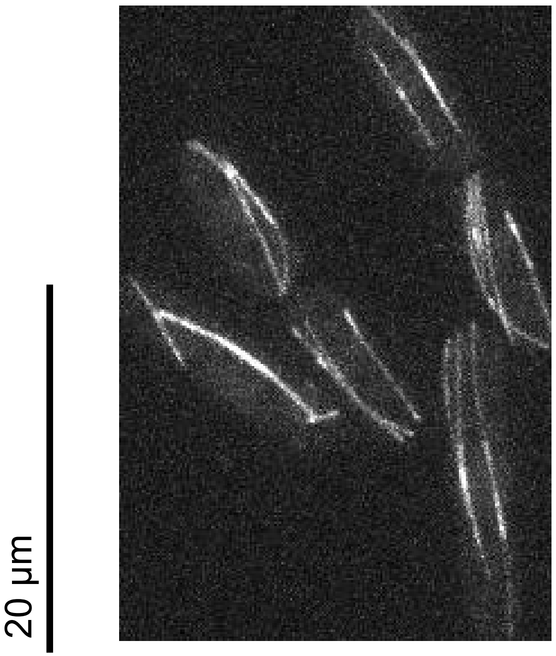




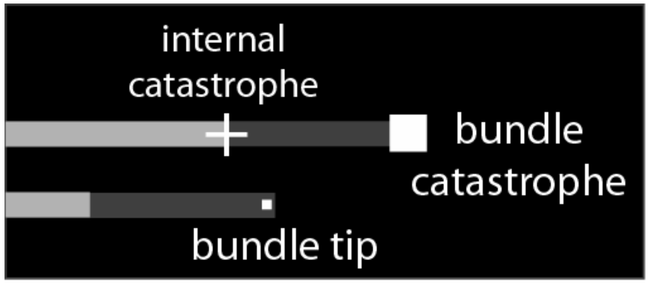
Enhanced Catastrophe Rate at Cell Ends in Interphase Fission Yeast Cells: IN RESPONSE TO FORCE?

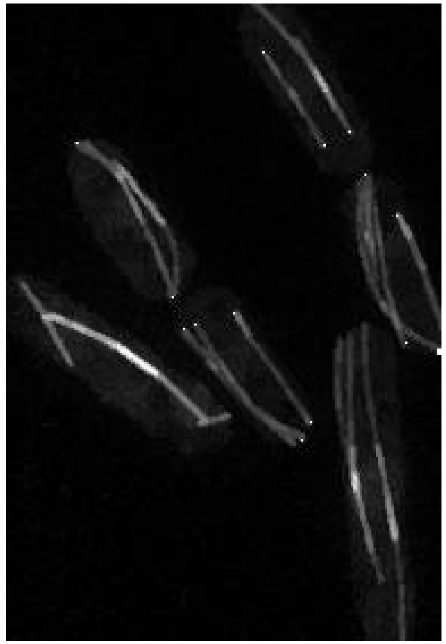
Detection of MT catastrophes by automated movie analysis

- GFP-tubulin
- 3D spinning disk confocal microscopy
- Maximum projections of 3D stacks, dt=8 sec



40 x real time





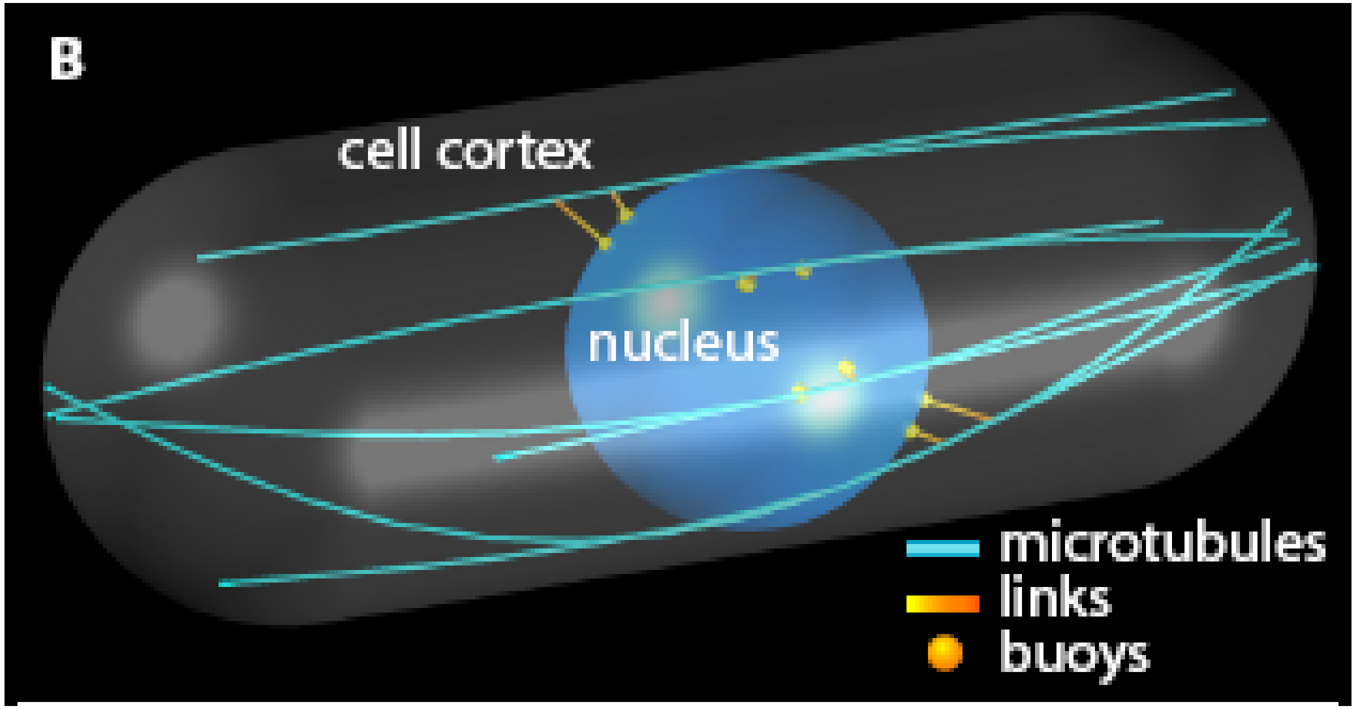
100s of cells
10.000s of catastrophes

Detect:

- Leading catastrophes
- Internal catastrophes
- Microtubule ends

CYTOSIM

Francois Nedelec, EMBL Heidelberg



Full 3D simulation taking into account:

- Measured dynamic parameters in fission yeast
- Confinement effects, cell shape
- Mechanical effects

Tutorial:

a) Introduction

Polymerization forces, Ratchet model

b) Force measurements in vitro

c) Positioning in Microfabricated chambers

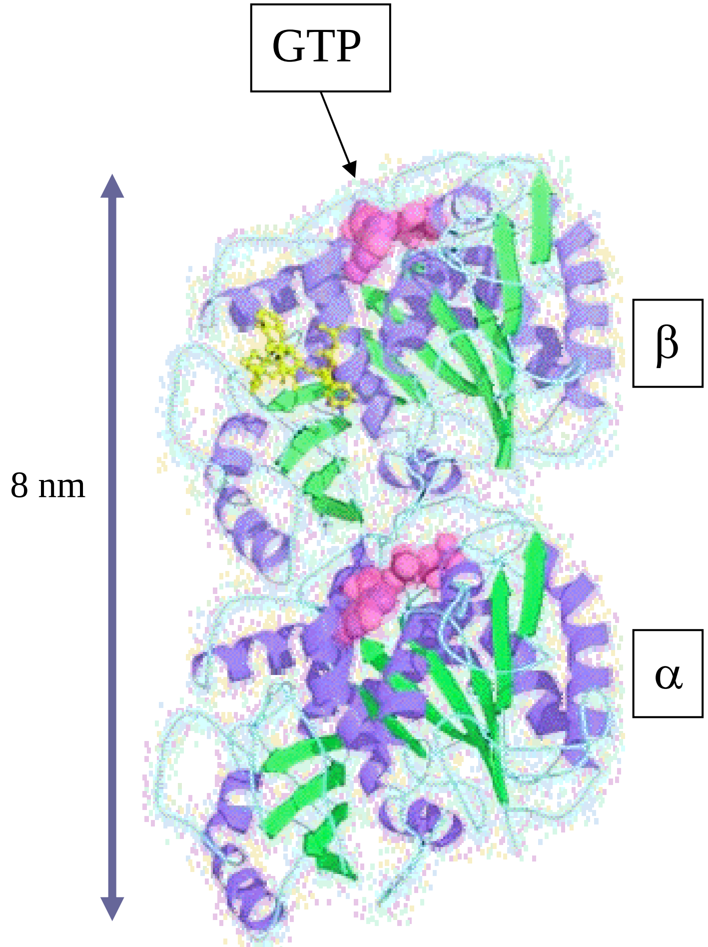
Simple model

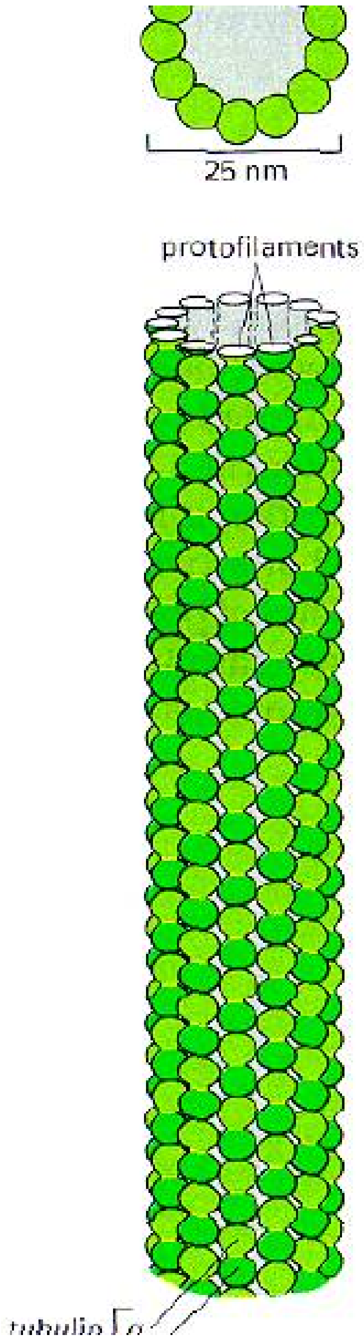
d) Forces and catastrophes in Fission Yeast

Results:

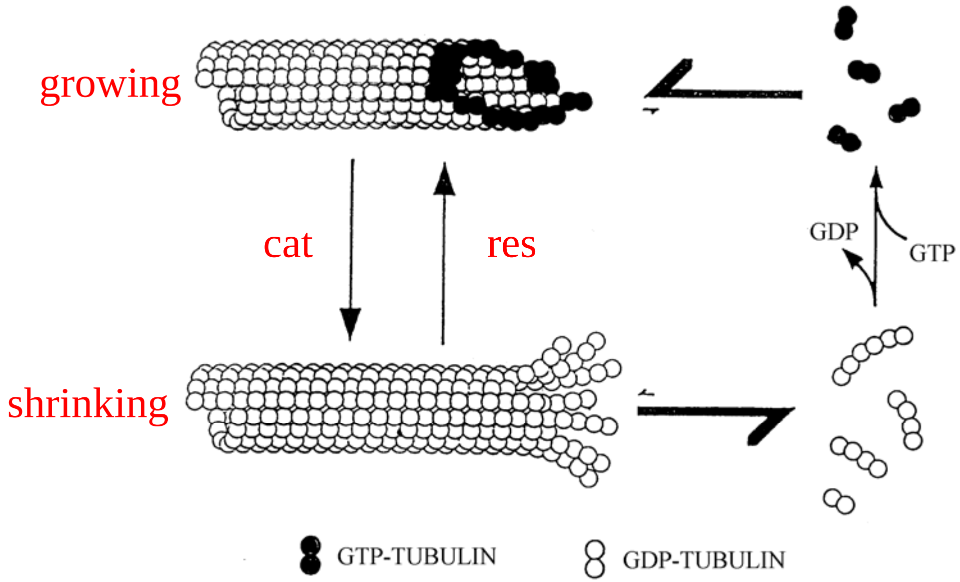
Summary of some results

Microtubule structure

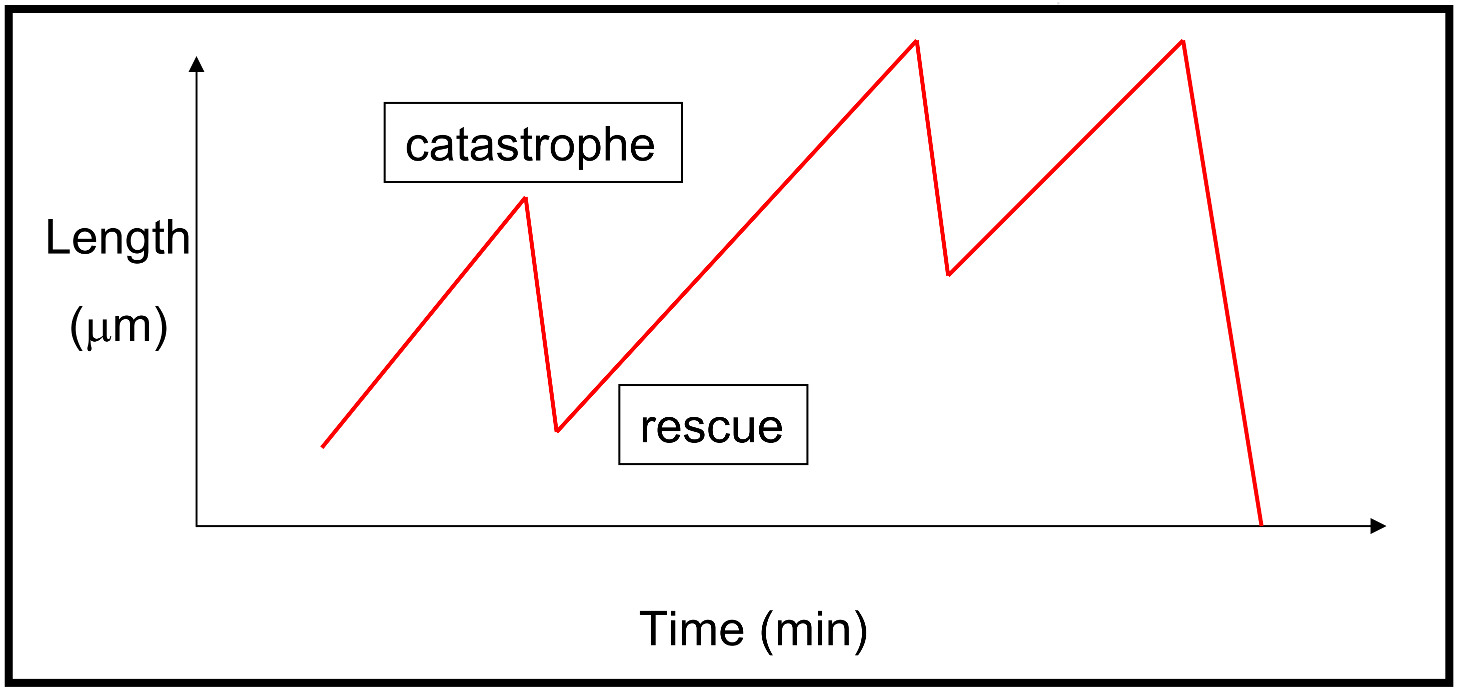




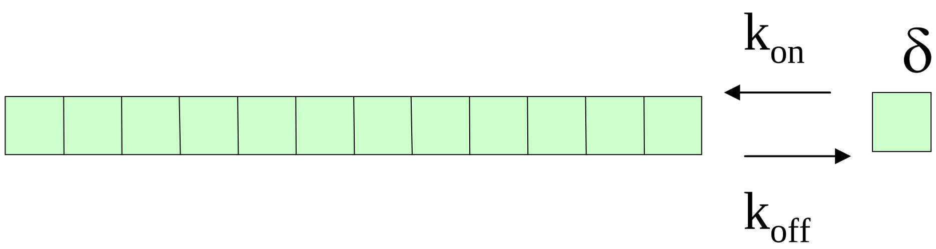
…. and dynamics

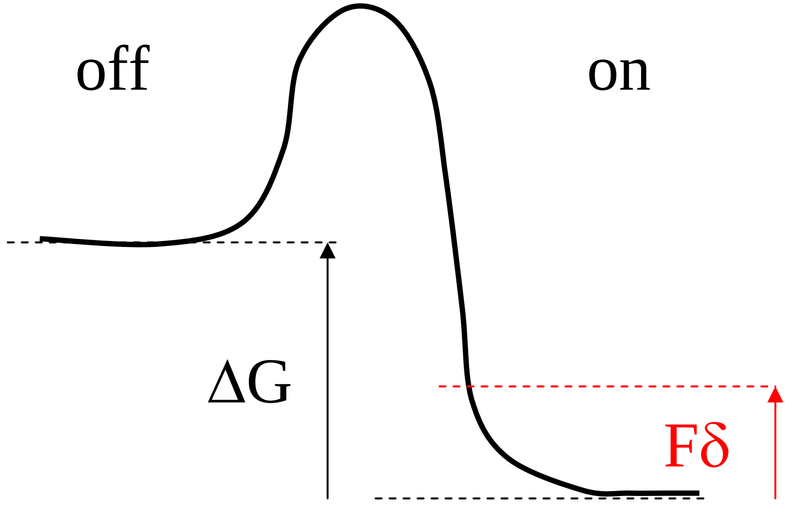


GTP hydrolysis needed
- for dynamics
- NOT for growth



A single filament





$$\boxed{V = \delta\left(k_{on}c - k_{off}\right)}$$

$$\frac{k_{on}c}{k_{off}} = \exp\left(\Delta G / k_B T\right)$$

With an applied force F:

$$\frac{k_{on}c}{k_{off}}(F) = \frac{k_{on}c}{k_{off}}\exp\left(-F\delta / k_B T\right)$$

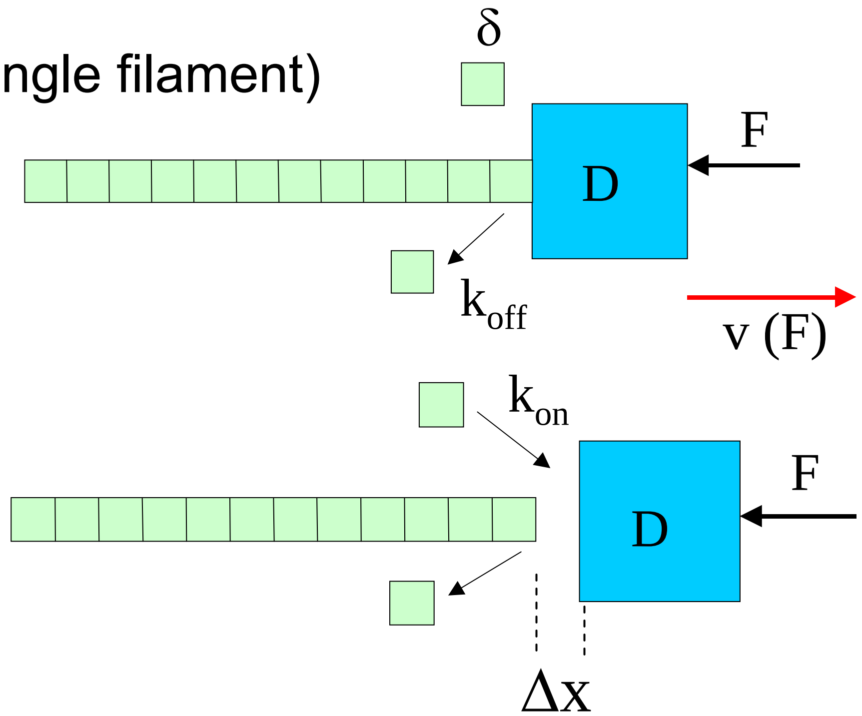
$$\boxed{V(F) = \delta\left(k_{on}c e^{-\alpha F\delta / k_B T} - k_{off} e^{(1-\alpha)F\delta / k_B T}\right)}$$

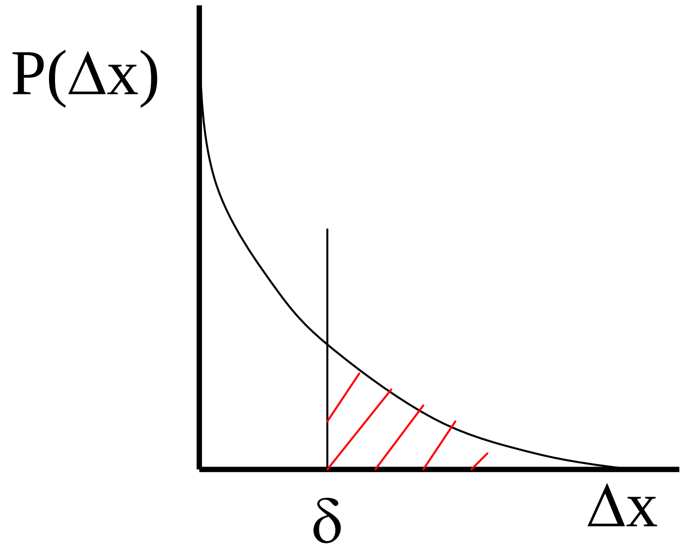
$$0 \le \alpha \le 1$$

$$\boxed{F_{\max} = \frac{k_B T}{\delta}\ln\left(\frac{k_{on}c}{k_{off}}\right)}$$

Pushing force: “Brownian ratchet”

(single filament)





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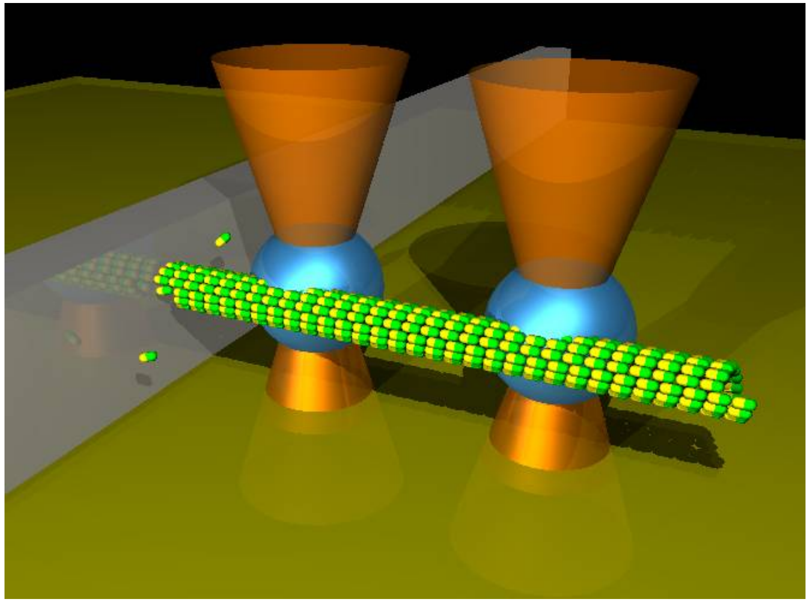
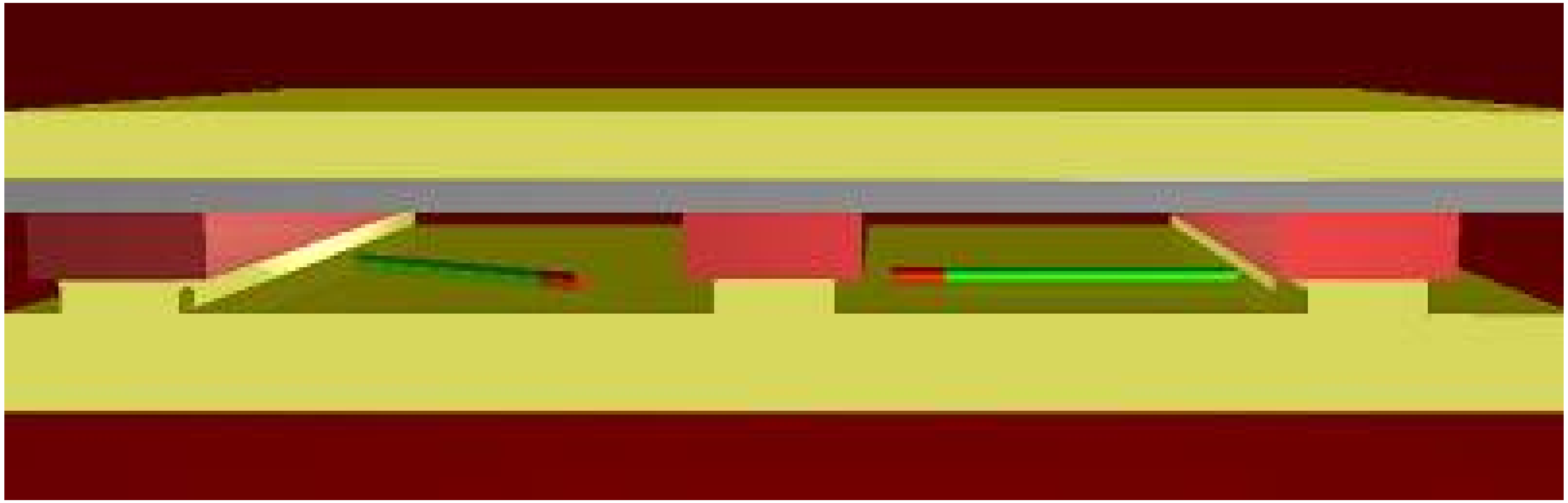
$$V\left(F\right) = \delta\left(k_{on} c e^{-F\delta/k_B T} - k_{off}\right)$$

$$F_{\max} = \frac{k_B T}{\delta} \ln\left(\frac{k_{on} c}{k_{off}}\right)$$

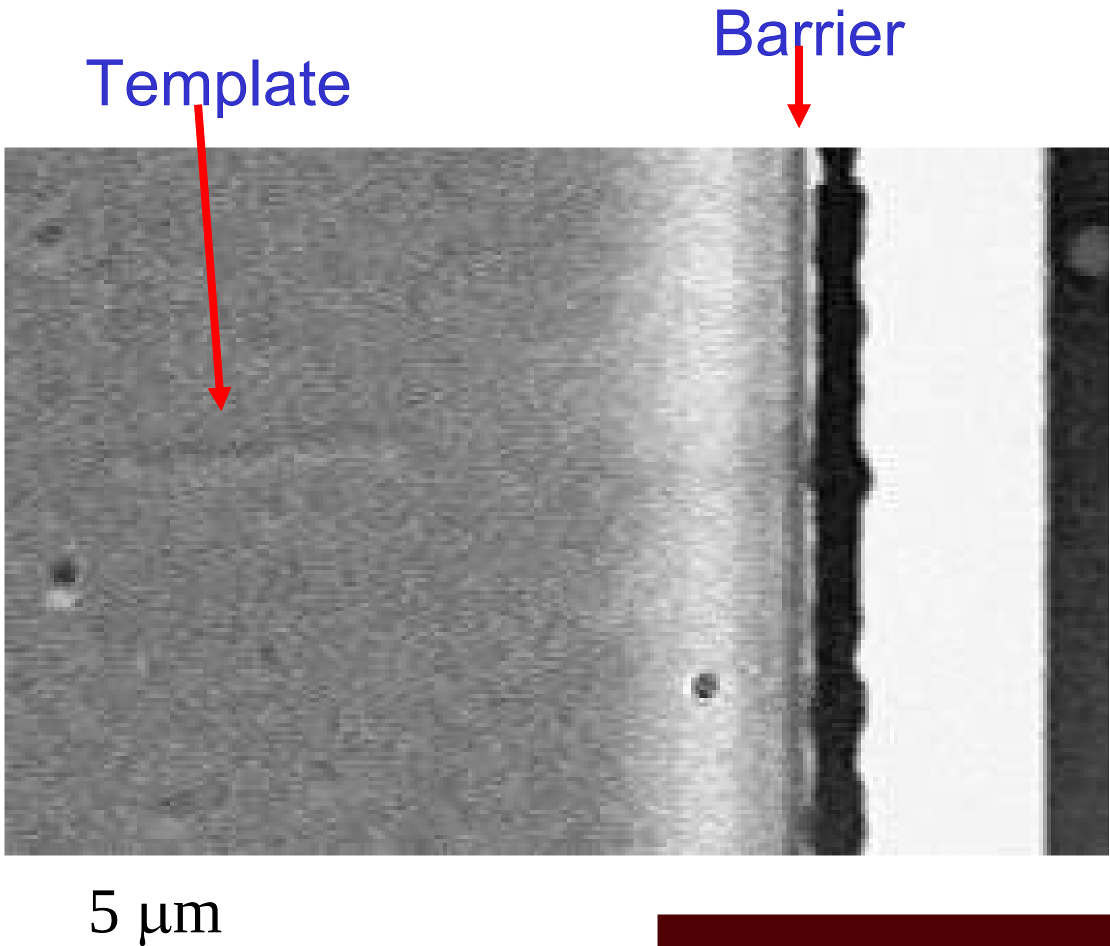
Peskin et al. 93

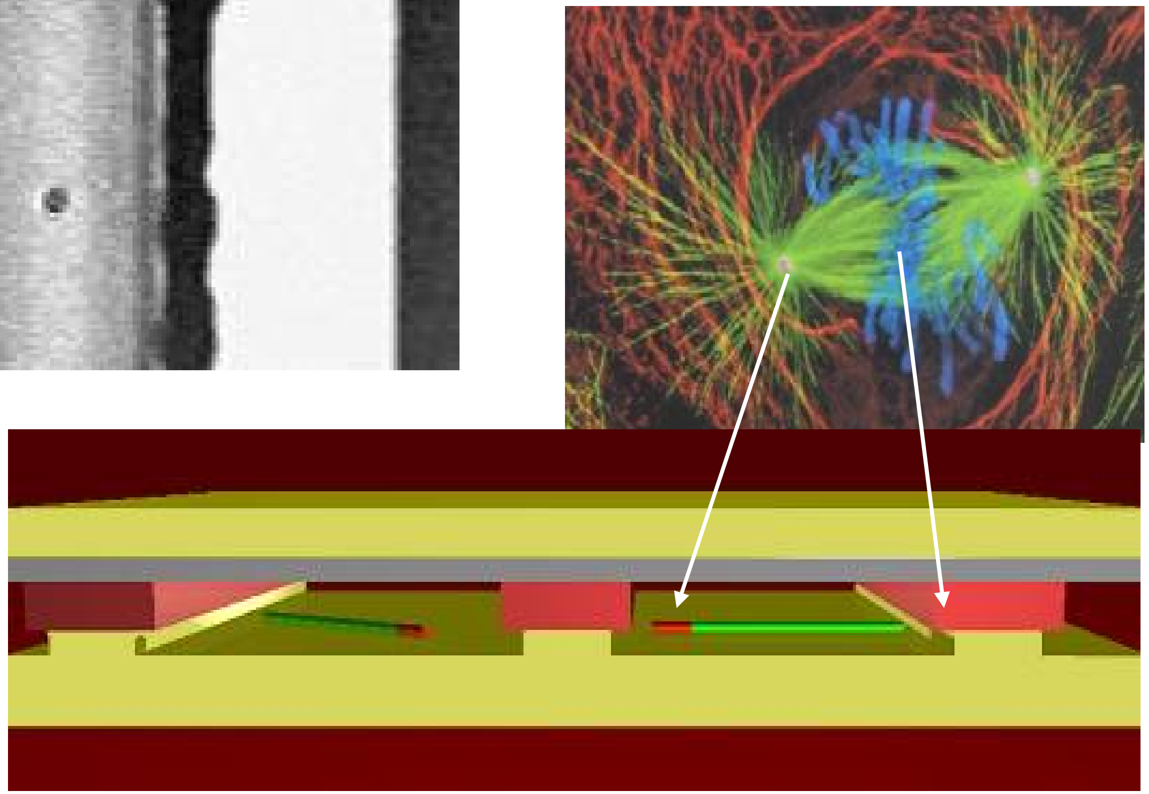
Single MT force generation in vitro

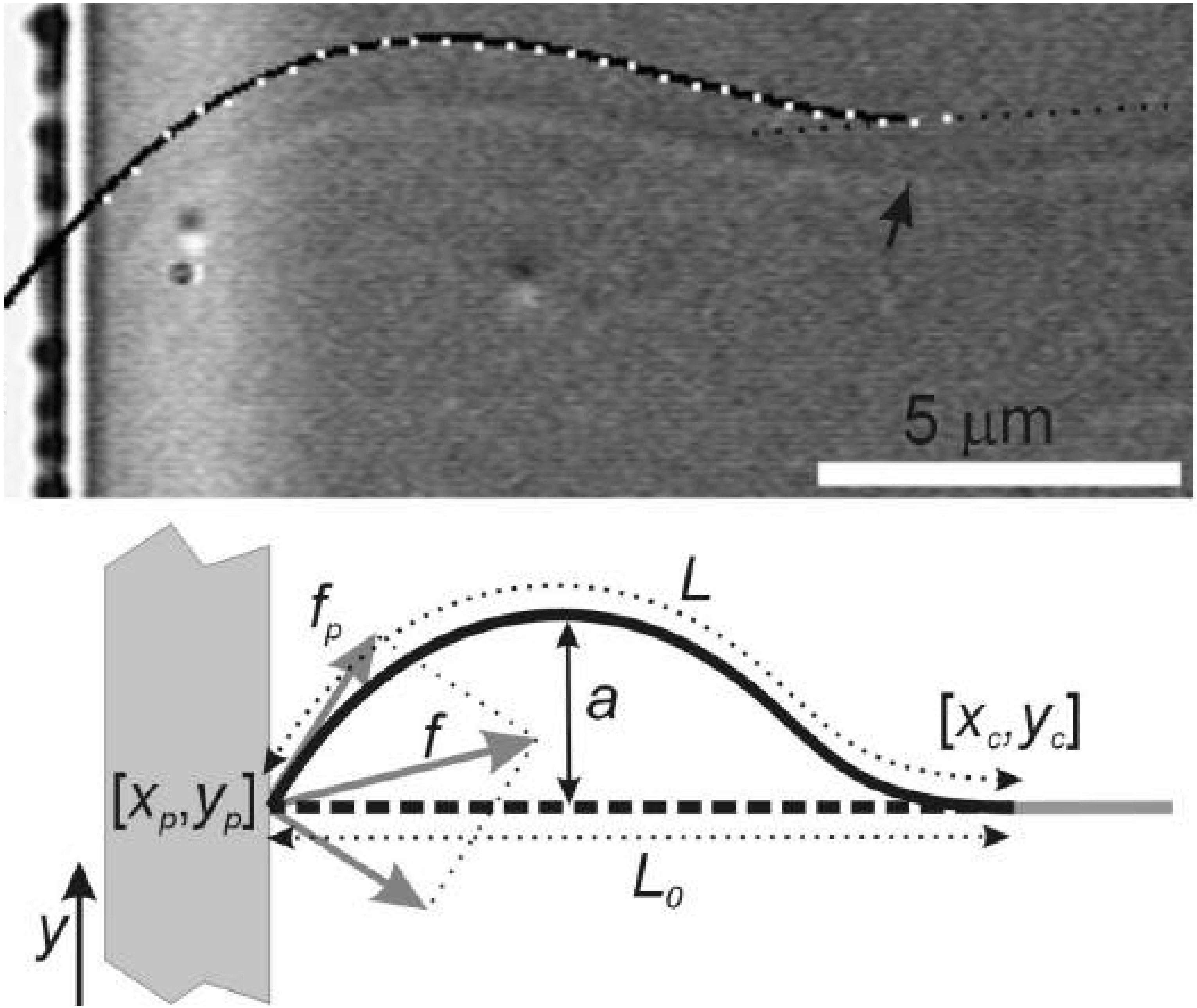




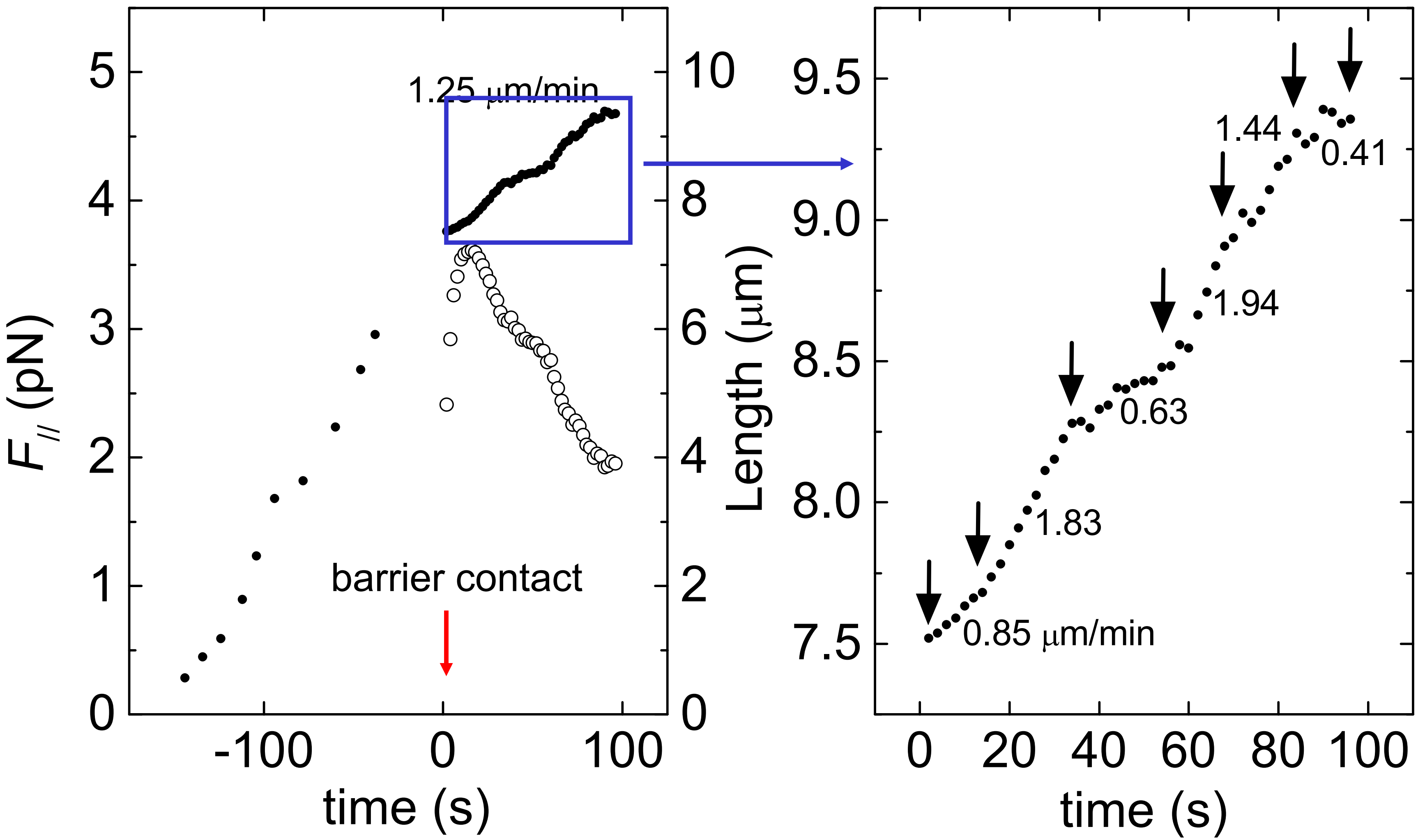
Buckling experiment






5 μm
L
f_p
a
$[x_c, y_c]$
f
$[x_p, y_p]$
L_0
y

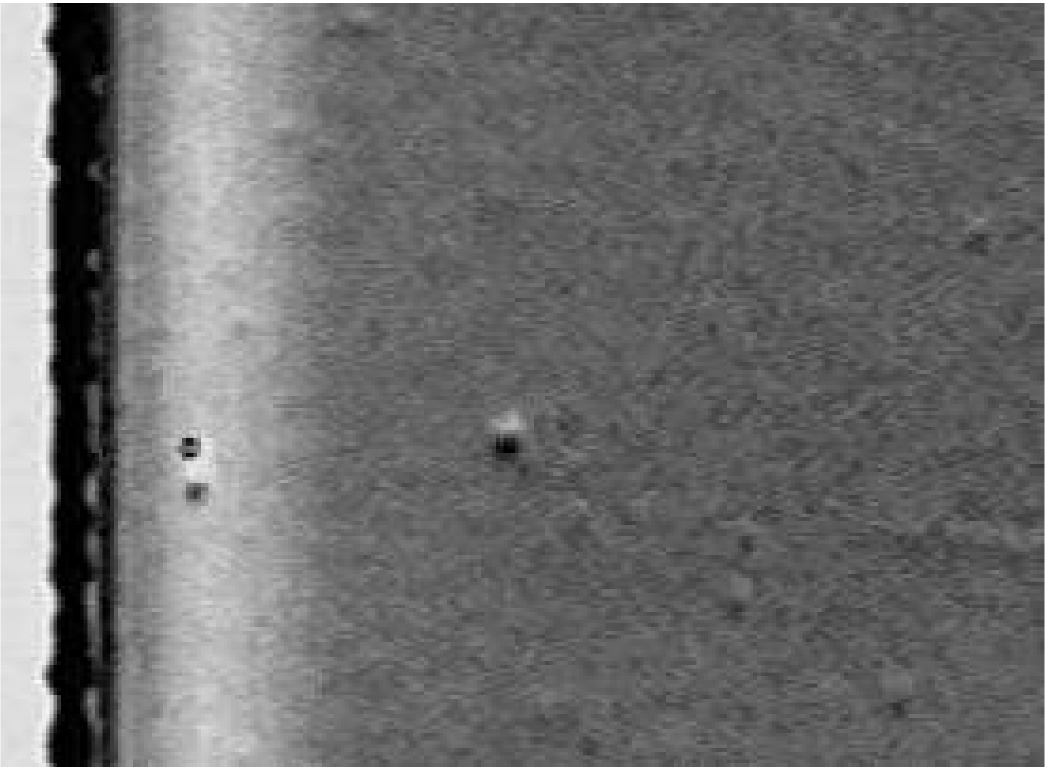
Individual MT growth: V_0 = 2.8 μm/min



Buckling experiment

MT length > 5 μm

Small force: few pN



Growth slows down

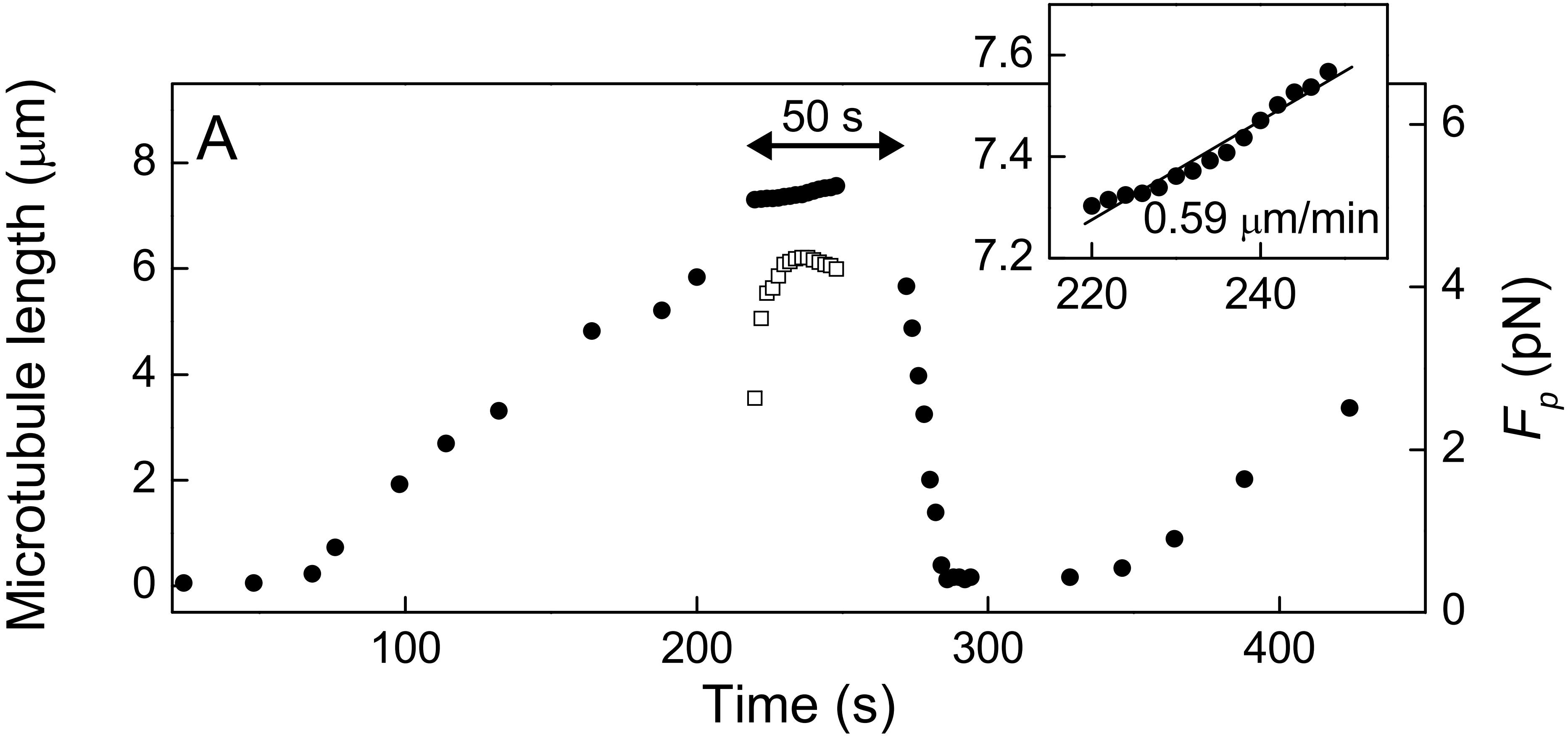
MT length < 5 μm

Large force: > 10 pN

Growth stalls,
Fast catastrophes

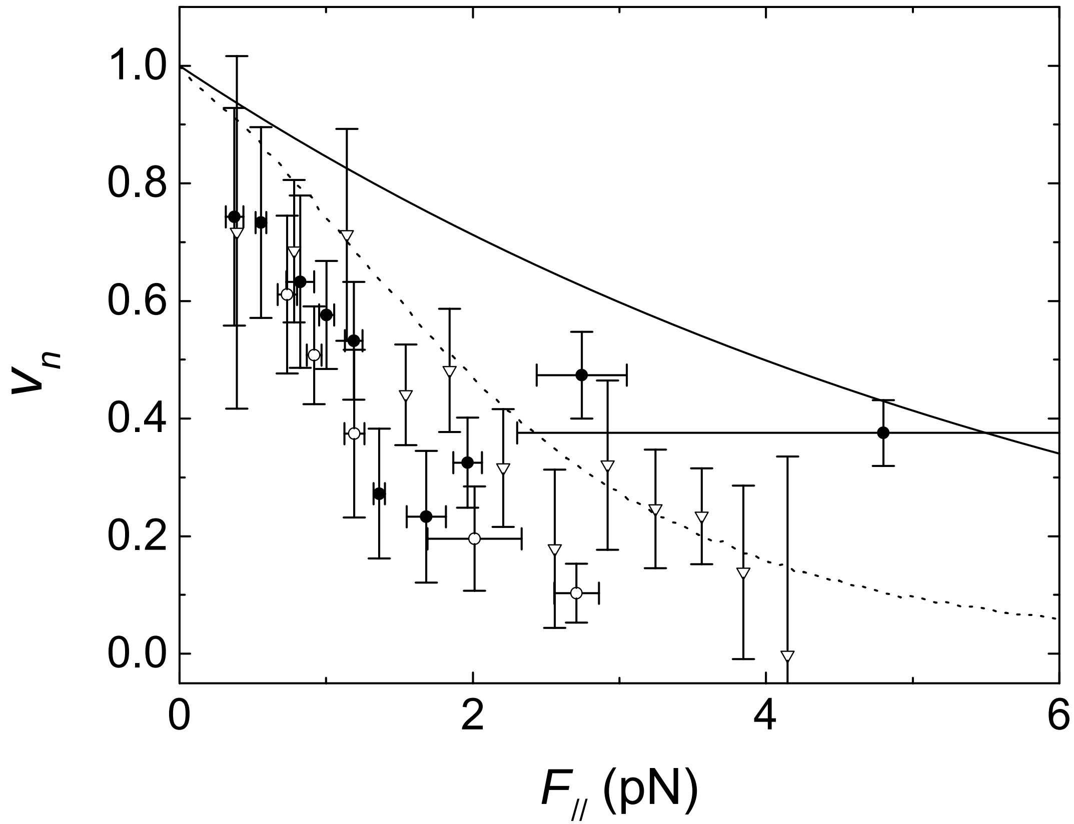
Scale bar 5 μm

Catastrophe times under force

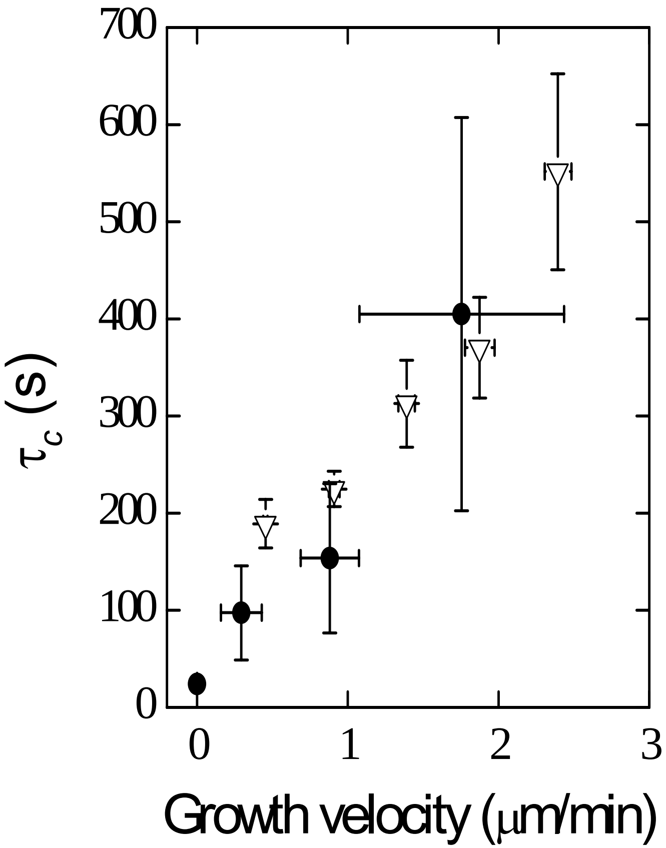


Dynamics: Response to Force

(normalized) Growth velocity



Catastrophes

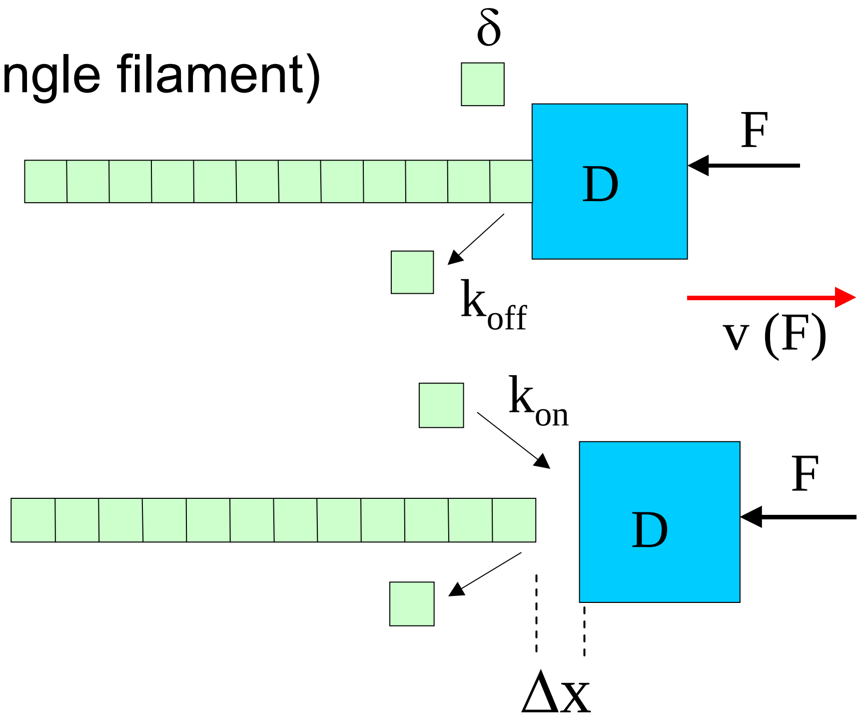


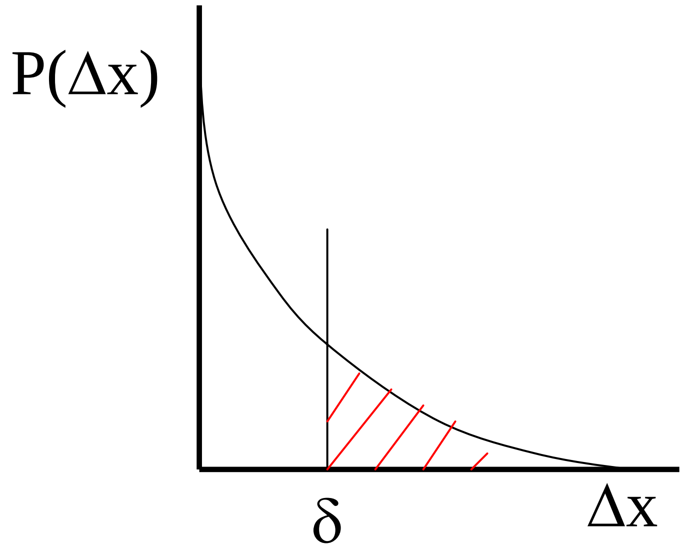
Janson & Dogterom, PRL 2004

Janson et al., JCB 2003

Pushing force: “Brownian ratchet”

(single filament)





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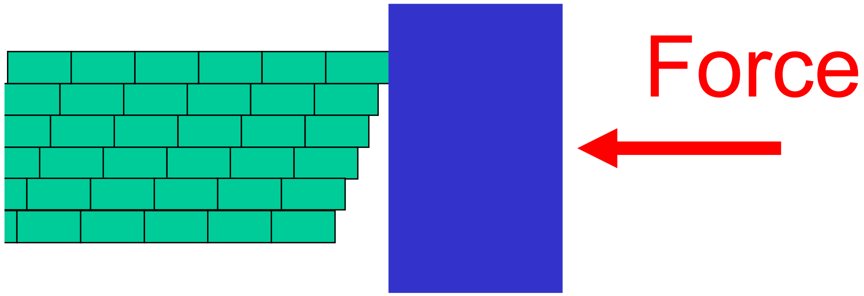
$$\boxed{F_{\max} = \frac{k_B T}{\delta} \ln\left(\frac{k_{on} c}{k_{off}}\right)}$$

Peskin et al. 93

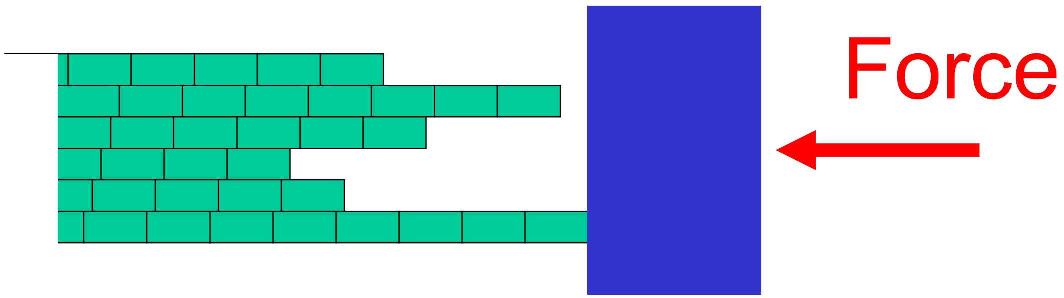
Multifilament Brownian ratchet ?

"Optimal" growth



$$\Delta x = \frac{\delta}{N}$$

Independent growth



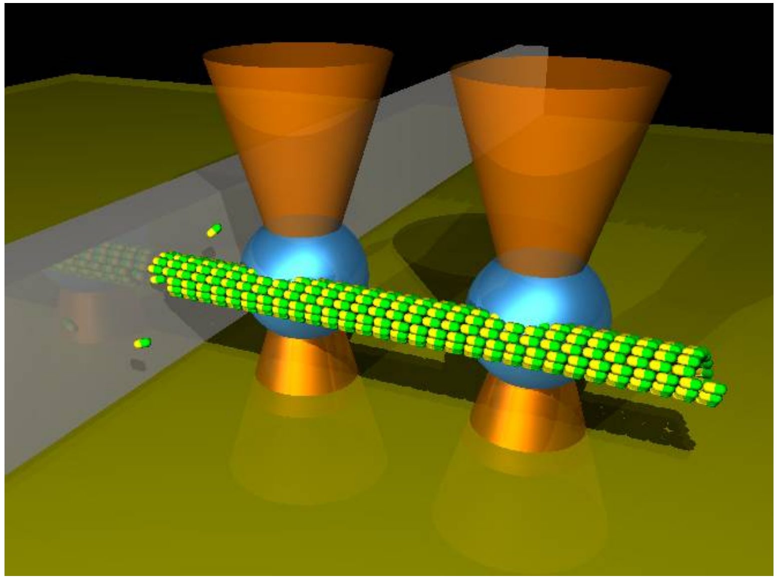
$$0 \leq \Delta x \leq \delta$$

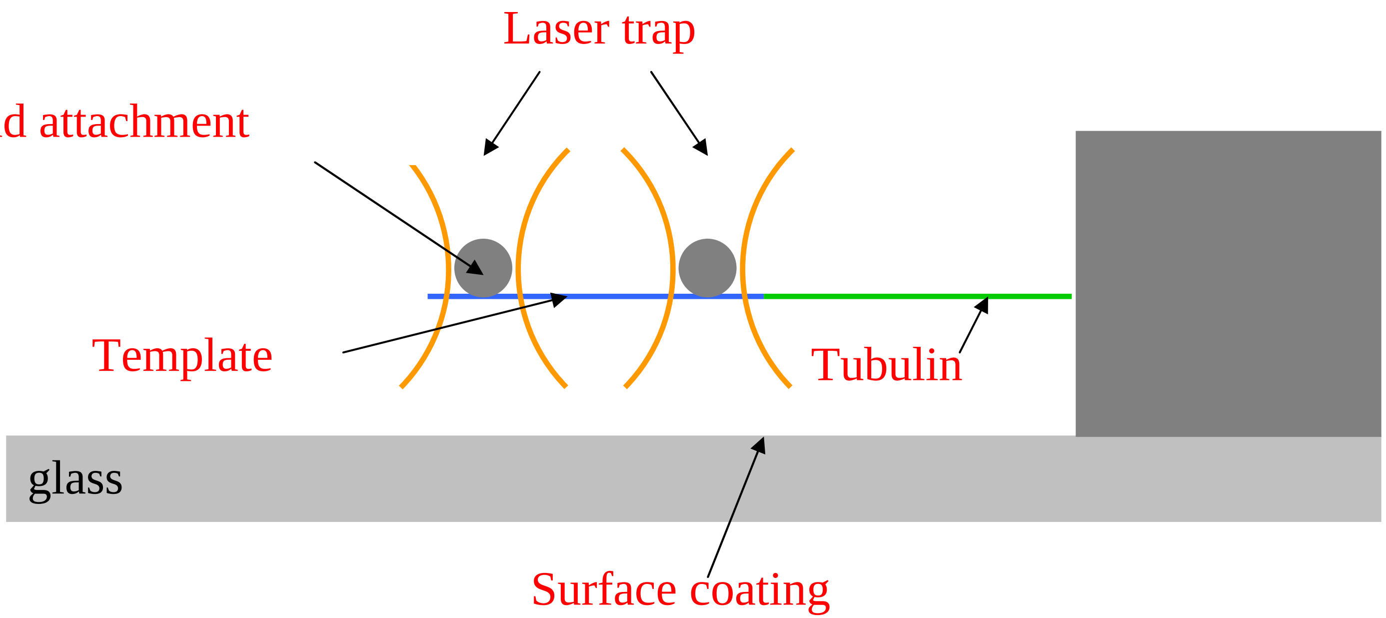
Mogilner & Oster, EBJ, 99; van Doorn et al, EBJ, 00

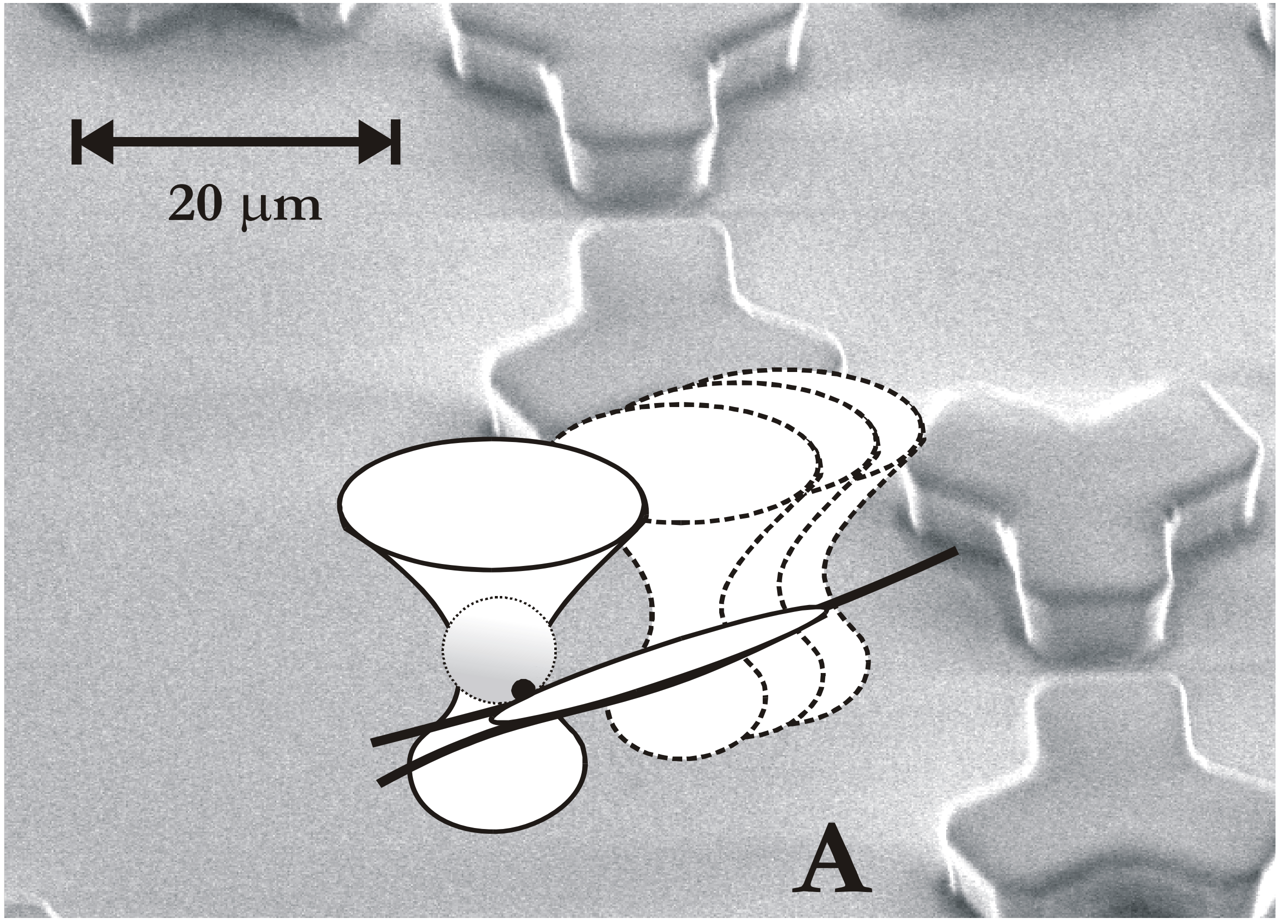
STALL FORCE ?

DYNAMICS AT MOLECULAR SCALE ?

LASER TWEEZERS



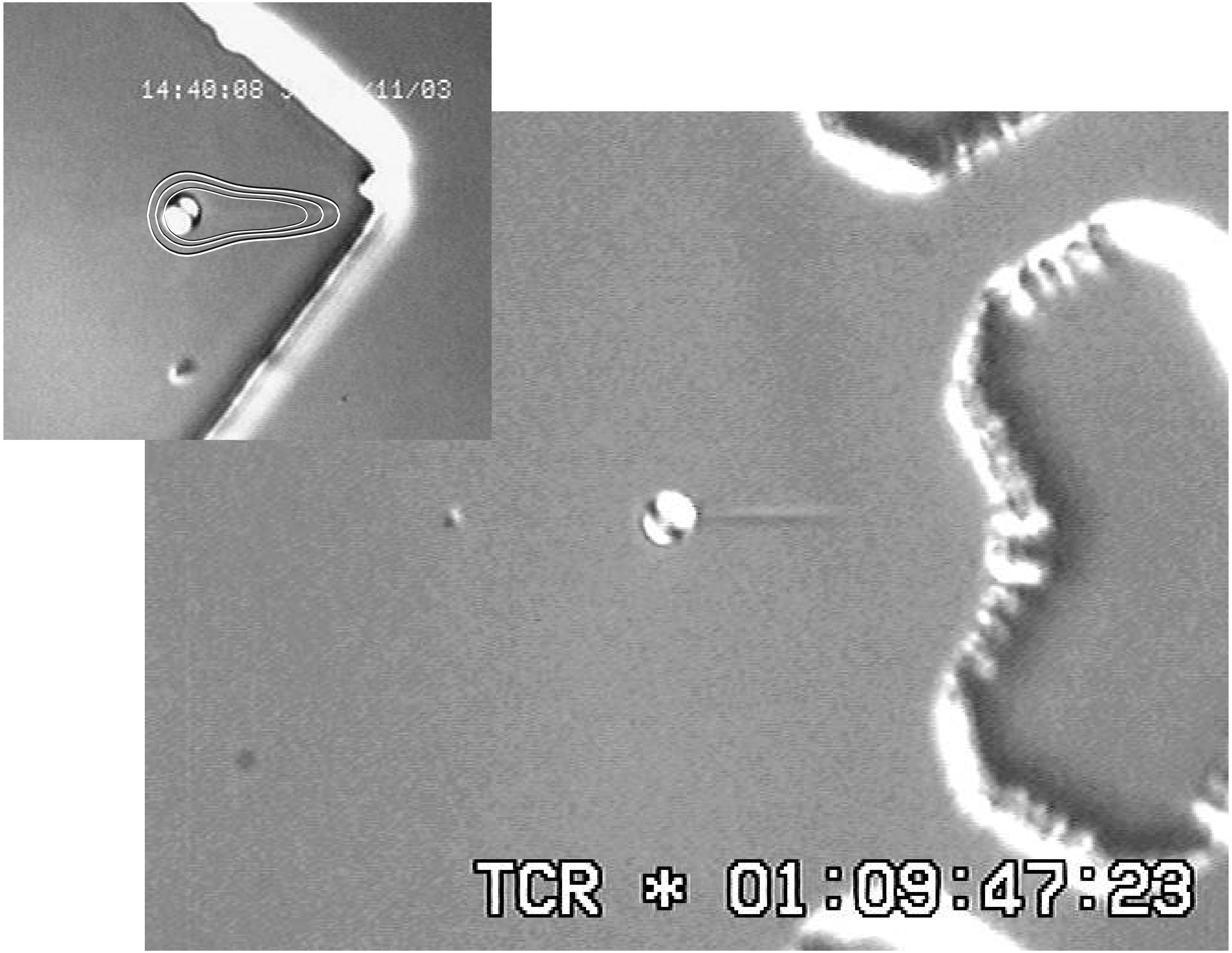




"Key hole" trap:

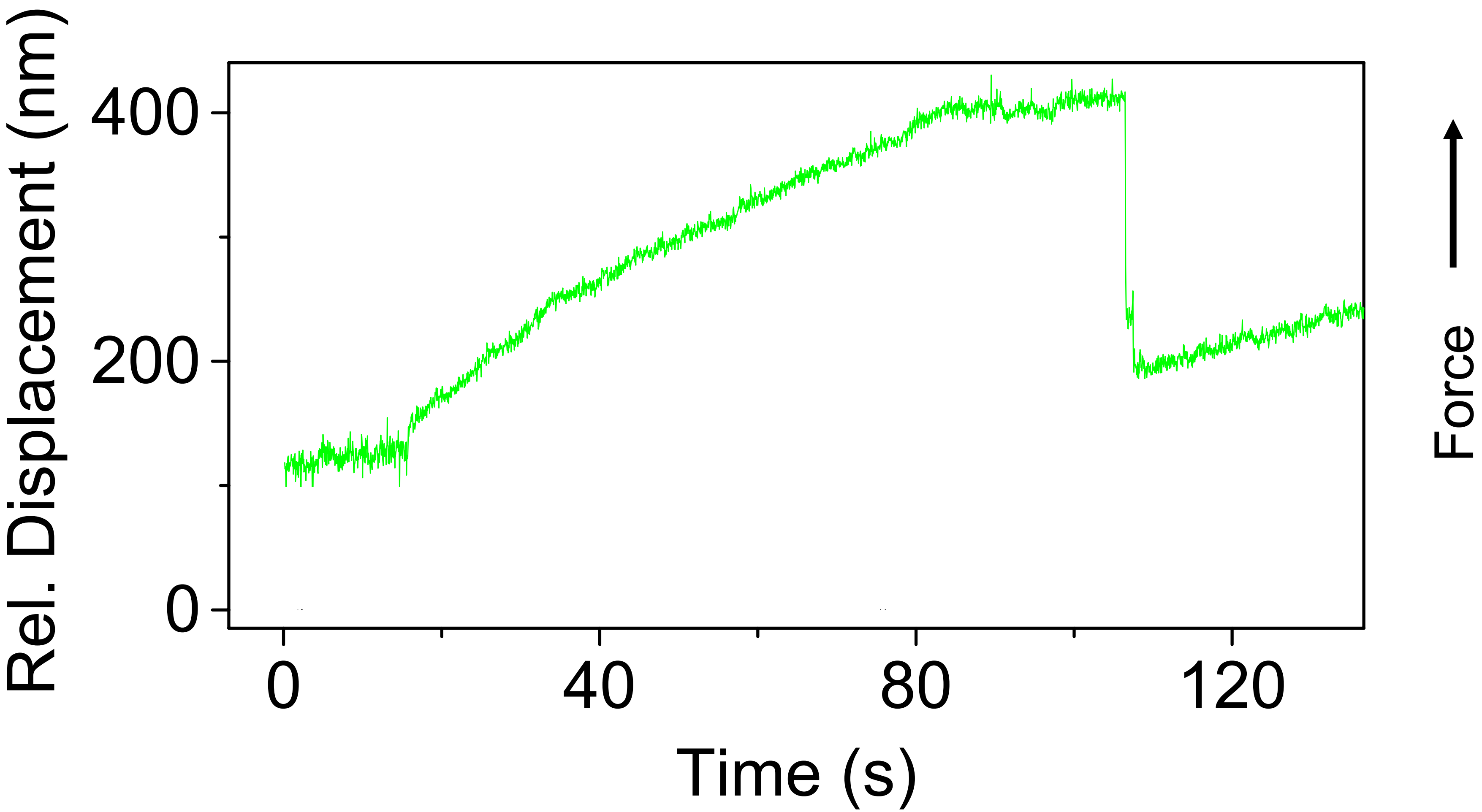
Kerssemakers et al, APL 2003

Barriers:
Schek et al, BPS (2003)

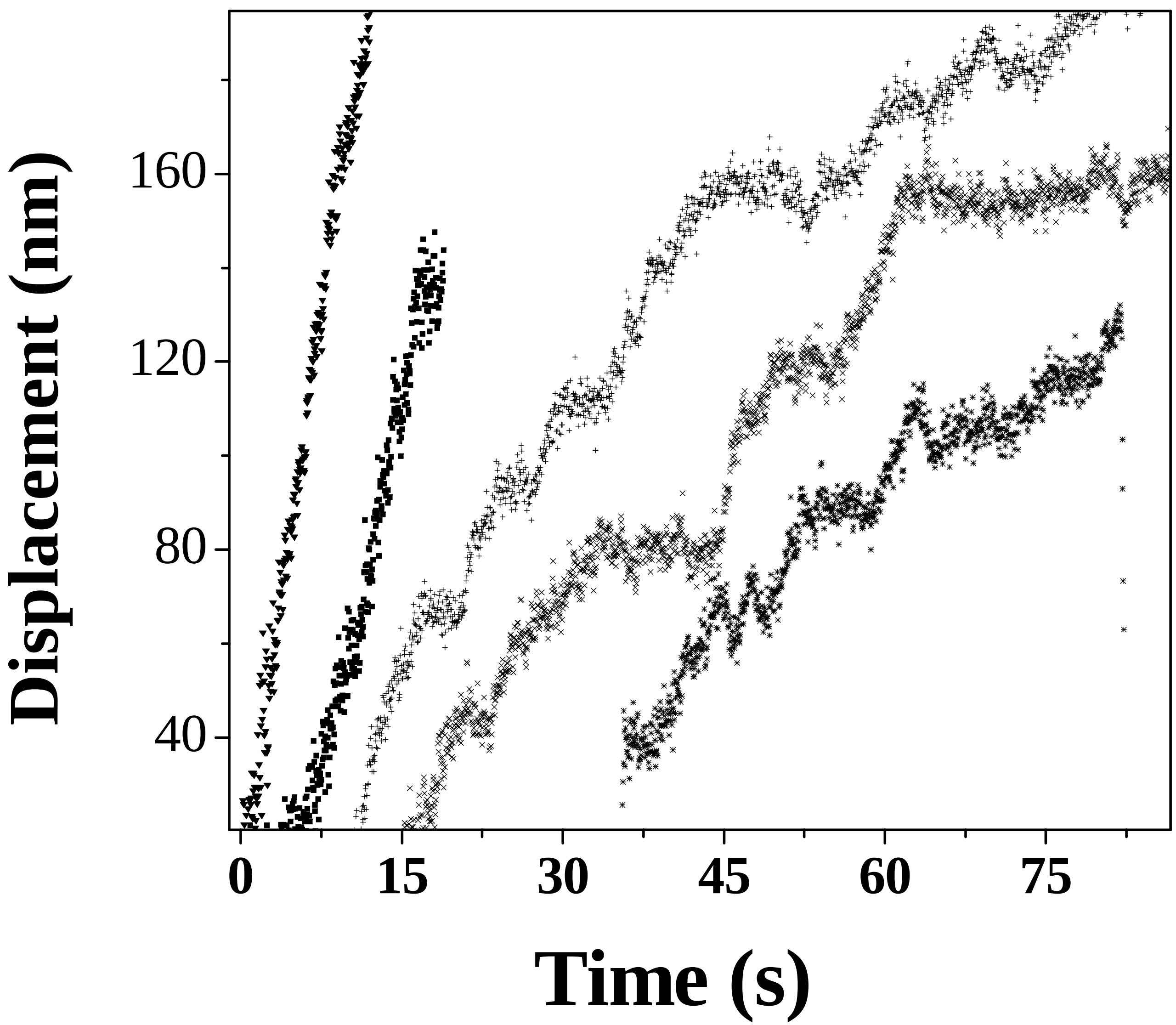

14:40:08
TCR * 01:09:47:23

In optical trap, as MT grows:

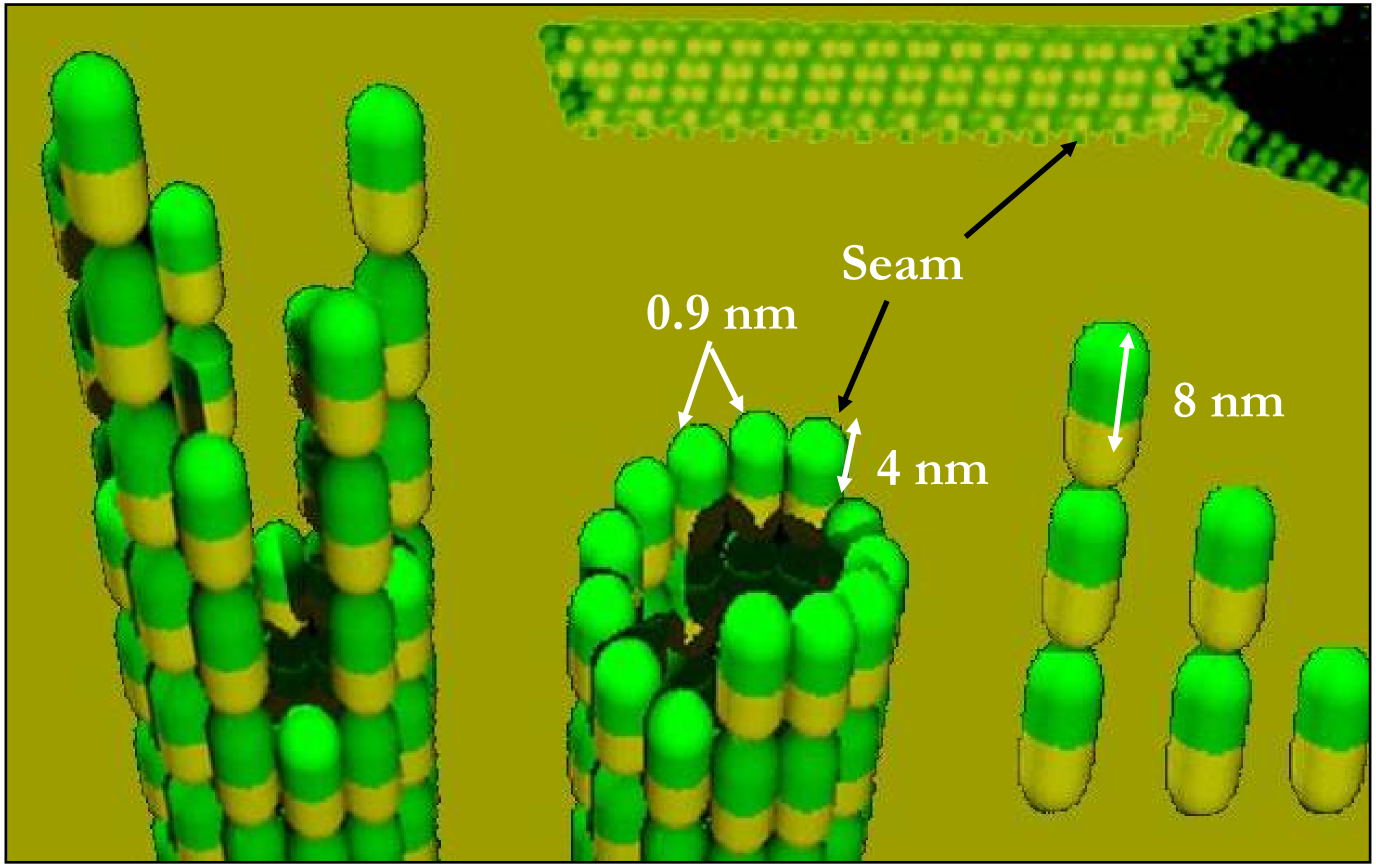
Force increases, Velocity decreases, and stalls ?



Towards molecular resolution ?

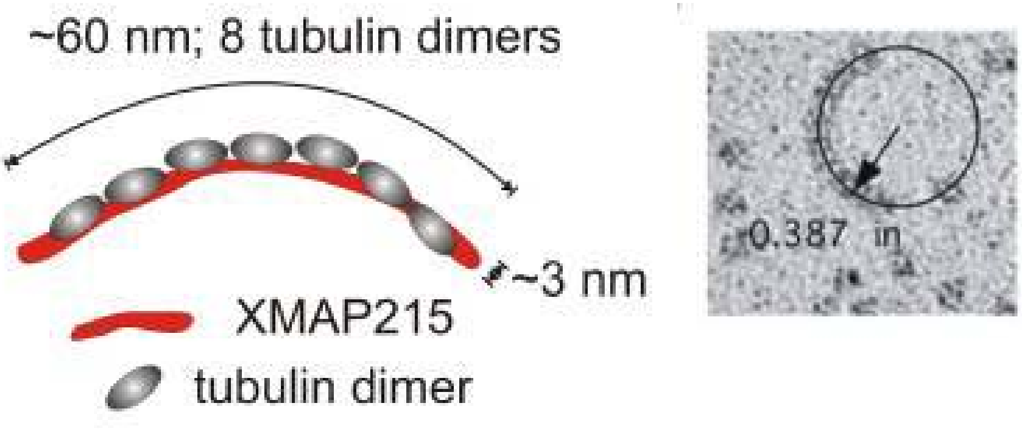


Some fast length increases larger than dimer size ????



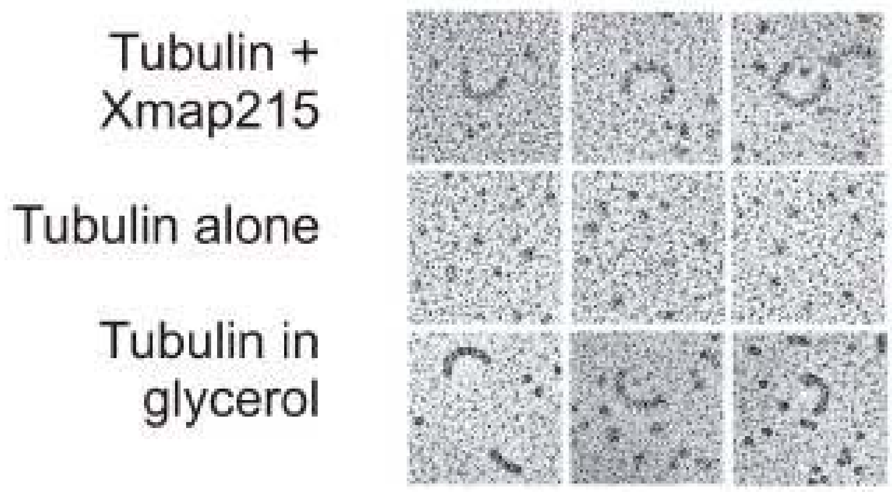
MT regulator: Xmap215*

- Can bind 7 to 8 tubulin dimers



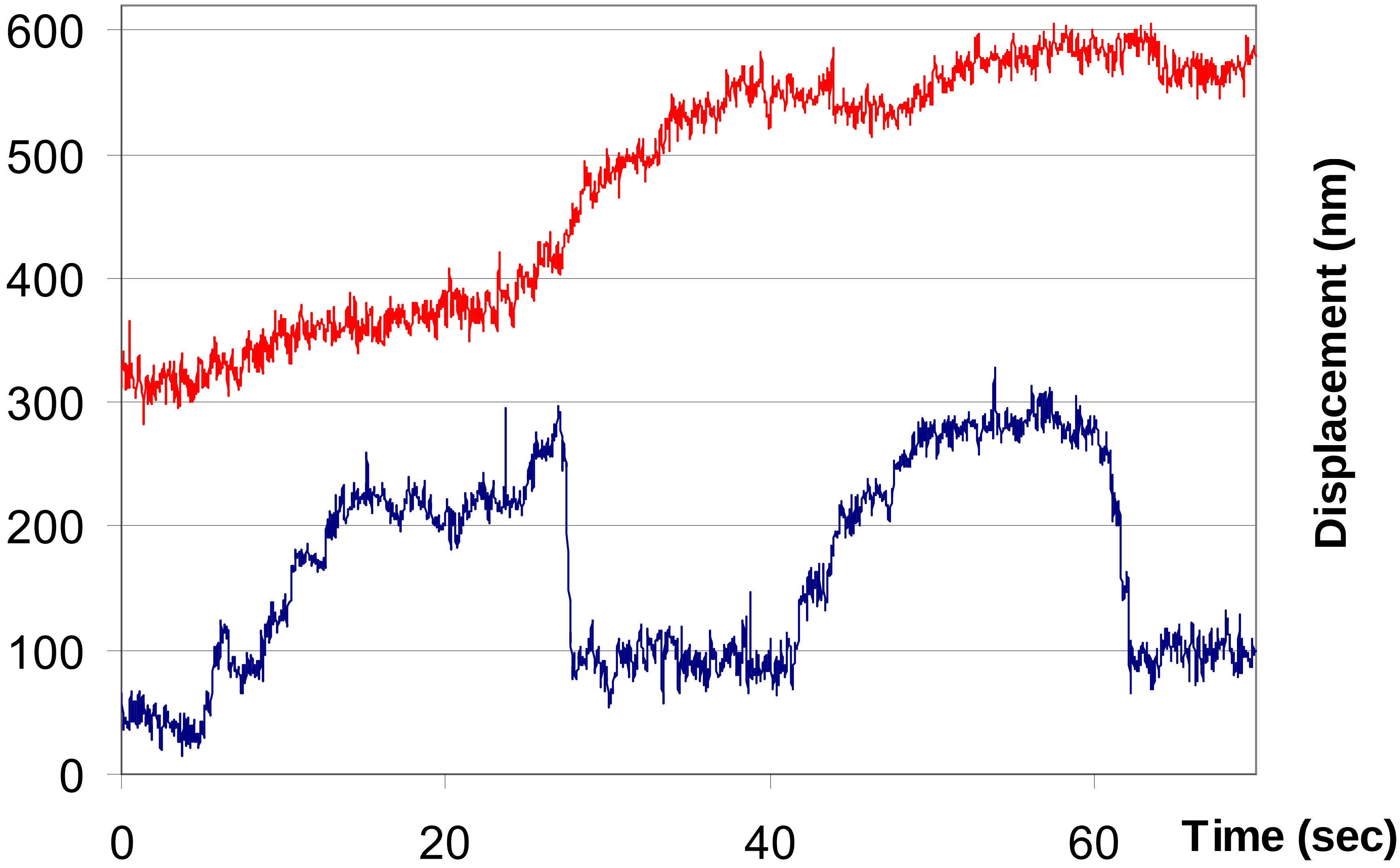
- Enhances growth of microtubules

  Mechanism ??



*Gift Tim Noetzel/Tony Hyman

With XMAP215: large step-like features



→ XMAP215 enhances growth by 'templating' ?

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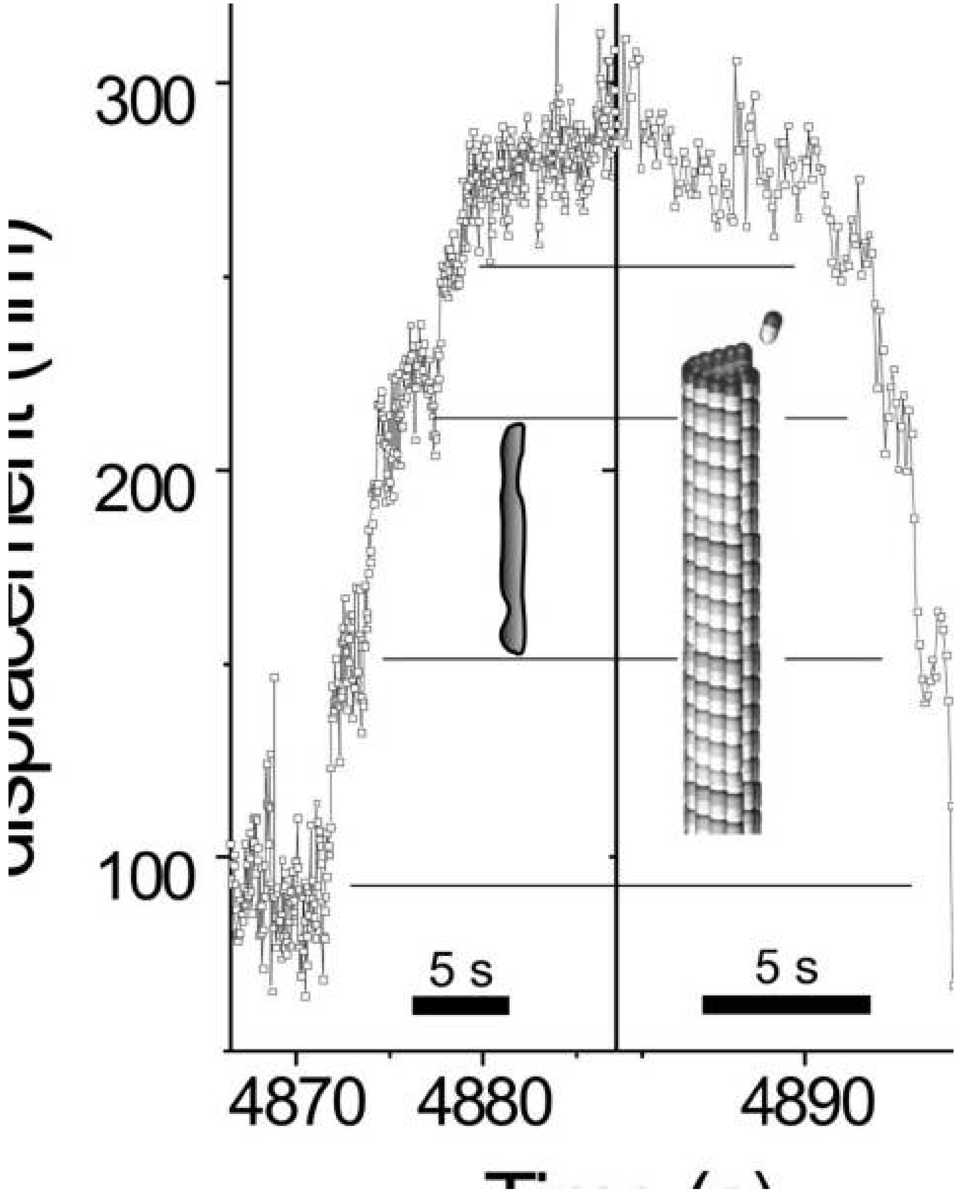
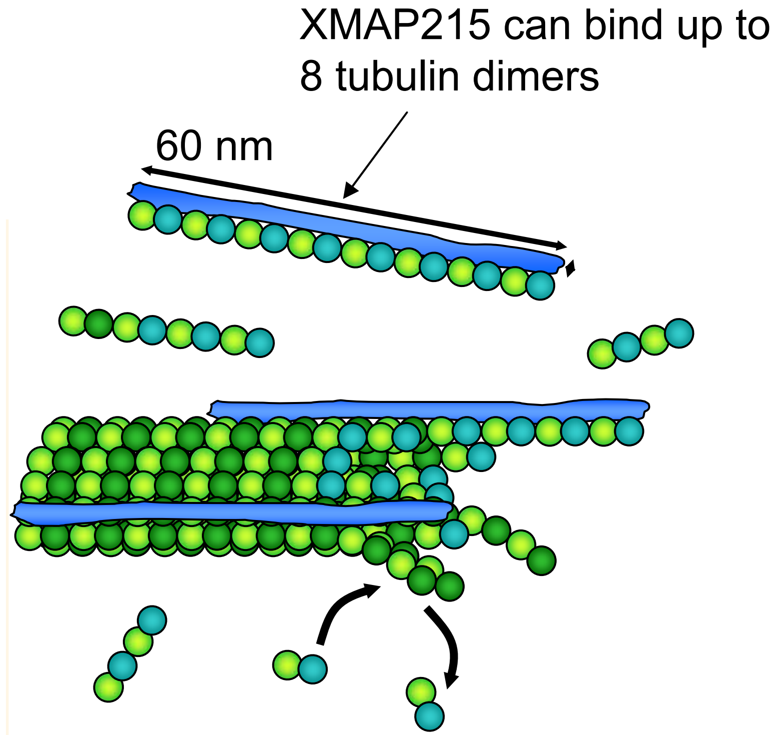
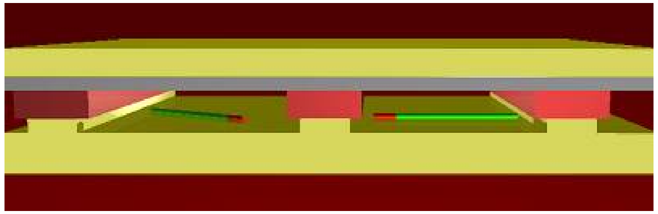




With Tim Noetzel / Tony Hyman, MPI-CBG Dresden

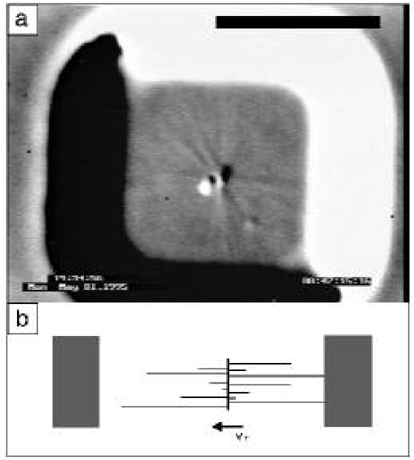
Kerssemakers et al, Nature, 2006



PROGRAM

1) Force generation capabilities on single MT level

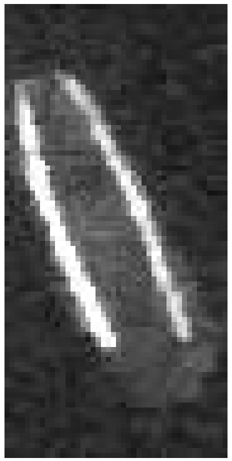
   Force-regulation of dynamics



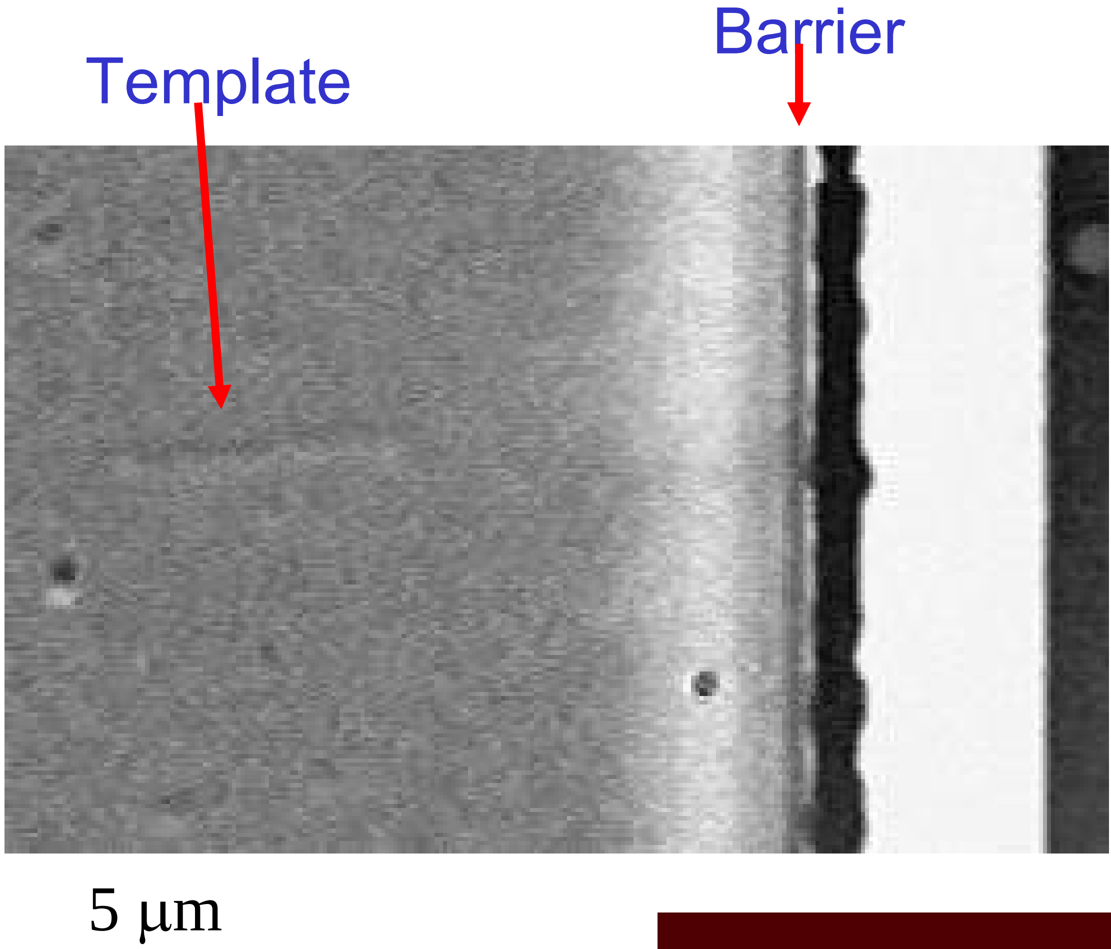
2) Positioning in simple model(s) (experiments)

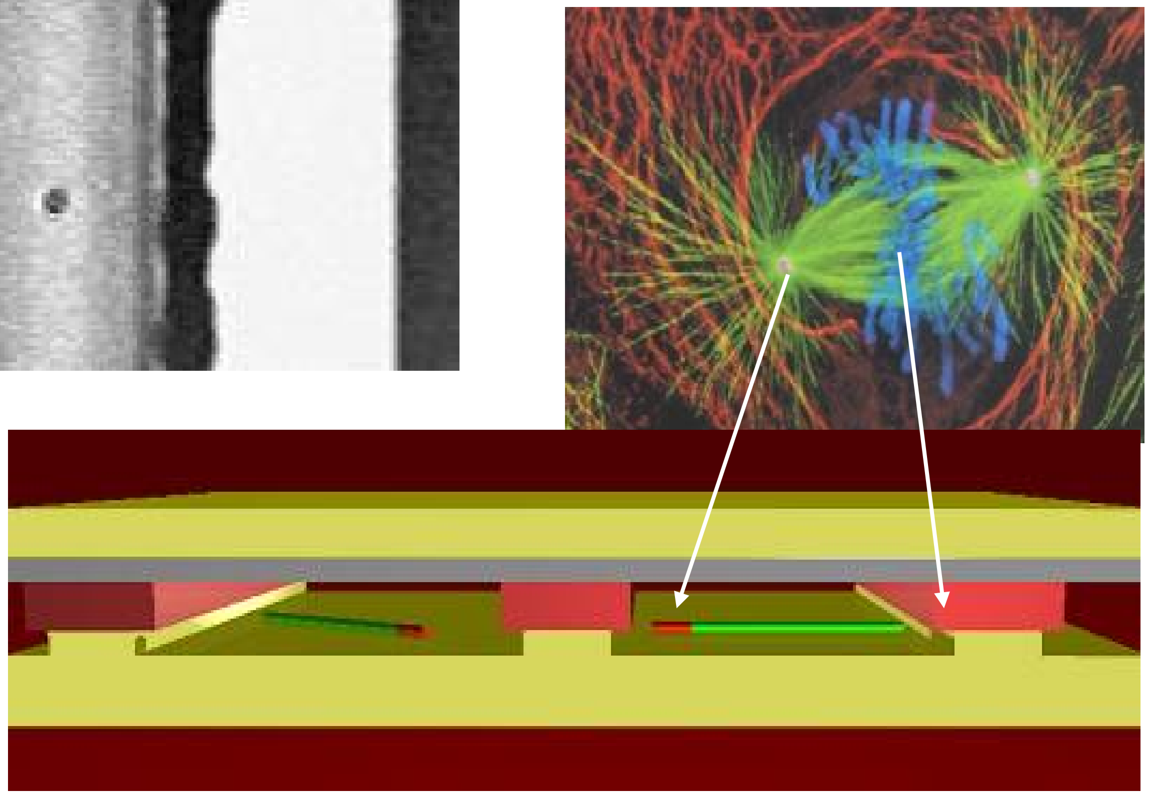
3) Force generation and positioning in vivo

   - Regulation ?
   - Fine tuning ?



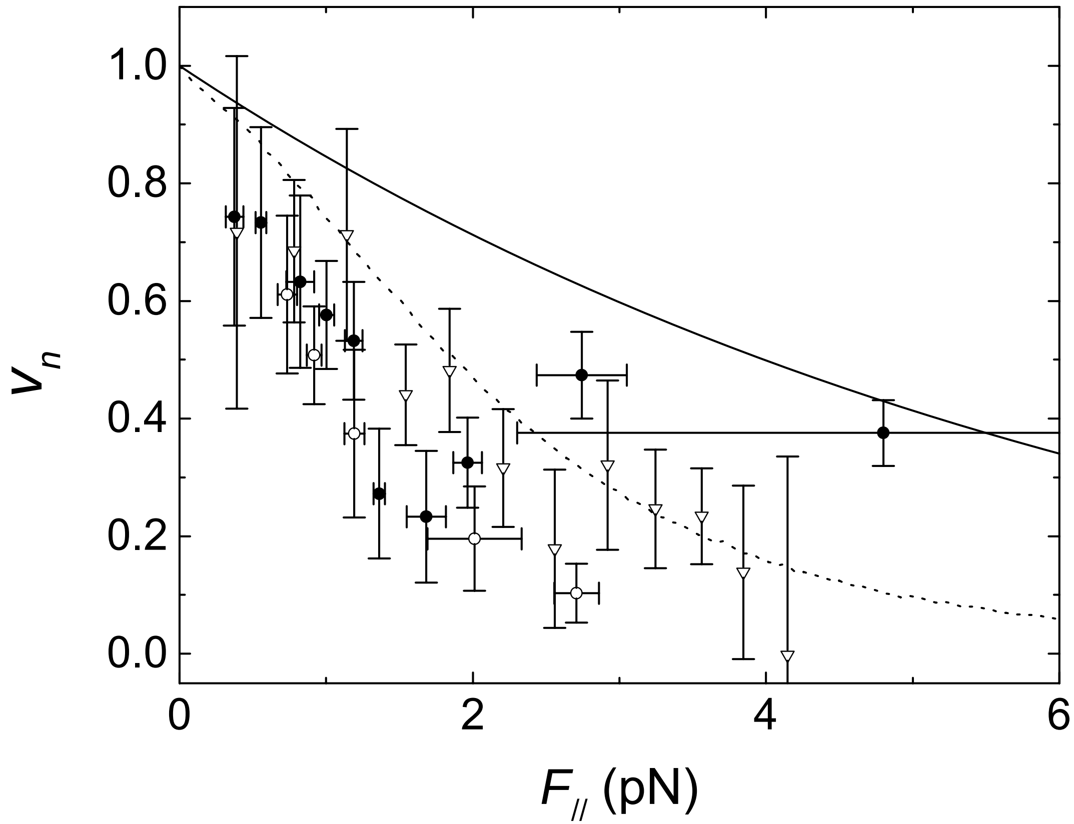
Buckling experiment



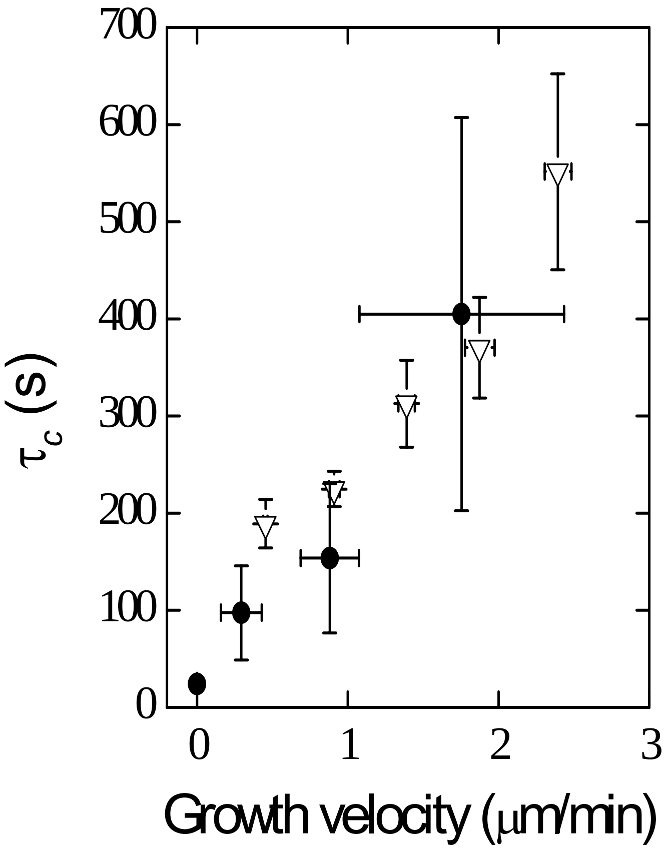


Dynamics: Response to Force

(normalized) Growth velocity



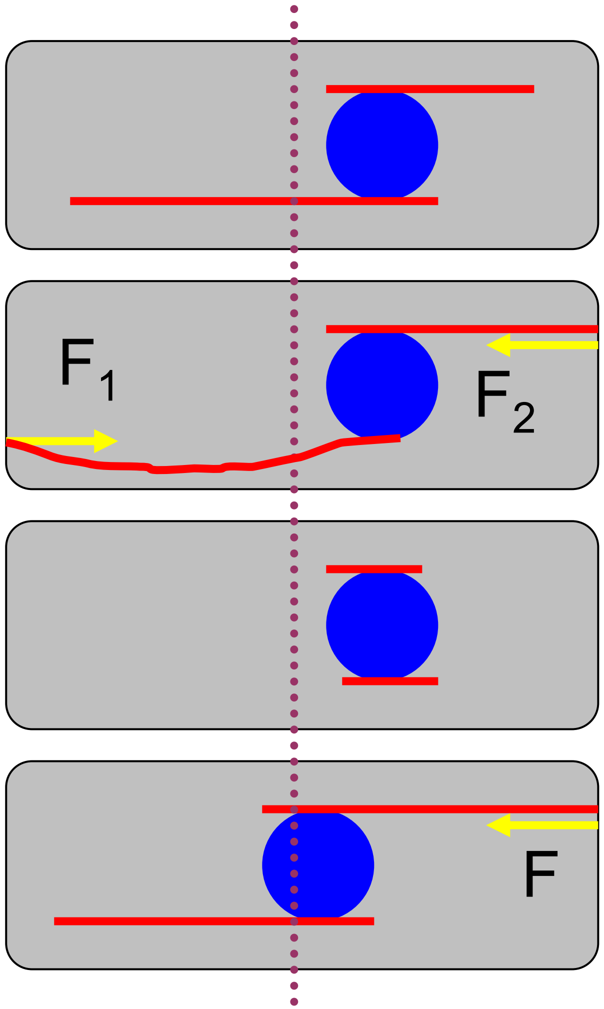
Catastrophes

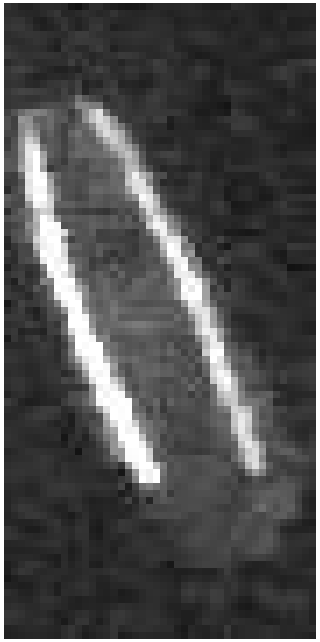


Janson & Dogterom, PRL 2004

Janson et al., JCB 2003

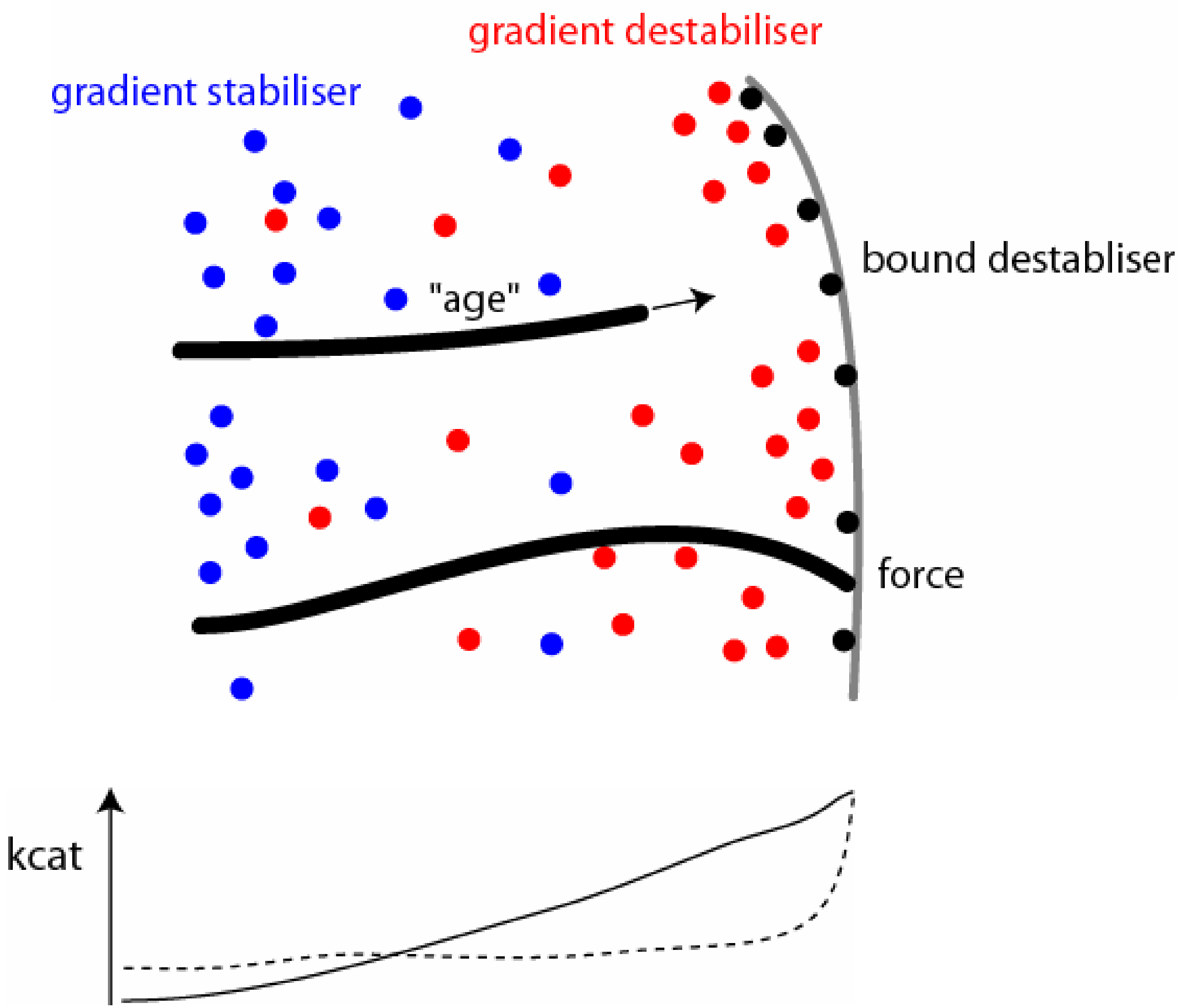
NUCLEAR POSITIONING IN INTERPHASE FISSON YEAST





Enhanced Catastrophe Rate at Cell Ends in Interphase Fission Yeast Cells: IN RESPONSE TO FORCE?

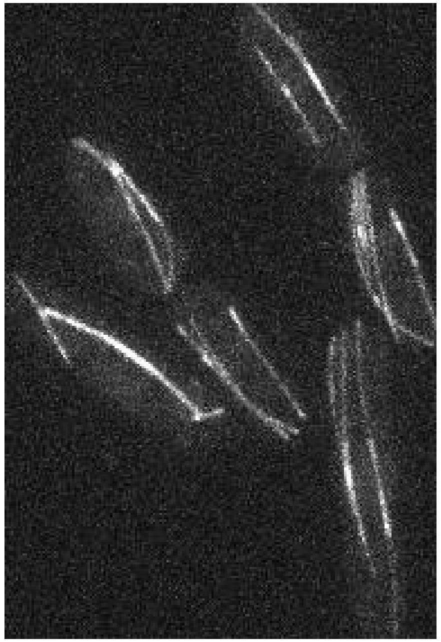
Possible ways to enhance the catastrophe rate near cell boundary



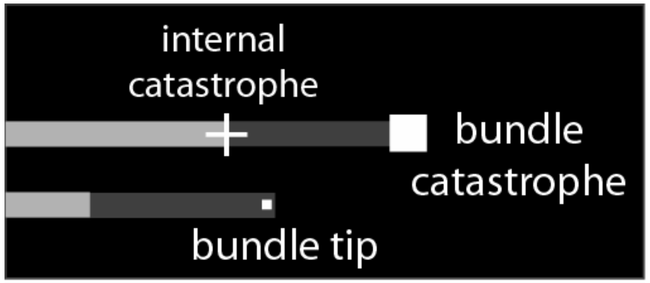
Detection of MT catastrophes by automated movie analysis

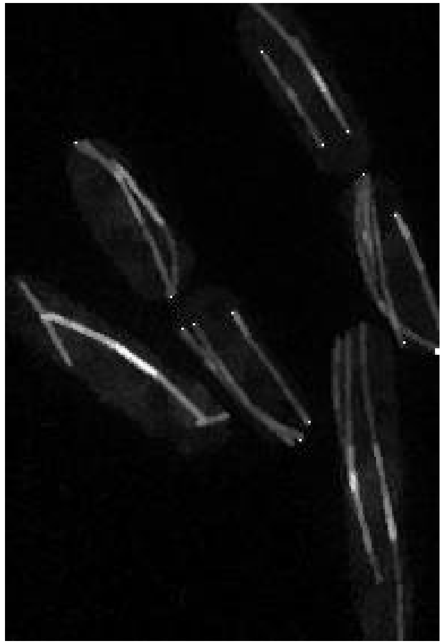
- GFP-tubulin
- 3D spinning disk confocal microscopy
- Maximum projections of 3D stacks, dt=8 sec

20 µm



40 x real time





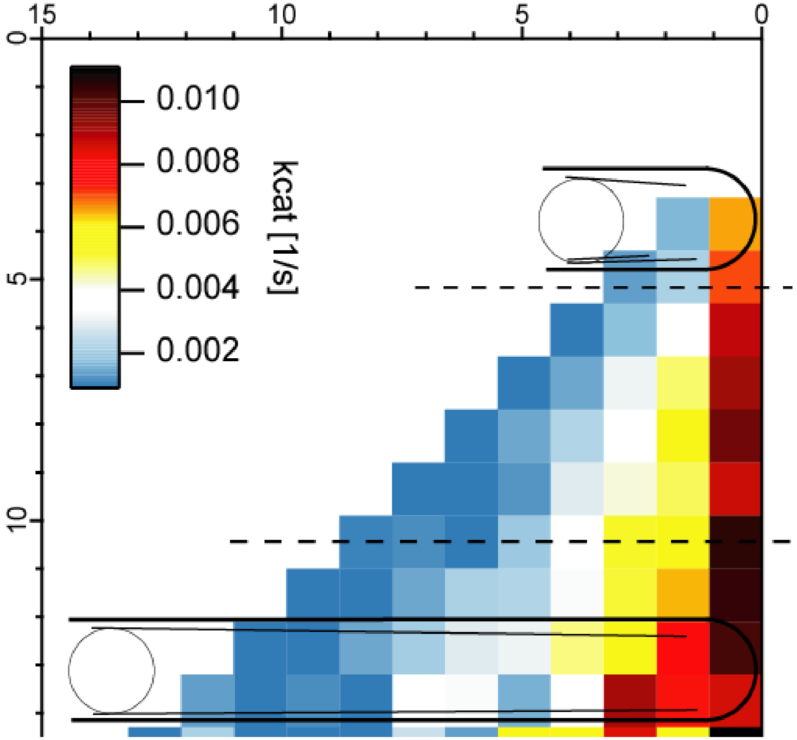
Detect:

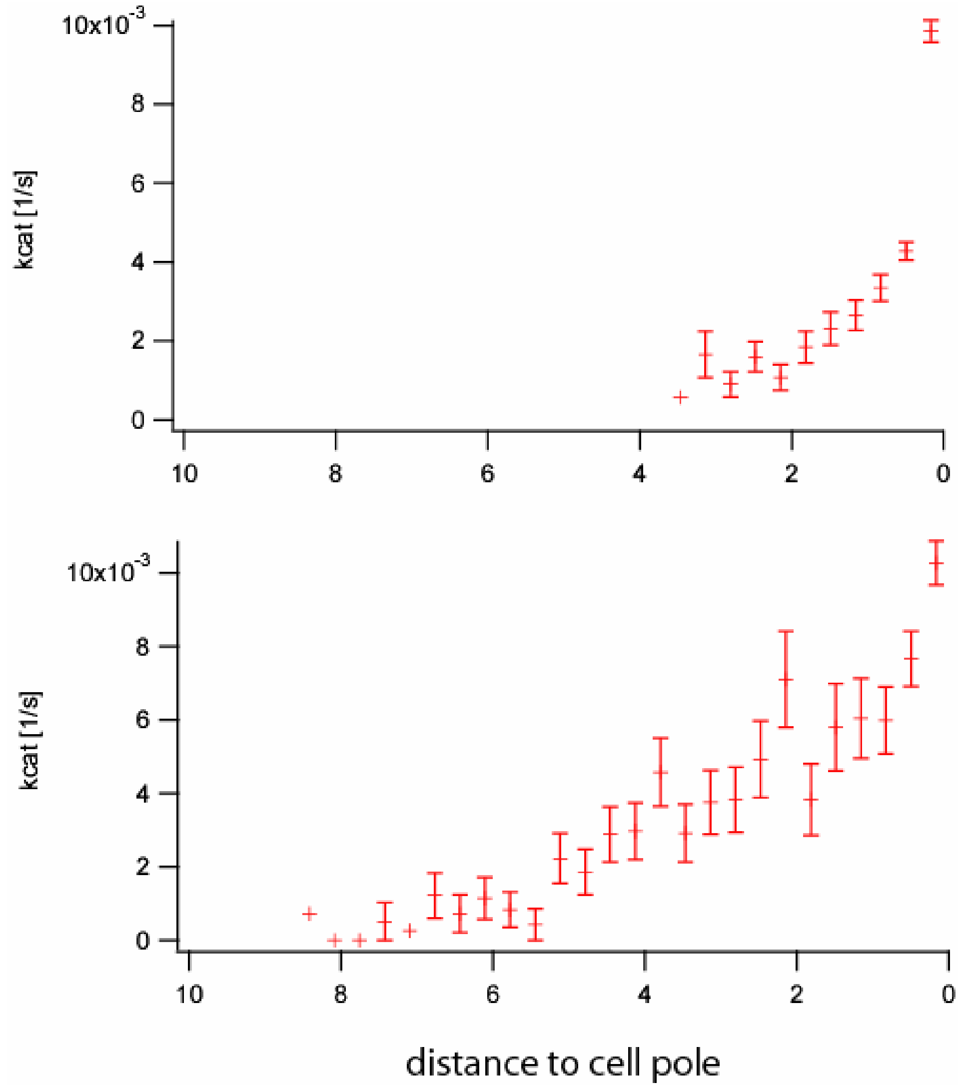
- Leading catastrophes
- Internal catastrophes
- Microtubule ends

100s of cells
10.000s of catastrophes

Towards cell end (μm)

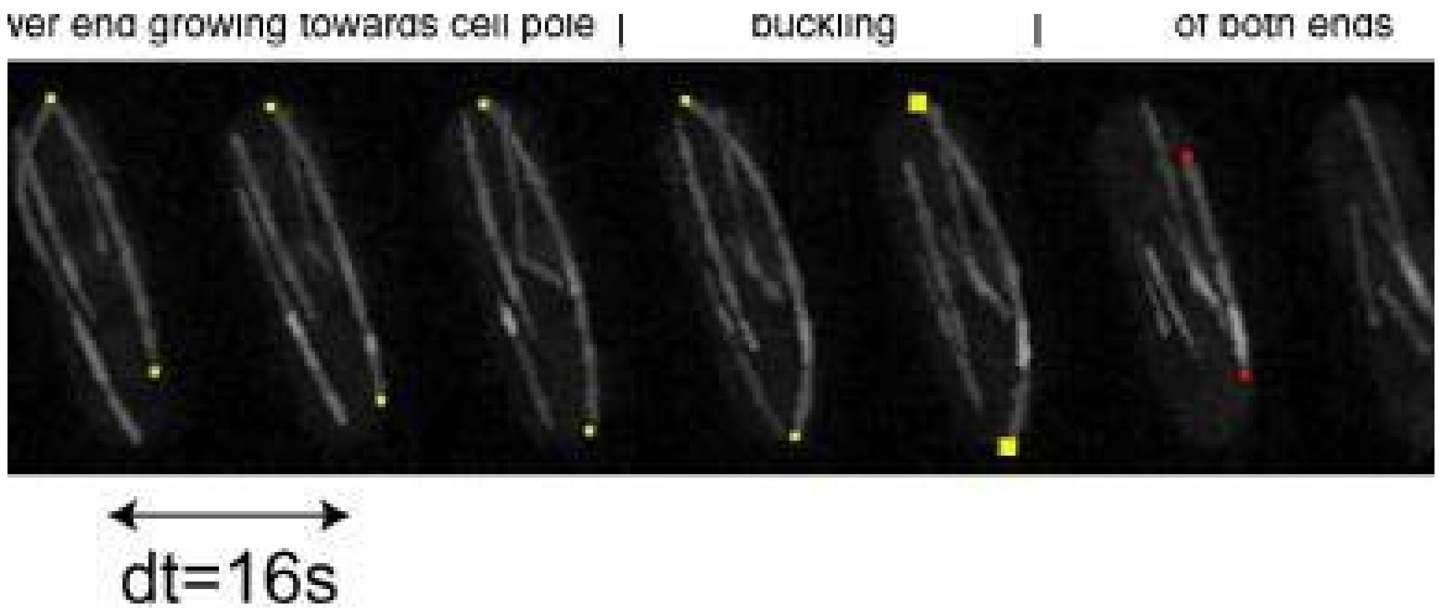
Cell size (μm)

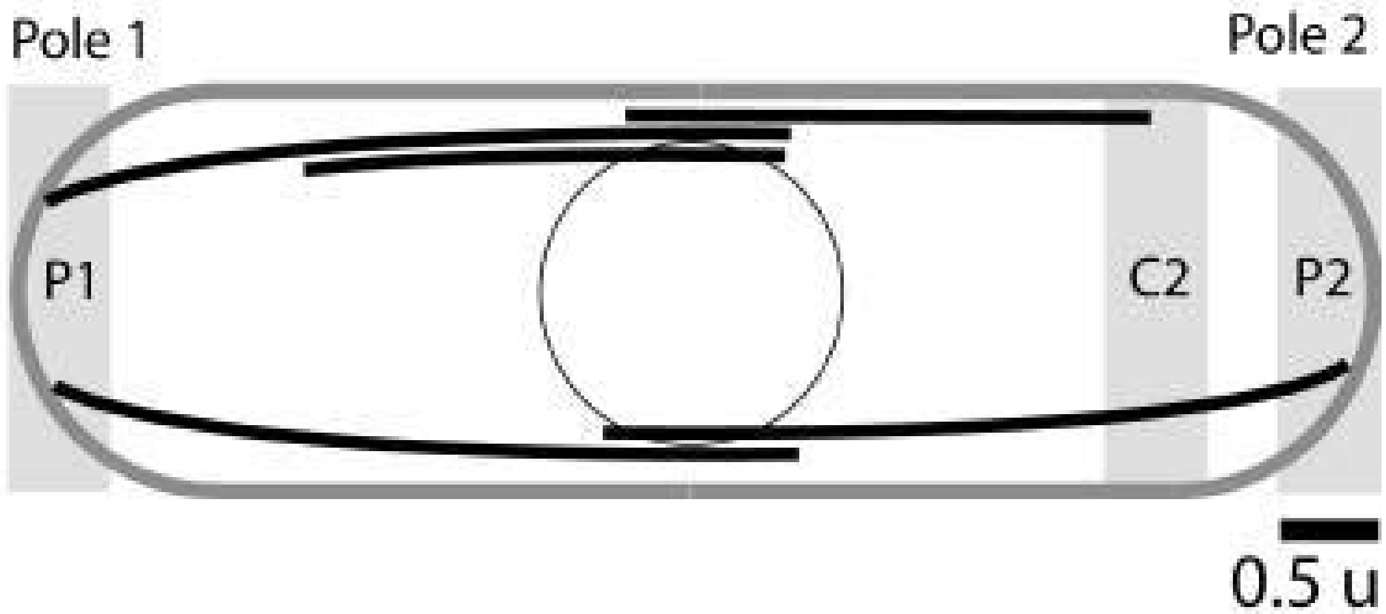




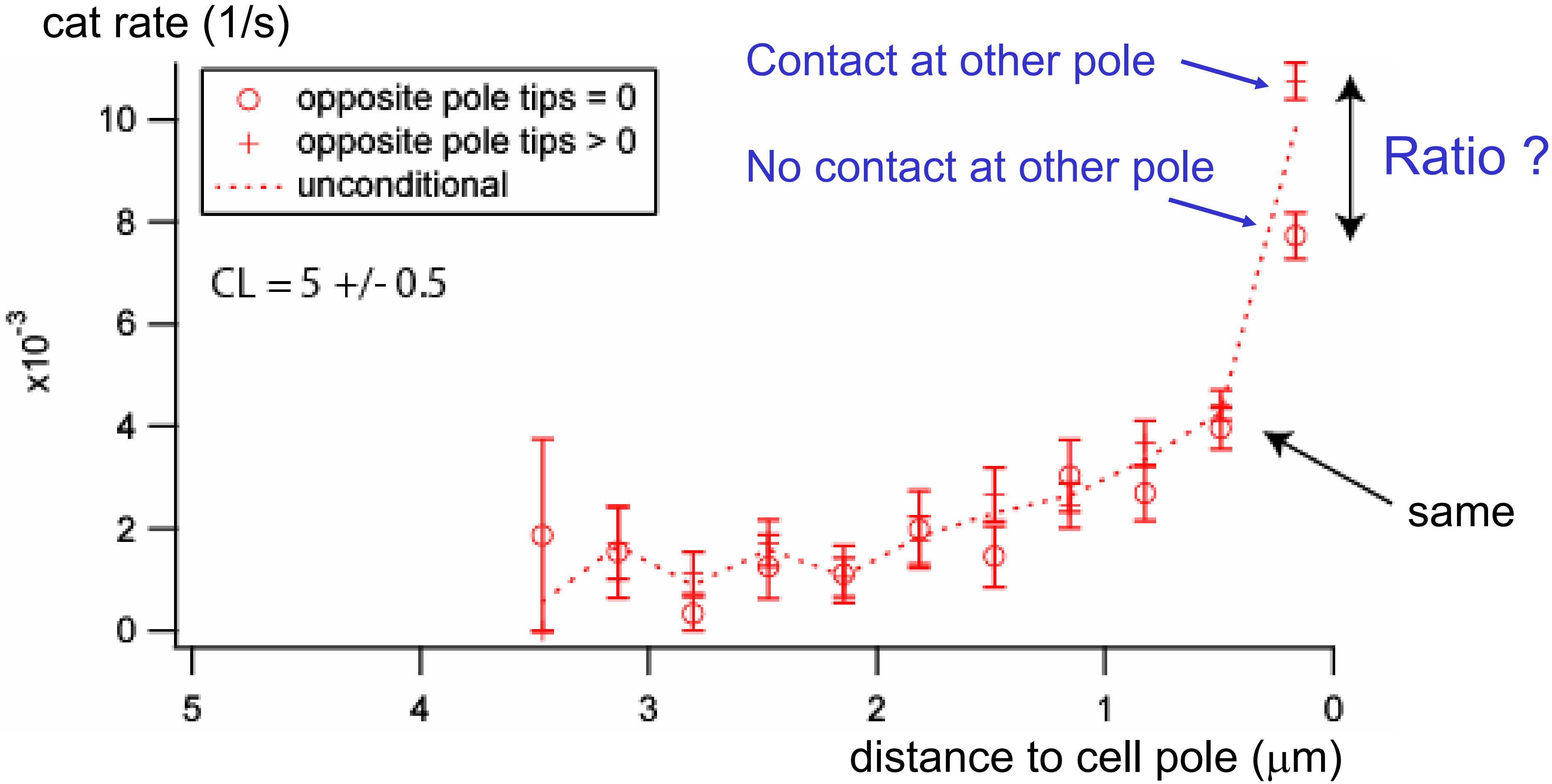
- Catastrophe rate increases with MT length
- Catastrophe rate enhanced at cell ends

Correlated catastrophes when contact at both ends



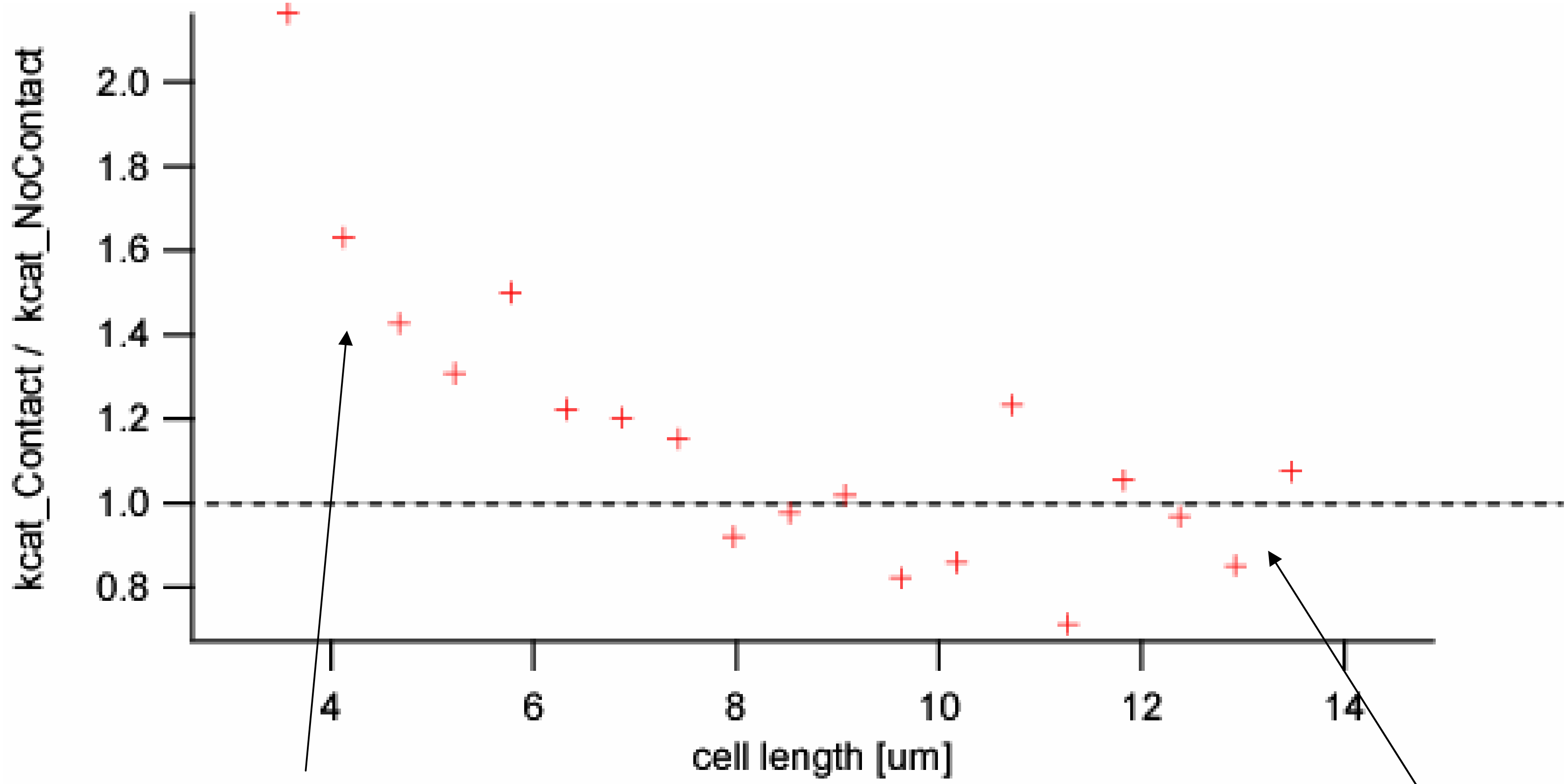


Evidence that the abrupt increase at cell end is due to compressive forces



“Force effect” decreases with cell length

Ratio



Short MTs feel high force:
catastrophes enhanced

Long MTs feel low force:
catastrophes unchanged

Conclusions:

In vitro:
- Microtubules slow down under force leading to fast catastrophes

In vivo (interphase fission yeast):
- Catastrophe rate higher for longer MTs
- Additional enhancement at cell boundaries due to force

Polymerization forces can alter microtubule dynamics locally, thus contributing to MT organization in cells

Acknowledgements

Optical trap:

Koen Visscher (Arizona)

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Yeast Experiments:

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Thomas Surrey (EMBL)

Francois Nedelec (EMBL)

XMAP215:

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Axonemes:

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GMPCPP:

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Dynein:

Ron Vale (UCSF)