Hungarian ELI project

NEW SZÉCHENYI PLAN

Lasers in Medicine and Life Sciences Advanced Summer School

16th July 2017

"Optical Tweezers"

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HUNGARIAN BIOPHYSICAL SOCIETY

ROLAND EÖTVÖS PHYSICAL SOCIETY (HUNGARY)

TÁMOP-4.1.1.C-12/1/KONV-2012-0005 project

HUNGARY'S RENEWAL

The project is supported by the European Union and co-financed by the European Social Fund.

Lecture Plan:

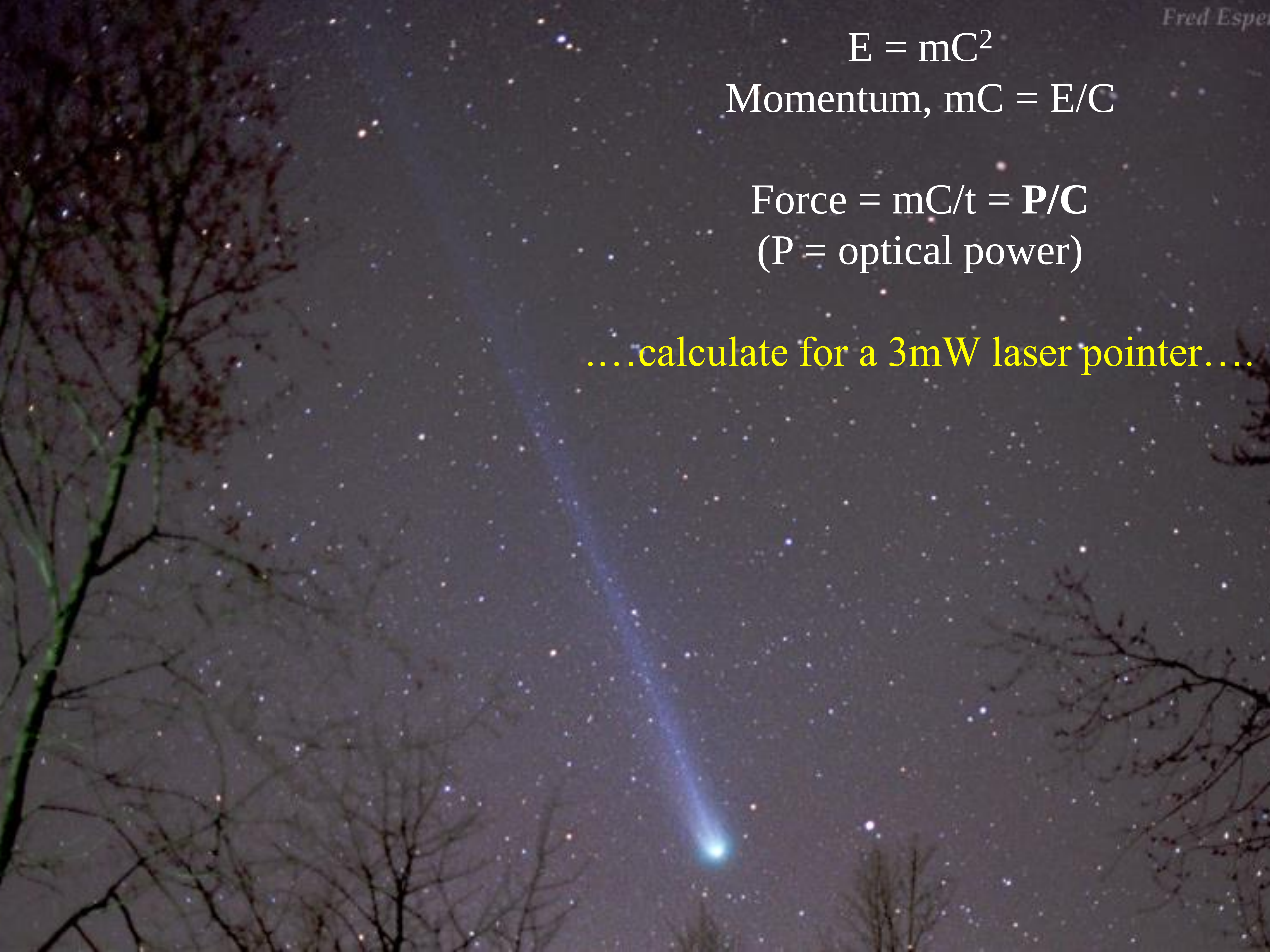
- Optical Tweezers have the correct mechanical properties to push and pull on single biological macro-molecules (picoNewtons and nanometres).

- The time-resolution is limited by the mechanical time-constant ~10kHz (recently improved to ~100kHz)

- It is possible to measure mechanical events powered by the energy from a single ATP molecule (only $10k_bT$).

- Optical Tweezers are easily compatible with other optical (laser-based) single molecule methods.

- Motor proteins are a model biological system for development of new biophysical methods and especially single molecule approaches.



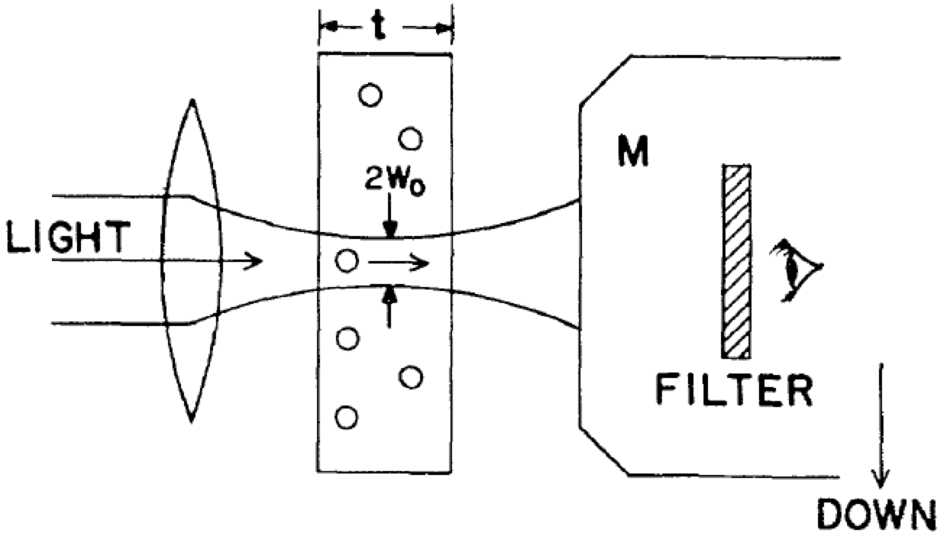
$E = mC^2$

Momentum, $mC = E/C$

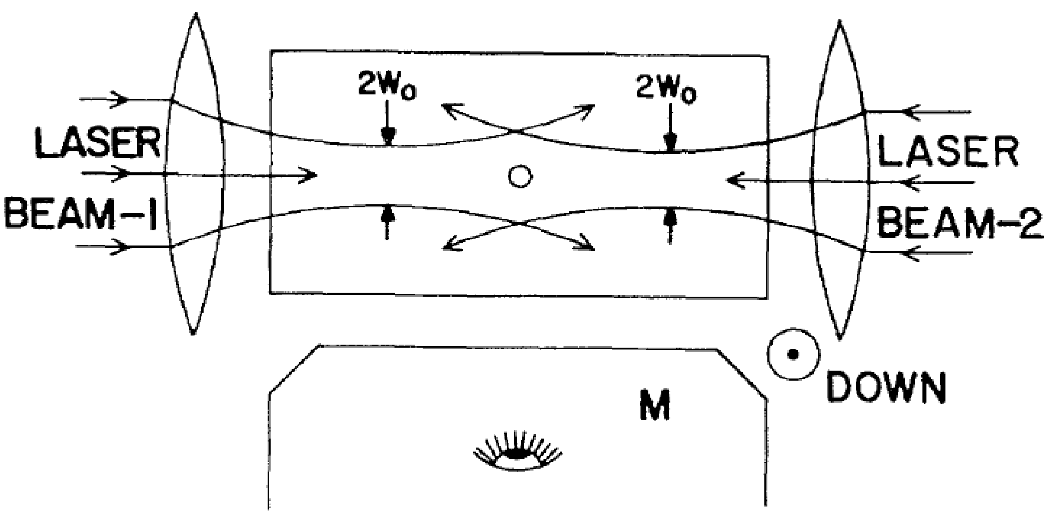
Force = mC/t = **P/C**

(P = optical power)

….calculate for a 3mW laser pointer….



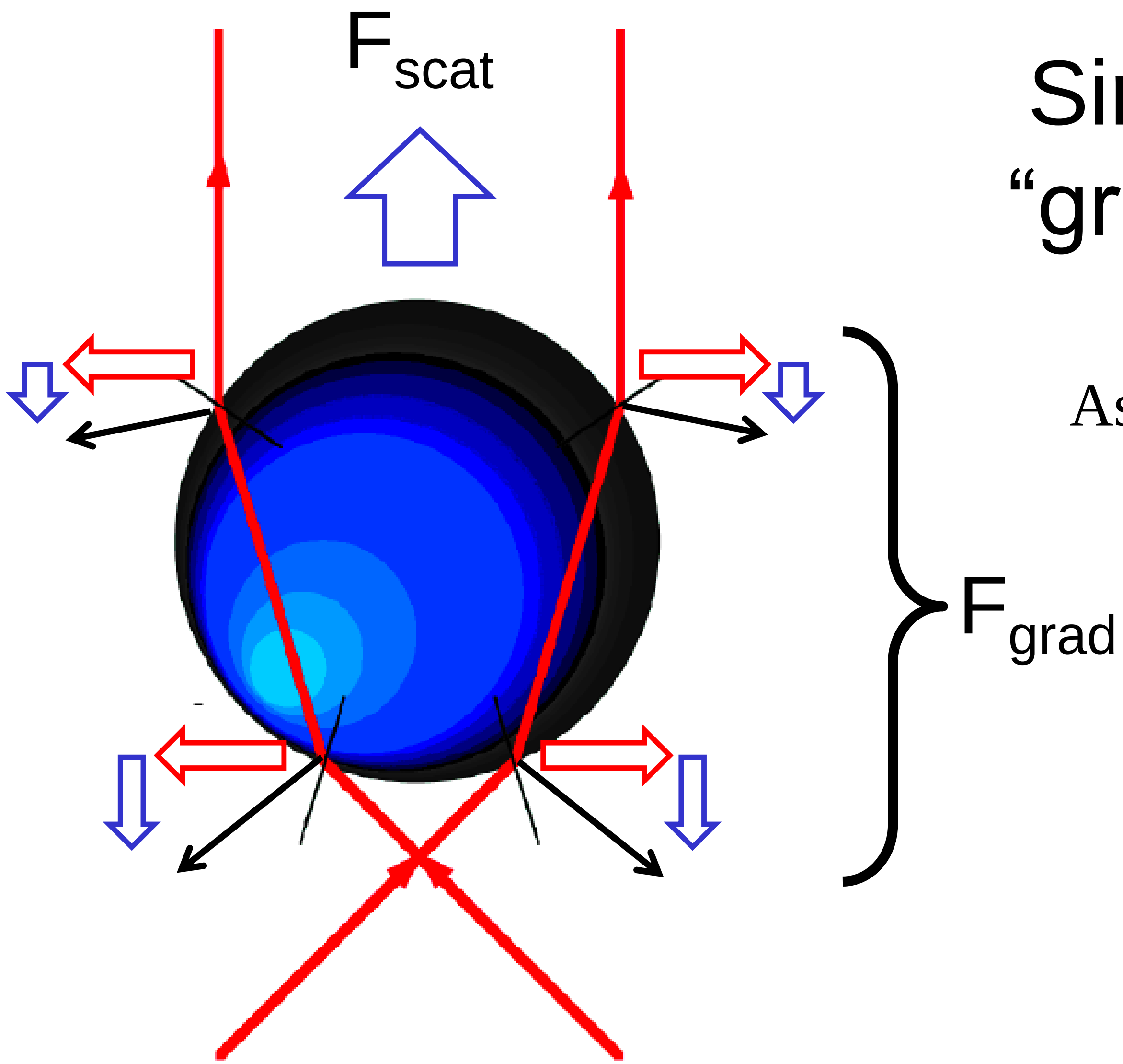
(a)



(b)

3-D trap using counter-propagating laser beams

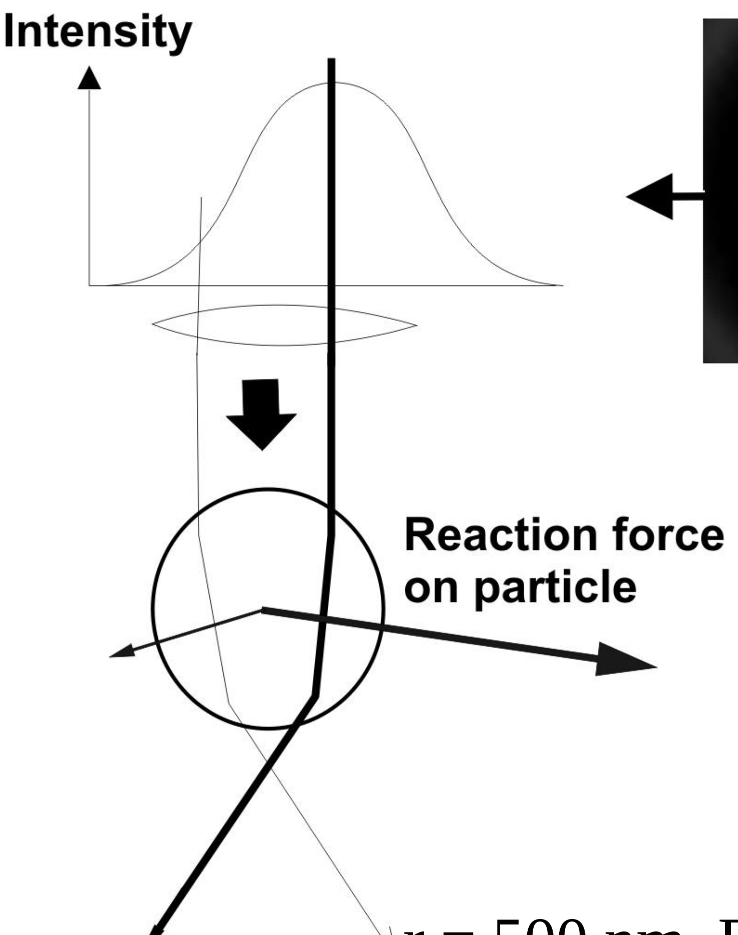
Ashkin & Dziedzic, 1971

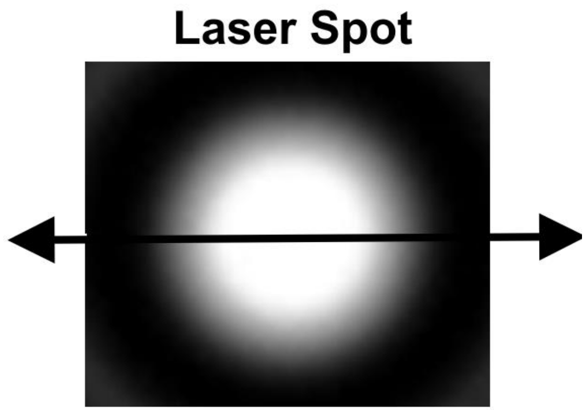


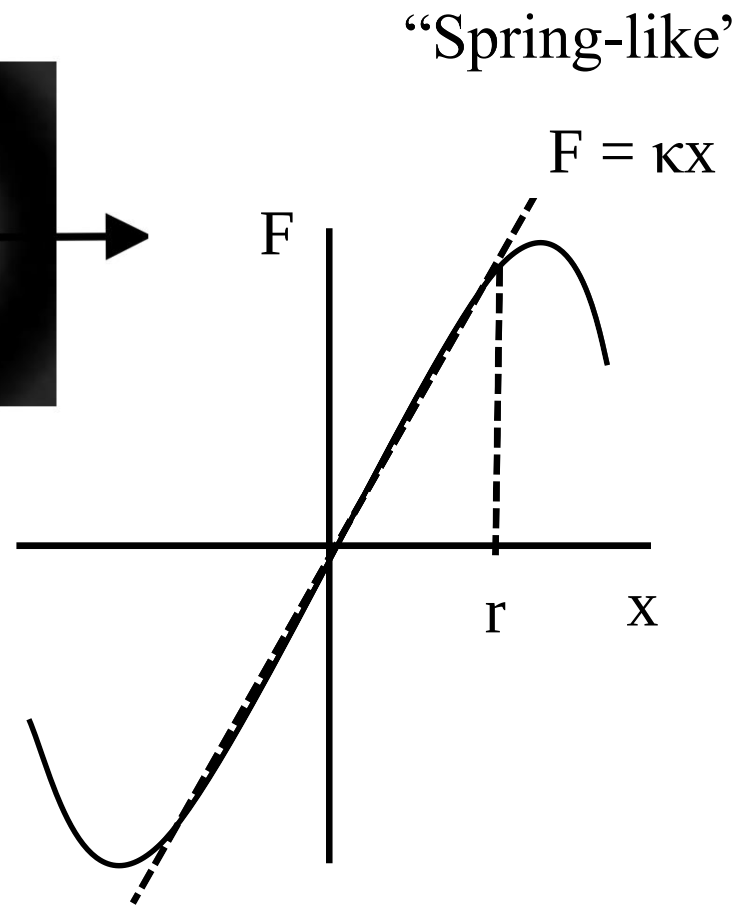
Single beam "gradient trap"

Ashkin *et al.* 1986

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

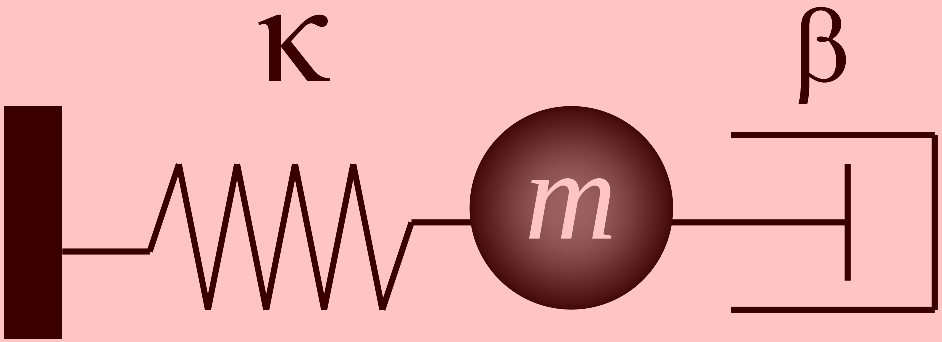






$r = 500$ nm, $F_{max} = 10$ pN   Typical: $\kappa = 0.02$ pN.nm^{-1}

Dynamic response



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

Stoke's drag

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

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

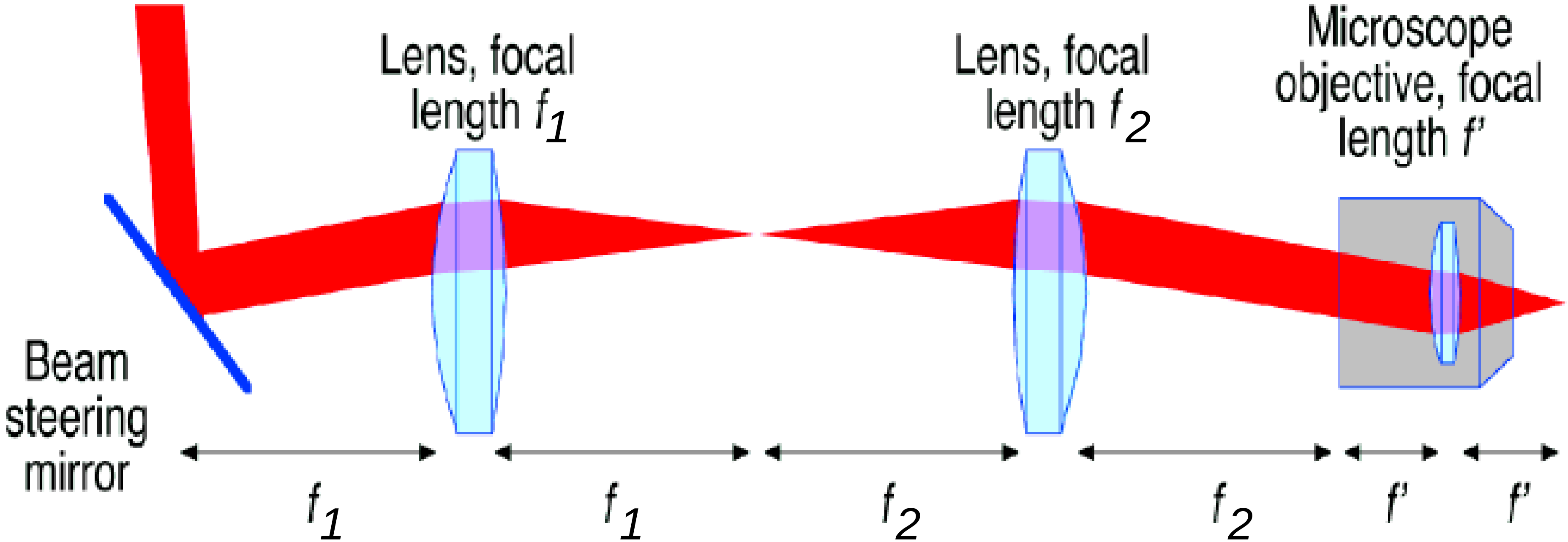
$$f_c = \frac{\kappa}{2\pi\beta} \quad < \mathbf{1\ kHz}$$

Typical values:

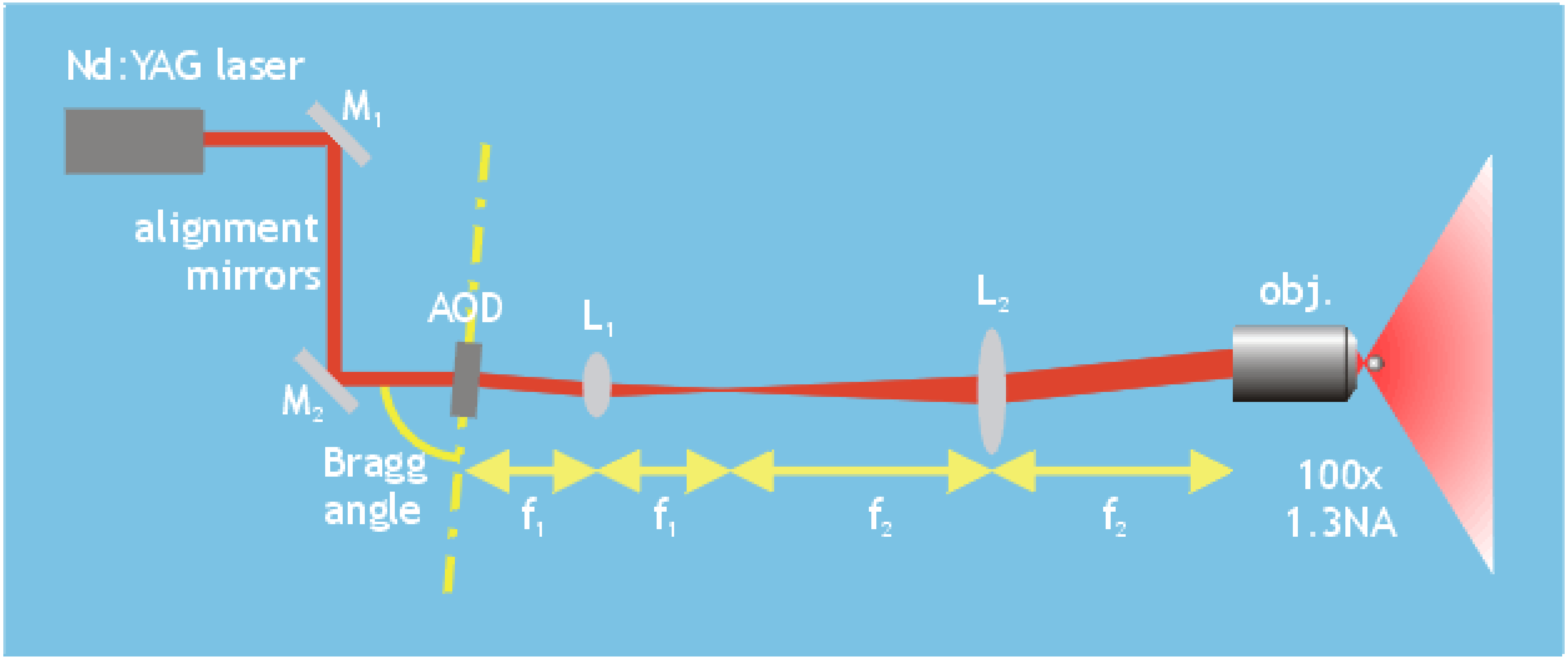
$m = 5\text{x}10^{-16}$ kg

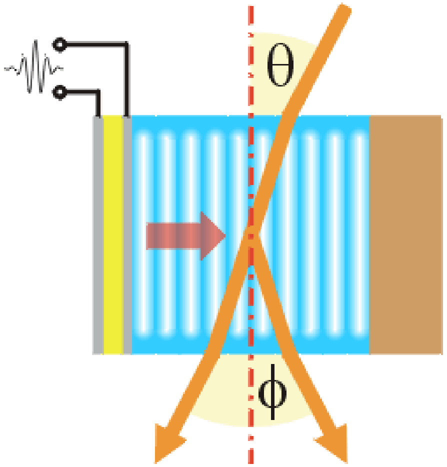
$\beta = 1\text{x}10^{-8}$ N.s.m^{-1}

$\kappa \sim 1\text{x}10^{-5}$ N.m^{-1}



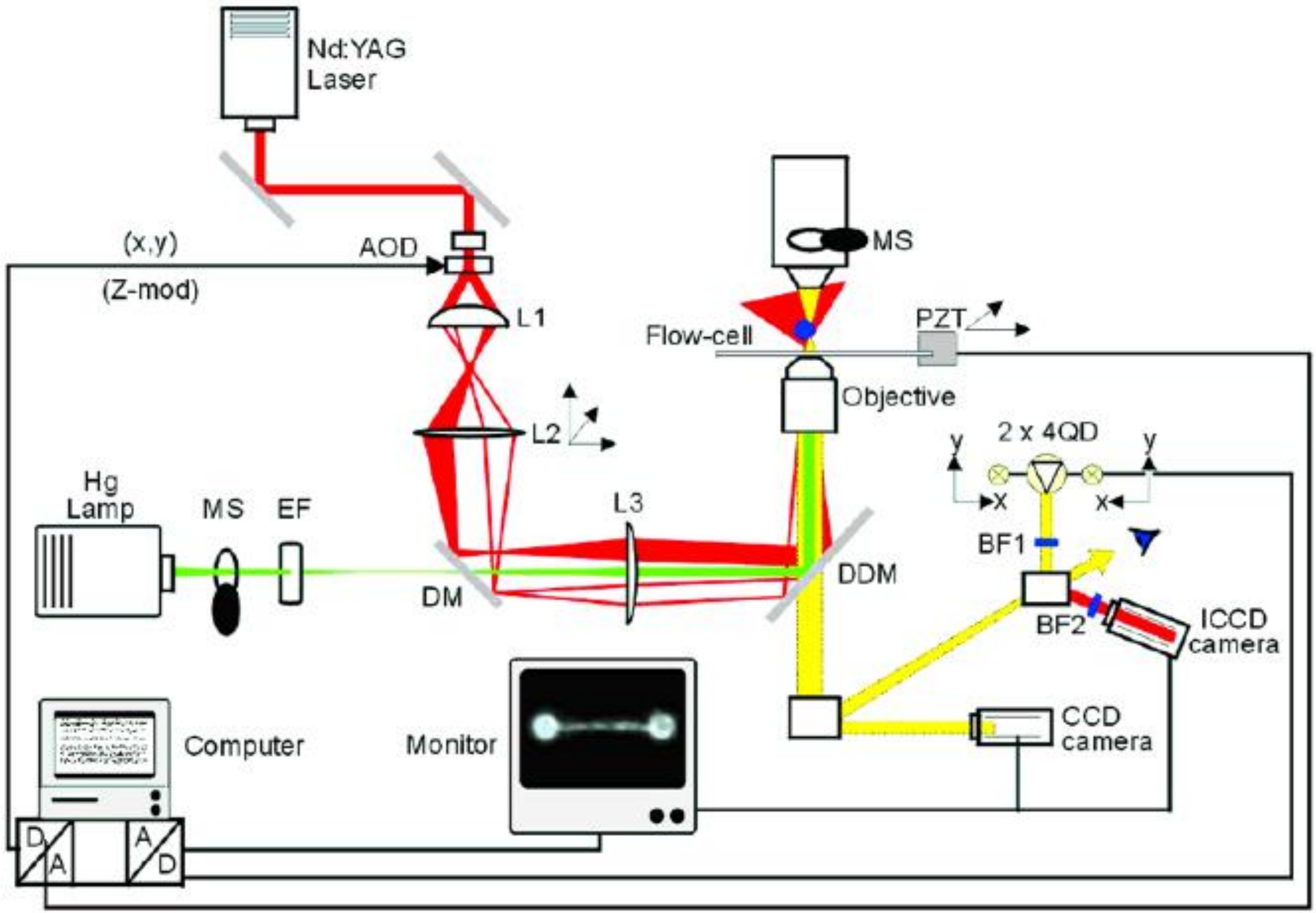
Molloy & Padgett (2002) *Contemporary Physics* **43:**241-258

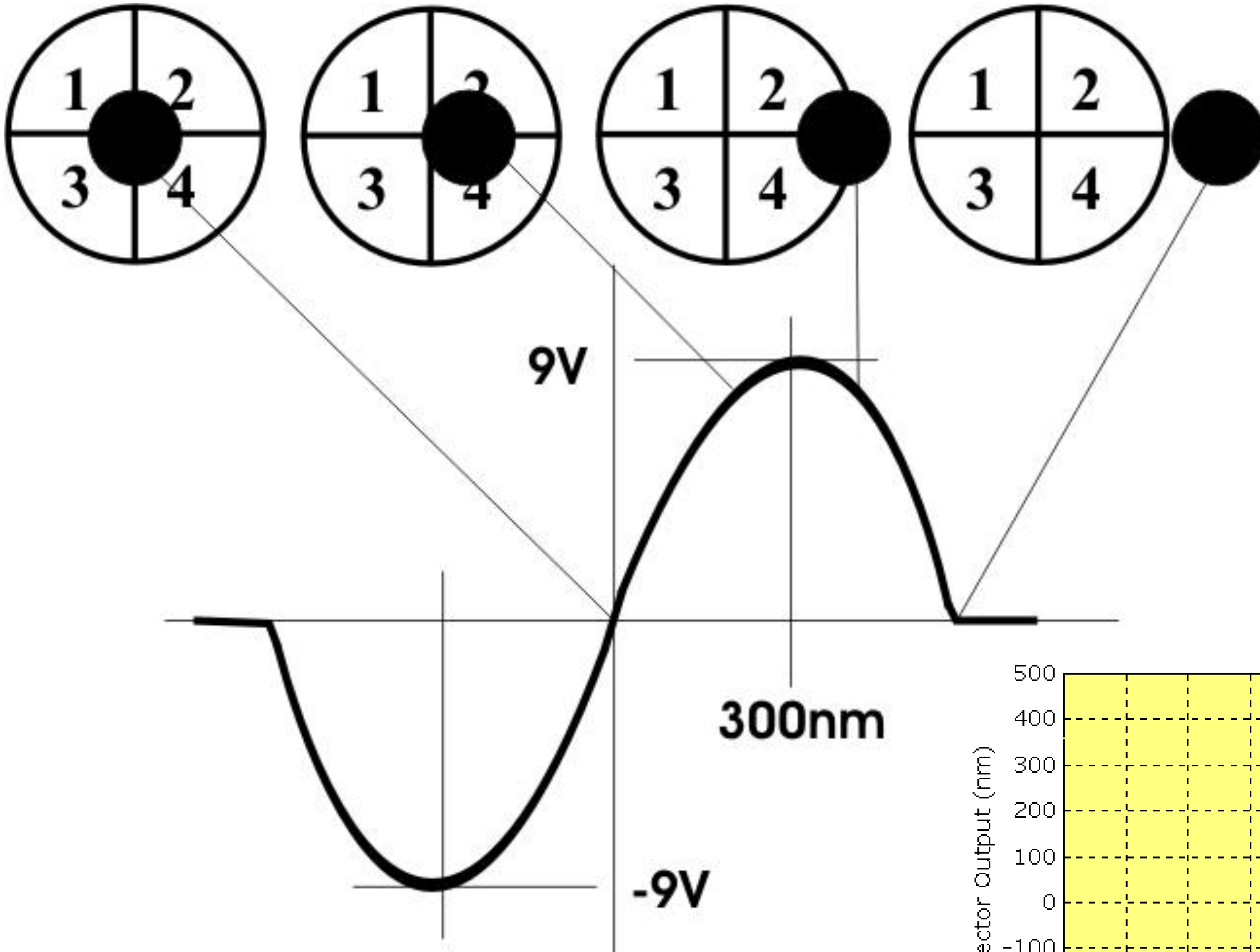


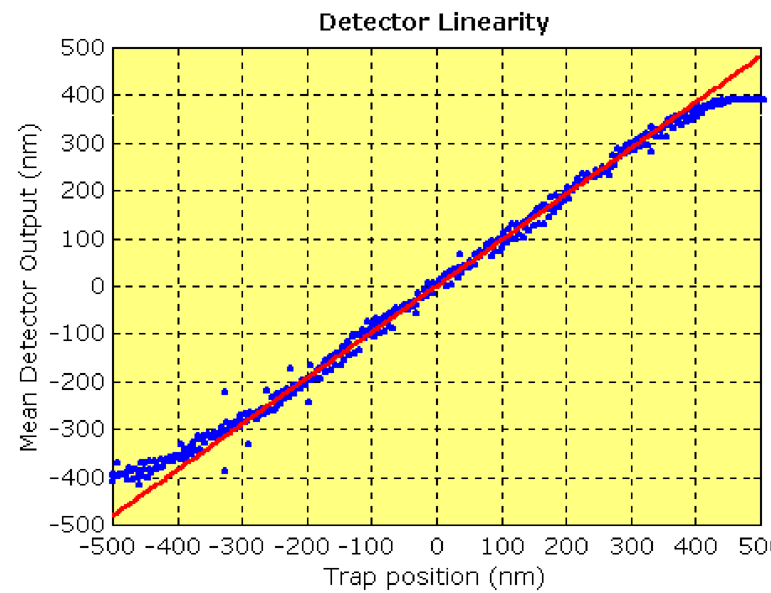


Move Laser beam very rapidly using Acousto-Optic Device "AOD"

Realistically – things are a bit more complicated!

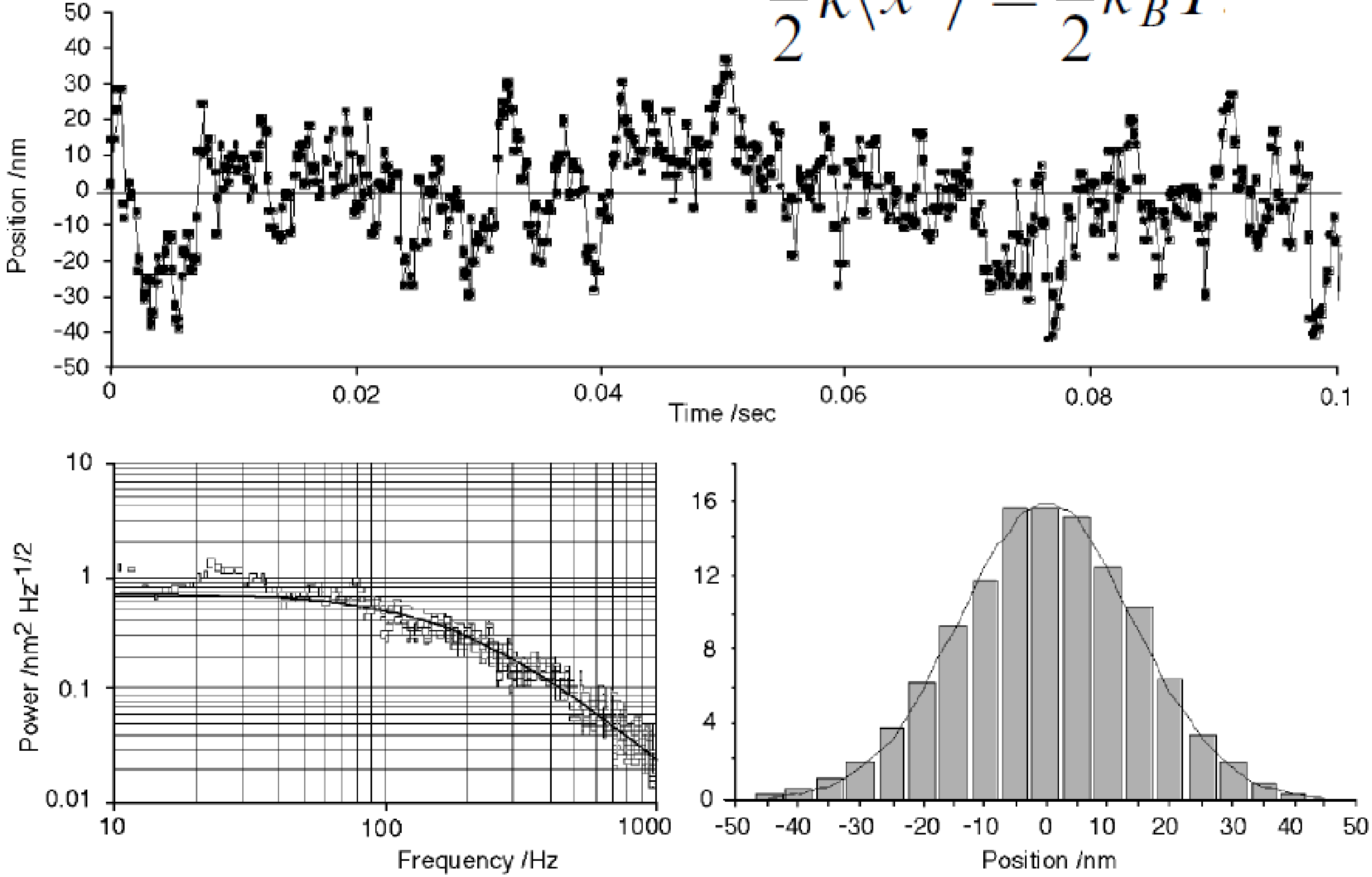



1
2
3
4
9V
300nm
-9V


Detector Linearity
Mean Detector Output (nm)
500
400
300
200
100
0
-100
-200
-300
-400
-500
-500 -400 -300 -200 -100 0 100 200 300 400 500
Trap position (nm)

Thermal motion of an optically trapped particle

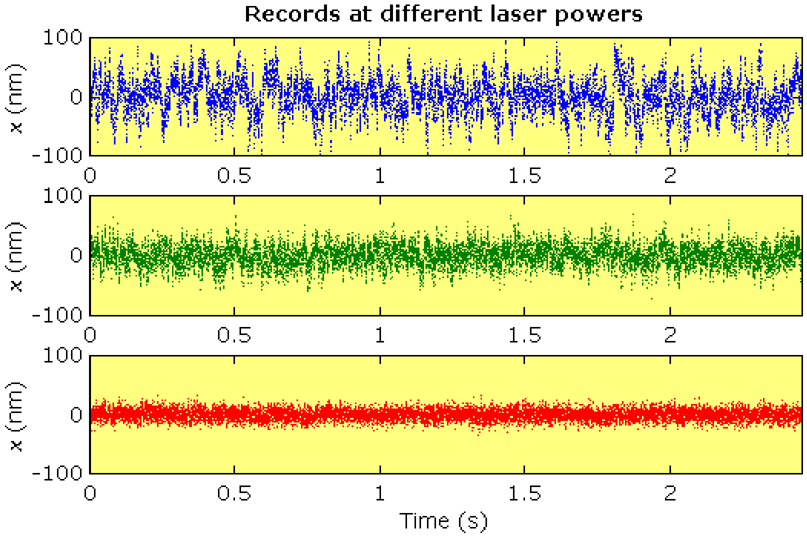
$$\frac{1}{2}\kappa\langle x^2\rangle = \frac{1}{2}k_B T$$

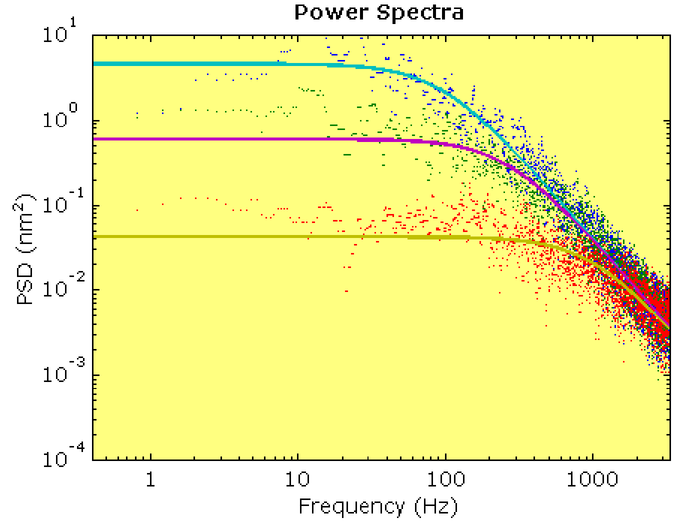


Thermal noise is ~ 14 nm r.m.s.

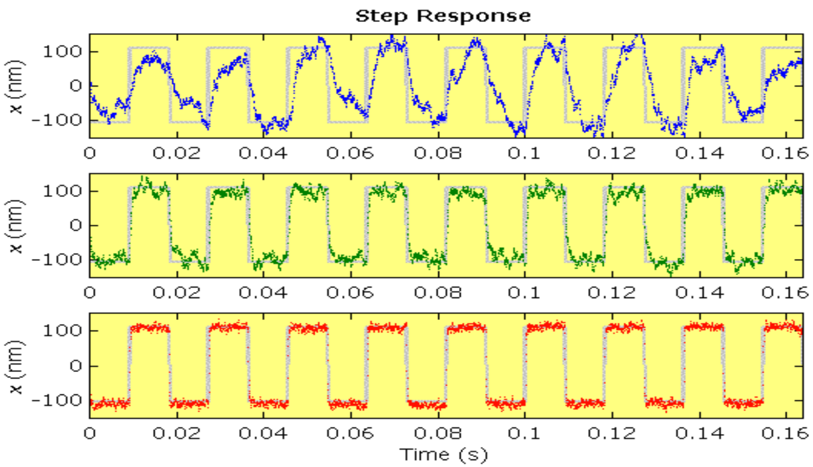
Calibrate optical trap stiffness

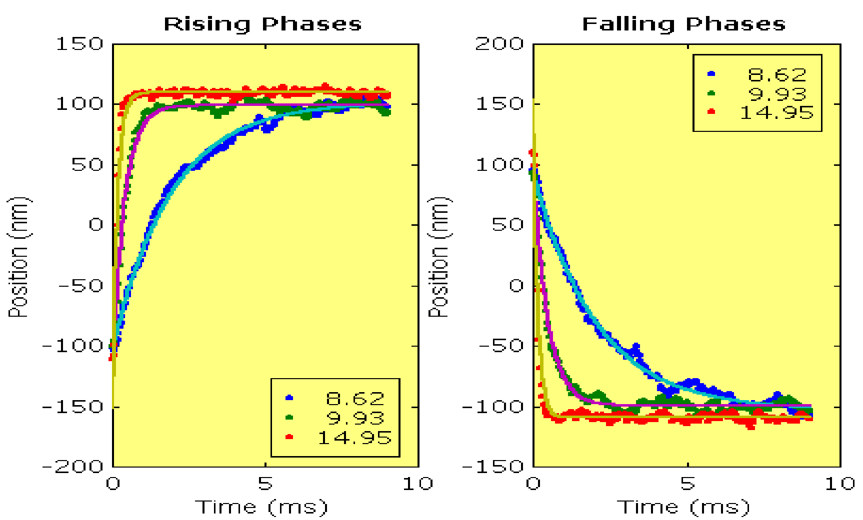
1) Record thermal noise

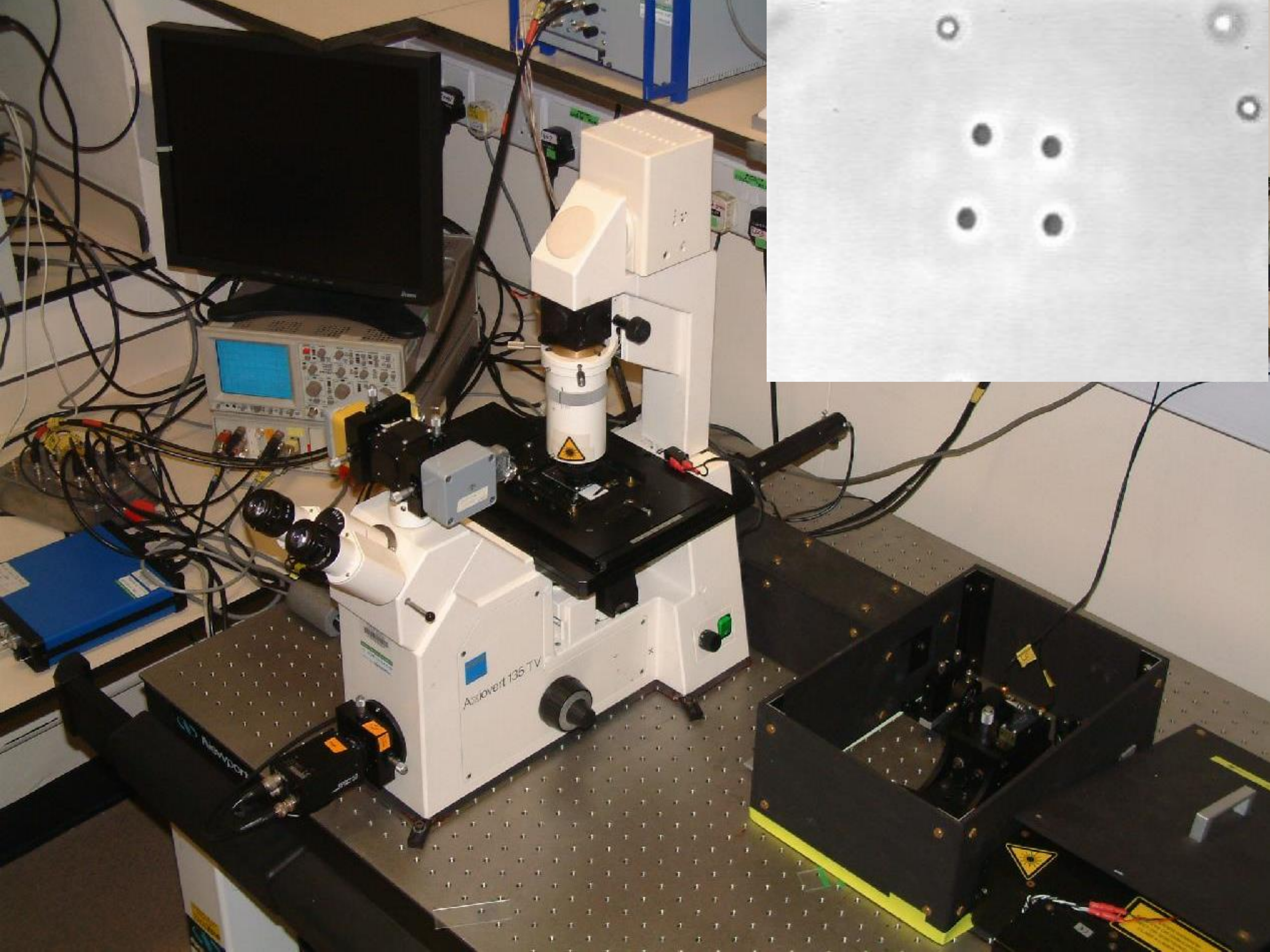


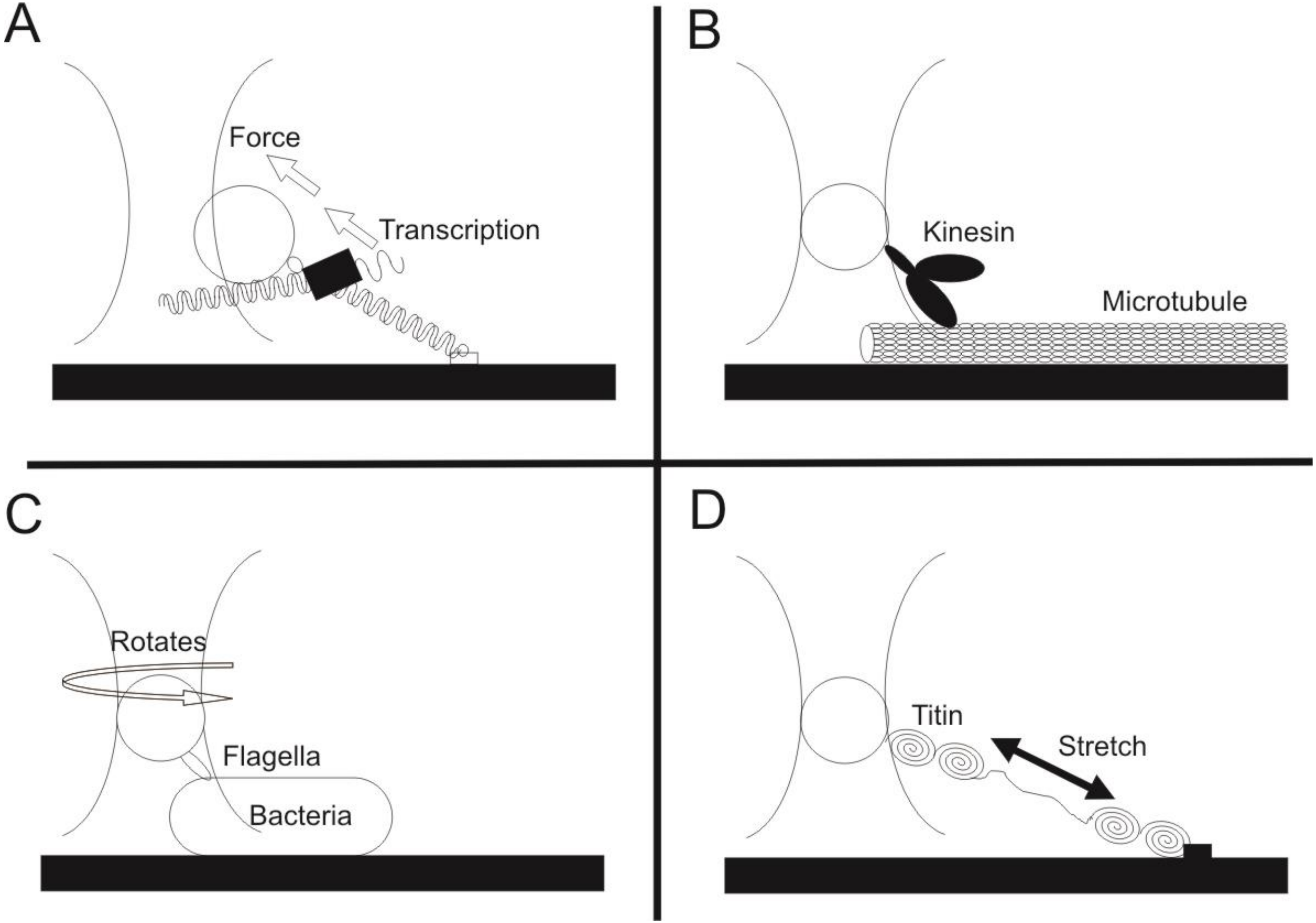


2) Impose viscous drag






Axiovert 135 TV


A
Force
Transcription
B
Kinesin
Microtubule
C
Rotates
Flagella
Bacteria
D
Titin
Stretch

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 calculations:

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

{ $1 pN.nm = 1x10^{-21}$ Joules }

SINGLE MOLECULE TECHNOLOGIES:

- **Some single molecule methods have built-in *gain* (or signal amplification)**

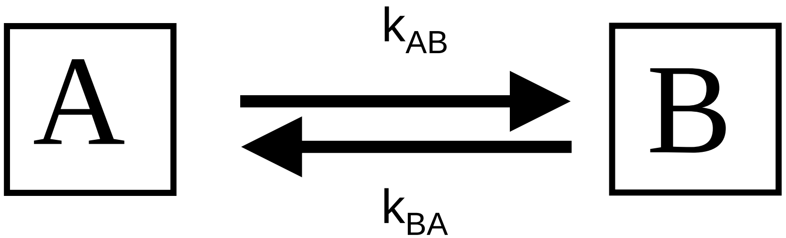
  - **Electrical measurements:** – opening of a single ion channel allows thousands of ions to flow across a membrane – this can be measured without greatly affecting the state of the channel

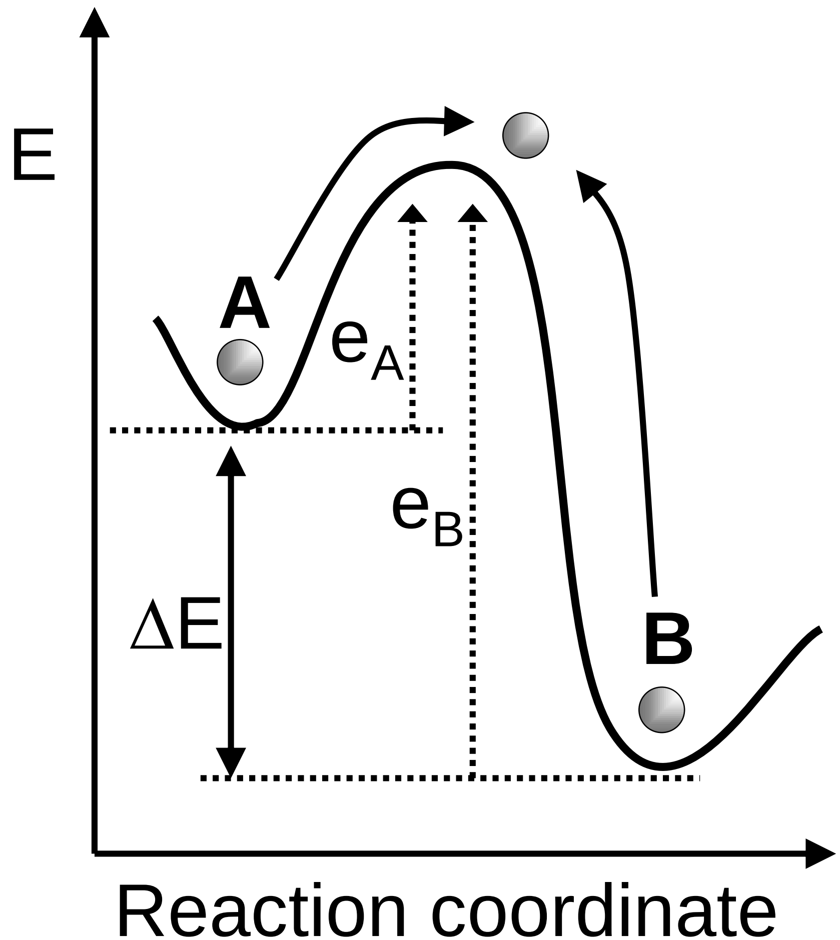
  - **Optical methods:** – A single fluorophore can emit millions of photons and output does not (usually) affect the mechanical or chemical properties of the system being studied.

Mechanical Studies “no built-in gain” - ***DIFFICULTY 6.6***

- <u>Optical Tweezers</u>
  - Low force regime (e.g. “conformational” changes)
  - Total spatial control in 3-dimensions
  - Protein-Protein & Protein-Ligand interactions
- <u>MagneticTweezers</u>
  - Low force regime (only z-axis control)
  - Ability to apply torque (twist)
  - DNA topology and DNA-protein interactions
- <u>AFM</u>
  - High force regime (e.g. unfolding)
  - Imaging (e.g. surface profiling + other methods)
  - Protein-Protein & Protein-Ligand interactions

SINGLE MOLECULE DATA SETS

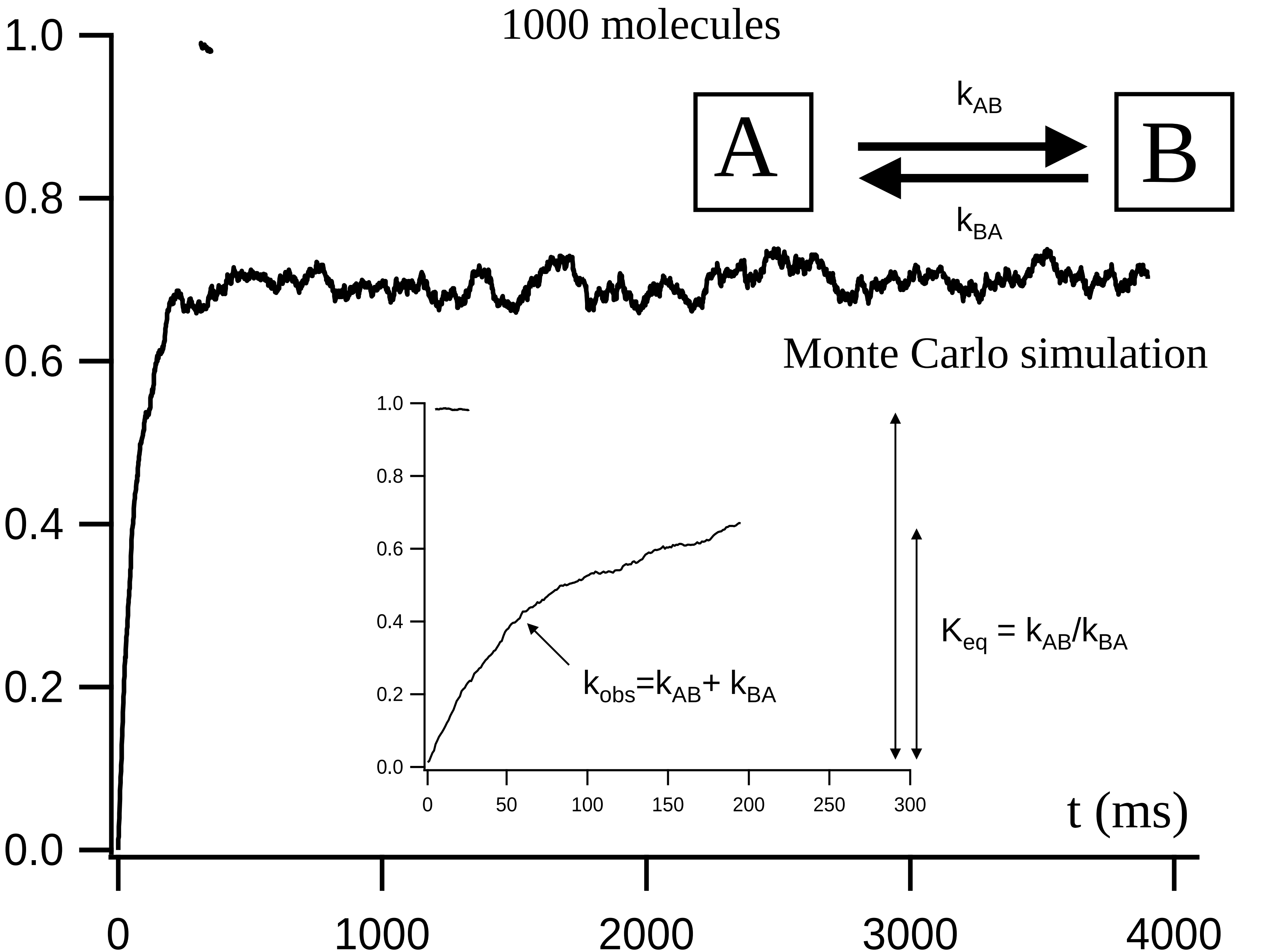




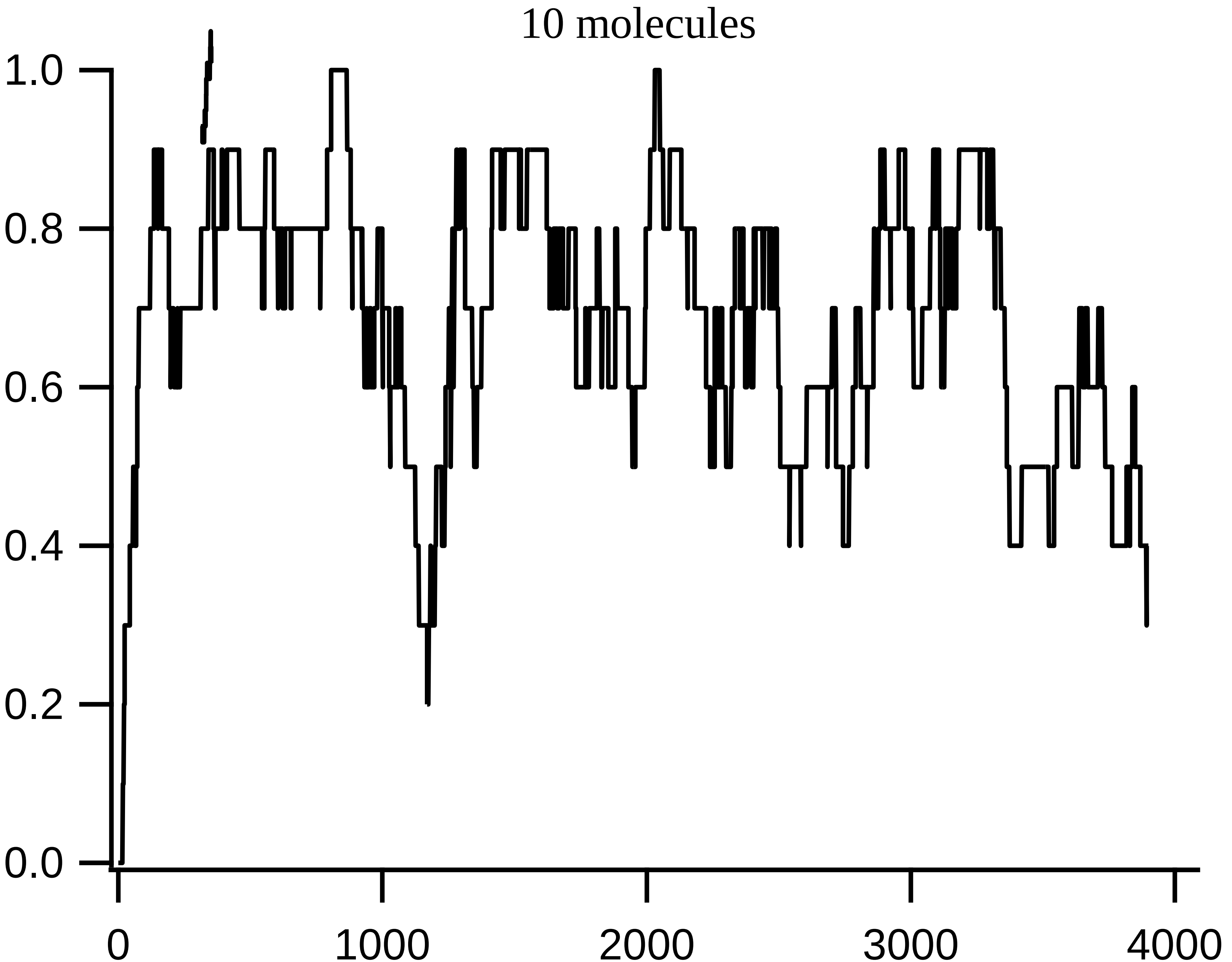
Transition state theory describes the kinetic properties of the system

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

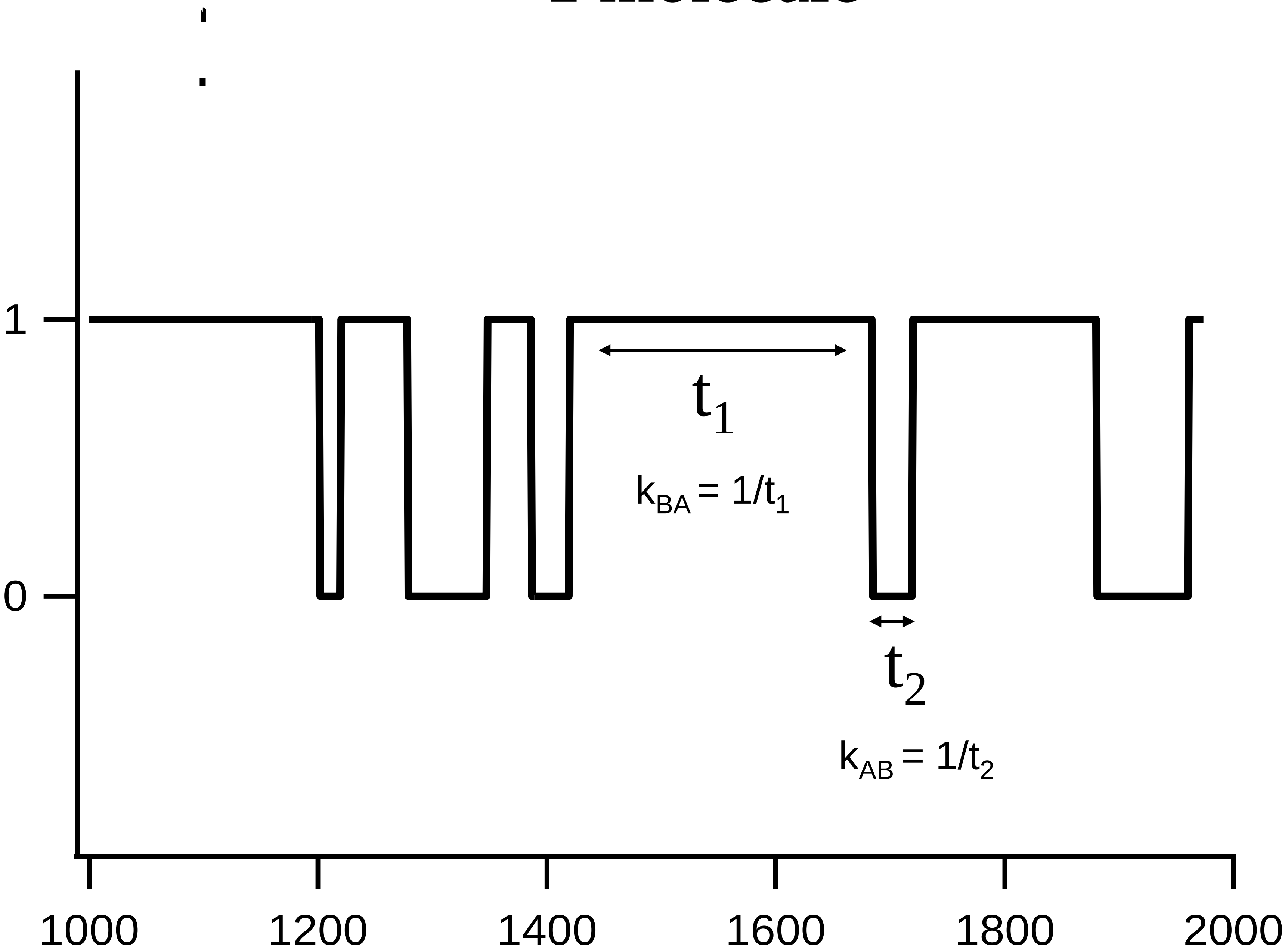
$$K = \frac{k_{AB}}{k_{BA}} = e^{\frac{-(e_B - e_A)}{k_b T}} = e^{\frac{-\Delta E}{k_b T}}$$



1000 molecules
A
B
k_{AB}
k_{BA}
Monte Carlo simulation
$K_{eq} = k_{AB}/k_{BA}$
$k_{obs}=k_{AB}+ k_{BA}$
t (ms)
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

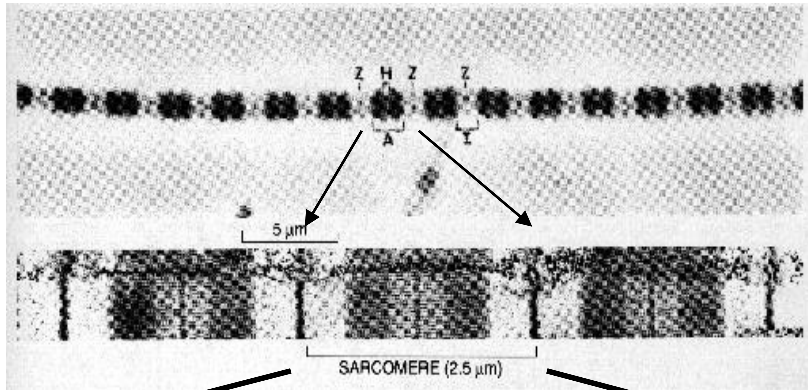
1 molecule



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

Filament sliding causes muscle to shorten:

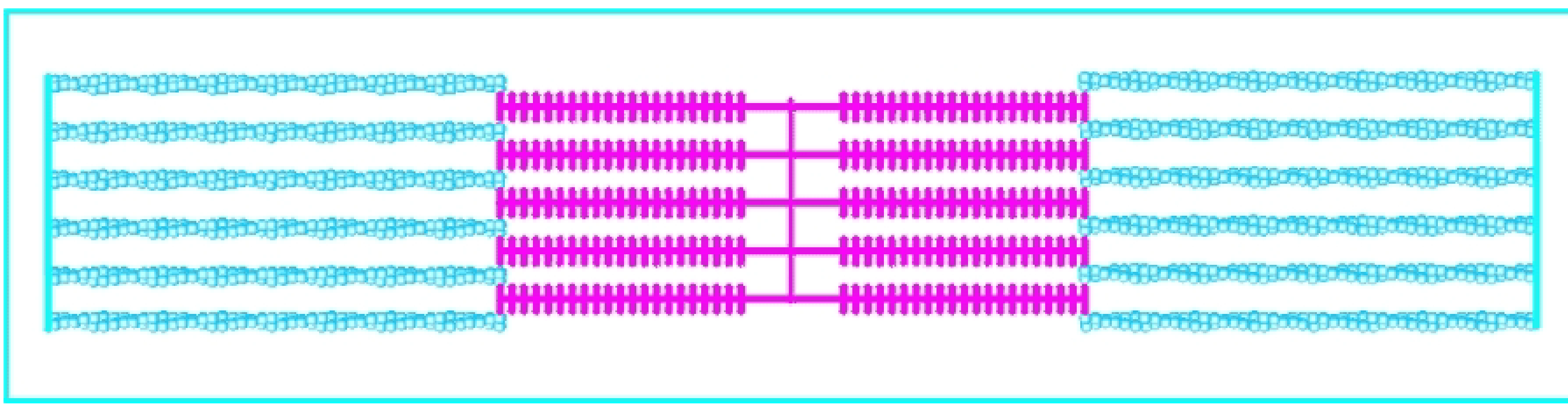
myofibril



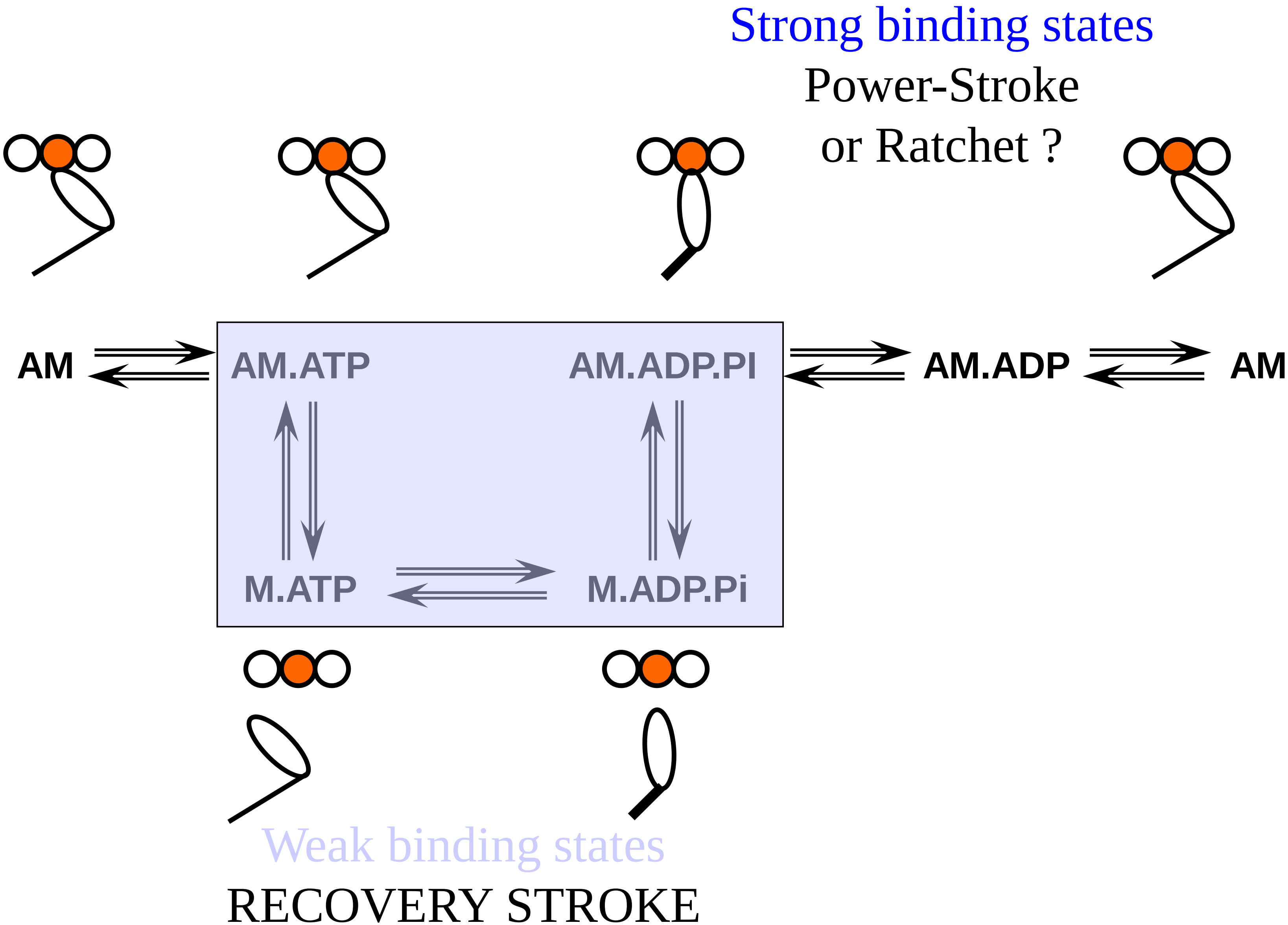
Light micrograph

Electron micrograph

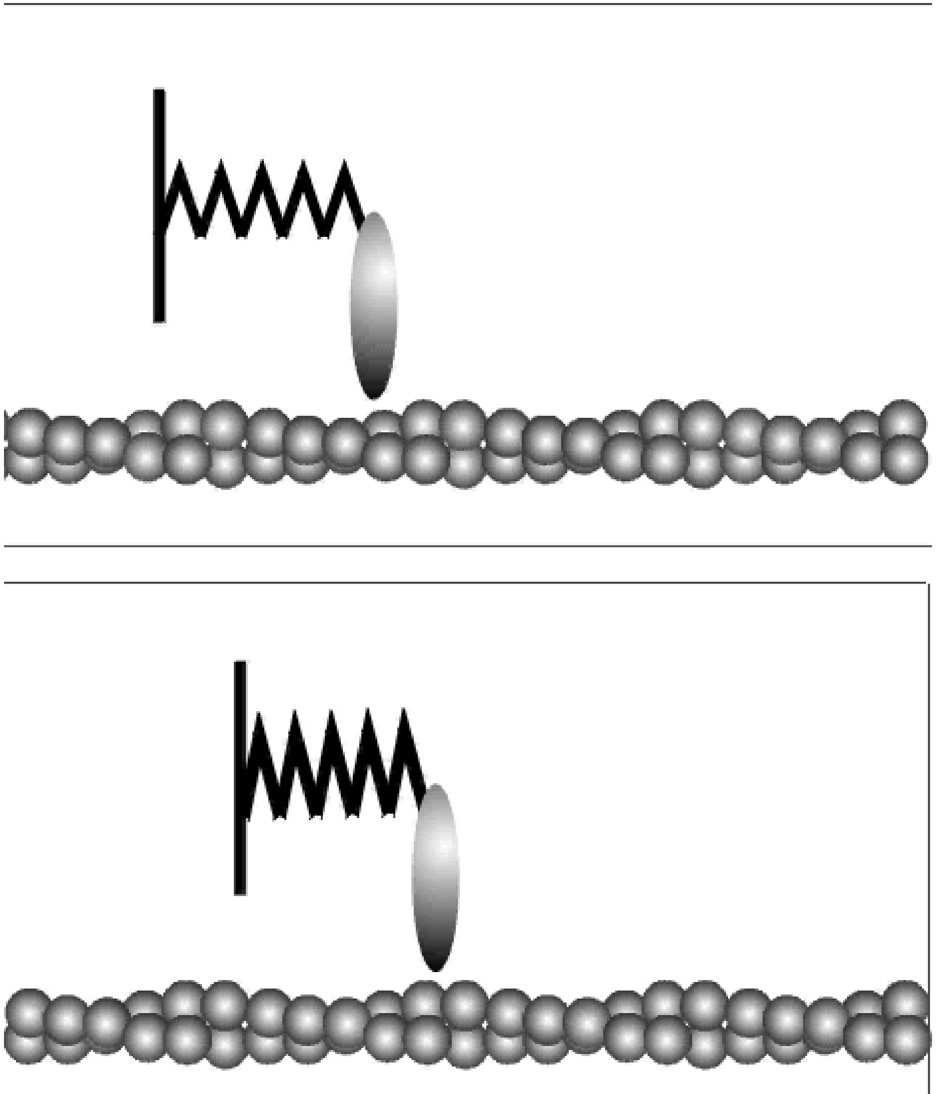
sarcomere



Acto-myosin ATPase pathway



How do myosin motors actually produce force and movement?

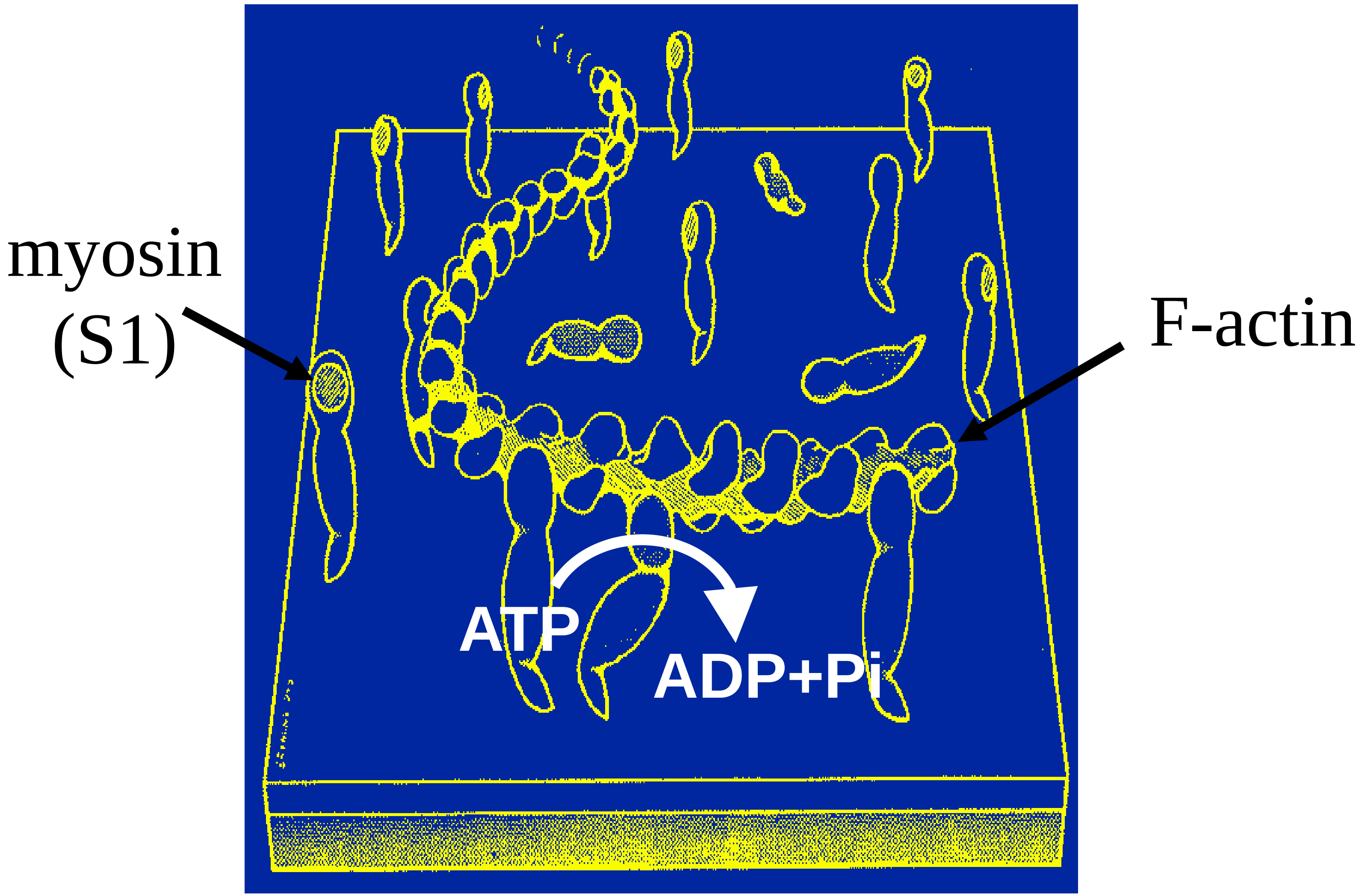


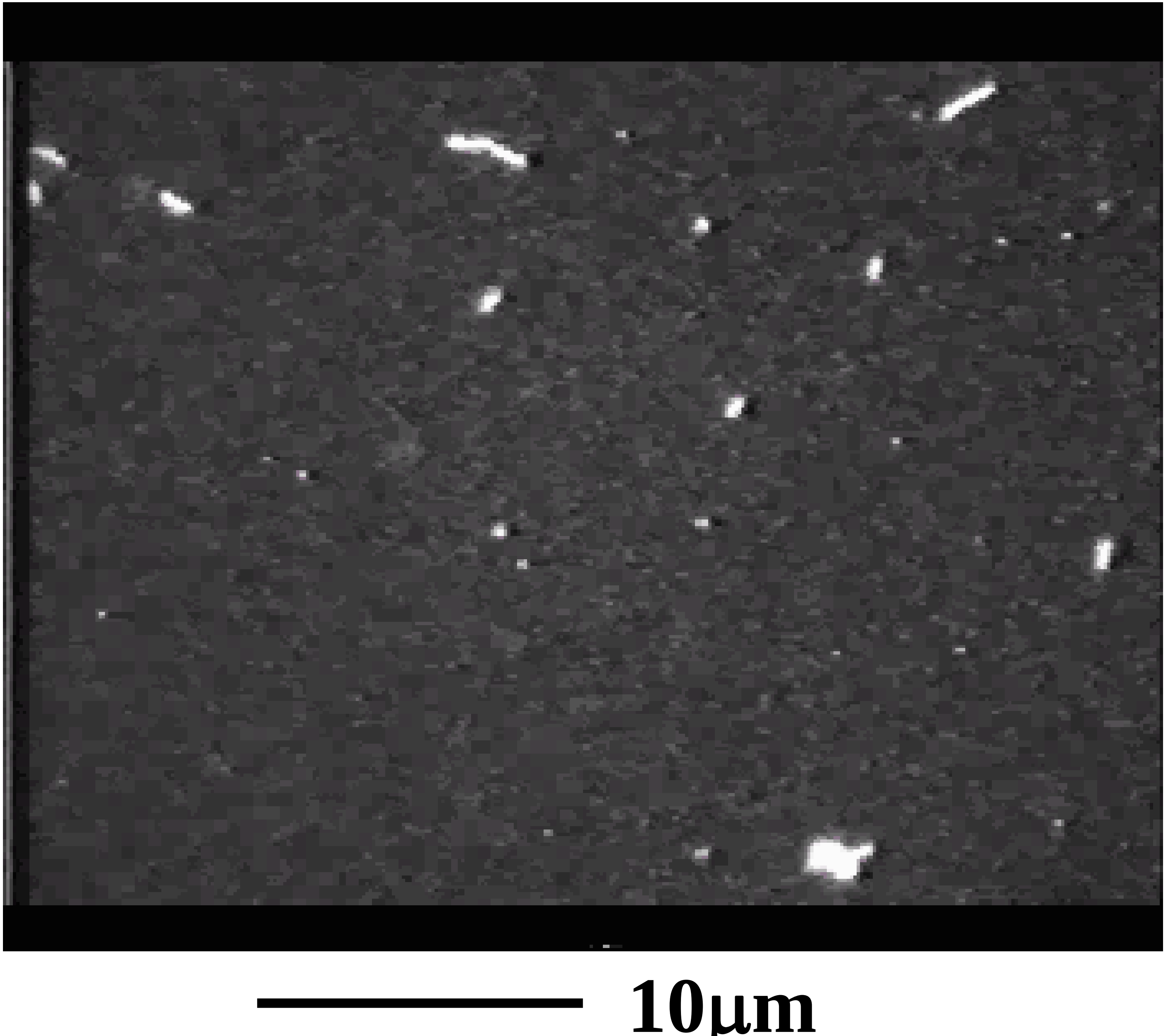
Thermal Ratchet

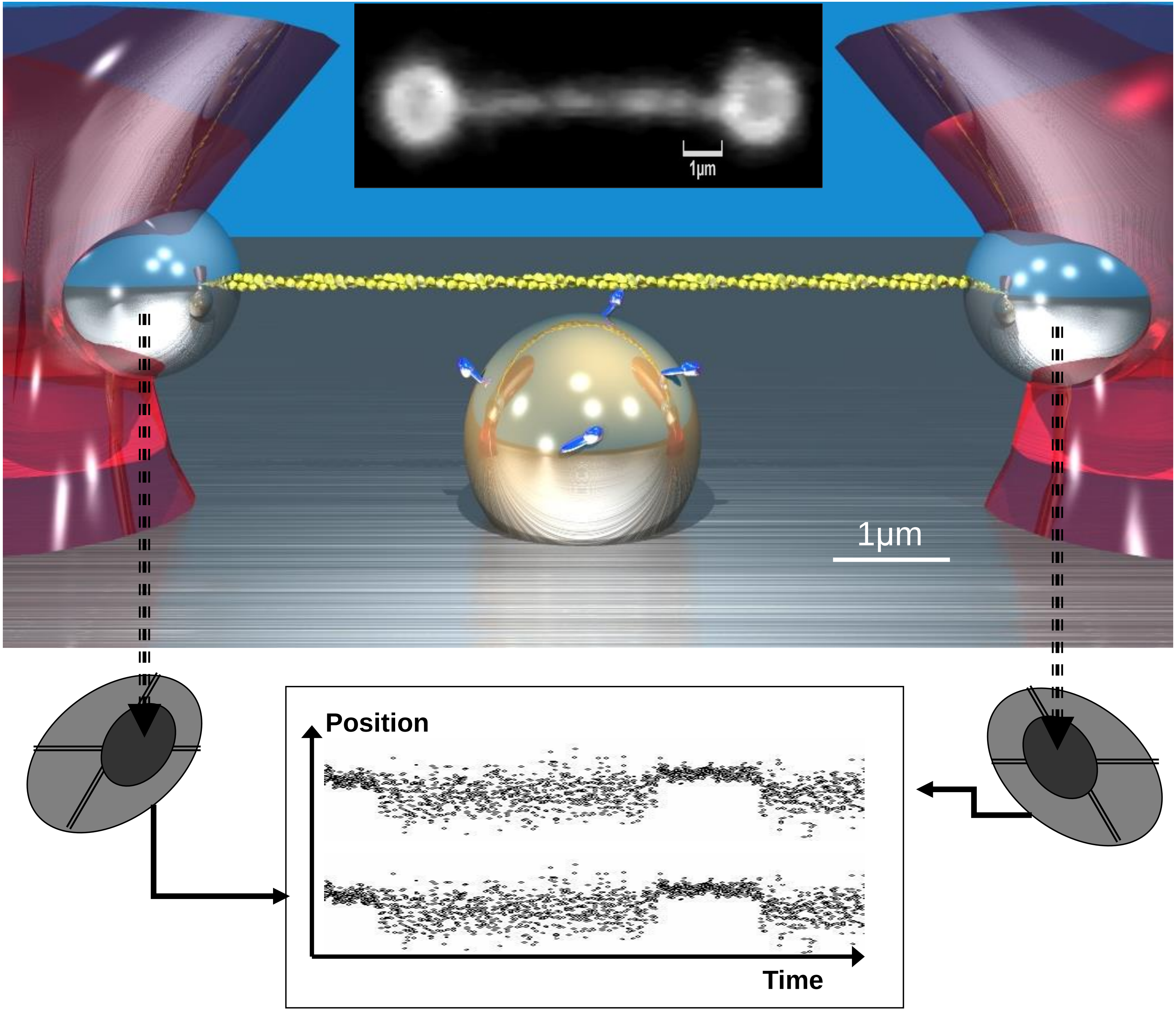
or

Powerstroke conformational change

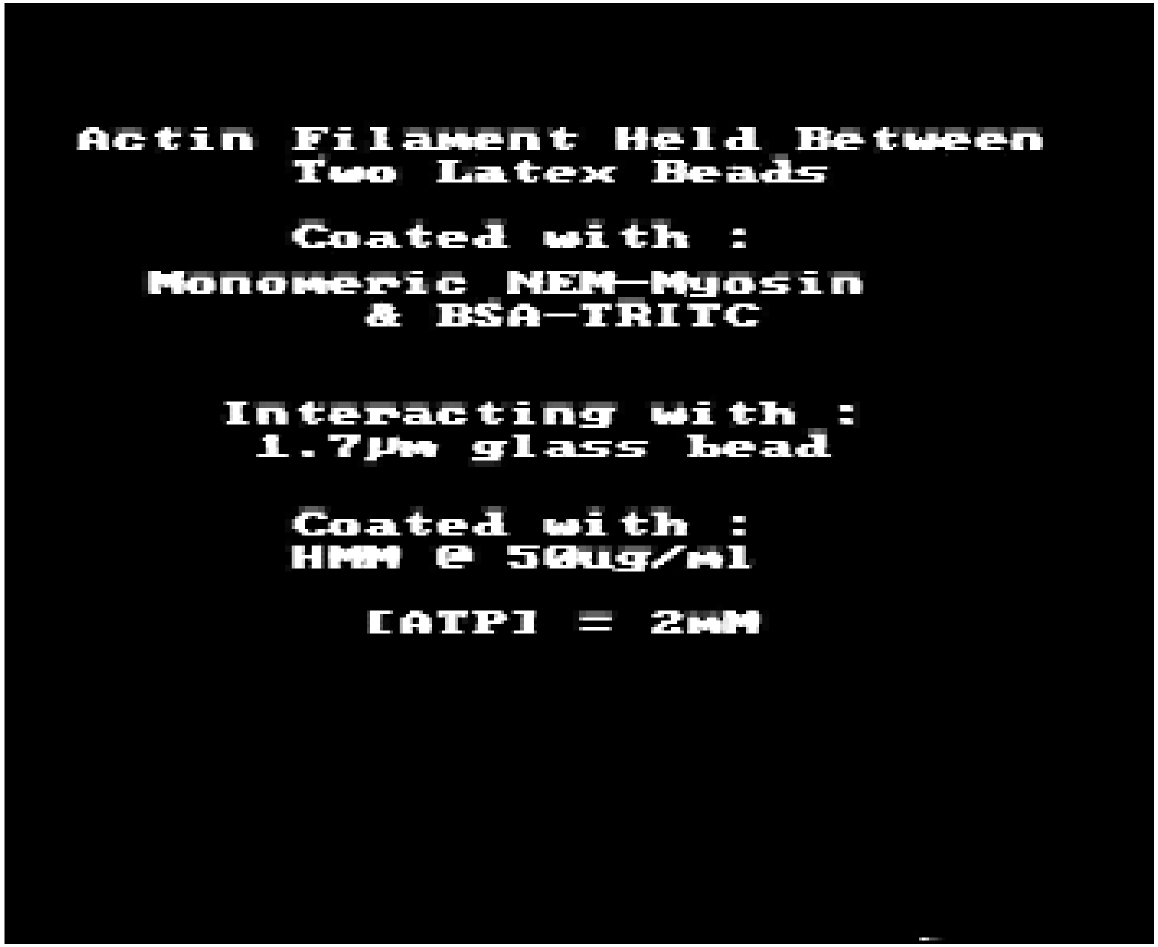
Acto-myosin *in vitro* motility assay :



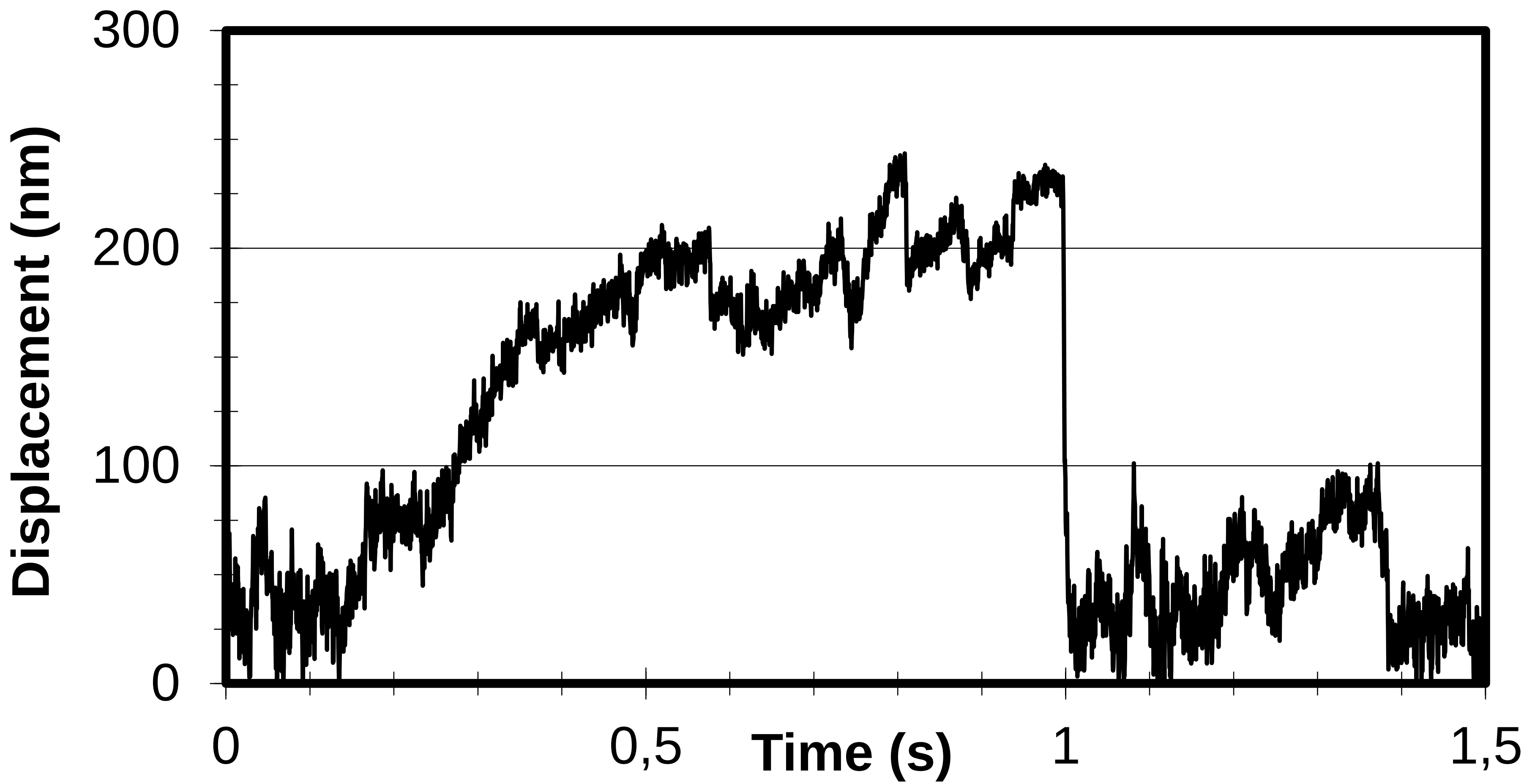

10μm


1µm
1µm
Position
Time

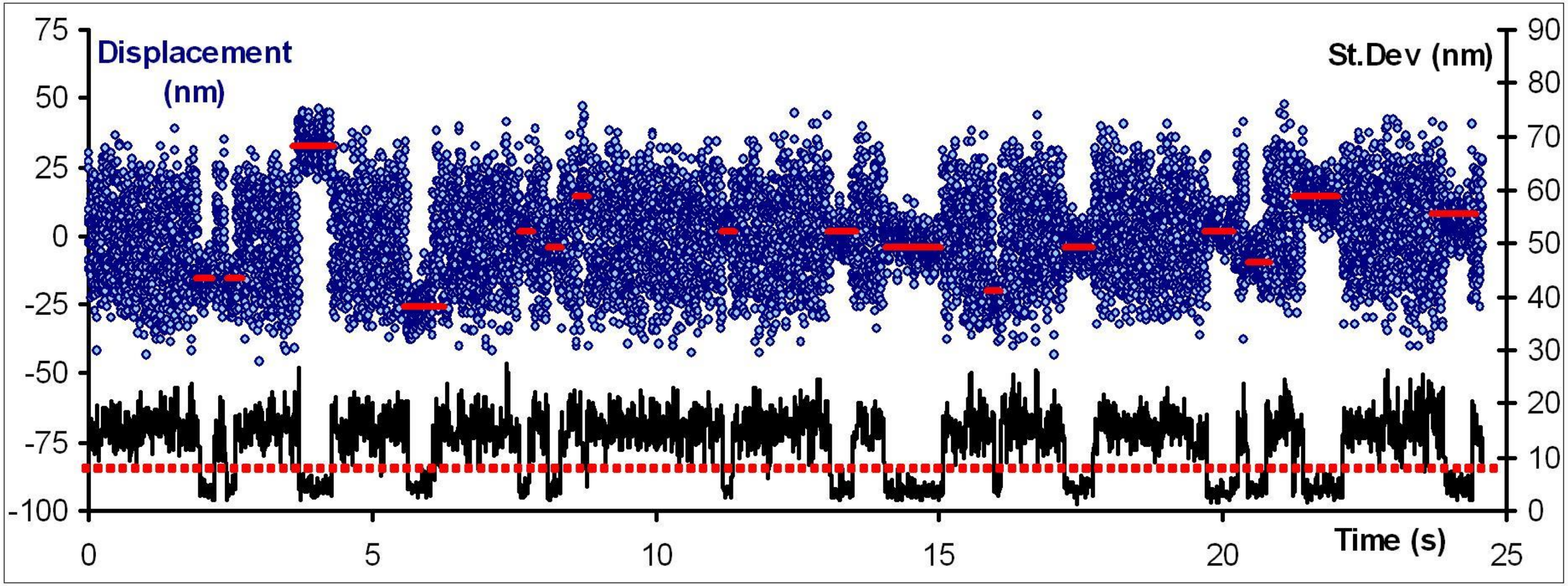
Optical trapping of acto-myosin



At <u>HIGH</u> myosin surface density many molecules work together to produce sliding.

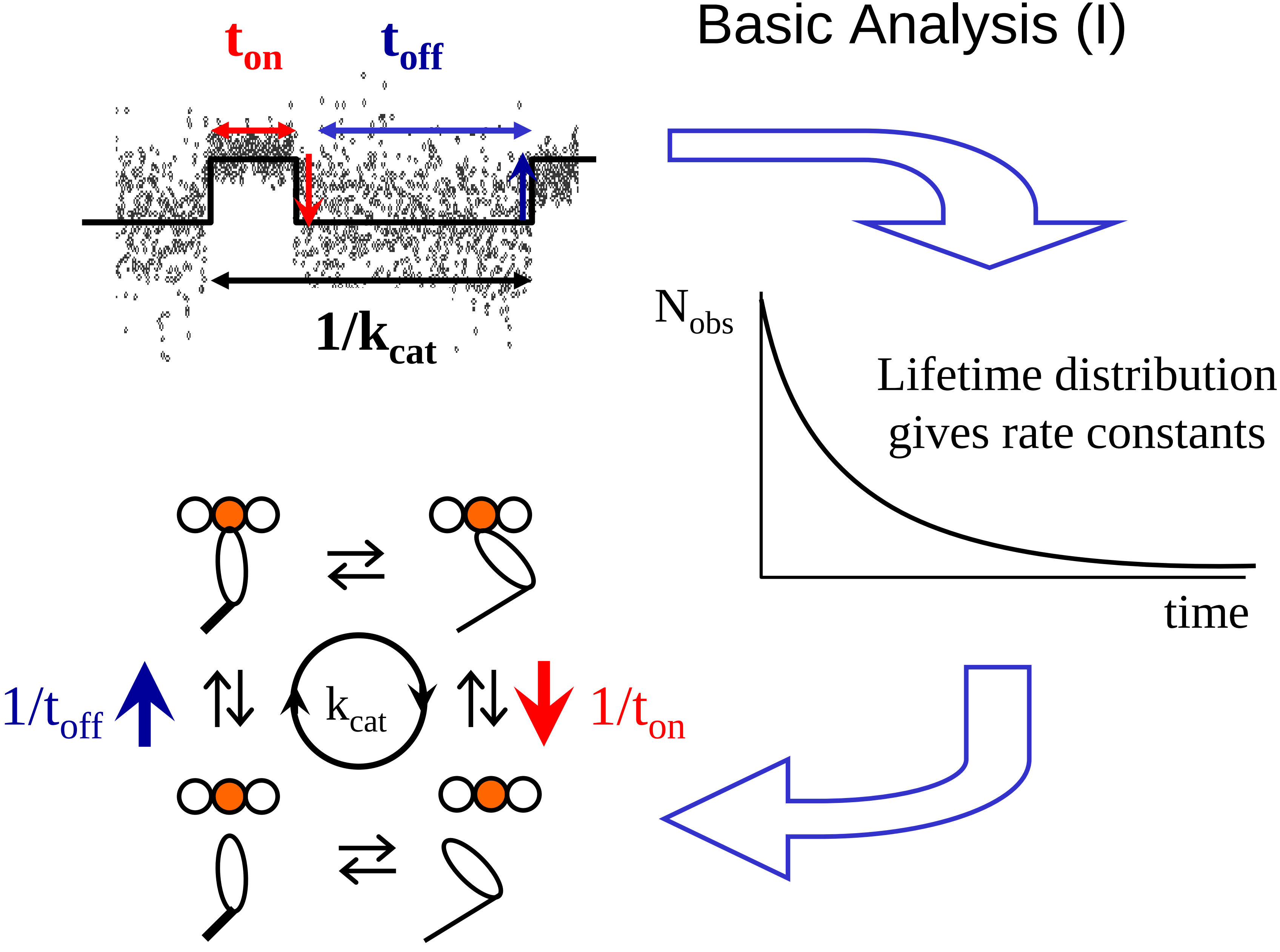


At LOW myosin surface density single binding interactions become visible.

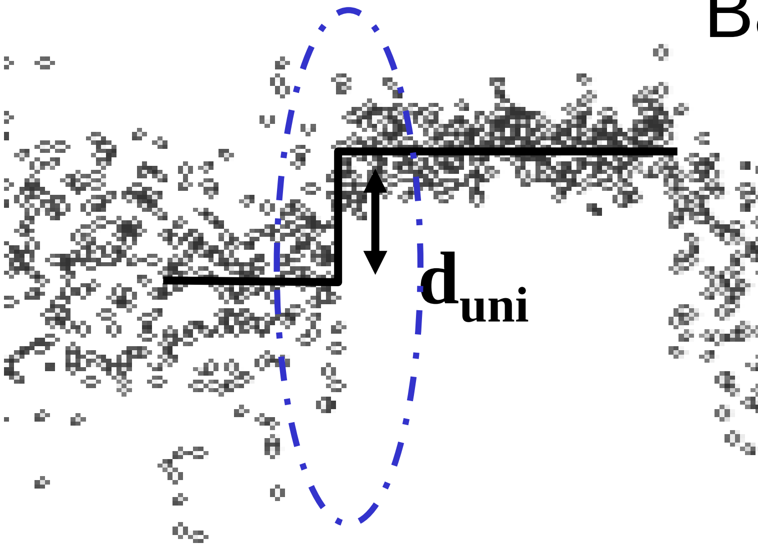


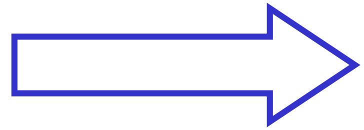
Note: The individual events are “mixed up” with the Brownian noise. But, when myosin binds the VARIANCE falls, this helps identify events.

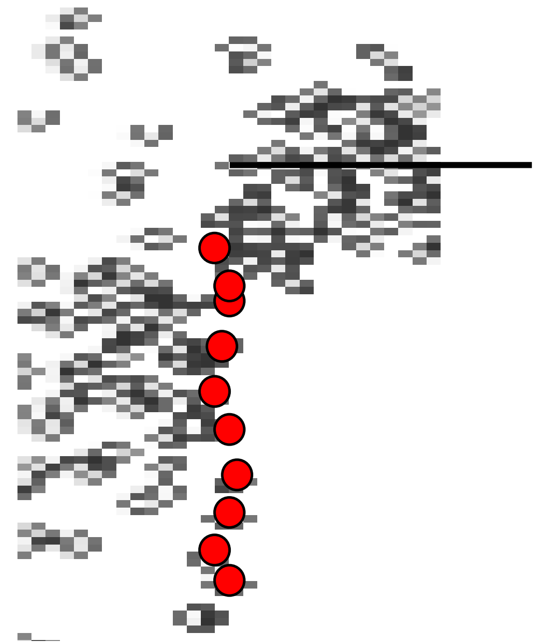
Basic Analysis (I)



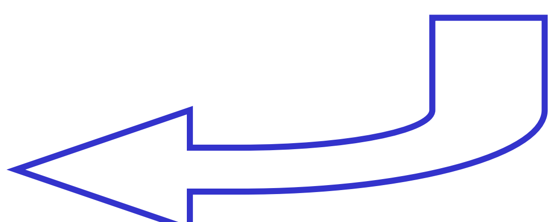
Basic Analysis (II)

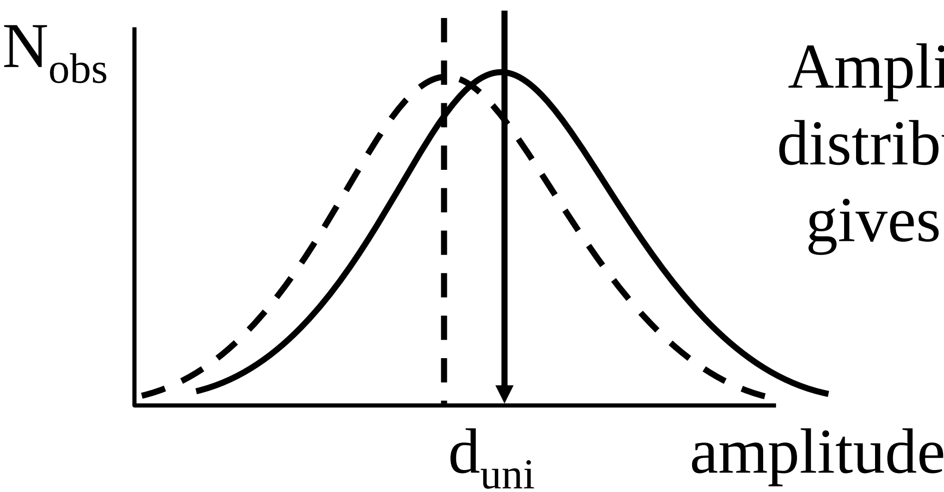






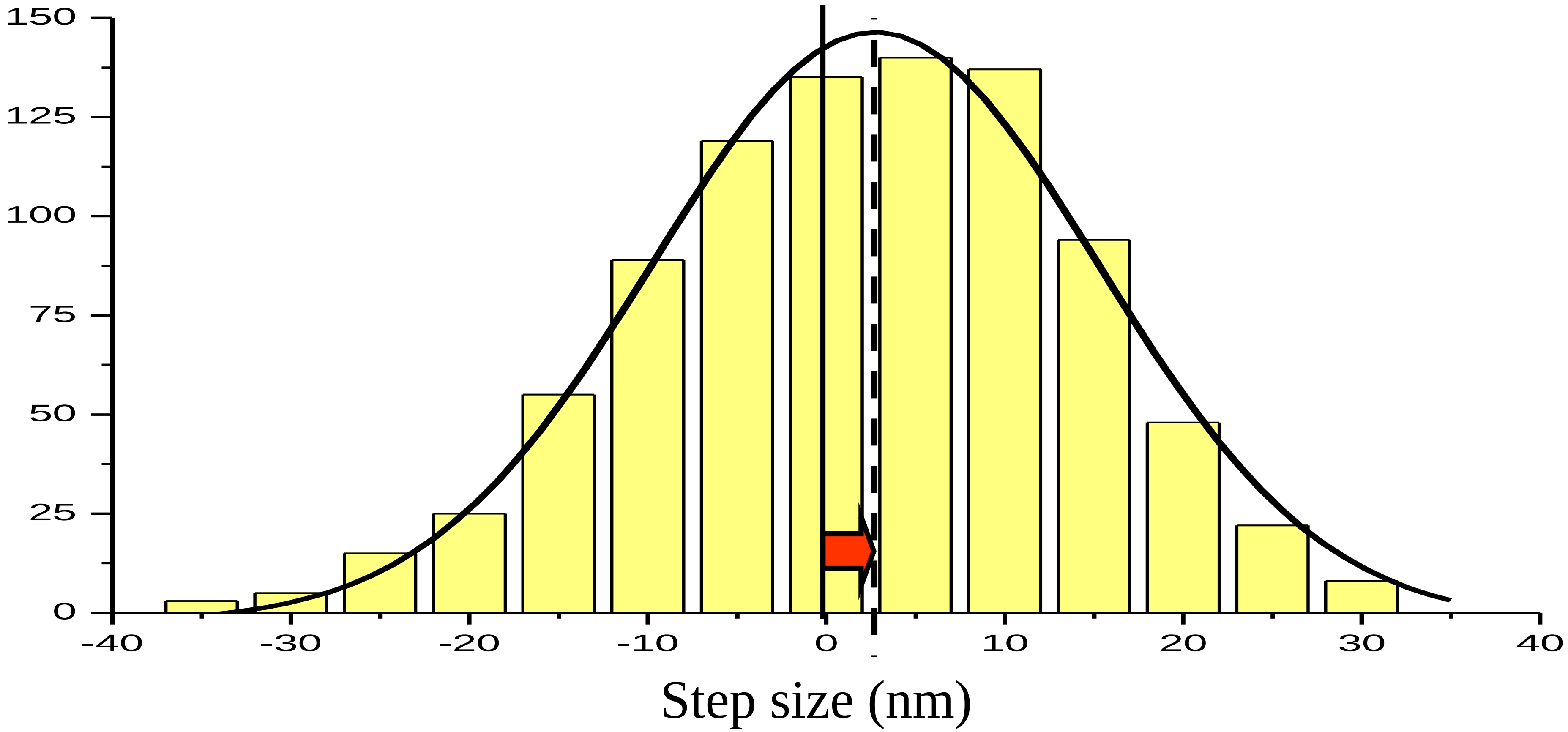
Start point is uncertain



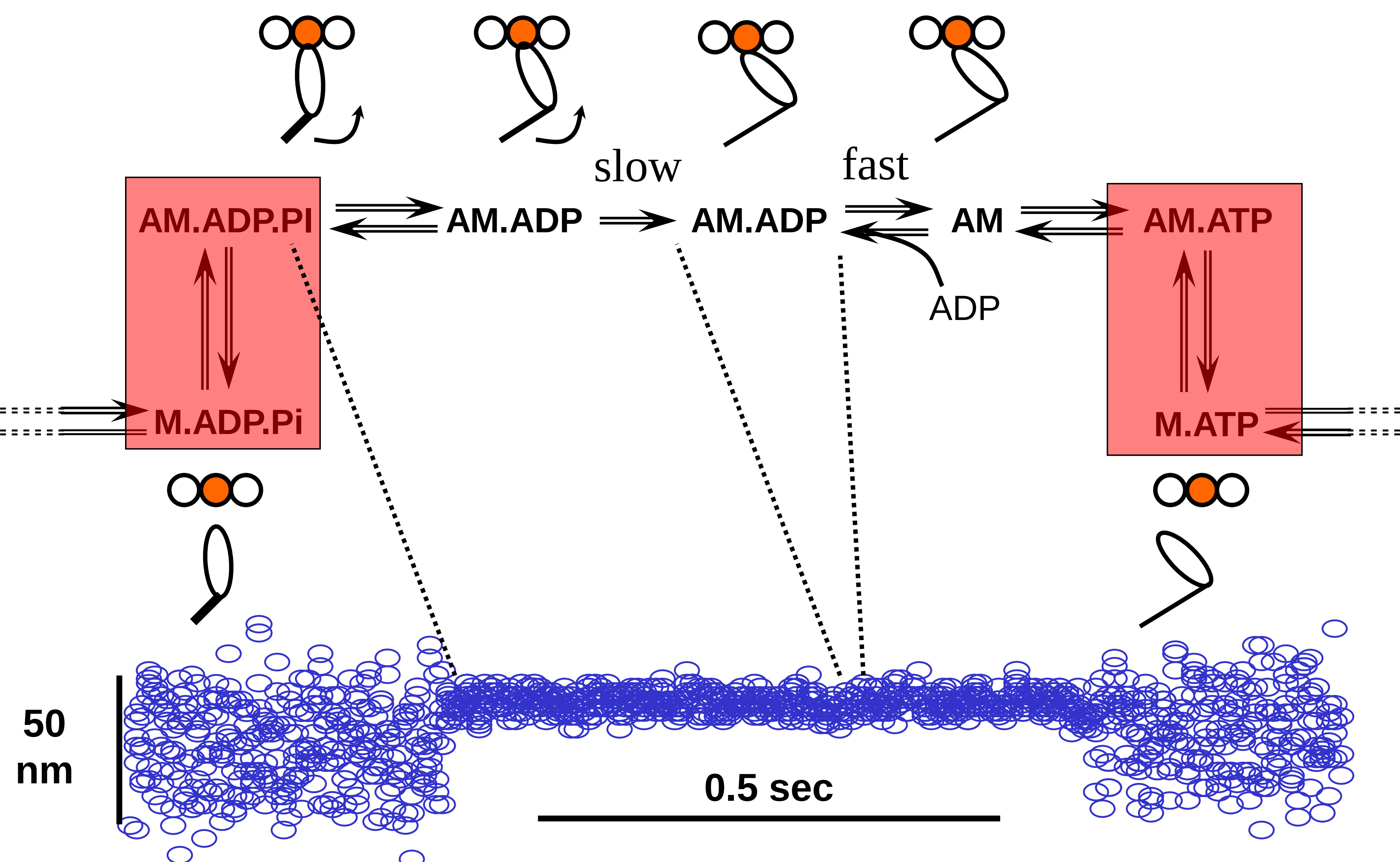


Amplitude distribution gives d_{uni}

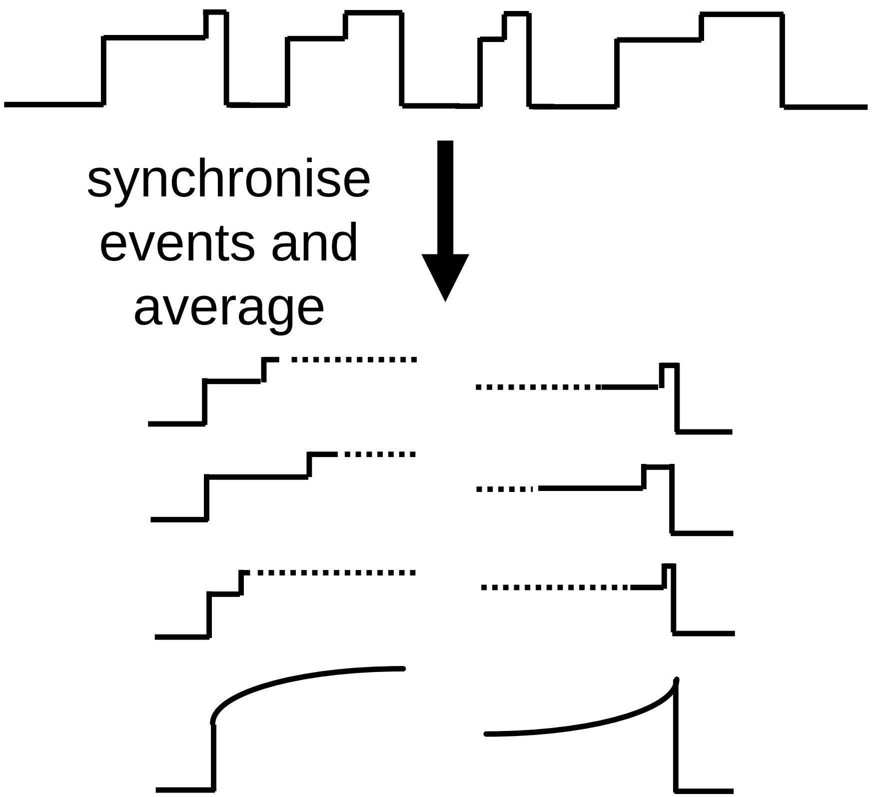
Size of the power-stroke

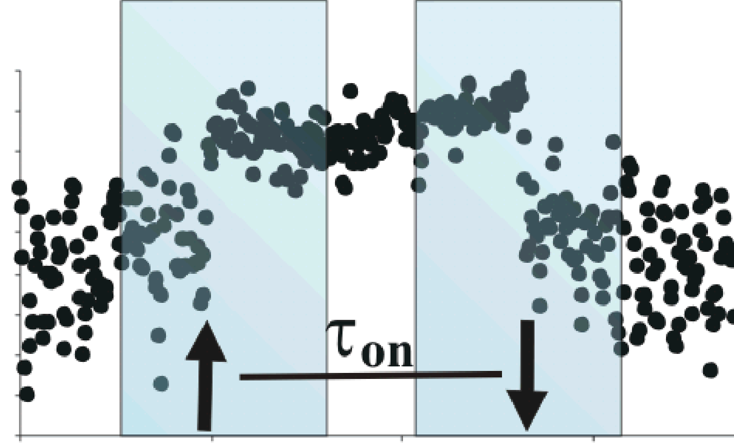


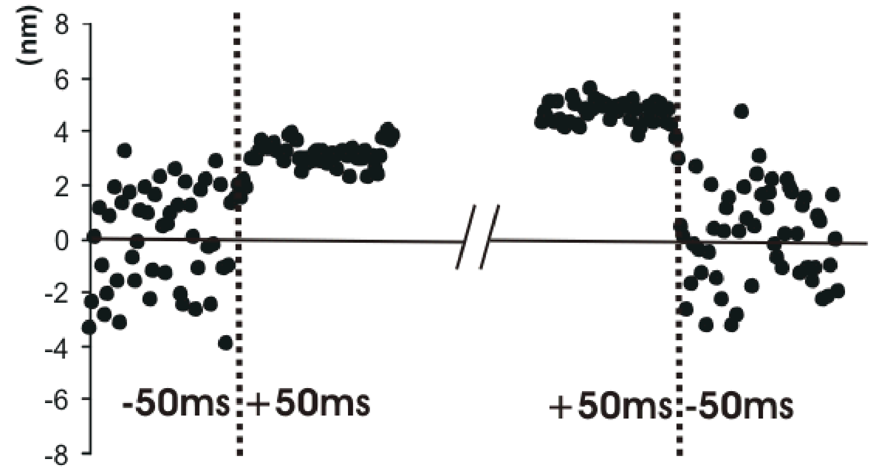
Mapping mechanics onto the Acto-myosin ATPase

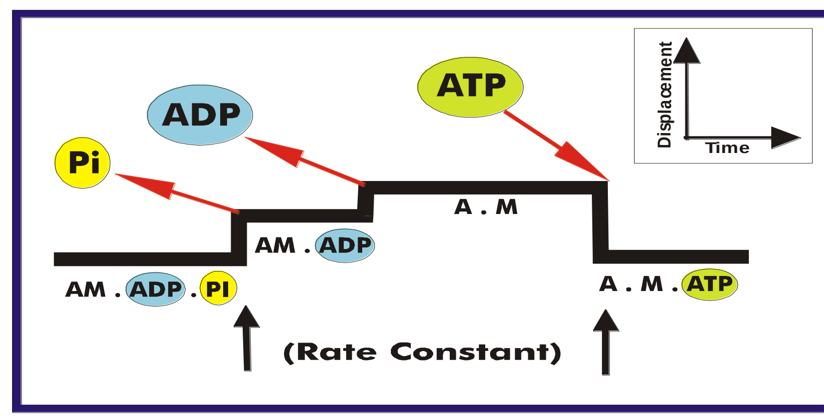


Ensemble Average



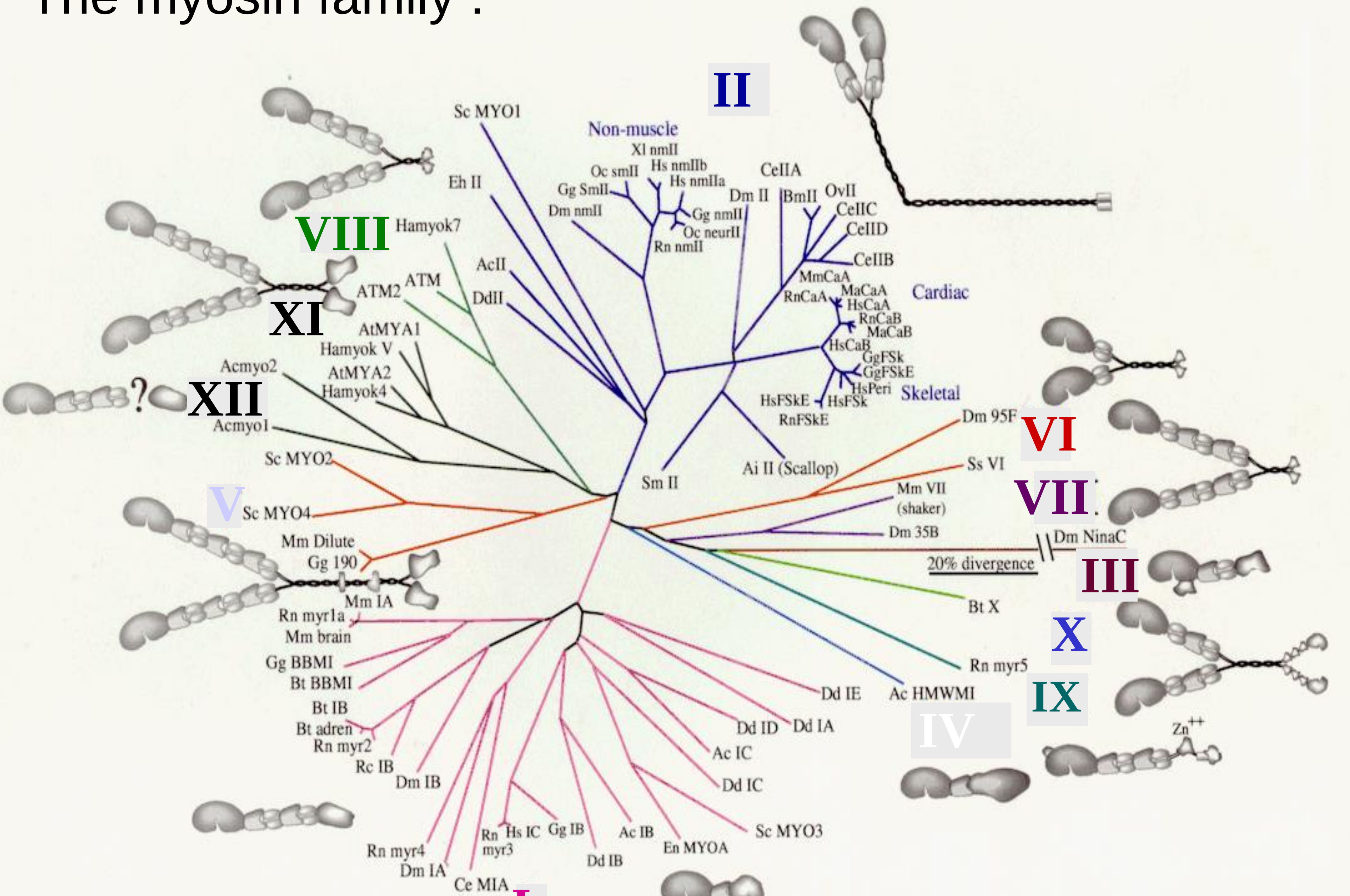






Veigel C, *et al*. (1999) *Nature* **398**:530-533.

The myosin family :



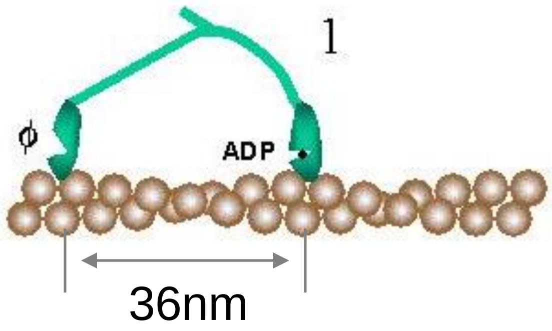
(Tony Hodge, LMB Cambridge)

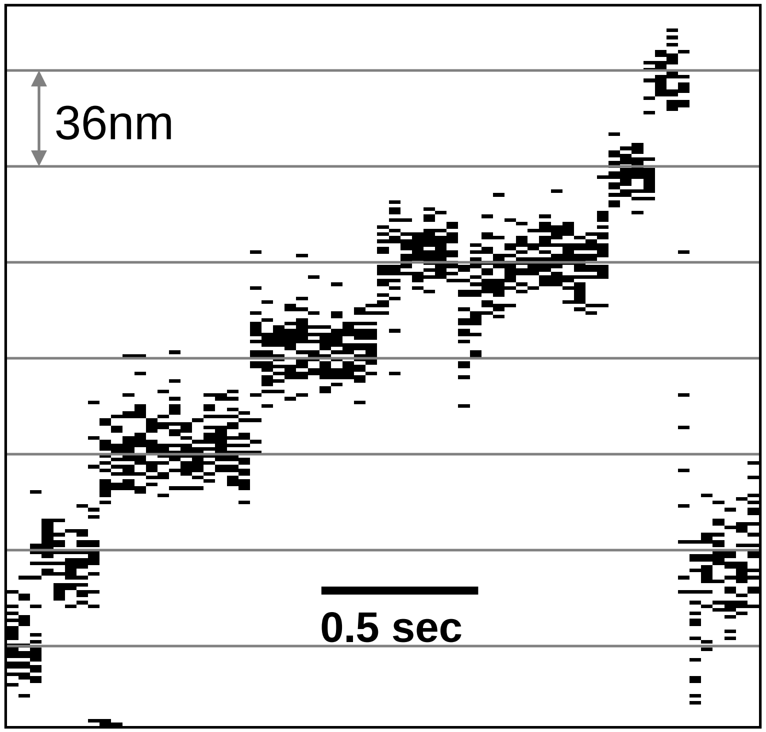
“Processive” and “Intermittent” motors

- 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 5, and most DNA processing enzymes are ***“Processive”*** motors and take many steps before detaching from their track. They **work as single molecules.**

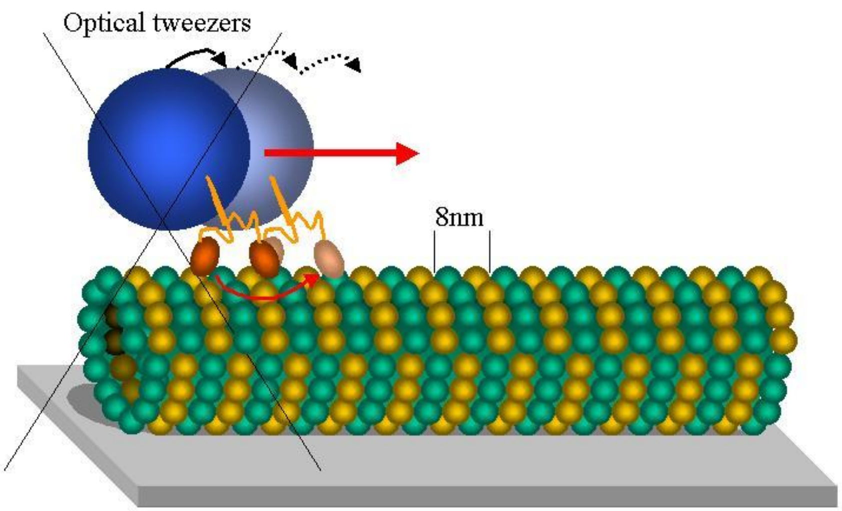
Myosin V

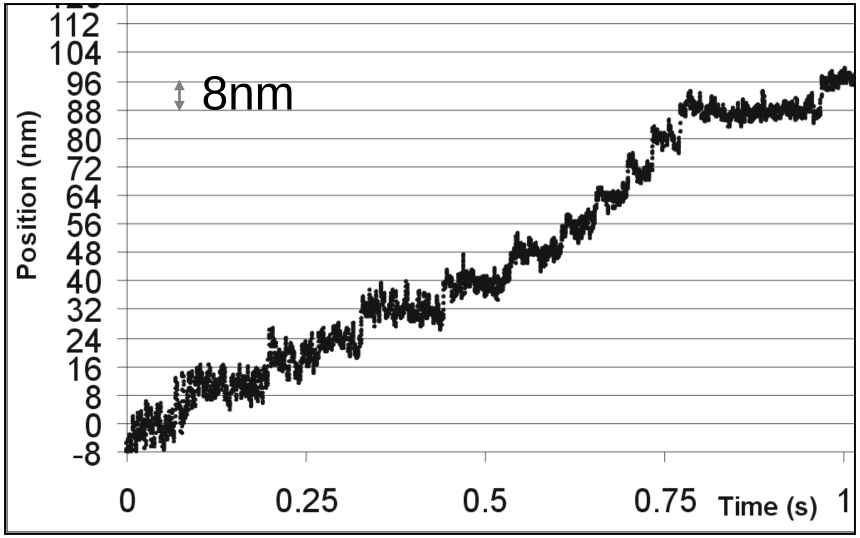




Veigel & Molloy

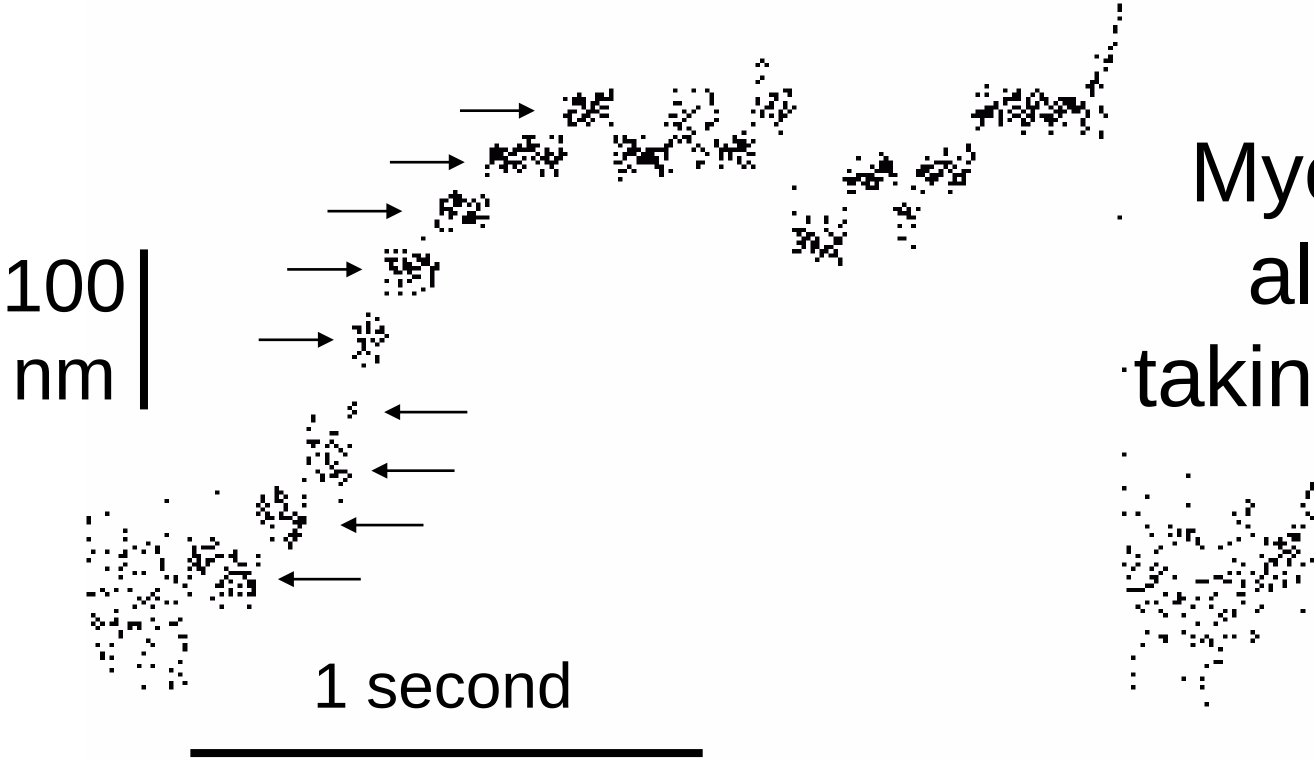
Conventional kinesin

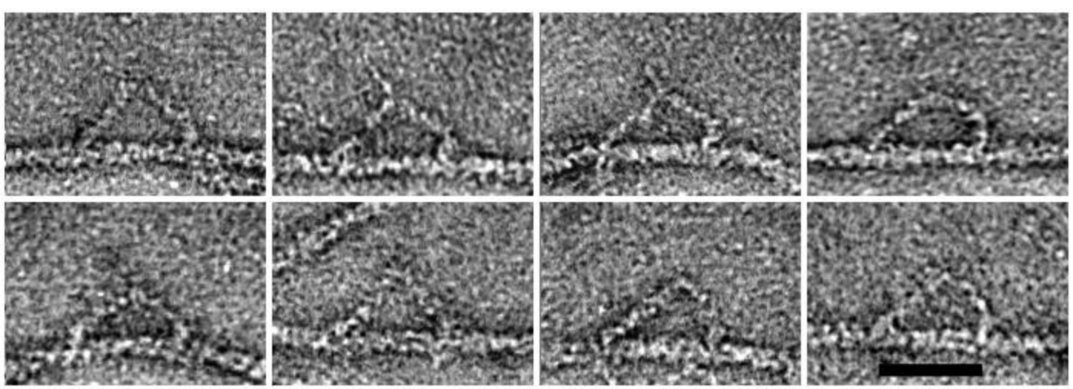


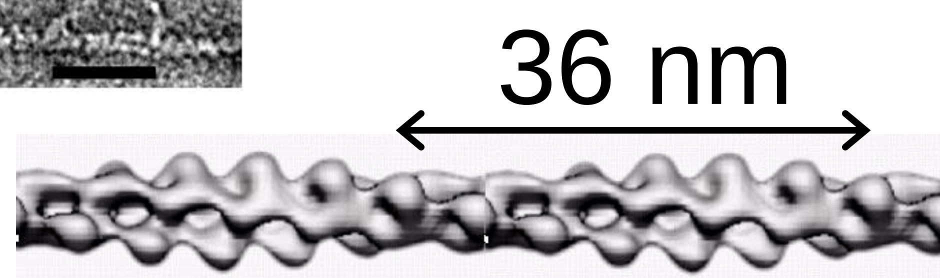


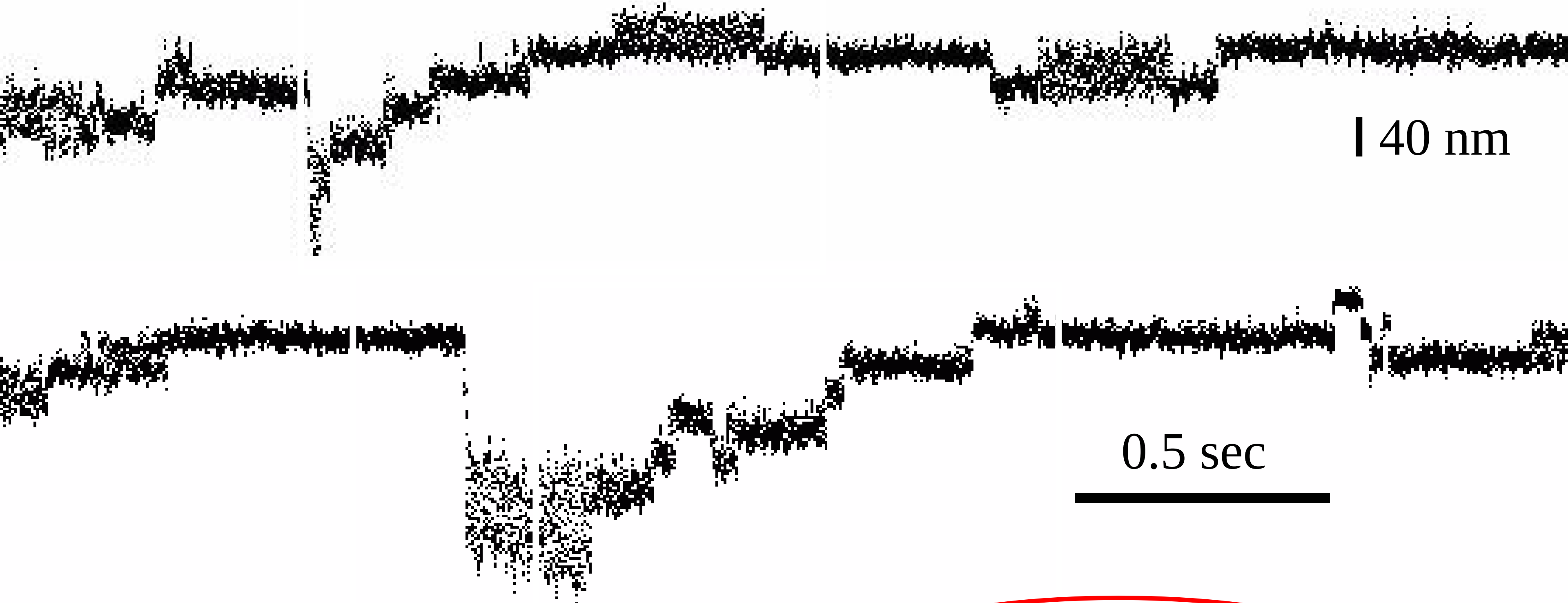
Carter & Cross

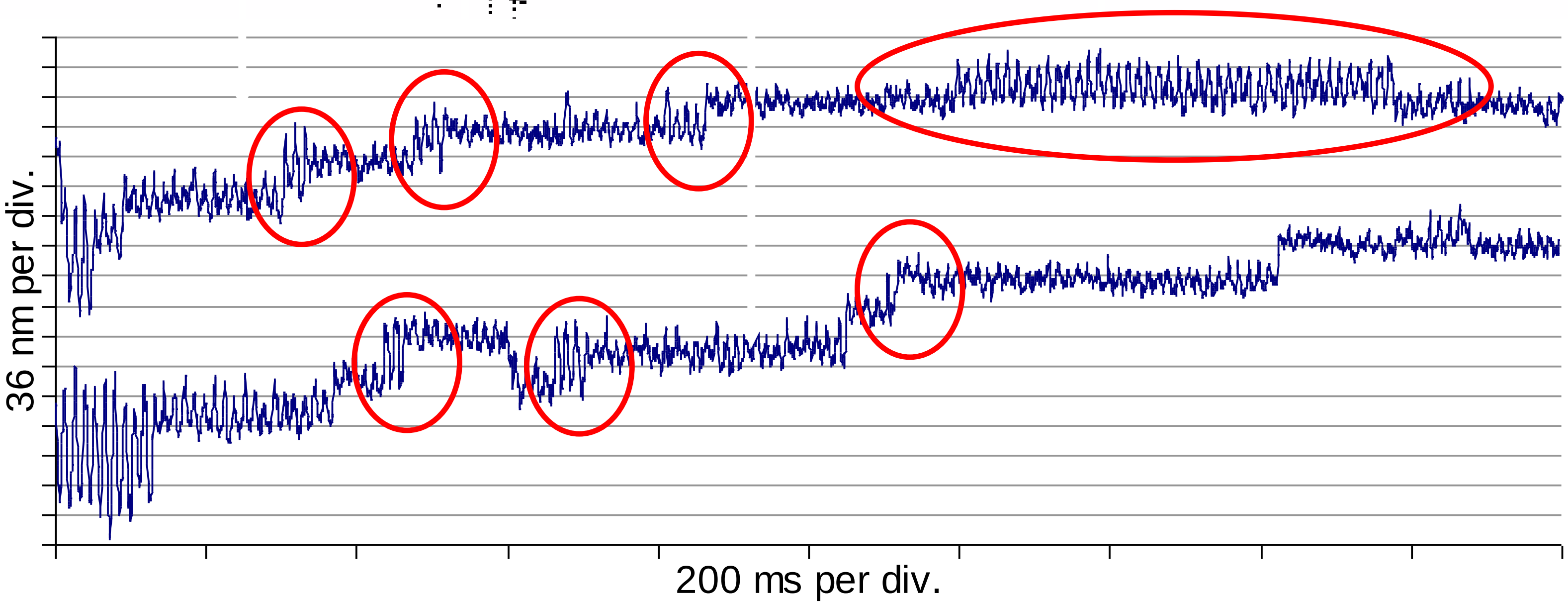
Myosin 5 walks along actin - taking 36nm steps





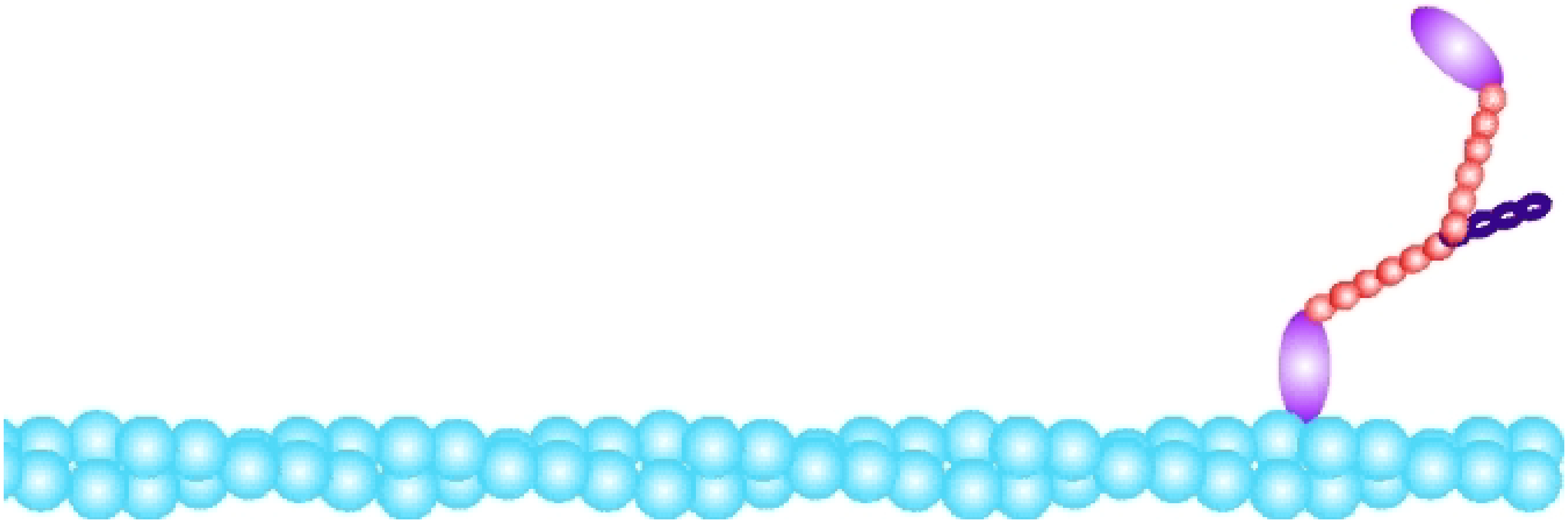






Veigel *et al.* (2002) *Nat. Cell Biol.* **4**:59-65.

How does myosin V walk??…….

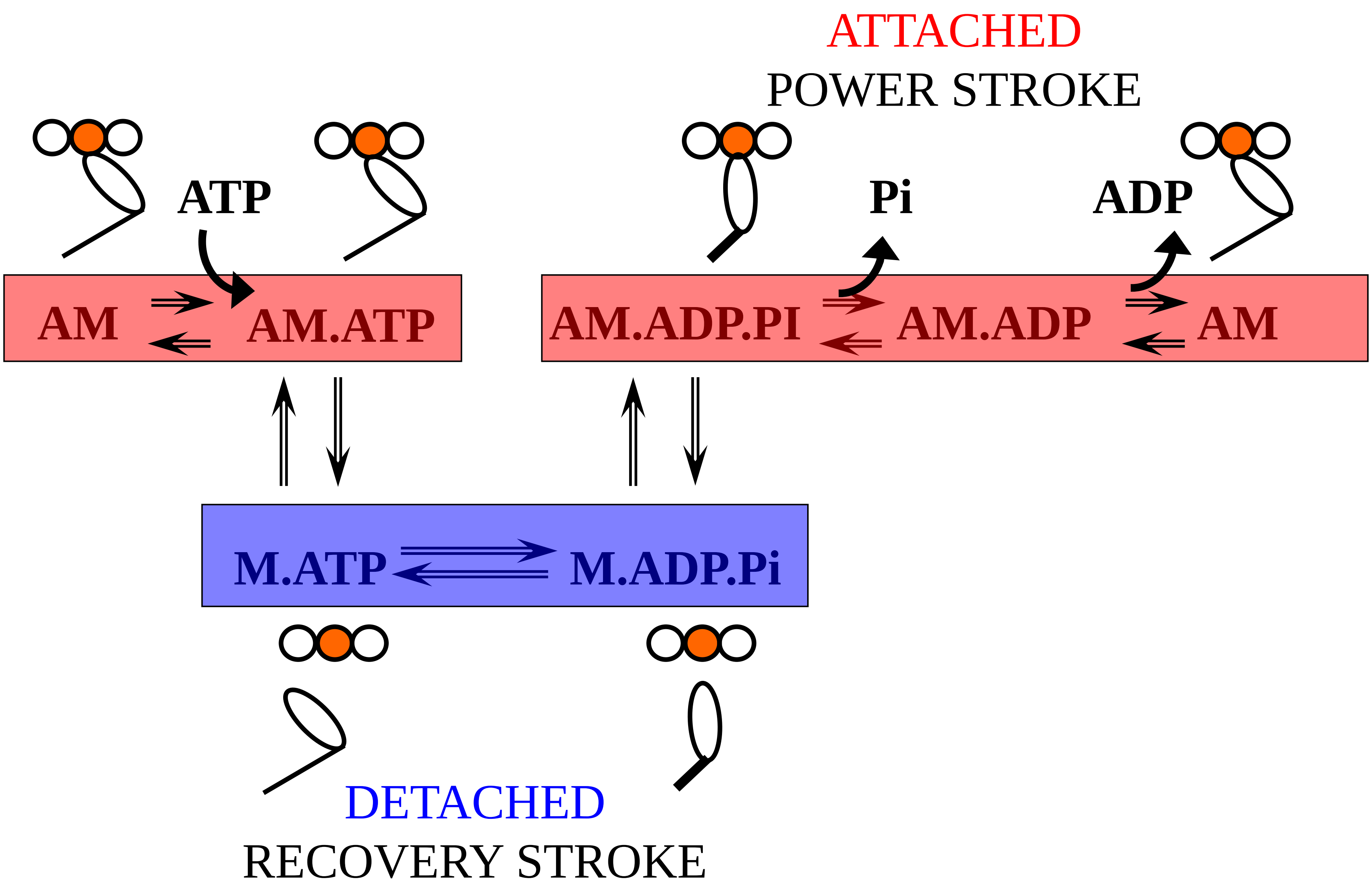


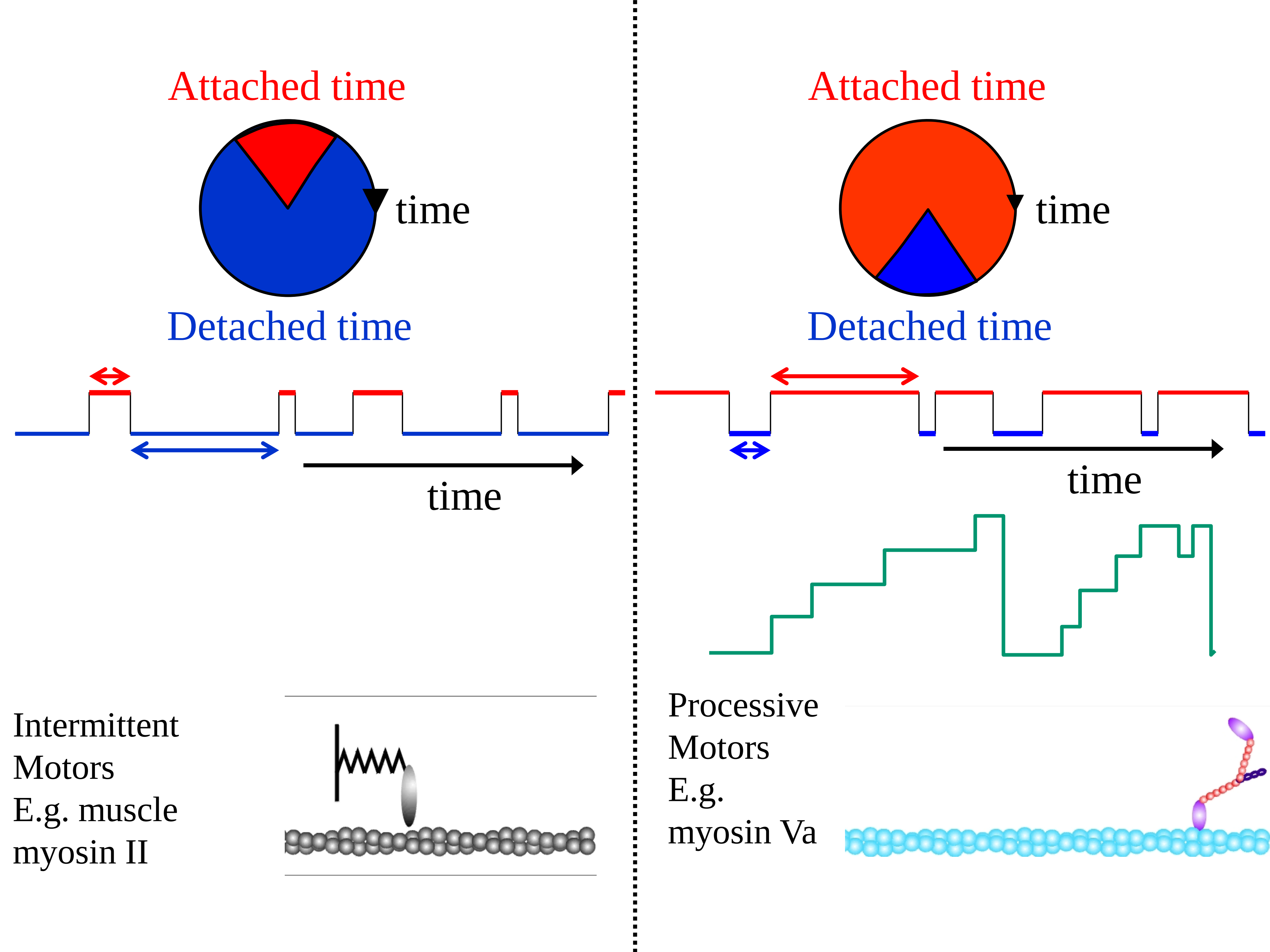
Lecture Overview:

- 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.

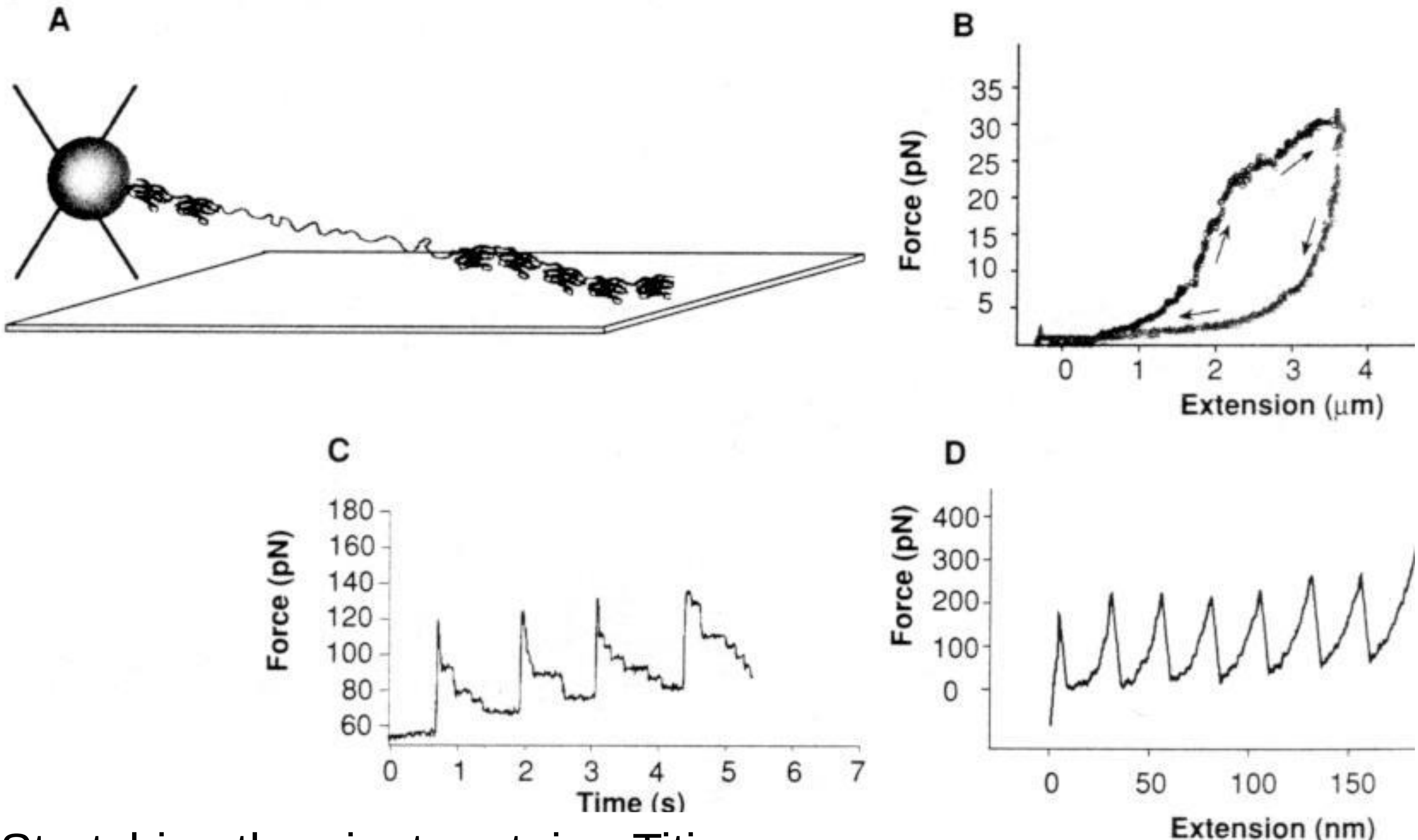
THE FUTURE………

- 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.


ATTACHED
POWER STROKE
ATP
Pi
ADP
AM
AM.ATP
AM.ADP.PI
AM.ADP
AM
M.ATP
M.ADP.Pi
DETACHED
RECOVERY STROKE

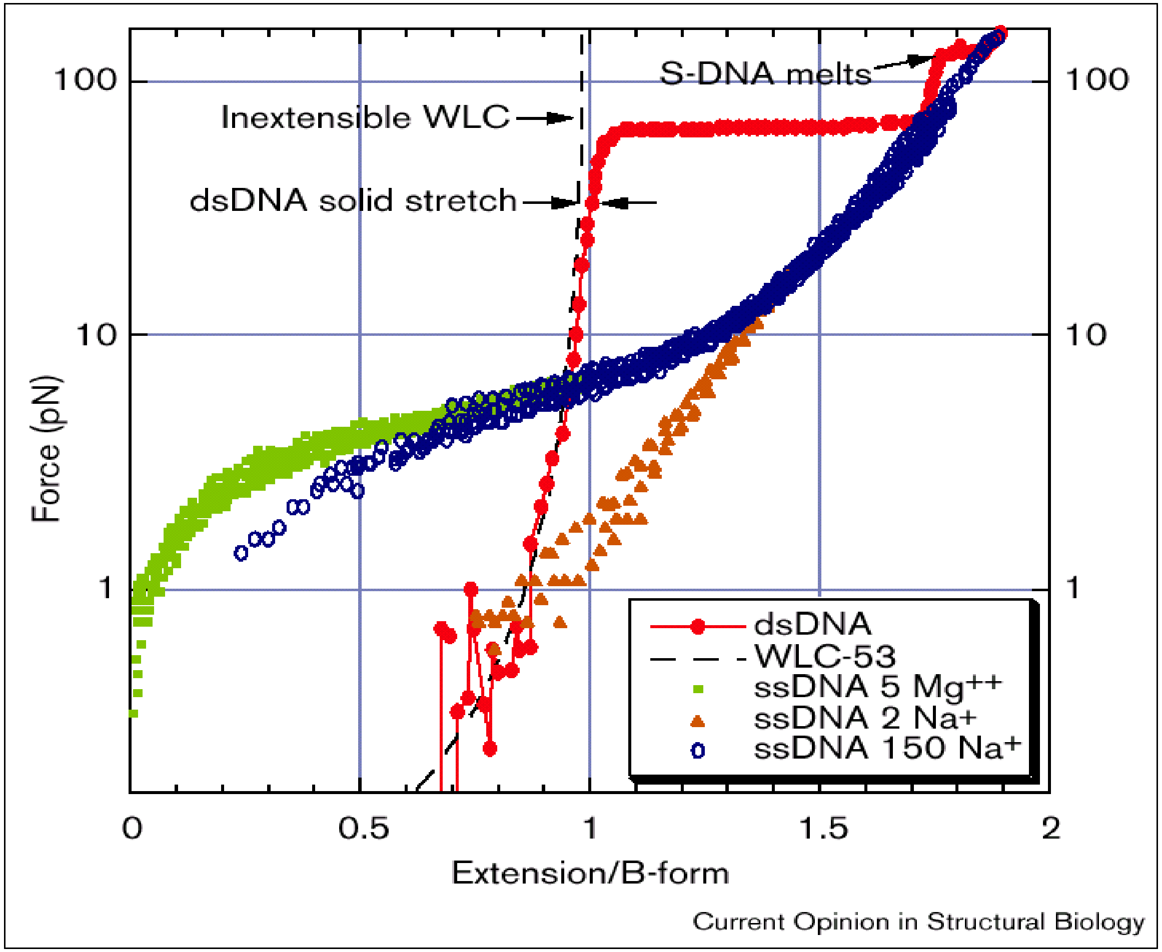

Attached time
time
Detached time
time
Intermittent
Motors
E.g. muscle
myosin II
Attached time
time
Detached time
time
Processive
Motors
E.g.
myosin Va

Optical tweezers can be used to stretch DNA and unfold proteins.



Stretching the giant protein - Titin
(Sleep *et al. Nature 1999*)

Stretching DNA with optical tweezers:



Single-molecule studies of DNA mechanics

Carlos Bustamante, Steven B Smith, Jan Liphardt and Doug Smith

Current Opinion in Structural Biology 2000, **10:**279–285

[Force/extension behavior of dsDNA and ssDNA. Different DNA molecules were pulled with force-measuring laser tweezers (Lambda phage DNA)]