“MECHANICAL PROPERTIES OF SINGLE MYOSIN MOLECULES ”

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EBSA – 2000

Collaborators/York group

Myosin-I

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Cell Biology, Scripps RI, USA

Myosin II

J. Kendrick-Jones,

R.T. Tregear

LMB, Cambridge, UK

Myosin V

F. Wang

J.R. Sellers

NHLBI, NIH, Bethesda, USA

Cy3-ATP

J. Ecclestone

NIMR, Mill Hill, UK

Optical Trappers

Single molecule imaging

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M.L. Bartoo*

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M. Tyreman

York Molecular Motors group

John C. Sparrow

David C.S. White

Goal of our current work:

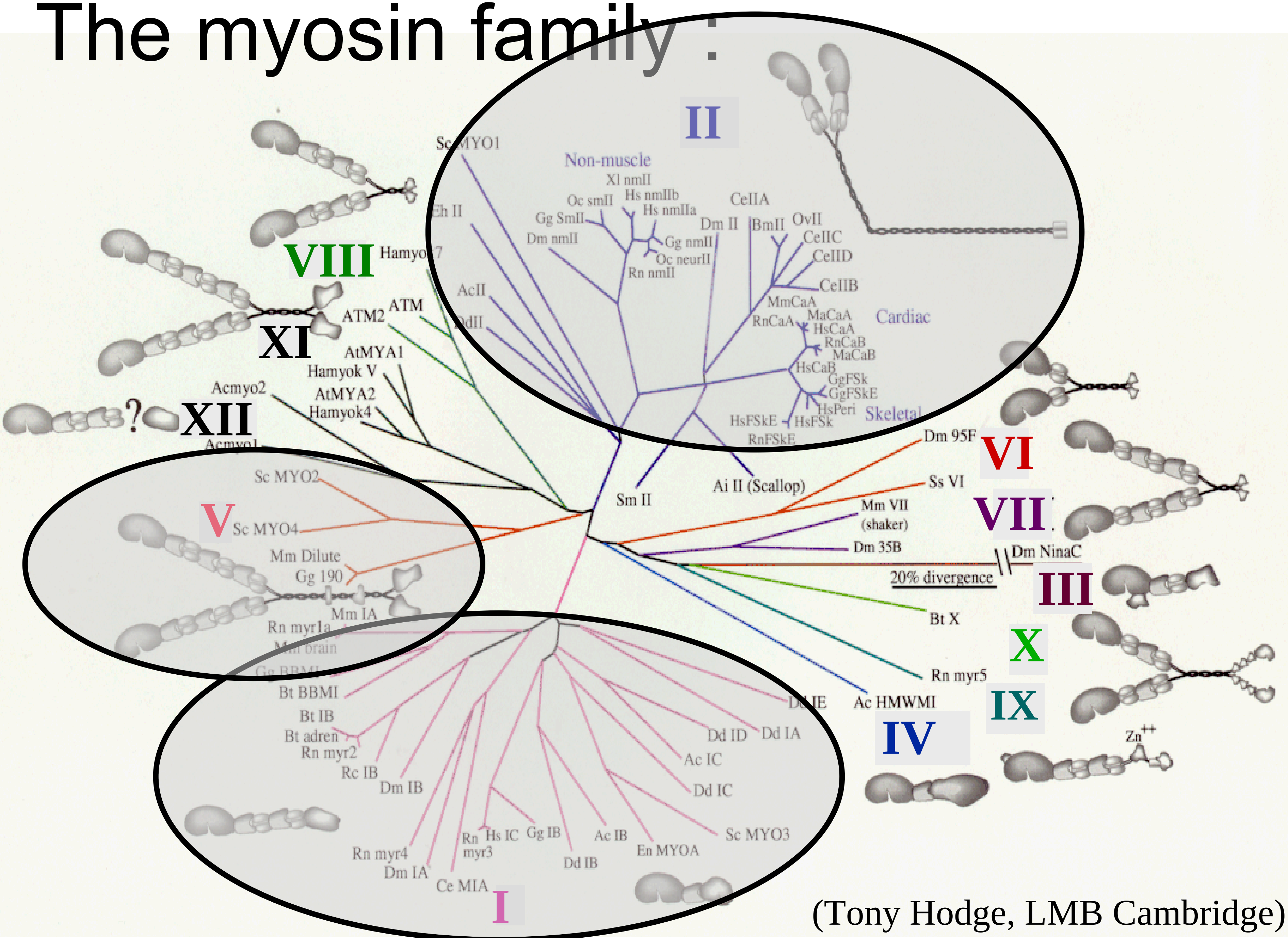
- Use single molecule technologies to study the molecular mechanism of force production by molecular motors.

- Relate the mechanical properties of different motors to their functional role in the living cell.

Background:

- Many forms of cellular motility are driven by the cyclical interaction of myosin with actin, coupled to the hydrolysis of ATP.
- The best understood “paradigm” is the acto-myosin-II found in skeletal muscle.
- There are now known to be 15 different families of myosin, each has a different cellular function. Most mammalian cells have about 10 different expressed isoforms.

The myosin family :



(Tony Hodge, LMB Cambridge)

<u>Myosin-II</u> - two-headed – filament forming

Non-muscle: myosin IIa and b

coiled-coil tail

2 IQ motifs

0.1 μm/sec

Muscle: cardiac, smooth, **skeletal**

coiled-coil tail

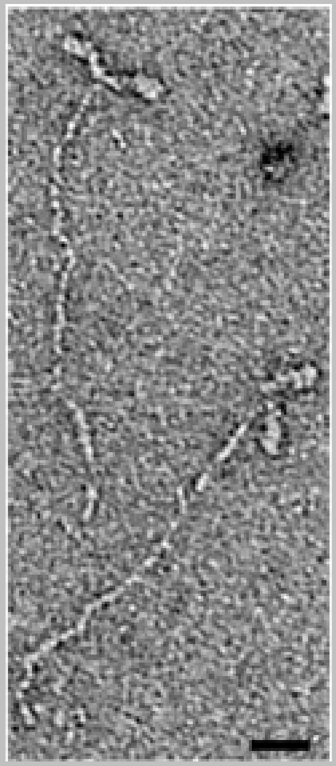
2 IQ motifs

limited proteolysis yields:

double headed HMM

single headed S1

6 μm/sec



20 nm

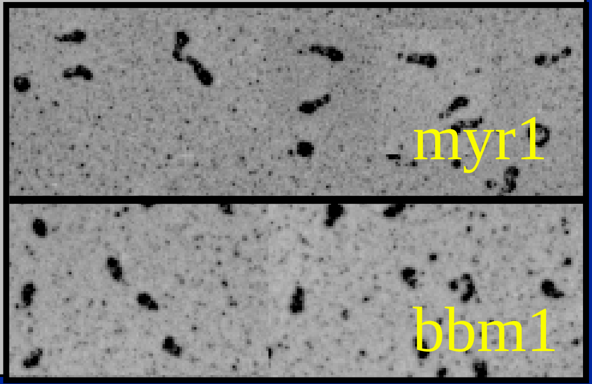
Myosin-I – single headed, membrane and/or actin binding

Subclass 1: human myosin Ic, rat myr3, Amoeboid
vesicle binding domain
SH3 tail domain - actin binding (?)
1 IQ motif

Subclass 2: myosin Iα, bbmI, rat myr1 (130kDa myosin I)
plasma membrane binding domain.
3-6 IQ motifs
0.05 μm/sec

Subclass 3: myosin Iβ, rat myr2 (110kDa myosin I)
plasma membrane (hair cell tip-link motor)
2 IQ motifs
0.05 μm/sec

Subclass 4: myosin Iγ, rat myr4
2 IQ motifs



Myosin-V - two-headed, membrane binding

Subclass a: chicken brain, mouse "dilute"

vesicle binding domain

(melanosome & synaptic vesicle transport)

6 IQ motifs (1 ELC?+5 calmodulin)

0.5 μm/sec

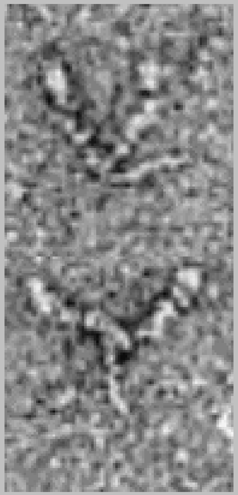
Subclass b: rat myr6, human Vb

Subclass c: human Vc

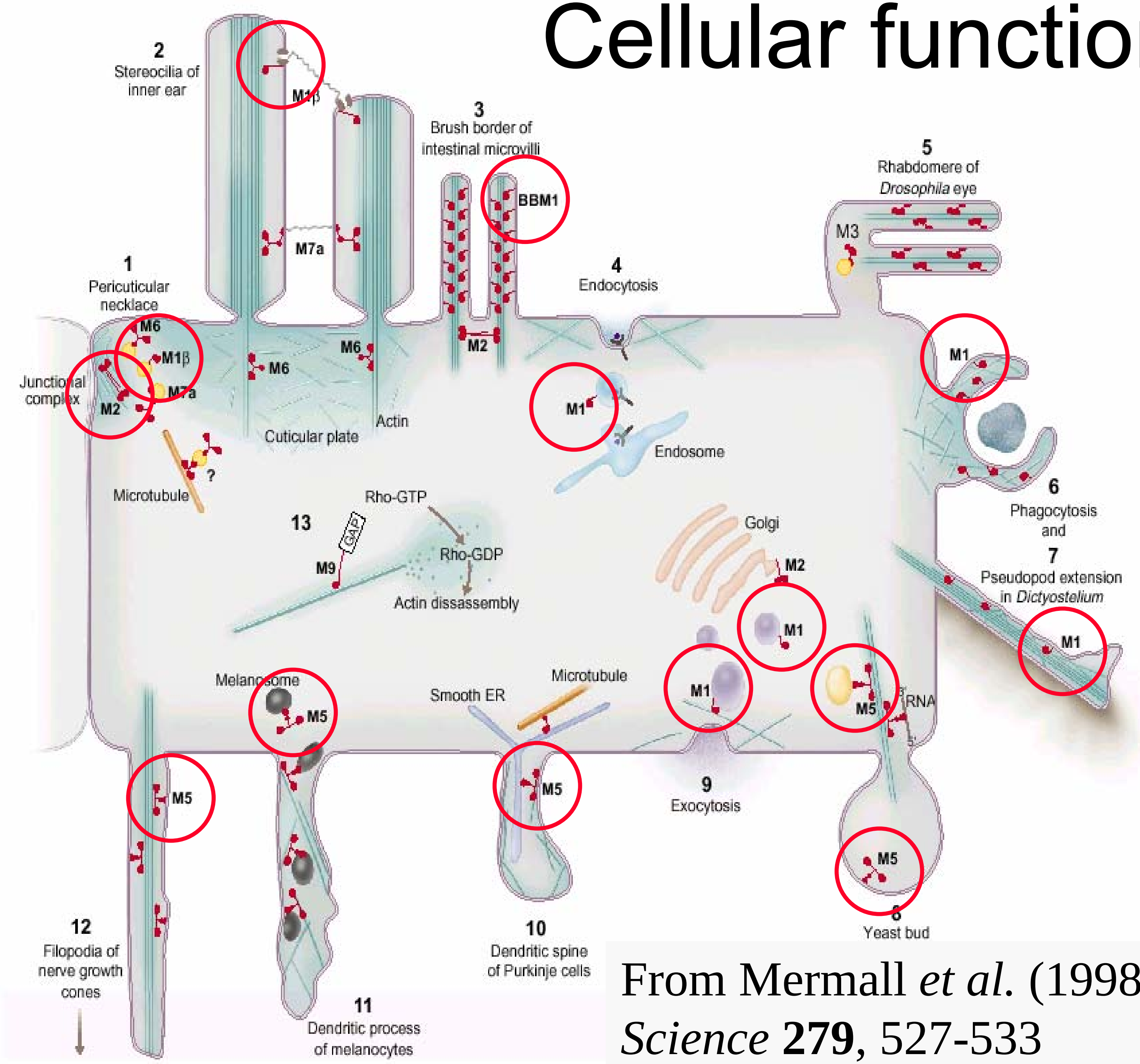
Closely related plant class VIII & XI

?Superfast processive motors?

60 μm/sec

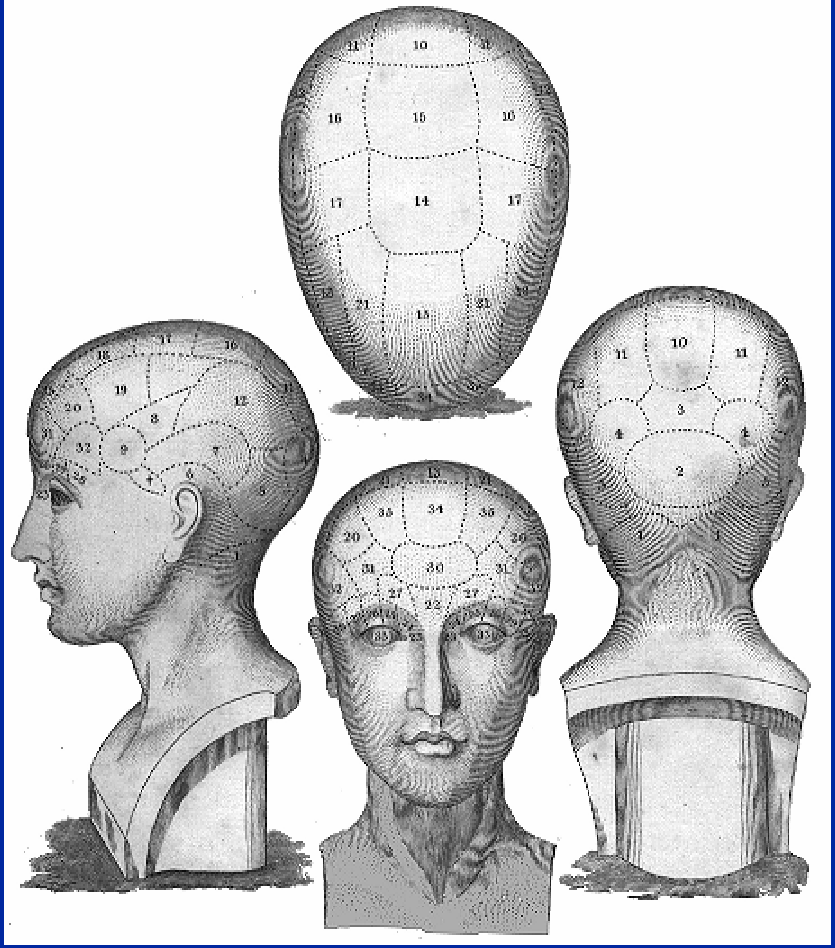


Cellular functions :



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

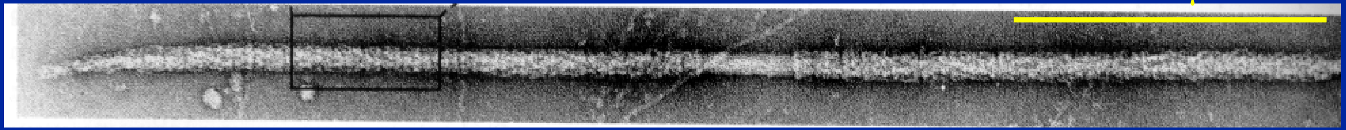
The surface structure of the myosin ***head*** determines its character.



The myosin ***tail*** specifies the "cargo" that is to be transported.

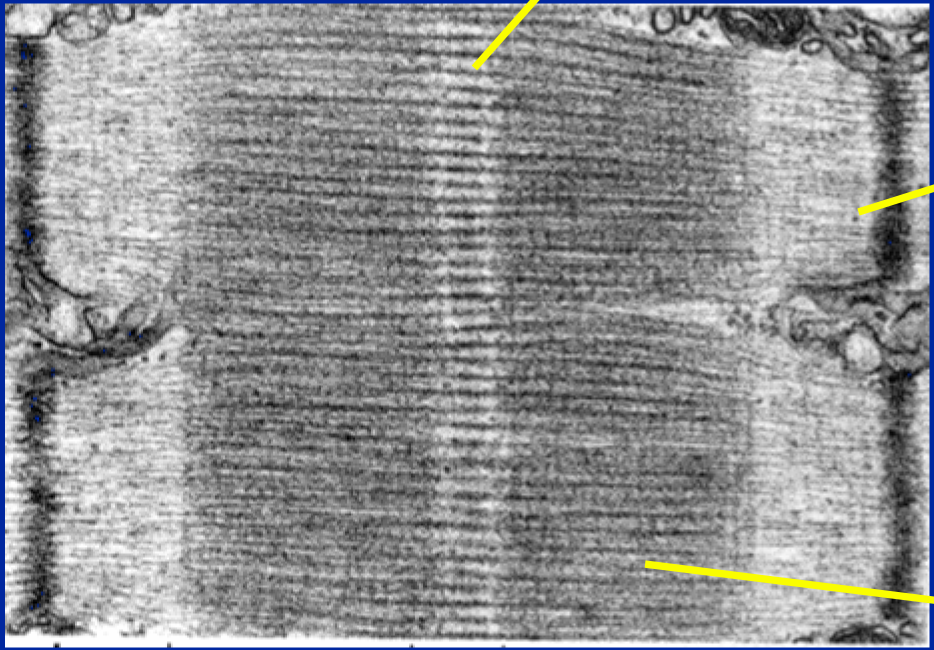
Acto-myosin in muscle :

0.5µm



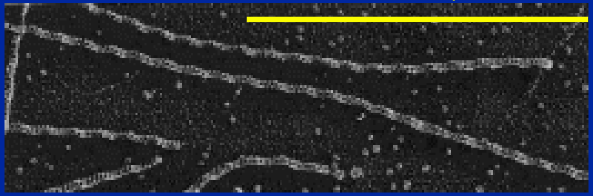
Myosin containing, thick filament

1 µm



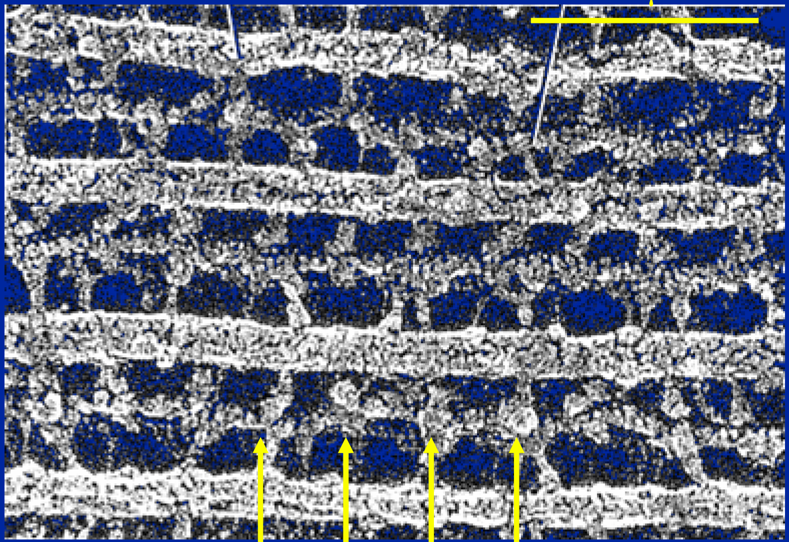
Sarcomere

0.5 µm



Actin containing, thin filaments

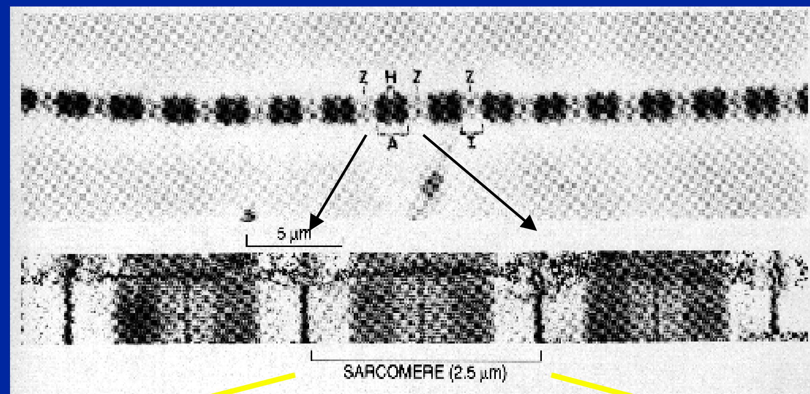
0.1 µm



acto-myosin “cross-bridges”

Filament sliding causes muscle to shorten:

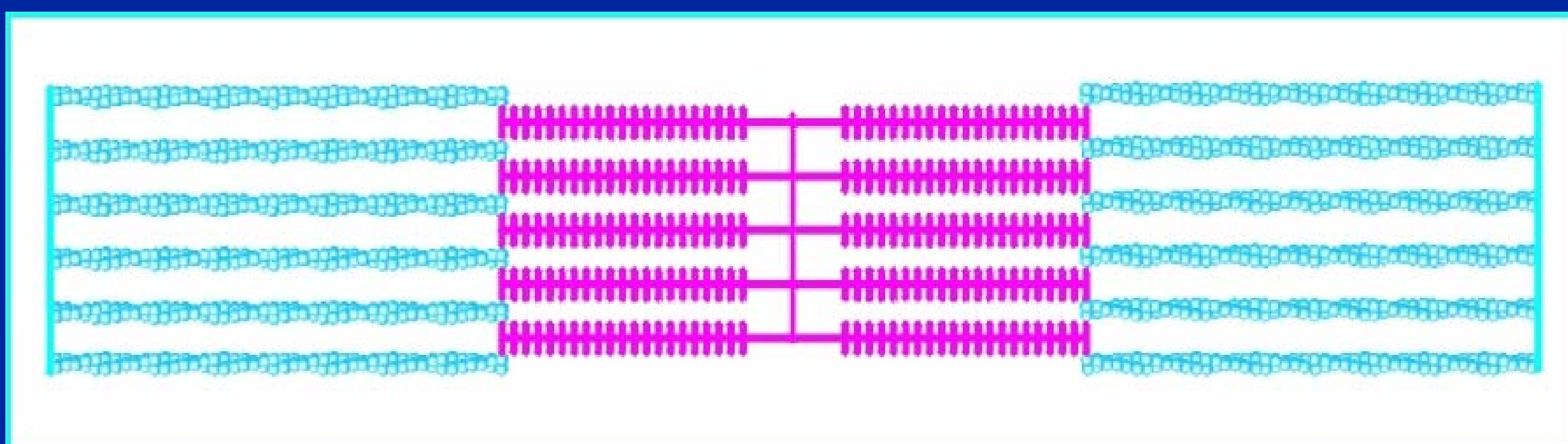
myofibril



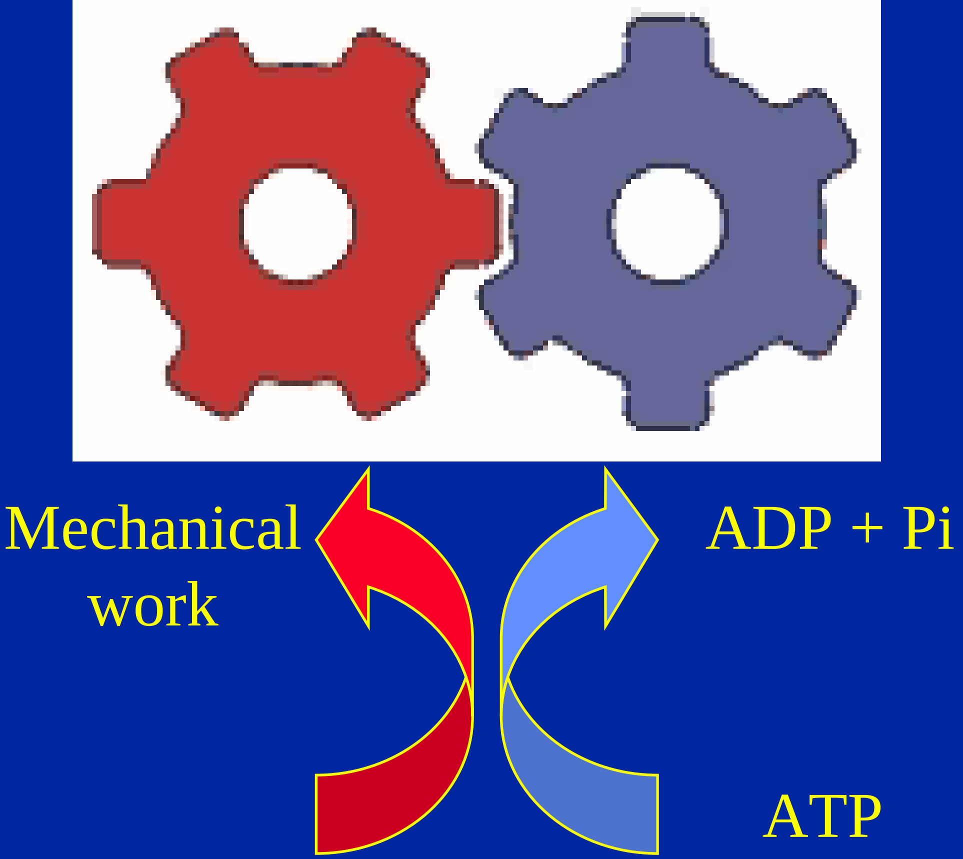
Light micrograph

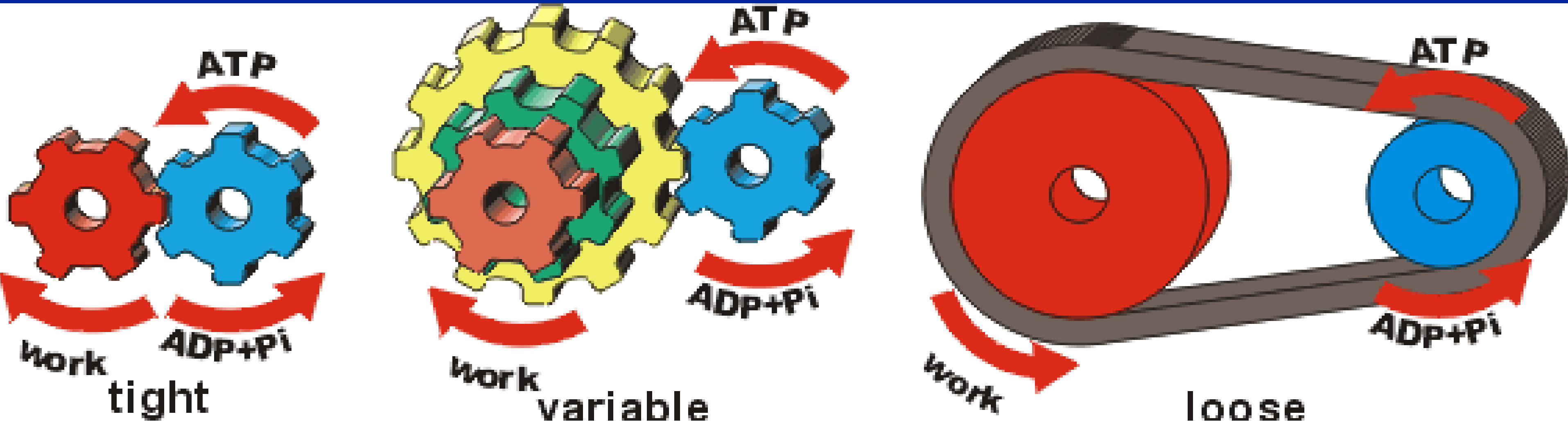
Electron micrograph

sarcomere

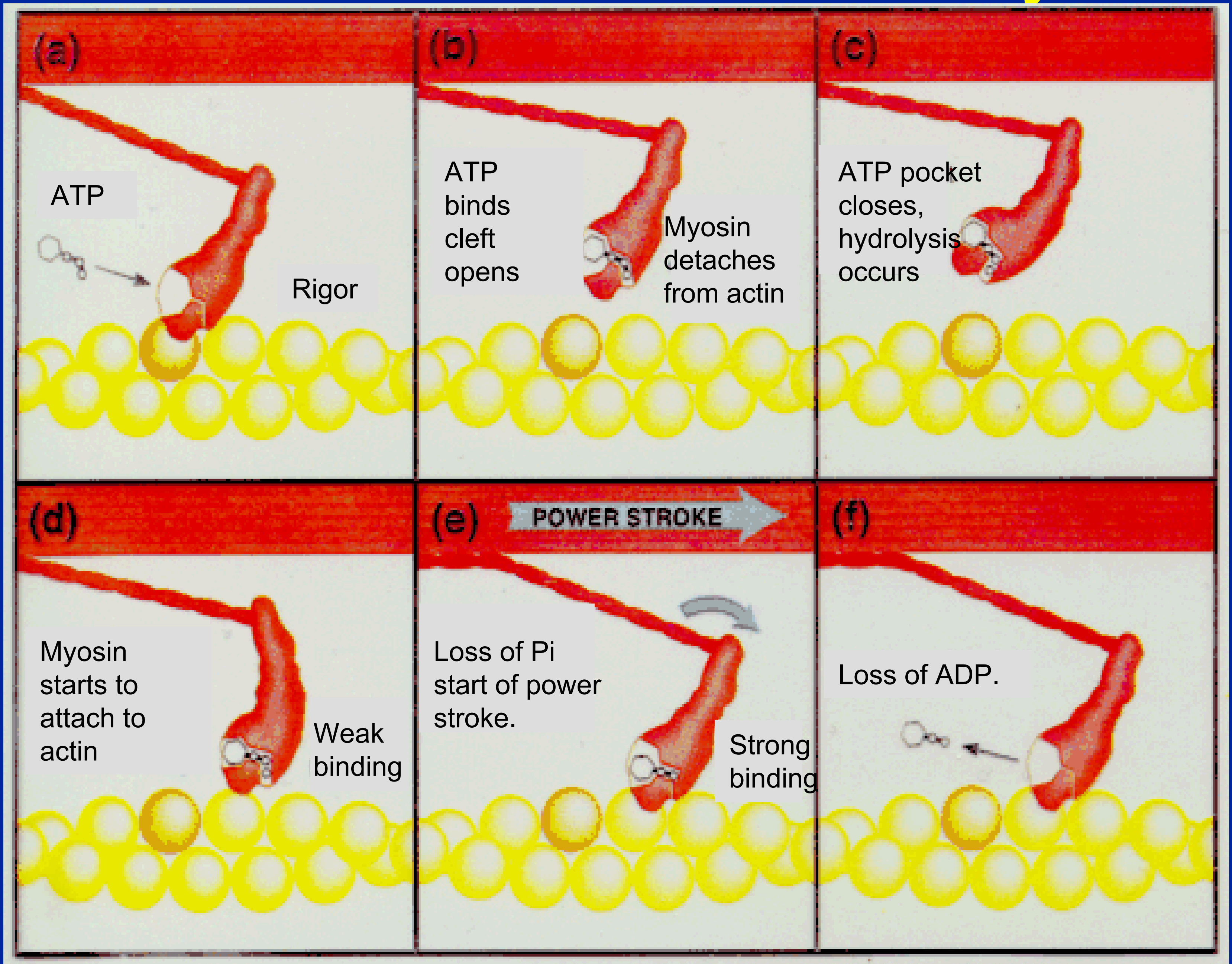


Mechanism of chemo-mechanical coupling

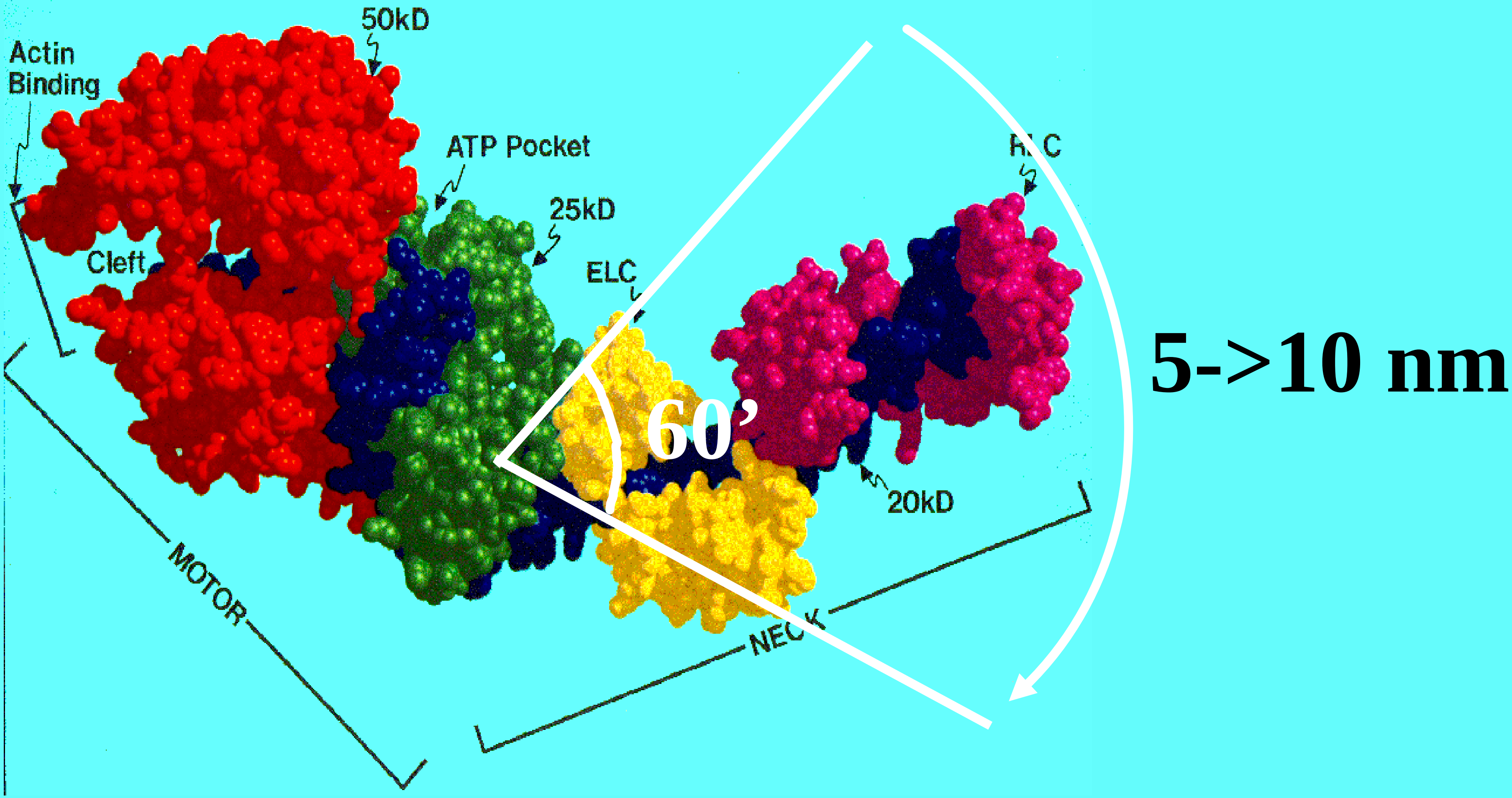




The chemo-mechanical cycle :



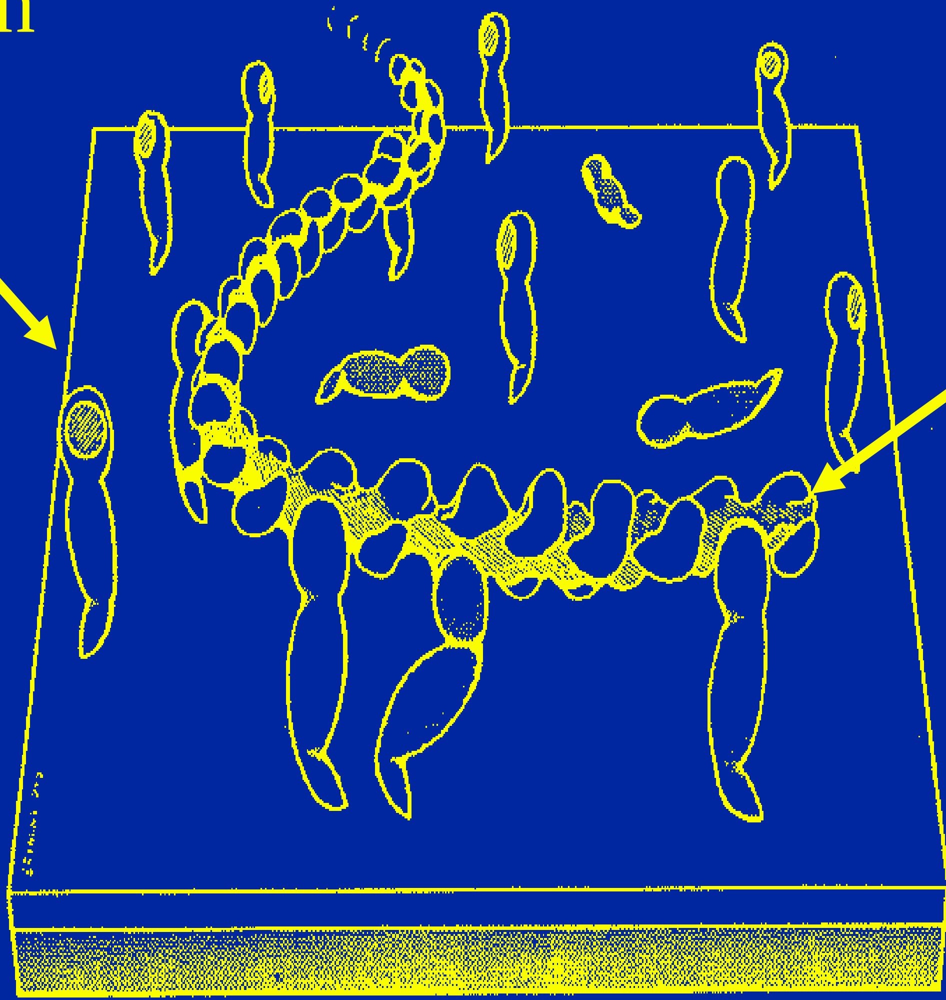
Myosin Subfragment-1



From Rayment *et al.*
(1993) *Science* **261**, 50-58

Acto-myosin *in vitro* motility assay :

myosin
(S1)



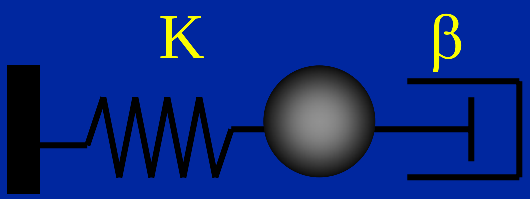
F-actin

10µm

Comet tails are produced by optical forces

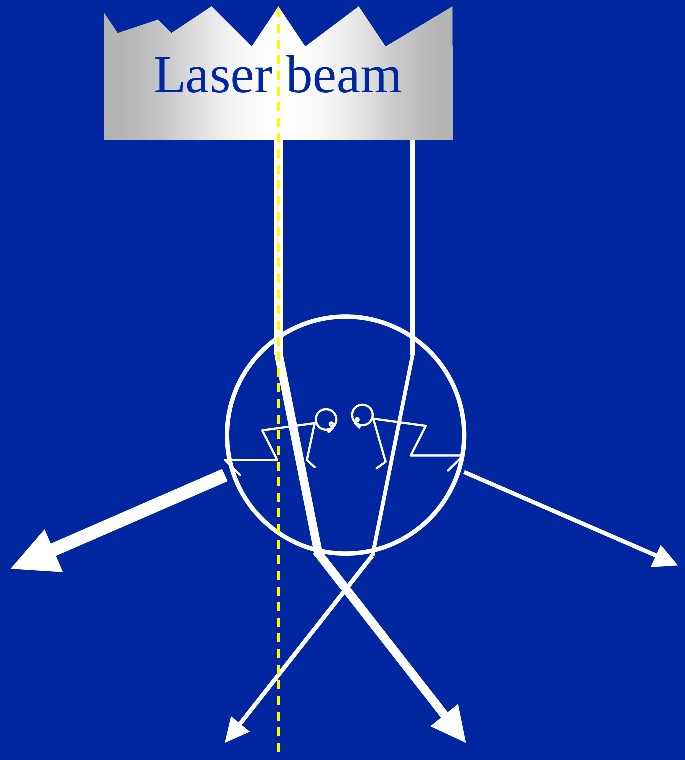
Optical tweezers:

- Optical tweezers are a form of nanotechnology – with a sensitivity that "bridges" the gap between AFM force spectroscopies and conventional spectroscopies.
- Mechanical energy differences on the same order as thermal energy, kT, **4pN.nm** can be measured (e.g. 1/10 the energy of a single ATP molecule and 1/100th the energy of a single photon).
- Resolution limited not by detection electronics but by thermal motion.
- The transducer is usually a 1μm diameter plastic bead
  - damping constant $\beta = 6\pi\eta r = 10^{-8}$ N.s.m^{-1}
  - stiffness= <0.1pN.nm stiffness (κ)
- Therefore:
- **Positional noise is 6nm** (r.m.s. $x = \sqrt{kT/\kappa}$),
- **Force noise is 0.6pN** ($= \sqrt{kT\kappa}$) and
- **Bandwidth = 1.5 kHz** ($f_c = \kappa/2\pi\beta$).



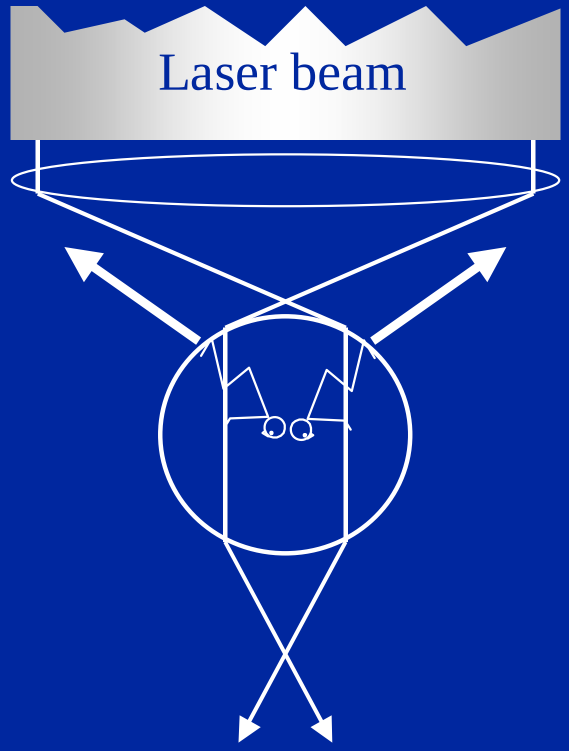
Optical tweezers :

• Lateral Trapping Force



• net force towards the centre of the beam and in the direction of light propagation

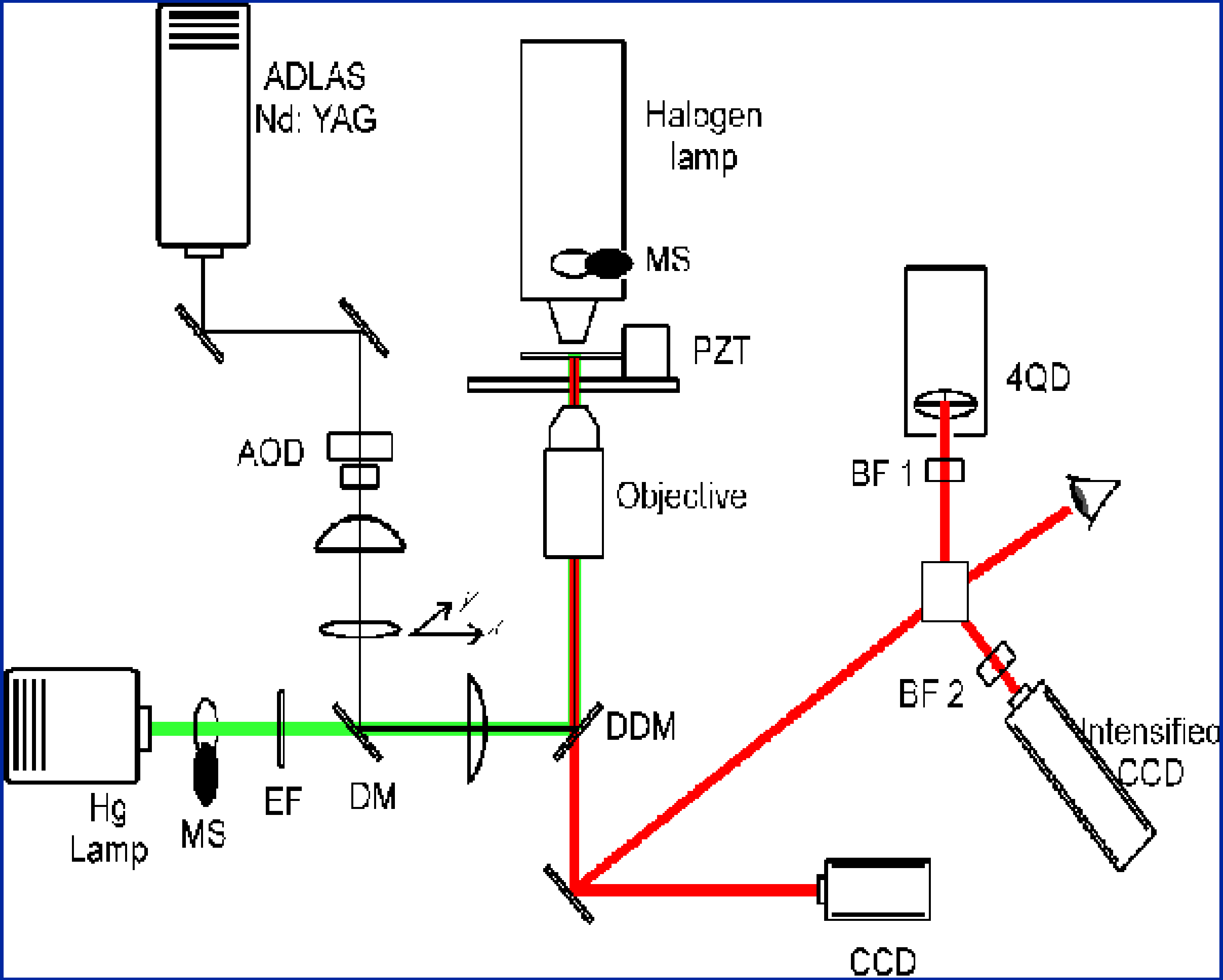
• Z-axis Trapping Force



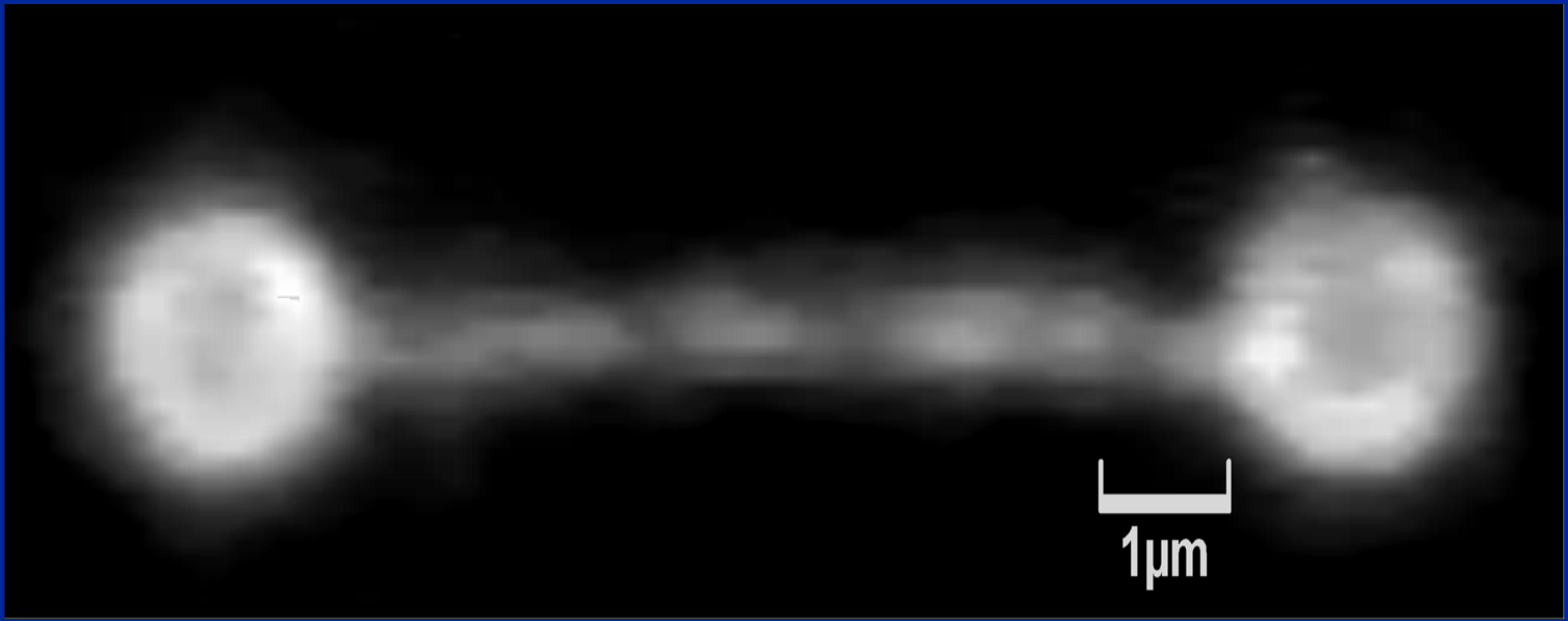
• Stable equilibrium; just below the focus

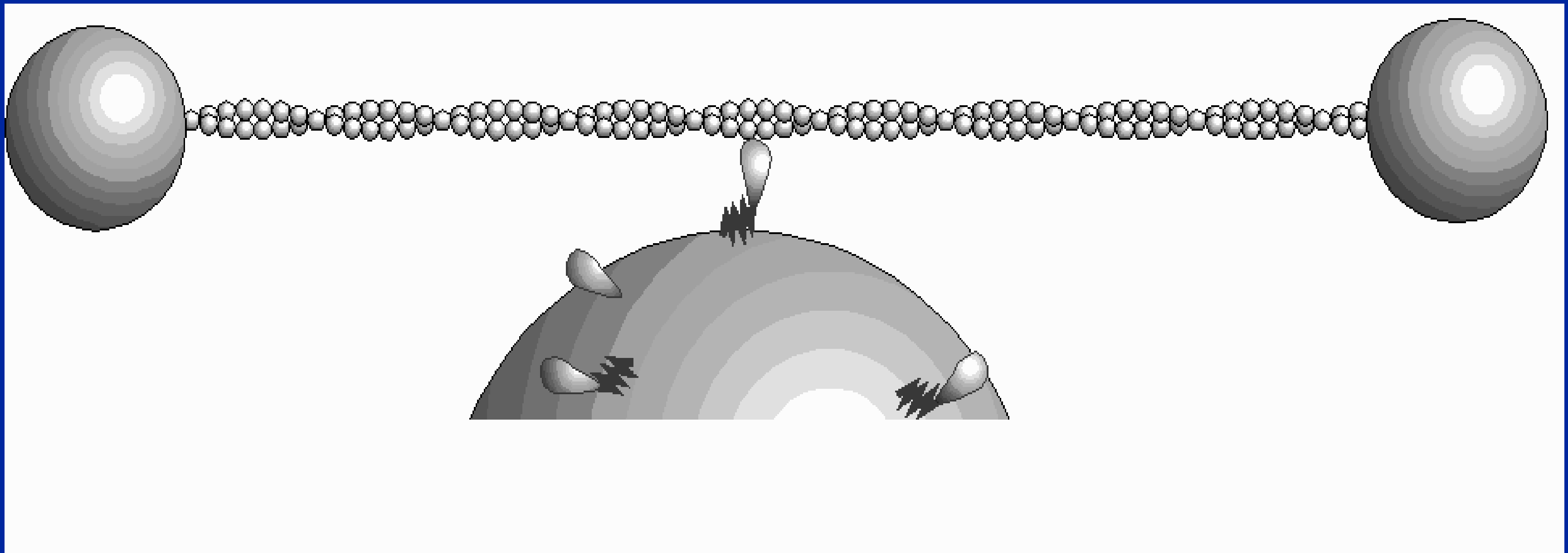
Optical tweezers

Optical tweezers “piconewton-nanometre” transducer.

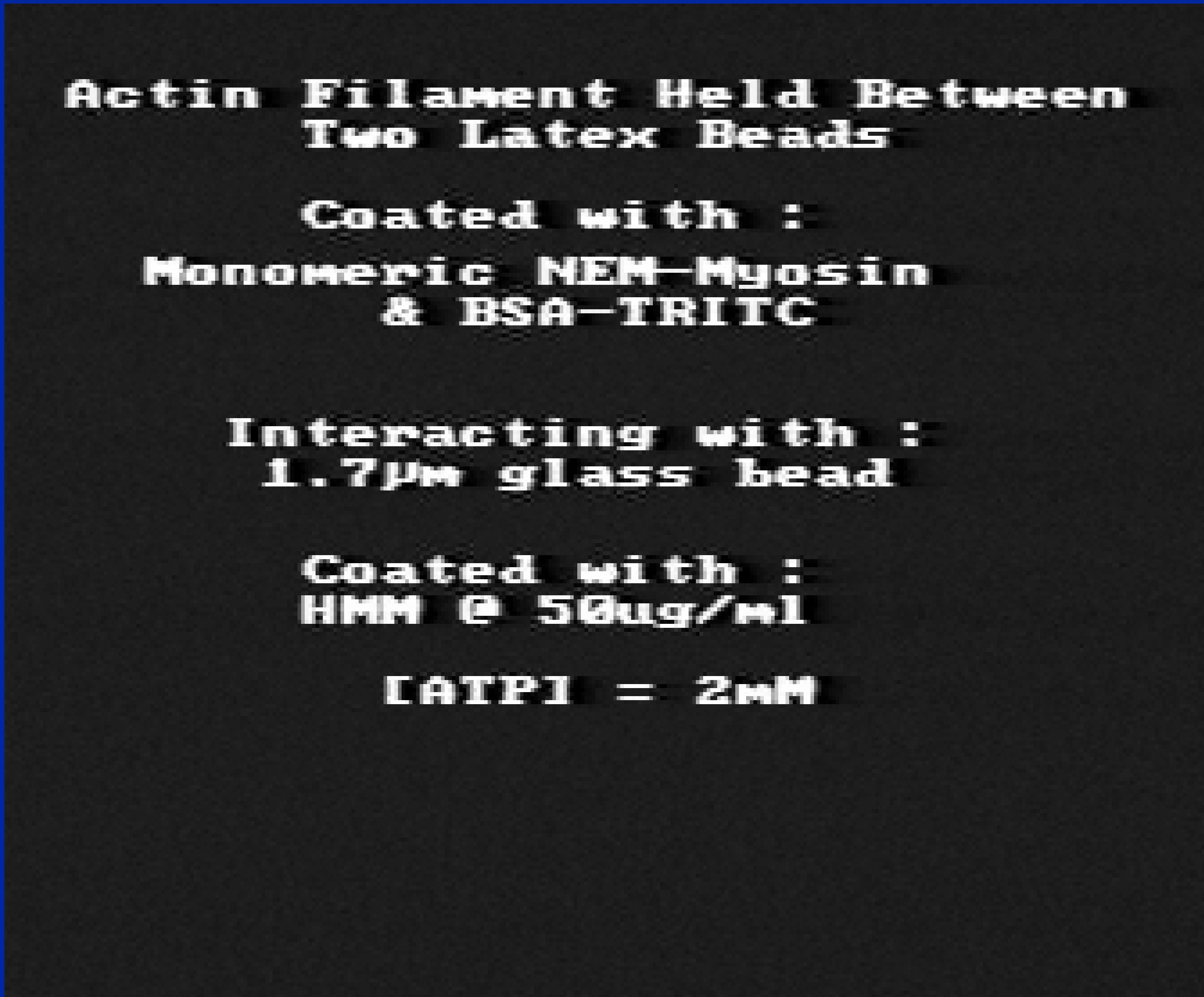


“Three bead” geometry :





5μm



5μm

Video clip

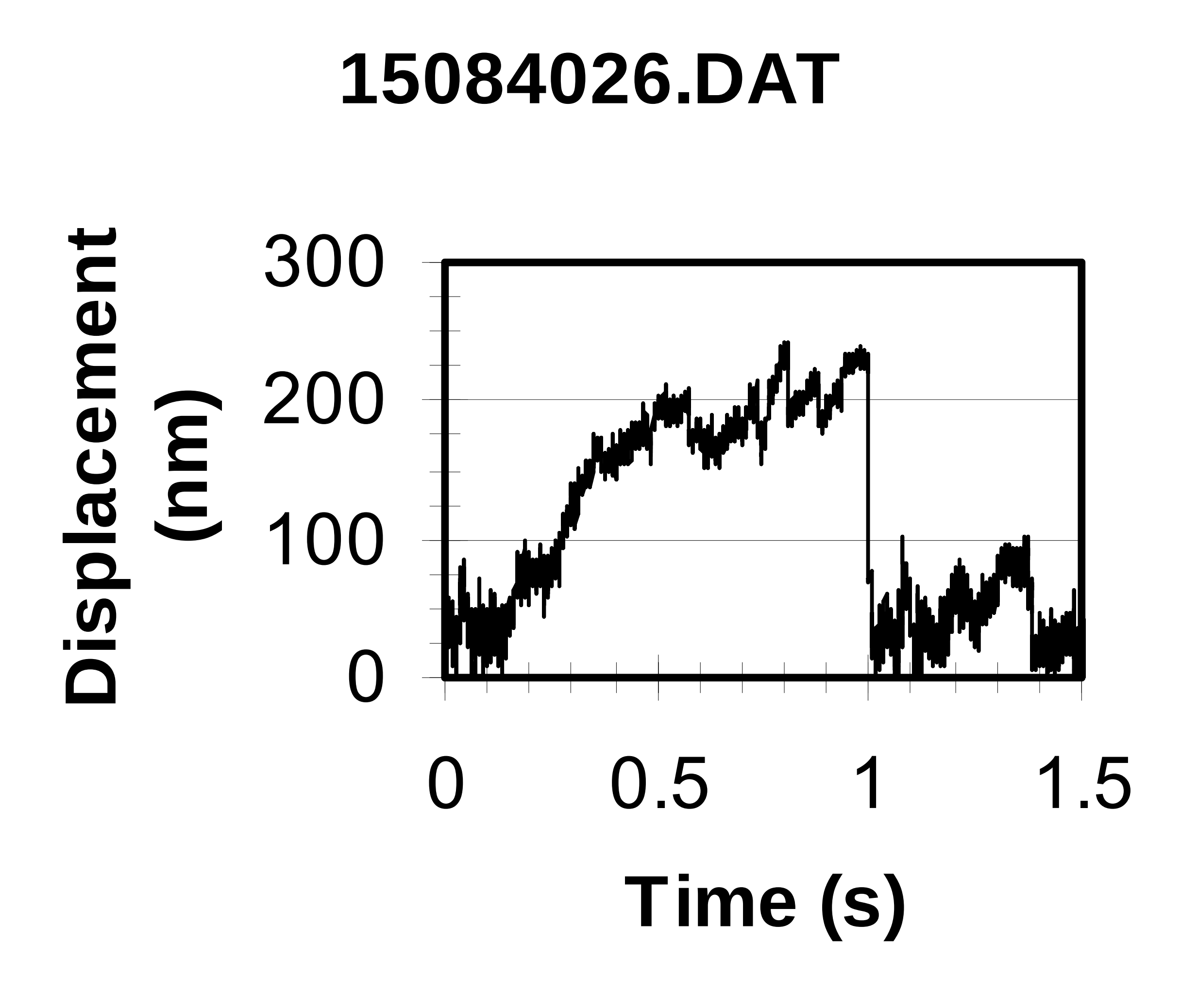
Mechanical Questions:

- Can we confirm the classical “swinging cross-bridge” using single molecule techniques?
- What new information can we obtain?

- How big is the “power stroke”?
- How stiff is the acto-myosin cross-bridge?
- Is one ATP used per cycle ? (efficiency)

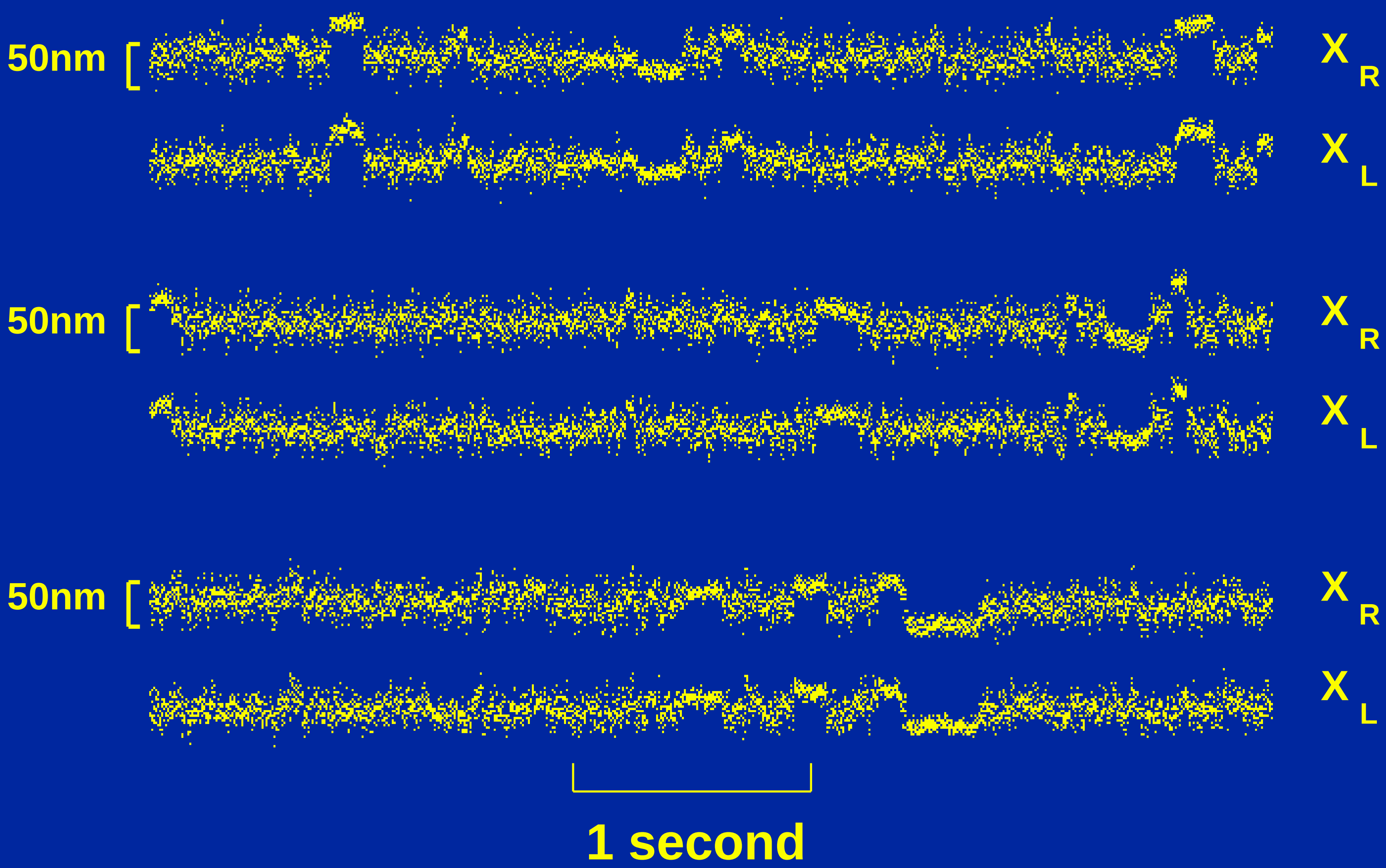
- What is the timing of force generation?
- “Thermal ratchet” vs. “power-stroke”
- What are the mechanical properties of unconventional myosins?

At high surface densities – many myosins act to produce sliding.

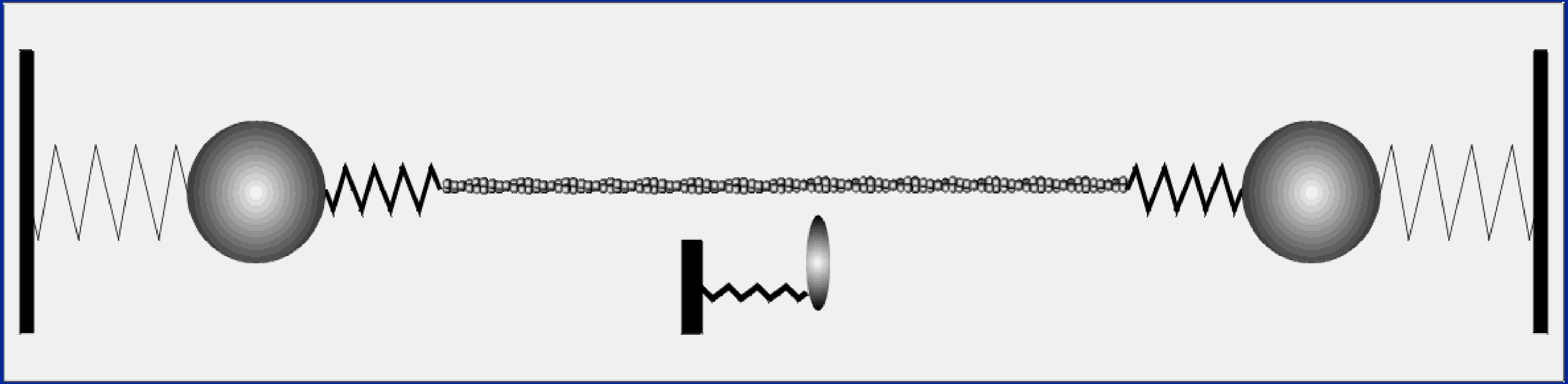


At low surface density, discrete mechanical events are observed. A single myosin head (S1) is sufficient for force and movement.

(skeletal muscle S1, 23°C, 10μM ATP)

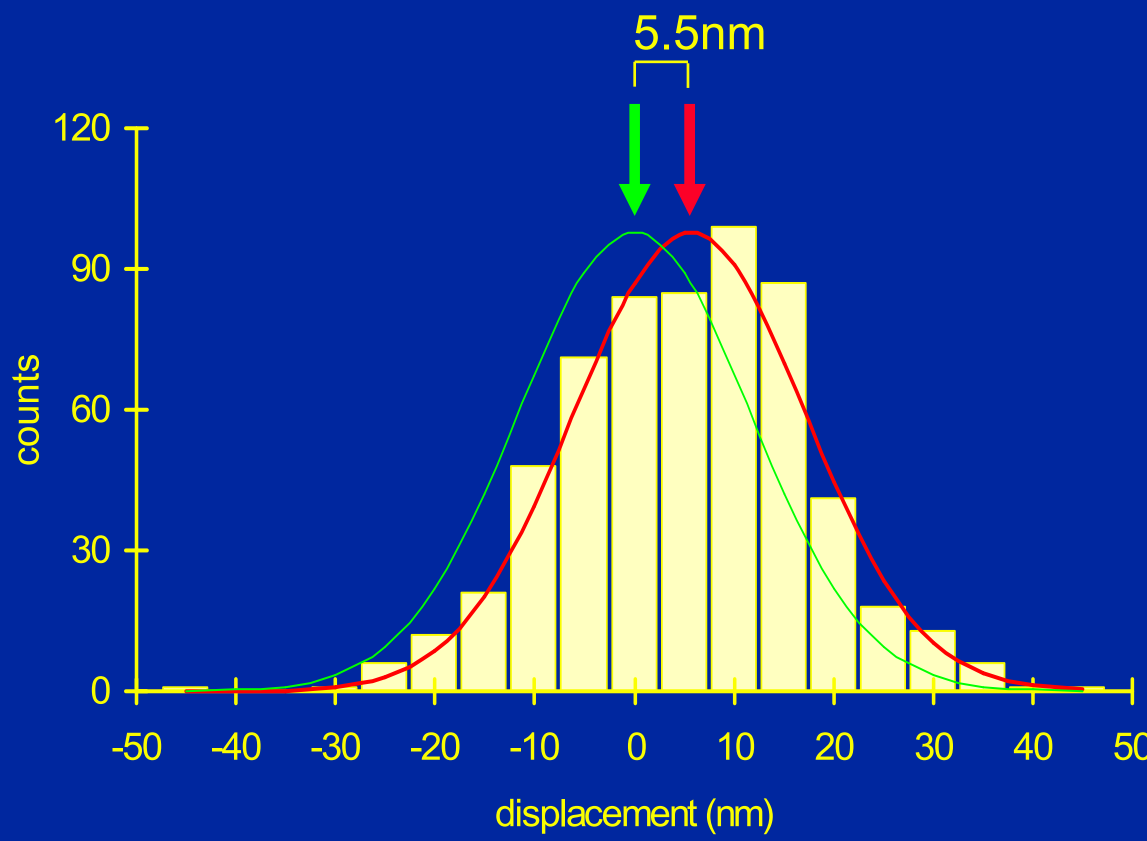


Thermal motion of the actin filament "randomises" step size measurements



The starting point of any individual measured event is uncertain.

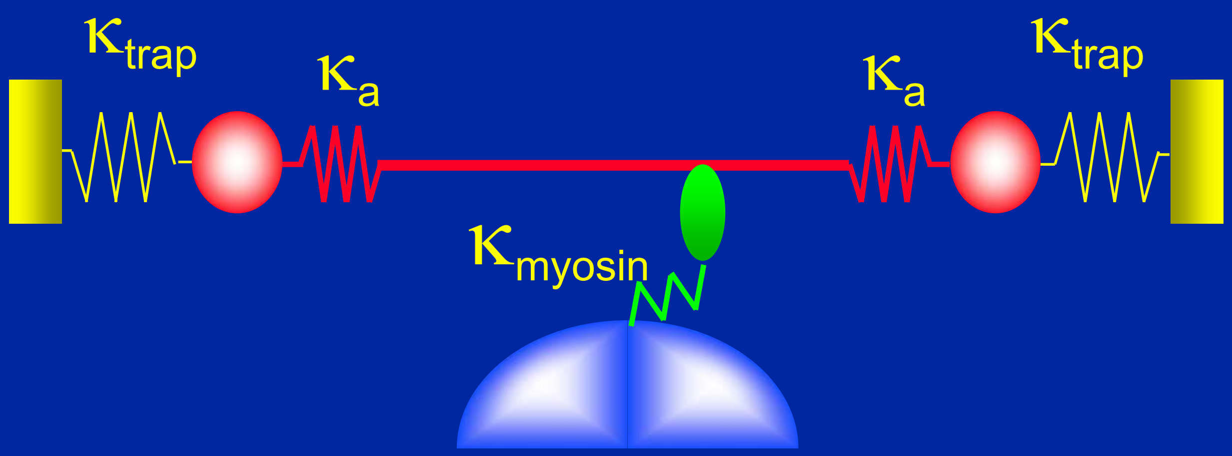
Unitary displacement for skeletal muscle myosin II.



$f(x) = a * \exp(-\kappa(x-x_o)^2 / 2kT)$

$\kappa = 0.027\text{pN/nm}, x_0 = 5.57\text{nm}$

Cross-bridge stiffness & series compliance

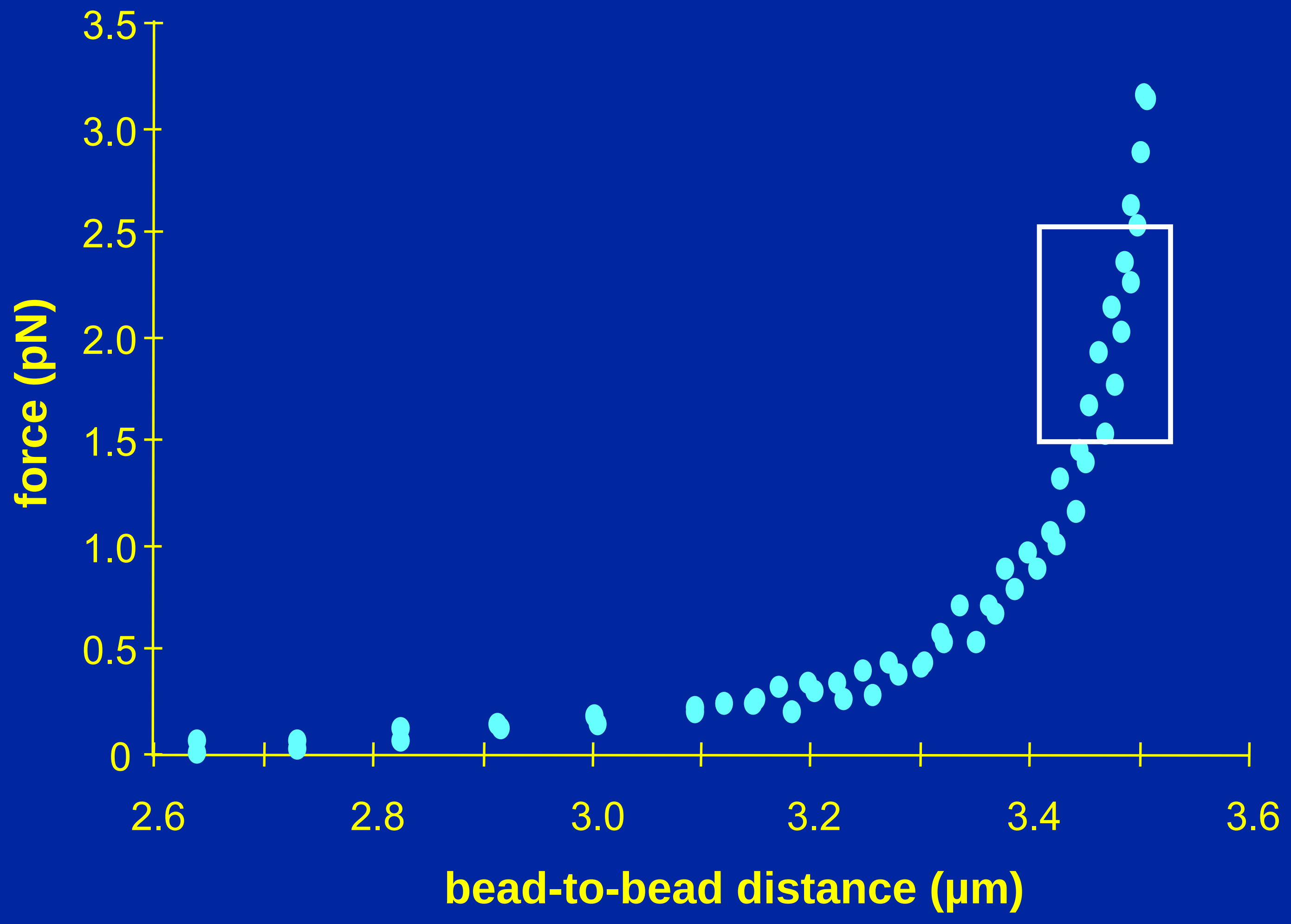


κ_{trap} stiffness of the trap

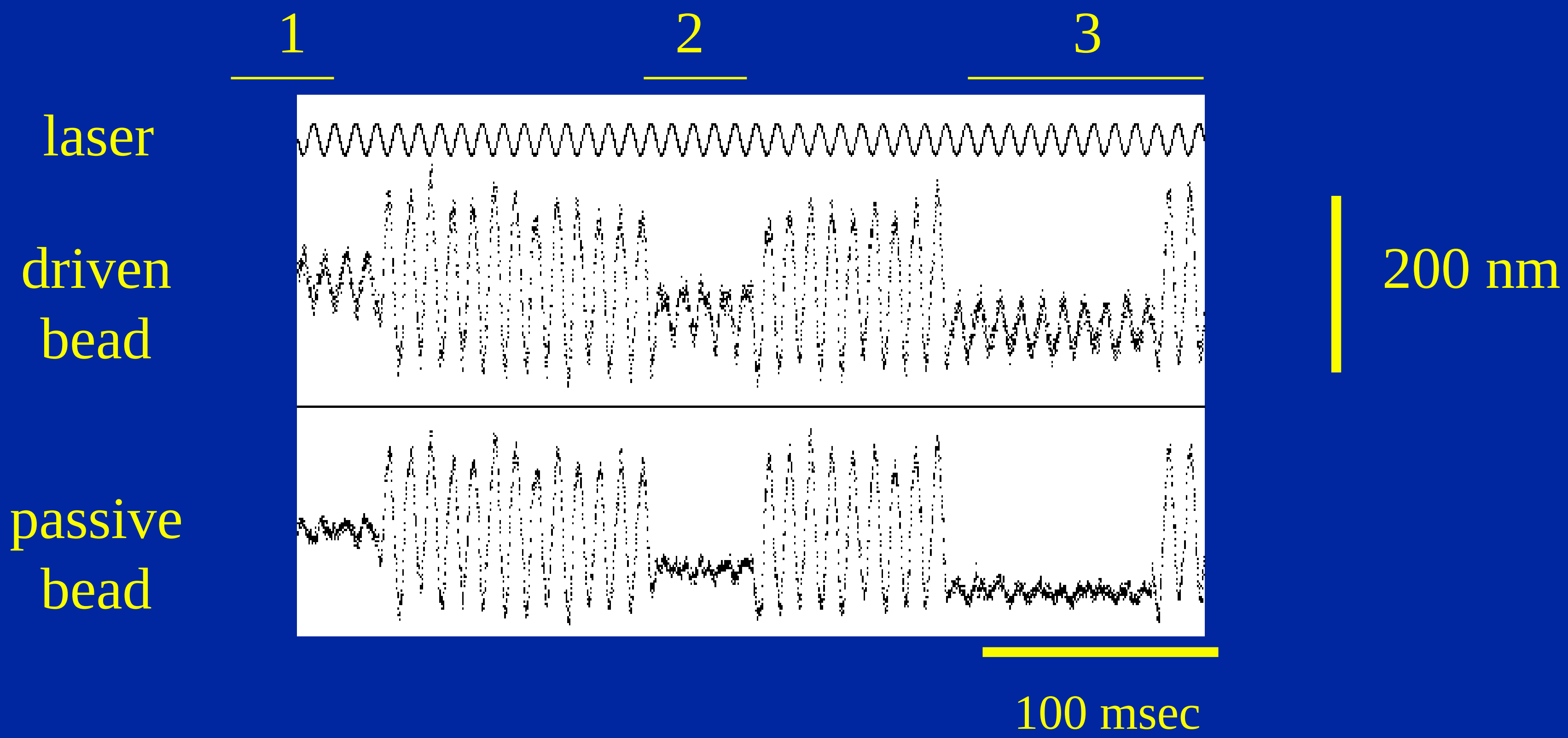
κ_a stiffness of the actin-to-bead connection

κ_{myosin} stiffness of the attached myosin

Force-Extension Curve of [Bead-Actin-Bead] = κ_a (measured by video microscopy)



Myosin Cross-bridge stiffness $\mathbf{k}_{\mathbf{myosin}}$



event	κ_a (pN/nm)	κ_{myosin} (pN/nm)
1	0.20 +/- 0.04	0.37 +/- 0.20
2	0.17 +/- 0.02	0.63 +/- 0.35
3	0.18 +/- 0.02	0.71 +/- 0.65

Veigel *et al.* (1998)
Biophys. J. **75**, 1424-1438

How much work is done by a single cross-bridge stroke?

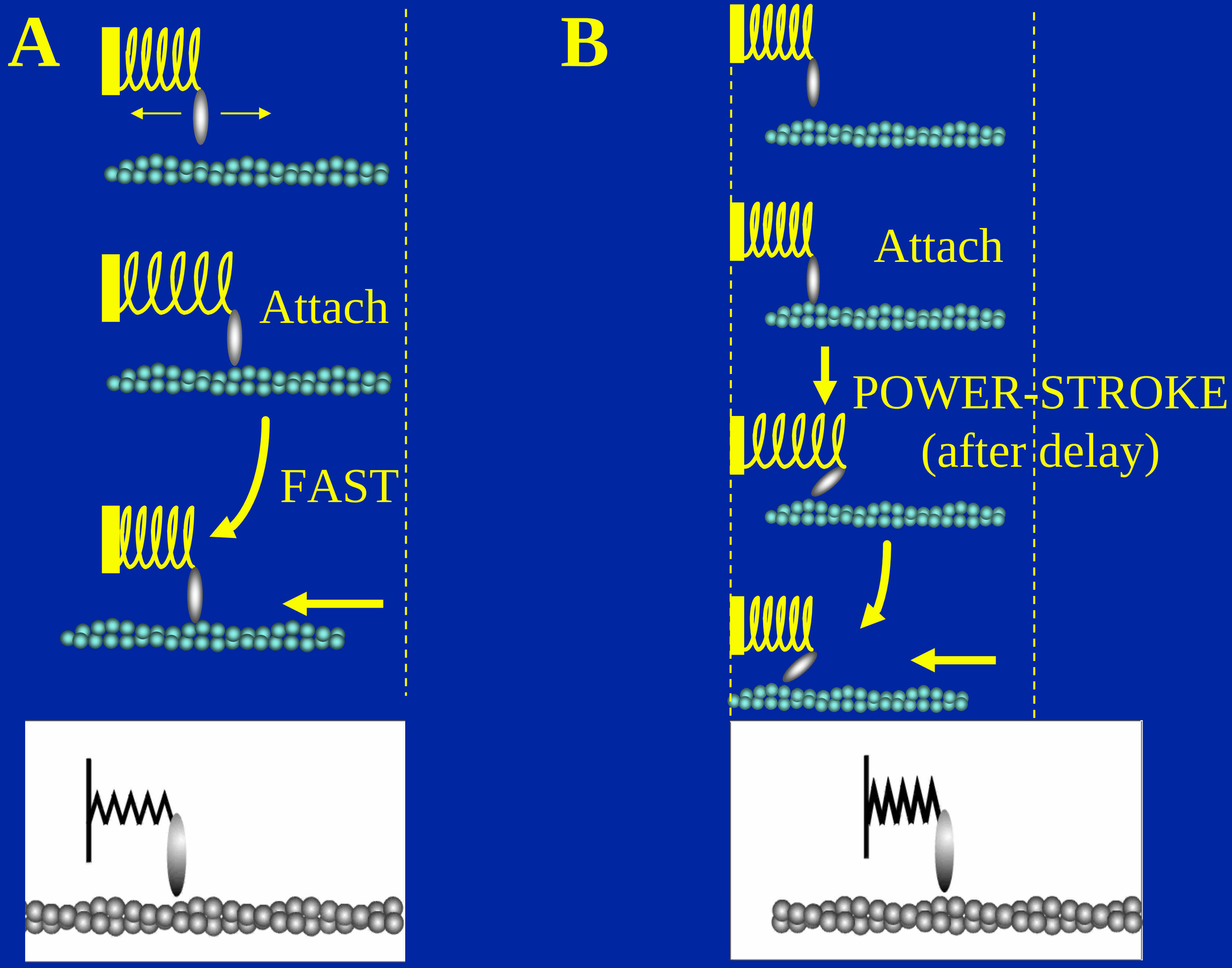
In vitro

- Work = $1/2kx^2$ = $0.5*0.7*5^2$ = **10 pN.nm**

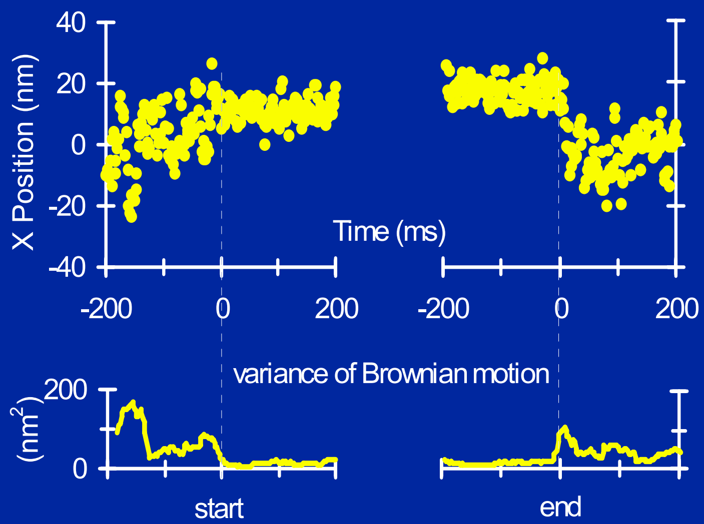
In Muscle:

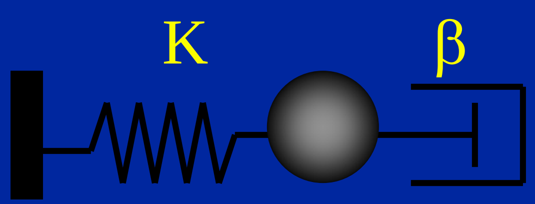
- G_oATP = 80pN.nm * 40% efficiency = **32pN.nm**

Thermal ratchet or Power stroke?



Time resolution is limited by the bandwidth of the Brownian motion :





$$f_c = \kappa / 2\pi\beta$$

time resolution ~ 20ms

ATPase:

Product release for different myosins

Average lifetimes: **(1mM ATP)**

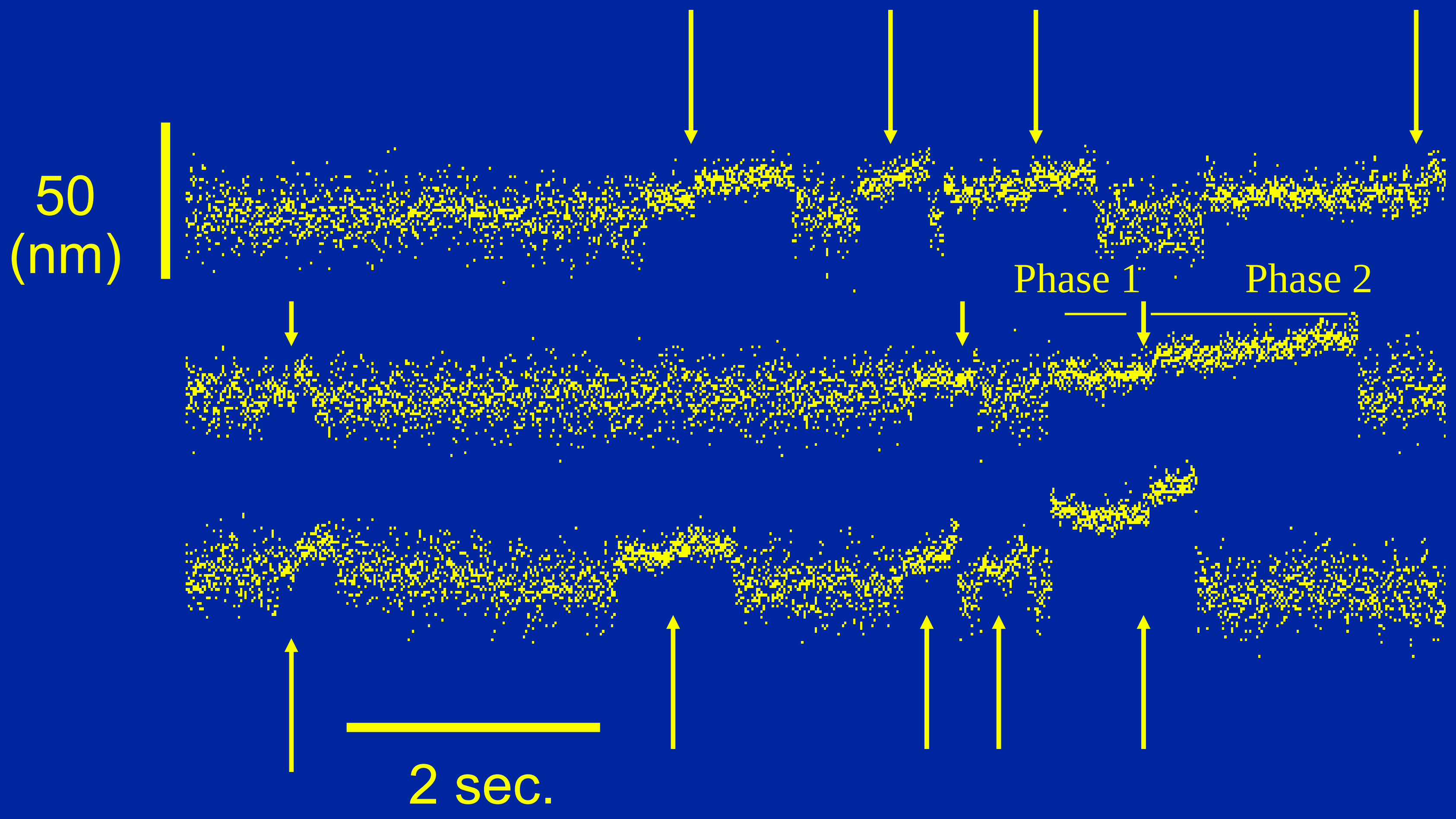
	AM.ADP.Pi	**AM.ADP**	**AM**
Skel. -II	<5ms	<2ms	<1ms
Smooth -II	<5ms	**~30ms**	<1ms
BBM1	<5ms	**~90ms**	~2ms
Myr1a	<5ms	**~350ms**	**~30ms**
Myosin Va	<5ms	**~90ms**	~1ms

Jontes *et al* (1997) *PNAS* 94, 14332-37

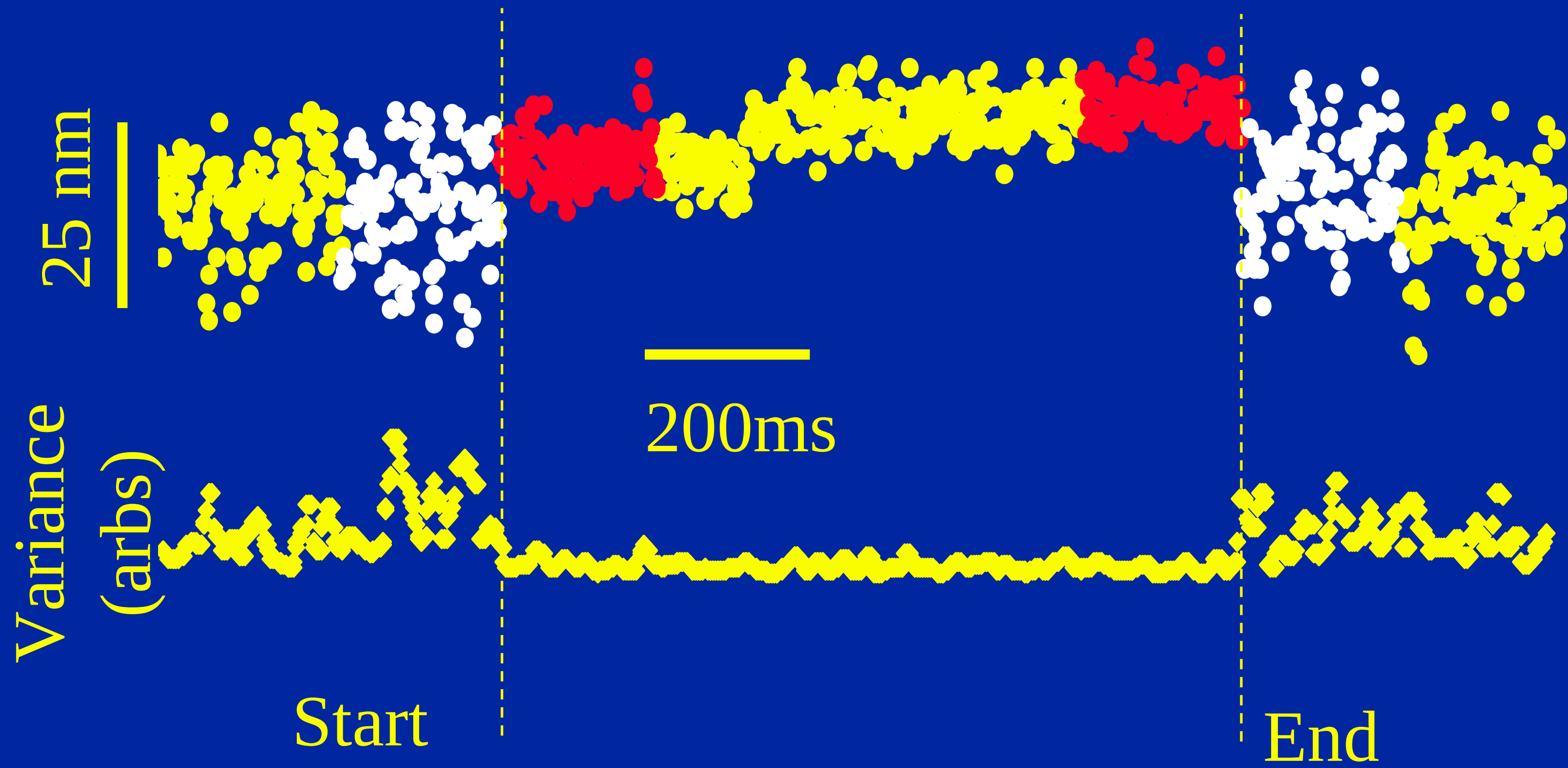
Cremo & Geeves (1998) *Biochemistry* 37, 1969-78

De la Cruz *et al* (1999) *PNAS* 96, 13726-31

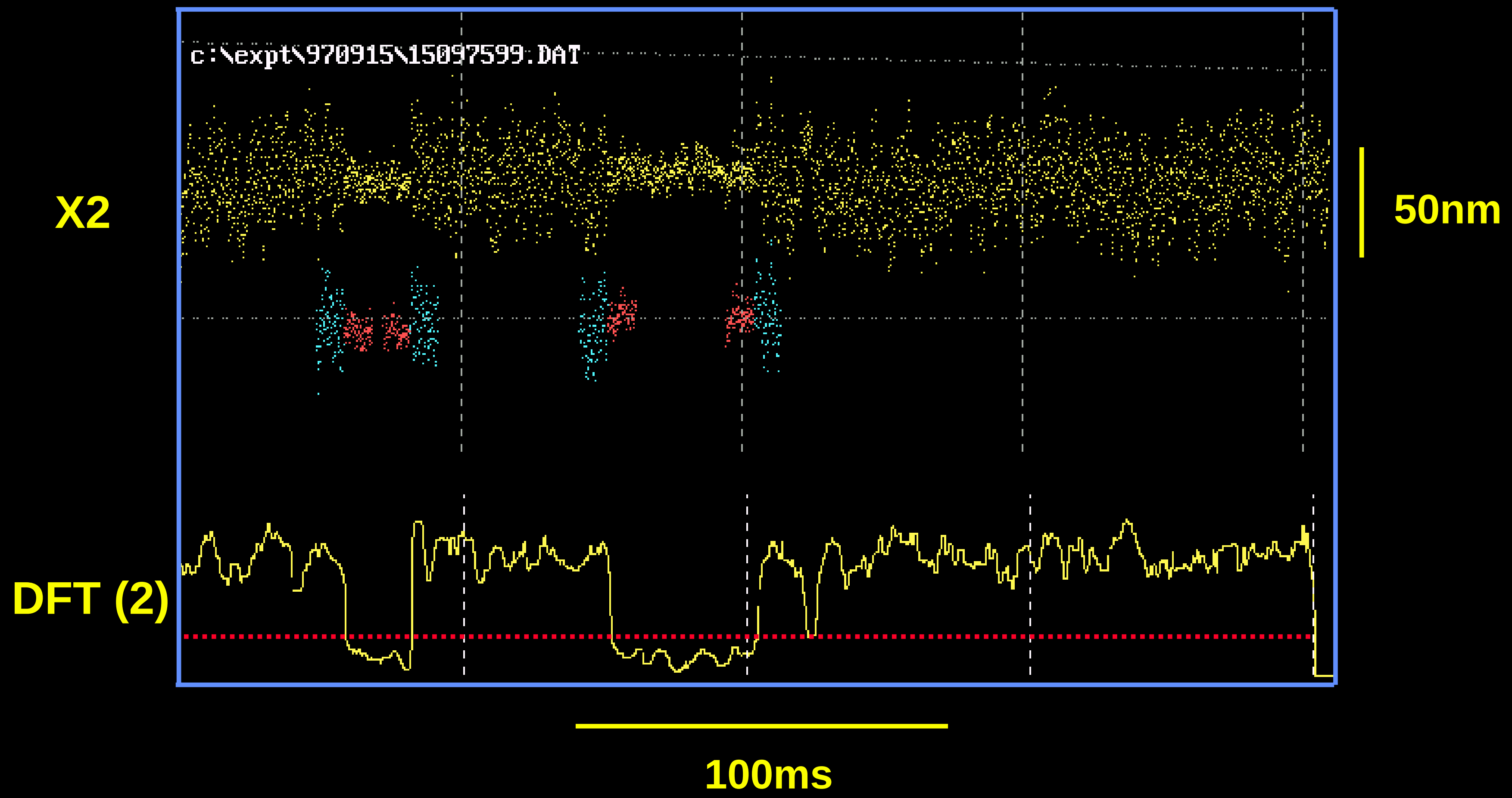
Myosin-I (Myr1 gene product)



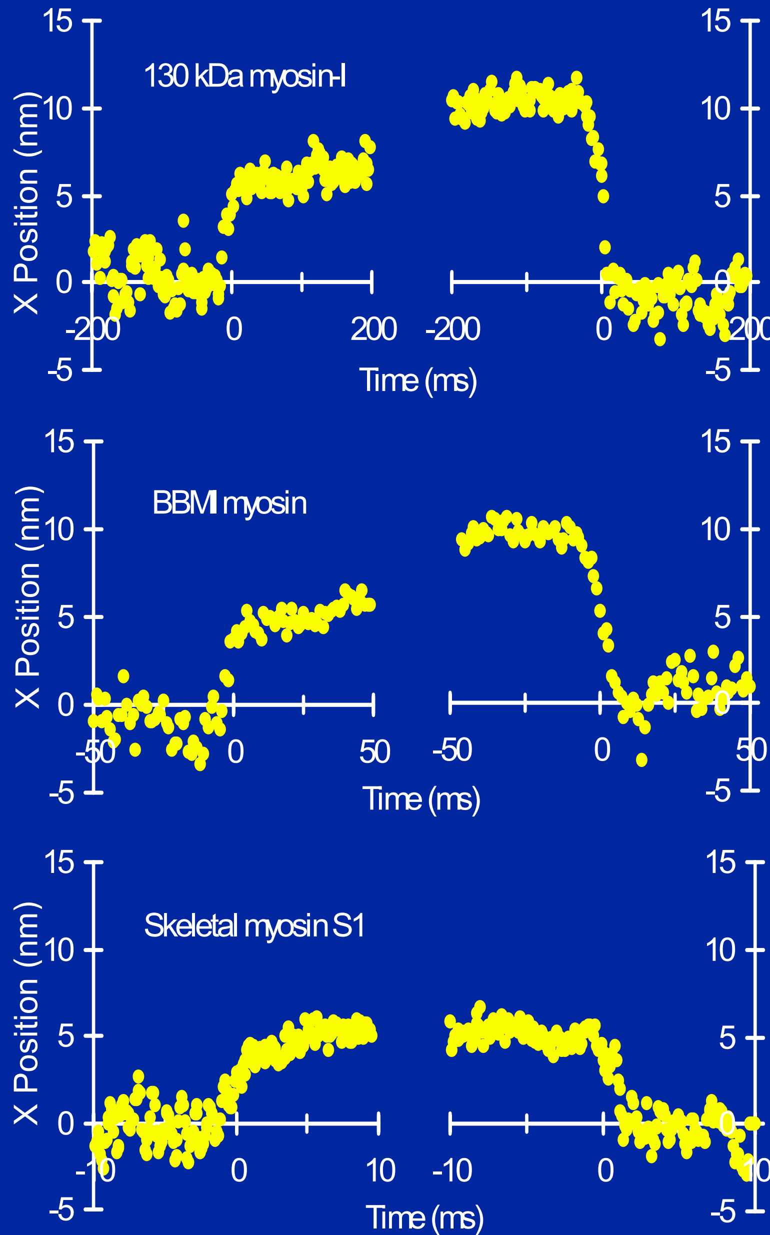
Data can be averaged by synchronising the start and end of many events.



Time resolution can be improved by oscillating one of the optical tweezers. At 1kHz, binding can be detected within 1ms.



Average time-course of displacement events



Lifetimes of phase 1 and phase 2

Myr-1	Phase 1			Phase 2		
[ATP]	**Mean (ms)**	SD	(n)	**Mean (ms)**	SD	(n)
3 μM	**299.7**	31.8	(114)	**305.5**	32.5	(113)
10 μM	**268.2**	23.9	(166)	**160.3**	16.8	(143)
30 μM	**282.4**	33.8	(91)	**80.0**	12.7	(89)
100 μM	**291.5**	30.9	(115)	**50.3**	9.2	(96)

BBM-I	Phase 1			Phase 2		
5 μM	**110.7**	10.8	(196)	**100.9**	10.1	(194)
50 μM	**120.7**	11.5	(195)	**44.8**	6.8	(157)

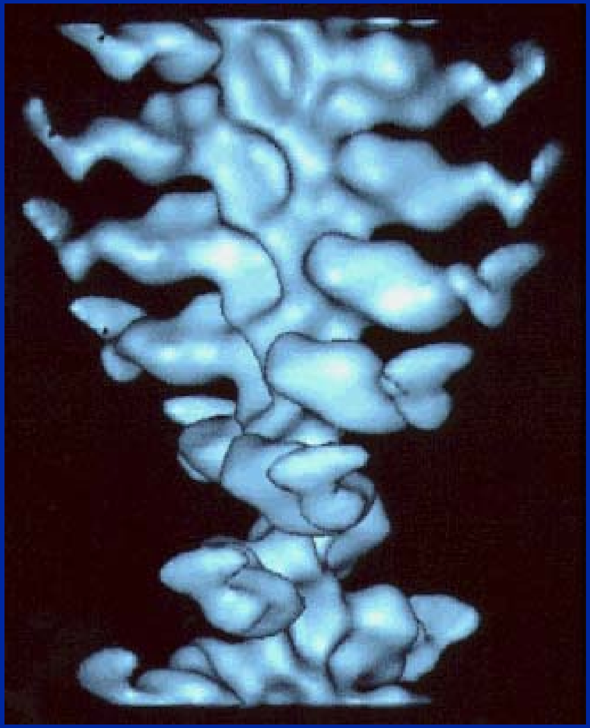
Skeletal S1	One Phase (only)		
	Mean (ms)	SD	(n)
6 μM	**75.1**	6.4	(326)
10 μM	**21.5**~	1.6	(269)
50 μM	**8.3**~	0.7	(332)
100 μM	**7.0**~	0.9	(149)

Myosin I

- A single-headed, myosin from brain and gut, involved in vesicle transport and membrane "tensioning".

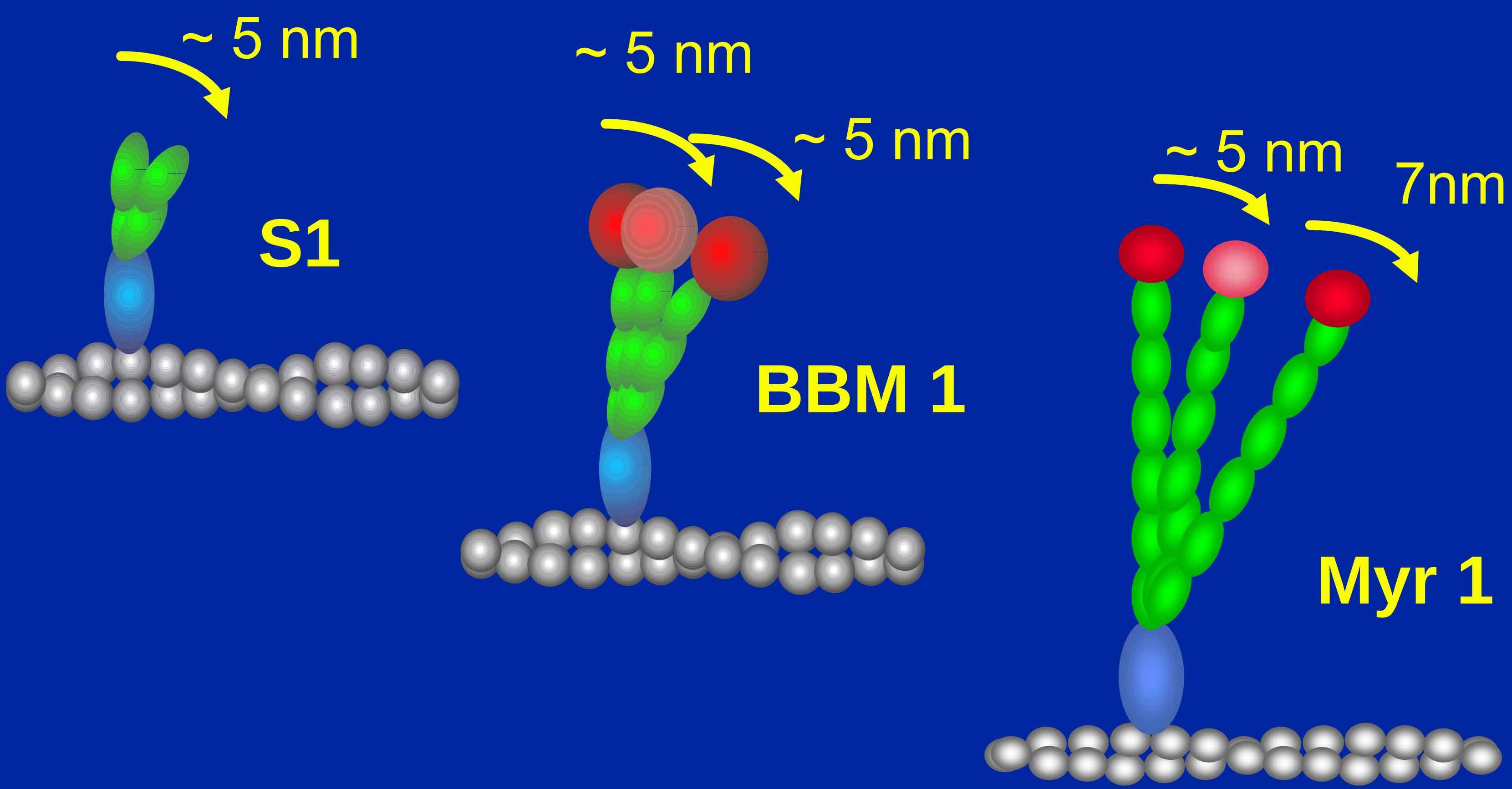
- Like smooth muscle myosin II there is an "ADP-induced" movement. Perhaps it also has a "latch state"

see
Jontes *et al.* (1995)
Nature **378**, 751-753

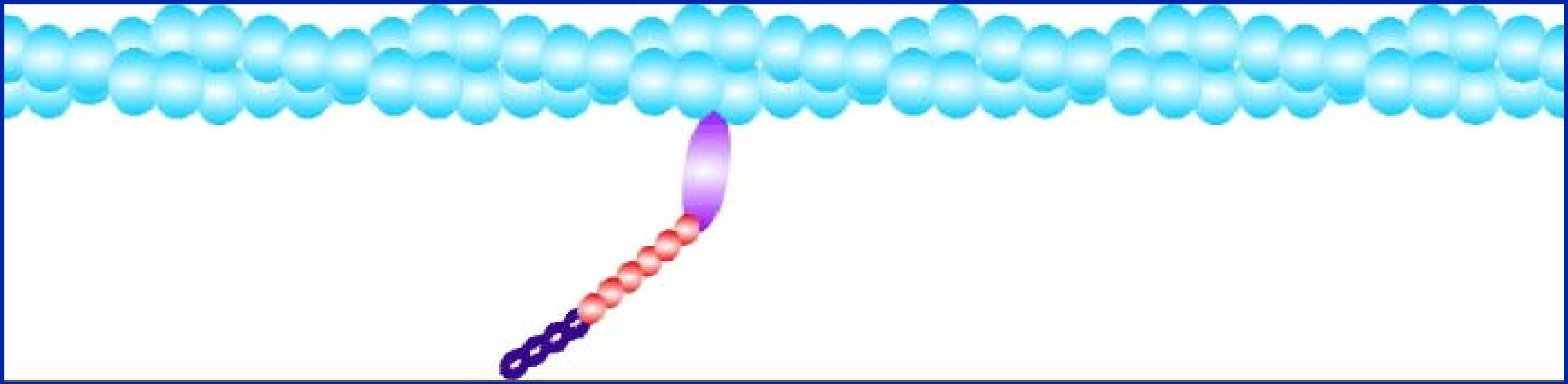


Whittaker *et al.* (1995)
Nature **378**, 748-751

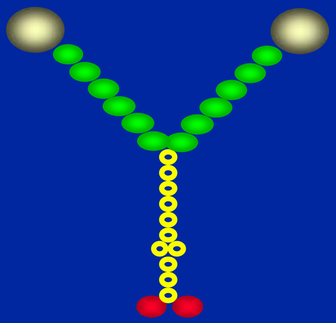
Summary:



Myosin I

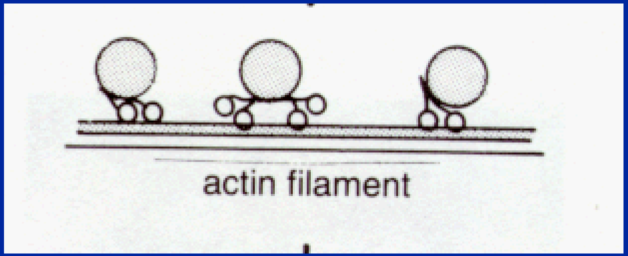


Myosin V



Structure:
2 heavy chains + 6 LC's, coiled-coil forming tail with globular domain for cargo binding

Function:
Transport and tethering of vesicles and organelles
e.g. ER vesicles in neurons, melanosome transport in melanocytes; mRNA transport in yeast



Mechanism of movement:
Is it a processive "porter" like kinesin?
or an intermittent "rower" like skeletal myosin II?

Processivity depends upon how you measure it!

Biochemical definition:

- >1 turnover per catalytic site per diffusional encounter.

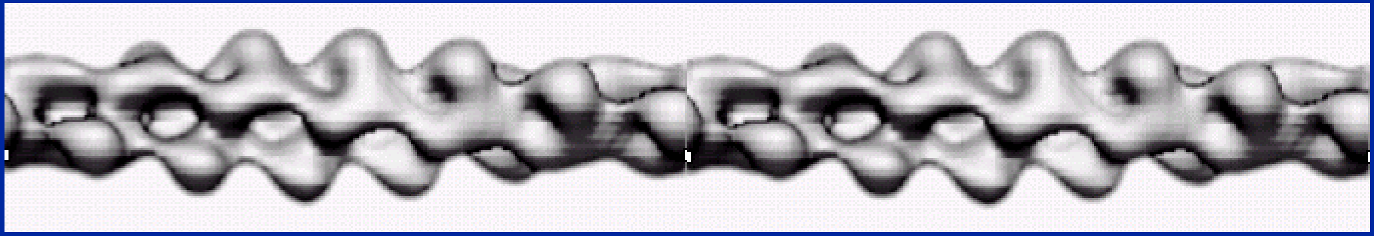
Mechanical definition:

- >1 mechanical step per attached period.

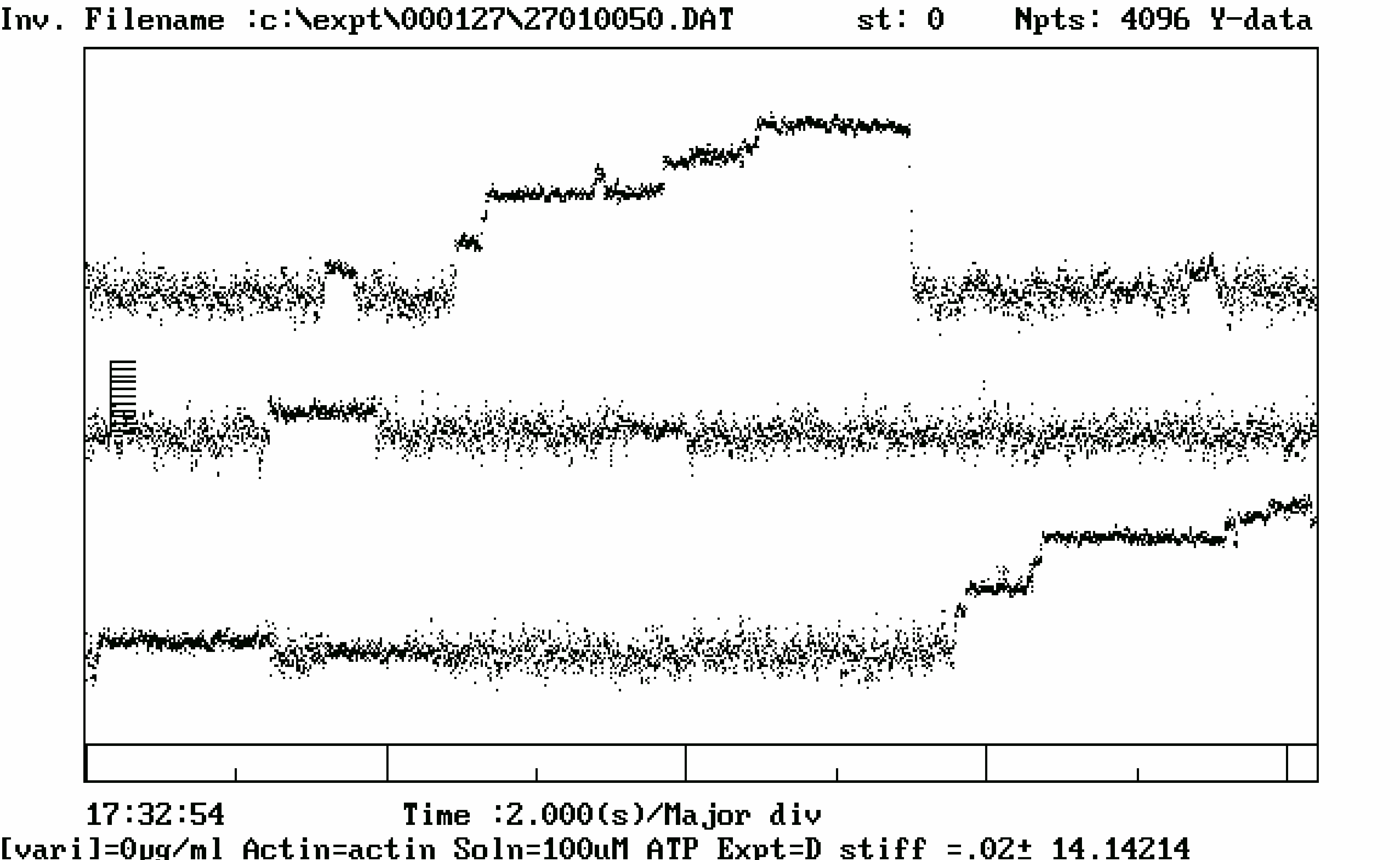
Note: <u>All</u> two-headed motors will be processive by this definition. Also, some single-headed motors might be processive.

Physiological definition:

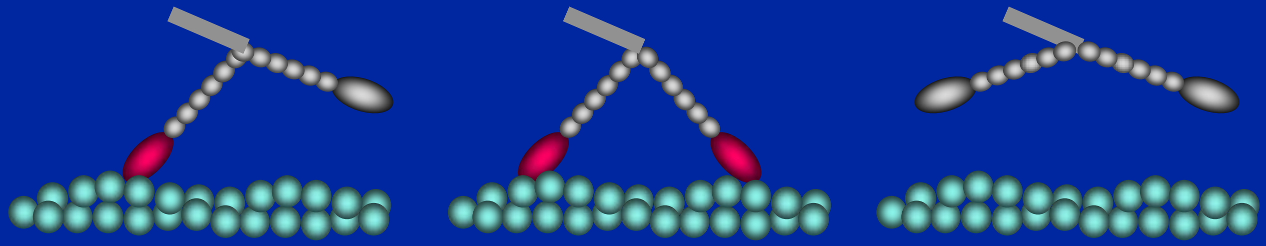
- a single molecule moves a significant distance (1/10th the diameter of a cell?) by making >10 (?) steps.



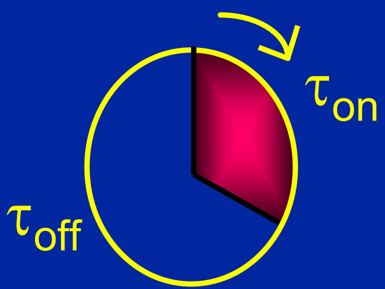
Myosin V is a processive motor:

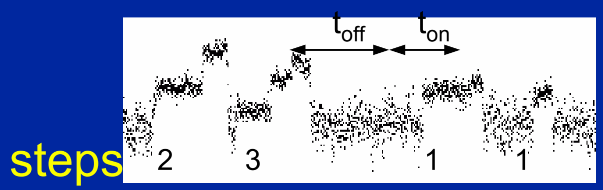


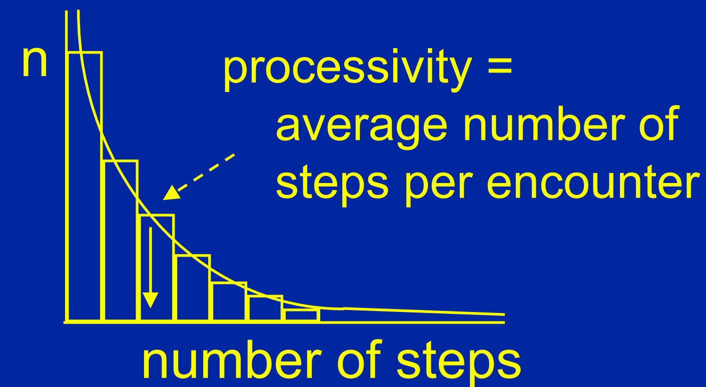
Myosin V and processivity:



$2pq$ p^2 q^2







Duty cycle $(p) = t_{on} / (t_{on} + t_{off})$

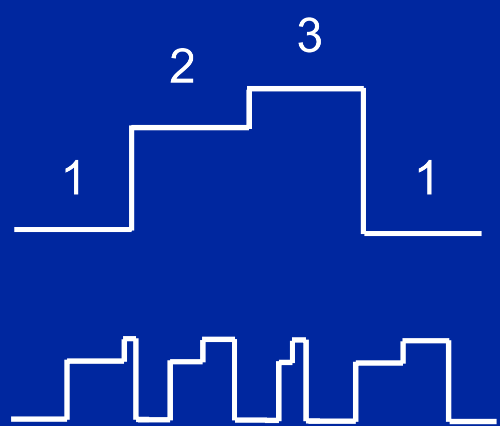
$(q) = (1 - p)$

If the heads work independently:

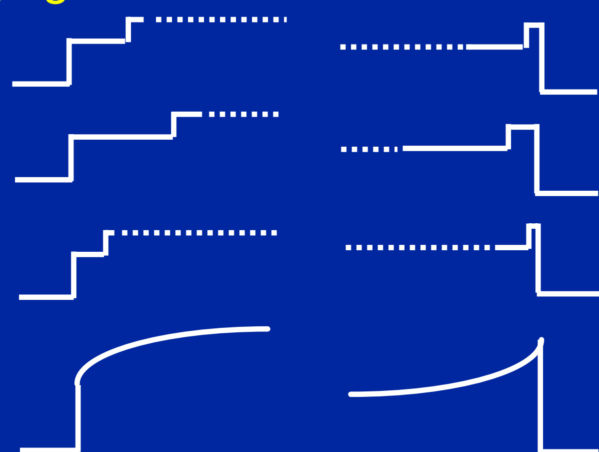
Processivity = $1 + \ln(2) / -\ln(2pq+p^2)$

(e.g. if duty ratio = 0.5 the processivity is 3.4)

Analysis of single step interactions shows that movement is generated in two-phases.

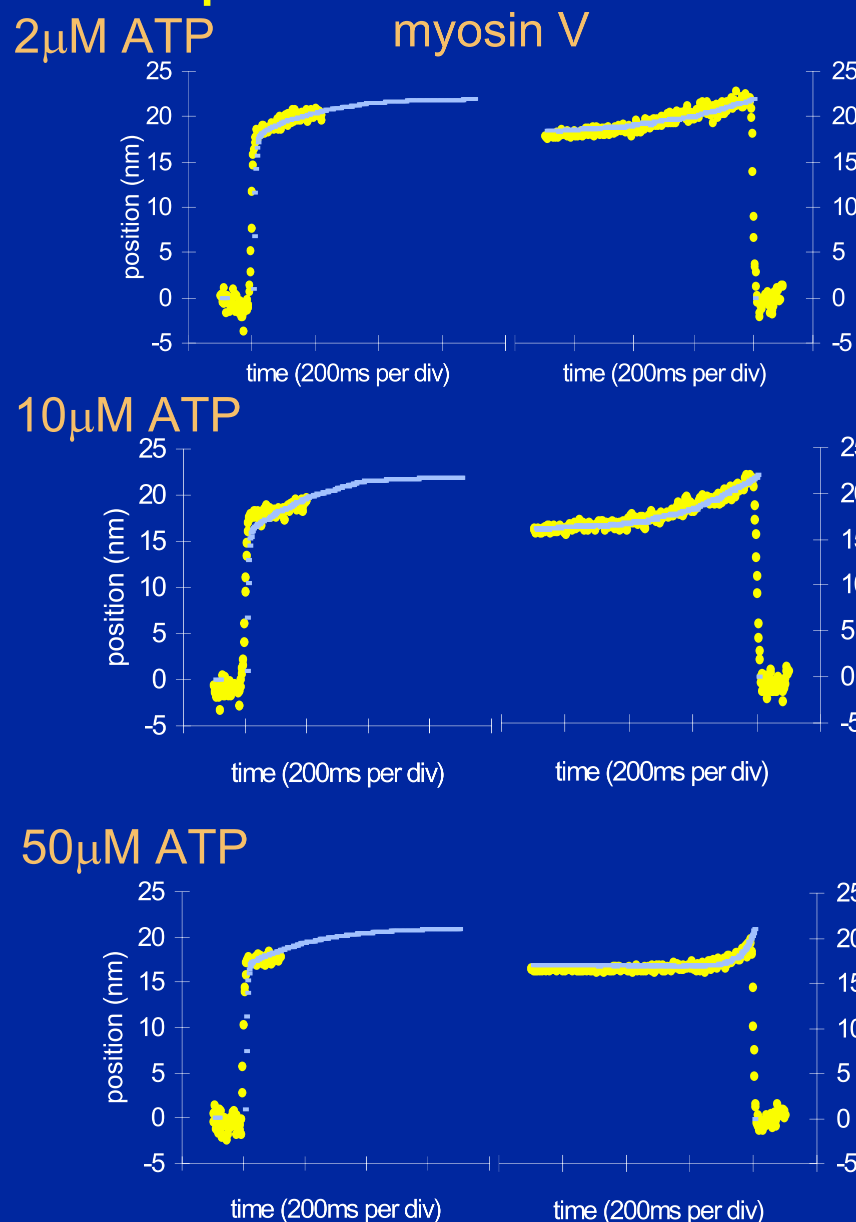


averaging: forward backward

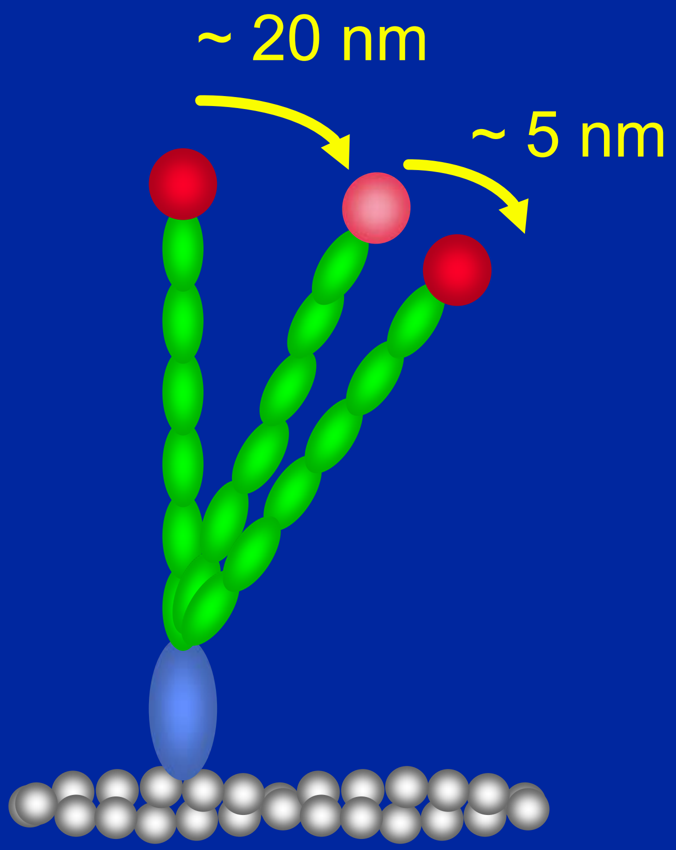


k 2 ⟶ 3 k 3 ⟶ 1

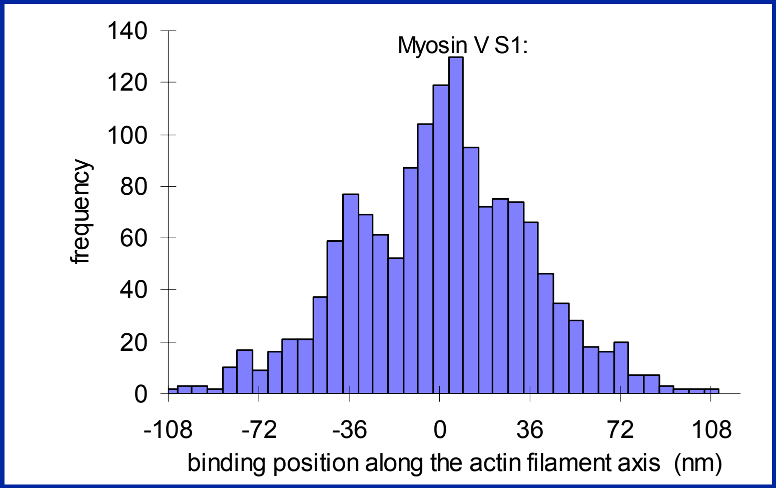
$5 s^{-1}$ $0.5 - 1.6\ \mu M^{-1}\ s^{-1}$

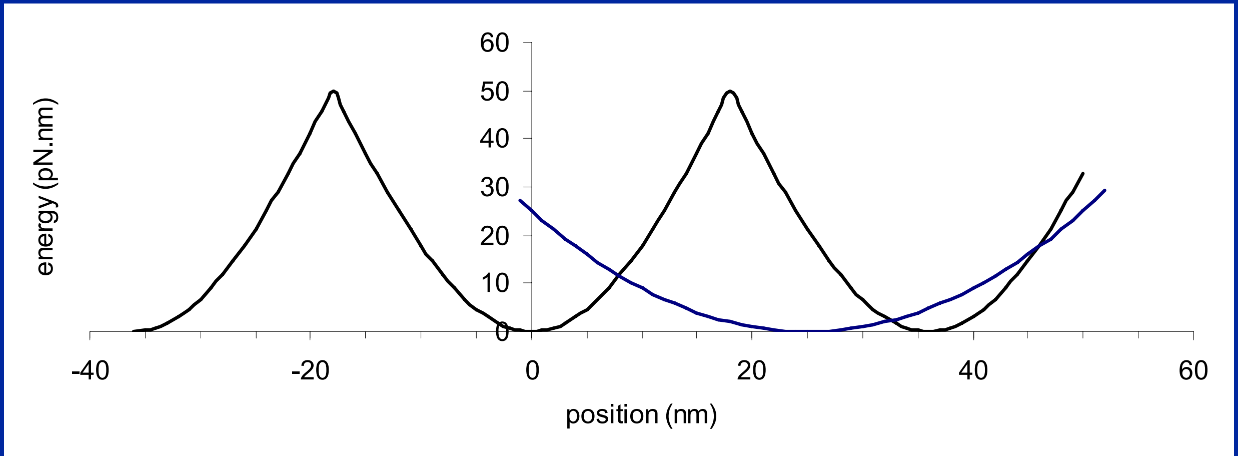


Myosin V also shows a two-phase power-stroke of ~25nm.

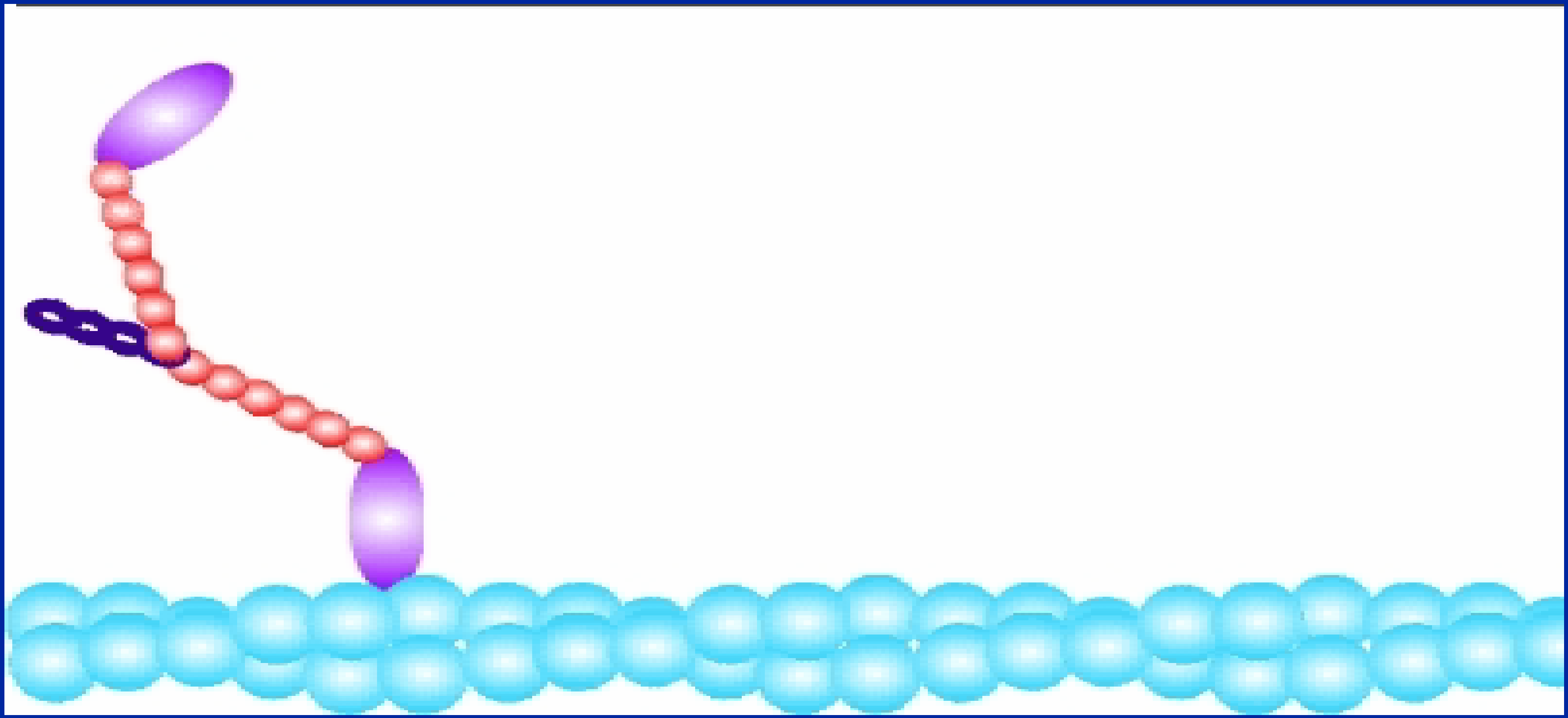


Walking is produced by a combination of powerstroke + biased diffusion.





Myosin V



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