LAMELIS ADVANCED SUMMER SCHOOL

“OPTICAL TWEEZERS”

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HUNGARIAN GOVERNMENT

European Union
European Social Fund

INVESTING IN YOUR FUTURE

Lecture Plan:

- Motivation for high-resolution mechanical studies in biology:
  "SINGLE MOLECULE STUDIES"

- What are optical tweezers and how do they work?

- Mechanical properties of optical tweezers and energy change in biological reactions:
  picoNewton forces and nanometre movements
  energy $< 10k_bT$

- Motor proteins (kinesin & myosin) as model systems for single molecule studies.

- Other laser-based single molecule methods e.g. TIRF microscopy.

Why work with individual molecules?

- Single molecule experiments can give unequivocal information about how enzymes work and can provide new insights into enzyme mechanism.
- Sequential steps that make up biochemical pathways can be observed directly. The chemical trajectory of an individual enzyme can be followed in space and time.
- There is no need to synchronise a population in order to study the biochemical kinetics
- Single molecule data sets can be treated in a wide variety of ways – e.g. can specifically look for heterogeneity in behaviour (ie strain dependence of rate constants, effects of membrane structure, etc).

Single molecule experiments:

Energy levels in biology :

1 Photon	= 400 pN.nm
1 ATP	= 100 pN.nm
1 Ion moving across a membrane	= 10 pN.nm
Thermal energy ($k_b T$)	= 4 pN.nm
($k_b T = RT/A$)	

{ $1\ \text{pN.nm} = 1 \times 10^{-21}$ Joules }

SINGLE MOLECULE TECHNOLOGIES:

PATCH CLAMP:

A single ion channel admits >5000 ions when it opens and the resulting current flow (picoAmps) can be measured relatively easily using a high input-impedance transistor.

(Energy $\equiv$ 50,000 pN.nm = 12,000 k_bT)

FLUORESCENCE MICROSCOPY:

A single fluorophore emits >10,000 photons per second and can be imaged quite easily using modern cameras (and seen by the naked eye).

(Energy $\equiv$ 1×10^6 pN.nm/video frame = 250,000 k_bT)

Mechanical Studies have no "built-in" gain

SINGLE MOLECULE MECHANICS:

Molecule motors consume 1 ATP molecule for each step taken.

(Energy ≡ 50 pN.nm = 10 k_bT (i.e. very challenging))

Optical Tweezers

Protein-Protein

Protein-Ligand interactions

Magnetic Tweezers

DNA topology

DNA-protein interactions

AFM

Protein (un)folding

Protein-Protein & Protein-Ligand interactions

Optical Force:

$$E = mC^2$$

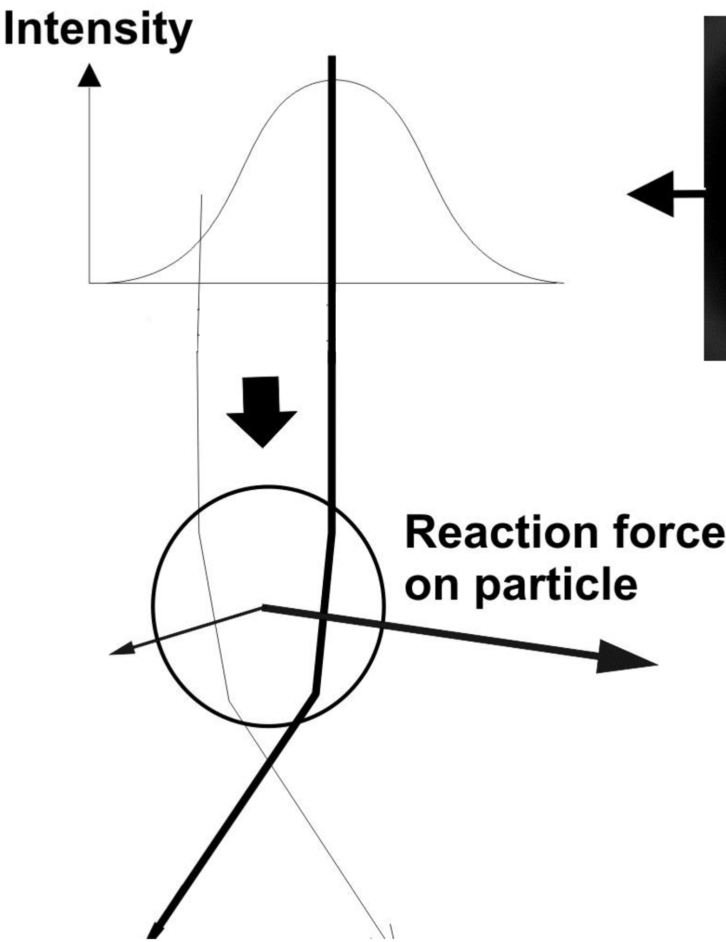
$$\text{Momentum, } mC = E/C$$

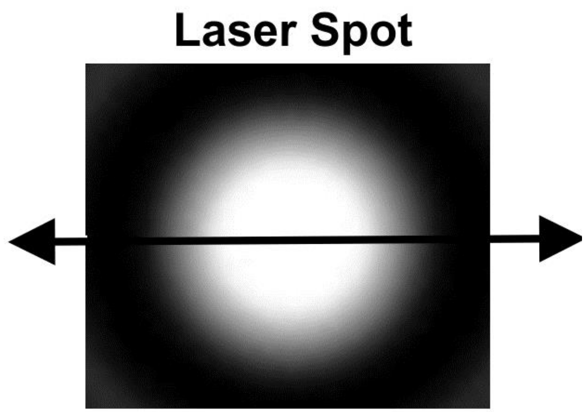
$$\text{Force} = mC/t = \mathbf{P/C}$$

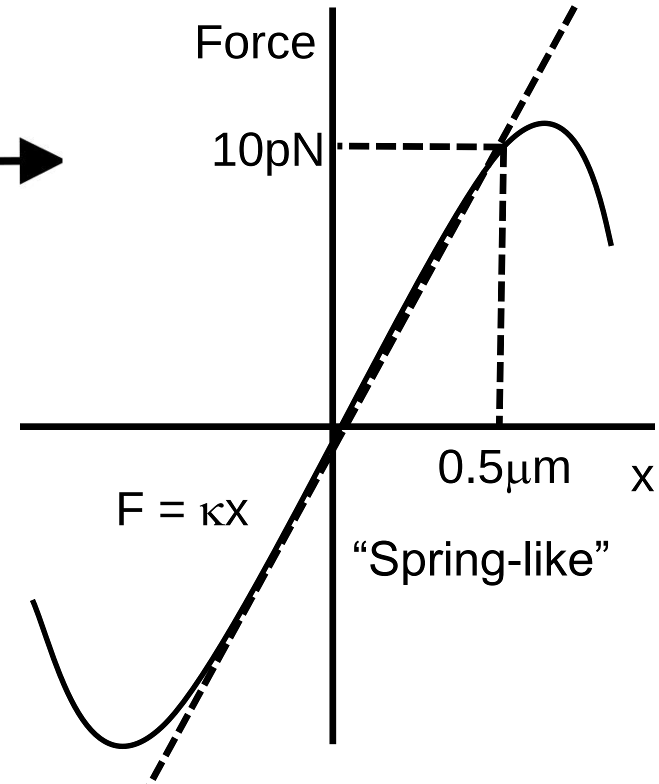
(P = optical power)

….calculate the force produced by a 3mW laser pointer….

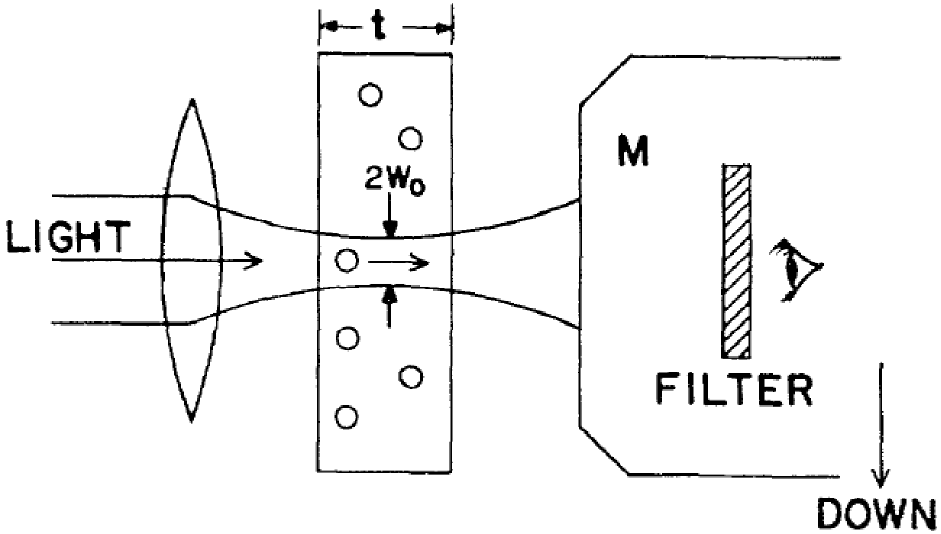
Laser beam has Gaussian intensity profile.
Restoring force is proportional to displacement



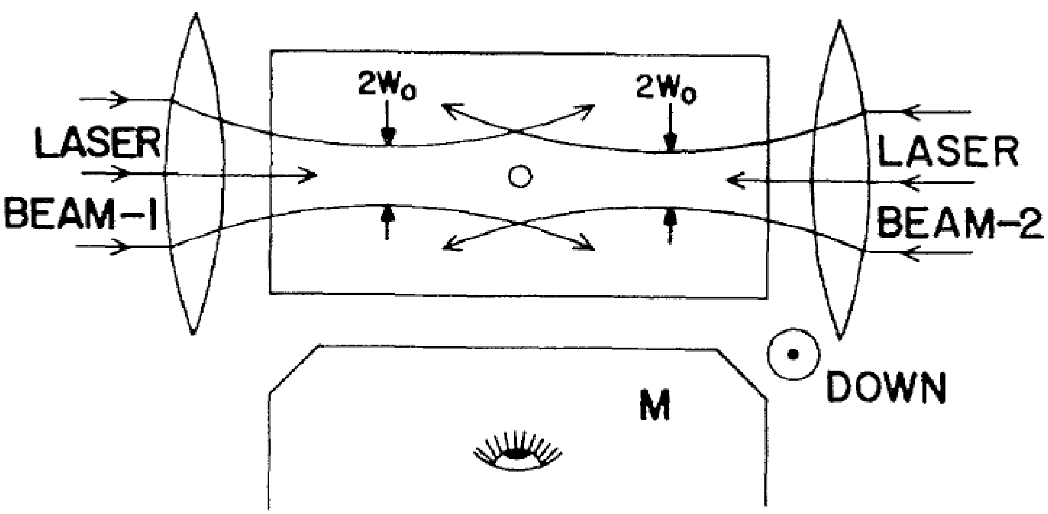




Stiffness, κ = 0.02 pN.nm^{-1}
~10x softer than protein



(a)



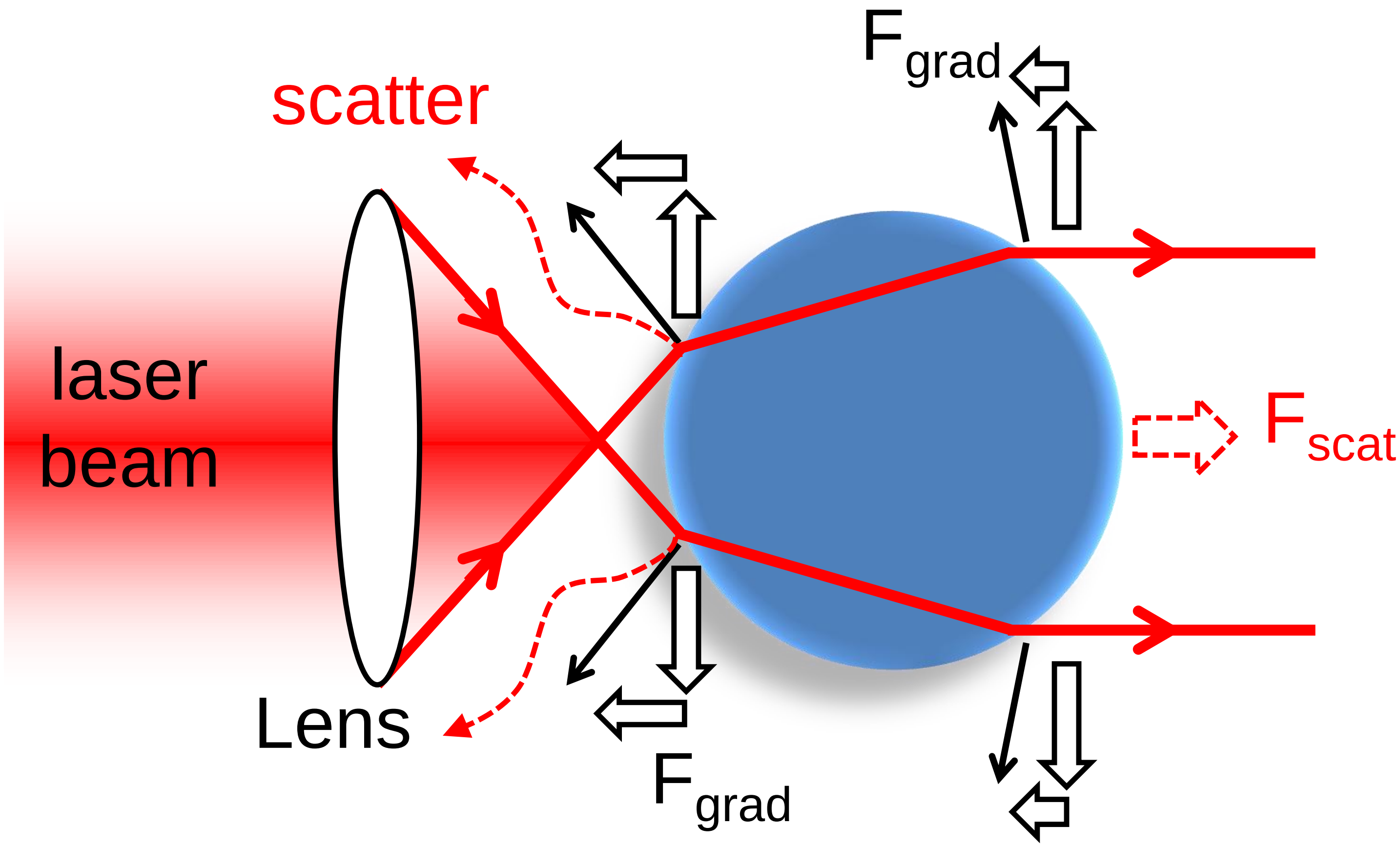
(b)

3-D trap using
counter-propagating
laser beams

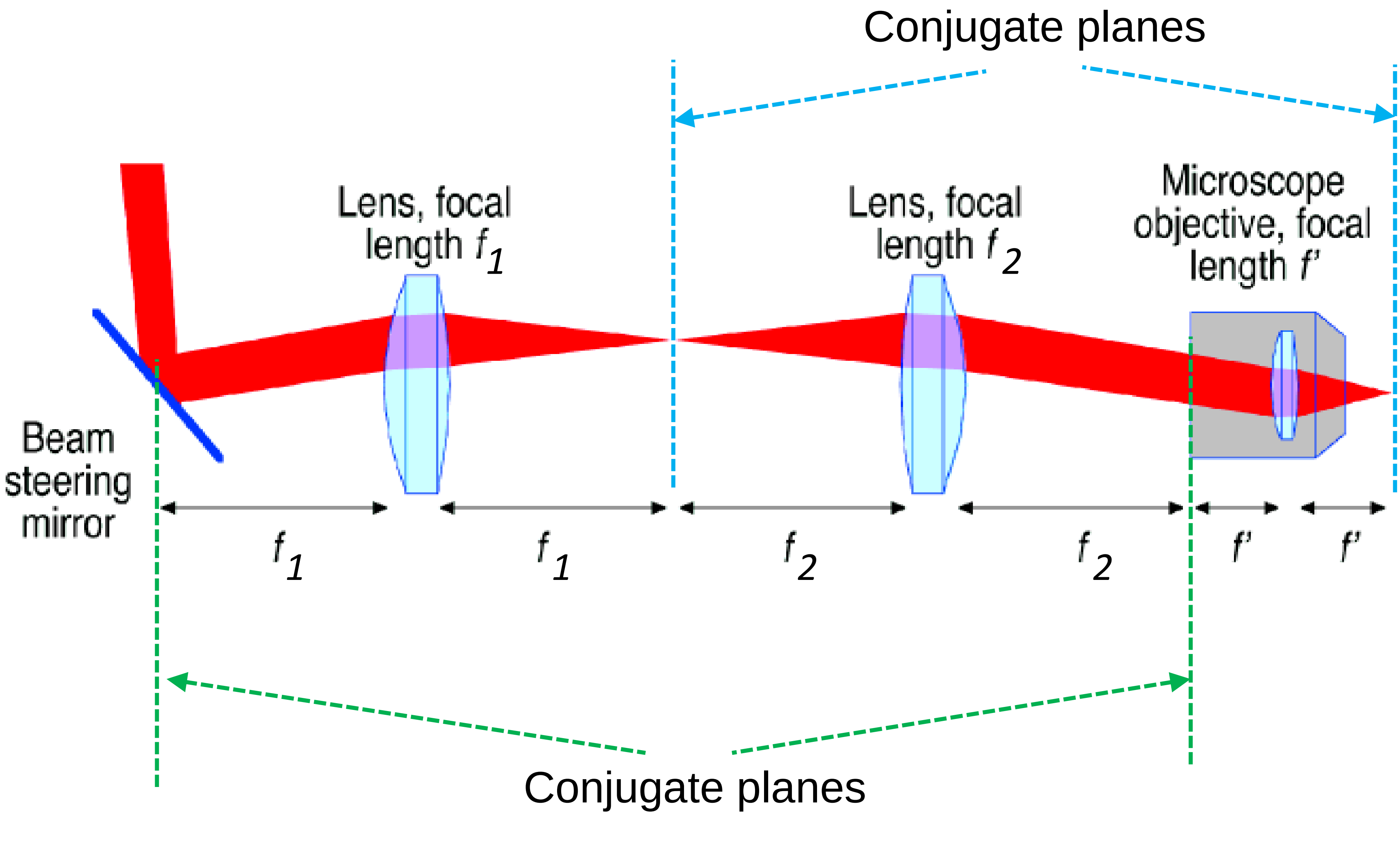
Ashkin & Dziedzic, 1971

Single beam "gradient trap"

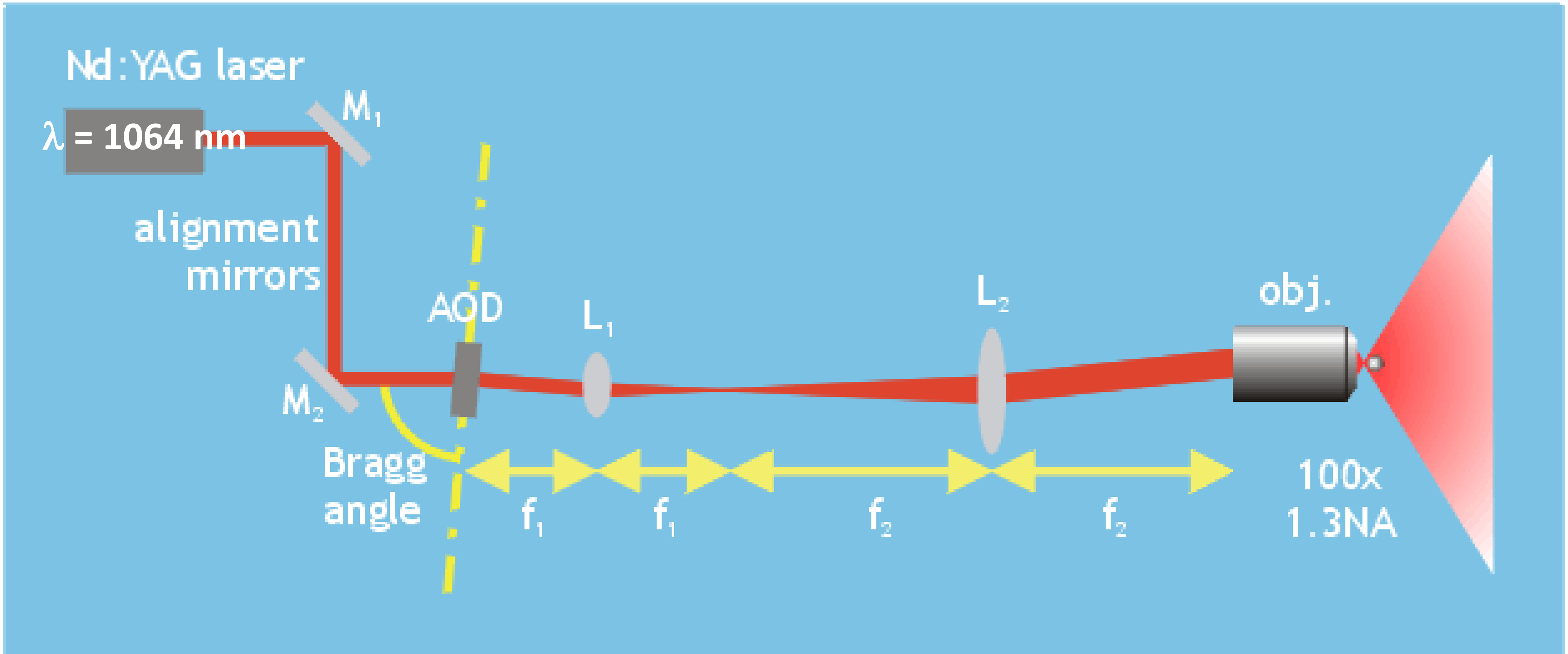
Ashkin *et al.* 1986

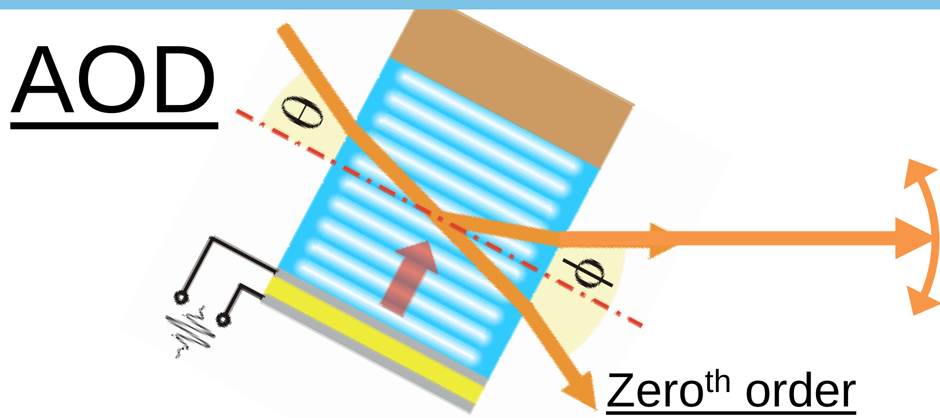


Optical "Trap" when: $\sum F_{grad} = \sum F_{scat}$



Molloy & Padgett (2002) *Contemporary Physics* **43:**241-258





First order:

$\sin(\phi) = (\lambda/d) = 1.064\ \mu m / 15\ \mu m$

ϕ = 75 mRads

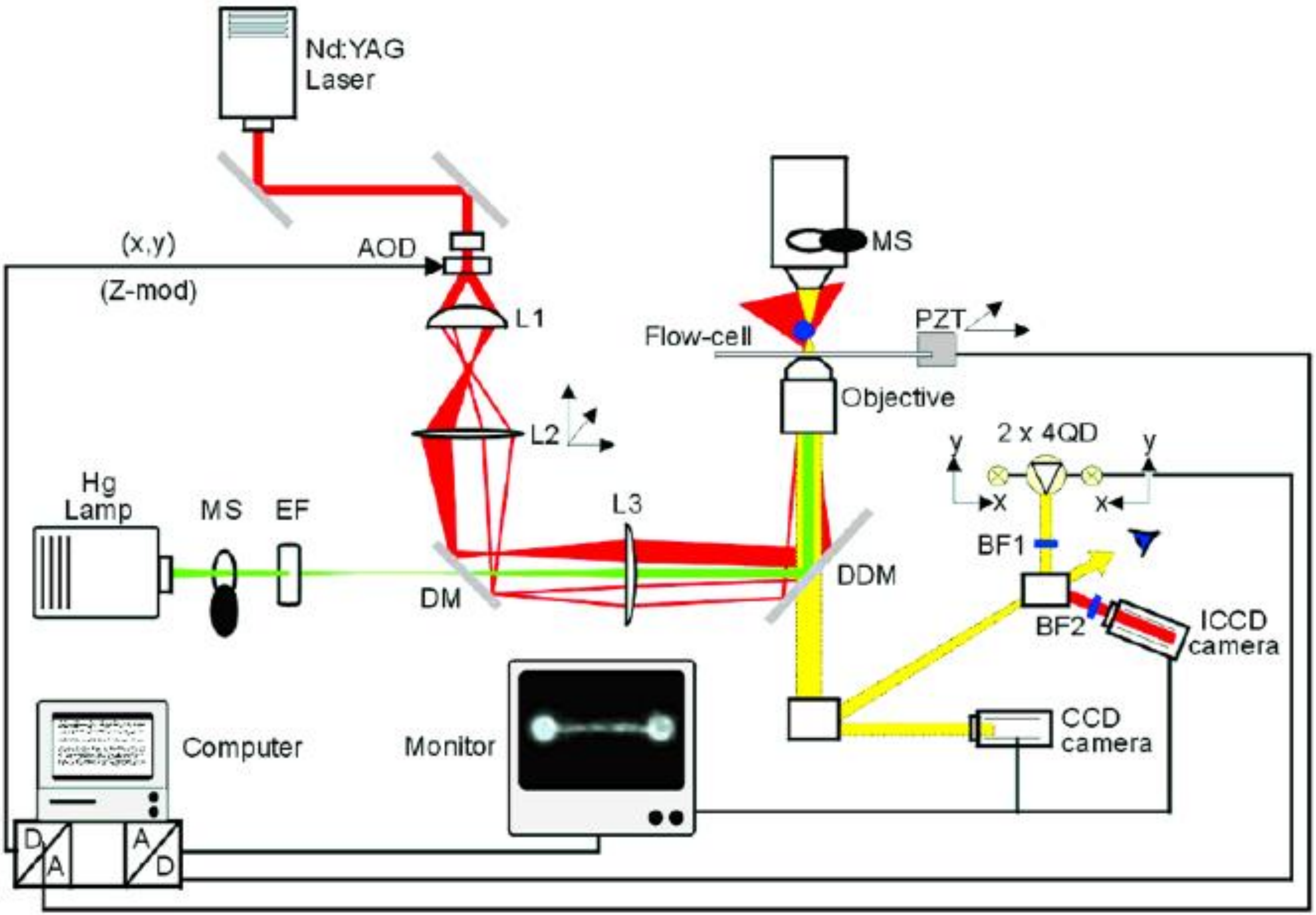
$\Delta\phi$ (+/- 10%) ~ 15 mRads

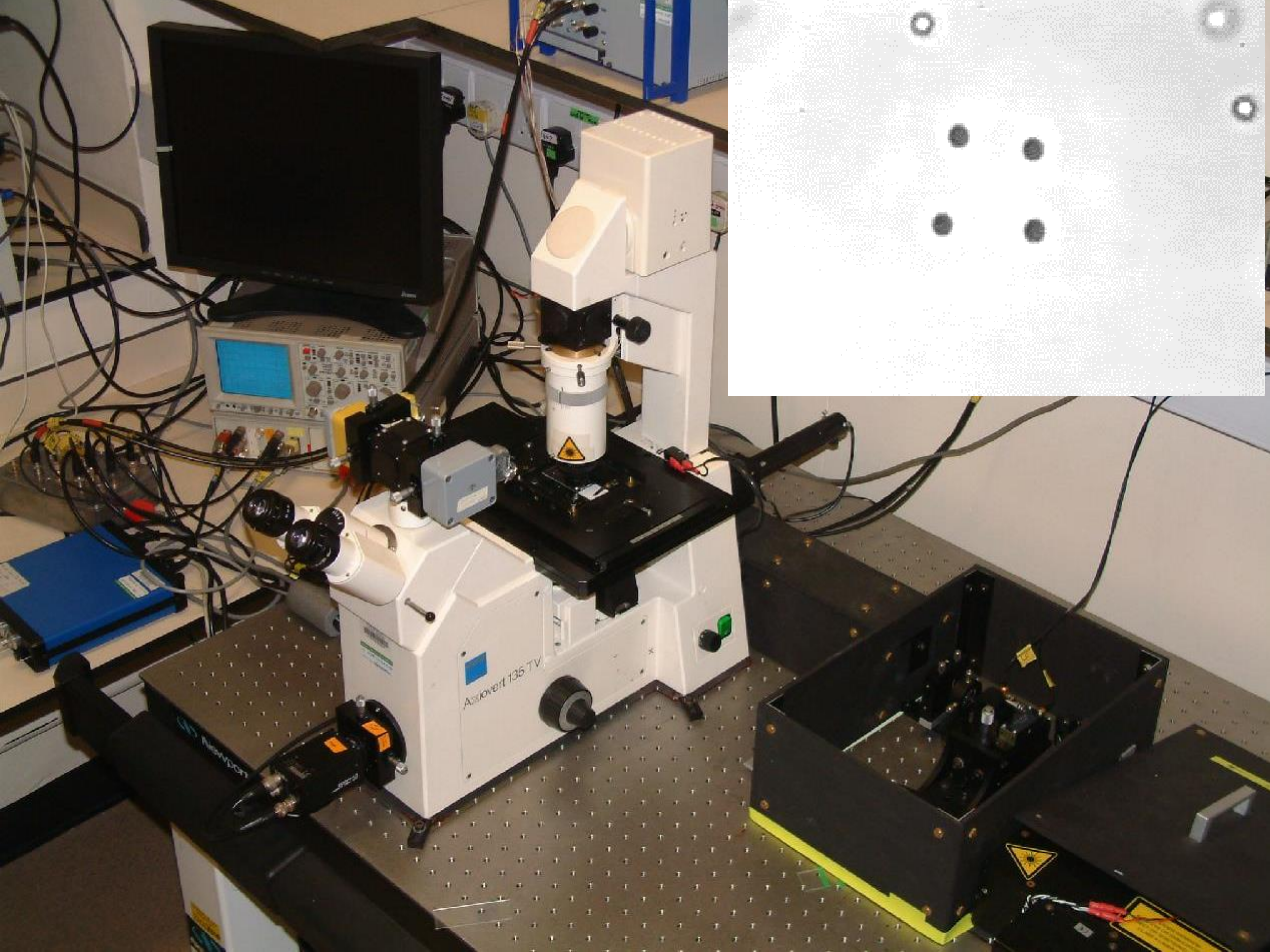
travelling acoustic wave

acoustic velocity (TeO_2) = 800 $m.s^{-1}$

freq. ~ 50 MHz (spacing, d ~15 μm)

Realistically, it's a bit more complicated

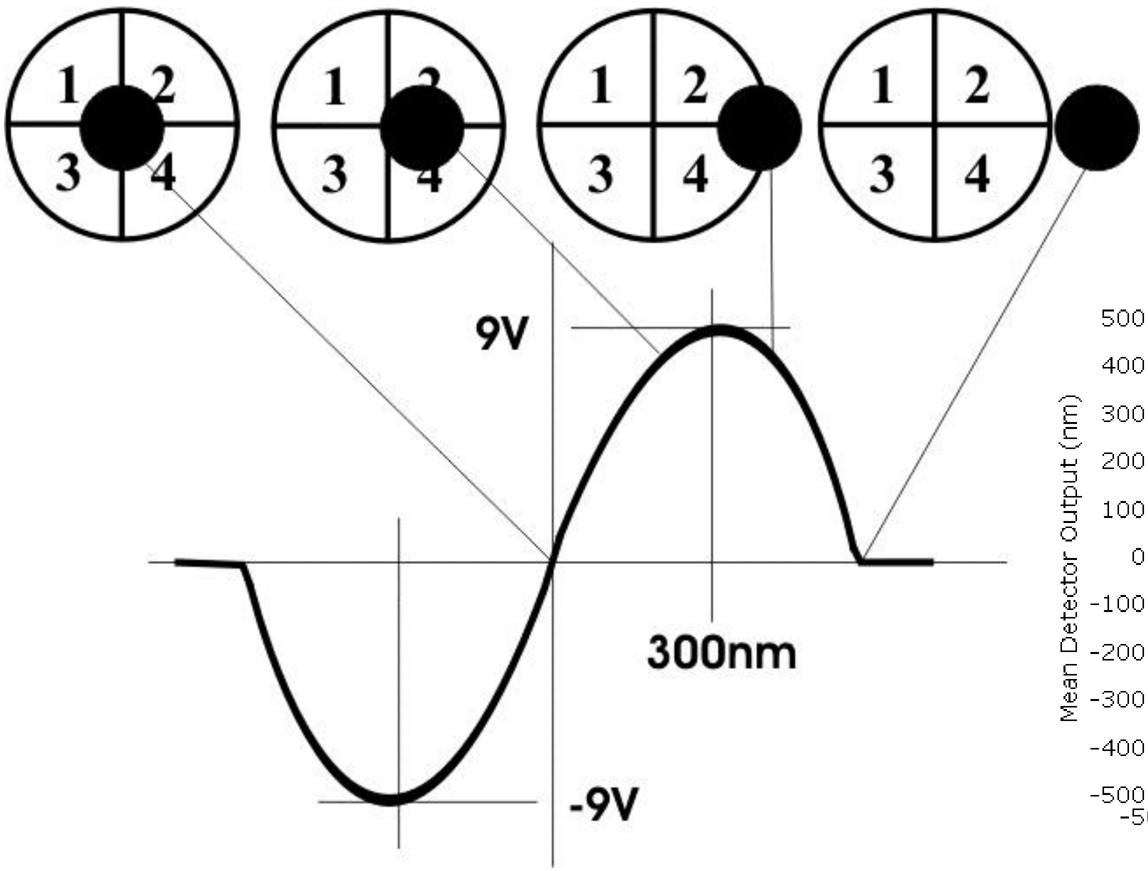


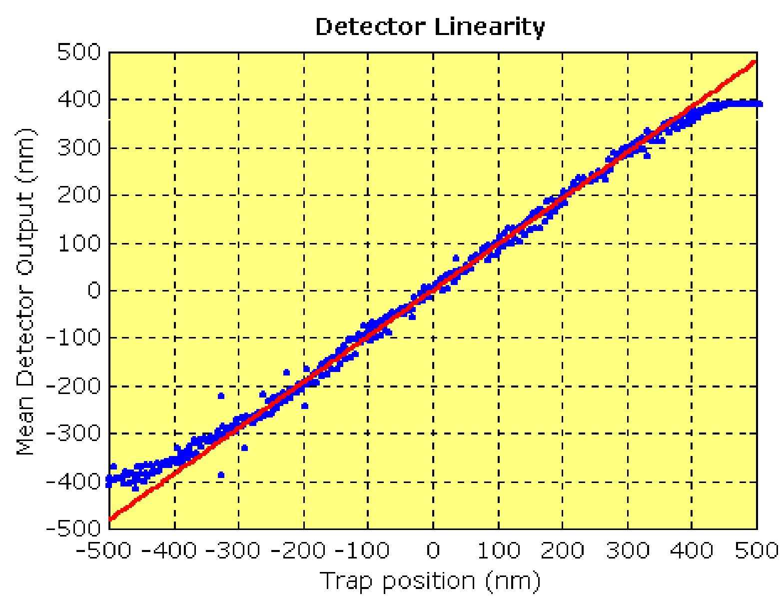

Axiovert 135 TV

In order to measure forces we need to measure the position of the optically trapped particle with very good accuracy (<1 nm) and good time resolution (>1 kHz).

Most instruments use 4-quadrant photodiodes.

However, modern (inexpensive) CMOS cameras operate at 5kHz (over a limited field of view) and are now commonly used to “centroid” the bead image.





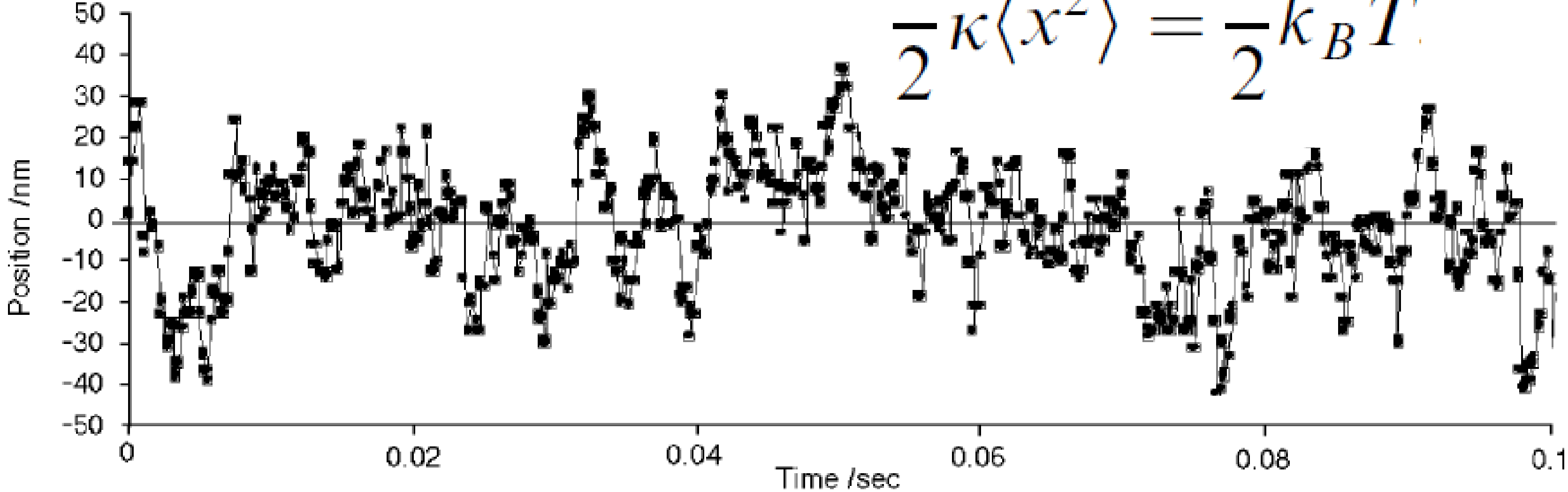
Super-resolution imaging
Super-resolution mechanics

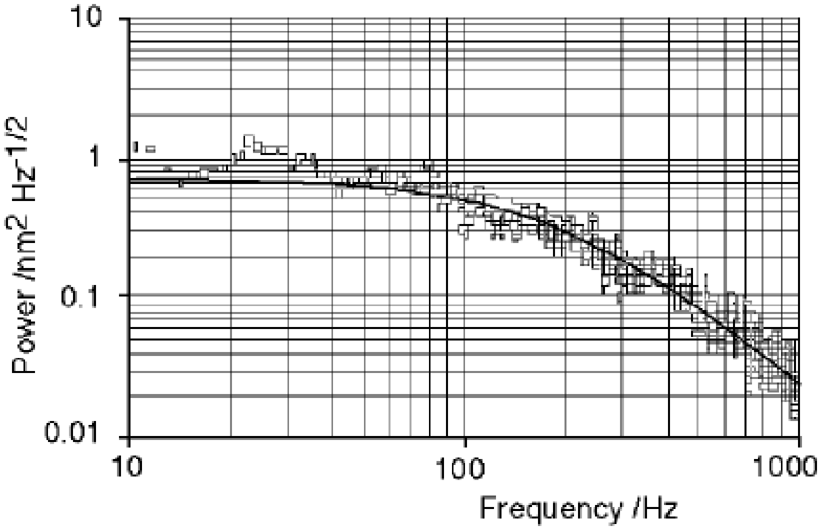
Super-resolution optical imaging requires statistical analysis of the spatial distribution of photons arising from a diffraction-limited source. We need to characterise the spatial autocorrelation function (the “Airey disc”)

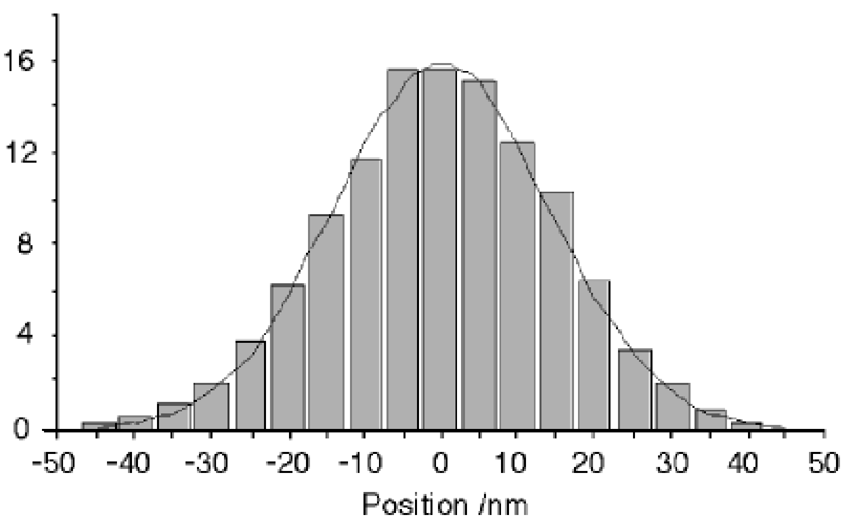
Super-resolution mechanics requires statistical analysis of the temporal distribution of forces and movements arising from a thermal-noise-limited source. We need to understand the temporal autocorrelation function (the “power density spectrum”)

Thermal motion of an optically trapped particle

$$\frac{1}{2}\kappa\langle x^2\rangle = \frac{1}{2}k_B T$$

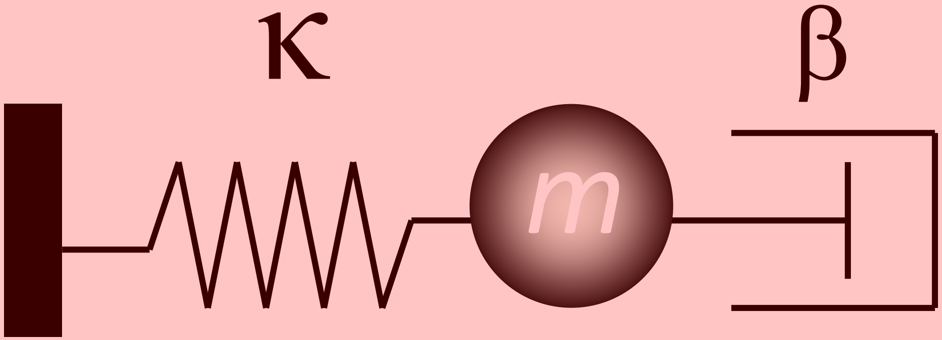






Thermal noise is ~ 14 nm r.m.s.

Dynamic response



$$m\frac{\delta^2 x}{\delta t^2} + \beta\frac{\delta x}{\delta t} + \kappa\delta x = 0$$

Stoke’s drag

$$\beta = 6\pi\eta r$$

$$f_{res} = \frac{1}{2\pi}\sqrt{\frac{\kappa}{m}}$$ > 50 kHz

$$f_c = \frac{\kappa}{2\pi\beta}$$ < 1 kHz

Typical values:

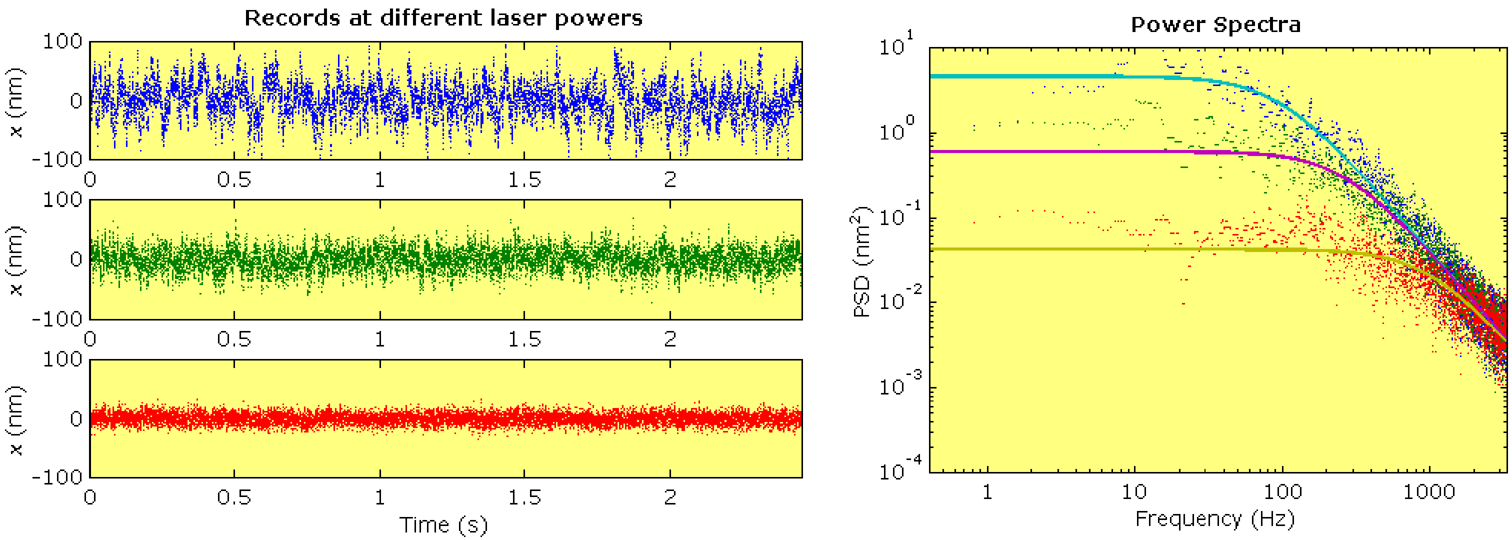
m = 5×10^{-16} kg

β = 1×10^{-8} N.s.m^{-1}

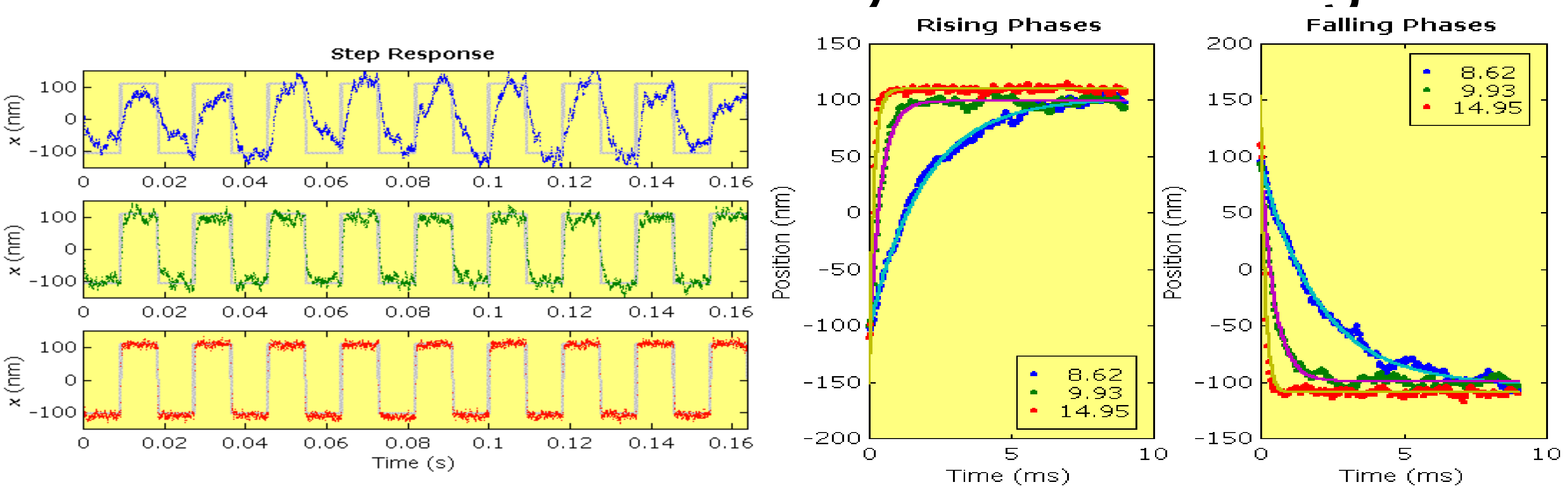
K ~ 1×10^{-5} N.m^{-1}

Calibration of optical trap stiffness

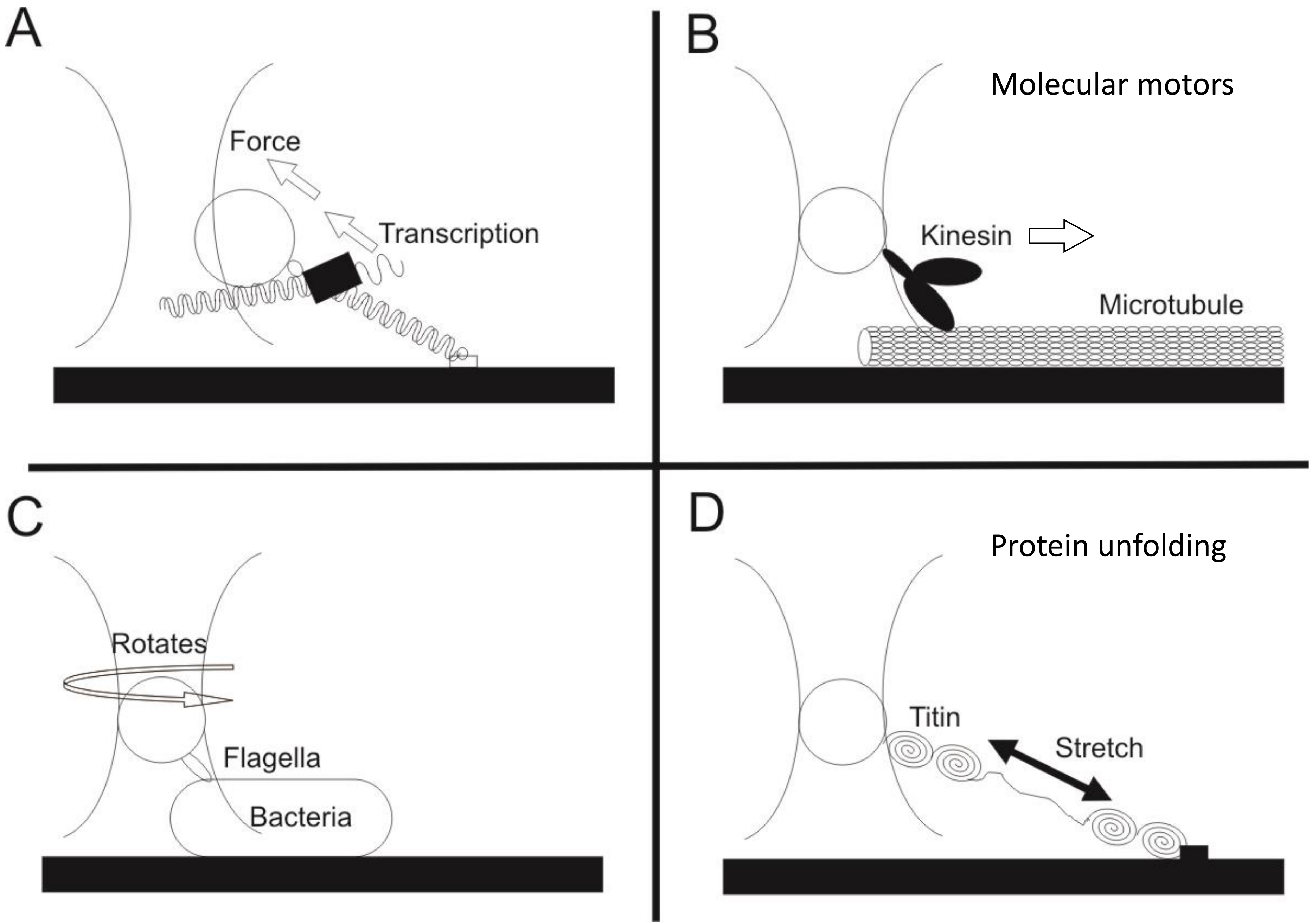
1) Brownian motion



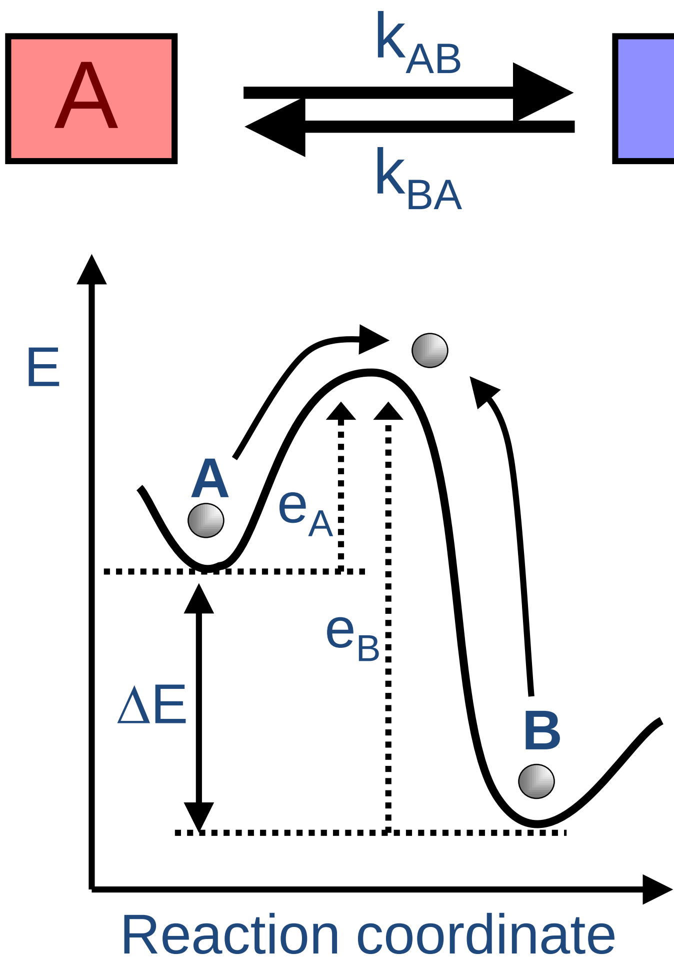
2) Bead deflection caused by viscous drag



Optical tweezers can be used to measure forces in biology



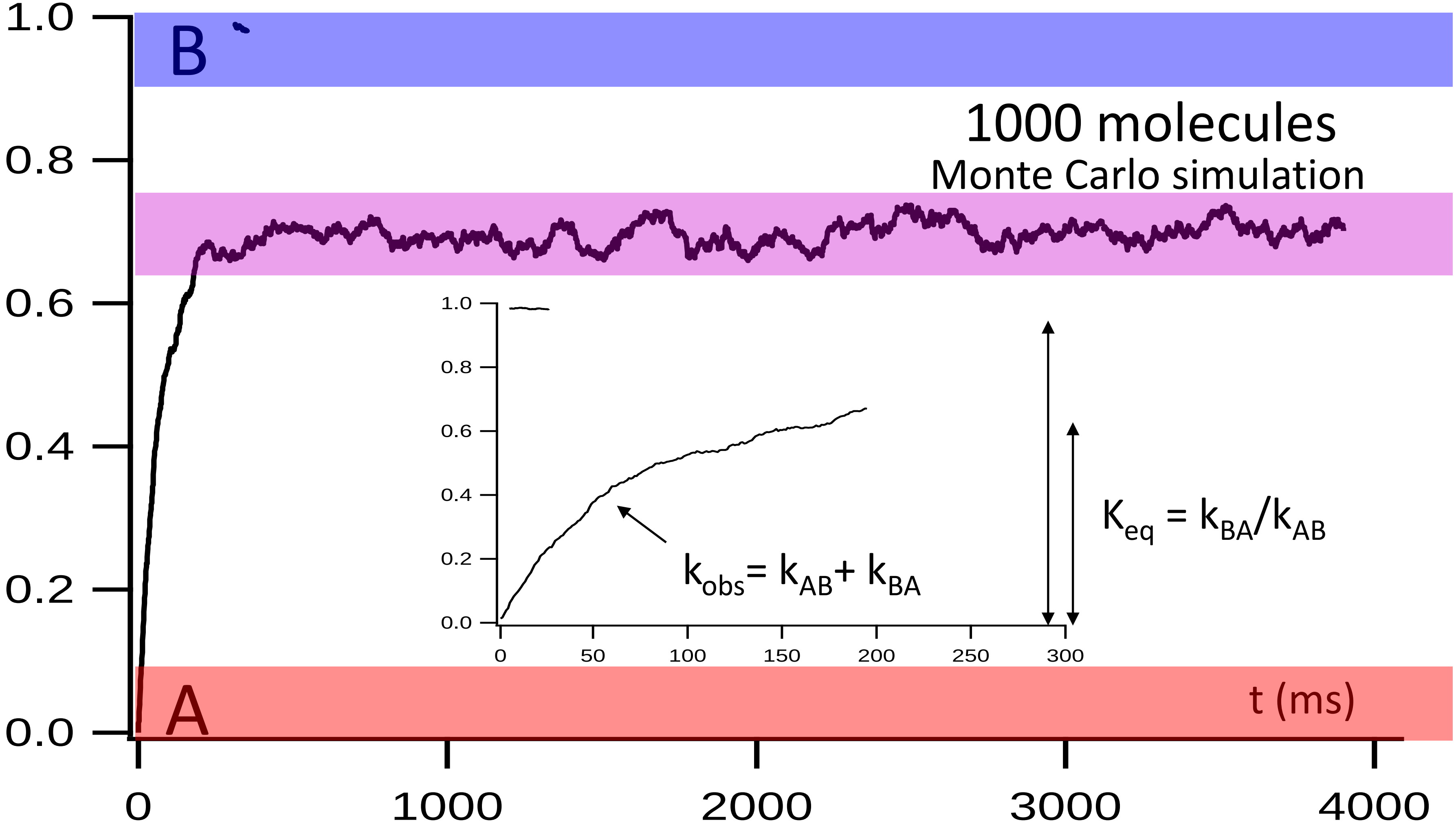
SINGLE MOLECULE DATA SETS

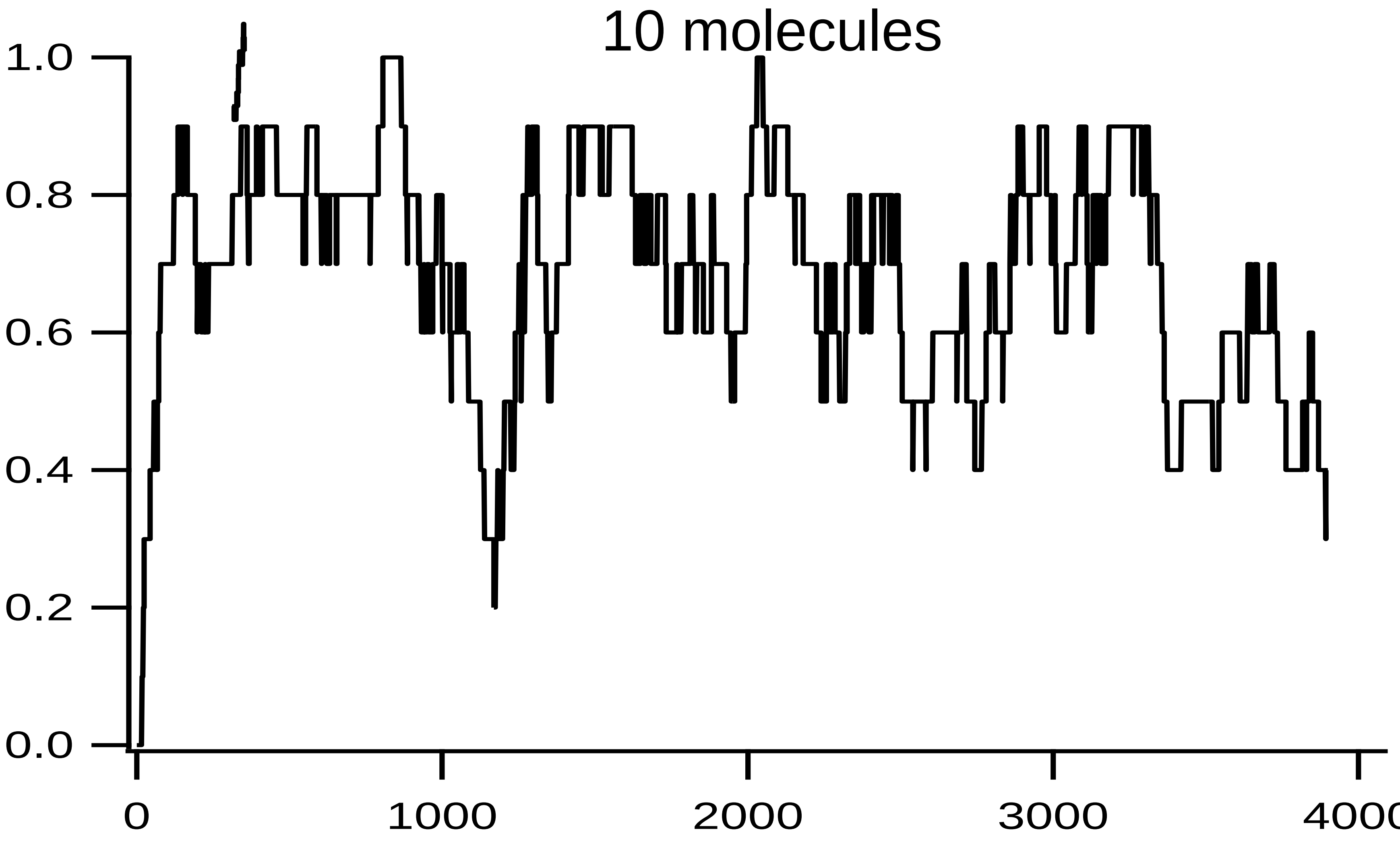


Transition state theory describes the kinetic properties of the system

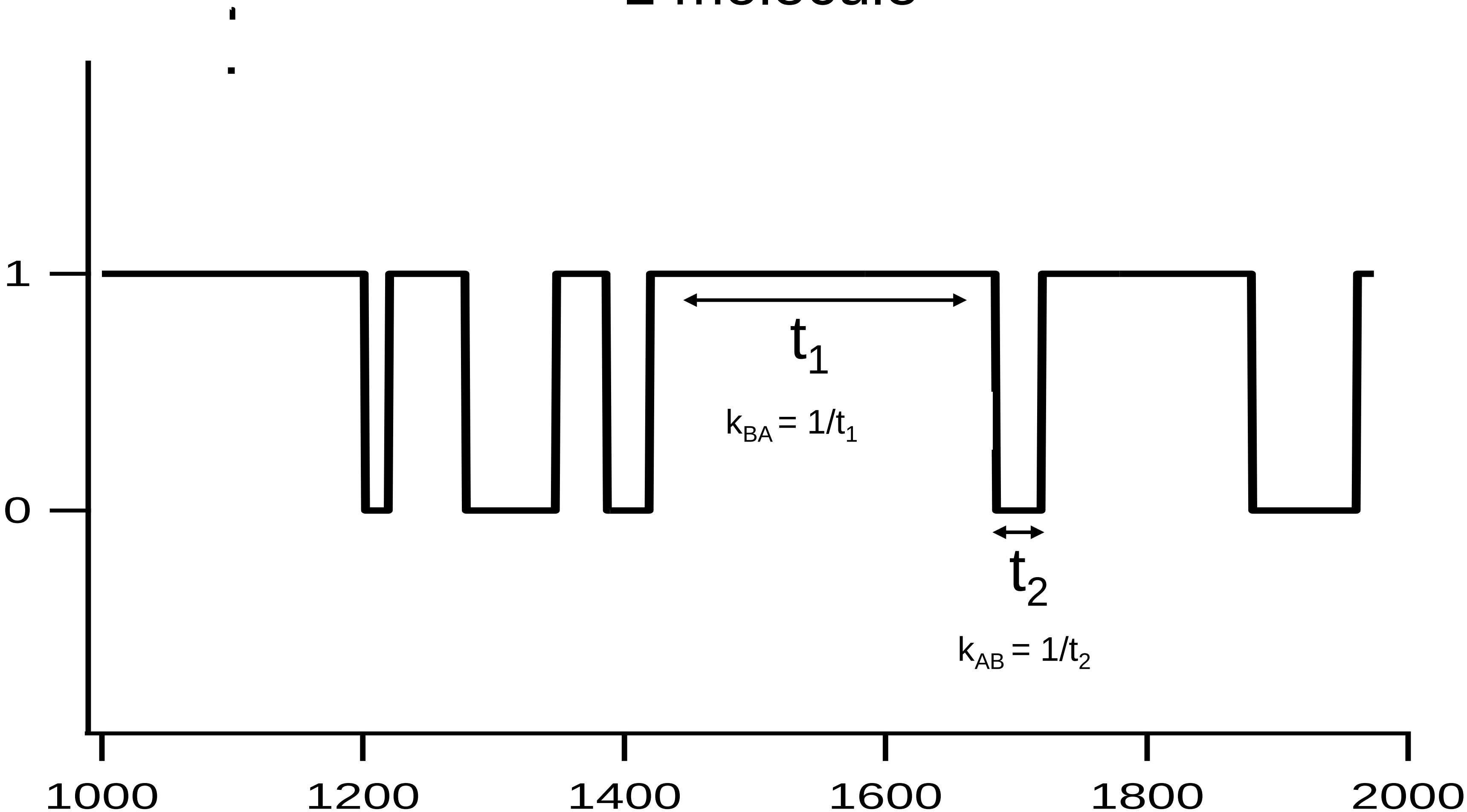
$$k_{AB} \propto e^{\frac{-e_A}{k_b T}} \qquad k_{BA} \propto e^{\frac{-e_B}{k_b T}}$$

$$K = \frac{k_{AB}}{k_{BA}} = e^{\frac{-(e_B - e_A)}{k_b T}} = e^{\frac{-\Delta E}{k_b T}}$$


1000 molecules
Monte Carlo simulation
B
A
t (ms)
$k_{obs} = k_{AB} + k_{BA}$
$K_{eq} = k_{BA}/k_{AB}$
1.0
0.8
0.6
0.4
0.2
0.0
0
1000
2000
3000
4000
0
50
100
150
200
250
300


10 molecules
1.0
0.8
0.6
0.4
0.2
0.0
0
1000
2000
3000
4000

1 molecule



- We want to measure the duration and amplitude of single molecule “Events”.

- “Events” have different names and forms:

  “open” and “closed”

  “on” and “off”

  “bound” and “free”

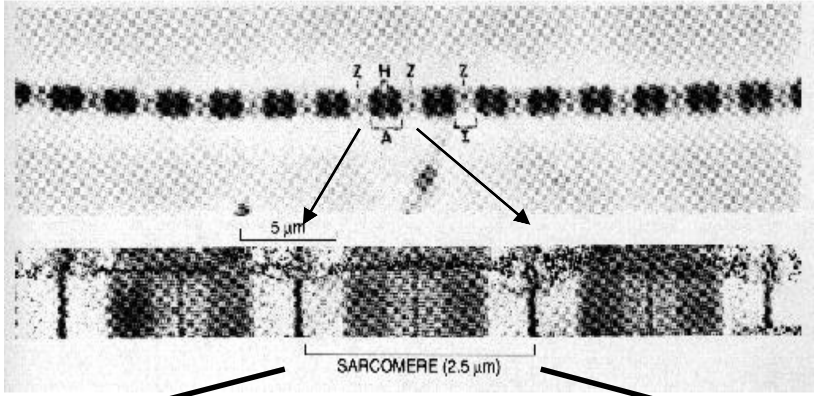
  etc..

- “Events” can be single chemical states or comprise “sub-states” which can only be revealed by careful kinetic or amplitude analysis.

How can we use optical tweezers to understand how molecular motors produce force and movement from ATP?

Filament sliding causes muscle to shorten:

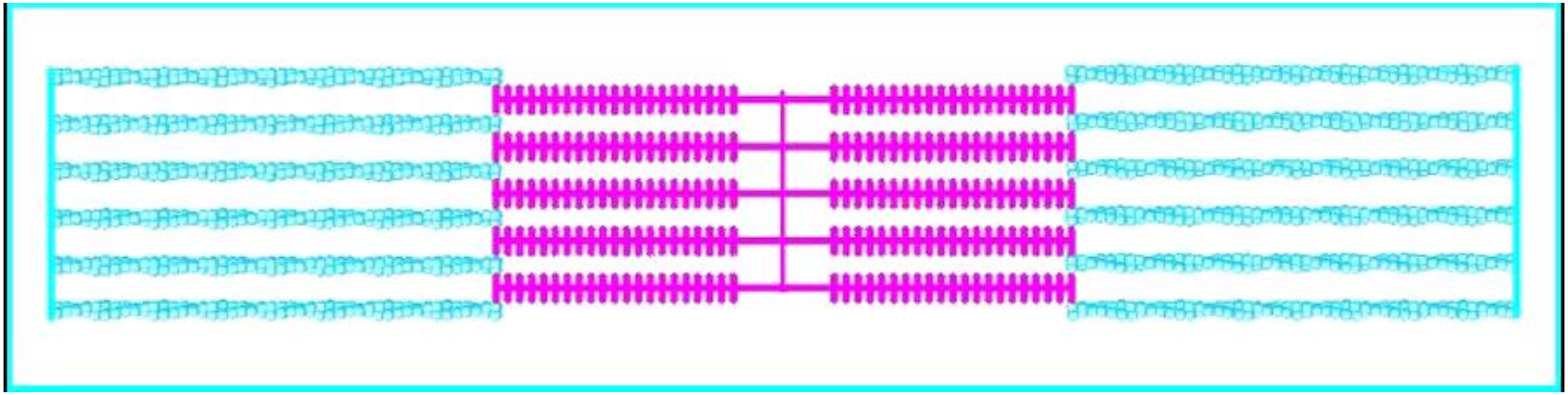
myofibril



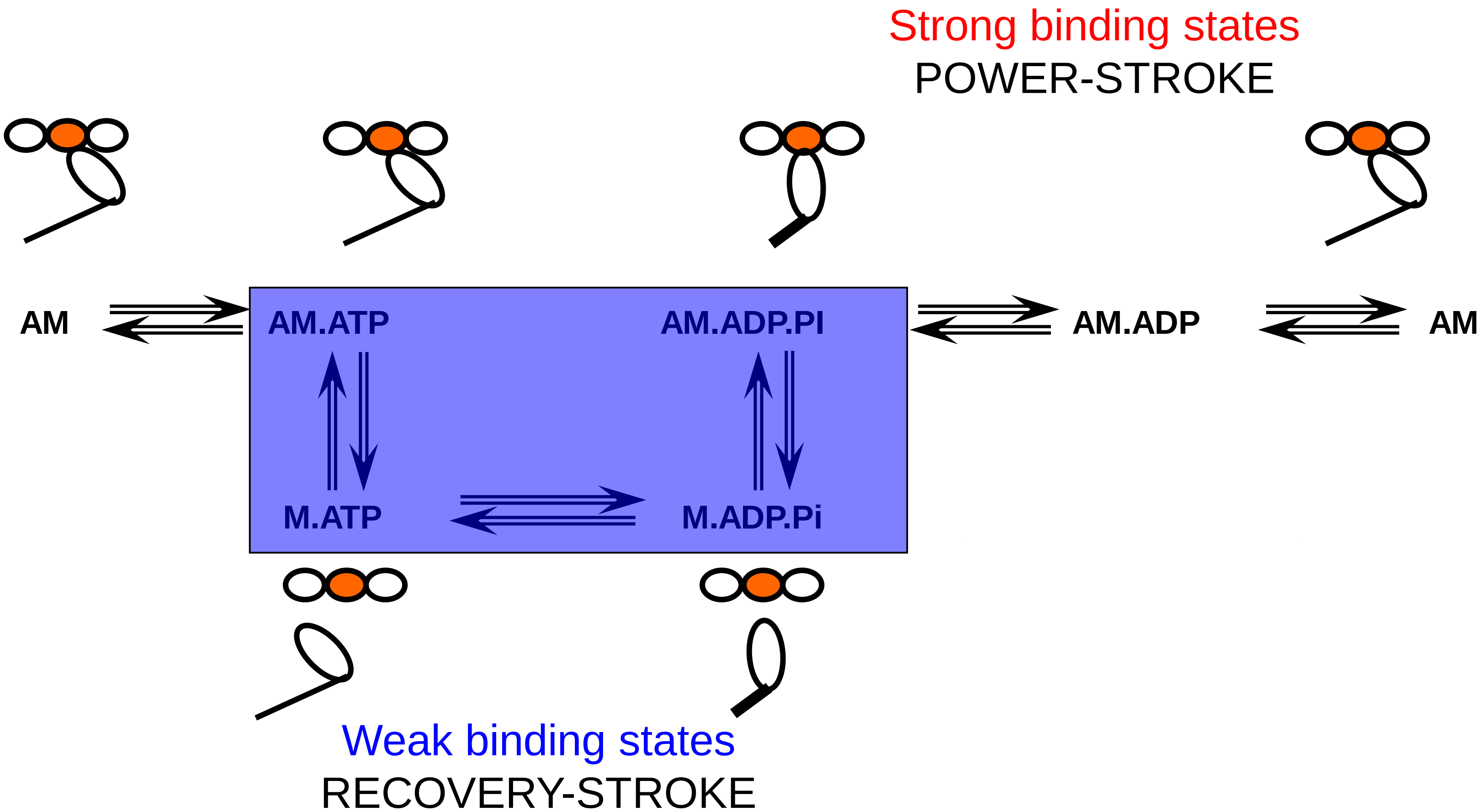
Light micrograph

Electron micrograph

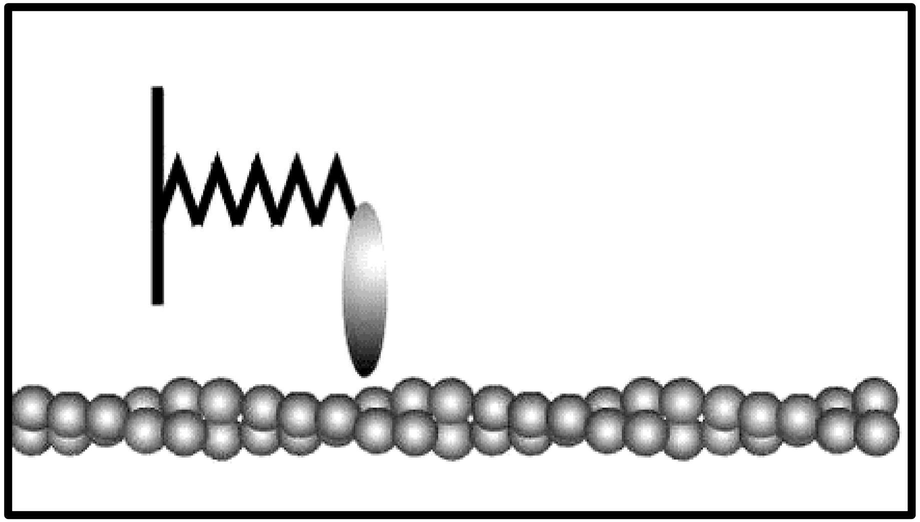
sarcomere



Acto-myosin ATPase pathway

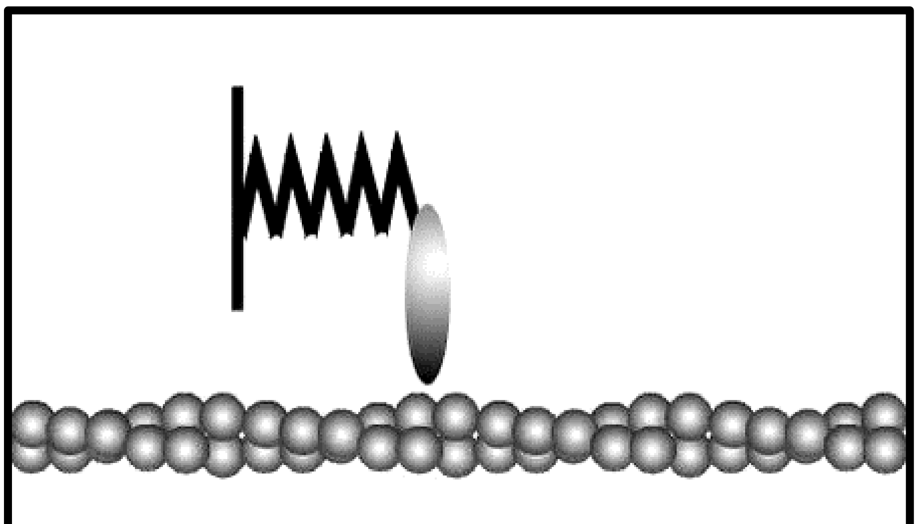


How do myosin motors actually produce force and movement?



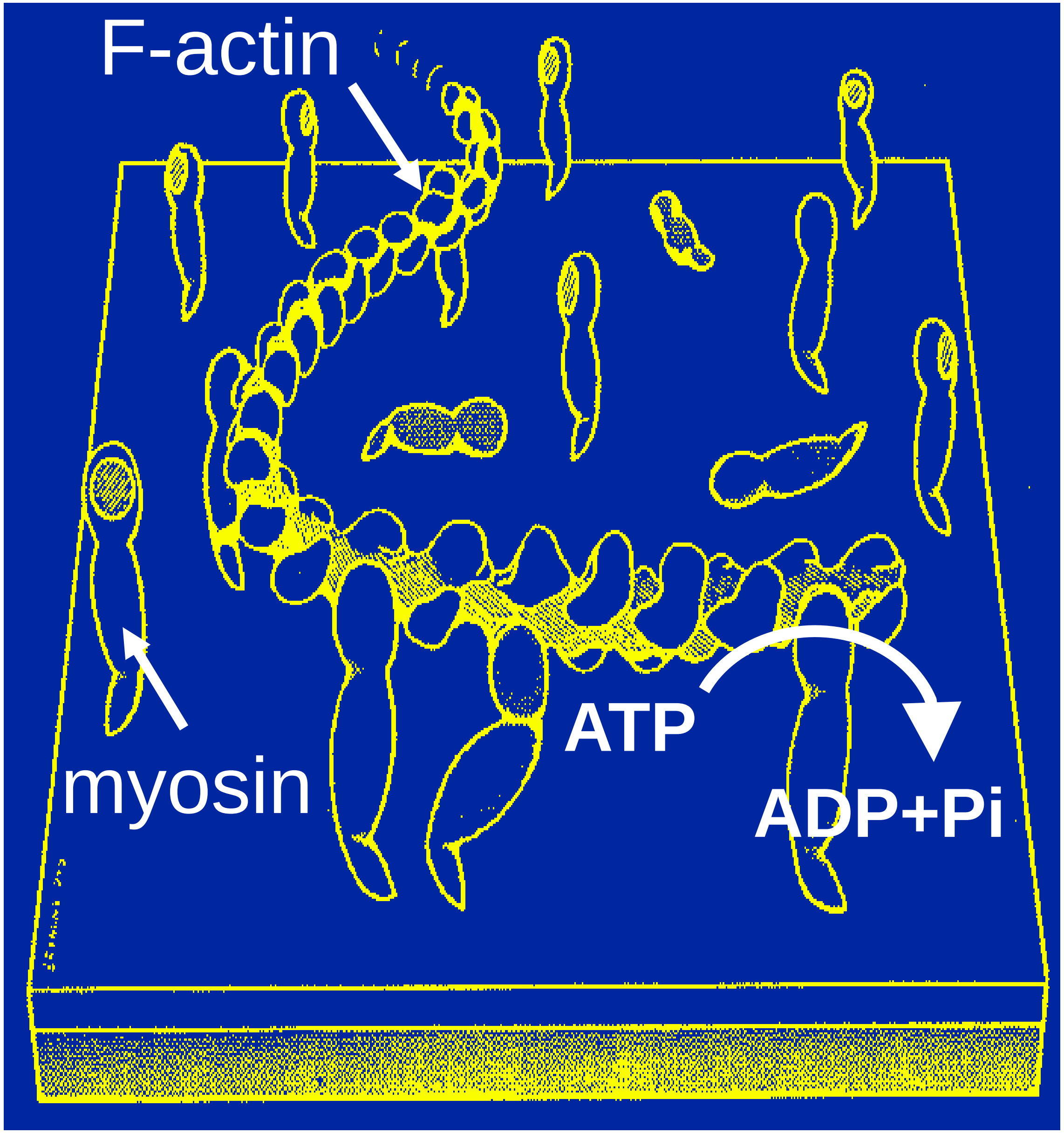
Thermal Ratchet

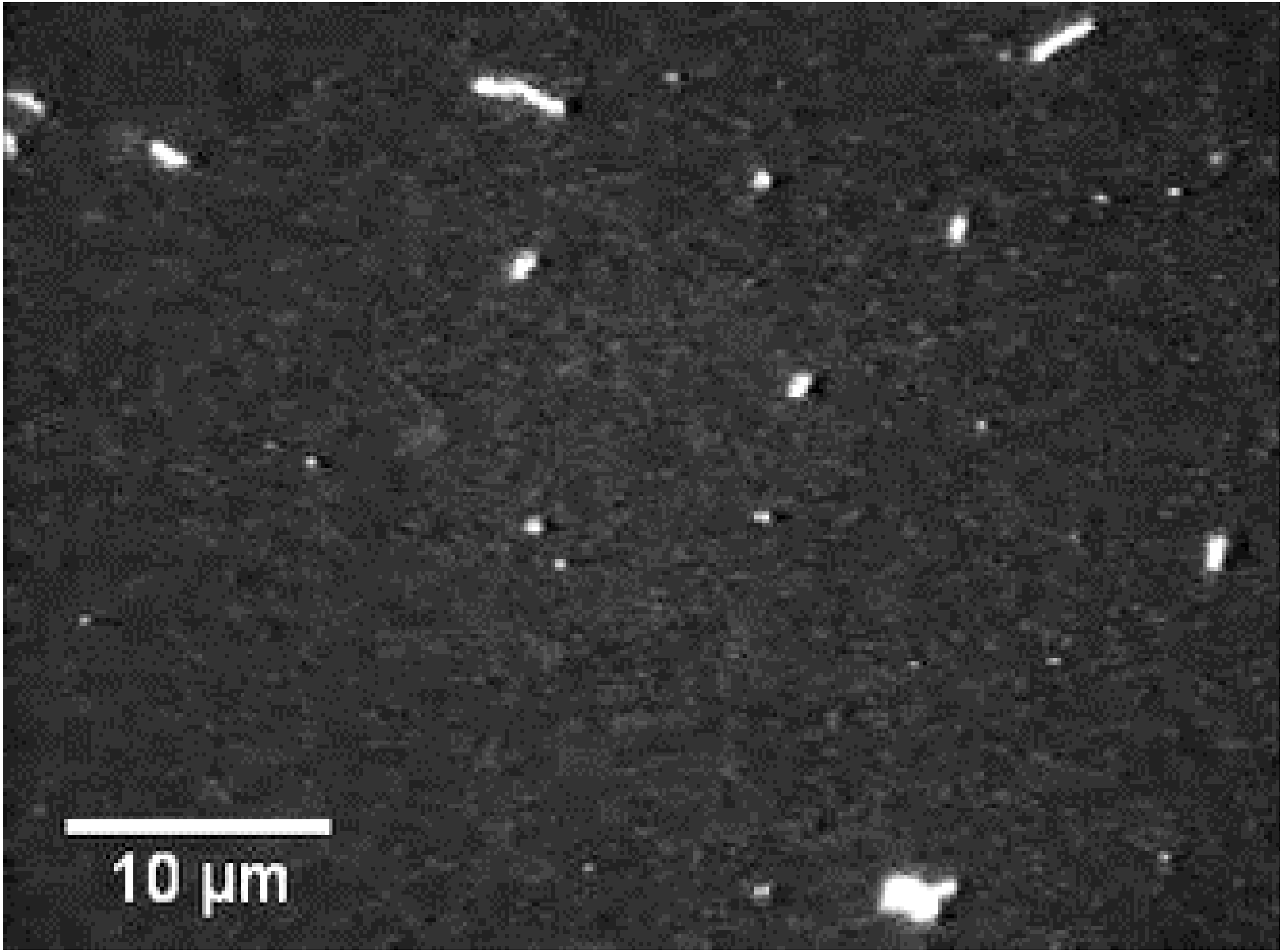
or



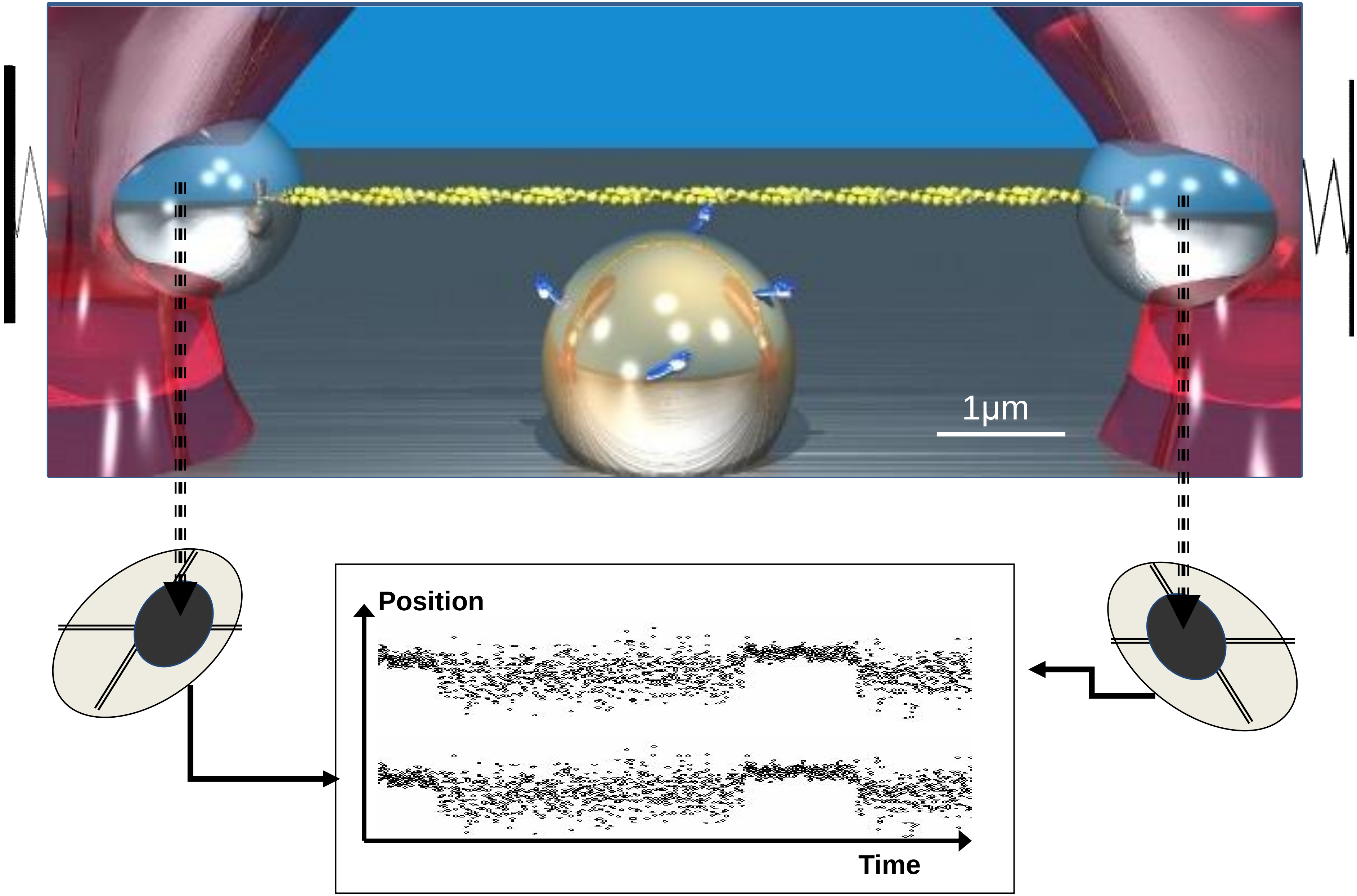
Power-stroke conformational change

Acto-myosin *in vitro* motility assay :




10 µm

High-resolution mechanical studies



Actin Filament Held Between
Two Latex Beads

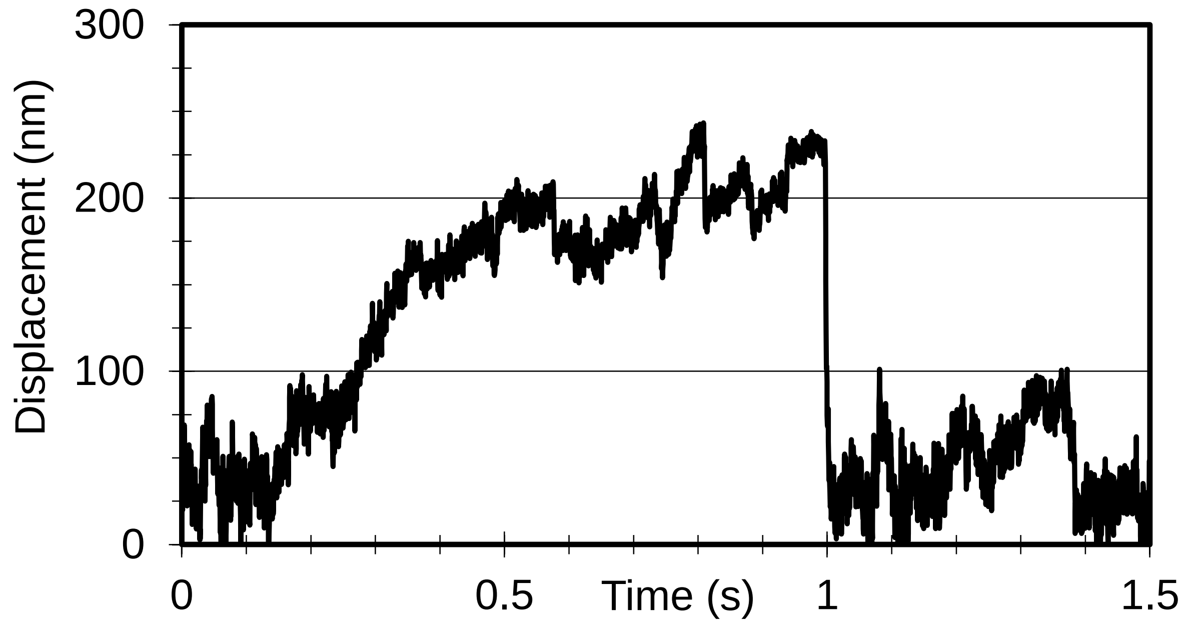
Coated with :

Monomeric NEM-Myosin
& BSA-TRITC

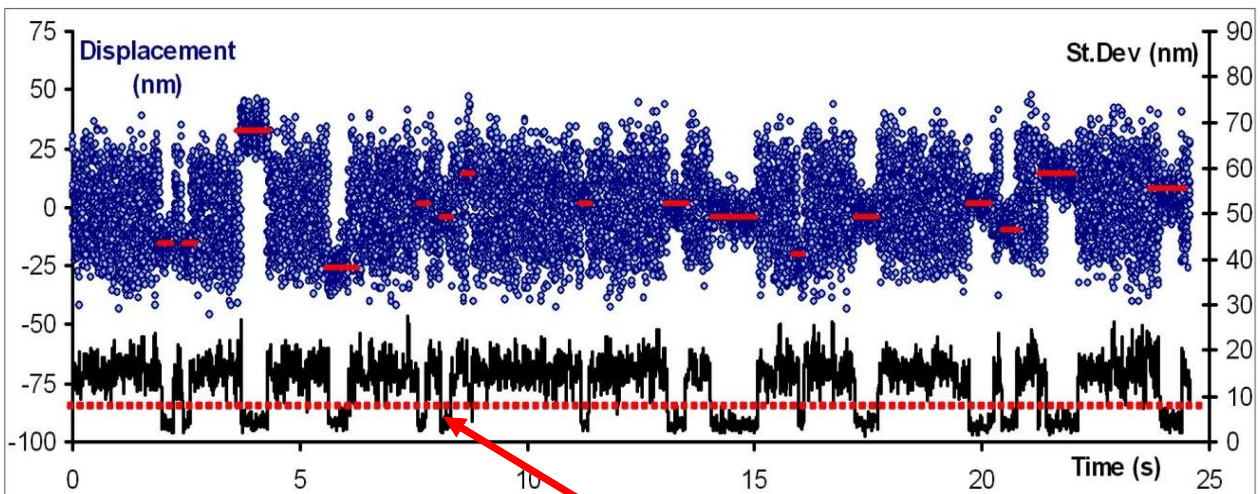
Interacting with :
1.7μm glass bead

Coated with :
HMM @ 50ug/ml

[ATP] = 2mM



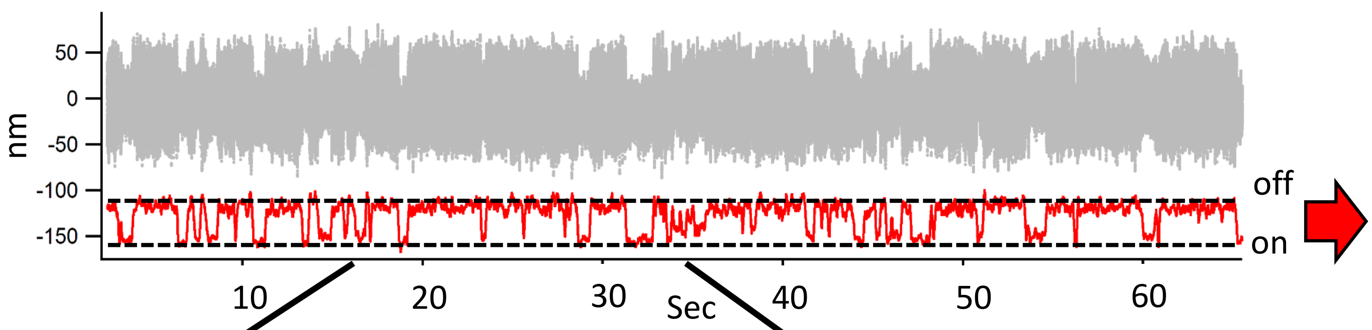
~20->50 molecules



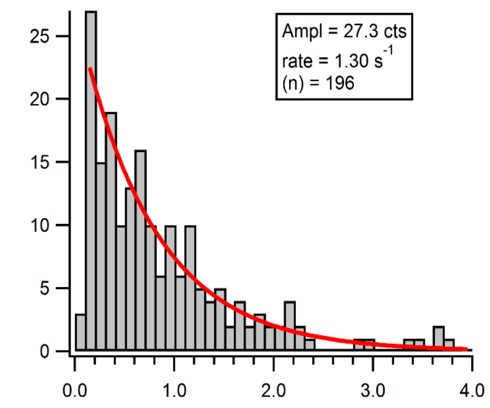
1 molecule

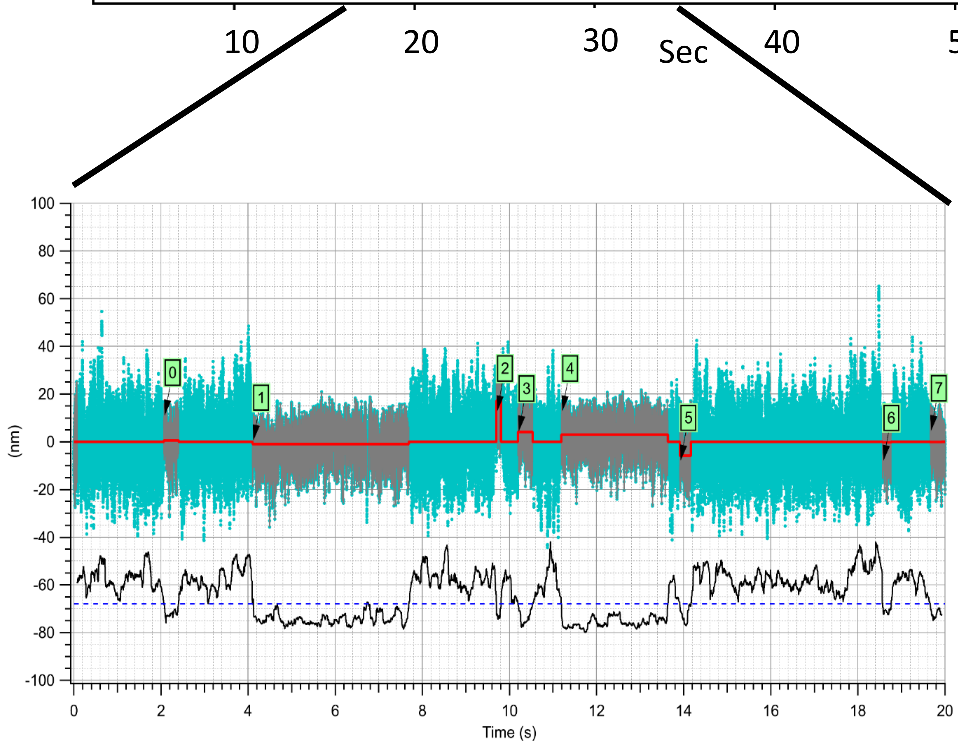
<u>Variance</u> tells us about the system STIFFNESS

Single molecule optical trapping

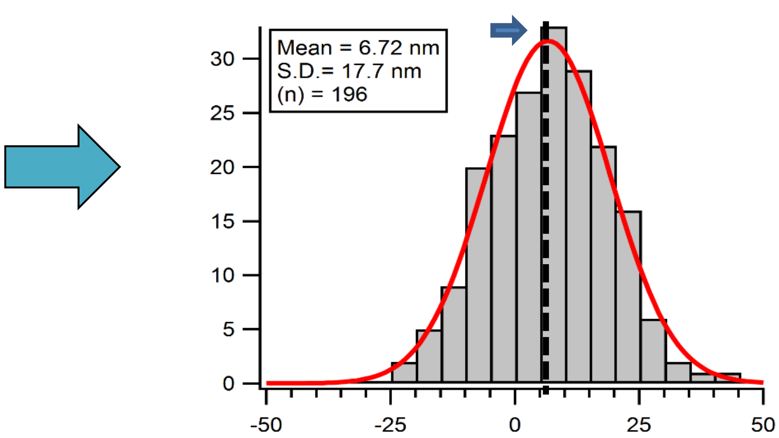


KINETICS



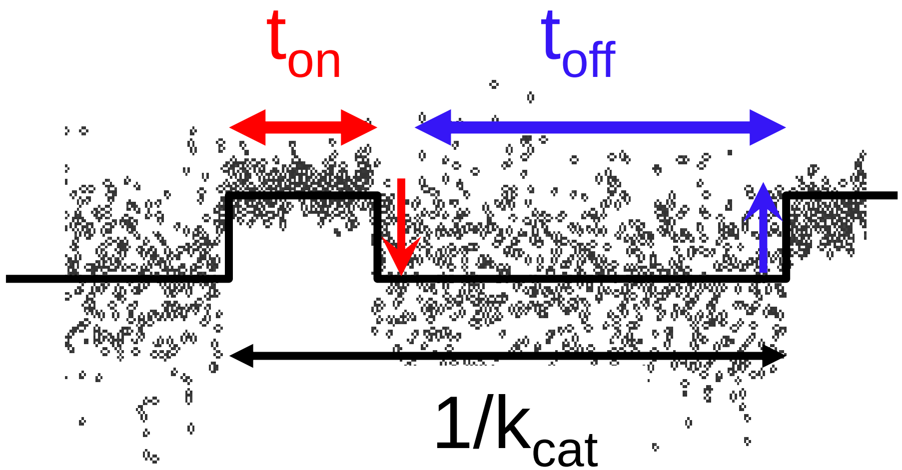


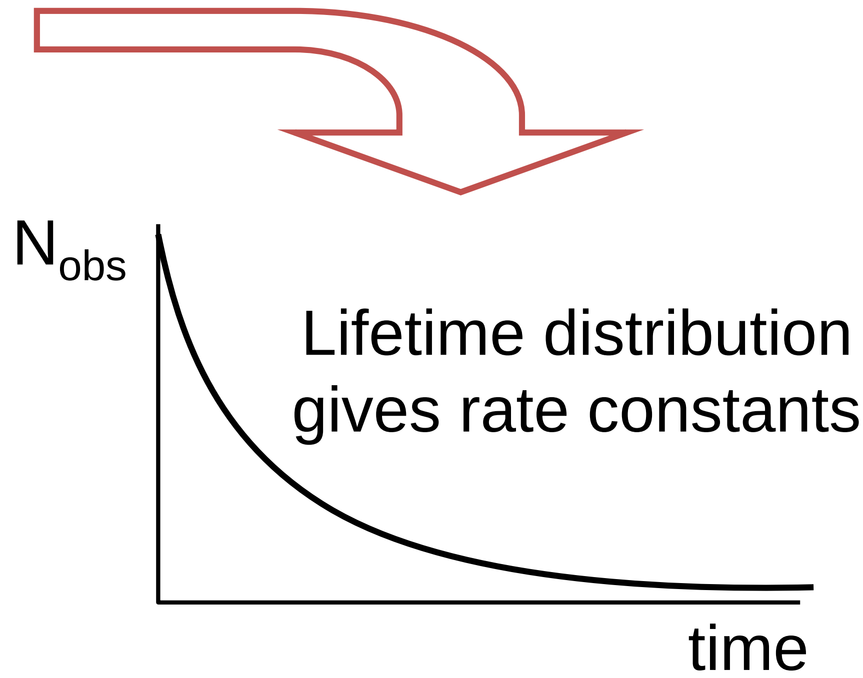
Amplitude of power-stroke

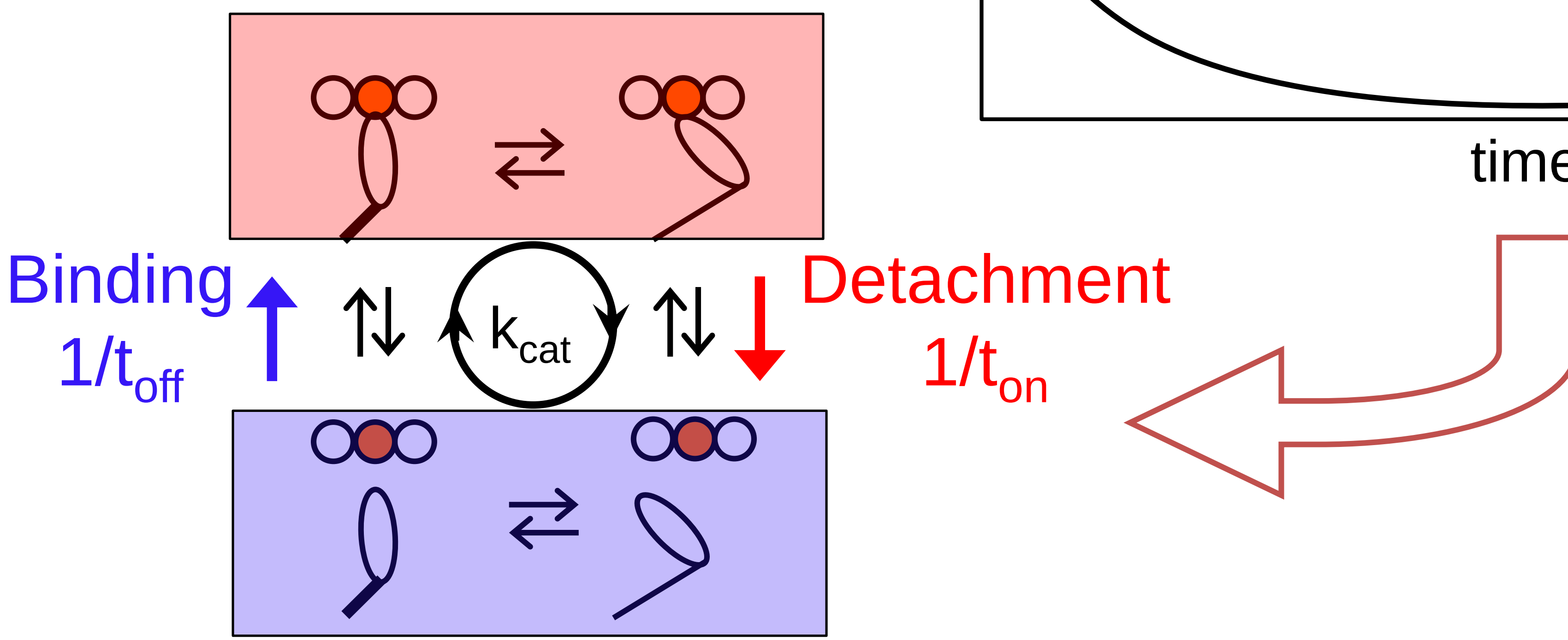


Molloy et al.(1995) Nature *378, 209-212*

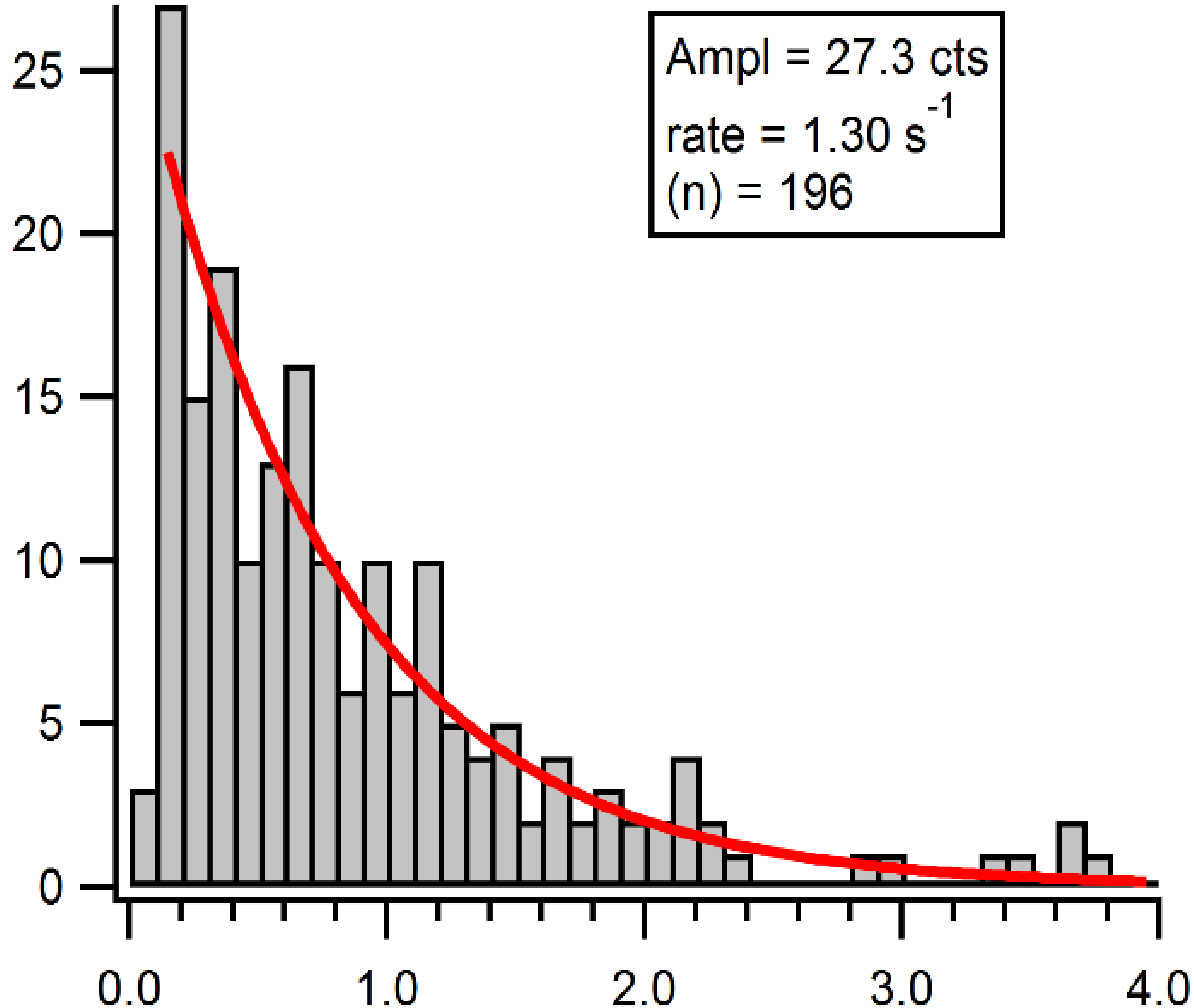
Basic Analysis (I)



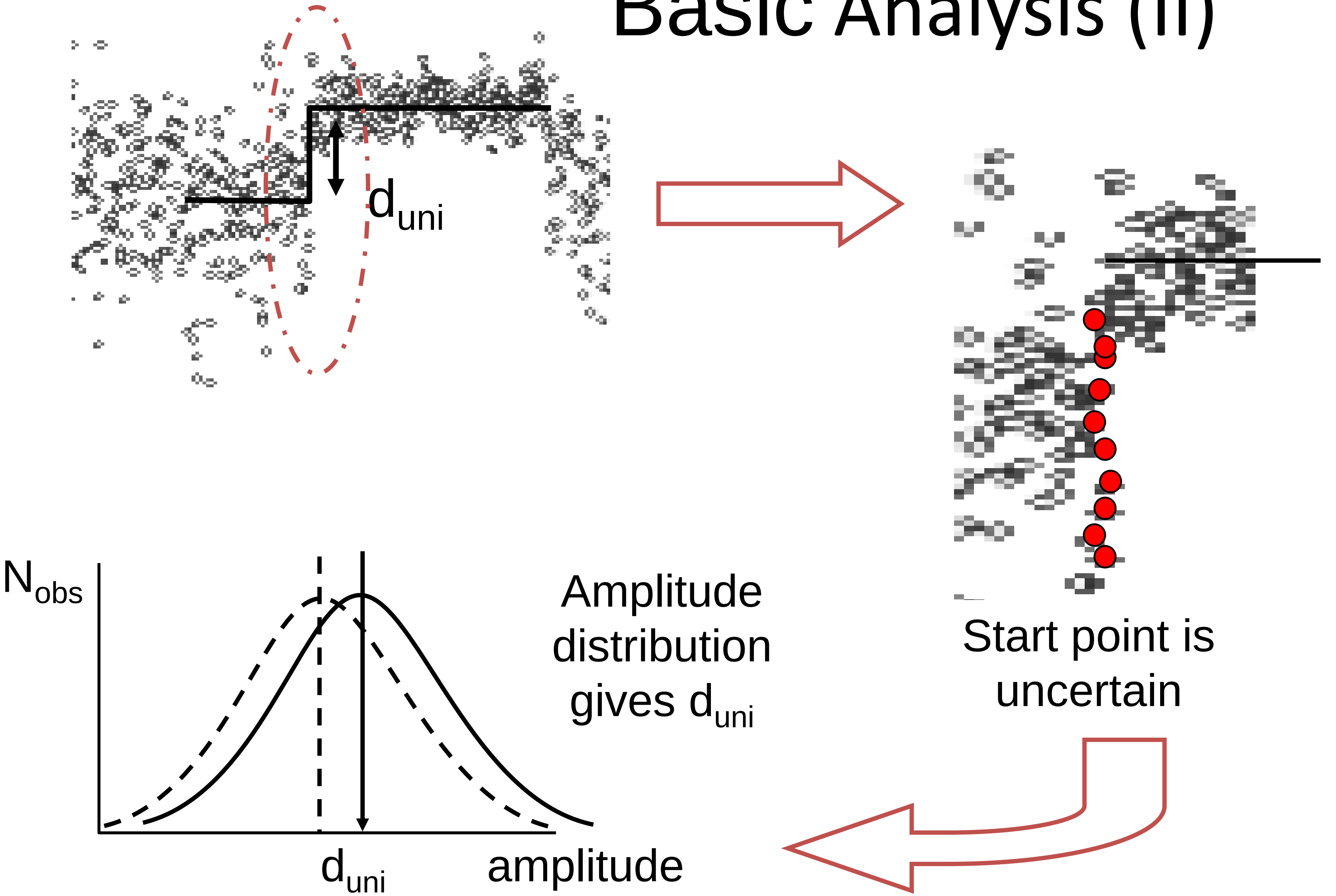




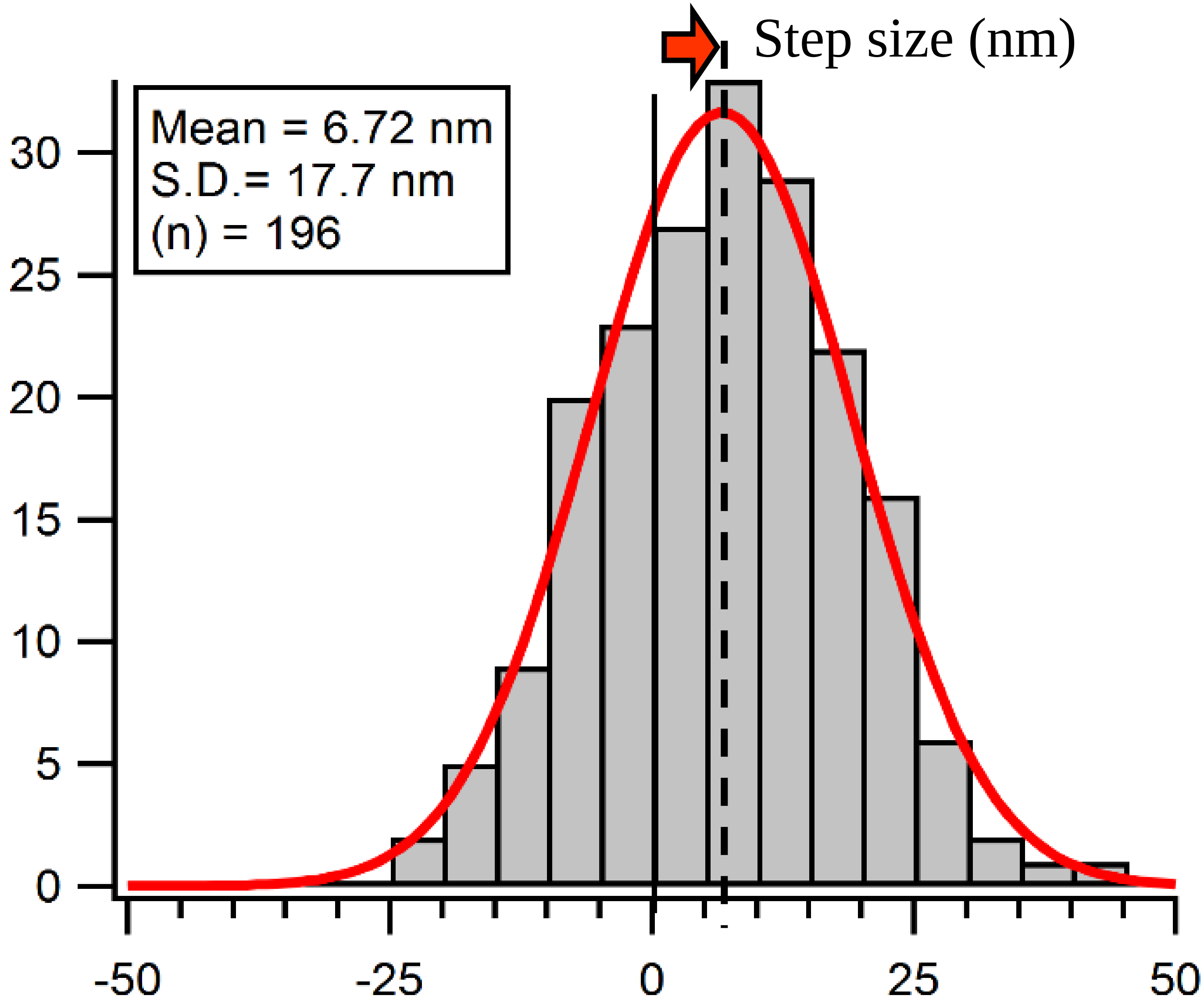
Detachment rate



Basic Analysis (II)



Size of the power-stroke



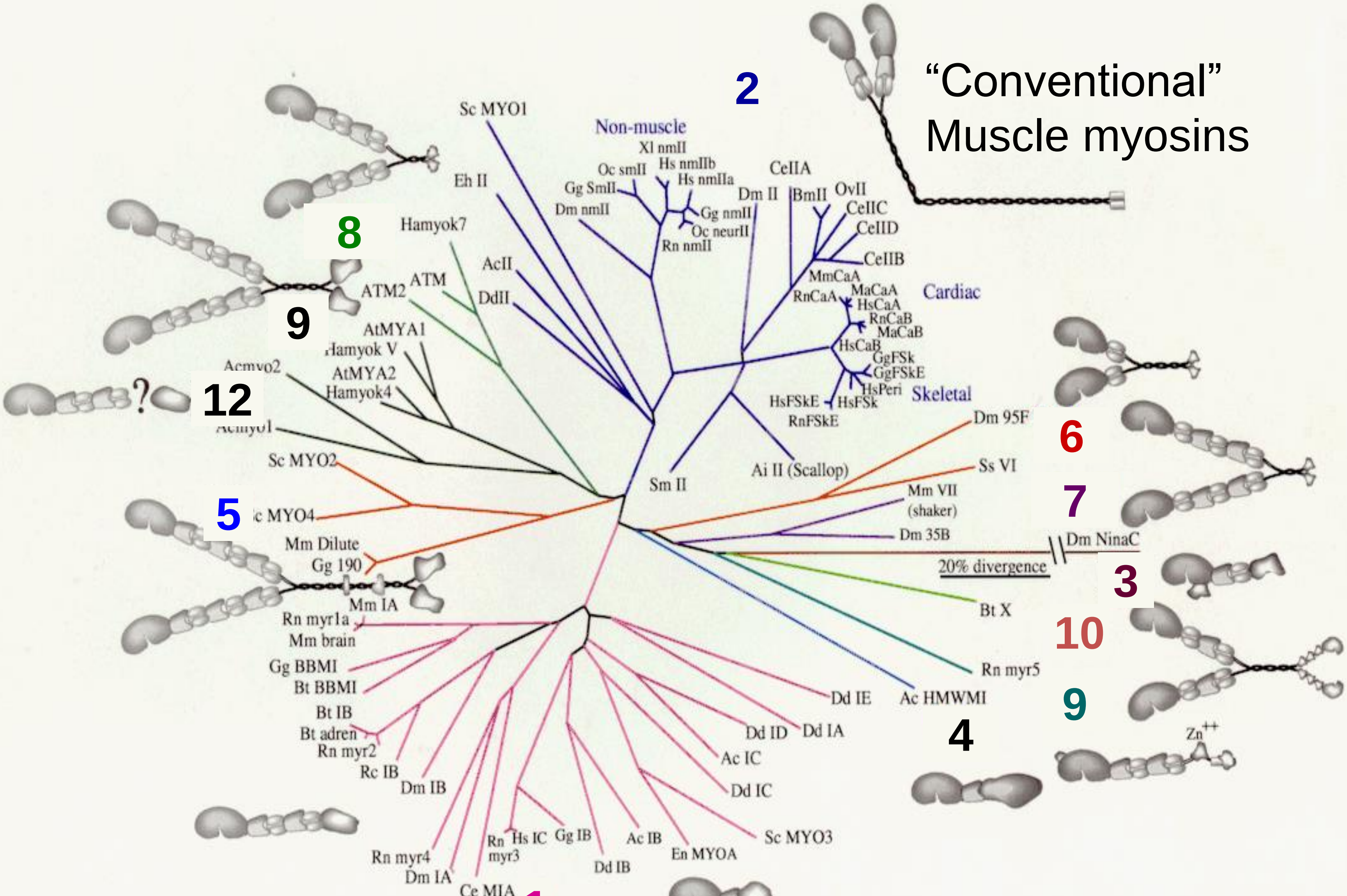
What have we learnt by studying single myosin molecules?

<u>Muscle myosin:</u>

Kinetics:	Binding event terminated by ATP binding 1 Powerstroke requires 1 ATP Event lifetimes are stochastic <10% of time bound to actin >90% unbound
Amplitudes:	Powerstroke produces 5->10 nm movement & 2->5 pN force Myosin stiffness is ~ 1pN.nm^{-1}
Efficiency:	30 pN.nm mechanical work ~ 30% ΔG ATP
Inter alia:	One myosin head is sufficient for force and movement Myosin binds preferentially at actin helical repeat distance Event lifetime is load-dependent Some myosins produce movement in two discrete phases i.e. Evidence for "different bound states"

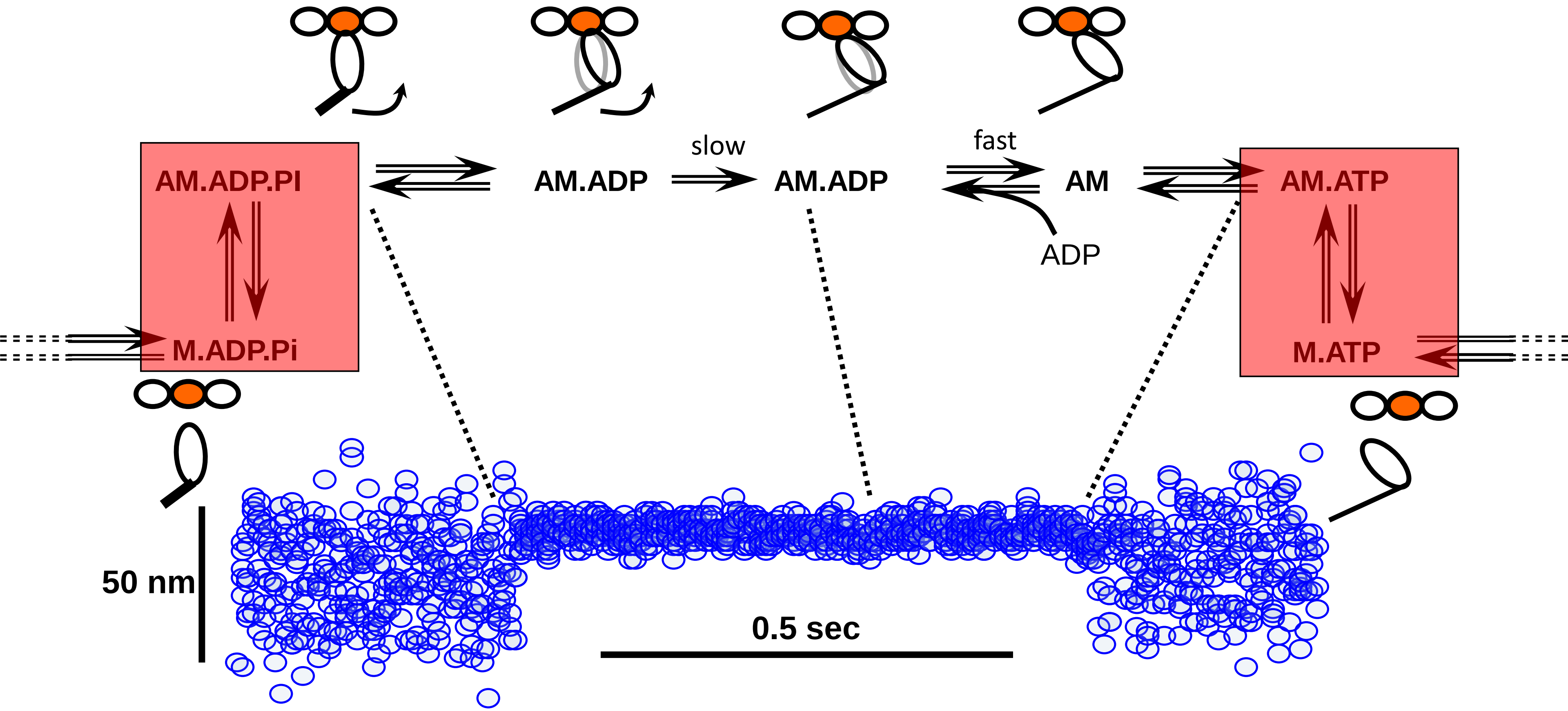
Structural and functional diversity

<u>The myosin family:</u>

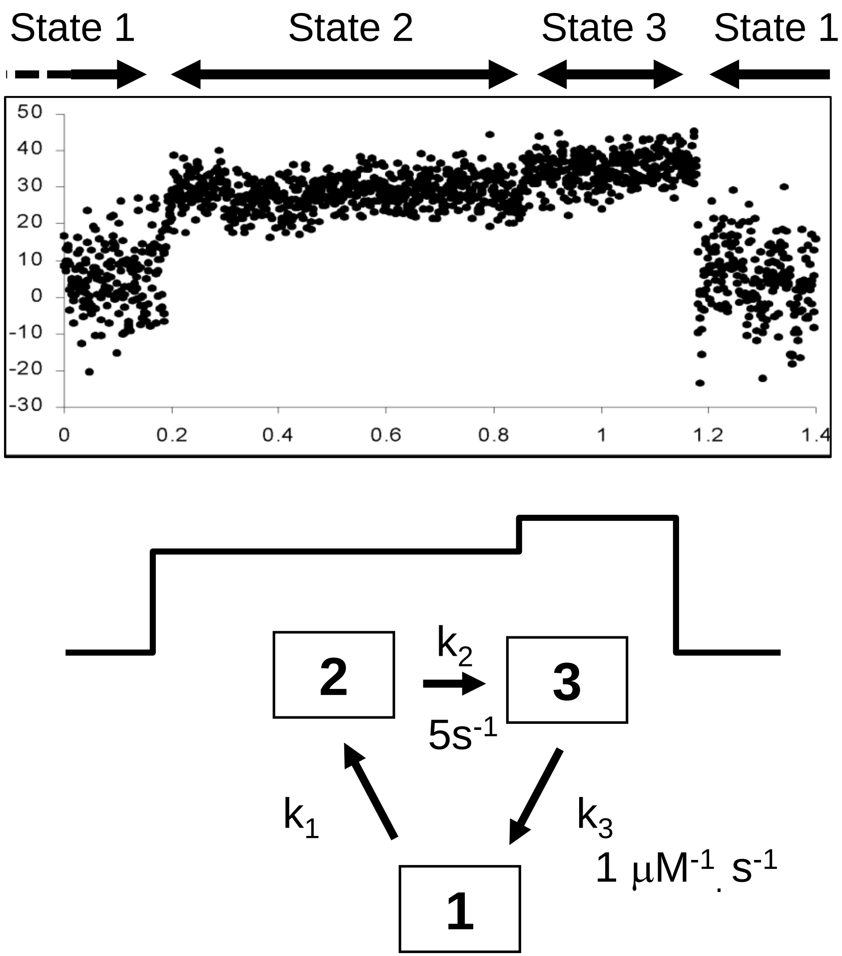


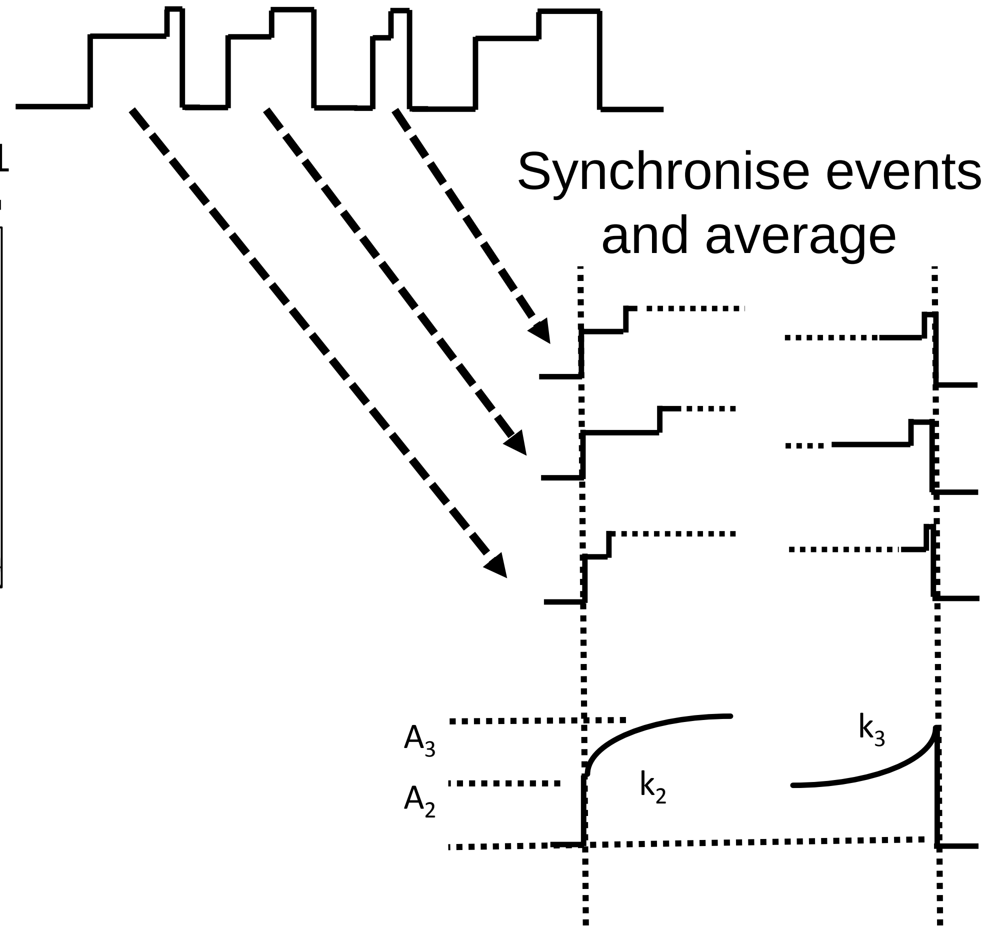
(Tony Hodge, LMB Cambridge)

Super-resolution mechanics myosin class 1 (a "tension sensor")



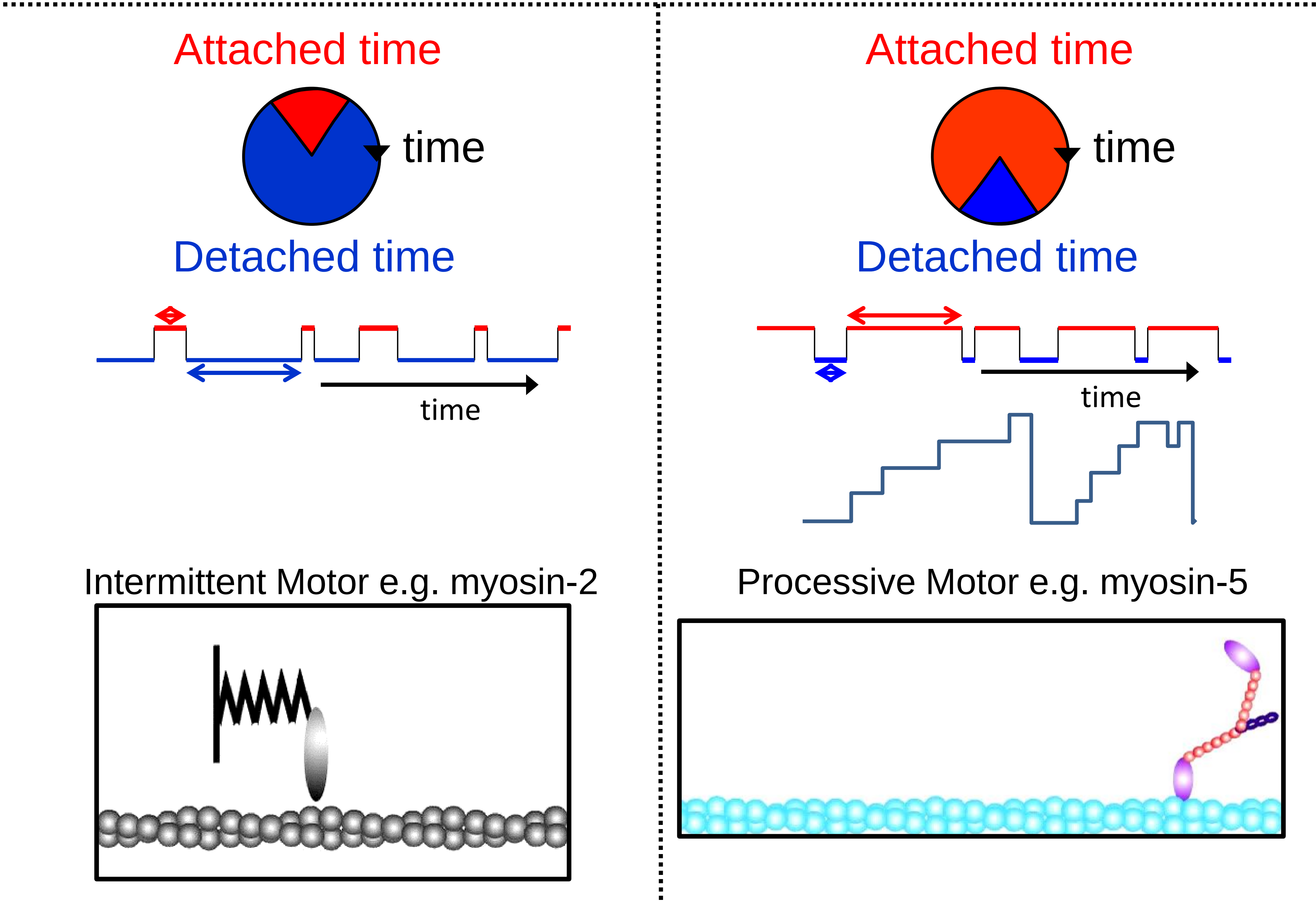
Ensemble averaging reveals "sub-states"





Veigel et al. (1999)
Nature *398, 530-533*

DUTY-CYCLE RATIO

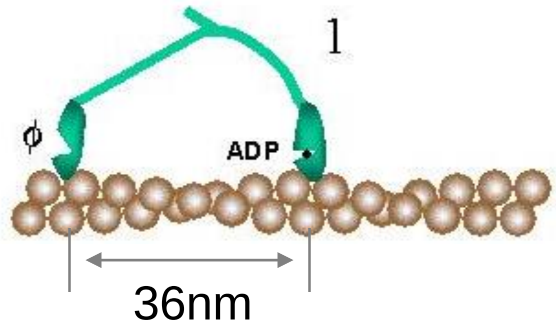


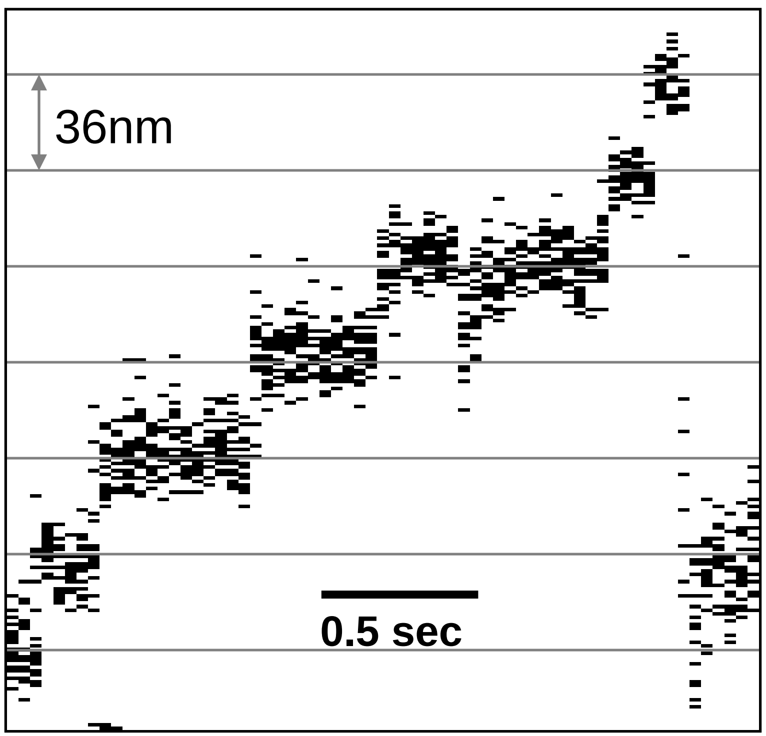
"<u>Processive</u>" and "<u>Intermittent</u>" motors

- ***"Intermittent"*** motors must **<u>work in teams</u>** to produce large movements and forces.

- ***"Processive"*** motors can take many steps before detaching from their track and they can **<u>work alone as single molecules</u>.**

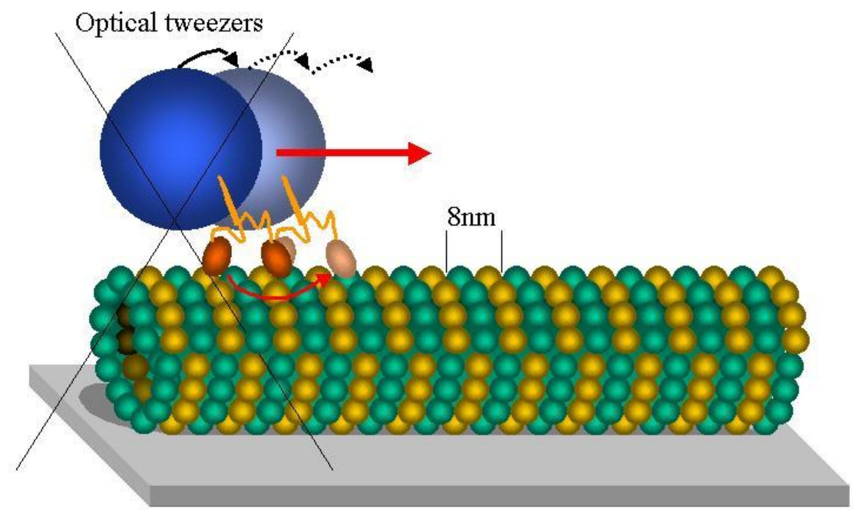
Myosin-5

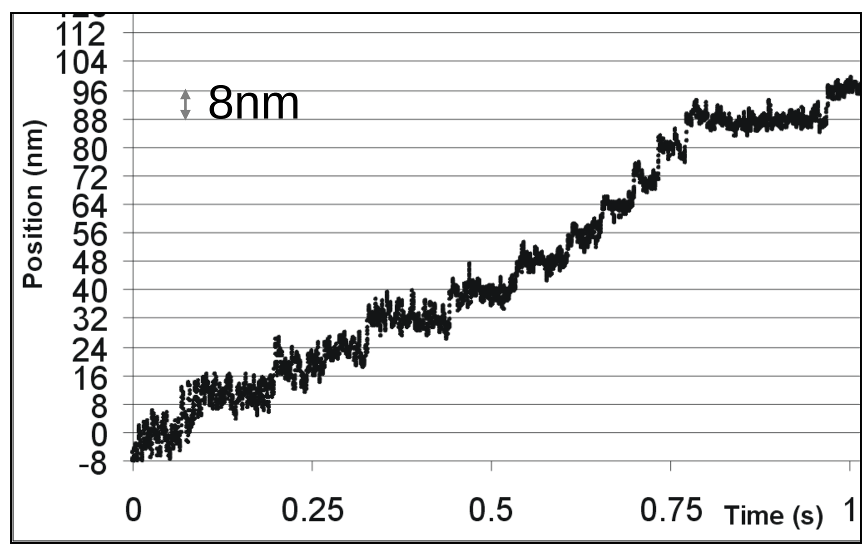




Veigel & Molloy

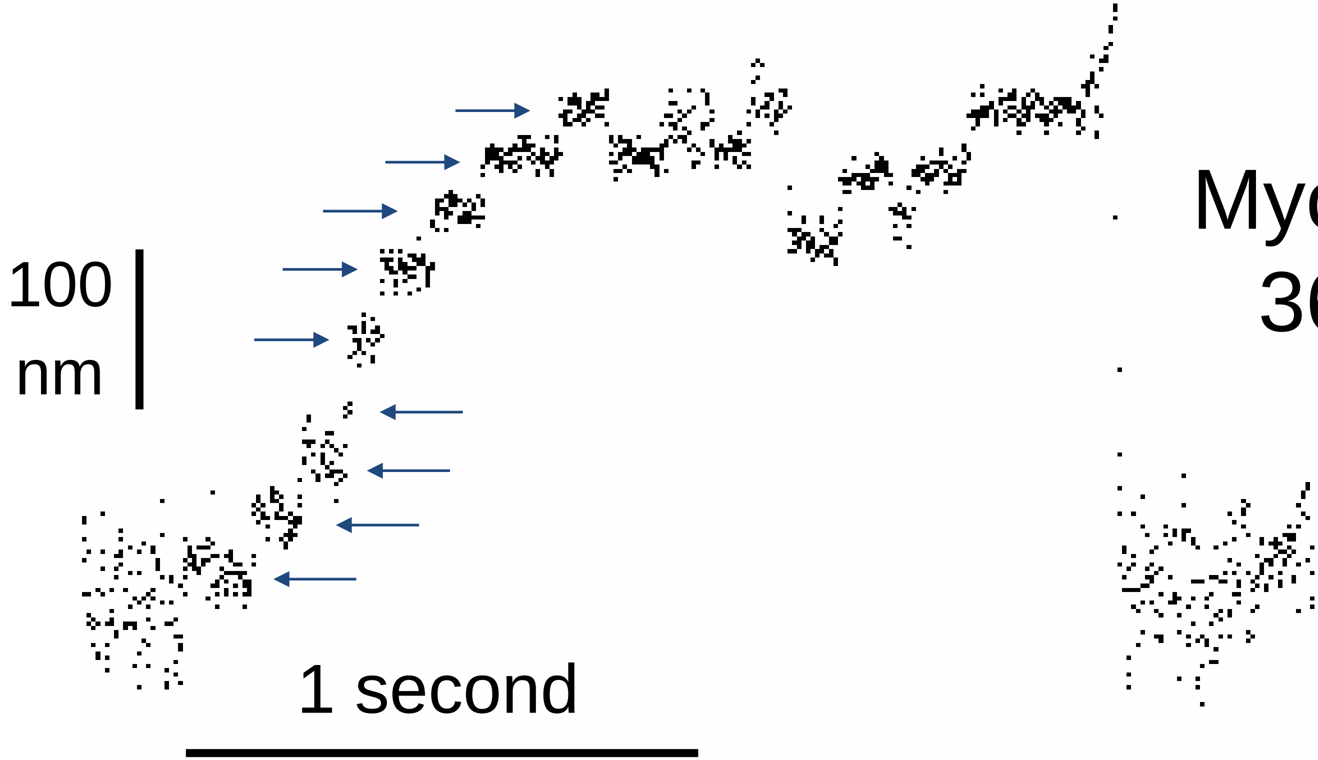
Conventional kinesin

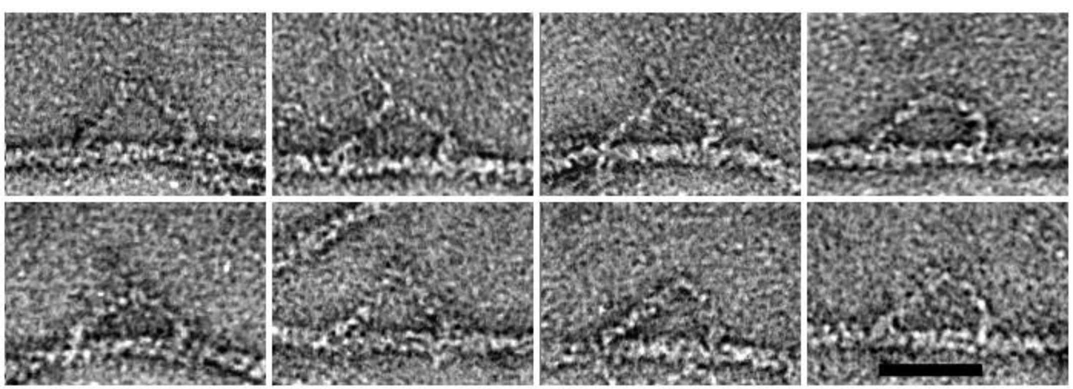


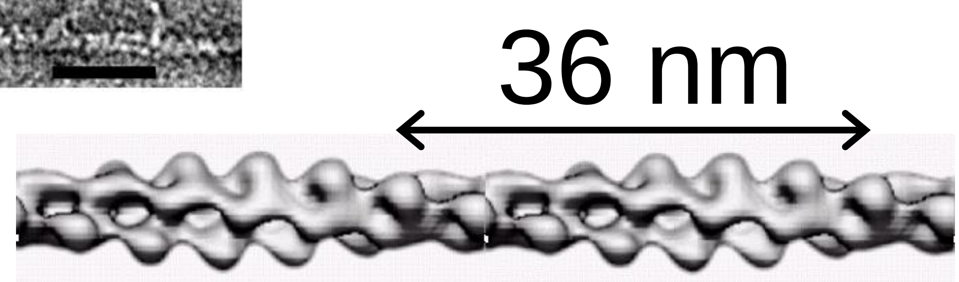


Carter & Cross

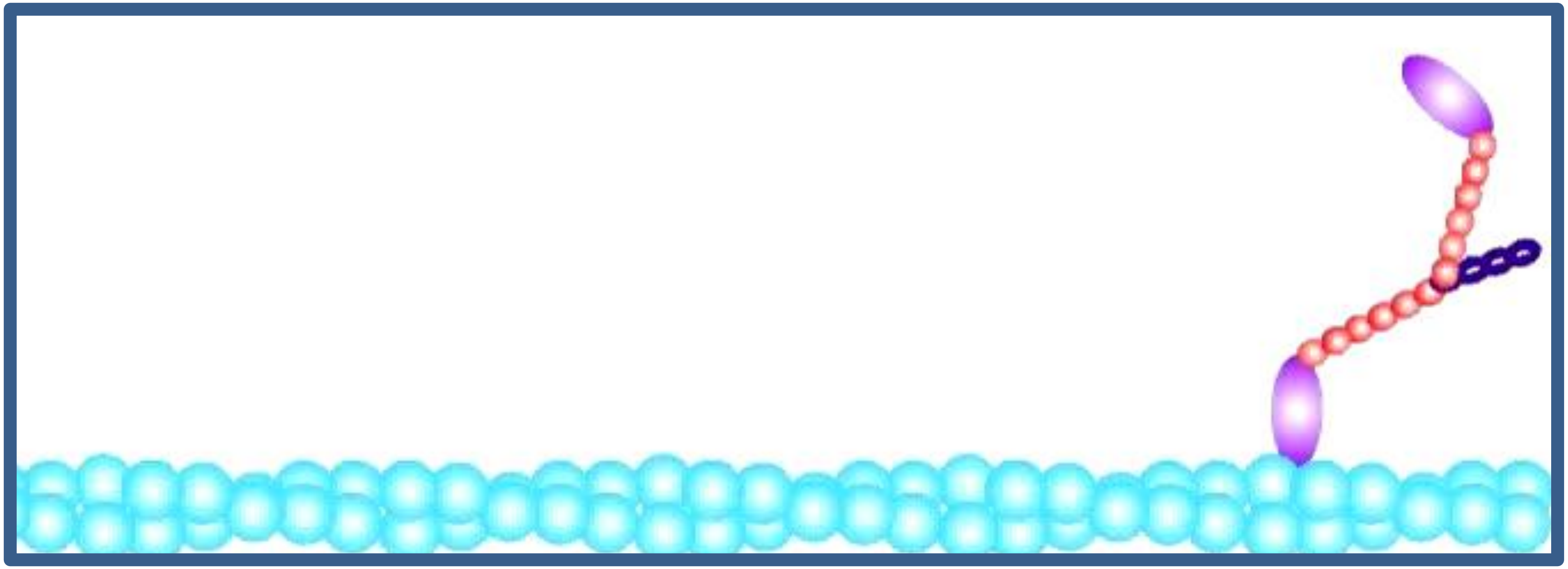
Myosin-5 takes 36nm steps

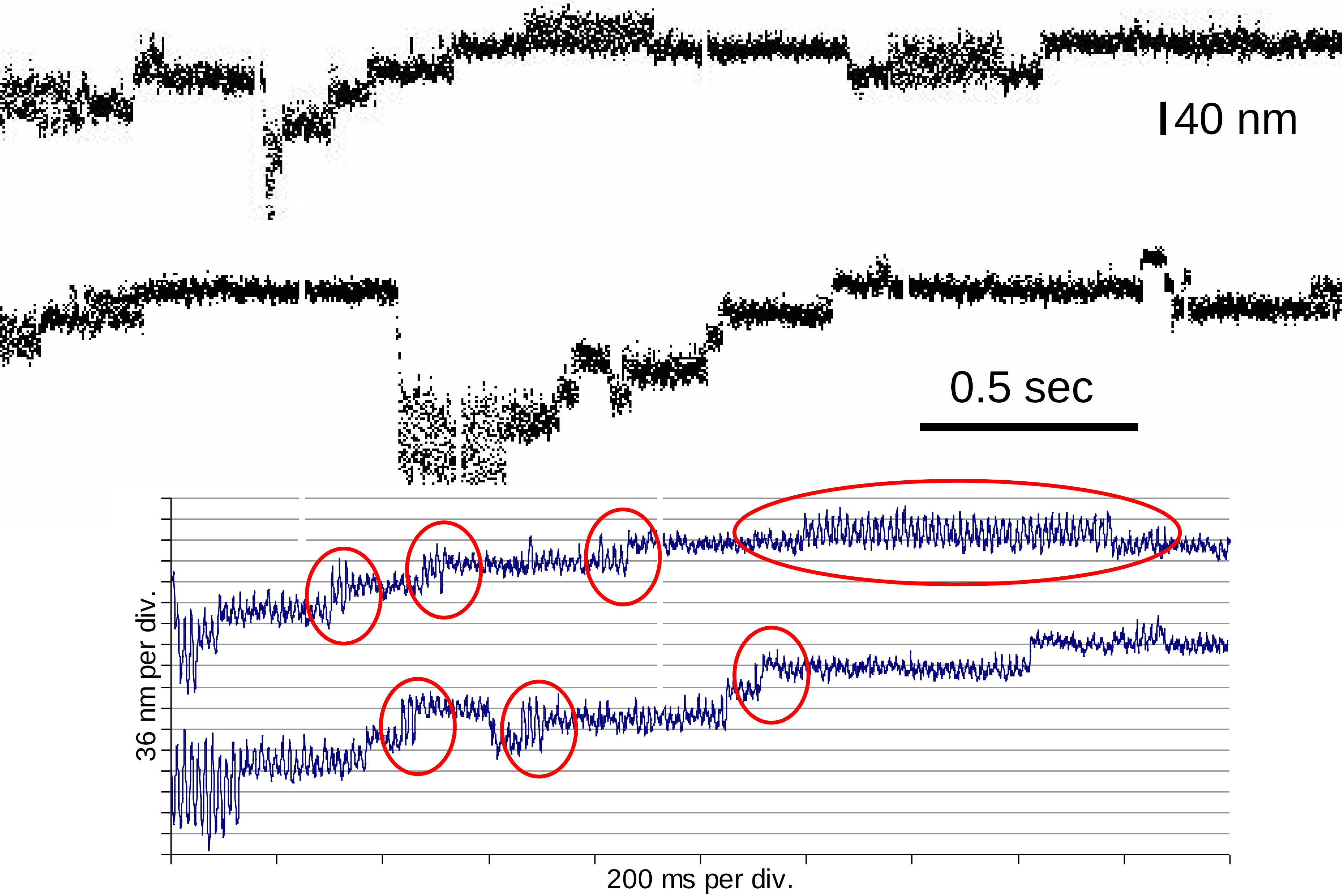






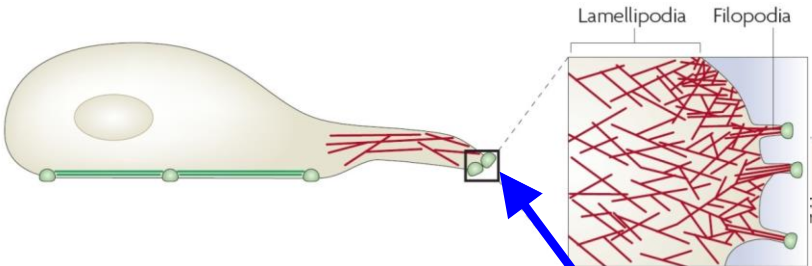
How does myosin-5 walk??…….

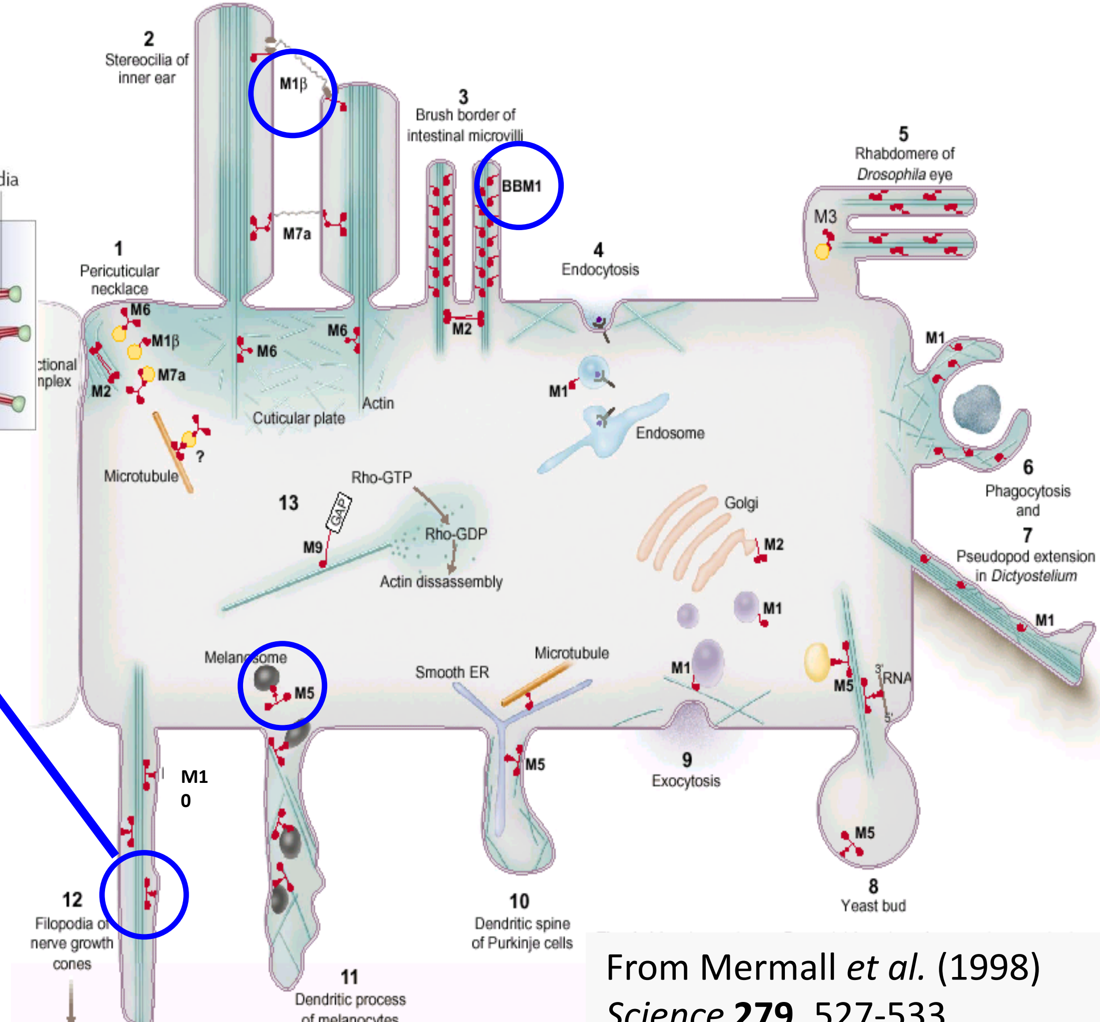




Veigel *et al.* (2002) *Nat. Cell Biol.* **4**:59-65.

Myosin Class-10





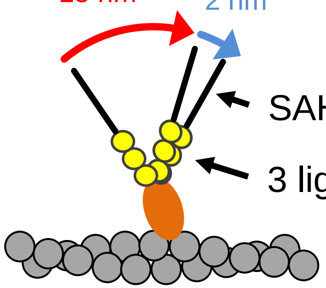
How does myosin-10 localise to the tip of the filopodium?

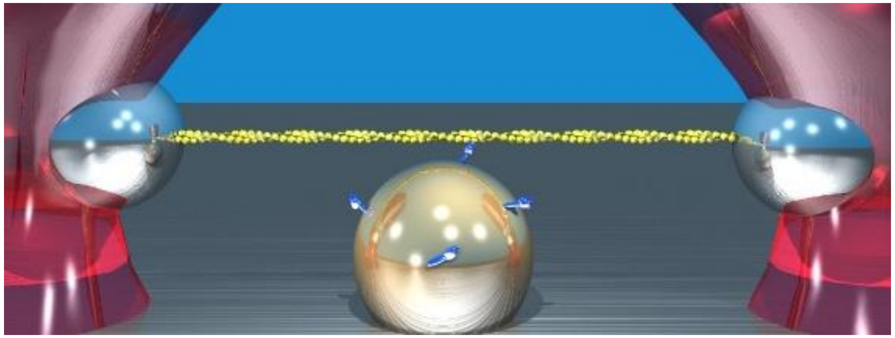
Can we observe single molecules inside a living cell?

From Mermall *et al.* (1998) *Science* **279**, 527-533

Myosin-10 can be both an intermittent, single headed motor and a processive dimeric motor.

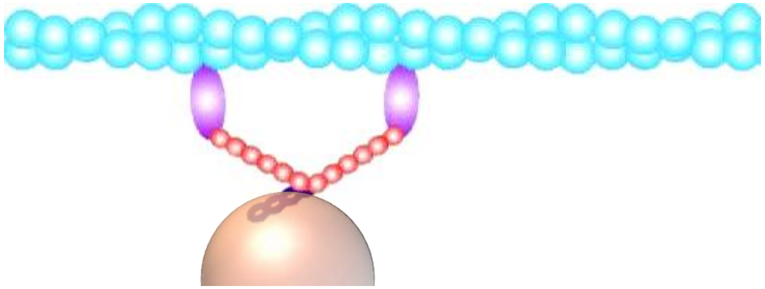
Monomeric form:

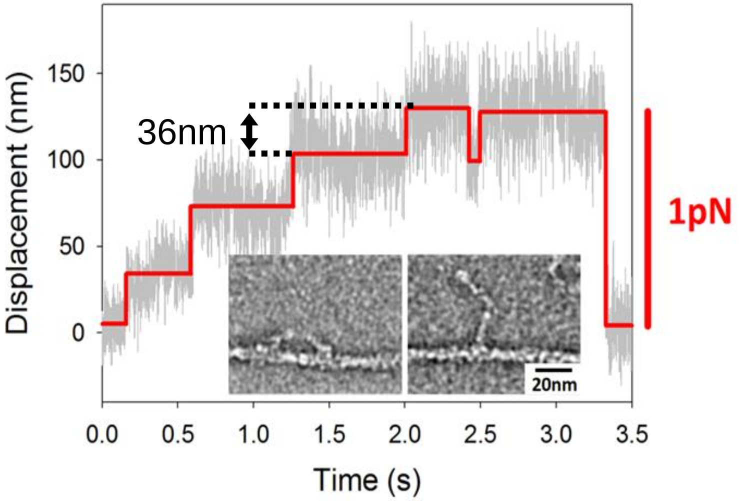




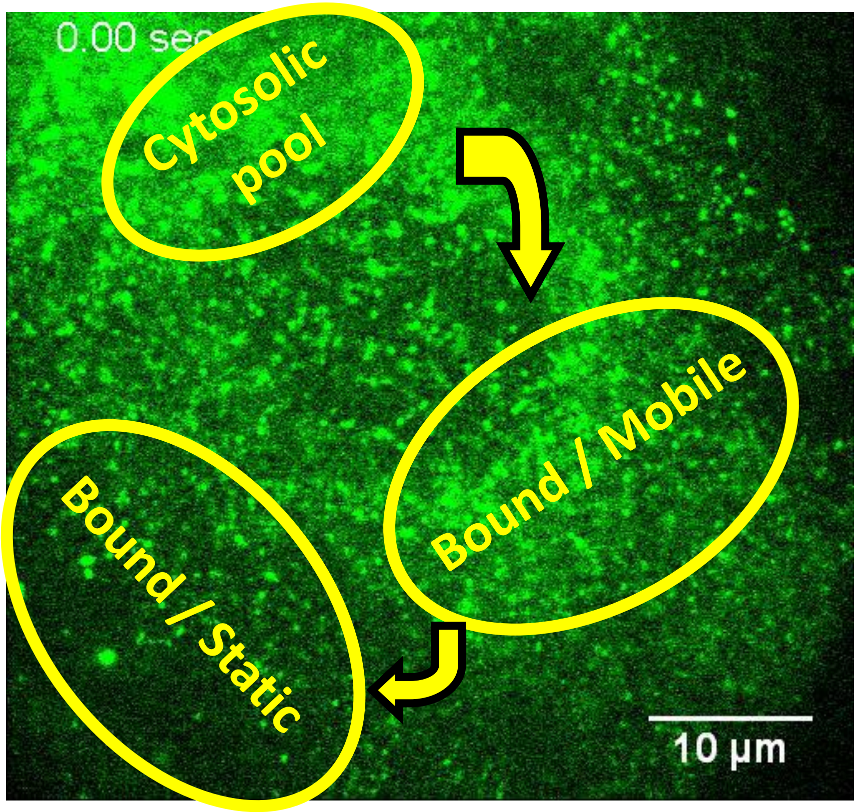
Takagi, Y., et al. (2014) PNAS 111, E1833-E1842

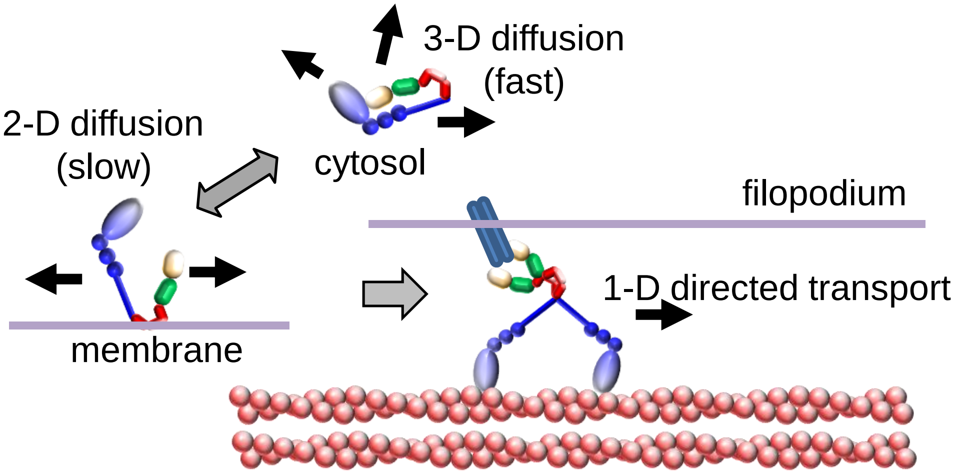
Dimeric form:

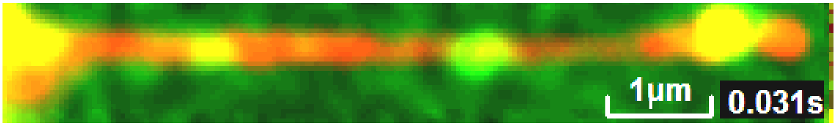


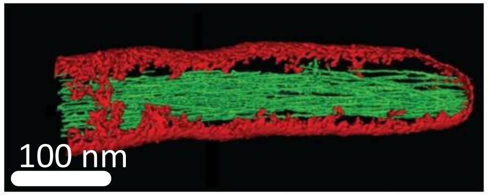


Using TIRF microscopy we can see individual molecules of myosin-10 moving inside a living cell

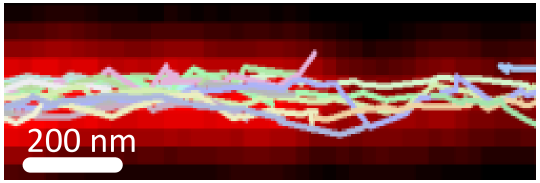








Cryo-electron tomography



Super-resolution optical microscopy

Baboolal, T.G. et al. (2016). J. Biol. Chem. 291, 22373-22385

Lecture Overview:

- Optical Tweezers are relatively simple to build and are compatible with standard laboratory microscopes

- They have a sensitivity and time-resolution suitable for studying biological macromolecules and cells

- They have contributed to our understanding of the mechanism and function of molecular motors (like kinesin, dynein and myosin) and also of DNA processing enzymes.

THE FUTURE………

- The advent of fast cameras, fast parallel processing, and more powerful lasers mean that time-resolution is now in the microsecond regime; and forces of ~100pN are possible - opening the possibility to study molecular dynamics and cellular mechanics.

THANK YOU FOR YOUR ATTENTION!

SZÉCHENYI 2020

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