# “Some single molecule studies of myosins”

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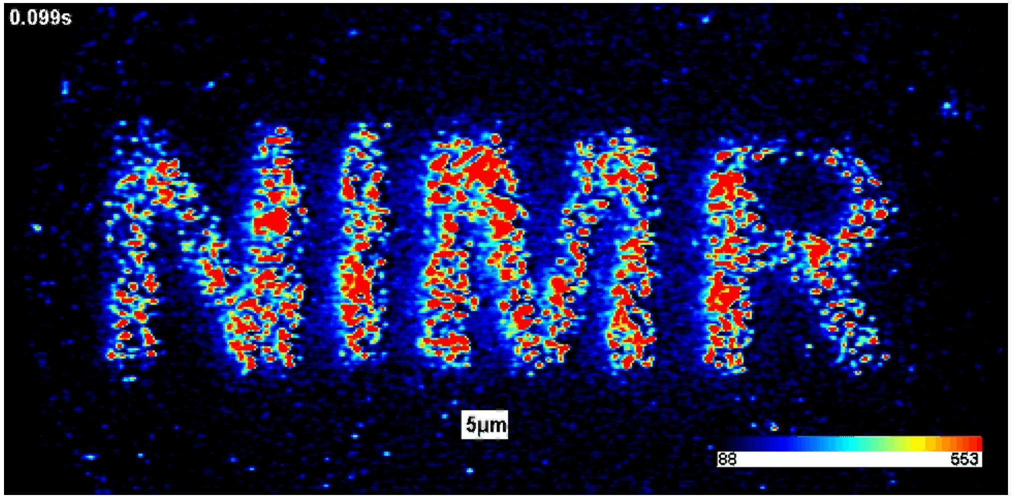
Division of Physical Biochemistry



# Motivation for the work

# Molecular motors are important in disease – e.g. red blood cell invasion by MALARIA

10μm

# Mechanical Properties of Red Blood cells

- Erythrocyte mechanical properties are well-studied

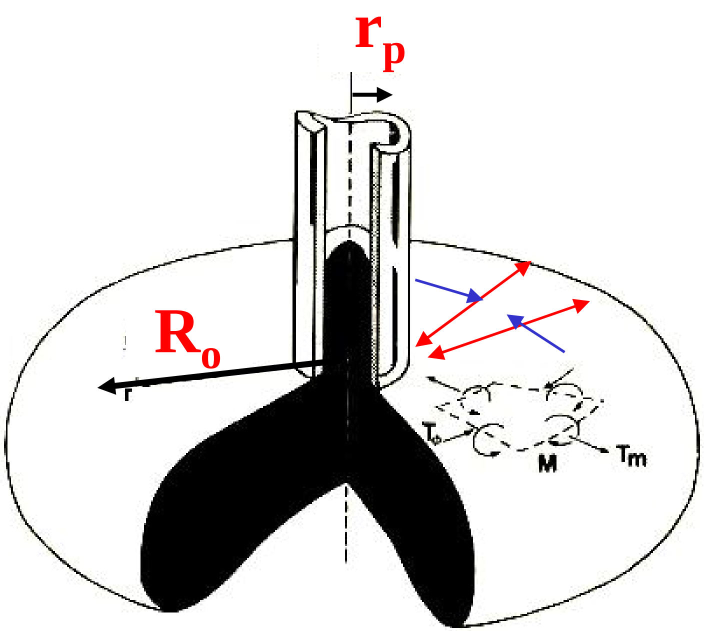
- Early work used fluid shear flow and osmotic compression
- Observation of membrane "flickering"

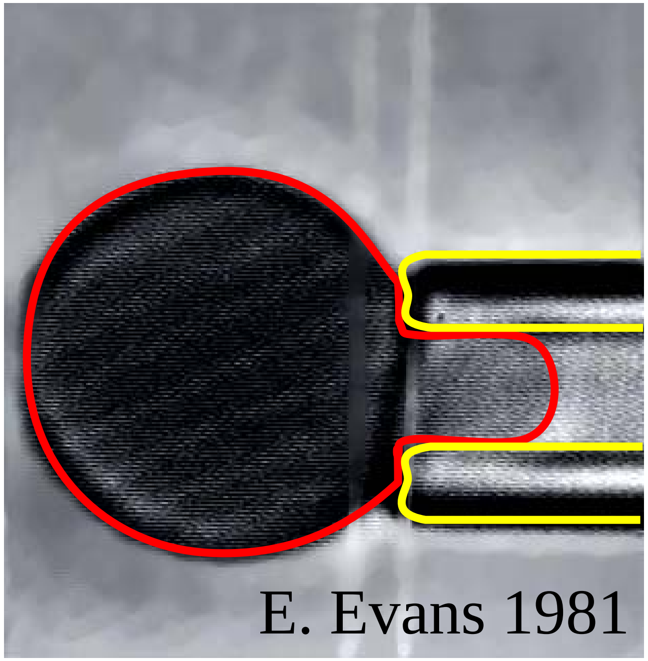
- More recently by direct manipulation by:
- Pipette aspiration (1980s)
- AFM (1990s)
- Optical tweezers (1990s)

- Biophysics is directly applicable to malarial invasion but little mention is made of this in the literature!

# Simple engineering treatments work well.

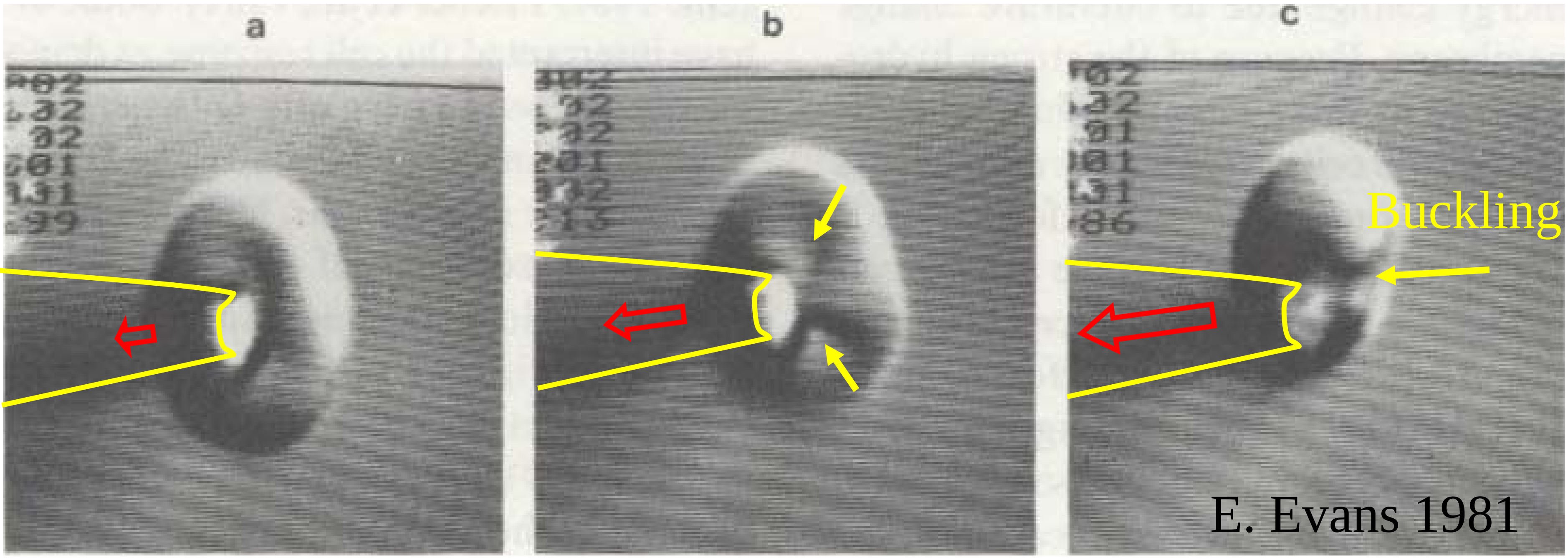
- Elastic sheet with boundary conditions:



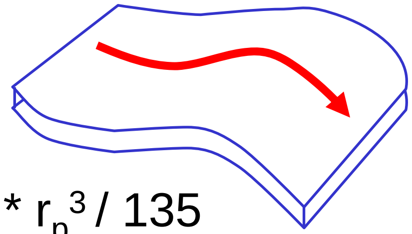




- Both extension and compression forces are generated
- Compression forces lead to buckling

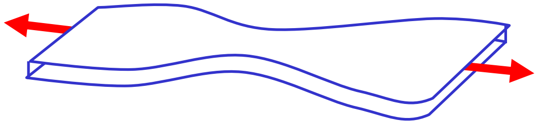
# The buckling force enables the membrane elastic modulus to be estimated.




Bending modulus (B)



Elastic Shear modulus (H)



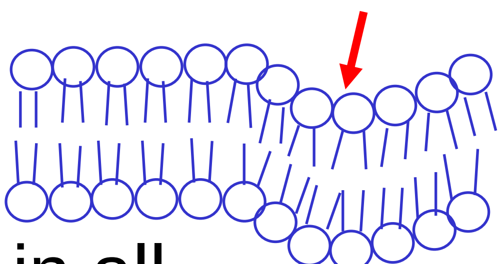
When $R_0=3r_p$ $B = \Delta P * r_p^3 / 135$

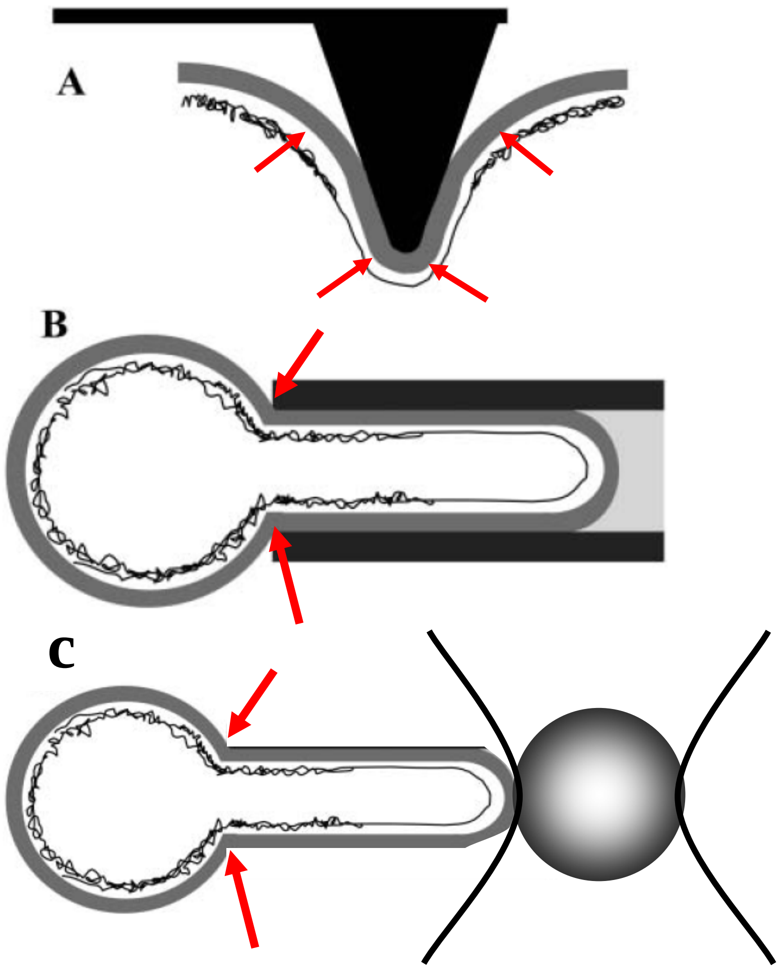
Experiment gives B = <u>200 pN.nm</u>

Experiment gives H = <u>0.002 pN.nm$^{-1}$</u>

# Similar estimates can be made using AFM, and Optical tweezers

- The lipid membrane will flow freely. But it will also exert a restoring force wherever it is bent (due to its bending stiffness)



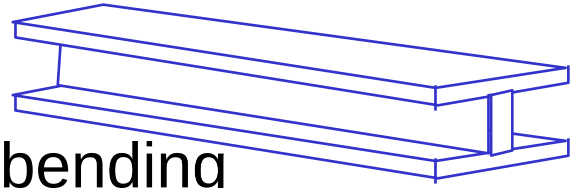



- However, in all experiments the underlying cytoskeleton is probably disrupted.

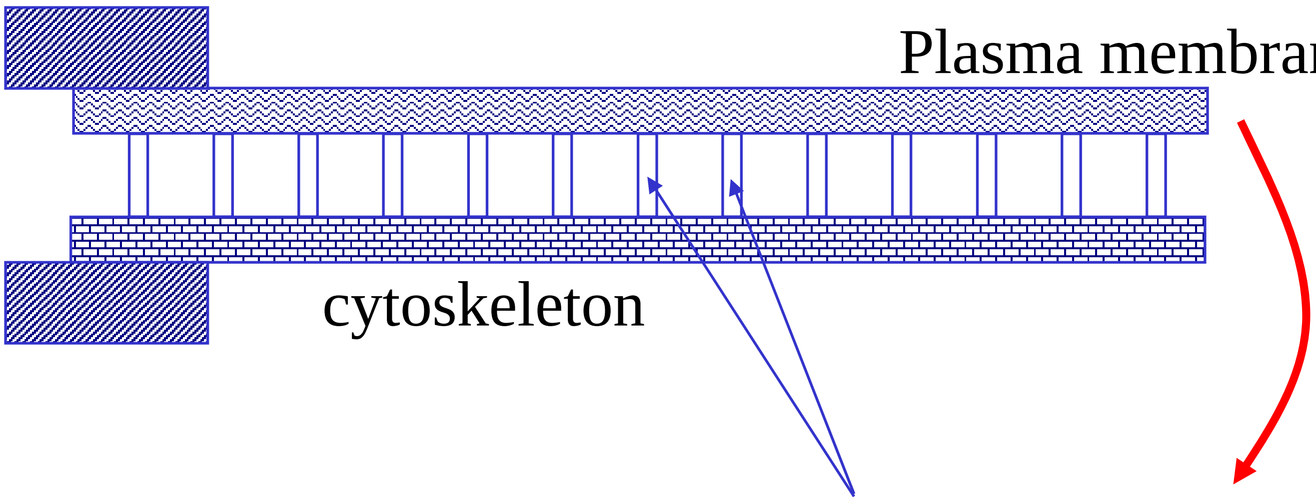
# The buckling force is in the nanoNewton range

# – this requires >10,000 myosin molecules

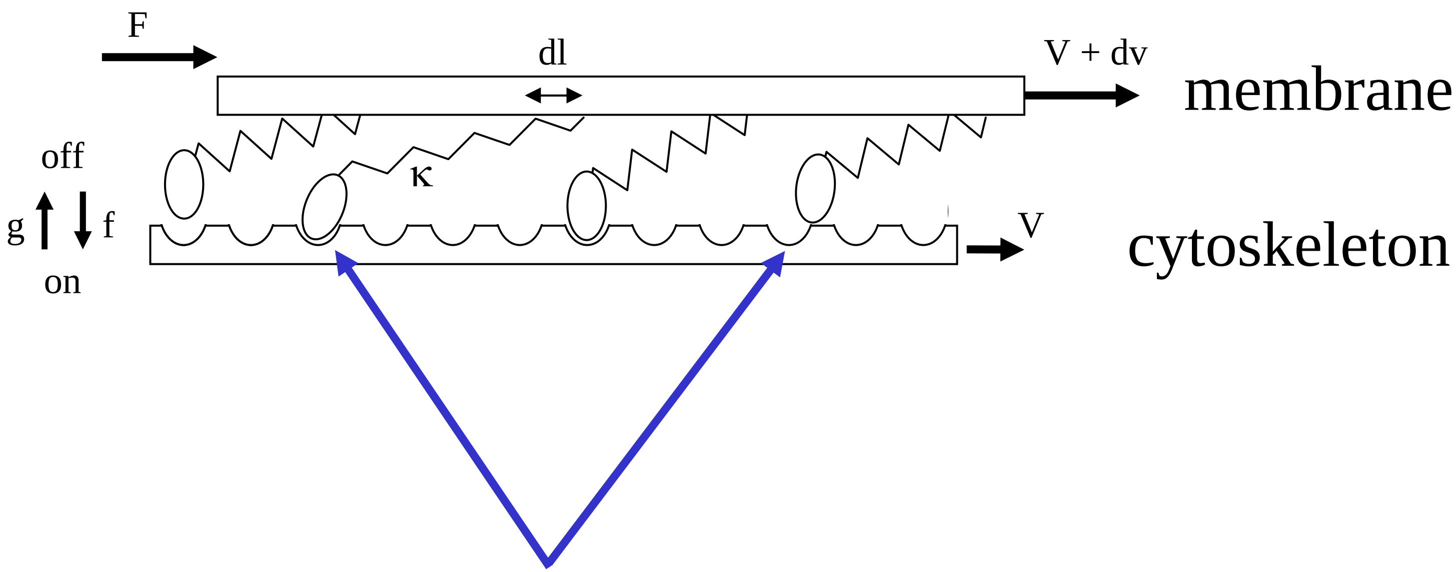
Gross mechanical properties of the plasma membrane depend upon underlying structures e.g. the cytoskeleton.



Classic "I-beam" structure – very stiff in bending



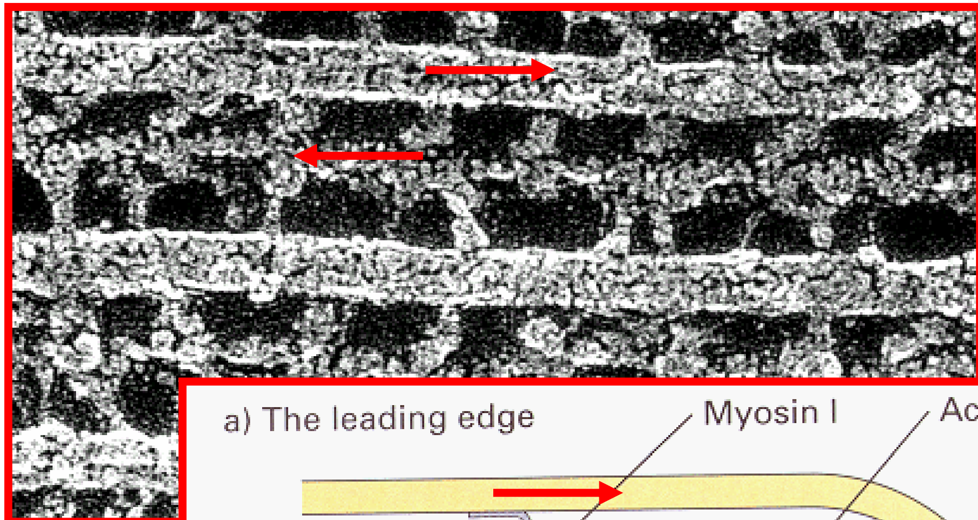
# The cross-links can be active or passive - but all are dynamic.



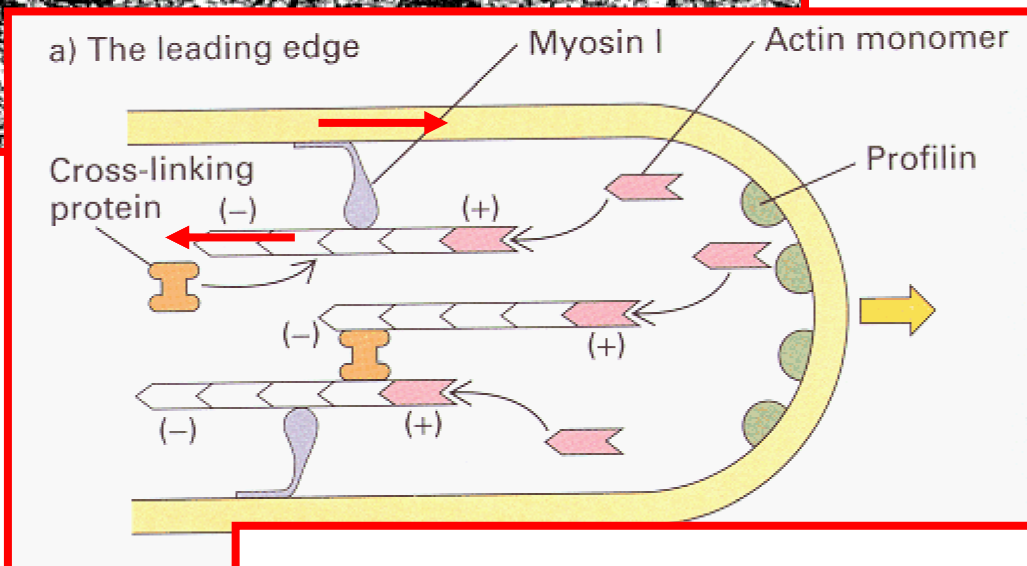

Cross-links might either……..

- remain bound and resist shearing – elastic response
- detach and then reattach giving rise to viscous drag force
- actively change shape and generate active forces (“cortical tension”)

# Muscle and membranes

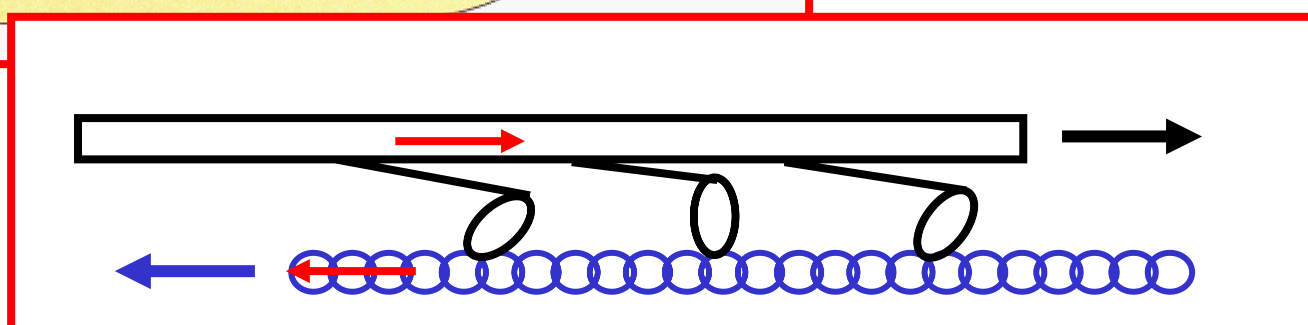


Muscle




Membrane

Model



# We are investigating the dynamic links made between the cytoskeleton and the plasma membrane.

- Active links power the invasion of a parasite
- Passive links resist the invasion of a parasite!

- They are responsible for rearrangement and control of cell shape (endocytosis, filopodia)

- They actively move "*integral membrane proteins*" involved in a variety of functions (e.g. receptors and channels)

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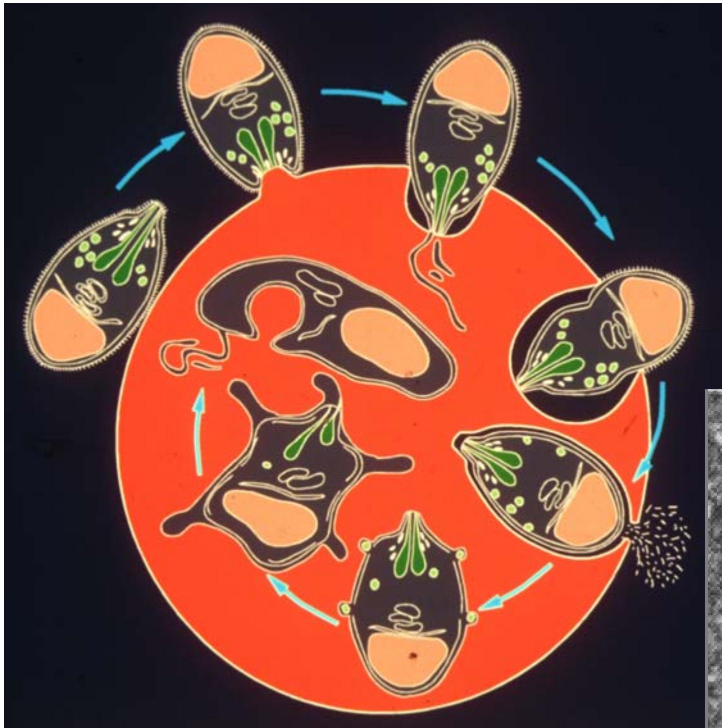
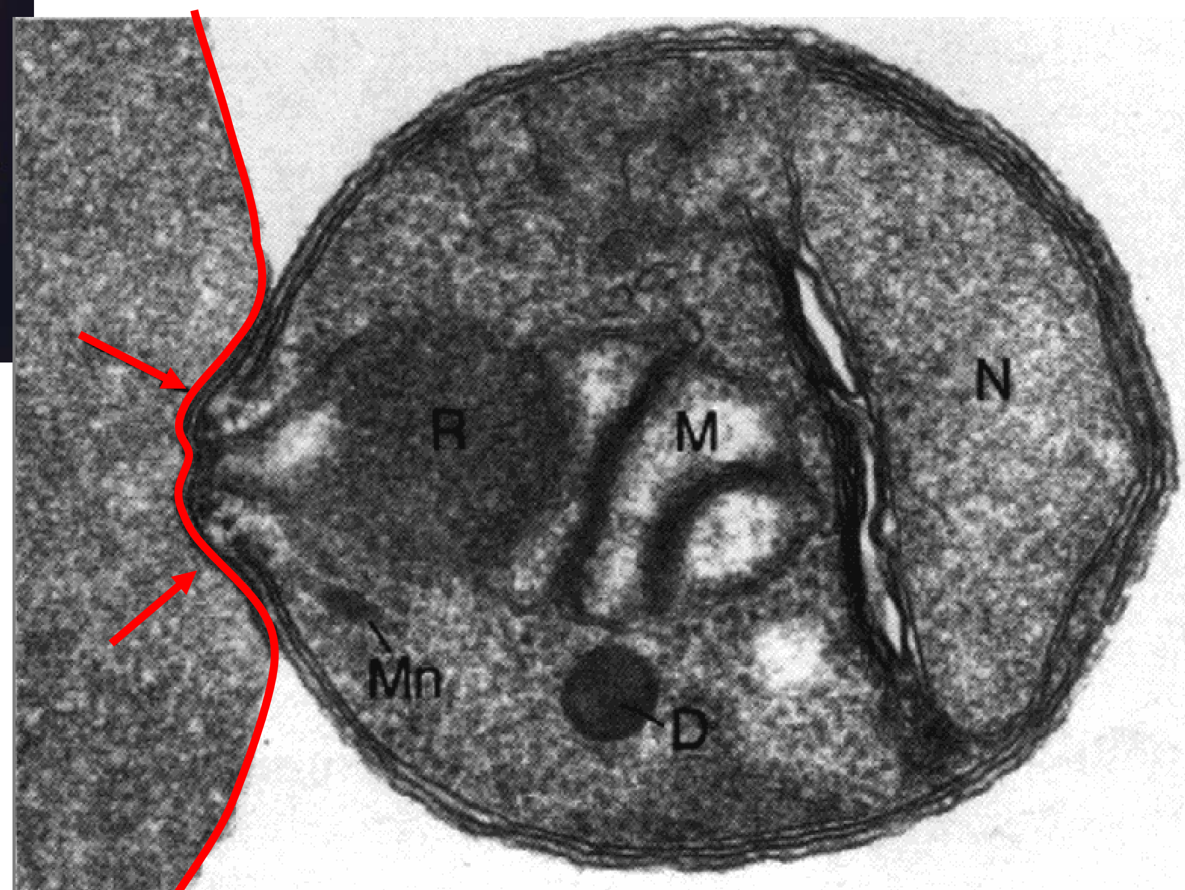
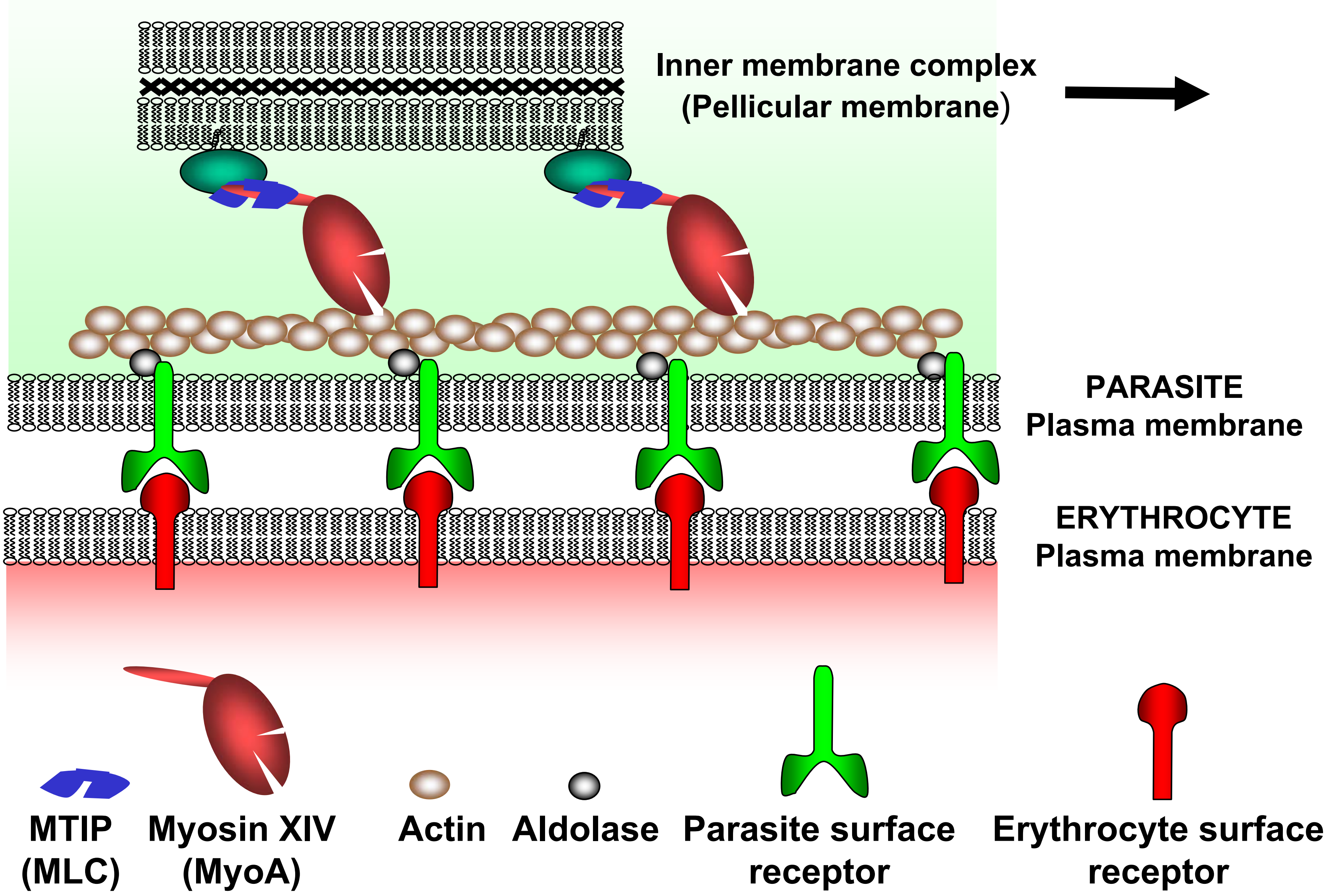
- E.G. Cortical tension can be reduced by expression of PH domains (that interfere with cross-links)

# Malarial invasion of the red blood cell

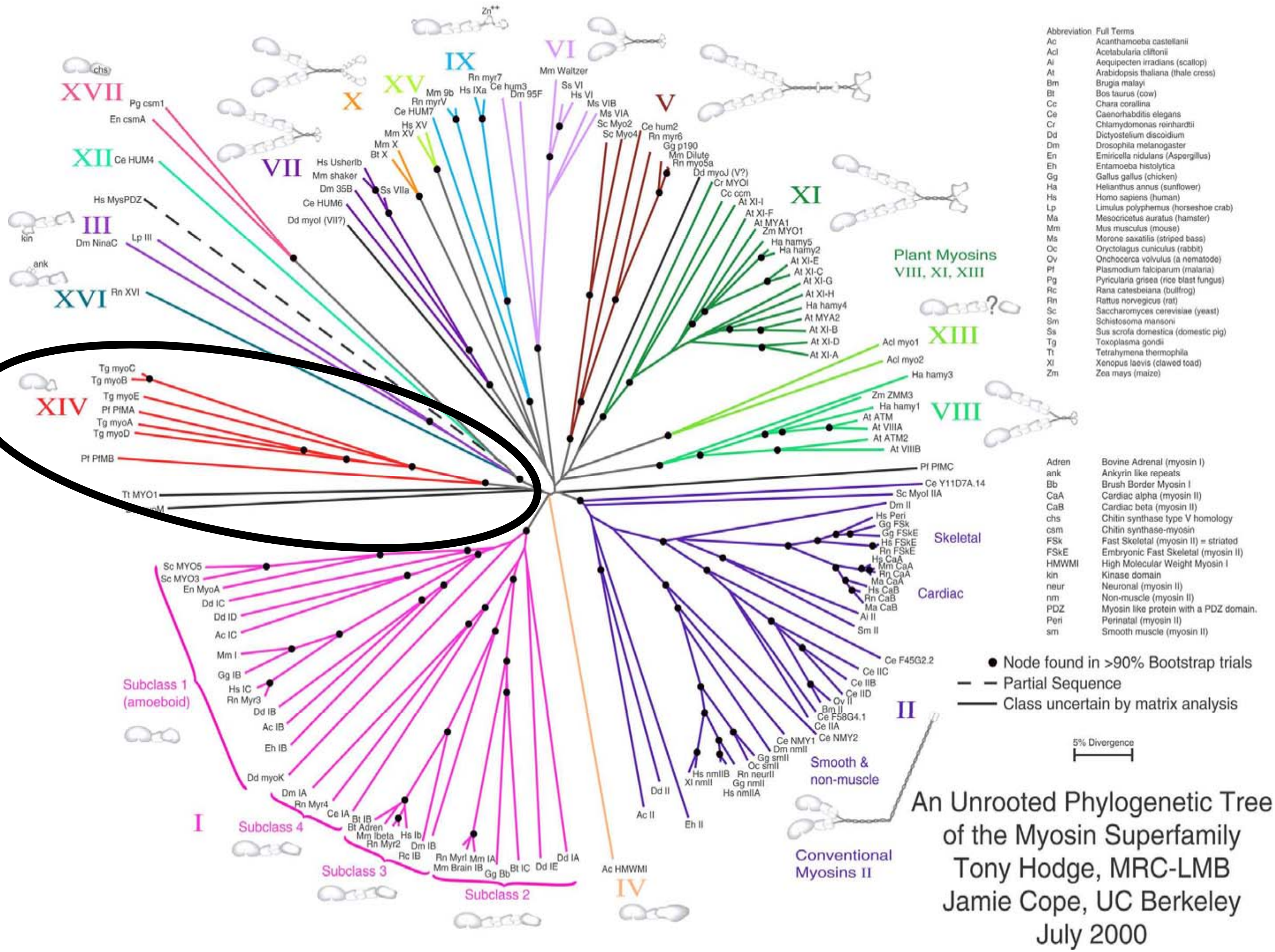




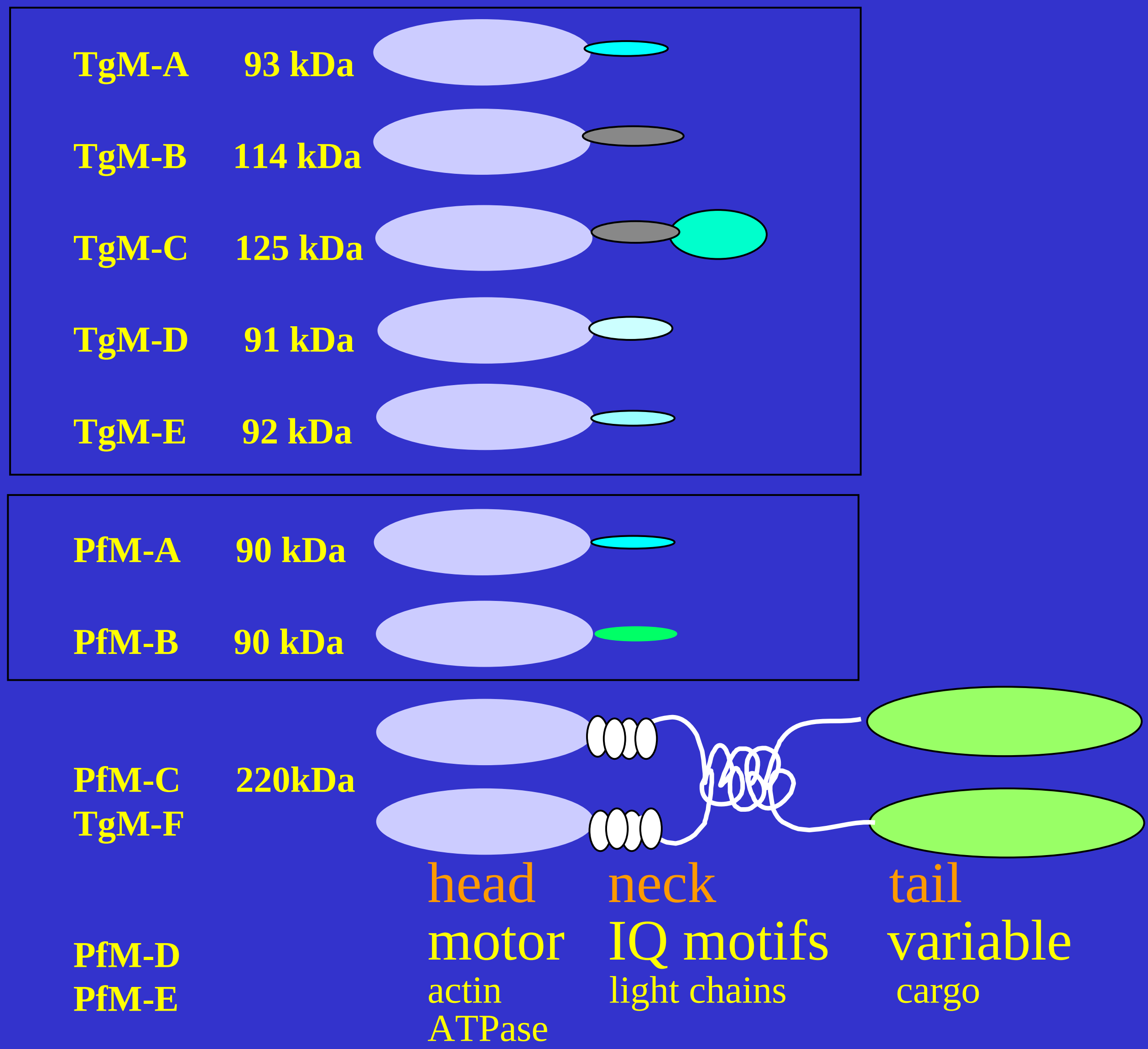
# Putative organisation of the motor complex



# Myosin Family Tree



# Class XIV Myosins



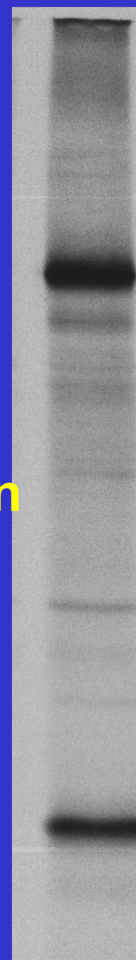

Courtesy of Dominique Soldati

# Native tissue Pfmyo-A purification

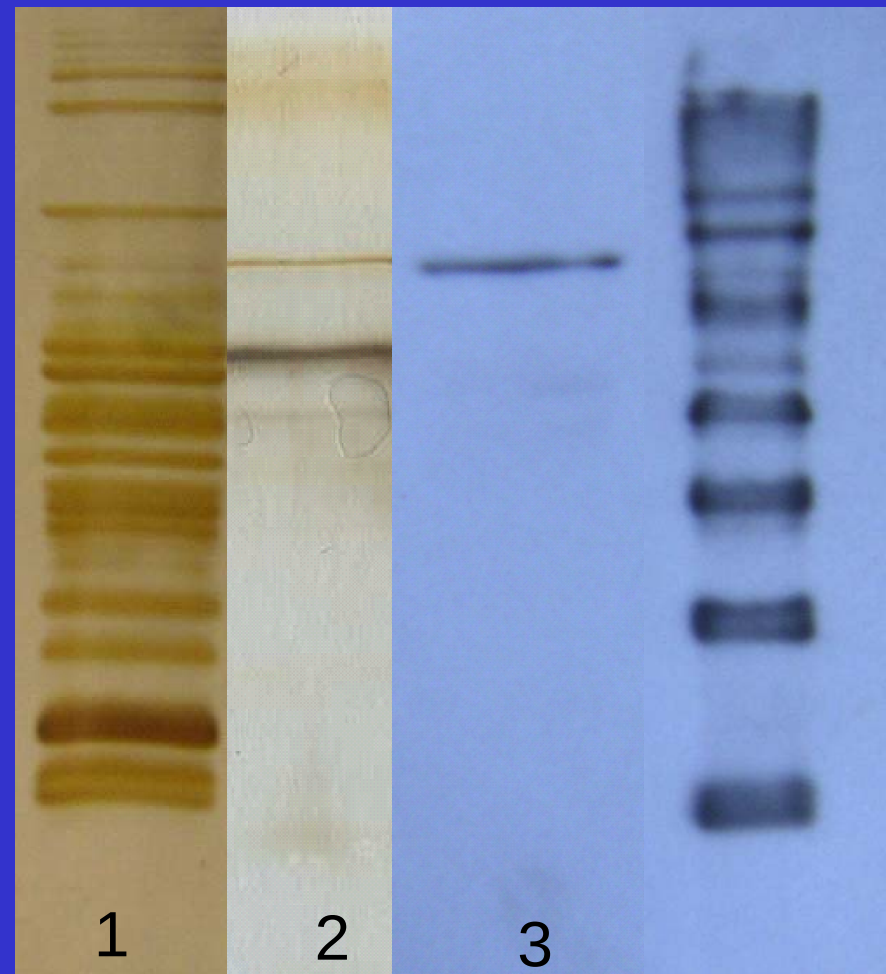
$S^{35}$

Pfmyo-A

MTIP

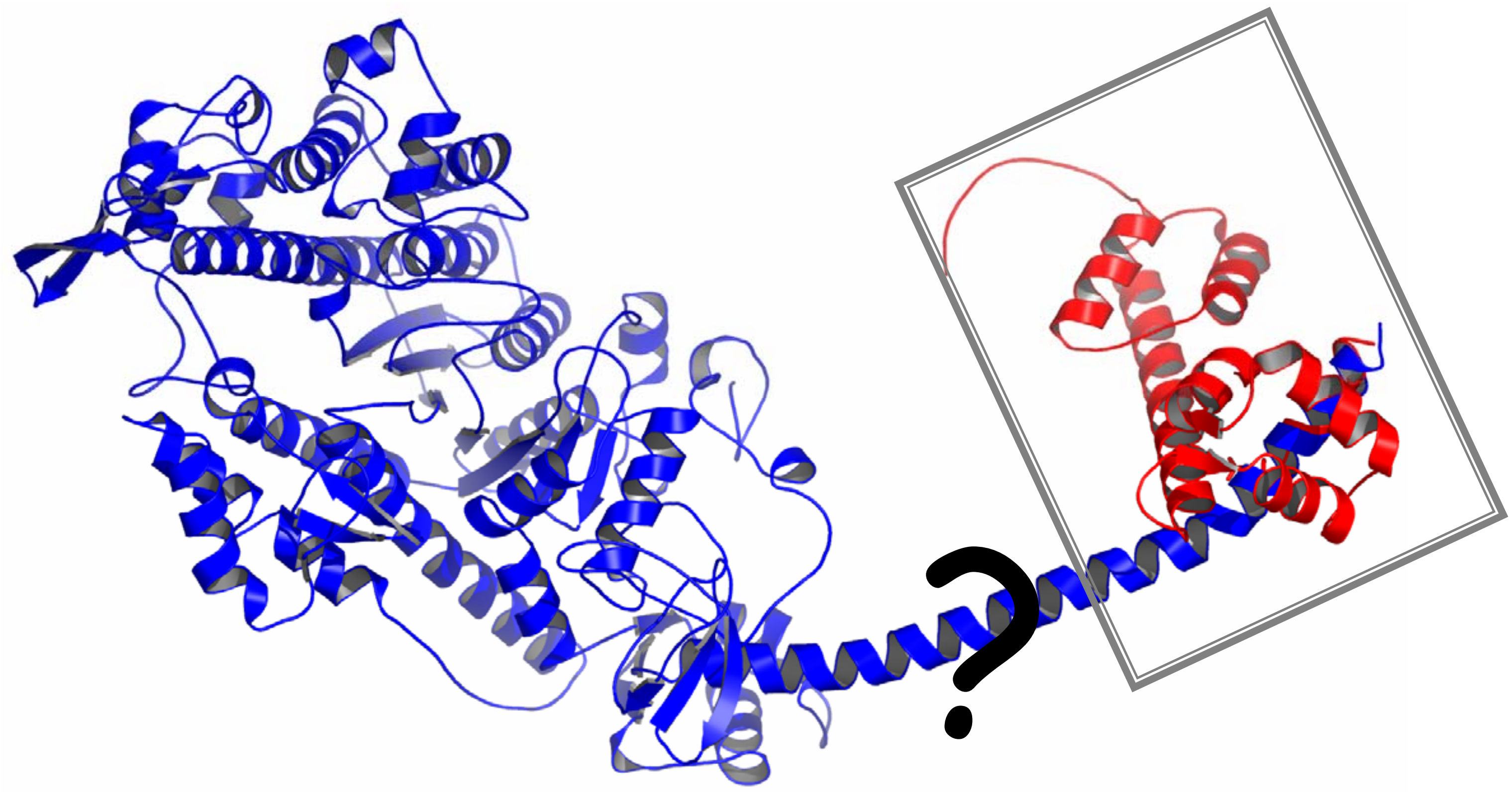


- MTIP immunoprecipitation
- Compete Pfmyo-A

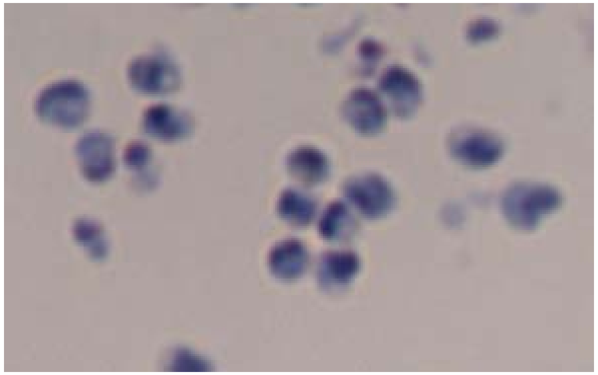



kDa
120
100
80
60
50
40
30

1 Crude cell lysate 2 Pfmyo-A after peptide competition 3 Pfmyo-A western



# Myosin XIV *in vitro* motility



Purified merozoites

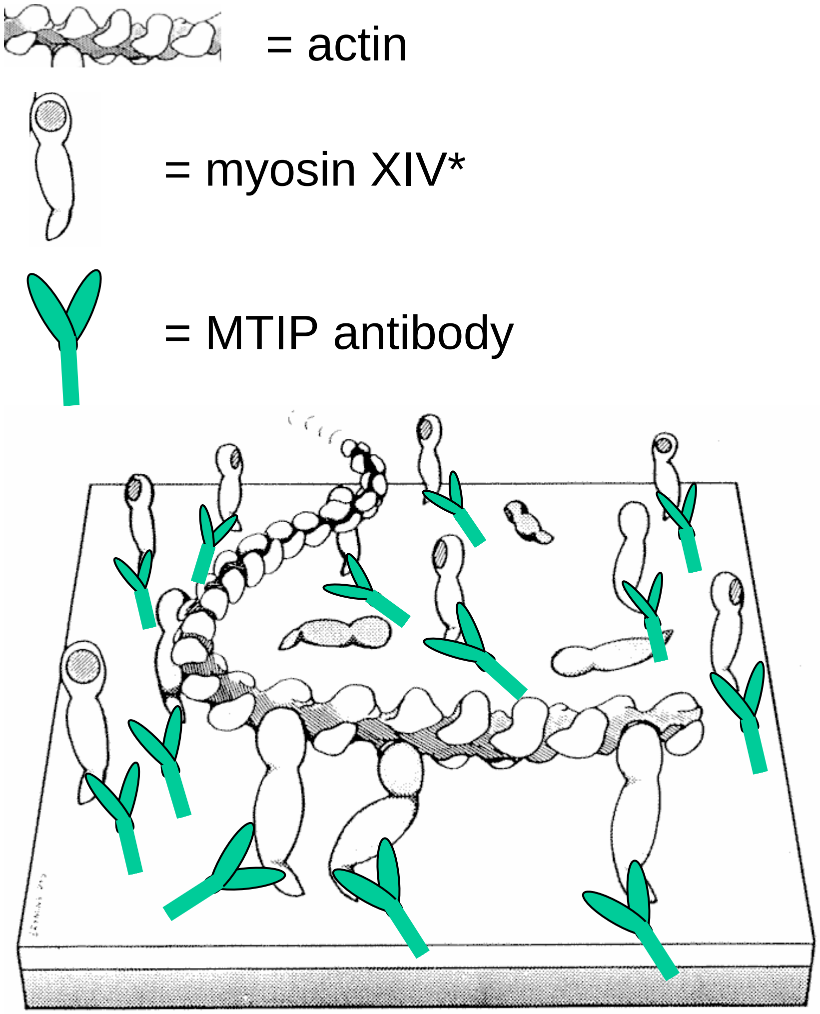
⬇

**Freeze thaw cycle**
**80µl hypotonic buffer**
**Protease inhibitors**

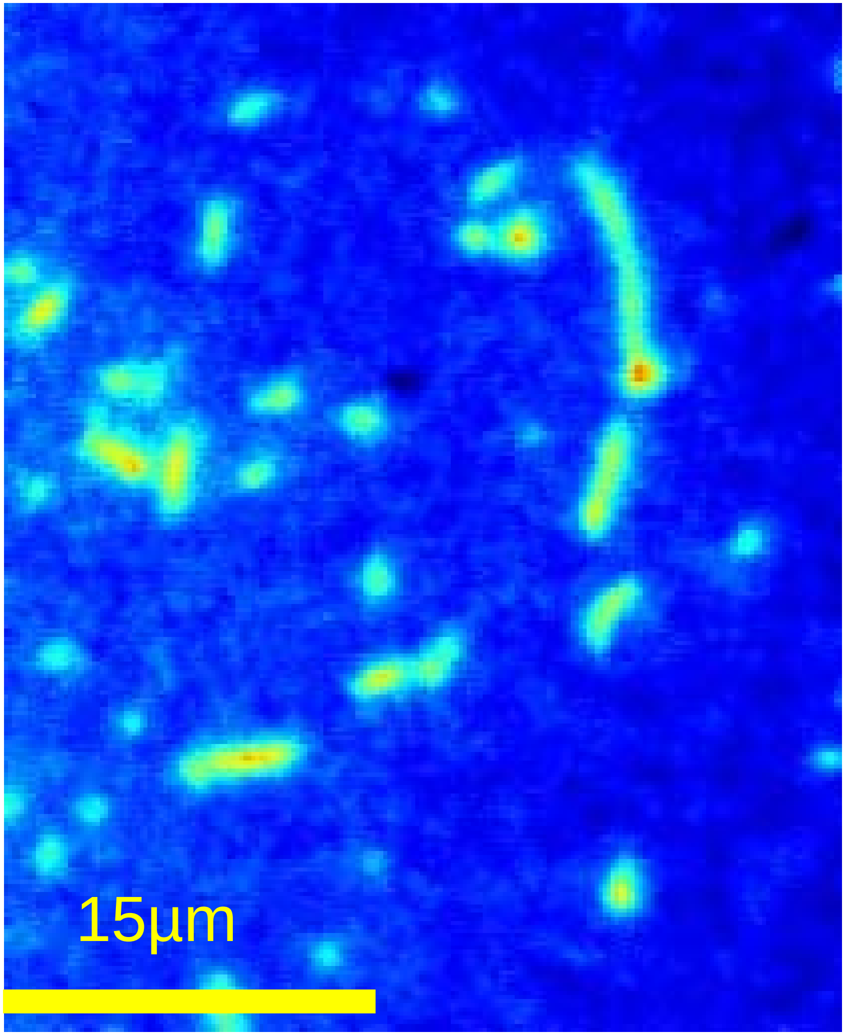
**100k spin**
**S/N removed**

⬇

*In vitro* motility assay

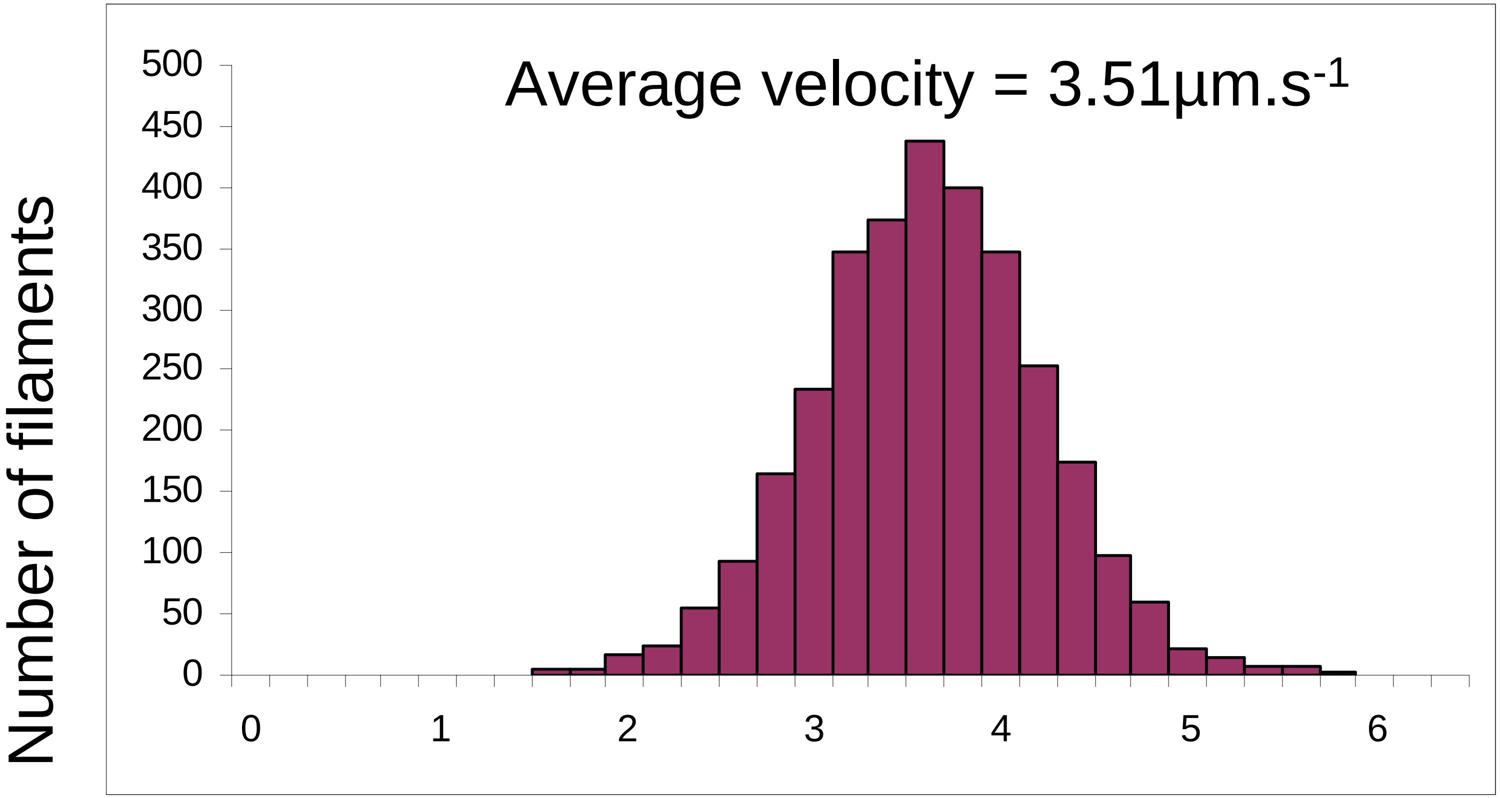


# Myosin XIV in vitro motility



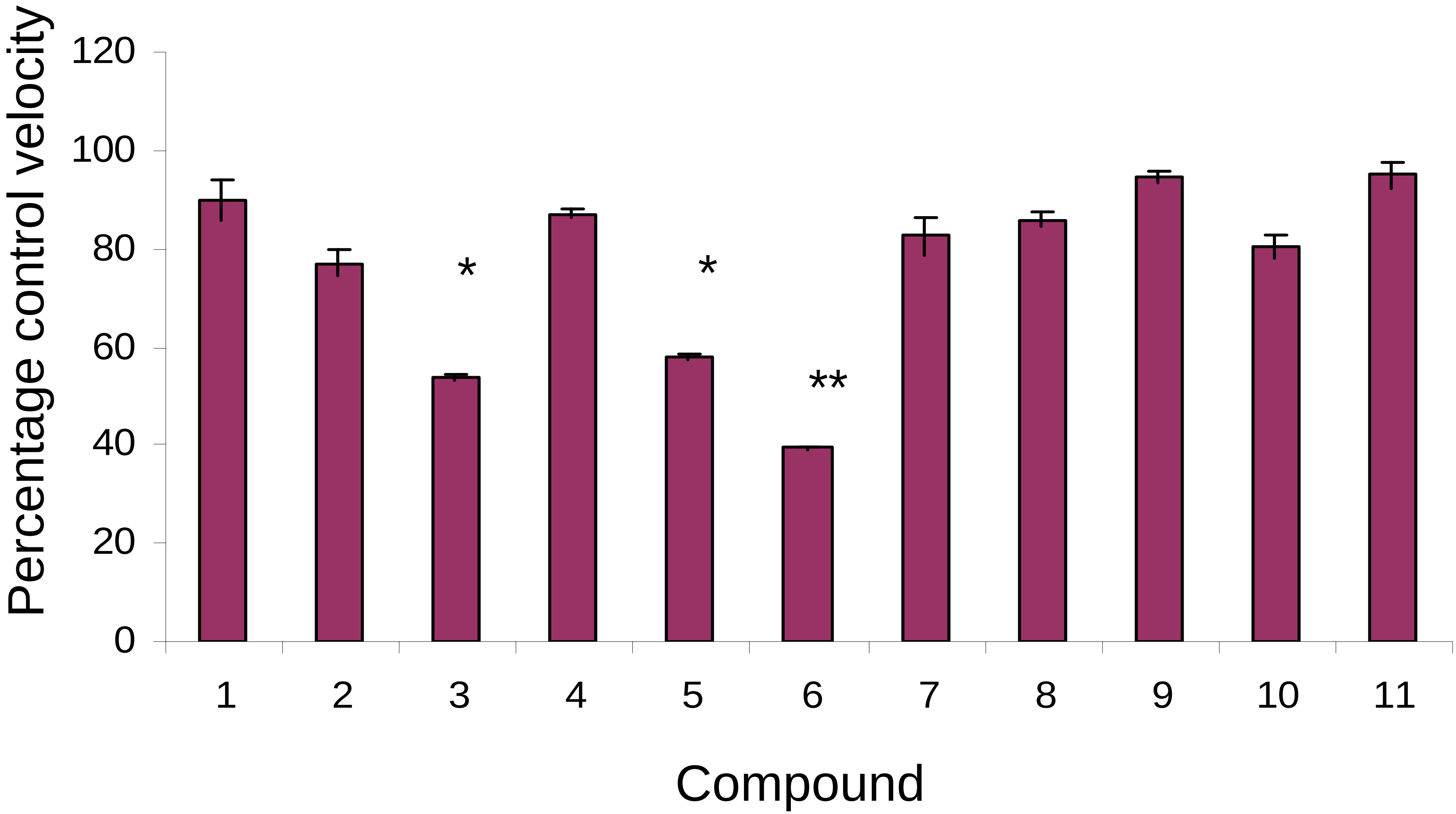

actin sliding velocity
= 3.51µm.s-1
At 25 º C

# Pfmyo-A *in vitro* motility




Filament velocity (µm.s$^{-1}$)

# Blebbistatin analogues



# A brief interlude on processive motors

# "Processive" and "Intermittent" motors

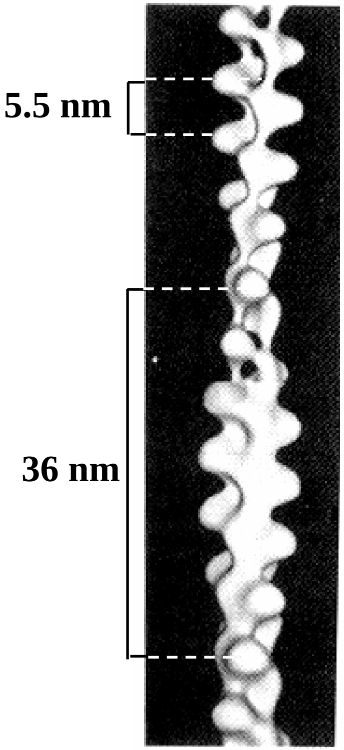
- Most myosins and many kinesins interact in an ***"Intermittent"*** manner with their track. They must **work in teams** to produce large movements and forces.

- kinesin 1, myosin V, and most DNA processing enzymes are ***"Processive"*** motors and take many steps before detaching from their track. They **work as single molecules.**

- Some motors (e.g. myosin VI) can modulate their properties.

## ACTIN FILAMENT
(repeats every 5nm and every 36nm)

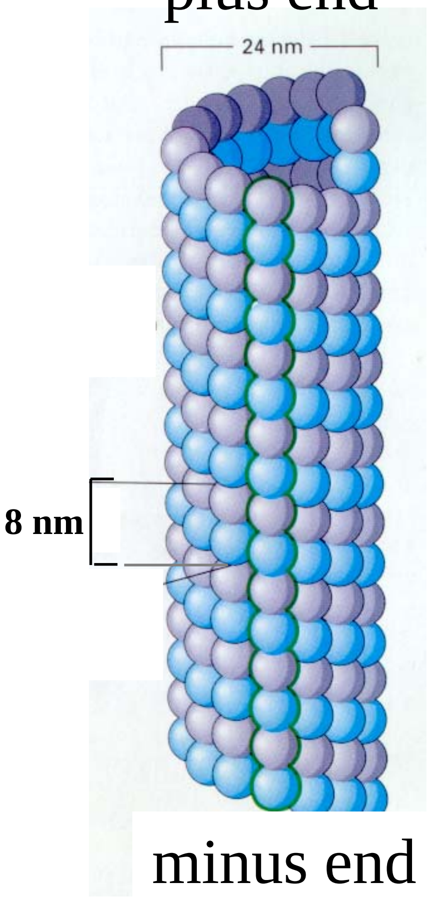
Minus end - pointed end




Plus end - barbed end

## MICROTUBULE
(repeats every 8nm)

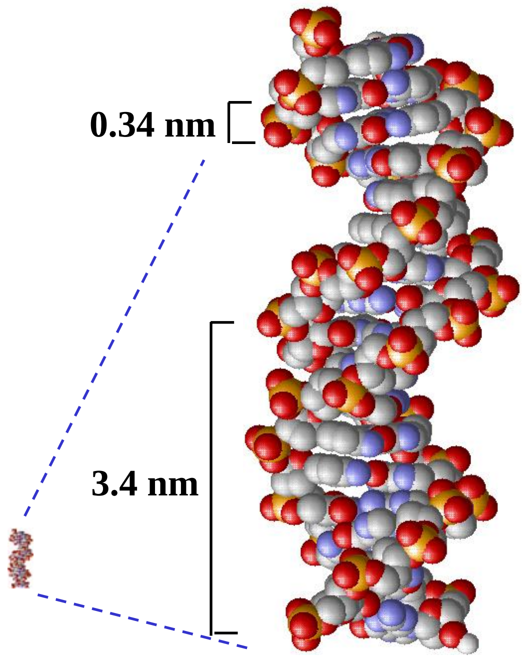
plus end




minus end

## DNA
(repeats every 0.34nm)

3’ & 5’

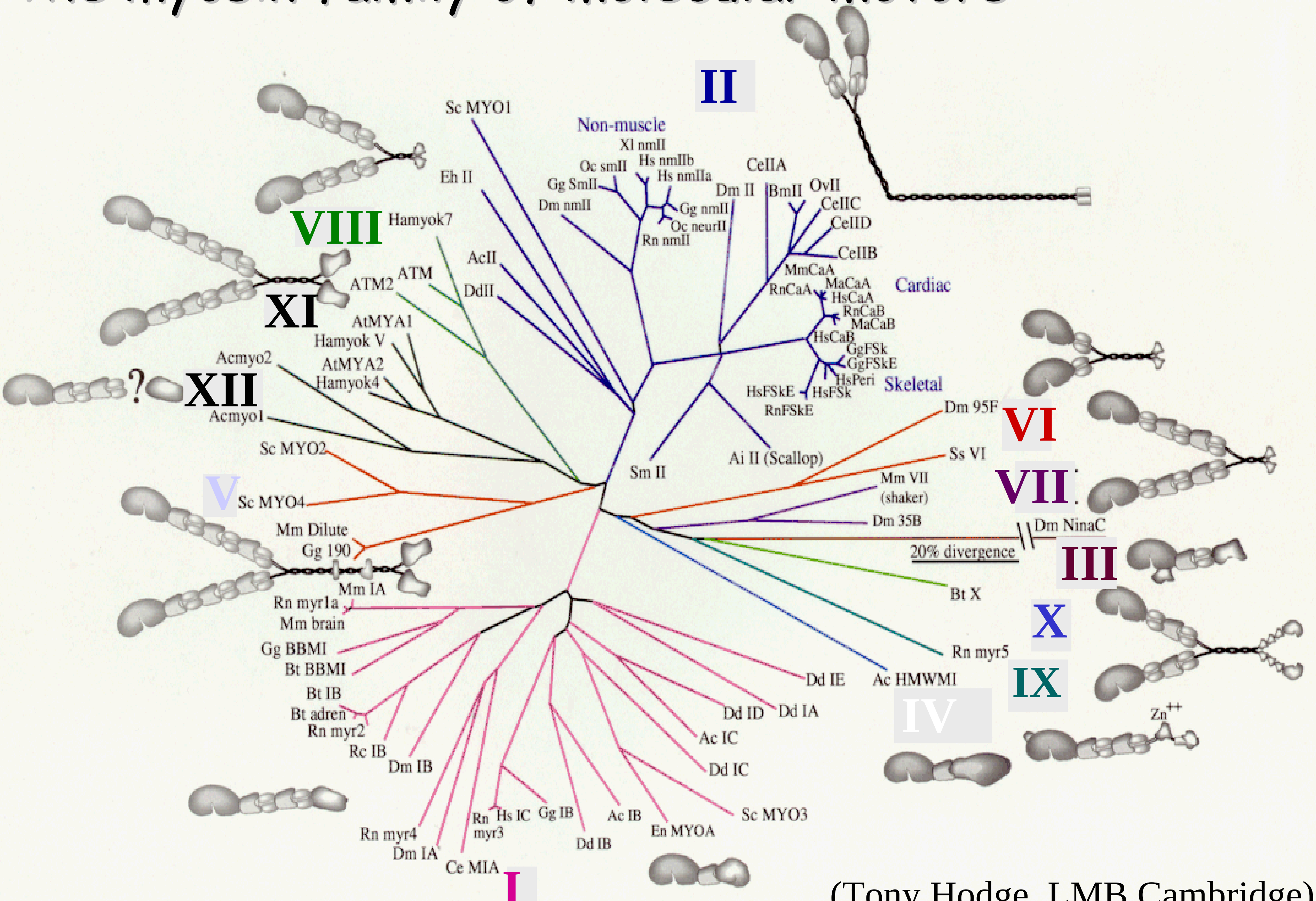



3’ & 5’

(not to scale)

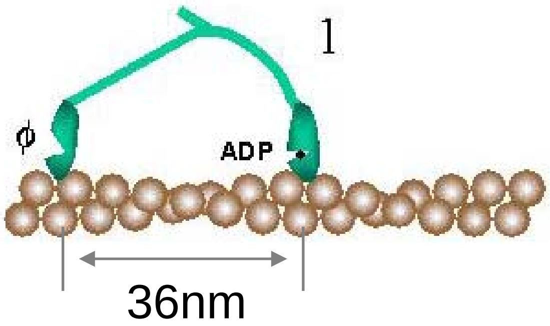
(Note: some cell motilities are driven by filament polymerisation)

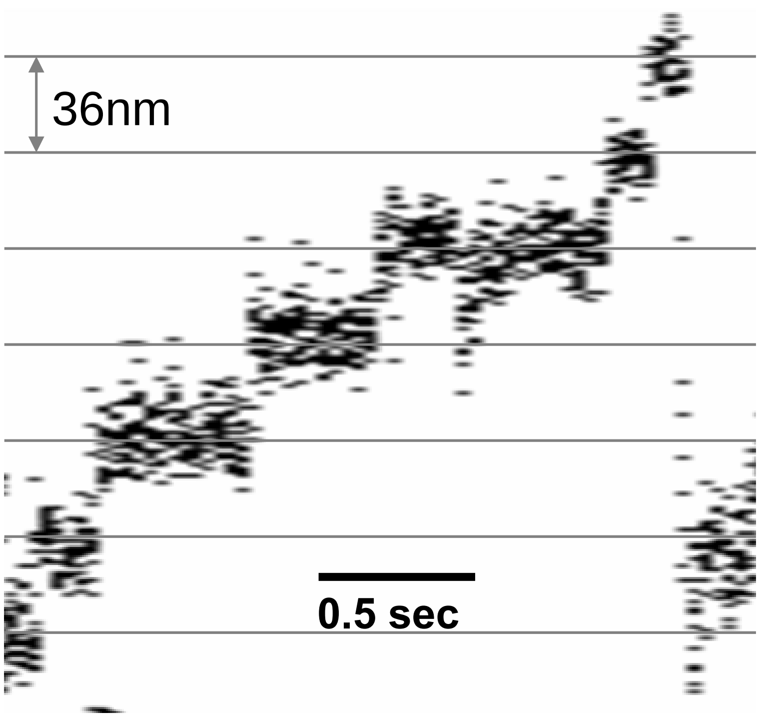
# The myosin family of molecular motors:




(Tony Hodge, LMB Cambridge)

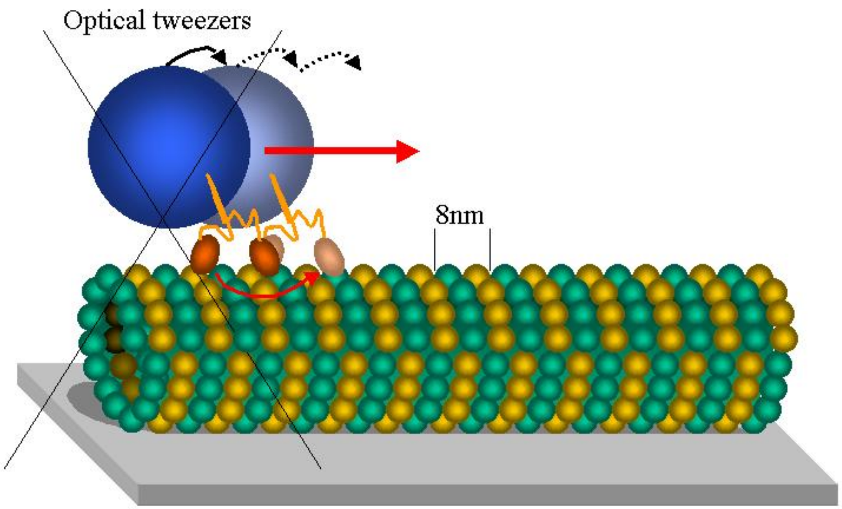
# Myosin V

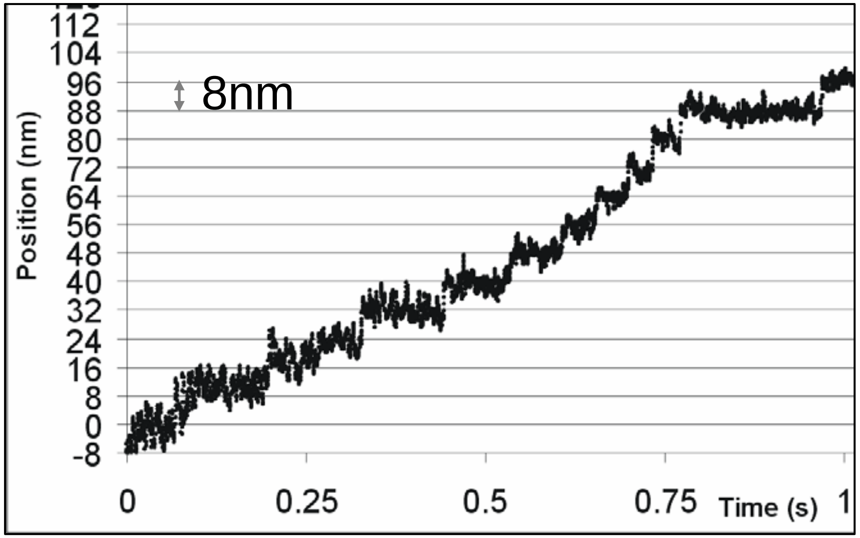



Veigel & Molloy

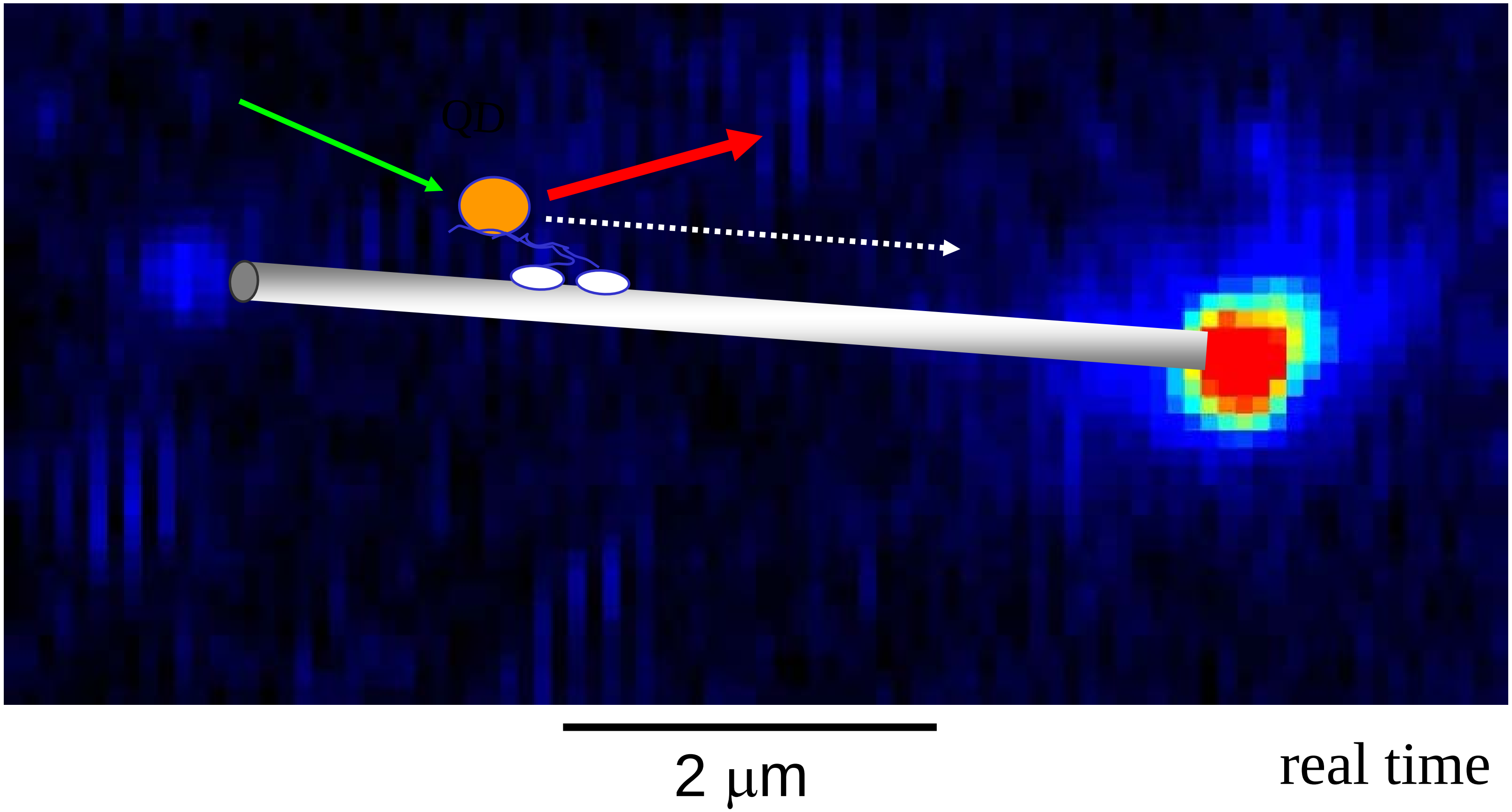
# Conventional kinesin

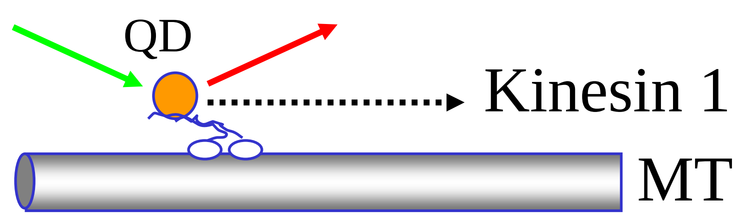



Carter & Cross

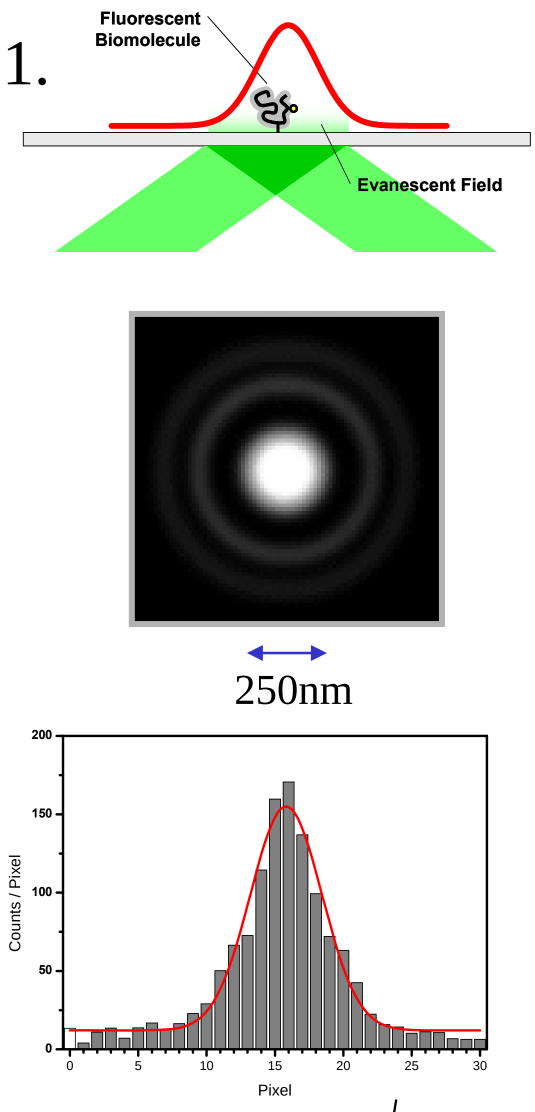
# MOVEMENT OF AN INDIVIDUAL KINESIN ON A MICROTUBULE VISUALISED USING A QUANTUM DOT



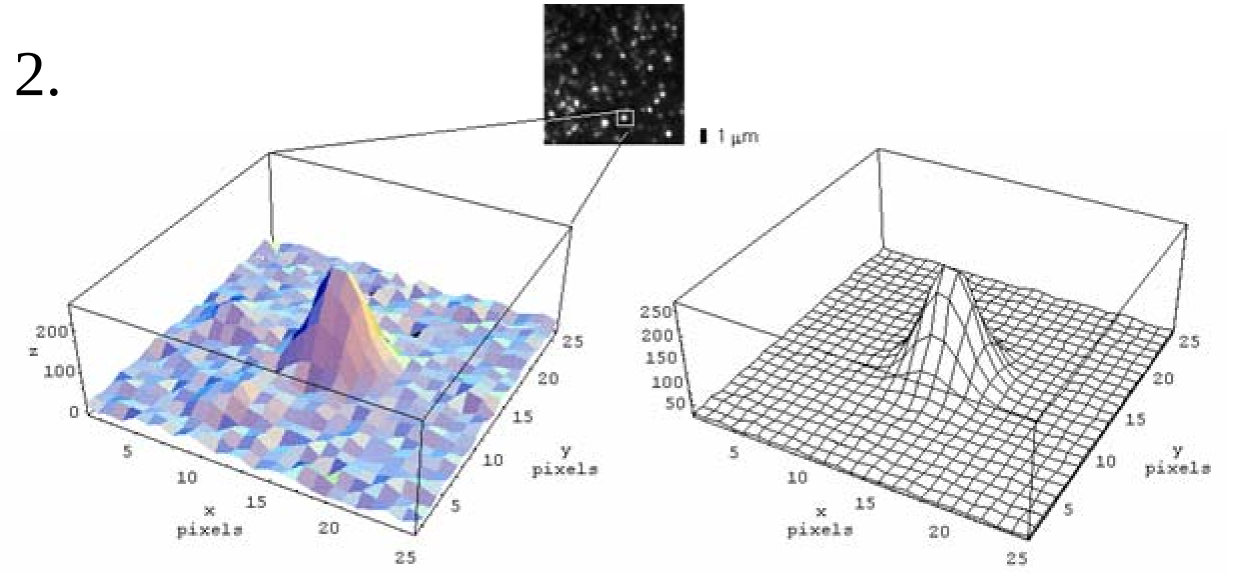



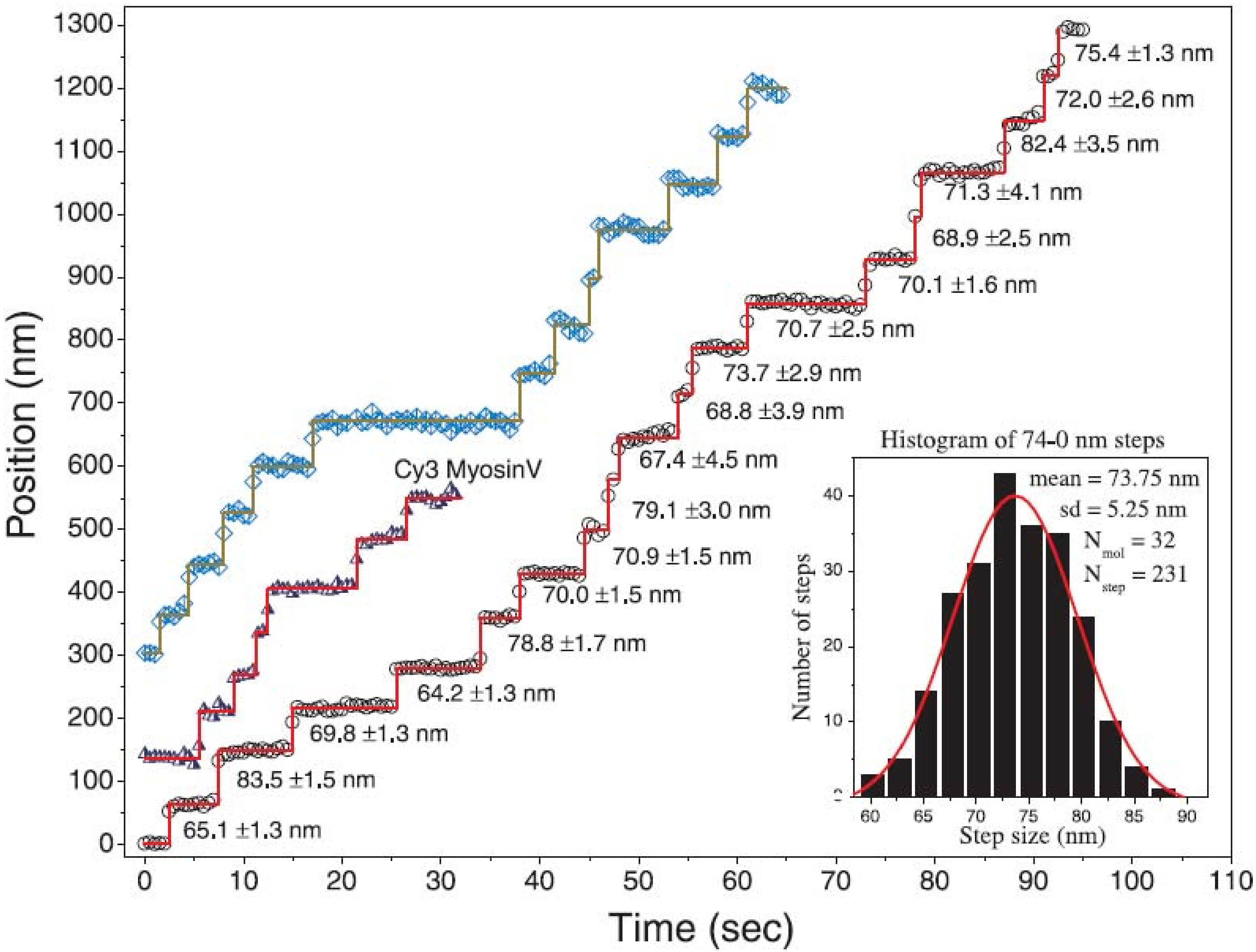

Mellor, Toba, Oiwa & Molloy
unpublished data

# Single fluorophore localisation

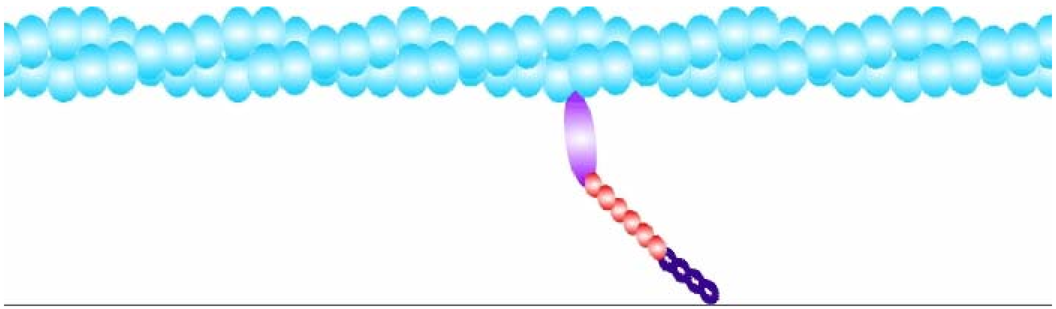



$$SEM = \sigma/\sqrt{n}$$

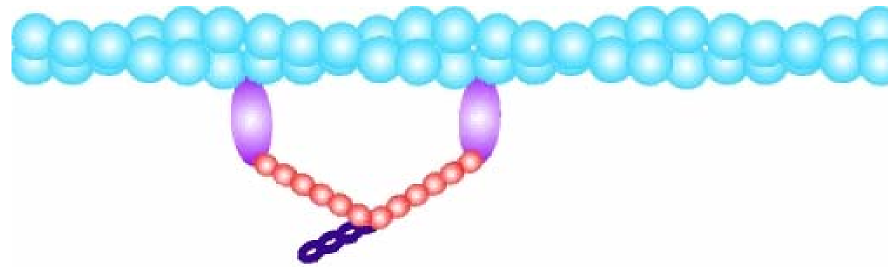




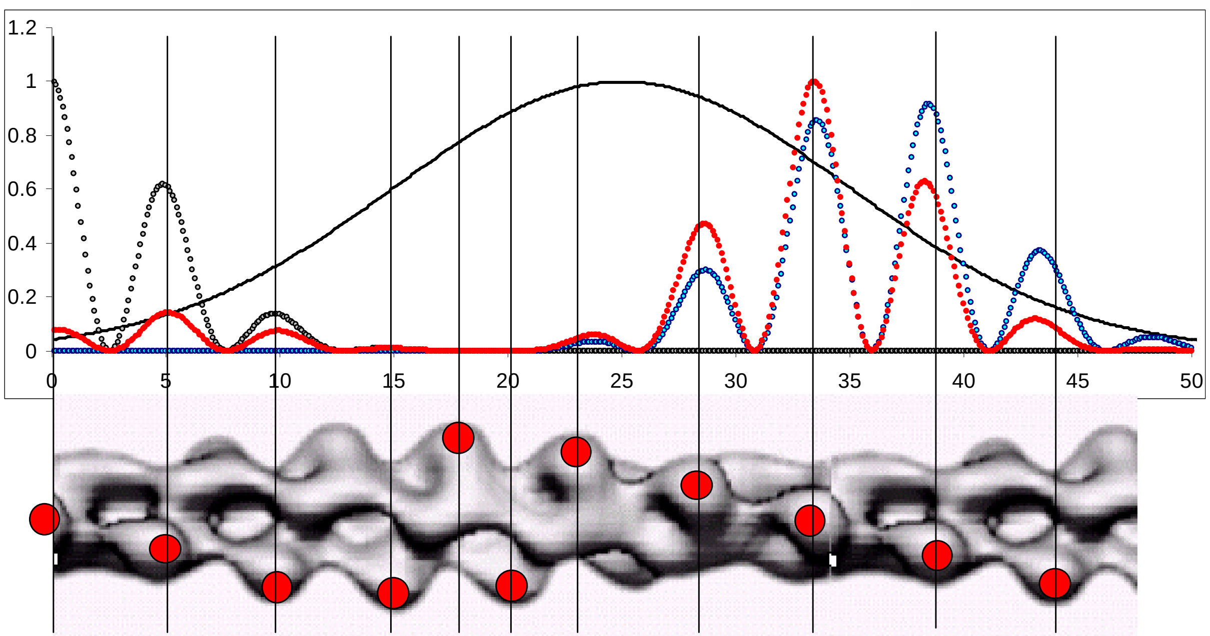

Yildiz, A., et al. (2003). "Myosin V walks hand-over-hand: Single fluorophore imaging with 1.5-nm localization." Science **300**(5628): 2061-2065.



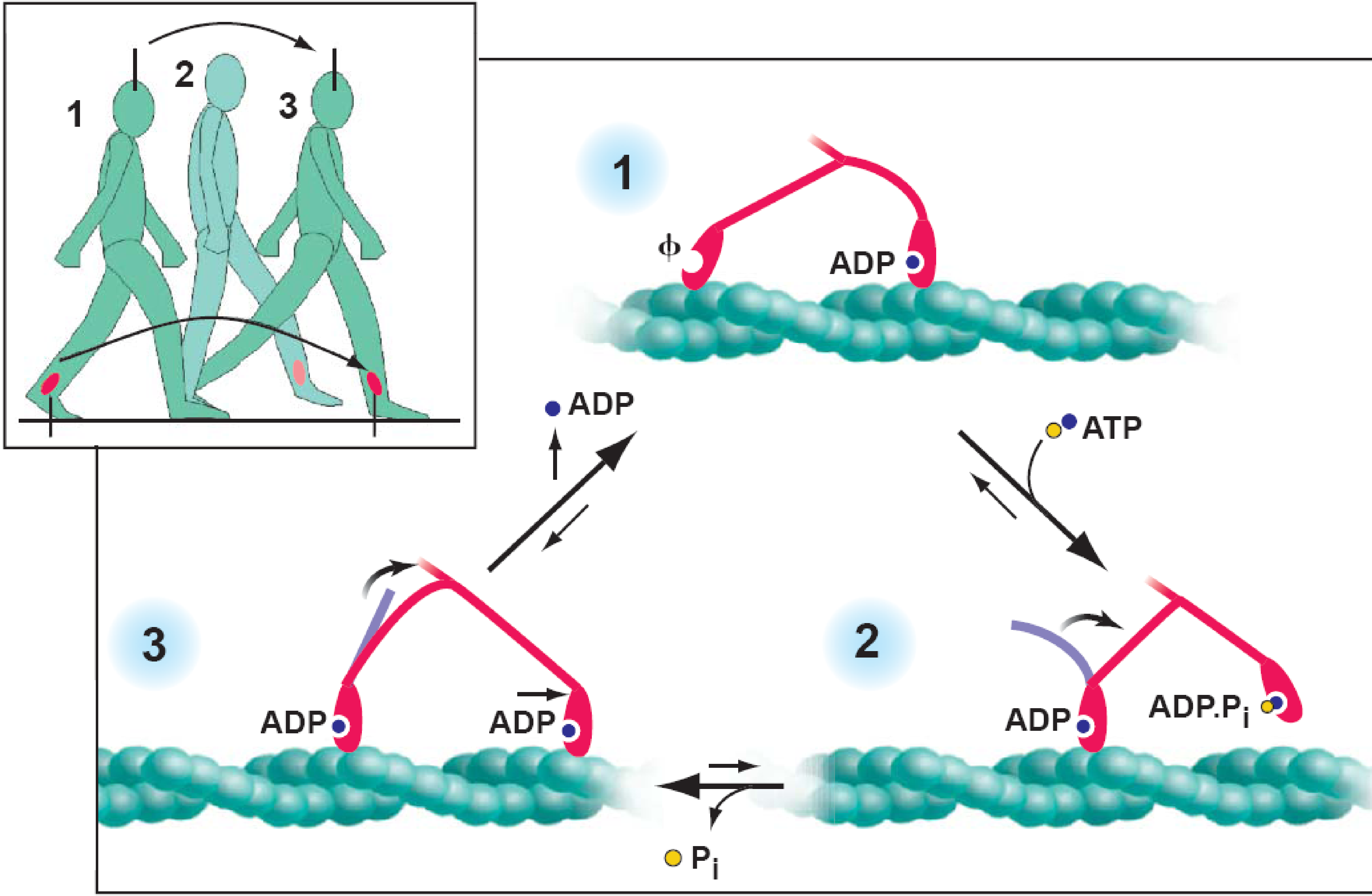
Intermittent

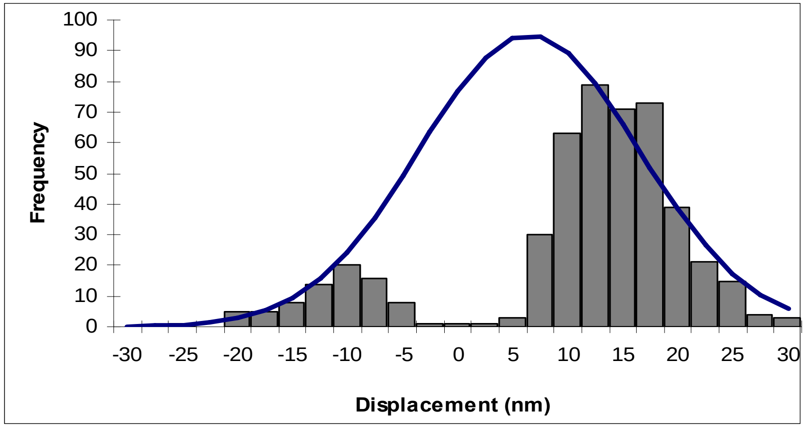


Processive



# Myosin V cycle

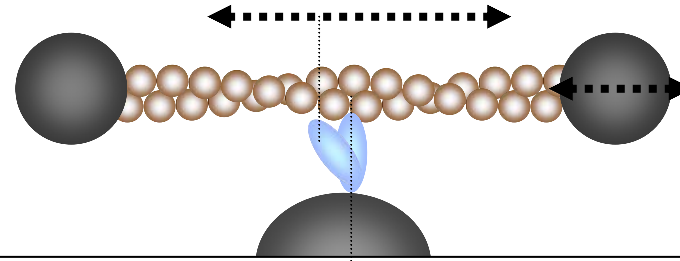


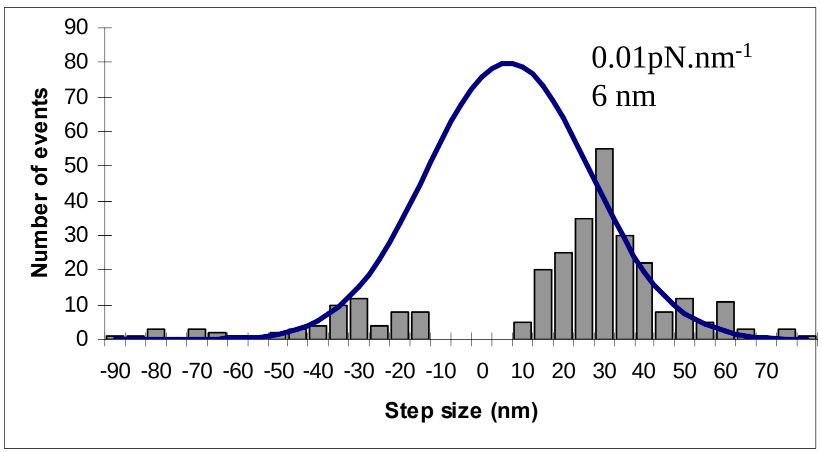



## Intermittent interactions

Myosin fixed – actin fluctuates in position

II

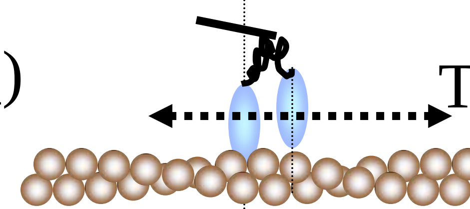




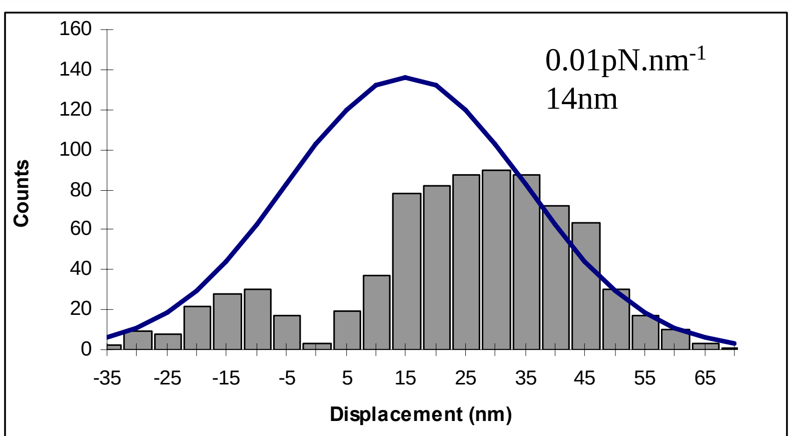

## Processive interactions

Actin fixed – free myosin head fluctuates

V(1IQ)



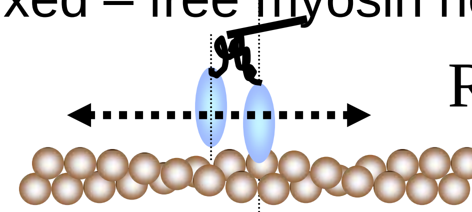
Tanaka *et al.*




## Processive interactions

Actin fixed – free myosin head fluctuates

VI



Rock *et al.*

# Many myosins exert forces on membranes

Examples:

Myosin Ib – membrane tensioning in the microvilli of the gut

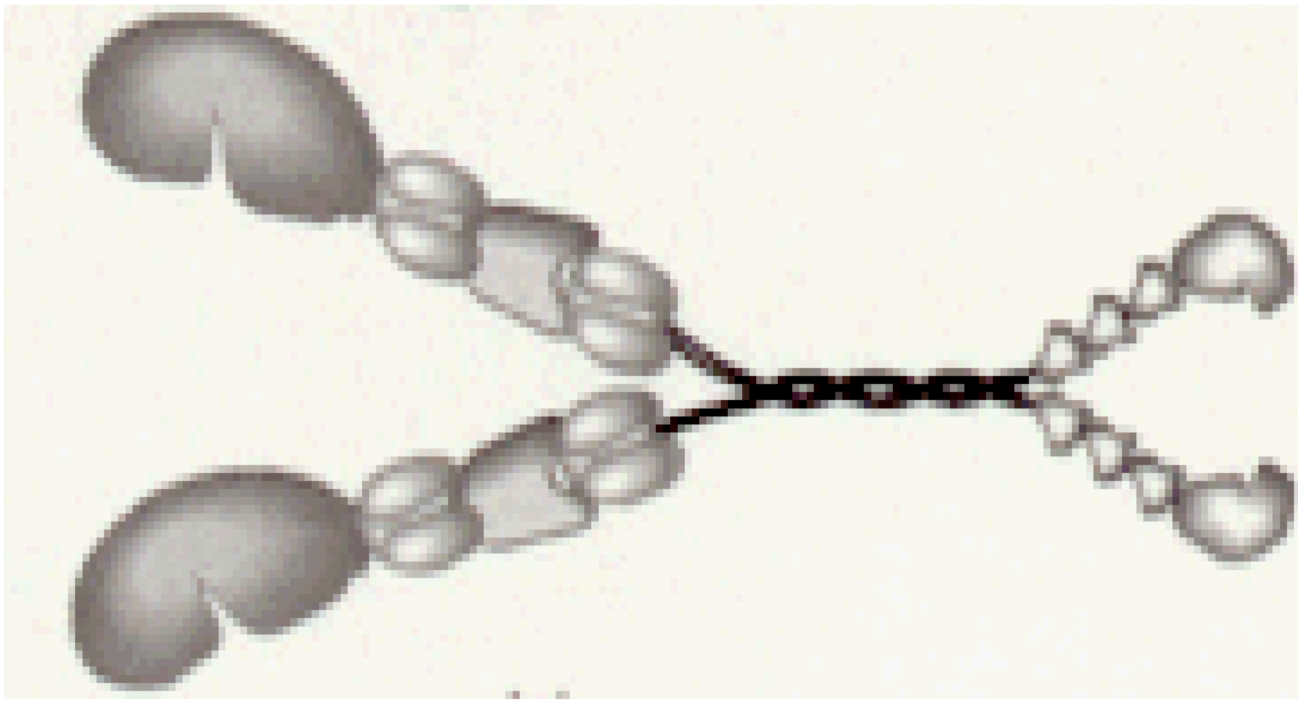
Myosin **Ic** – regulates the position of a sensory channel (in the ear)

Myosin VI – involved in endocytosis

Myosin **X** - inserts onto the plasma membrane via PH domains
(function is unknown)

Myosin **XIV** – responsible for motility of Apicomplexans
e.g. invasion motor of *Plasmodium falciparum* (malaria)

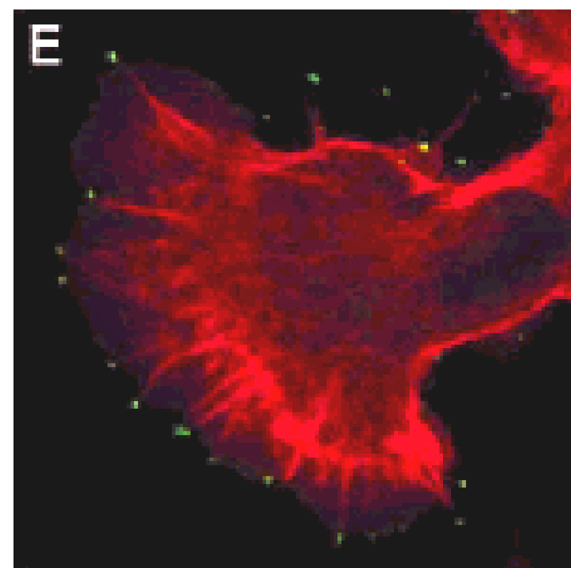
# Cellular targeting of Myosin X



Using Total Internal Reflection Fluorescence Microscopy

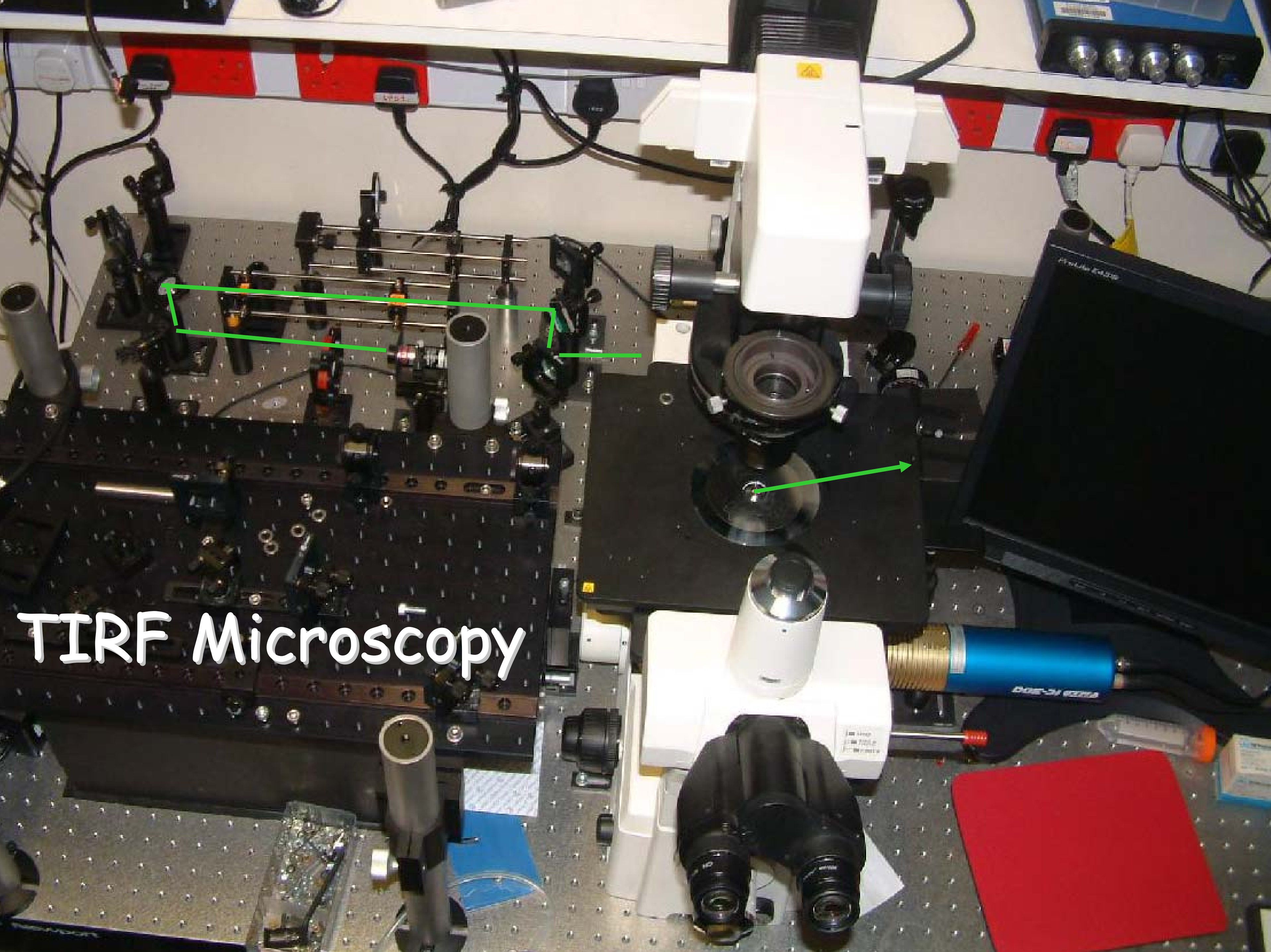
# How is myosin X targeted to its cellular location and what switches it on and off?

- The “head” of the molecule is the motor – the “tail” defines the cargo to be carried (and therefore its cellular function).
- Myosin X is targeted to the lamellipodium, to membrane ruffles and the tips of filopodial actin bundles.

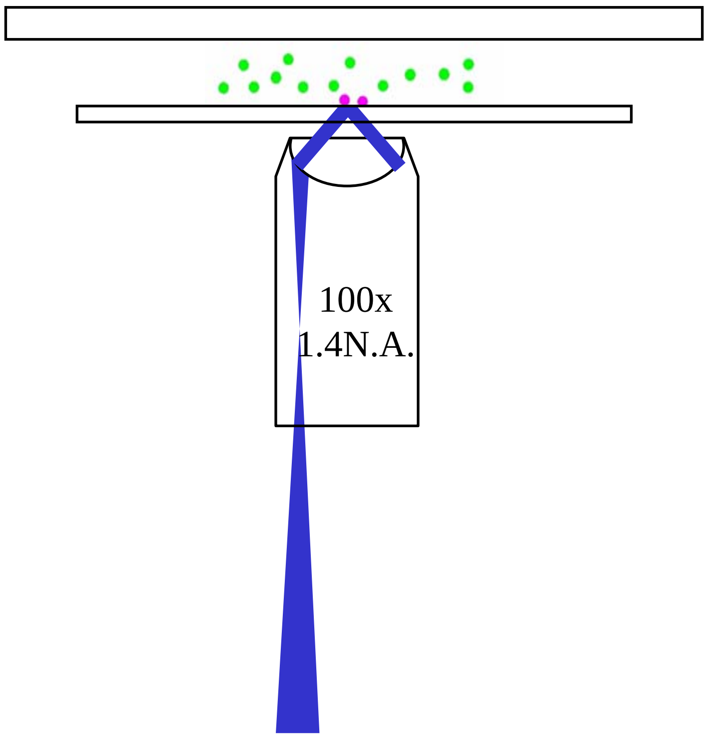


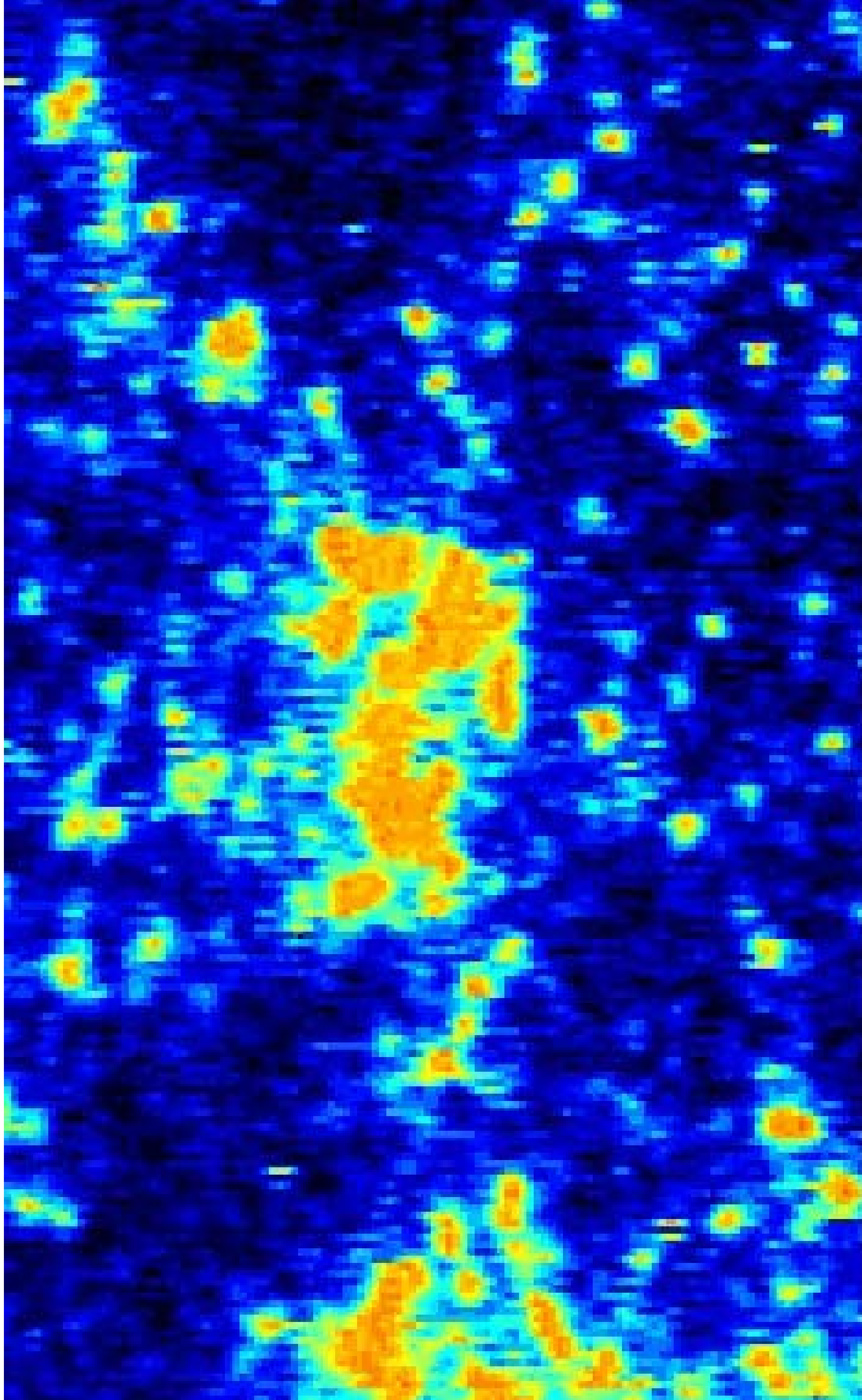

(Berg *et al., J. Cell Science,* **113**:3439-3451)

# TIRF Microscopy

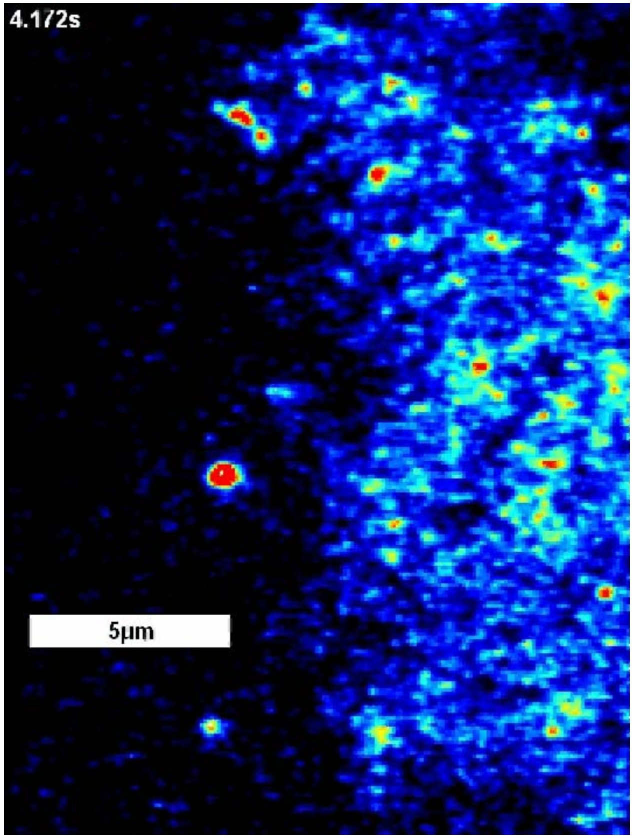


# TIRF

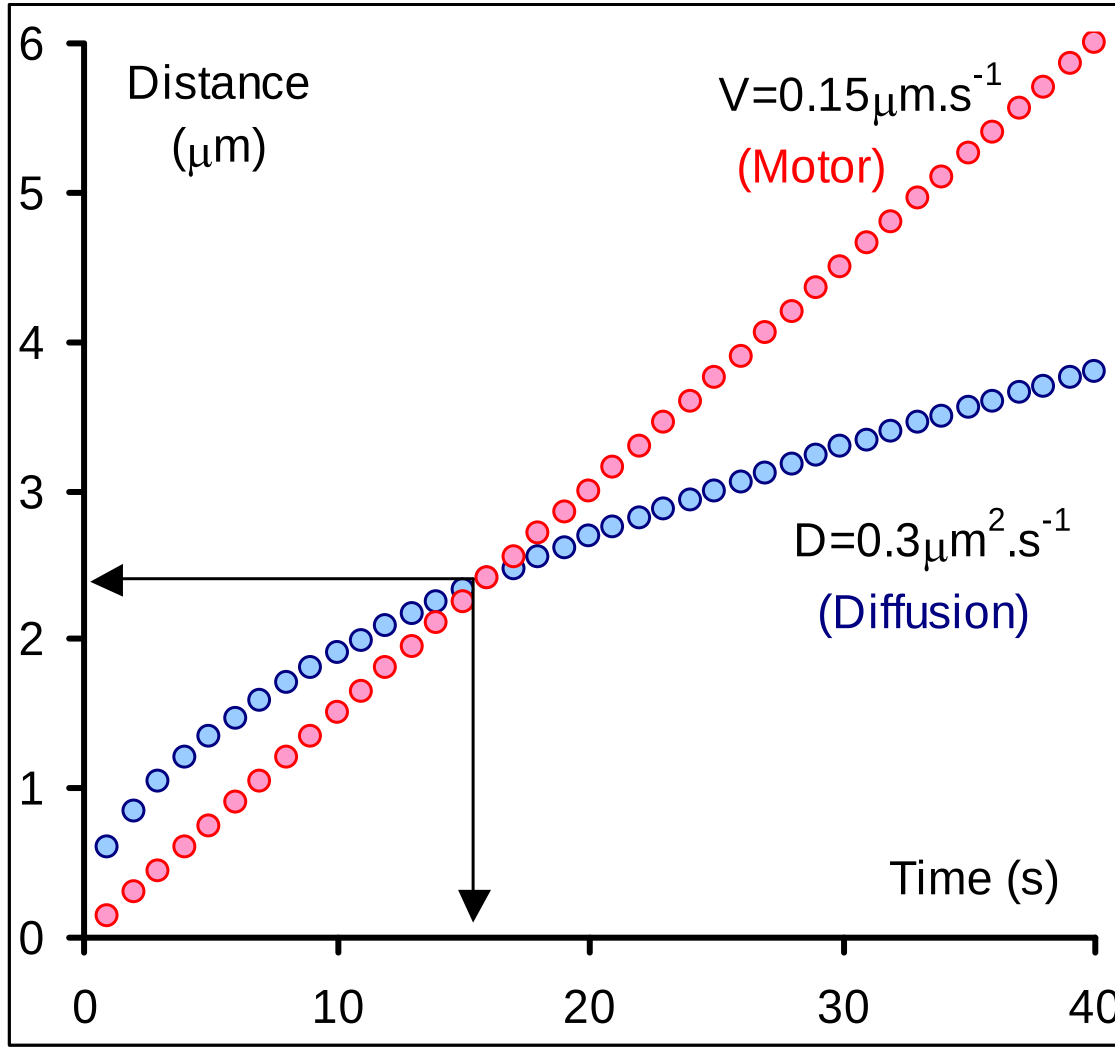


# Motor Properties:



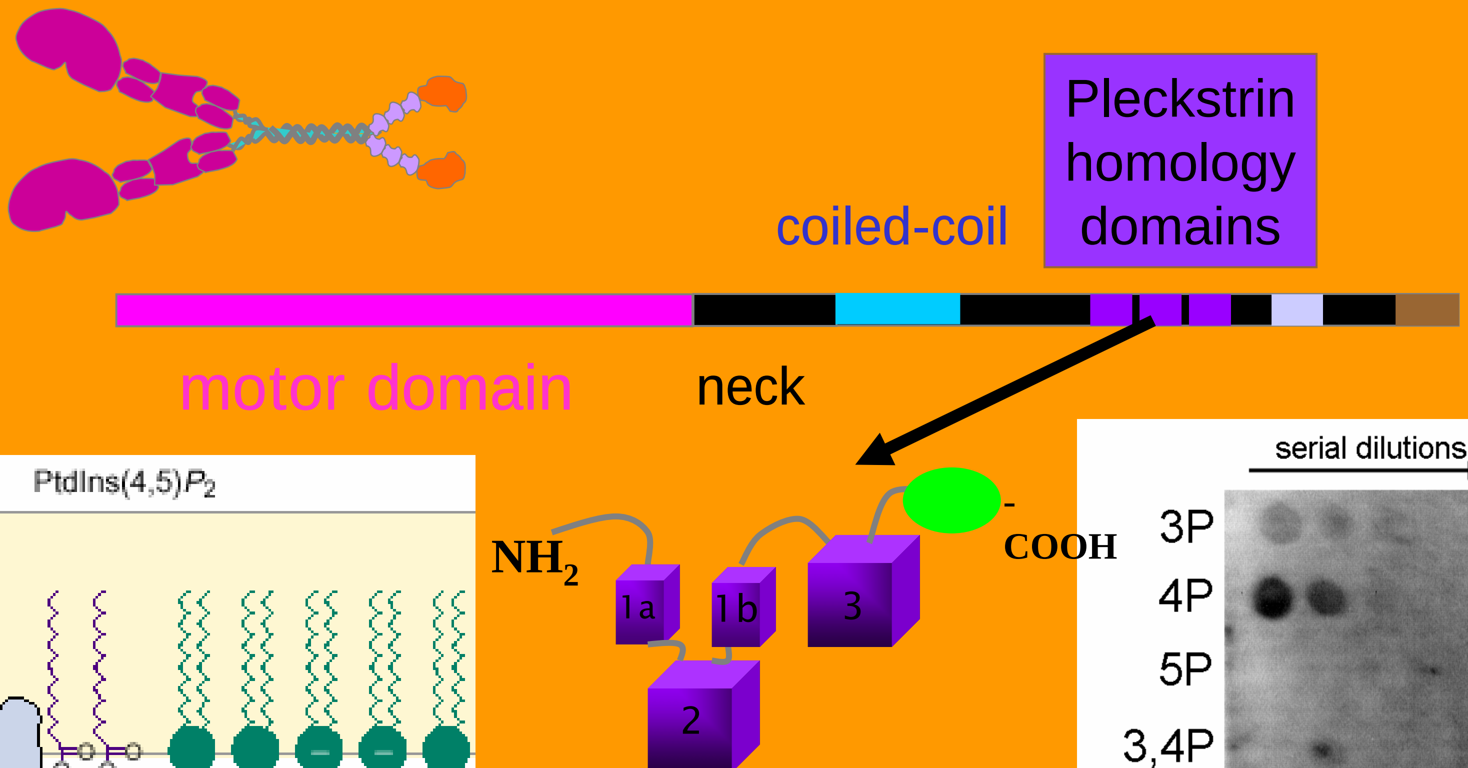

GFP-Myosin X expresssed in human endothelial cells

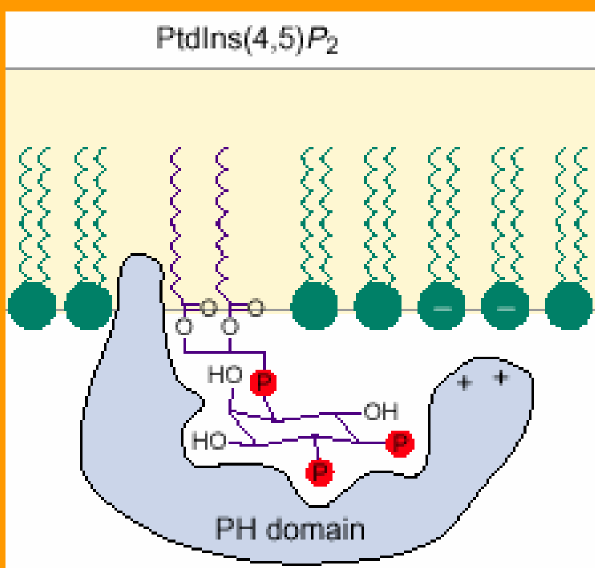



In vitro motility assay sliding velocity = 125nm $s^{-1}$

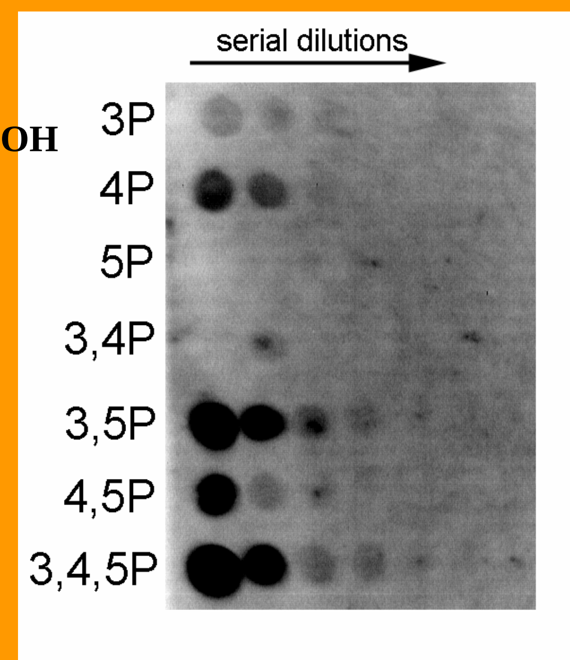
Myosin X coated vesicles move at ~ 140nm $s^{-1}$

# Myosin X has several membrane targeting domains in its tail.
# We have studied the PH domains.

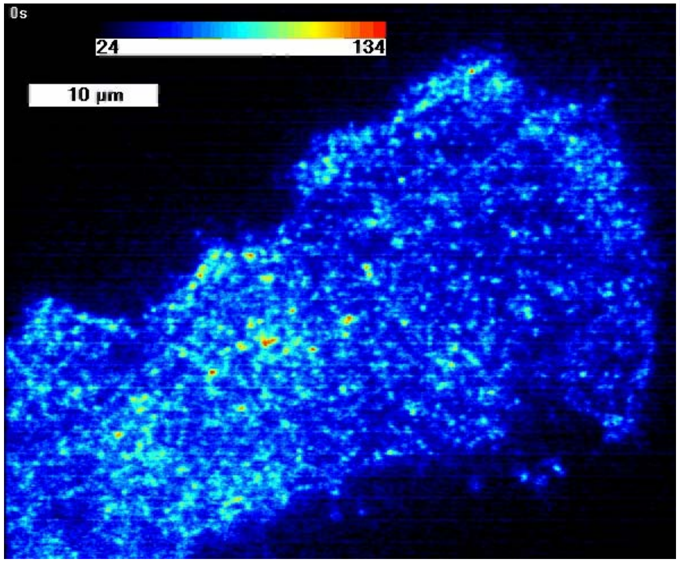


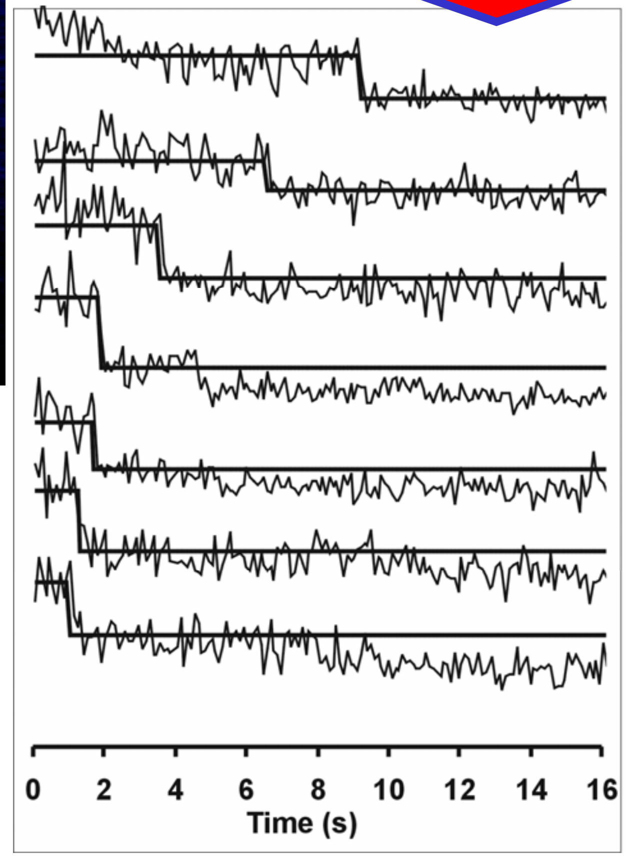



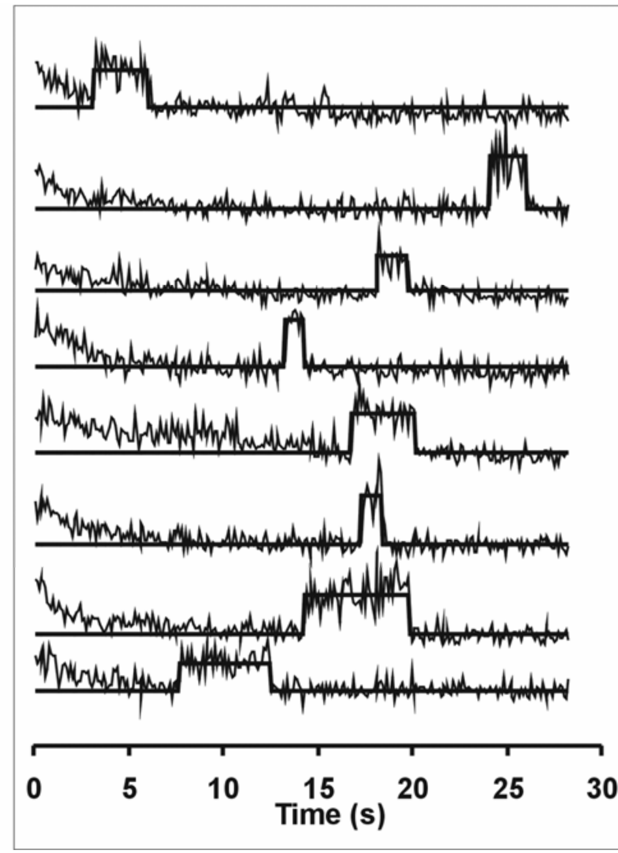

Balla *et al.* (2000) *TiPS*:**21**:238-241

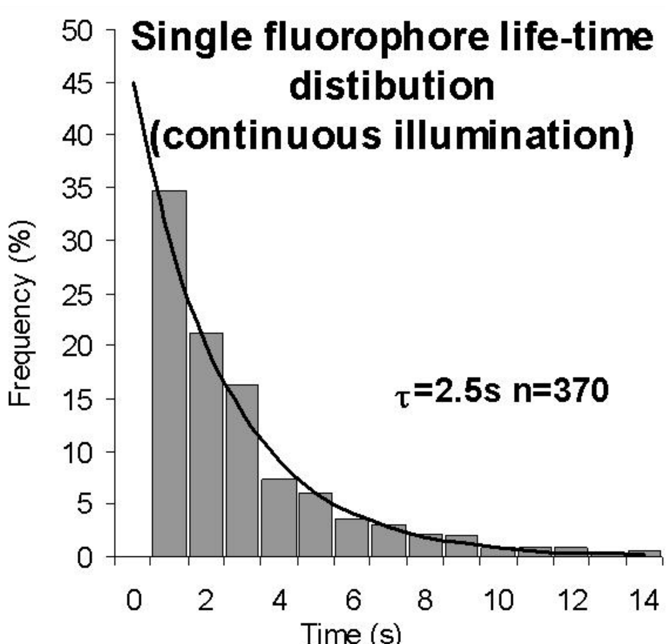


# Single fluorophore temporal trajectories



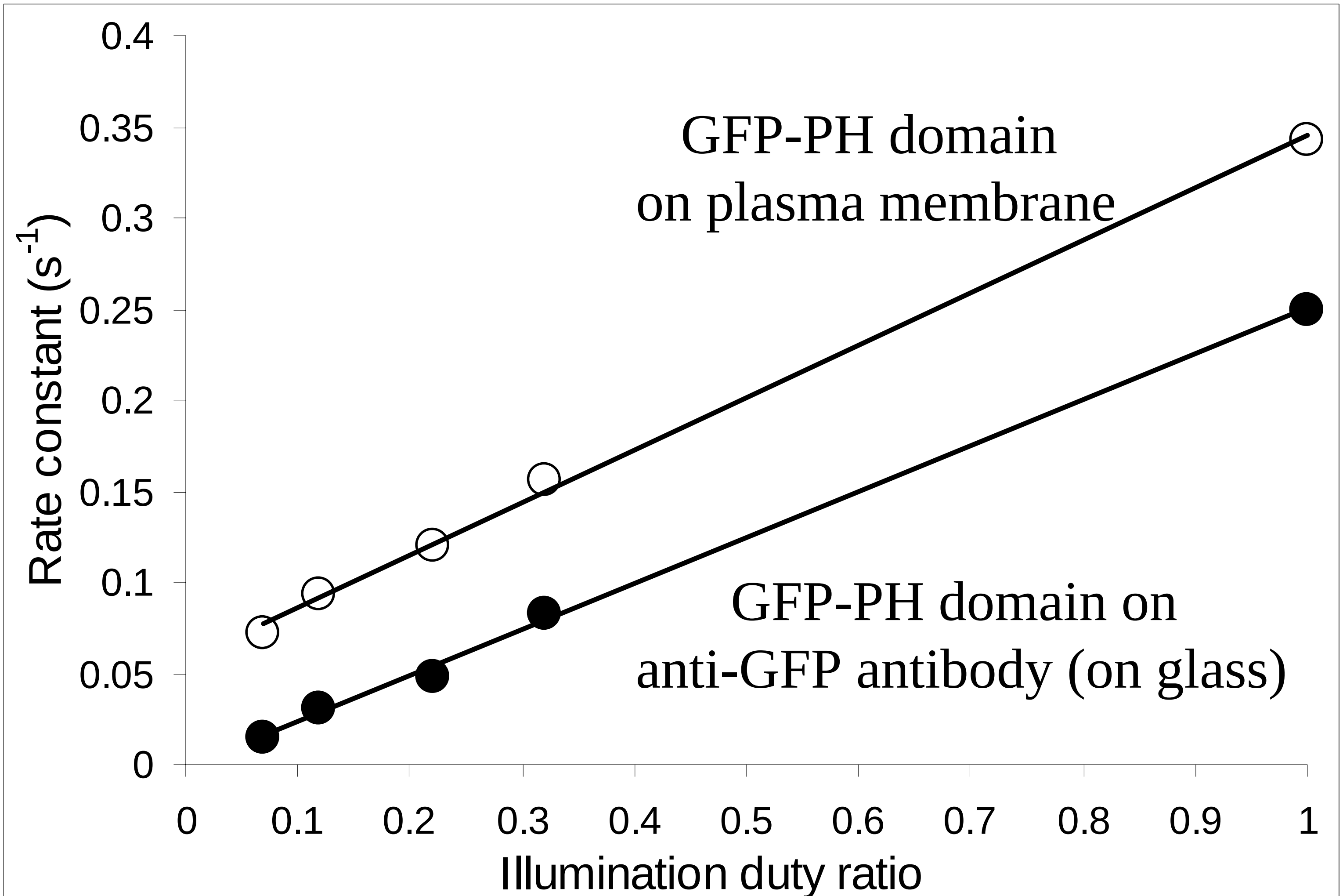



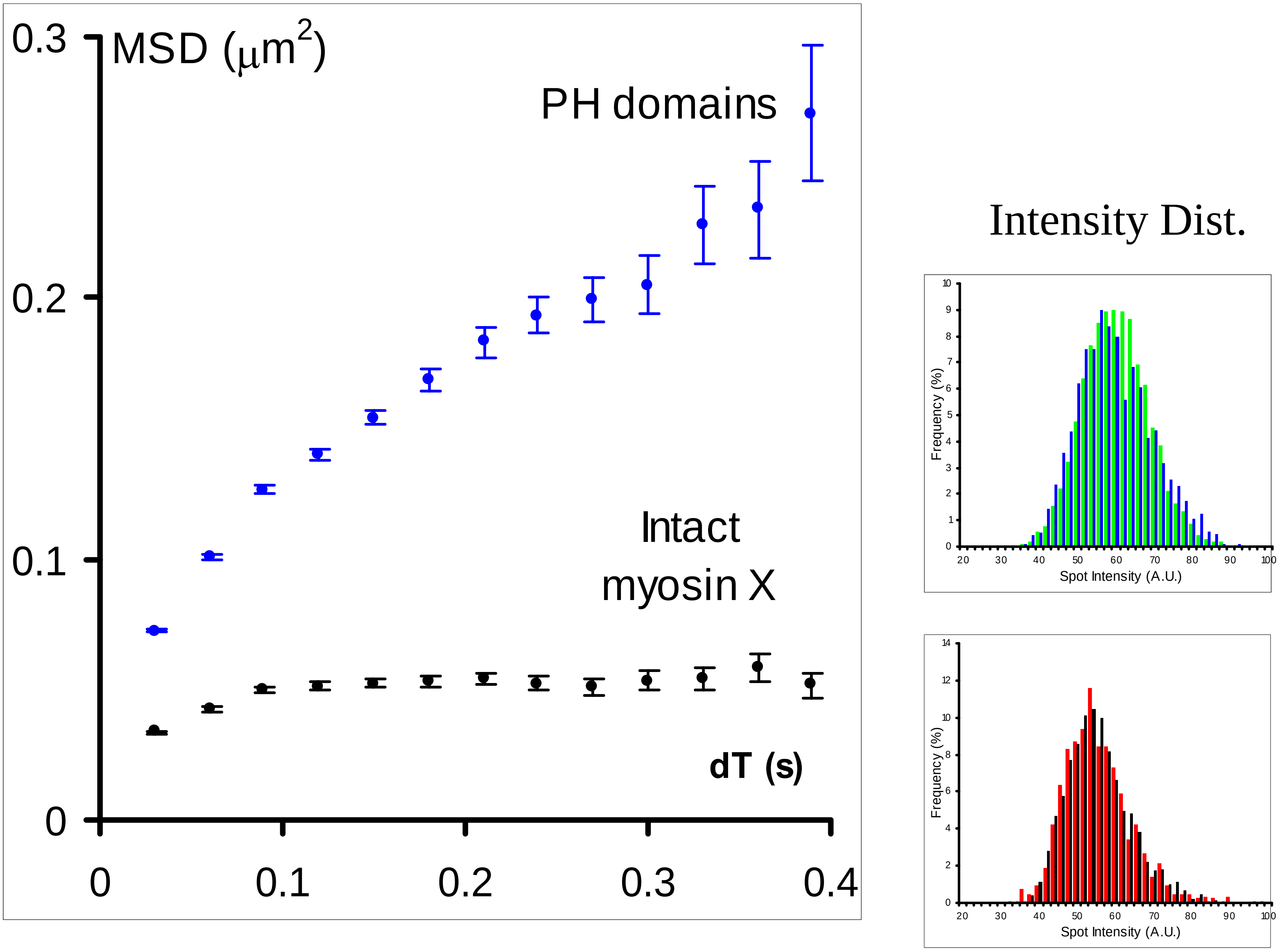




**NOTE: BLINKERS NOT SHOWN!**

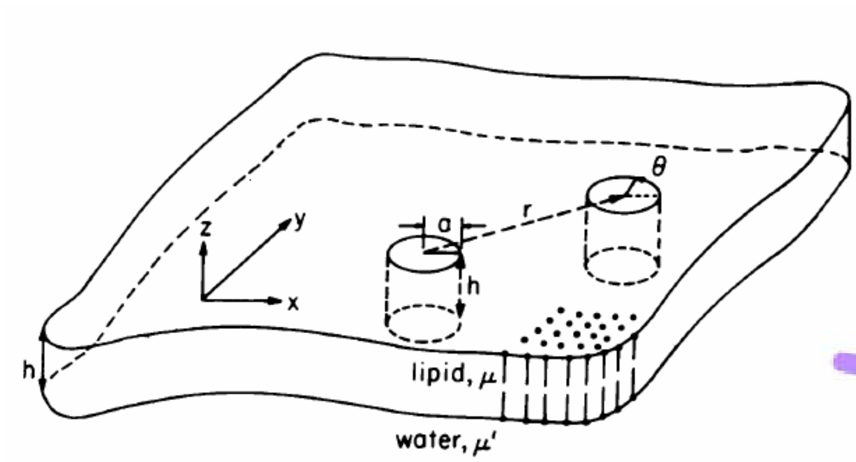
# Calculation of PH123 Dissociation Rate



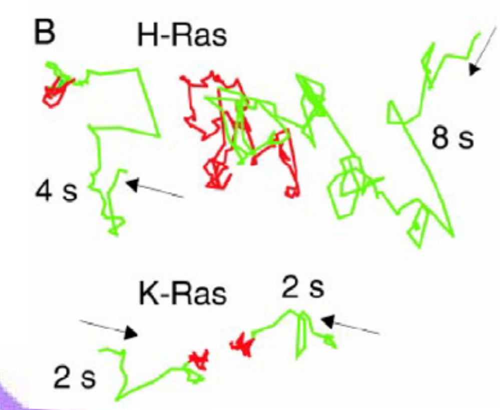

(average laser power)

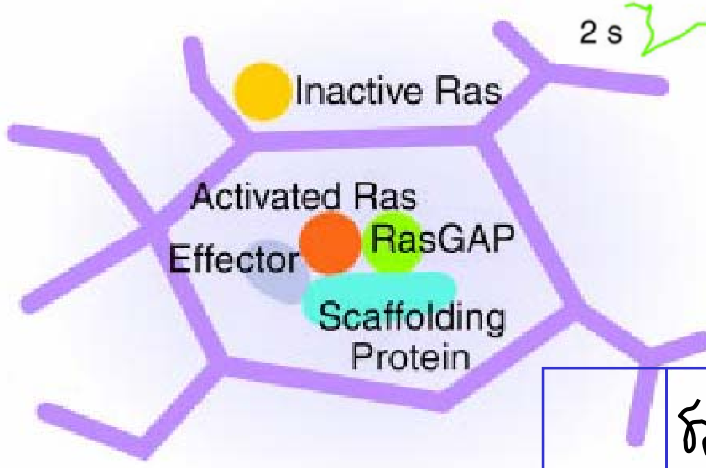

MSD (μm²)
PH domains
Intact
myosin X
dT (s)
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
Intensity Dist.
Frequency (%)
Spot Intensity (A.U.)
Frequency (%)
Spot Intensity (A.U.)

# Diffusion in membrane

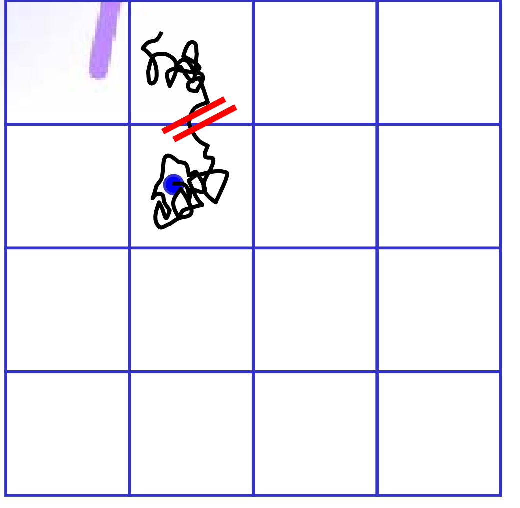



Saffman & Delbruck
PNAS, 1975

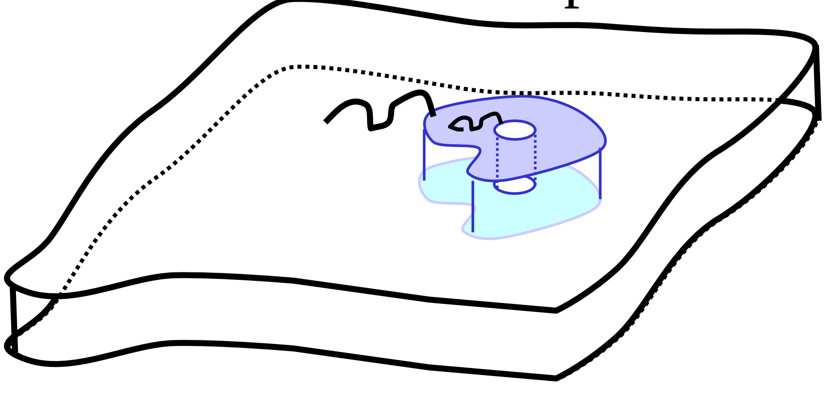


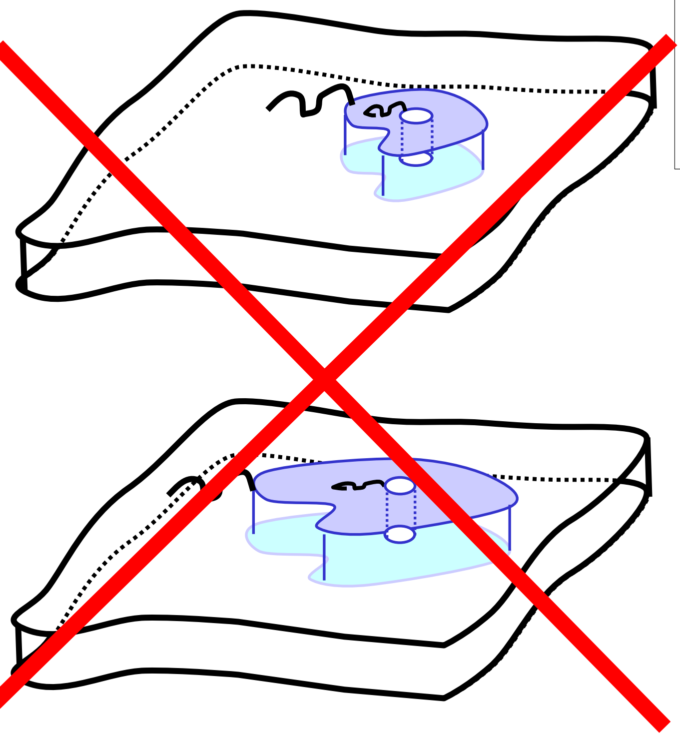

Membrane Fence
(energy barrier)

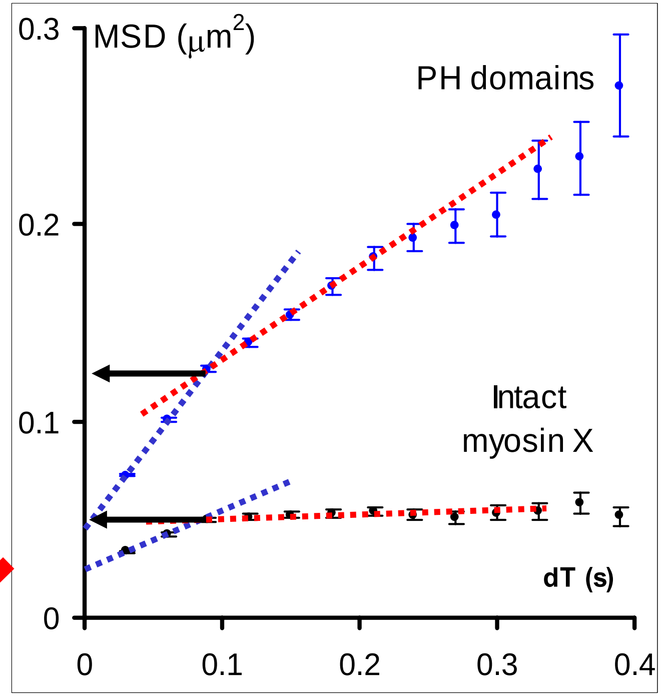


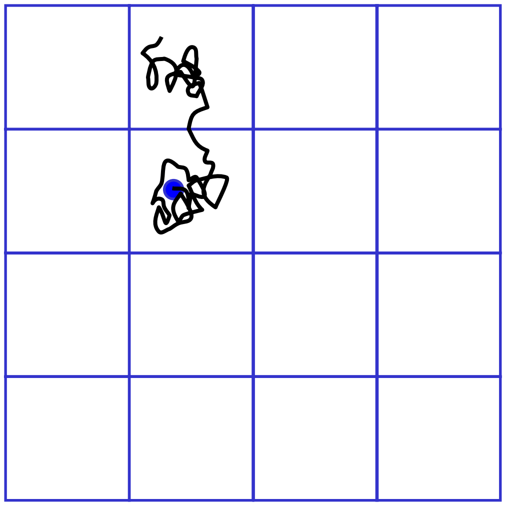
Lipid raft



Murakoshi .. Kusumi
PNAS, 2004

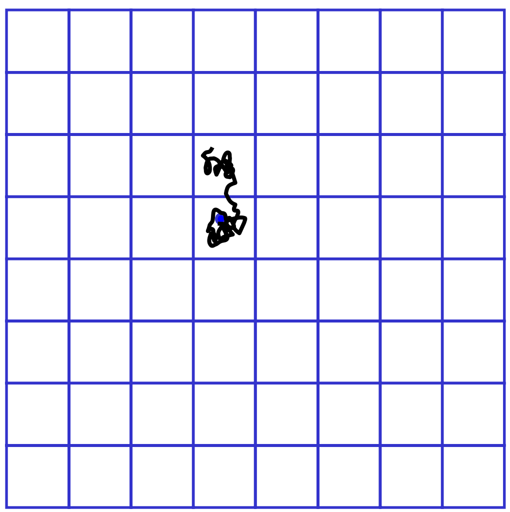




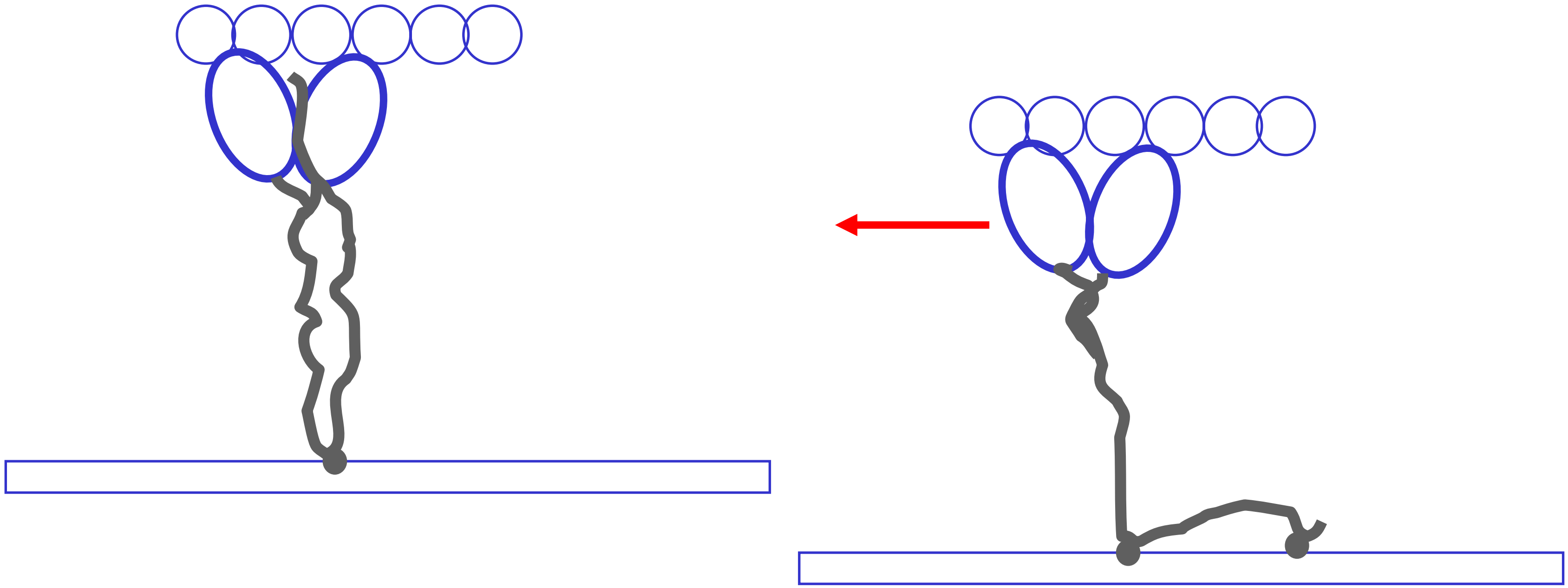
Cell size ~ 8nm

Cell size ~ 15nm



# Myosin X - On/Off switch

- Our model is that myosin X is switched off by its tail domain binding to the head when it is inappropriately localised.



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# Optical tweezers – single molecule mechanics