Acto-myosin:
from muscles to single molecules.

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Energy in Biological systems:

1 Photon = 400 pN.nm

1 ATP = 100 pN.nm

1 Ion moving across a membrane = 10 pN.nm

Thermal energy ($\frac{1}{2}k_bT$) = 4 pN.nm

{1pN.nm = 1×10^{-21} Joules}

At the level of cell biology (and smaller) mechanical systems are overdamped (Reynold's number <<1).

Reynold's number is the ratio of kinetic energy to viscous drag energy.

$$\textbf{K.E./Viscous work} = \tfrac{1}{2}mv^2/6\pi\eta avl$$

Where "**l**" is a characteristic length and $\mathbf{m} \propto \rho l^3$. Giving the ratio of energies: $\tfrac{1}{2}\rho l^3 v^2/6\pi\eta l^2 v$:

$$\textbf{Reynold's number} = \rho lv/\eta$$

However, this does not preclude resonant or oscillatory behaviours.

Diffusion can limit the rate of cell processes and even some chemical reactions in the cell.

$$D = k_b T / 6\pi\eta r$$

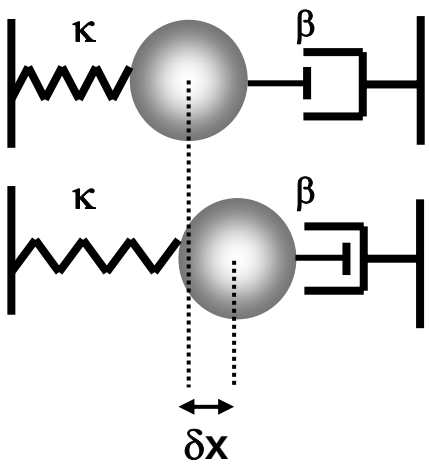
$$D = \tfrac{1}{2}\langle x^2\rangle / t \qquad (\langle x\rangle = \sqrt{2Dt})$$

Distance** moved is proportional to **square root of time.

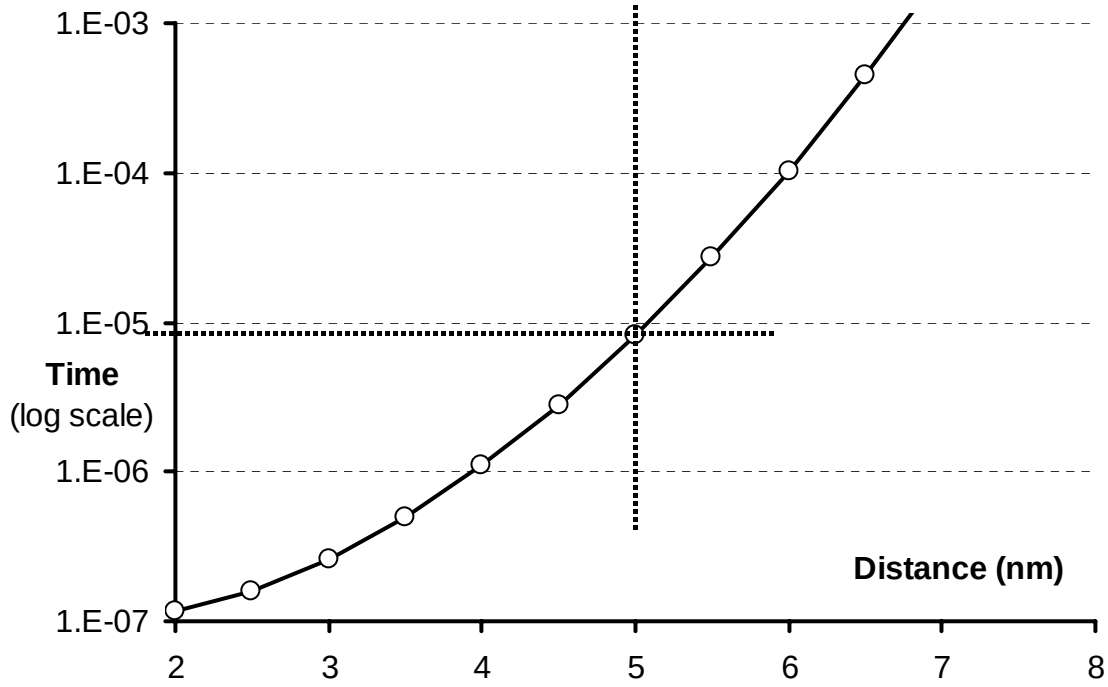
	Diffusion coefficient (cm^2/sec)	Time taken to diffuse 1 μm	10 μm	1 mm
small molecule	5×10^{-6}	**1 msec**	**0. 1 sec**	**16.7 min**
protein molecule	5×10^{-7}	**10 msec**	**1 sec**	**2.8 hr**
virus or vesicle	5×10^{-8}	**0.1 sec**	**10 sec**	**27.8 hr**
bacterium	5×10^{-9}	**1 sec**	**100 sec**	**11.6 day**
animal cell	5×10^{-10}	**10 sec**	**16.7 min**	**116 days**

ATP binding to acto-myosin is approx 10^7 $M^{-1}.s^{-1}$ = diffusion limited

Diffusion over an energy barrier



$$\tau \approx \frac{\beta}{\kappa} \bullet \sqrt{\frac{kT}{Q}} \bullet e^{Q/kT}$$



Kramers, HA (1940) *Physica* **7:**284-304

where :

β = viscous damping, from stoke's law = $6\pi\eta r$ **= 1.5×10^{-10} N.s**

Q = mechanical work done in stretching the spring by δ**x (nanometres) = ½κ(δx)²**

δ**x = 5 nm (distance to diffuse to next binding site)**

κ **= stiffness of myosin = 2pN.nm^{-1} (2×10^{-3} N.m^{-1})**

kT = thermal energy = 1.38×10^{-21} *** 300 = 4 pN.nm (or 4×10^{-21}J)**

τ **~ 10 microsceonds**

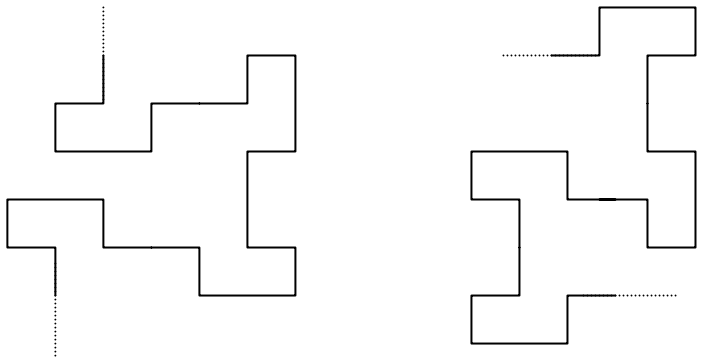
Numerous possibilities:

300 amino acids gives 20^{300} primary sequences

$\sim 10^{400}$

5 unique C-N bond angles per amino acid gives

$(10^{400})^5 = 10^{2000}$ conformations



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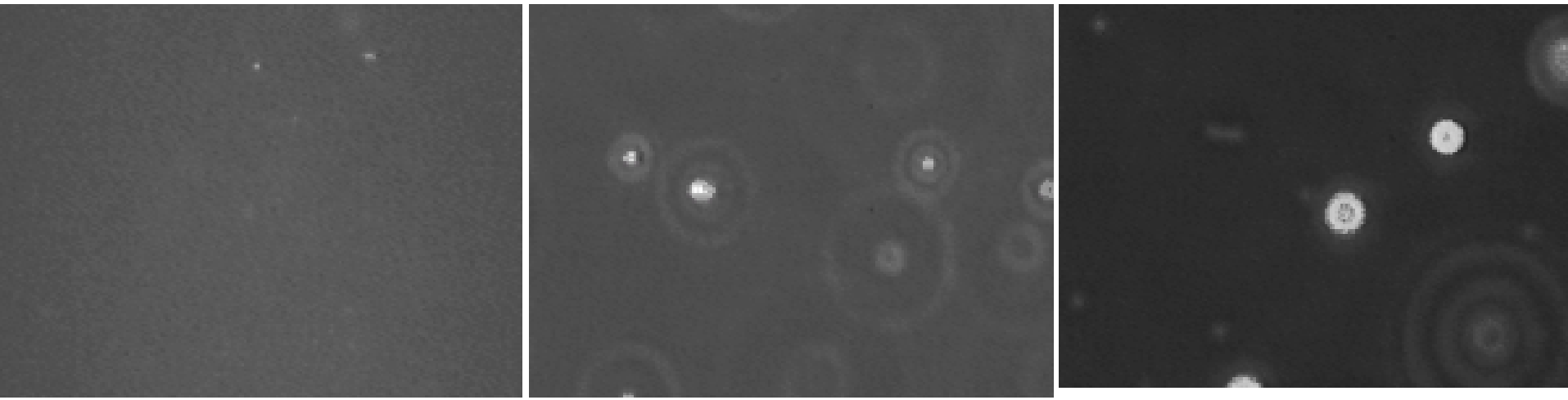
1) How many will fold correctly and how many will be functional?

2) Do folding and function go hand-in-hand?

Why do living things need motors?

- To compete in the modern world… diffusion is too slow and too random even things as small as bacteria and viruses need motors.



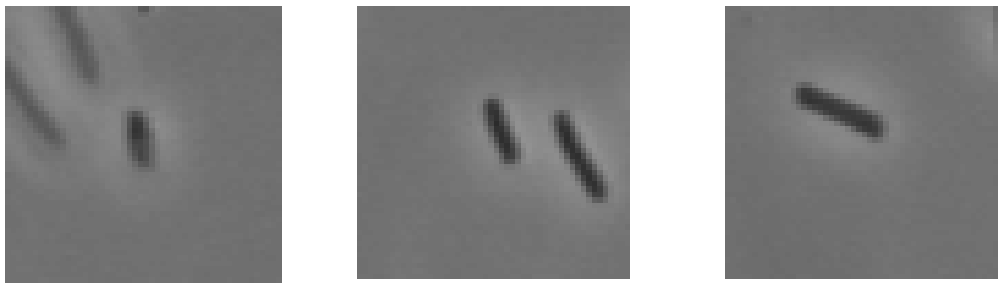
30nm beads

600 nm beads

2 μm beads

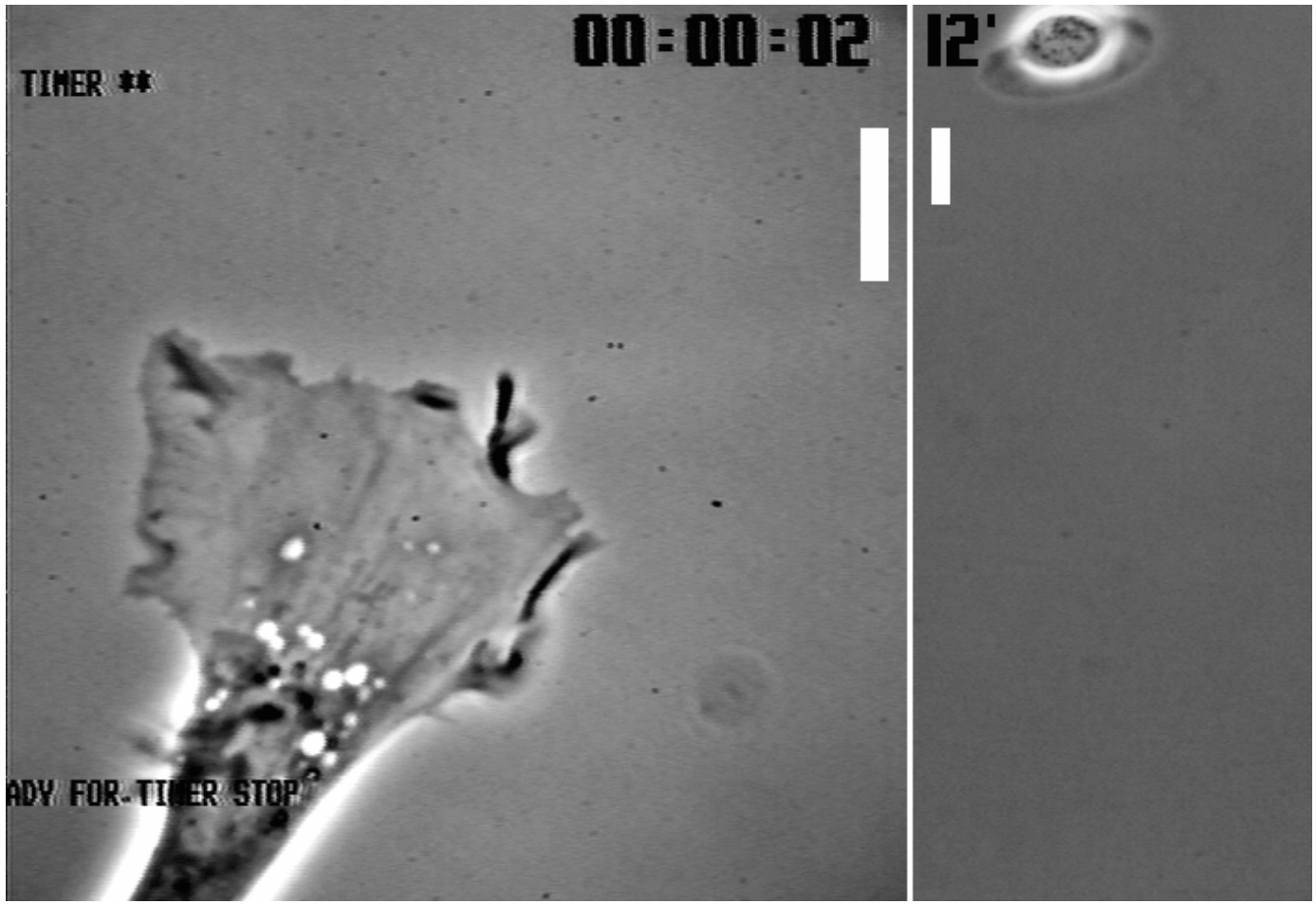
50um

Bacteria have true rotary motors:



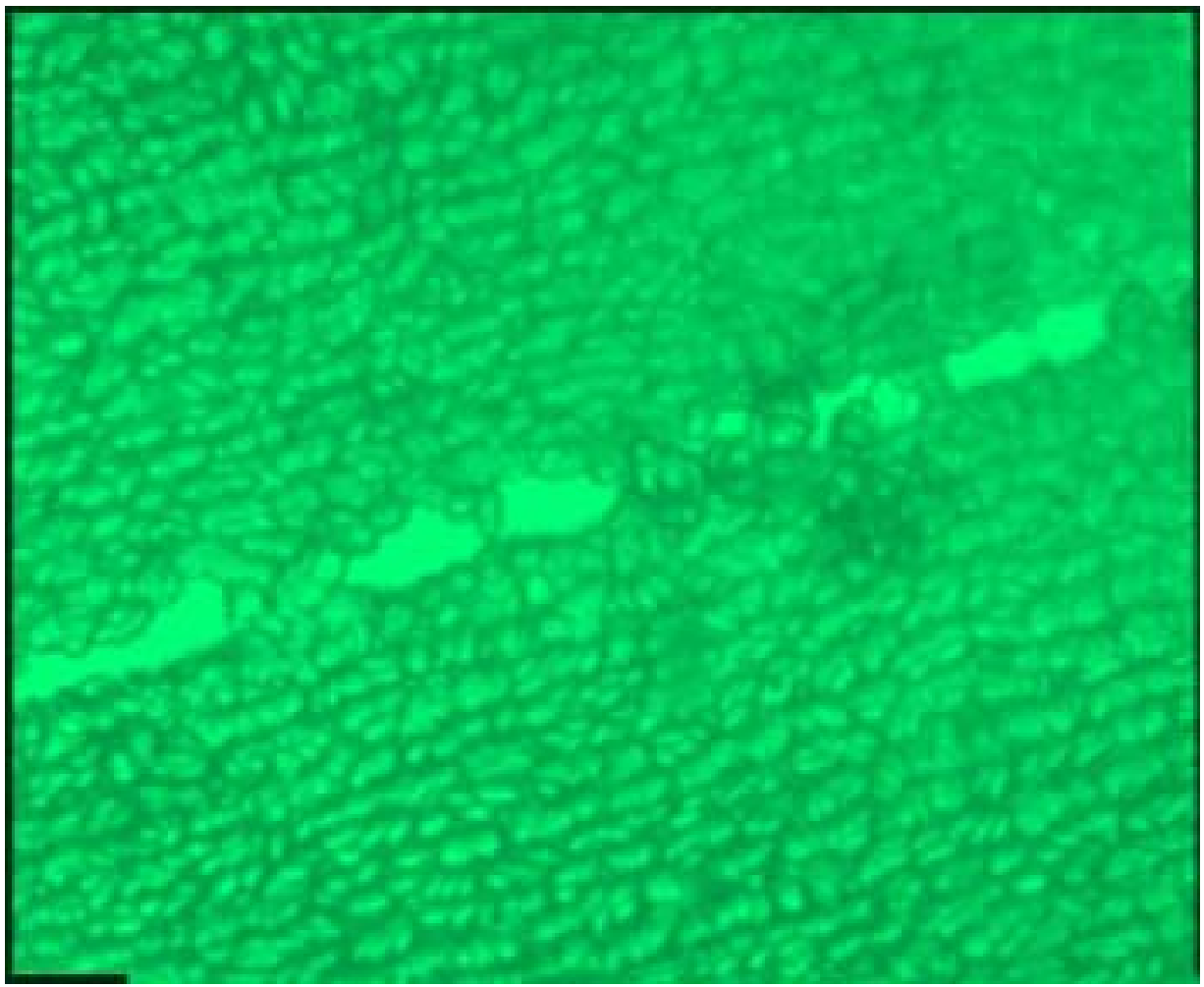
Tethered
E. coli bacteria

Animal cells use a wide variety of linear motors to power internal (motility) and external (locomotory) movements



Plants have motors too:

But none have muscles but some have microscopic contractile organelles

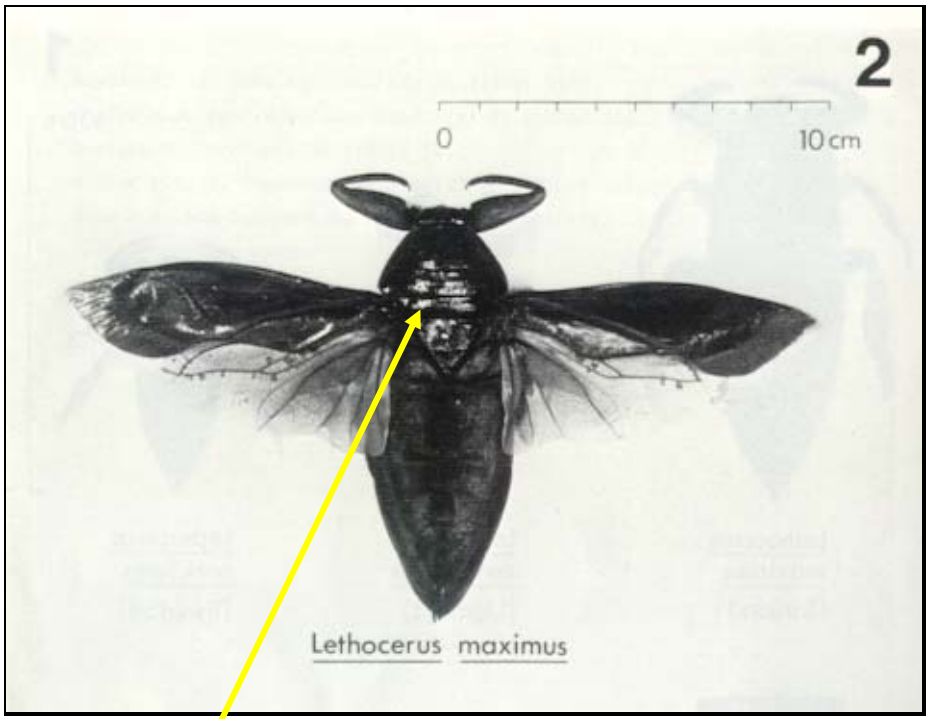


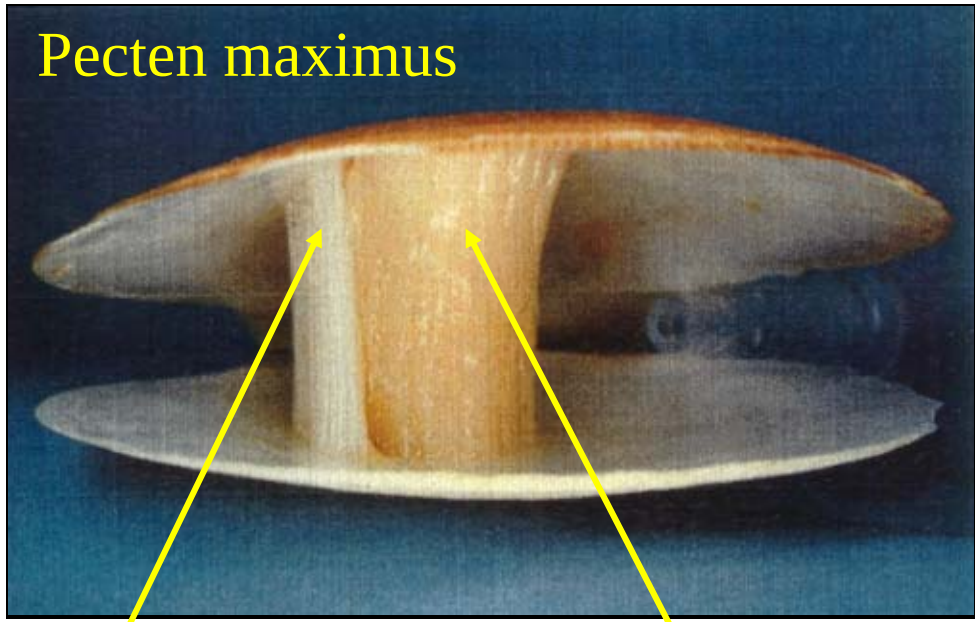
100μm

Up to $60\mu m.sec^{-1}$

Diversity of muscles gives insights into mechanism:

E.g. insect flight and Molluscan catch + adductor muscles:





Insect flight muscles

Catch muscle

Adductor muscle

Insect flight muscles form part of an self-oscillatory system.

$$f_{res} = \frac{1}{2\pi} \bullet \sqrt{\frac{k}{I}}$$

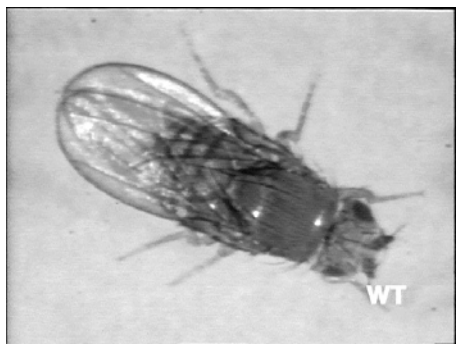
Where:

k = complex stiffness of the muscles

I = moment of inertia of the wings

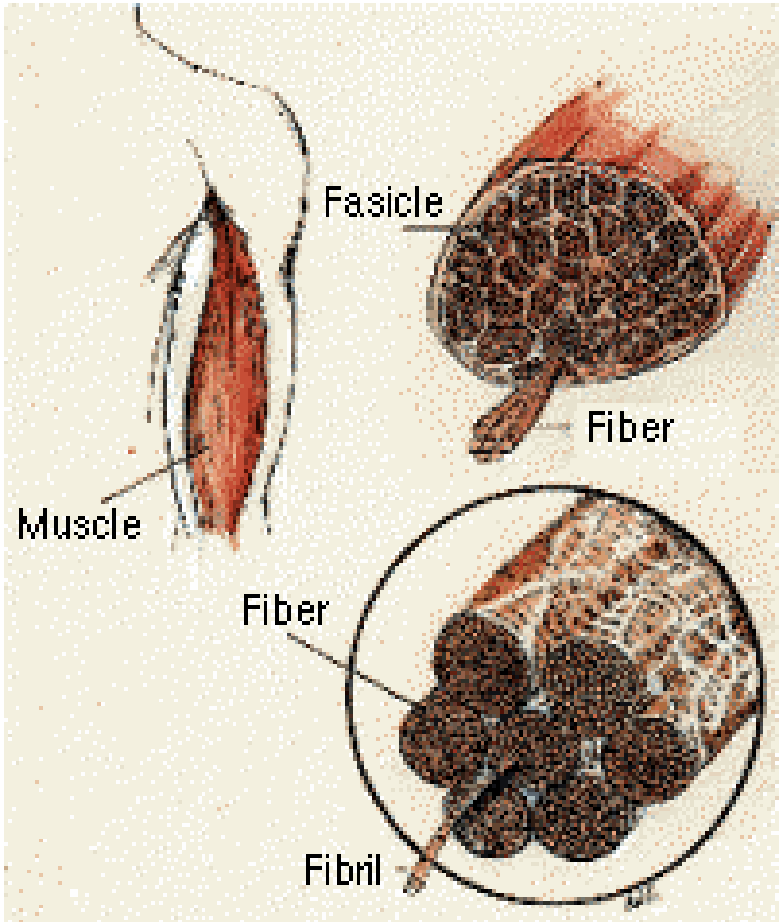
What happens if you mutate one amino acid in myosin?

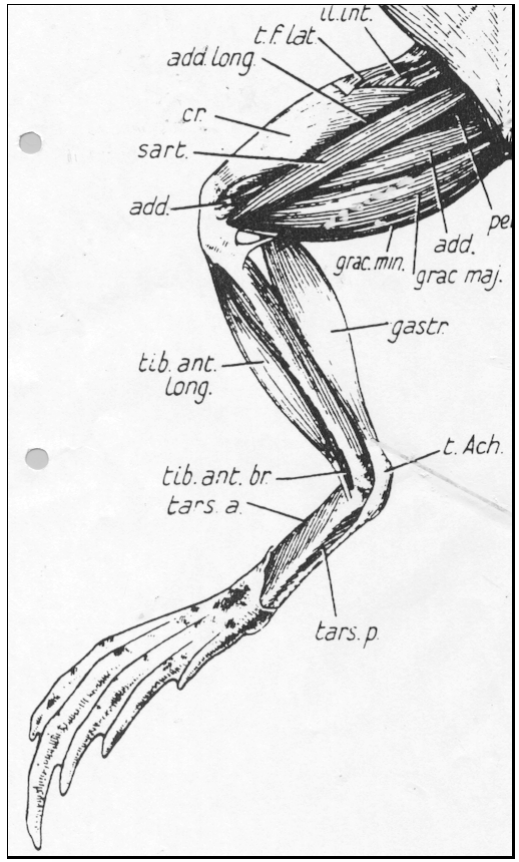
Wild Type



Mutant

Muscle contraction





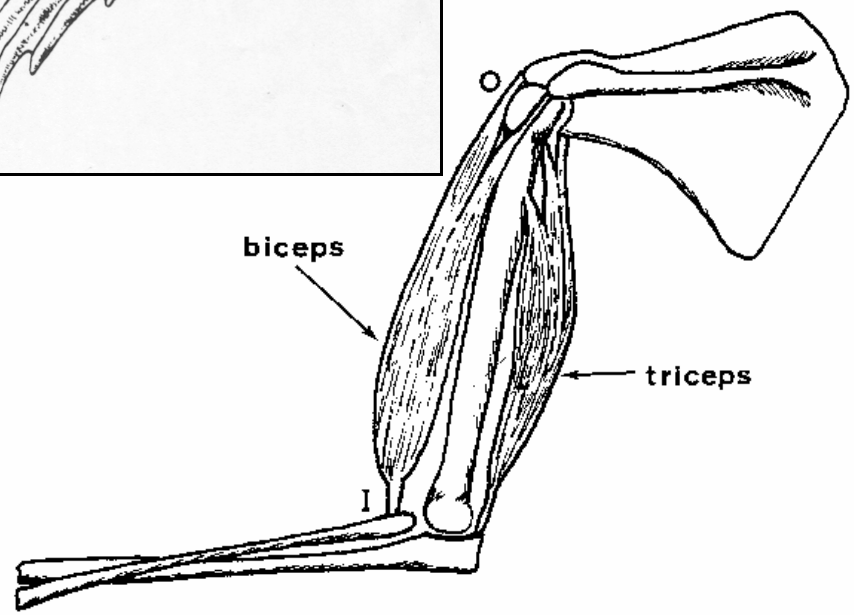
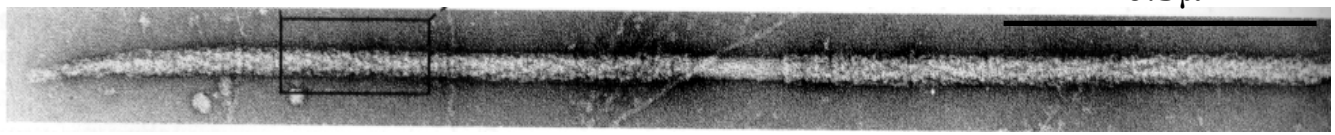
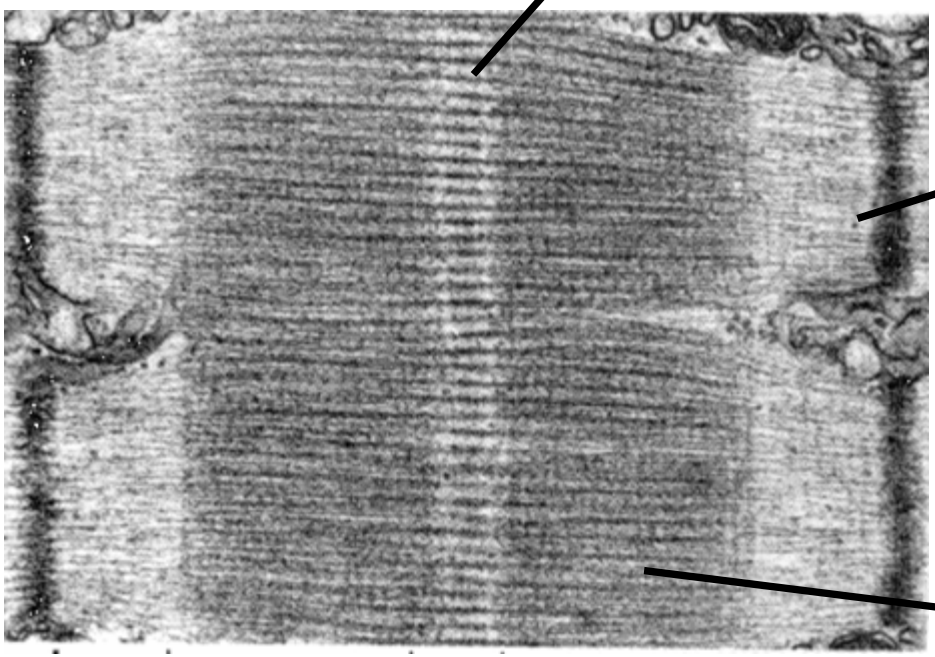


Figure 2.3 Indication of the antagonistic arrangement of pairs of muscles such as the biceps and triceps. (*O*: origin; *I*: insertion.)

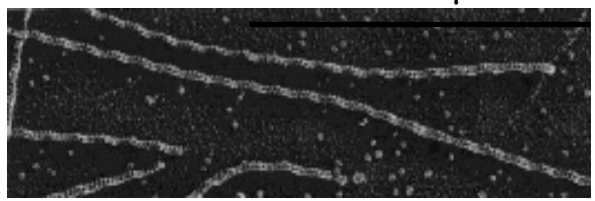
Acto-myosin in muscle :



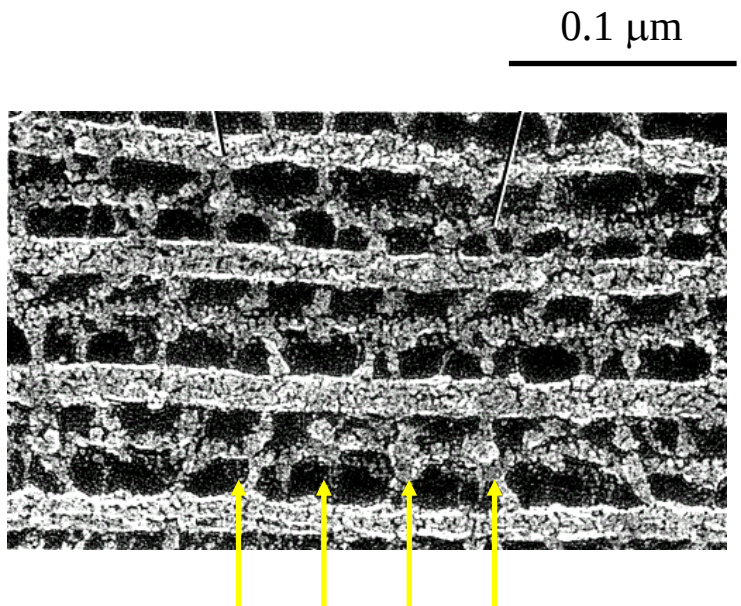
Myosin containing, thick filament



Sarcomere



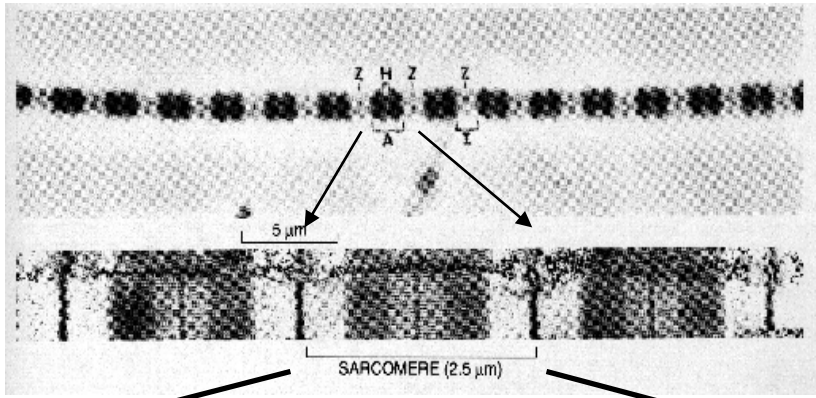
Actin containing, thin filaments



acto-myosin “cross-bridges”

Filament sliding causes muscle to shorten:

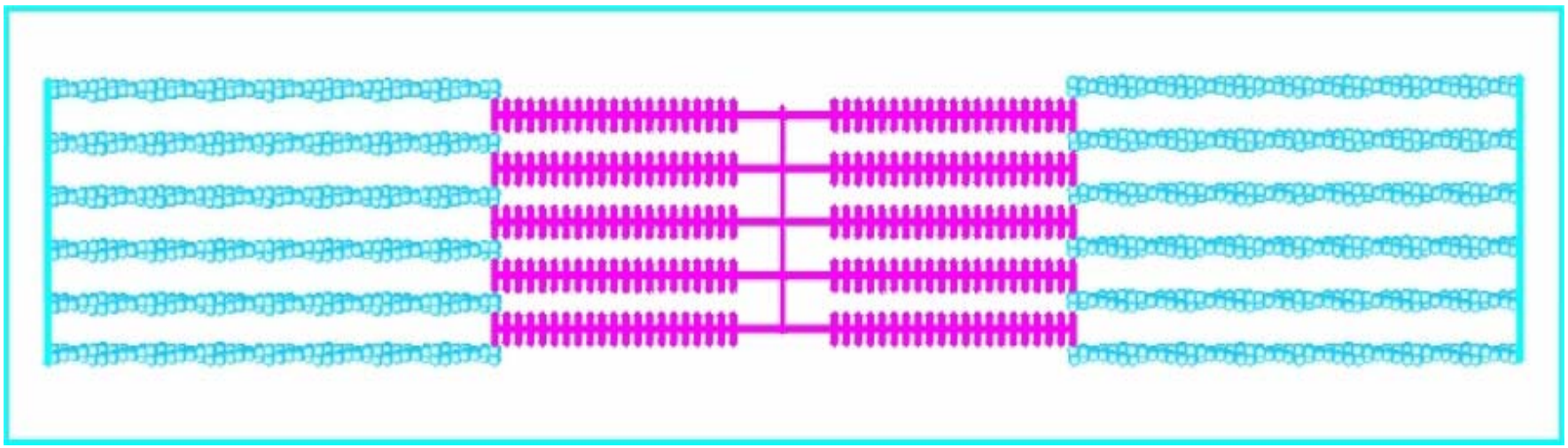
myofibril



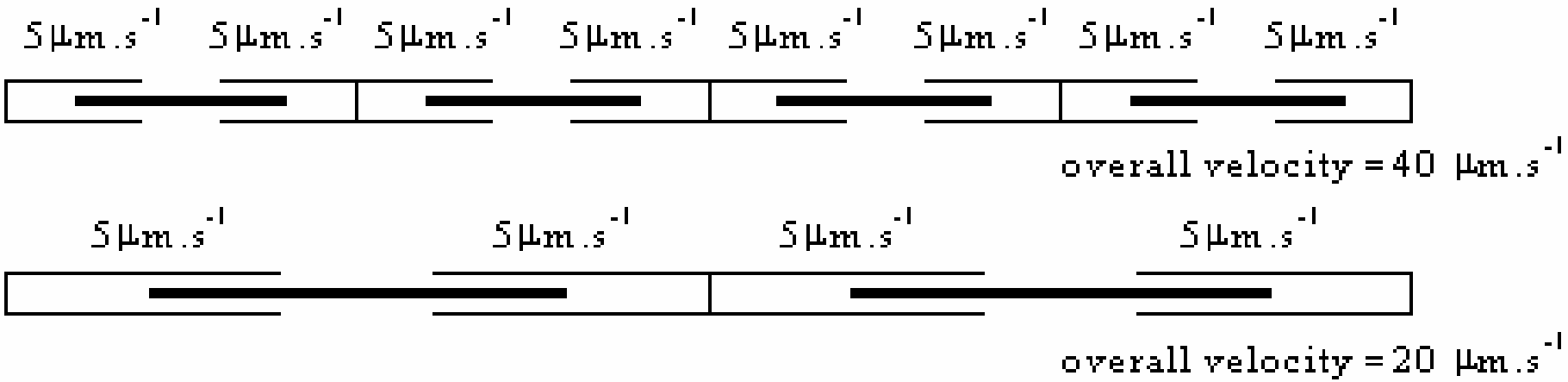
Light micrograph

Electron micrograph

sarcomere



Muscle learns to add:



Cross-bridges are independent force generators: AF Huxley, Gordon & Julian (1966)

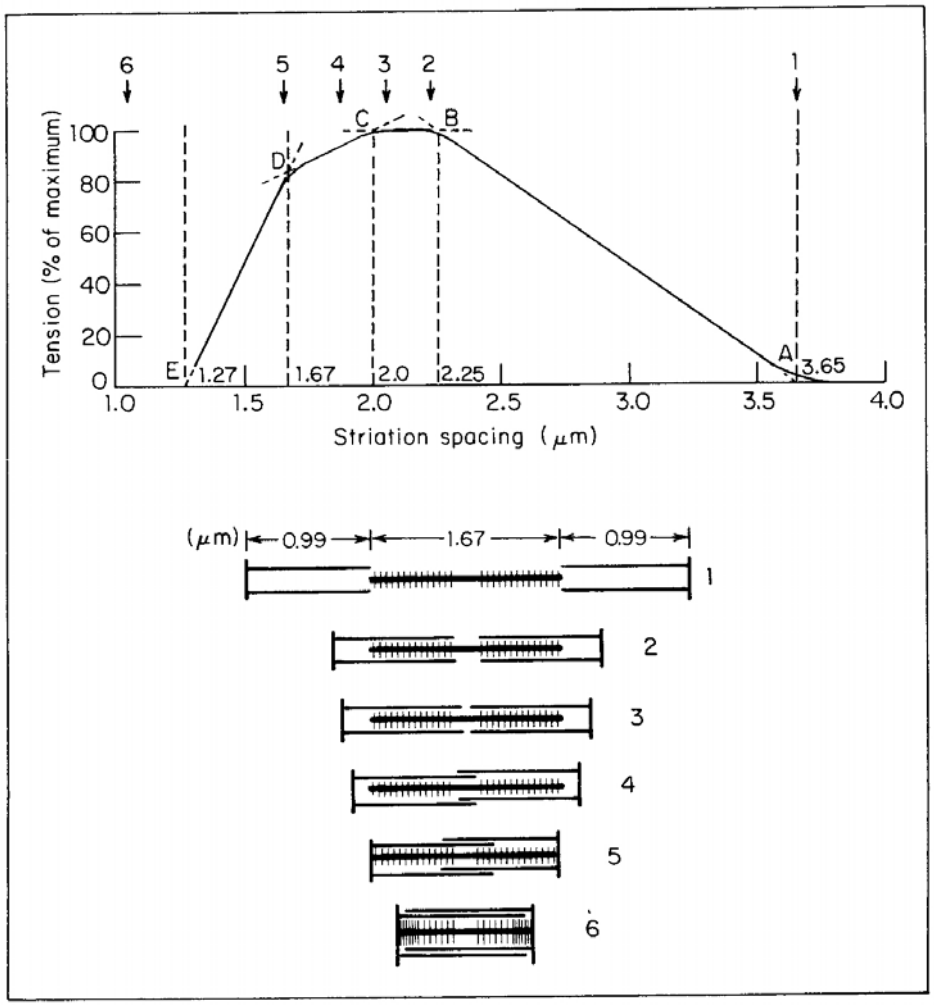
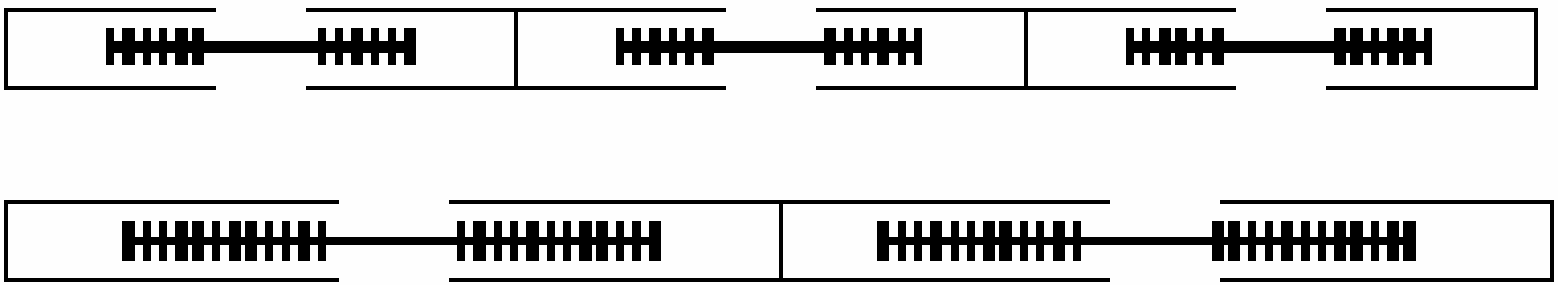


Fig. 3.7. Developed tension vs. length for a single fiber of frog semitendinosus muscle. The length of the segment was fixed for each measurement by the spot-follower servo. The sliding-filament diagrams in the lower part of the figure show the appearance of the sarcomere striation pattern at the lengths corresponding to the numbers in the force-length diagram. Modified from Gordon, Huxley, and Julian (1966b).

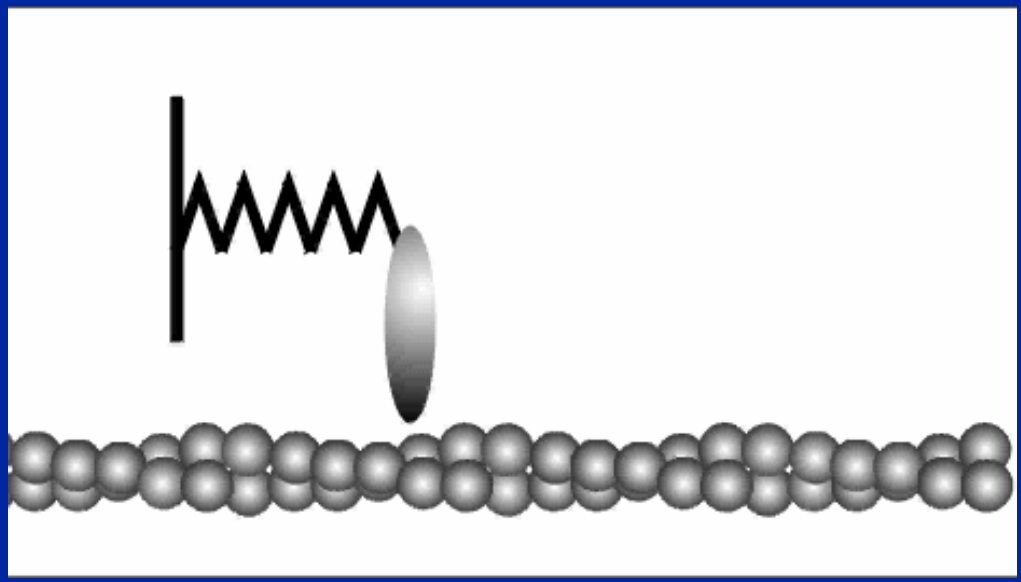
Lesson 1.

Muscle learns to compromise:

Since the molecular spacing on the thick filament is fixed – short sarcomeres generate smaller forces.

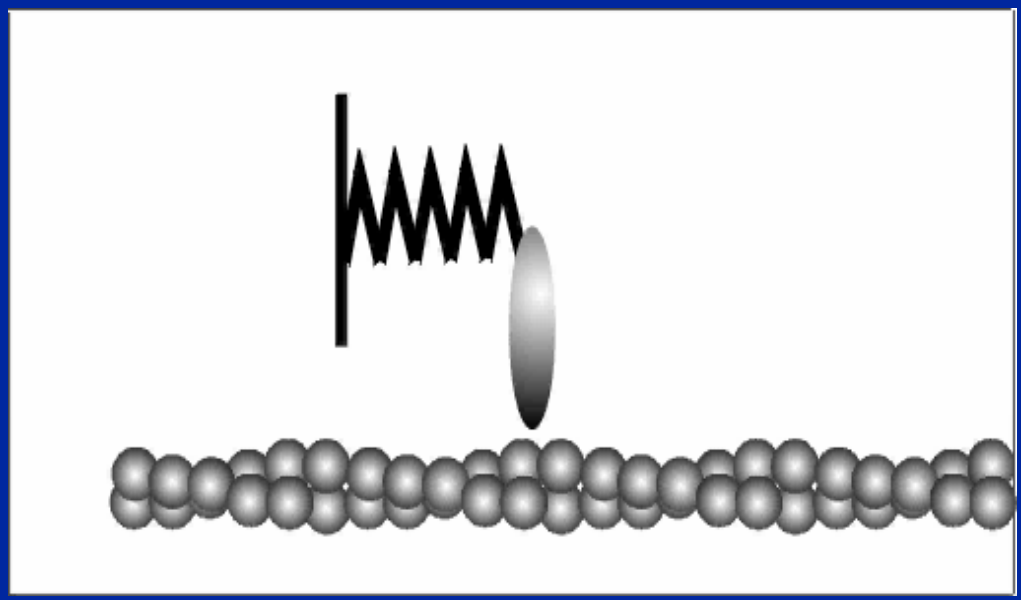


How do the myosin heads work?



Ratchet

or

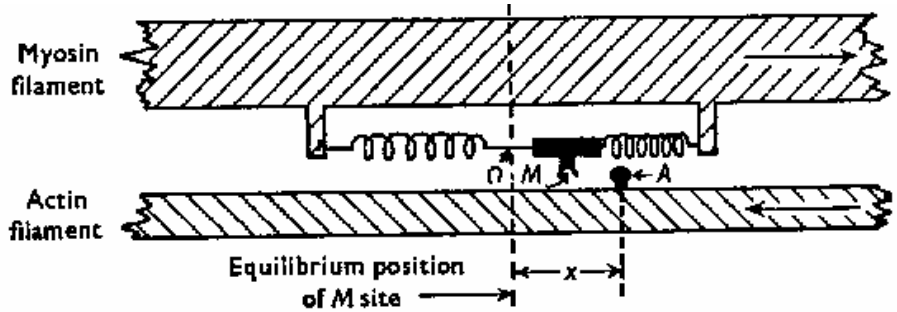


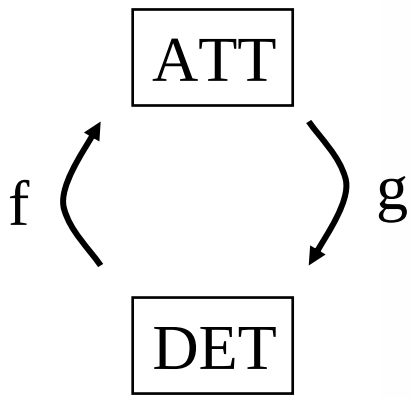
Powerstroke

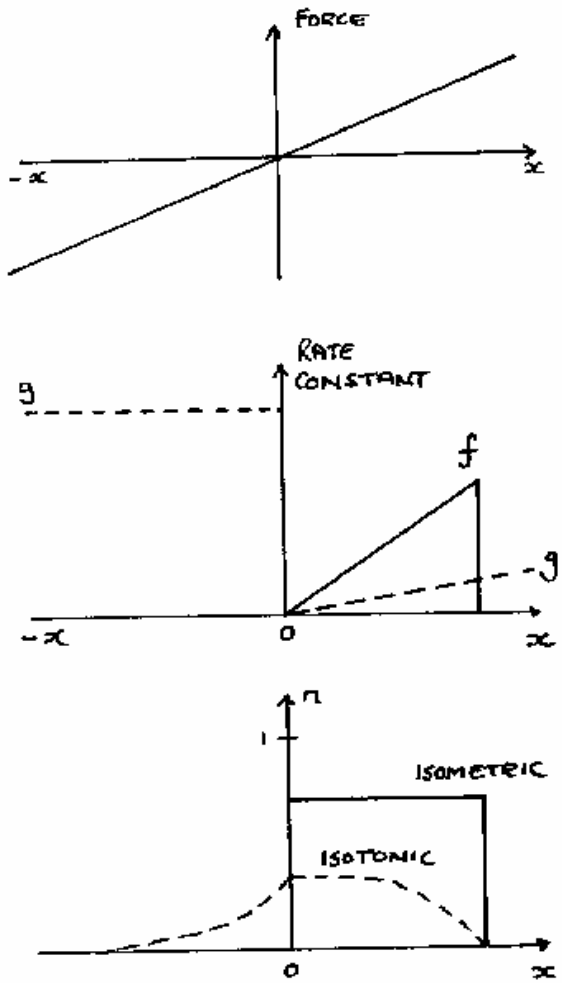
or BOTH?

The duty cycle:

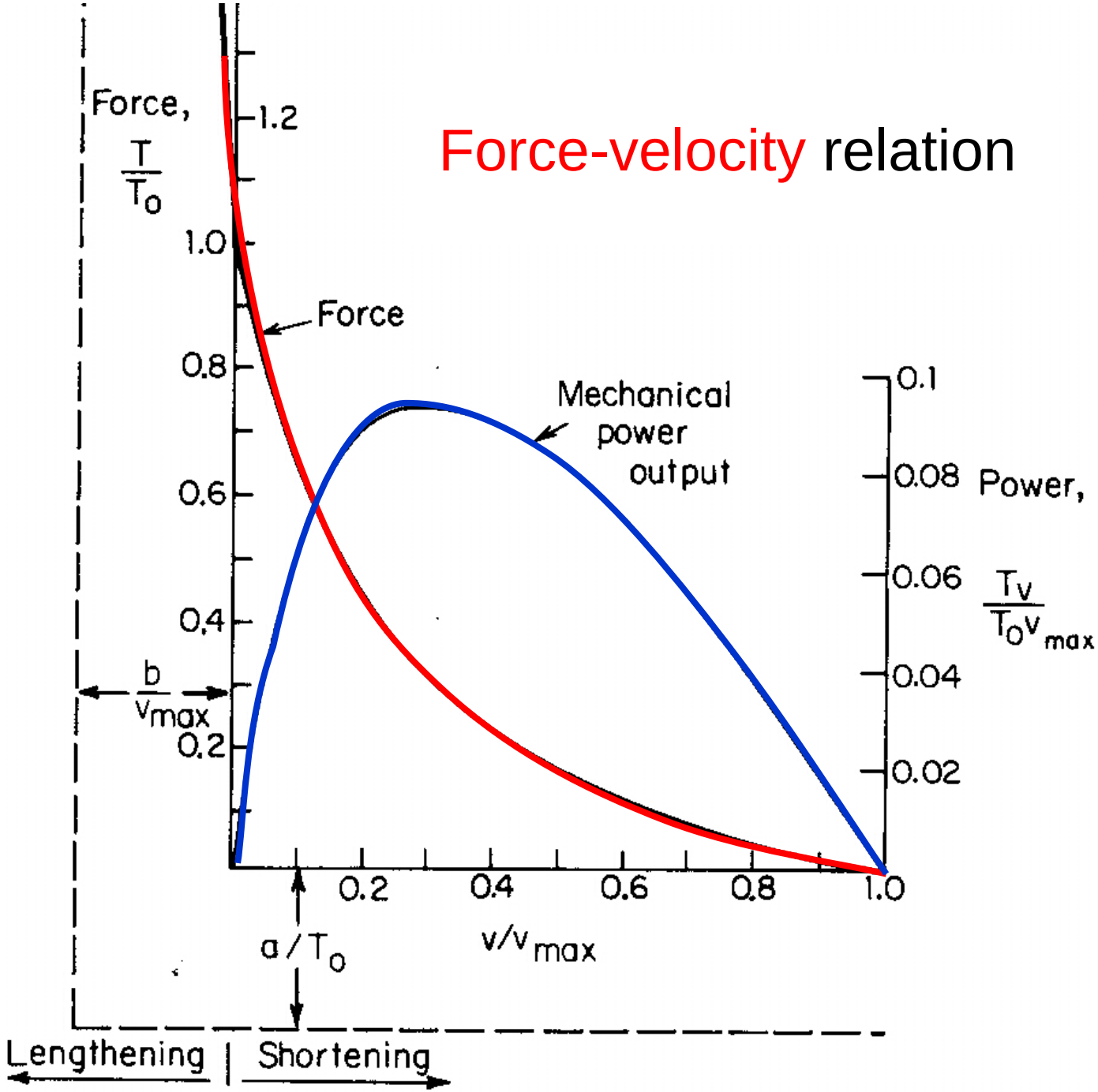
Huxley (1957)


Myosin filament
O
M
A
Actin filament
Equilibrium position of M site
x


ATT
f
g
DET

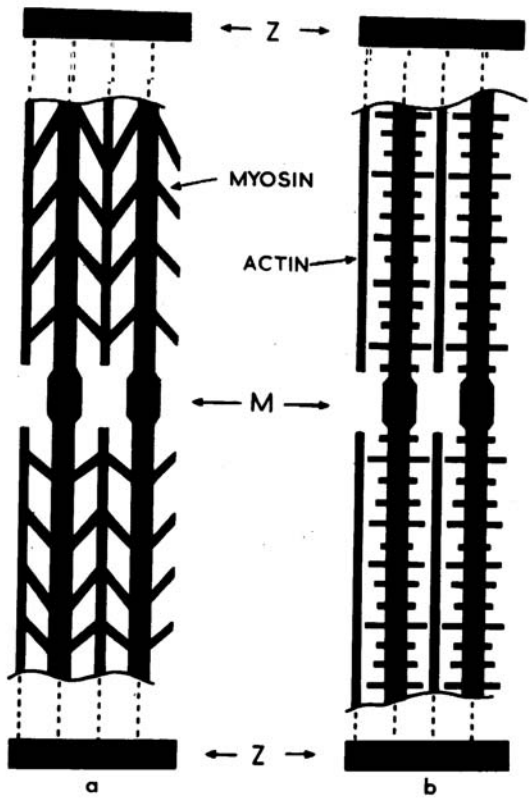

FORCE
-x
x
RATE CONSTANT
g
f
-g
-x
0
x
n
1
ISOMETRIC
ISOTONIC
0
x

Force-velocity relation



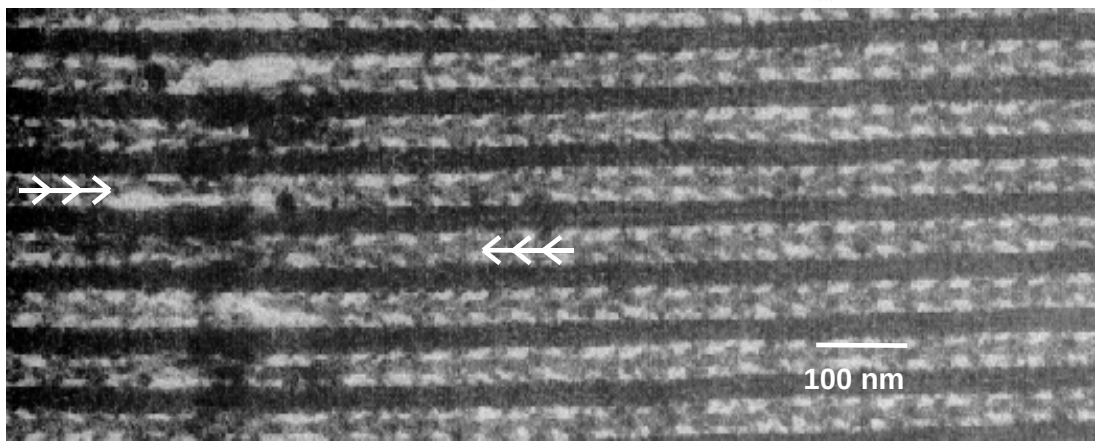
Swinging Cross-bridge hypothesis:

H.E. Huxley, 1969



Micrographs and x-ray diffraction of insect flight muscle

Reedy, Holmes and Tregear (1966)



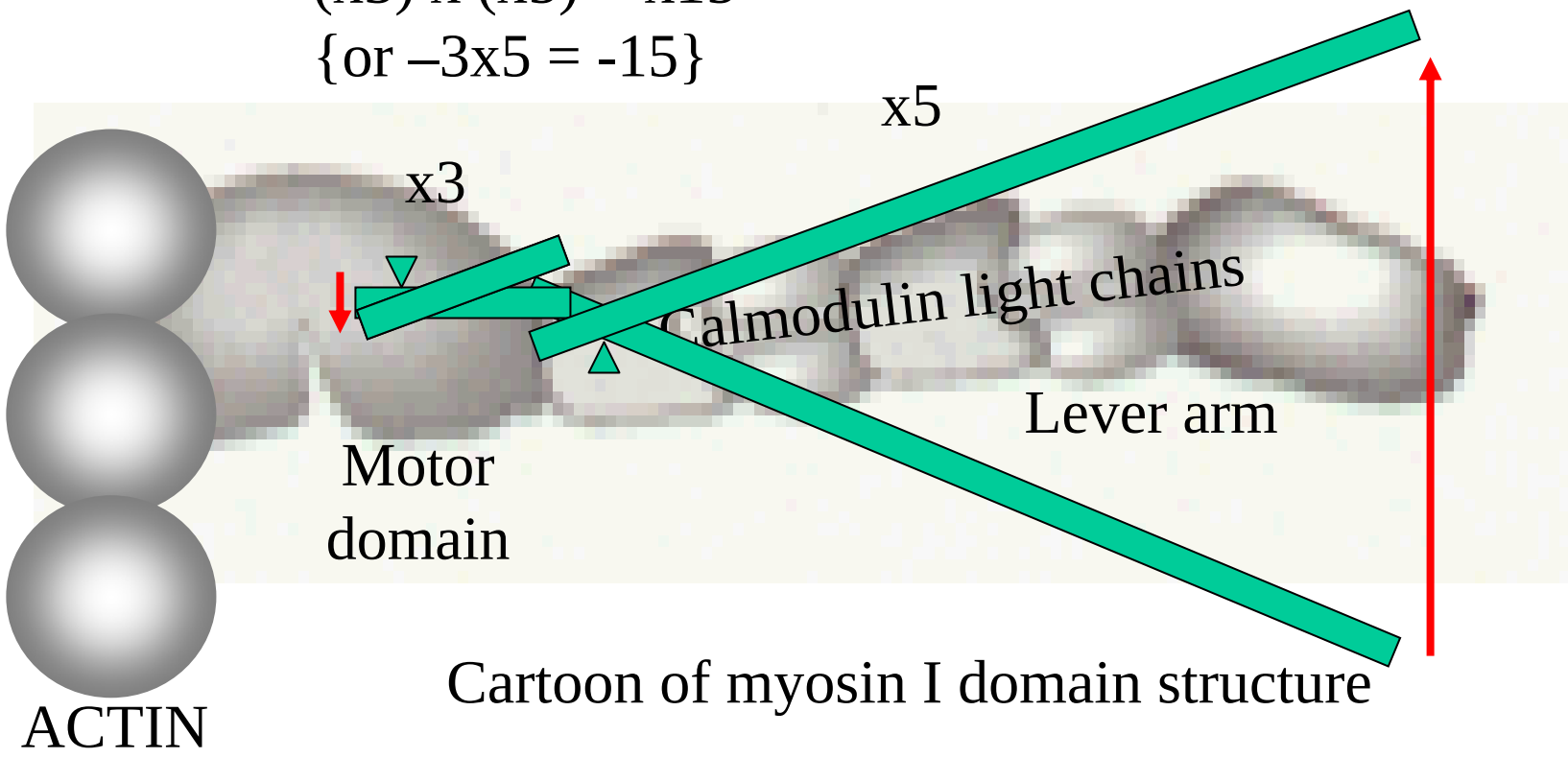
Myosin learns to multiply:

Two levers in series (impedance matching?)

Can also multiply by -1

(x3) x (x5) = x15

{or –3x5 = -15}

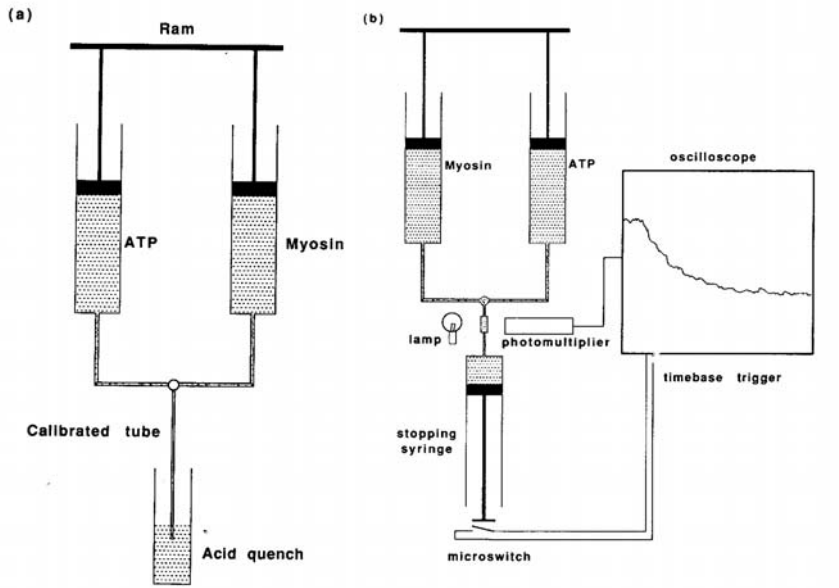


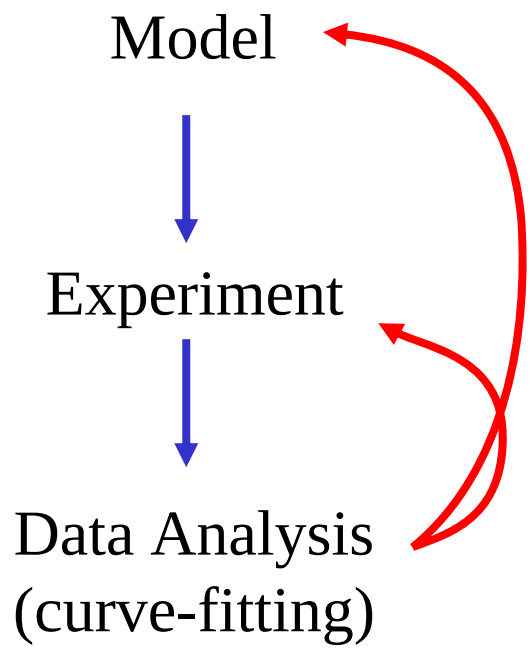
Cartoon of myosin I domain structure

Physical Biochemistry

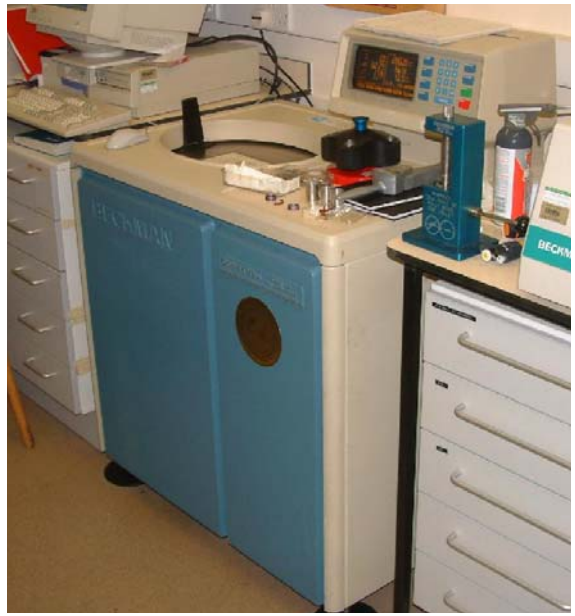
Biochemical pathways and Molecular mechanisms

$$E+S \rightleftharpoons E.S \rightleftharpoons E.P \rightleftharpoons E+P$$

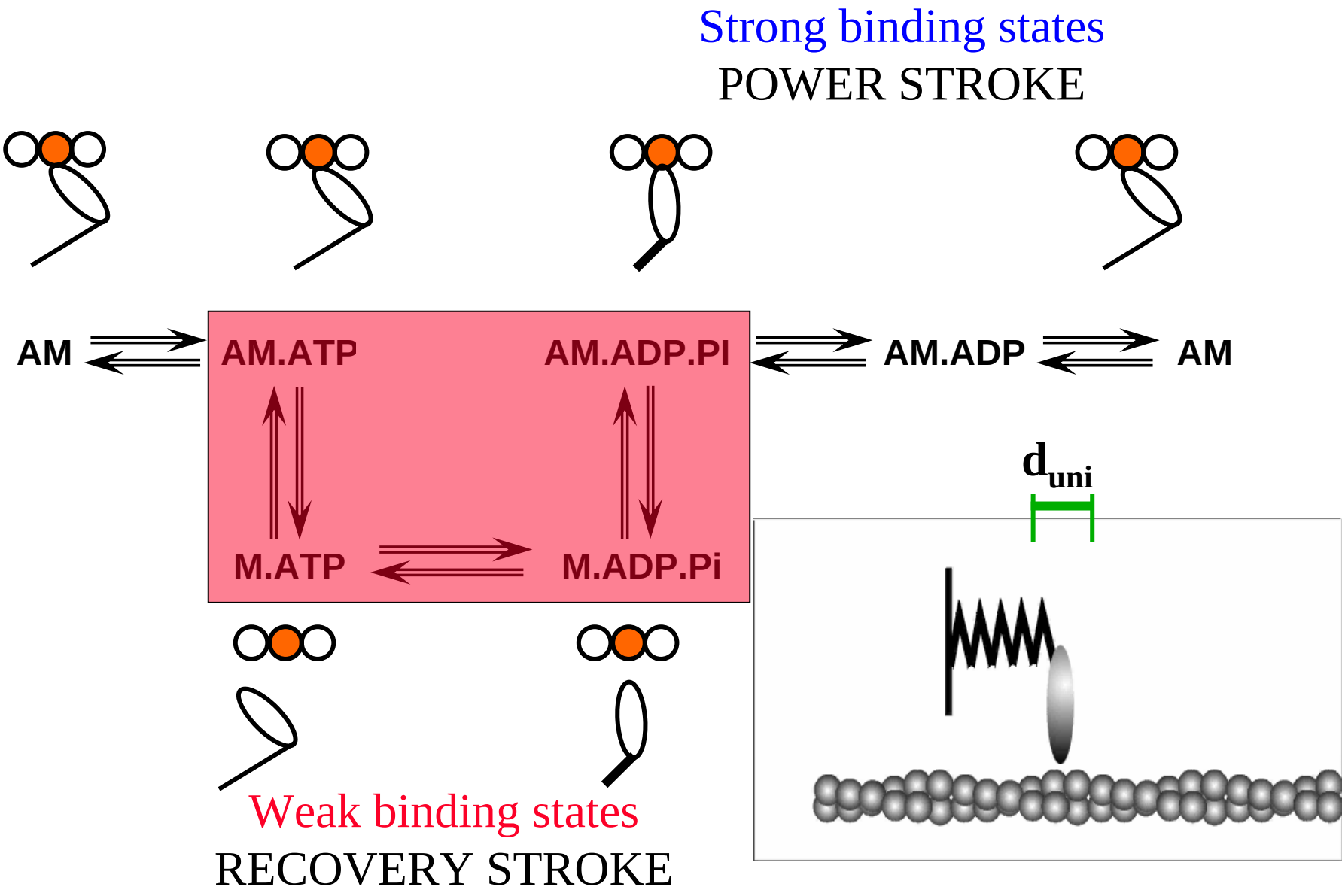




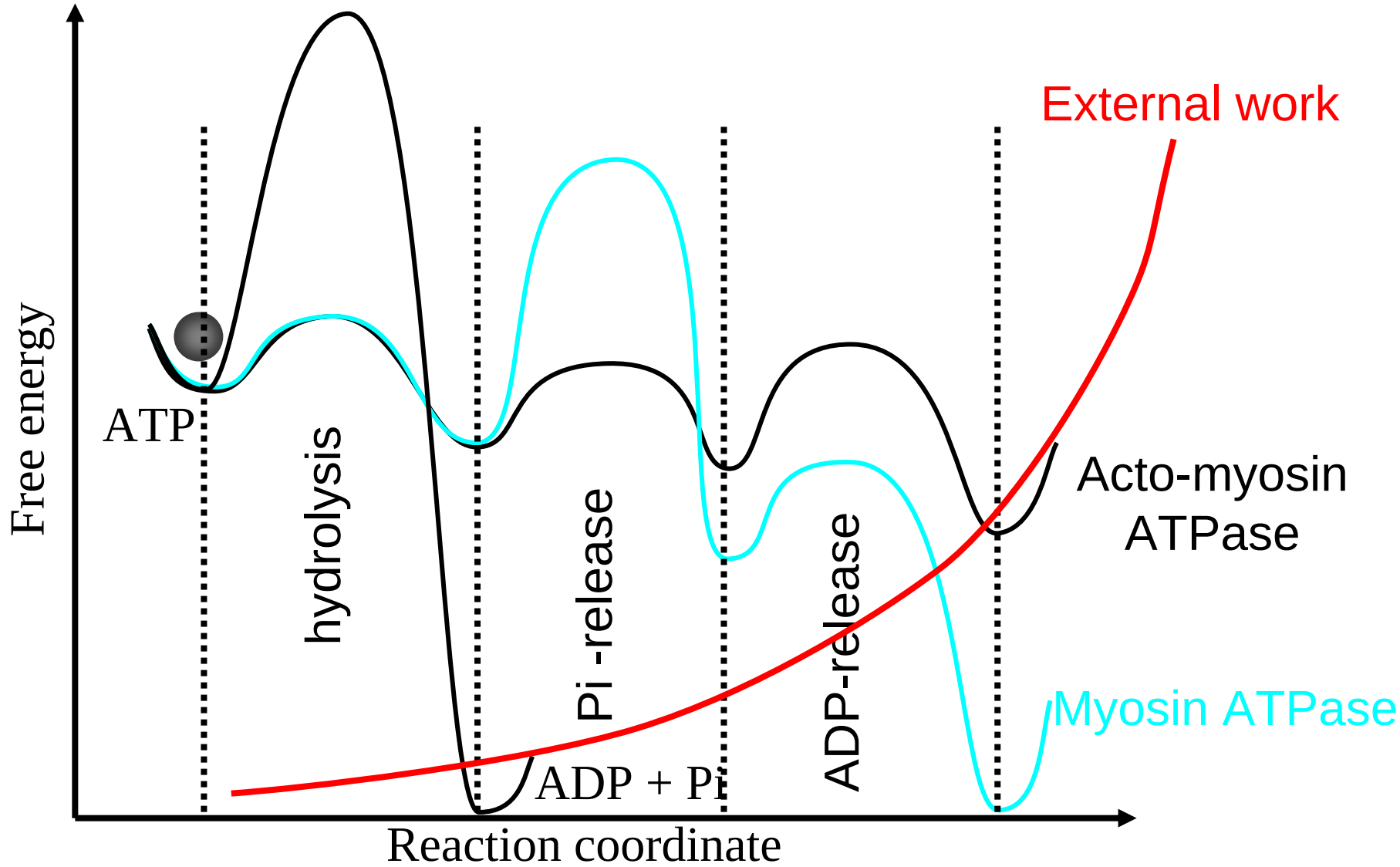
Molecular Characterisation



Acto-myosin ATPase pathway



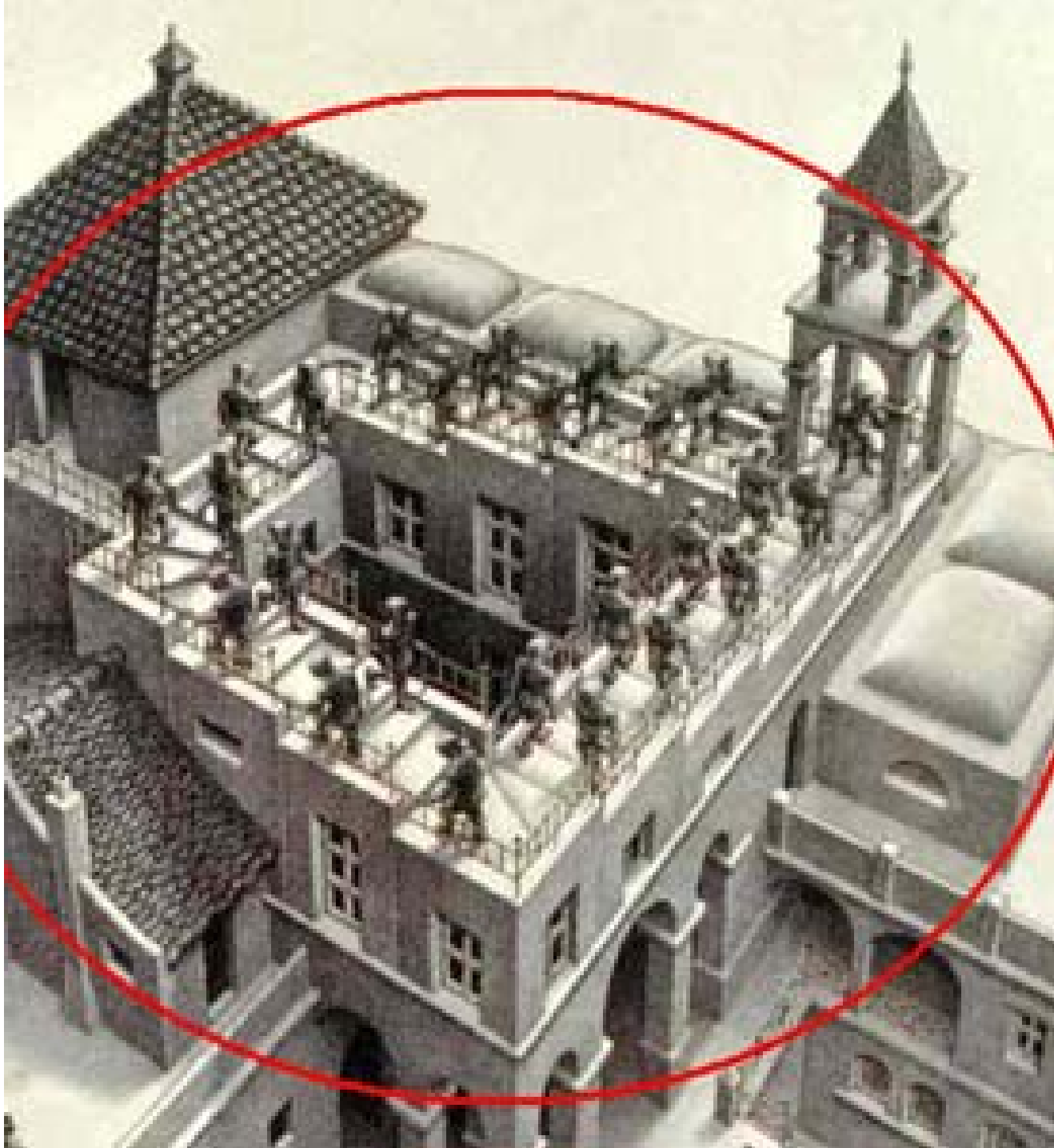
Chemical and mechanical free energy profile for the reaction pathway.



Keep it real!

Legalise it!

Respect,
Hill and others



Single MoleculeTechnologies

- Atomic Force Microscopy
- Patch-Clamp (ion channels)
- Electron Cryo-microscopy
- Total Internal Reflection Fluorescence
- Optical Tweezers

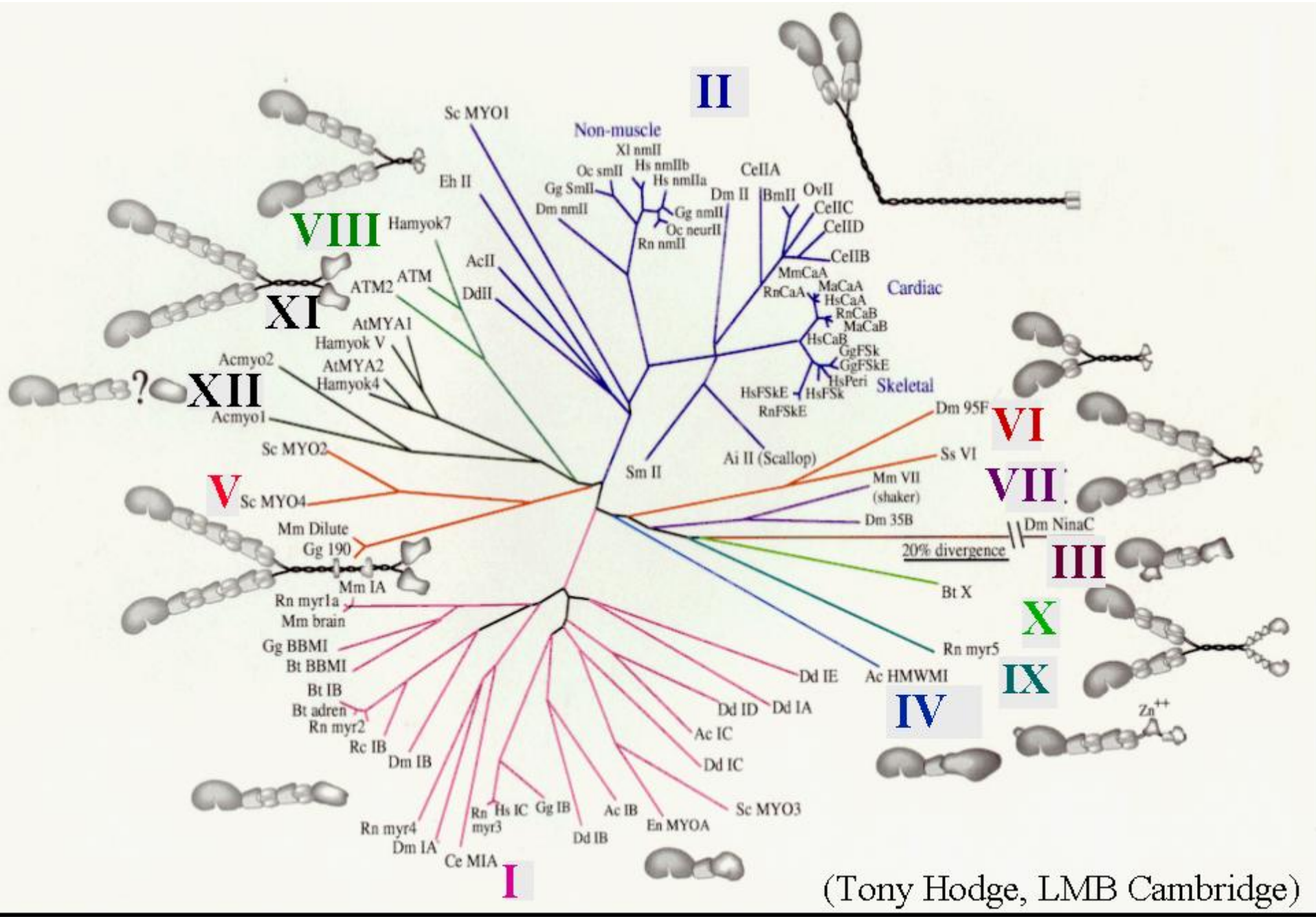
Why work with individual molecules?

- Single molecule experiments can give unequivocal information about how enzymes work and can provide new insights into enzyme mechanism.

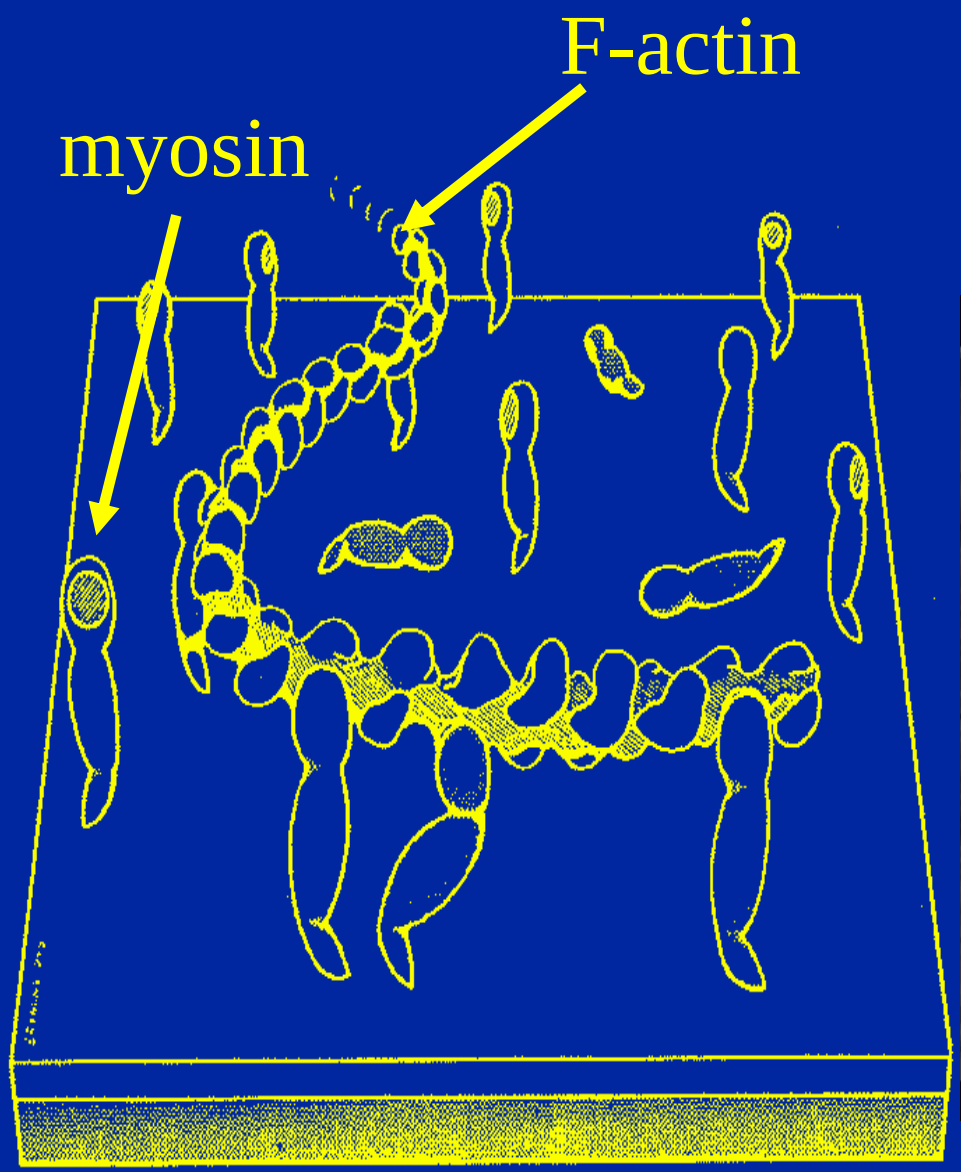
- Sequential steps that make up biochemical pathways can be observed directly. The chemical trajectory of an individual enzyme can be followed in space and time.

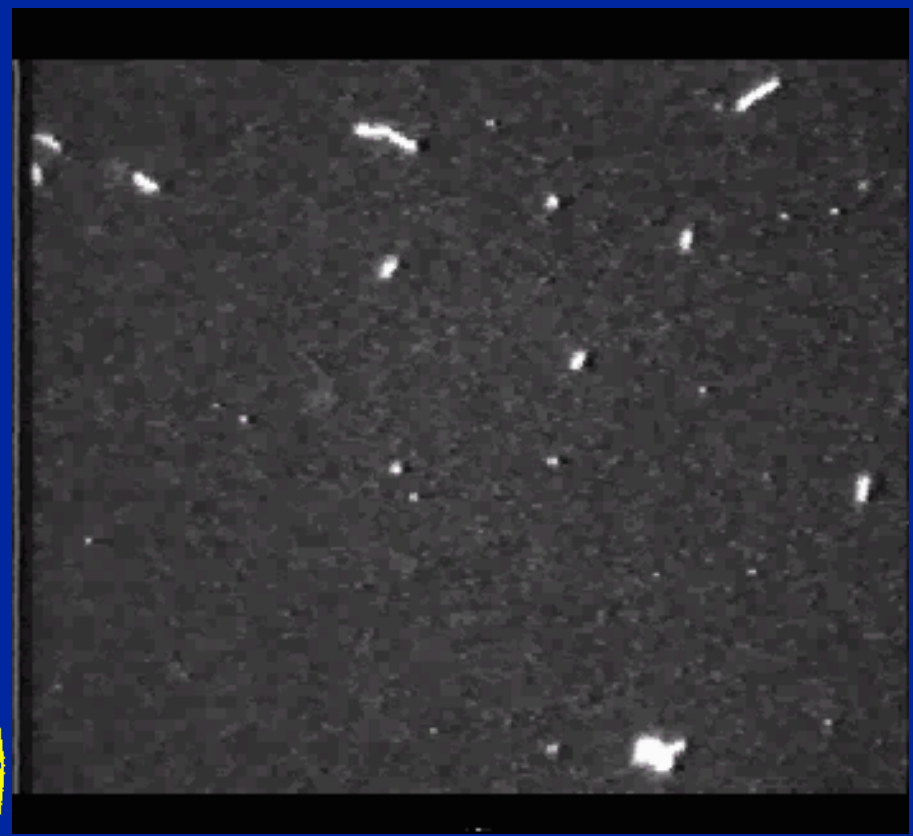
- There is no need to synchronise a population in order to study the biochemical kinetics

- 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).

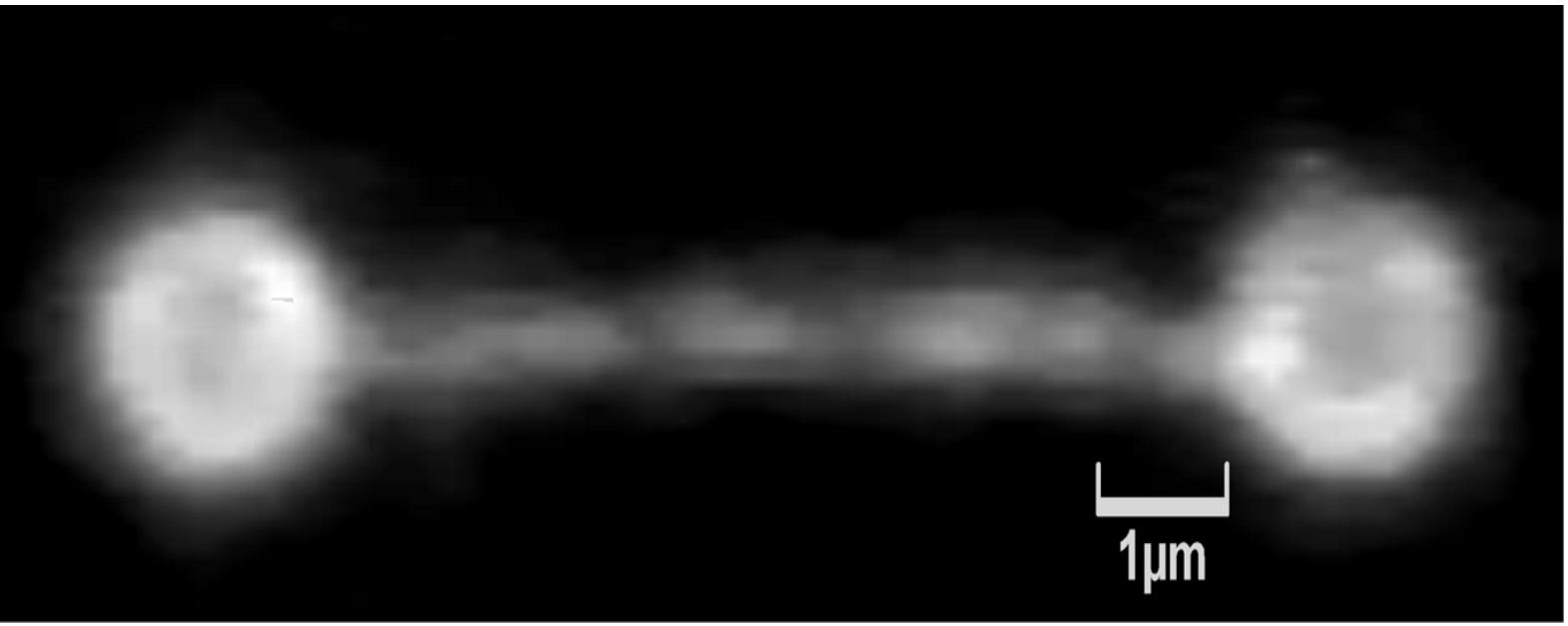

II
Sc MYO1
Non-muscle
Xl nmII
Oc smII
Hs nmIIb
Hs nmIIa
Gg SmII
Gg nmII
Dm nmII
Oc neurII
Rn nmII
Eh II
CeIIA
Dm II
BmII
OvII
CeIIC
CeIID
CeIIB
VIII
Hamyok7
AcII
DdII
ATM
ATM2
XI
MmCaA
RnCaA
MaCaA
HsCaA
RnCaB
MaCaB
HsCaB
GgFSk
GgFSkE
HsPeri
Cardiac
HsFSkE
HsFSk
RnFSkE
Skeletal
AtMYA1
Hamyok V
AtMYA2
Hamyok4
Acmyo2
XII
Acmyo1
Dm 95F
VI
Sc MYO2
Sm II
Ai II (Scallop)
Ss VI
VII
Mm VII
(shaker)
V
Sc MYO4
Dm 35B
Dm NinaC
Mm Dilute
Gg 190
20% divergence
III
Mm IA
Bt X
Rn myr1a
Mm brain
X
Gg BBMI
Bt BBMI
Rn myr5
Dd IE
Ac HMWMI
IX
Bt IB
Bt adren
Rn myr2
Rc IB
Dm IB
Dd ID
Dd IA
Ac IC
Dd IC
IV
Zn++
Sc MYO3
Rn myr4
Dm IA
Rn myr3
Hs IC
Gg IB
Ac IB
En MYOA
Dd IB
Ce MIA
I
(Tony Hodge, LMB Cambridge)

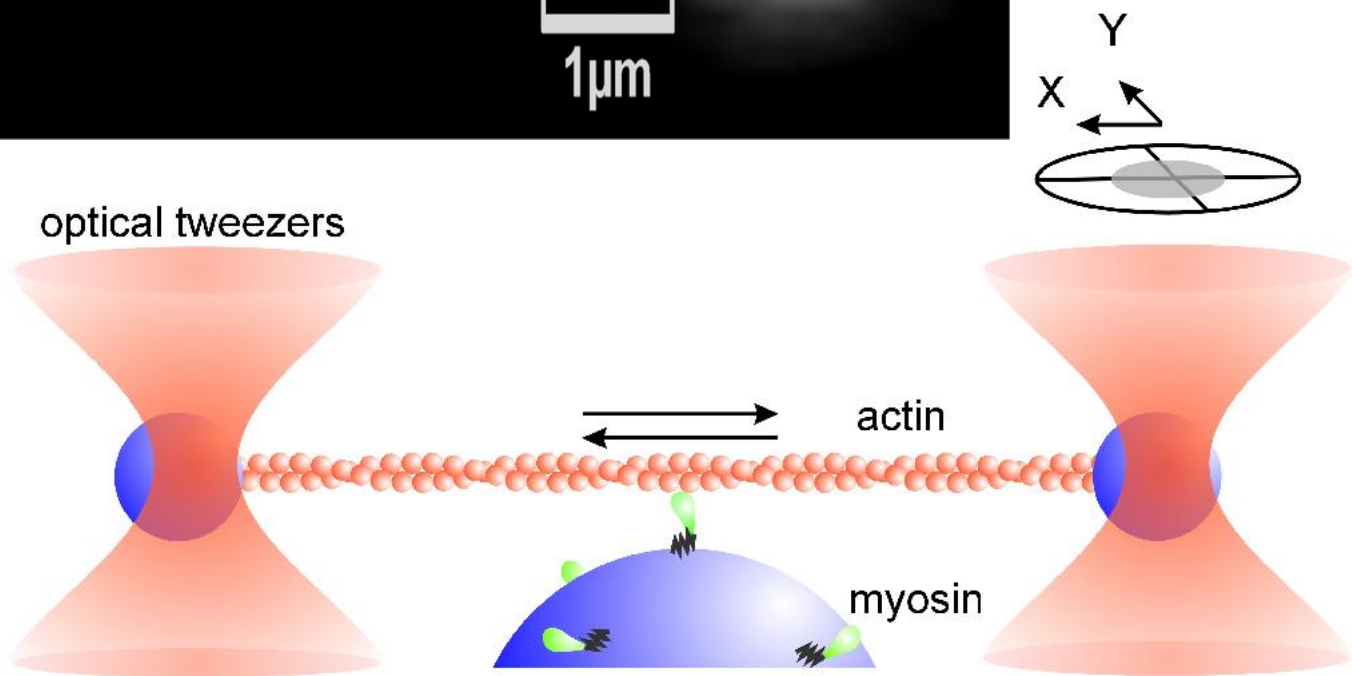
Acto-myosin *in vitro* motility assay :

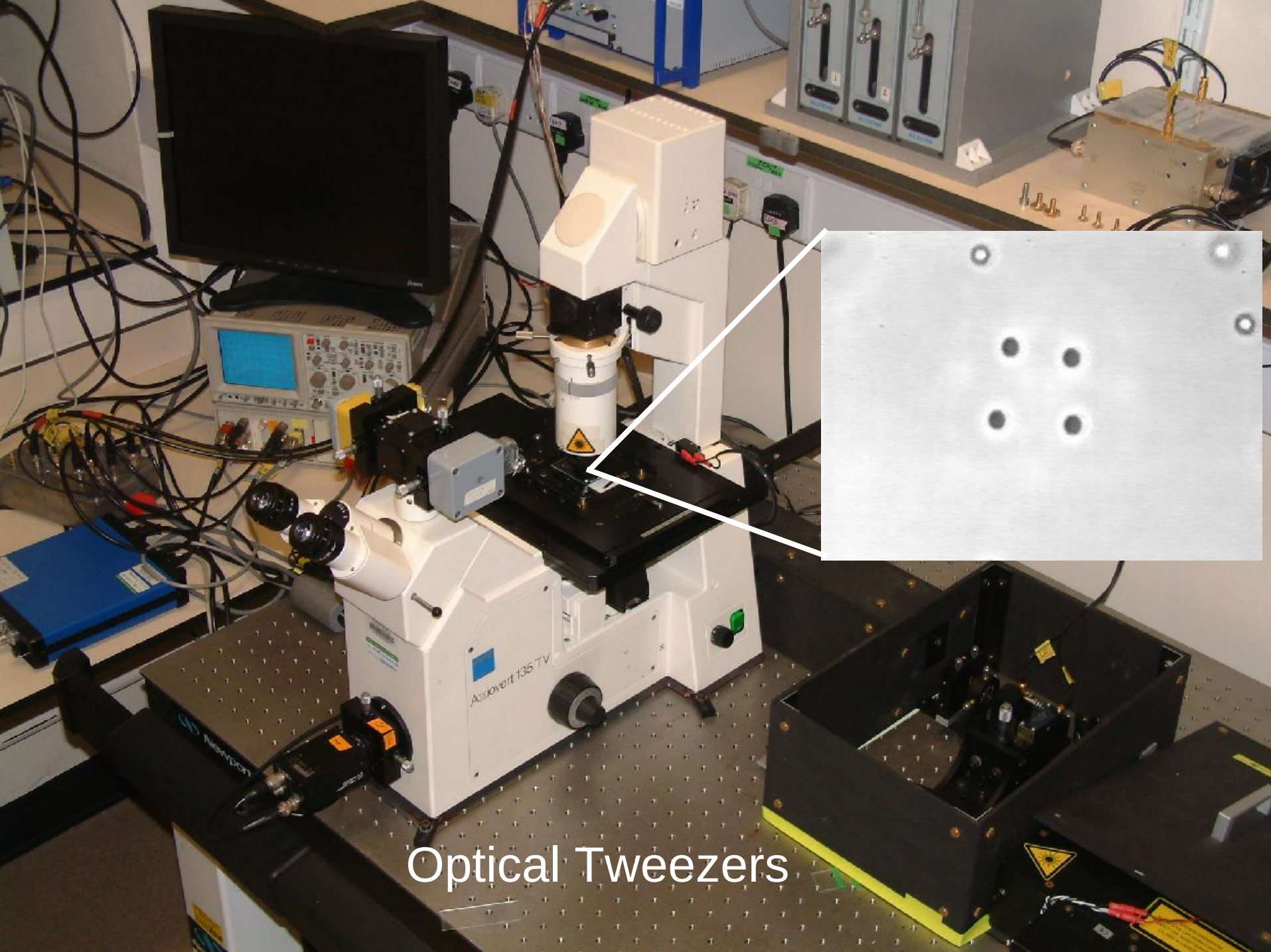




Optical tweezers – single molecule mechanics



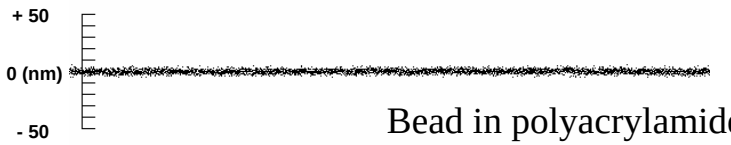




Optical Tweezers

Optical tweezers :

Time series data

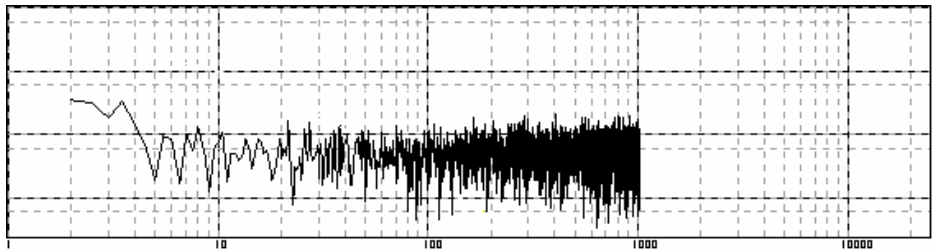


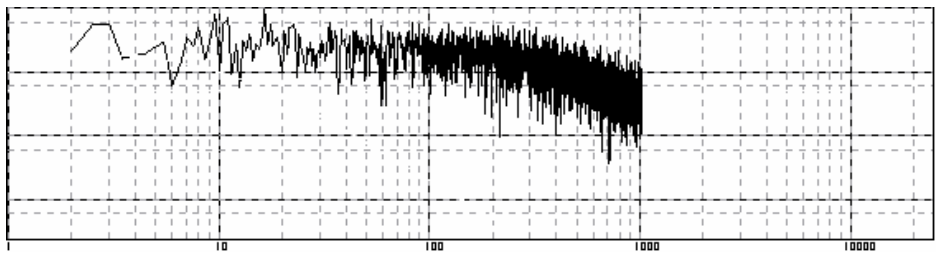
Bead in polyacrylamide

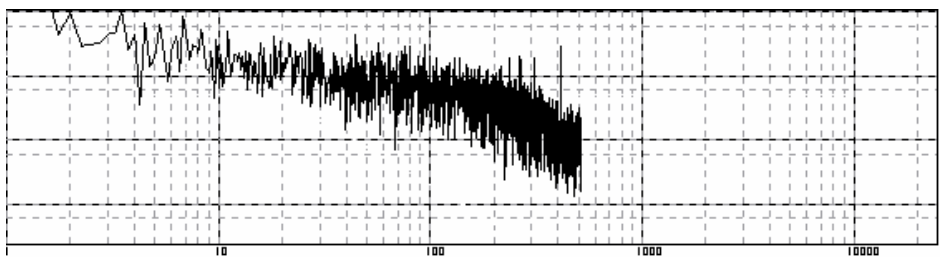
Bead in optical tweezer

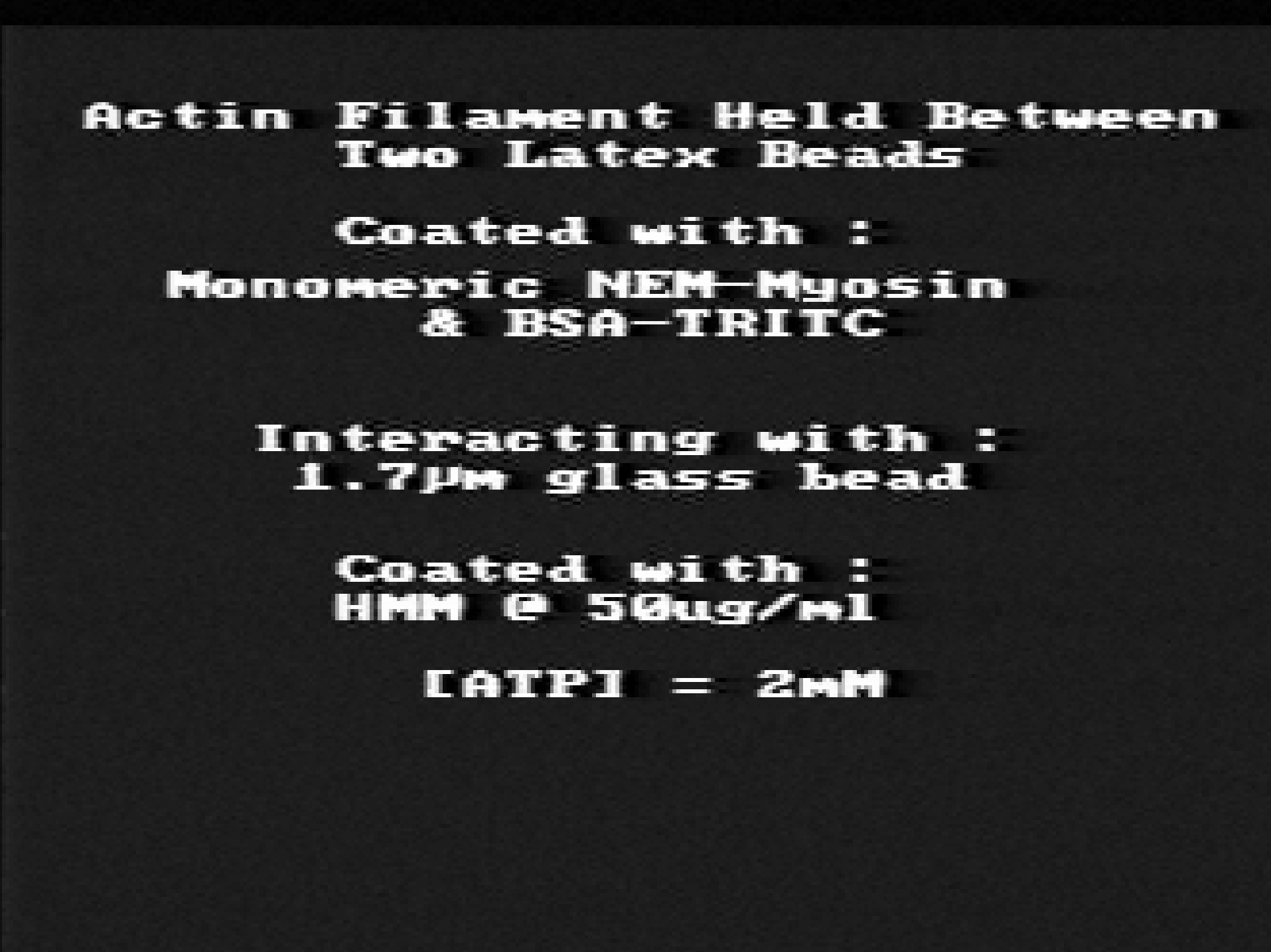
Bead in optical tweezer
+ acto-myosin interactions

Power Spectra



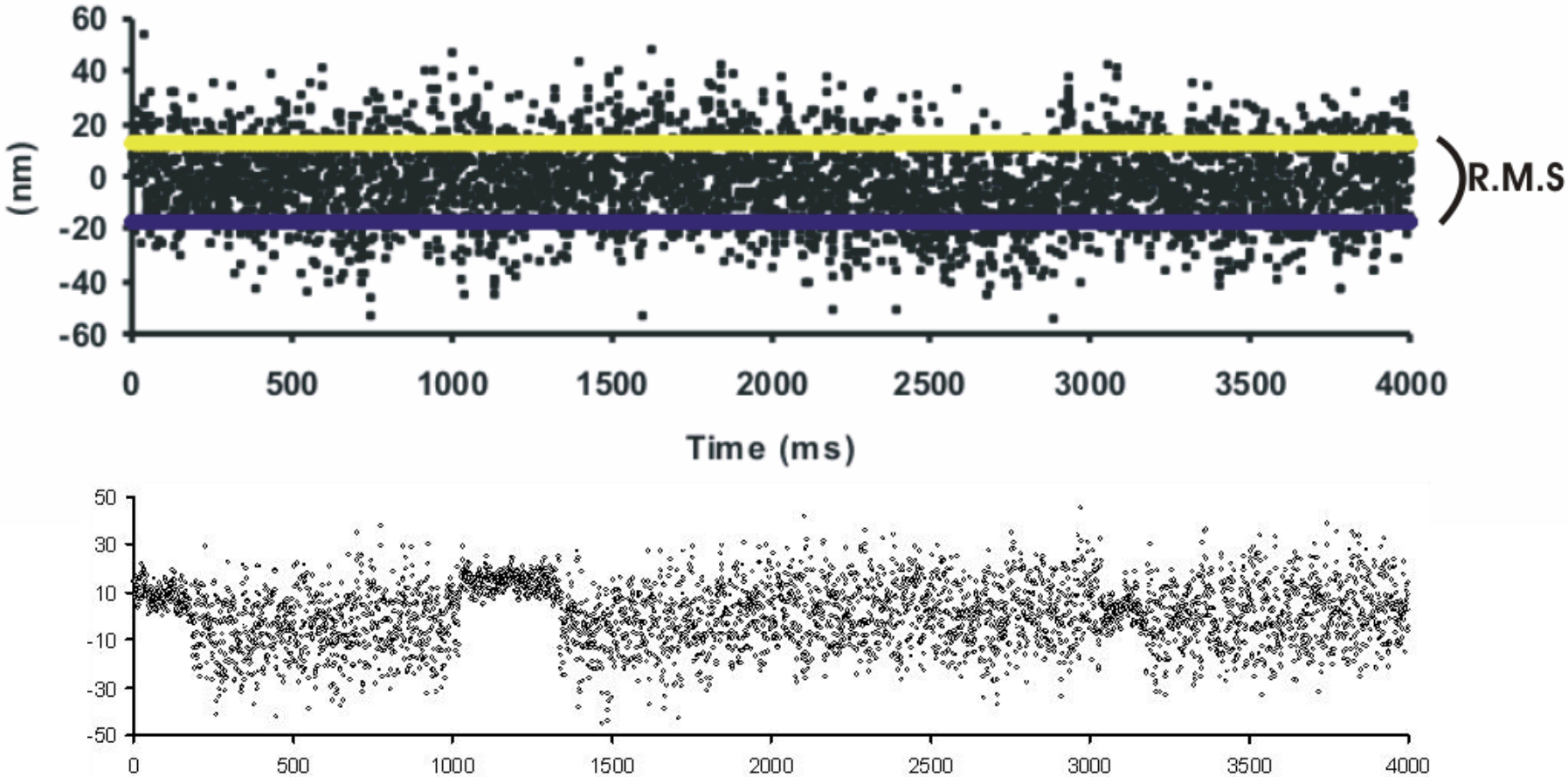




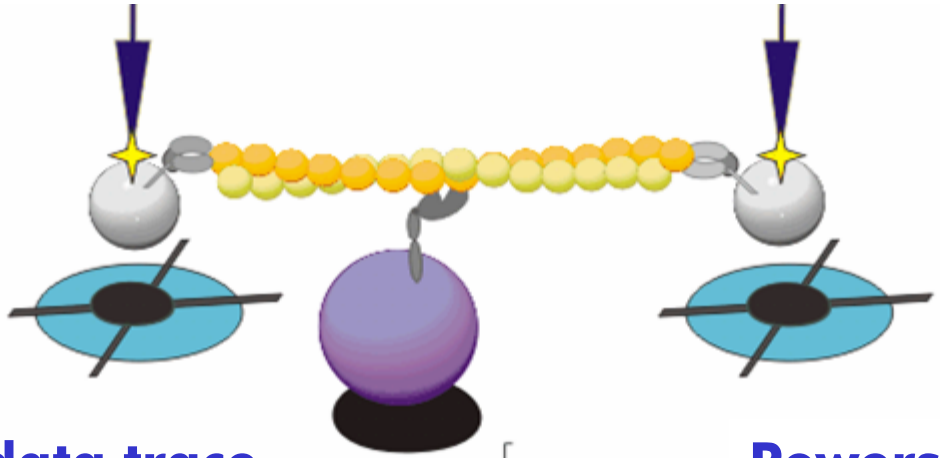

Actin Filament Held Between
Two Latex Beads
Coated with :
Monomeric NEM-Myosin
& BSA-TRITC
Interacting with :
1.7µm glass bead
Coated with :
HMM @ 50ug/ml
[ATP] = 2mM

Example data from optical tweezers experiment

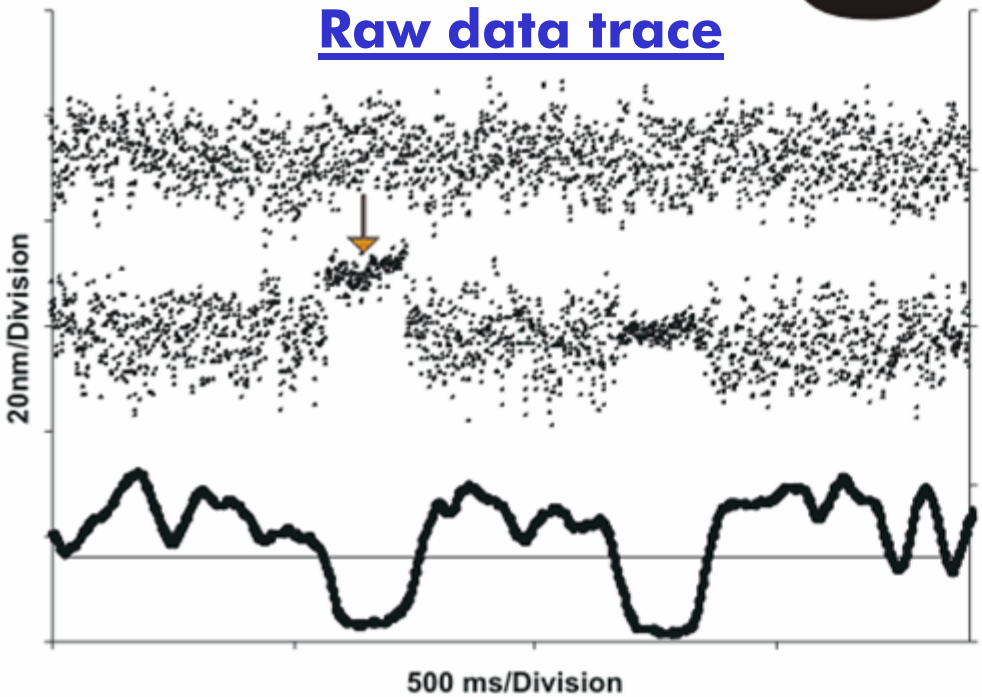
Thermal vibration of the bead in the tweezer: $1/2k_bT=1/2\kappa\langle x\rangle^2$



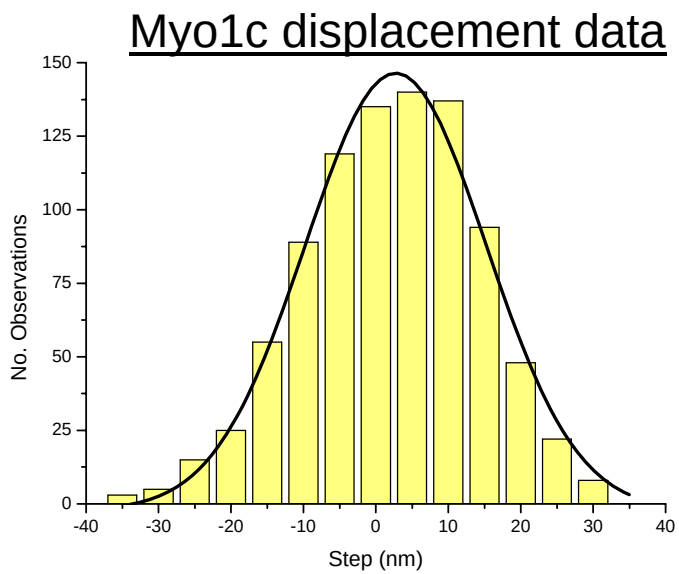
Size of the power-stroke



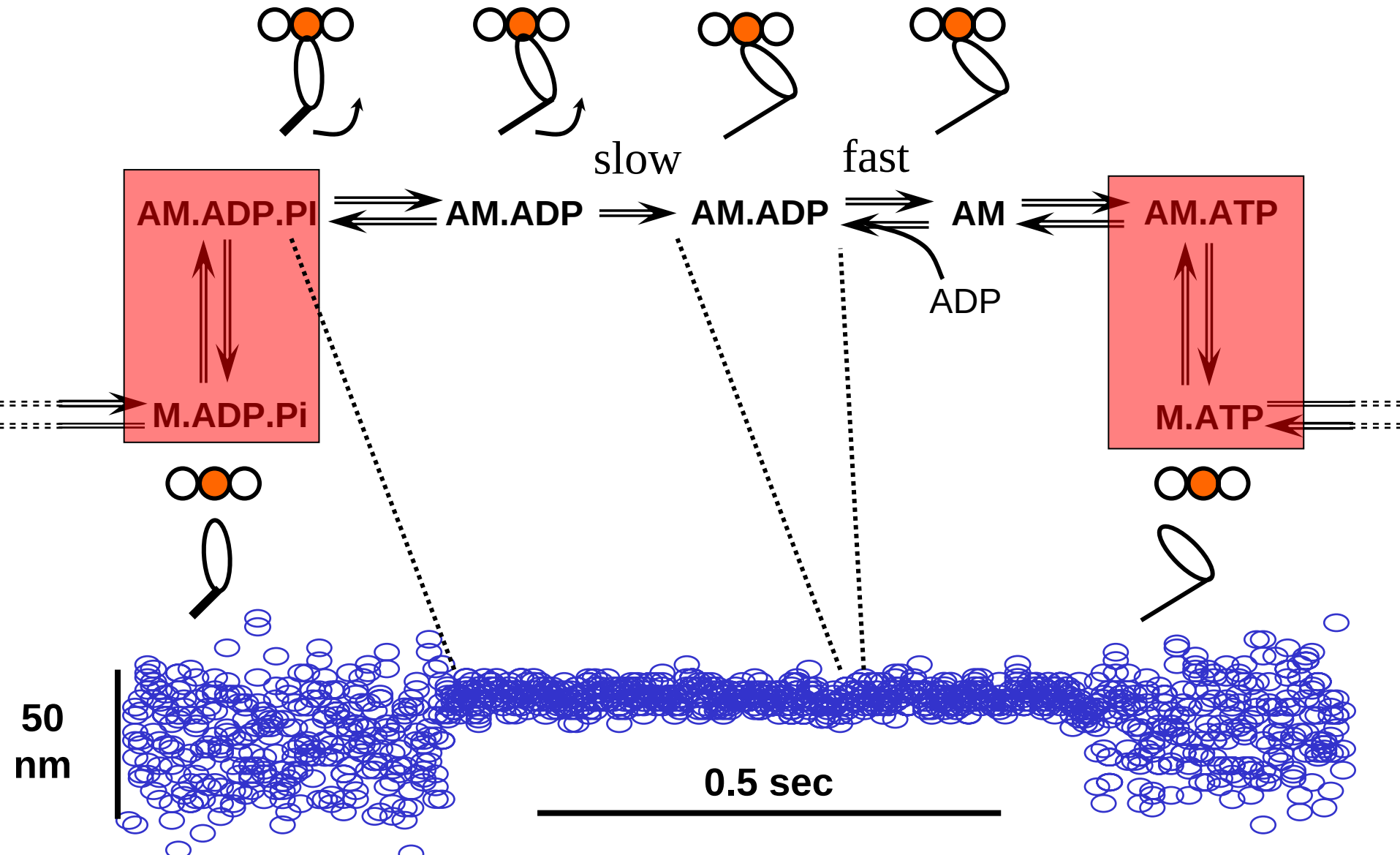
Raw data trace



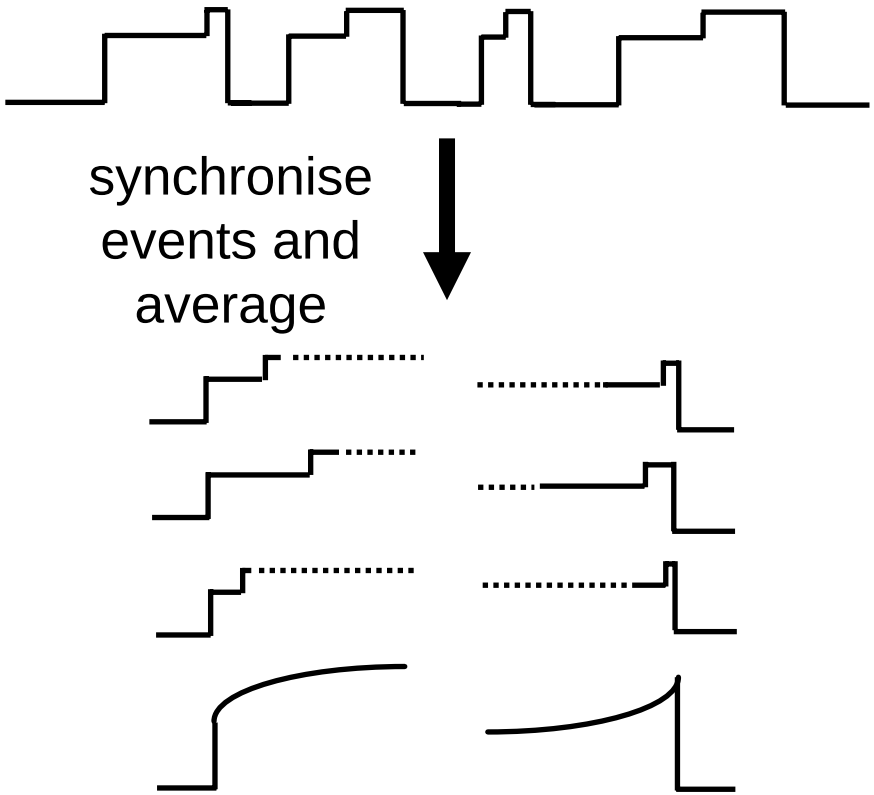
Powerstroke ~ 4nm

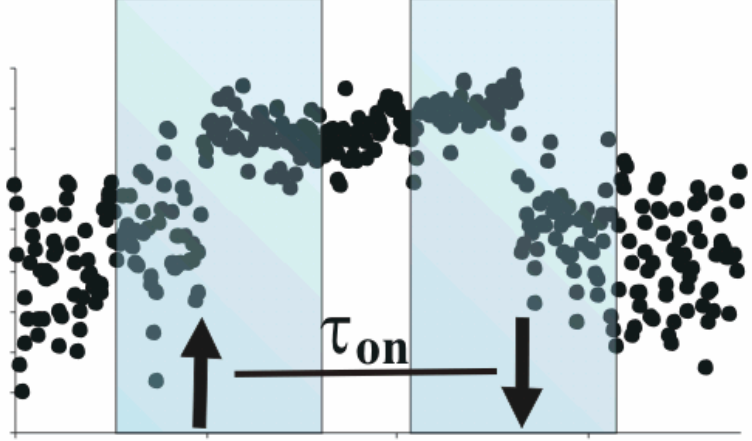


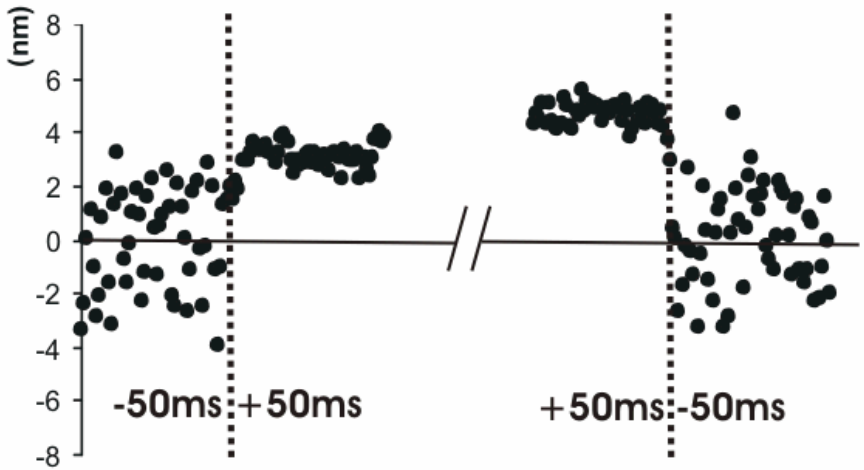
Acto-myosin ATPase

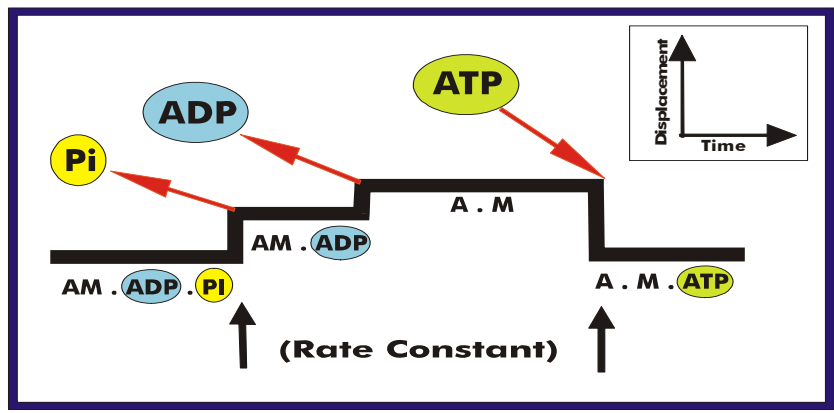


Ensemble Average



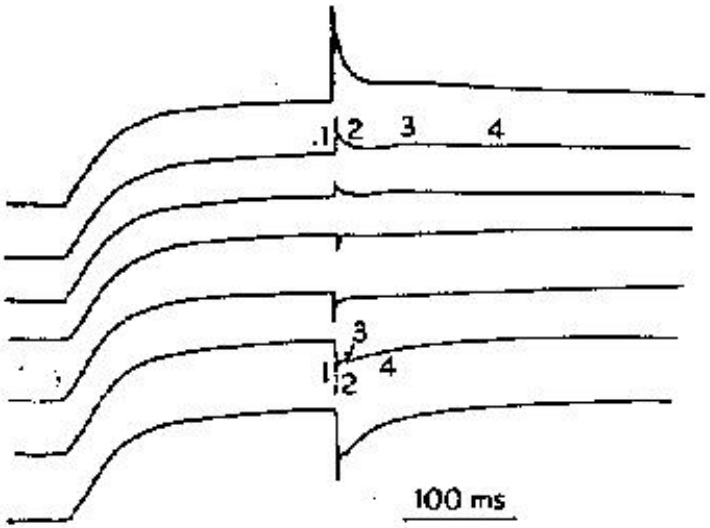


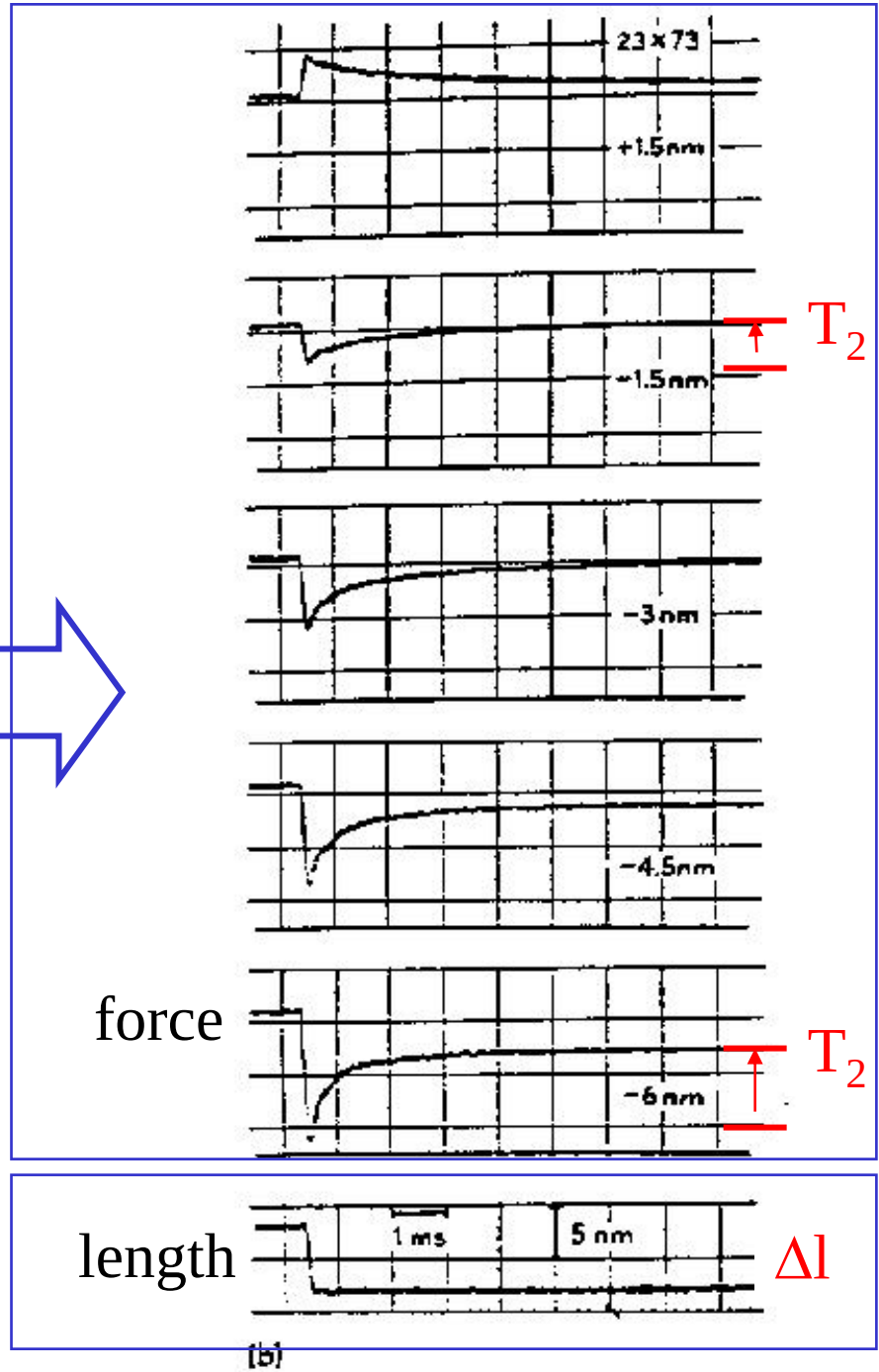




Rapid mechanical experiments using single muscle fibres:

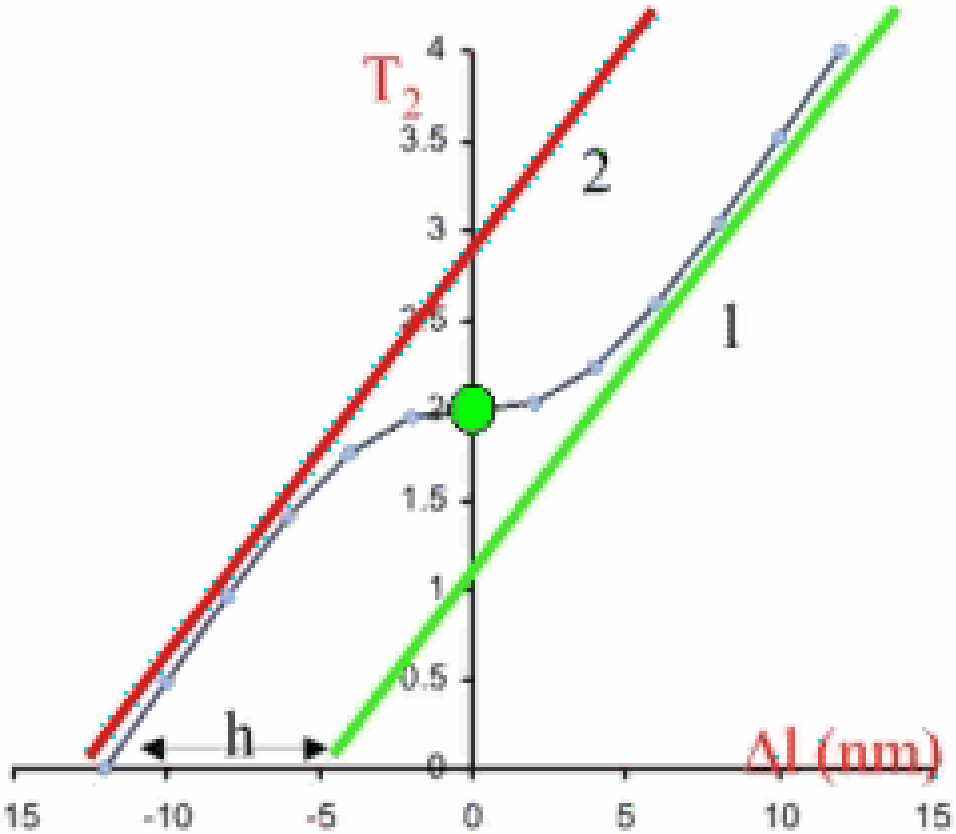
Experiment ->

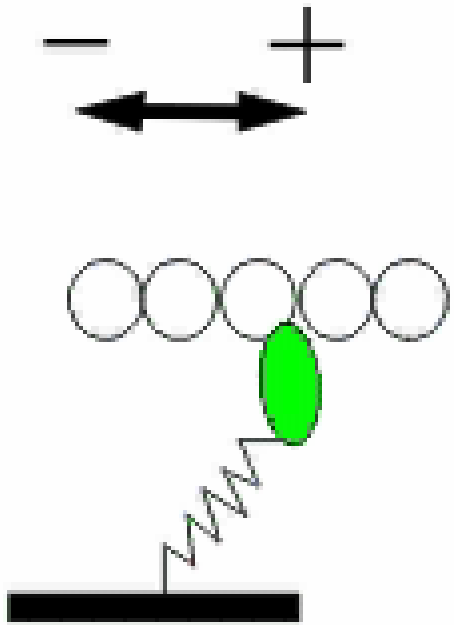




Huxley & Simmons (1971)

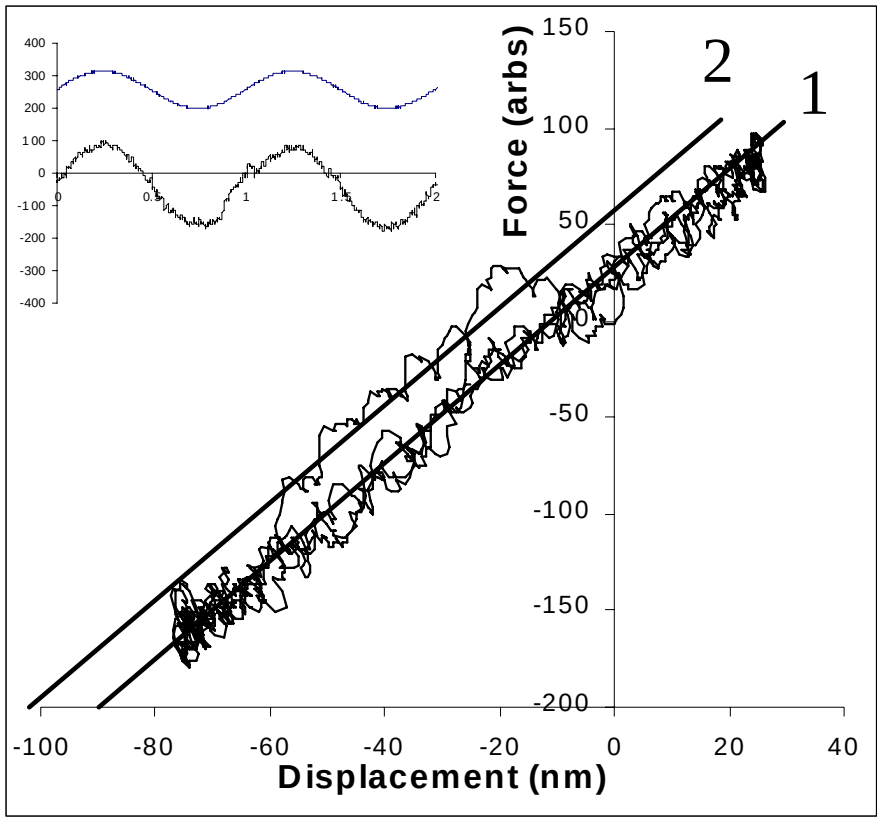
Huxley & Simmons (1971)

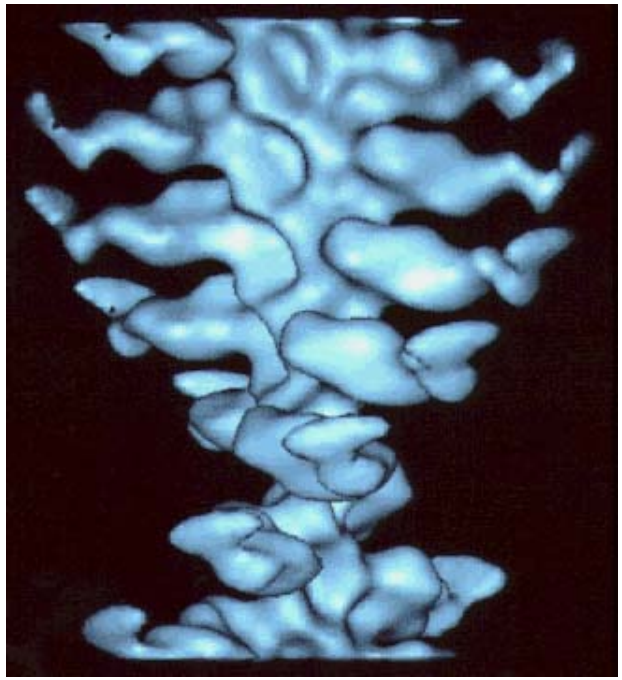




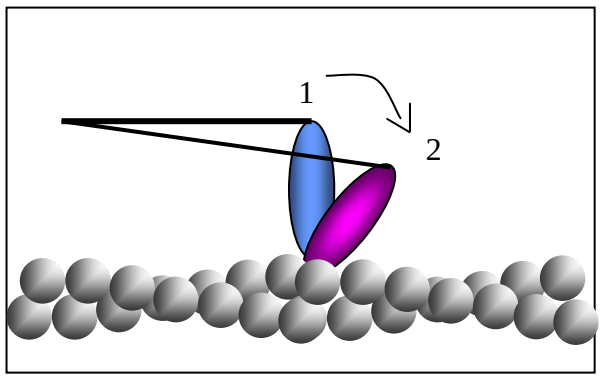
See Excel model!

Acto-myosin I subjected to controlled length change





(Jontes & Milligan)
BBM1



Force vs. Displacement

5μM ADP 10μM ATP