Chapter 13

Speeds of motors

Speeds of motors in vivo

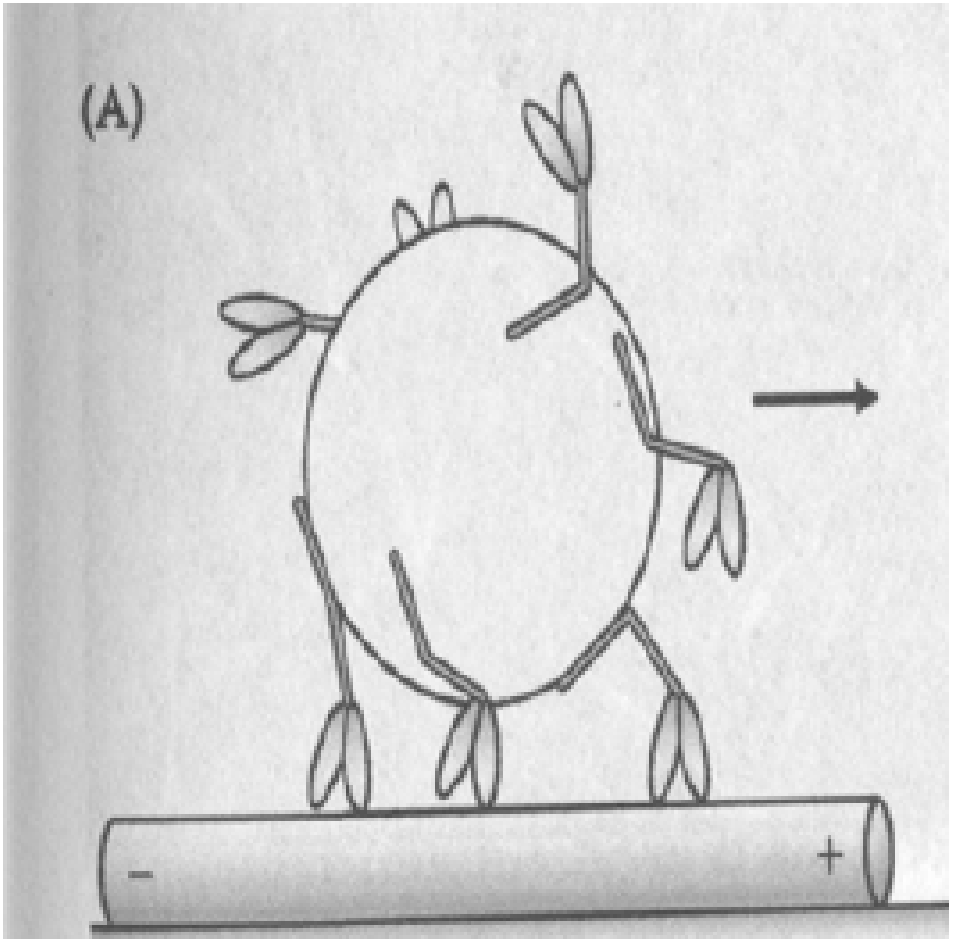
- Myosin IB – 200nm/s
- Cytoplasmic dynein – 1000nm/s
- Conventional kinesin – 1800nm/s

- Rowers vs. porters (myosin II vs. kinesin)

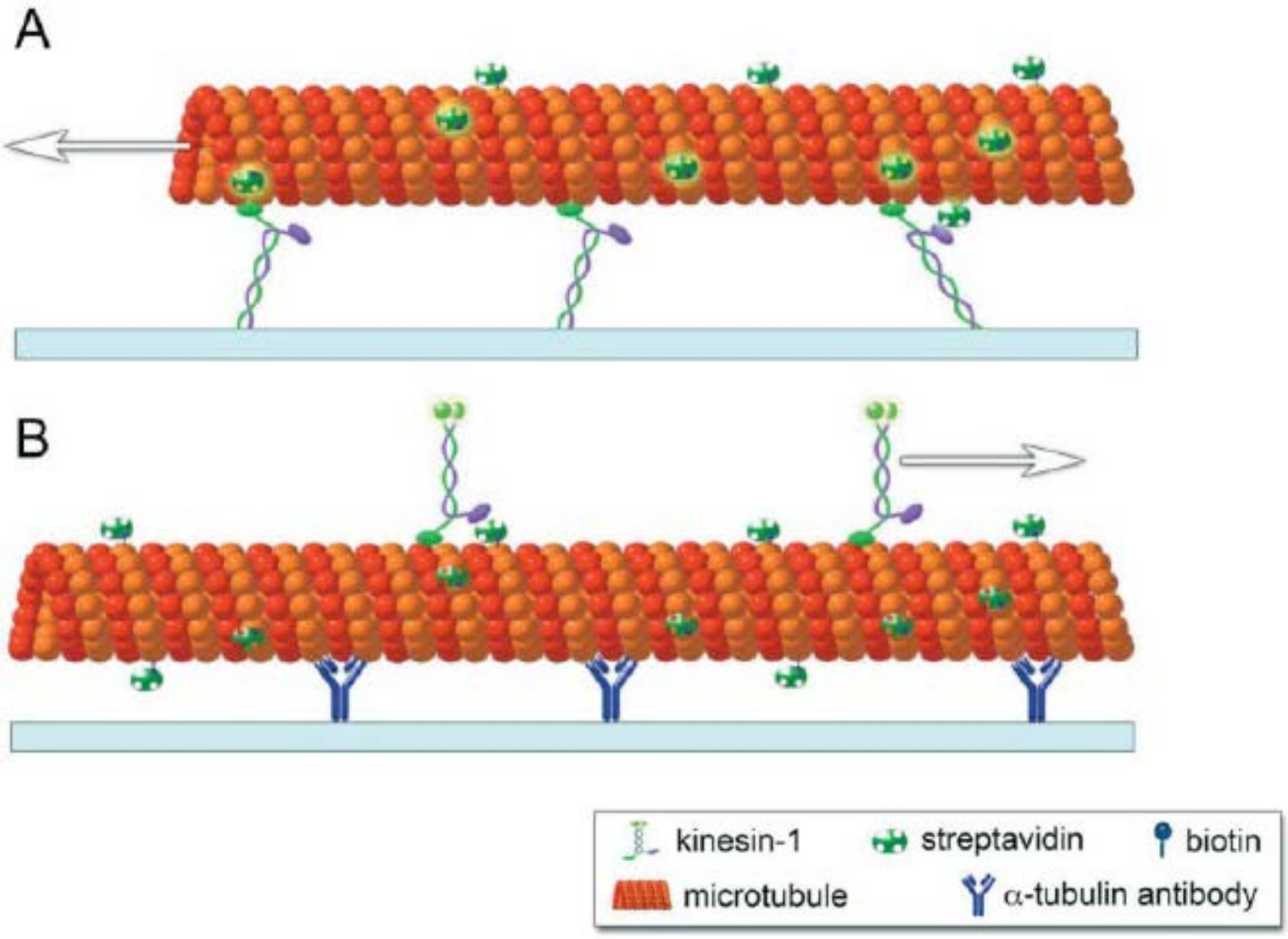
In vitro motility assays

- Sheetz and Spudich first observed the transport of fluorescent beads coated with purified myosin moving along actin.
- Bead Assay, Stepping Assay, Gliding assay

Bead assay



Gliding and stepping assays



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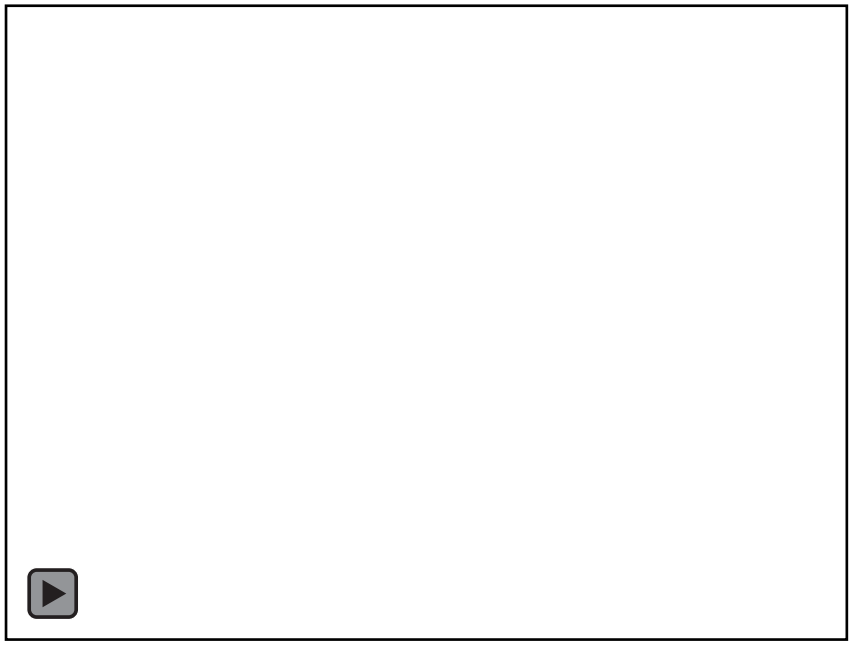

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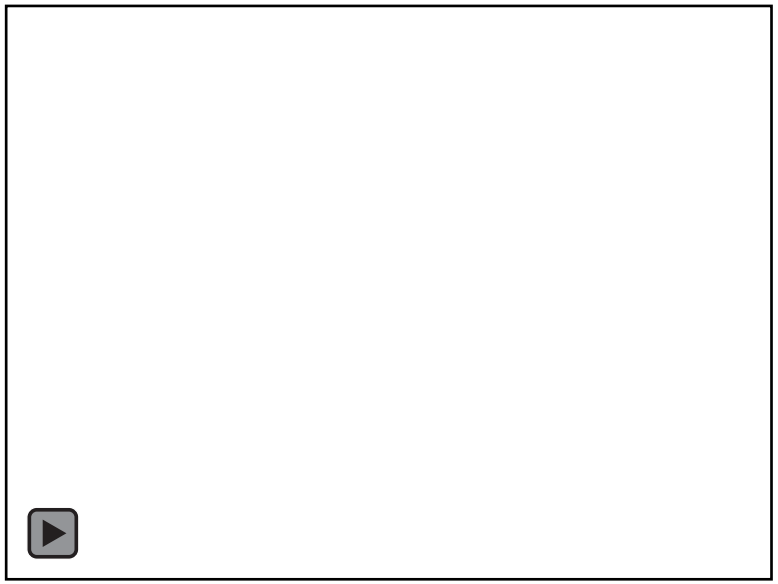

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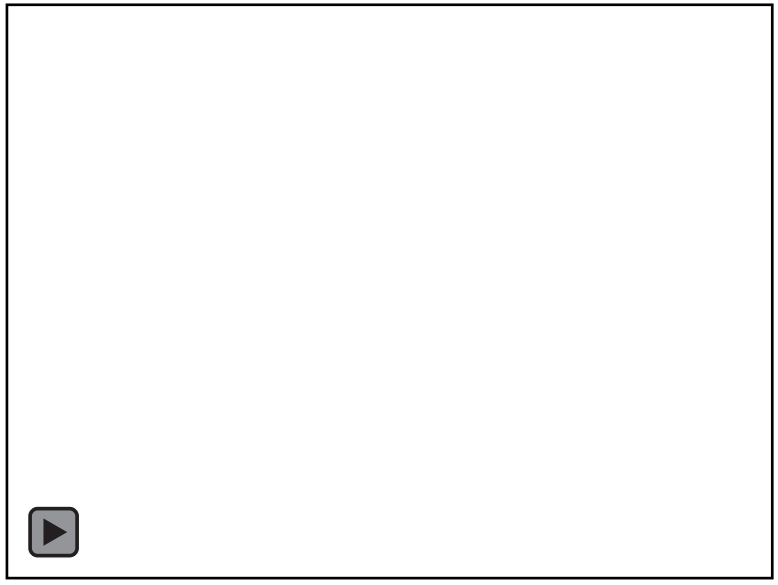
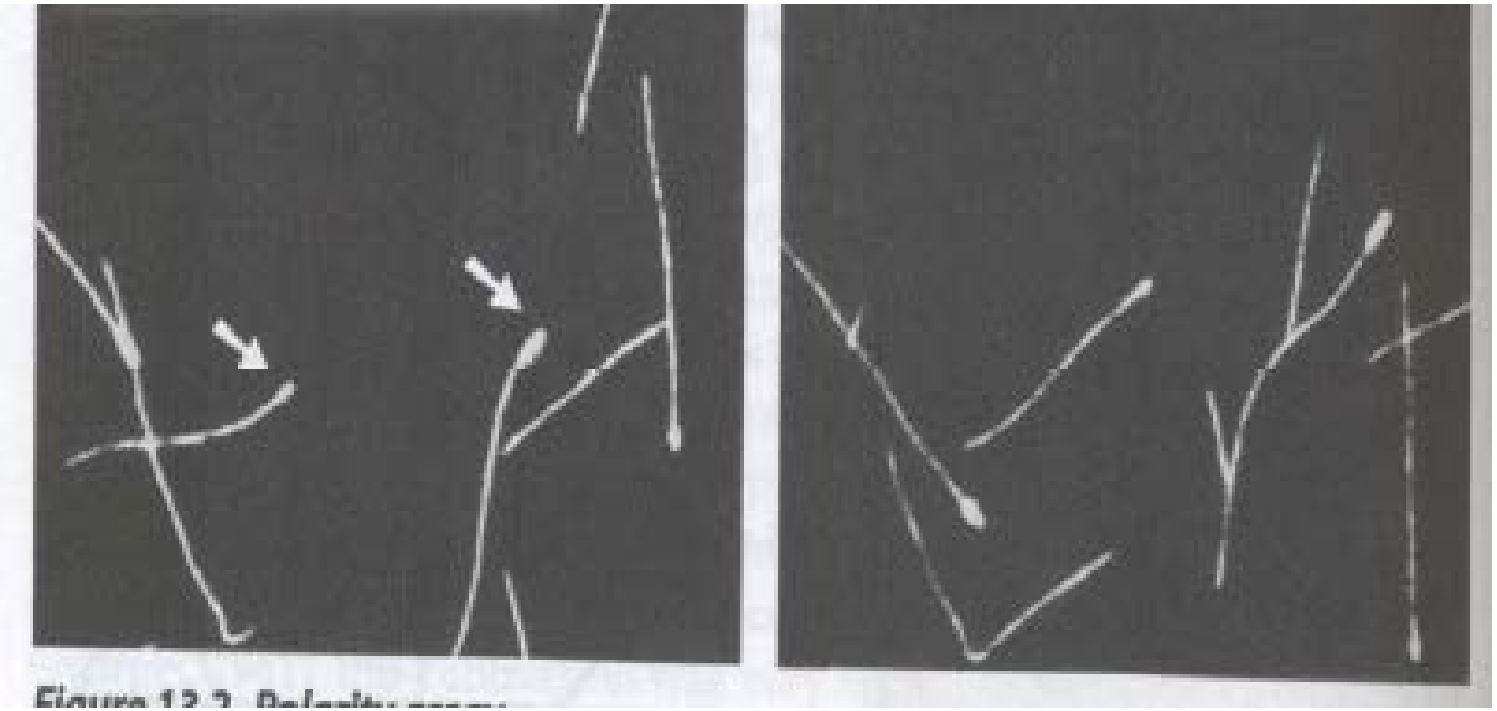


In vitro motility assays

- Polarity-marked microtubules
- Mechanical loads very small



Processive and non-processive motors

- Conventional kinesin is a processive motor, 100s of 8-nm steps
- Kinesin hydrolyzes about 125 molecules of ATP following initial binding to MT (biochemically verified) – 1 step per ATP
- Ncd is a non-processive, minus end directed motor protein

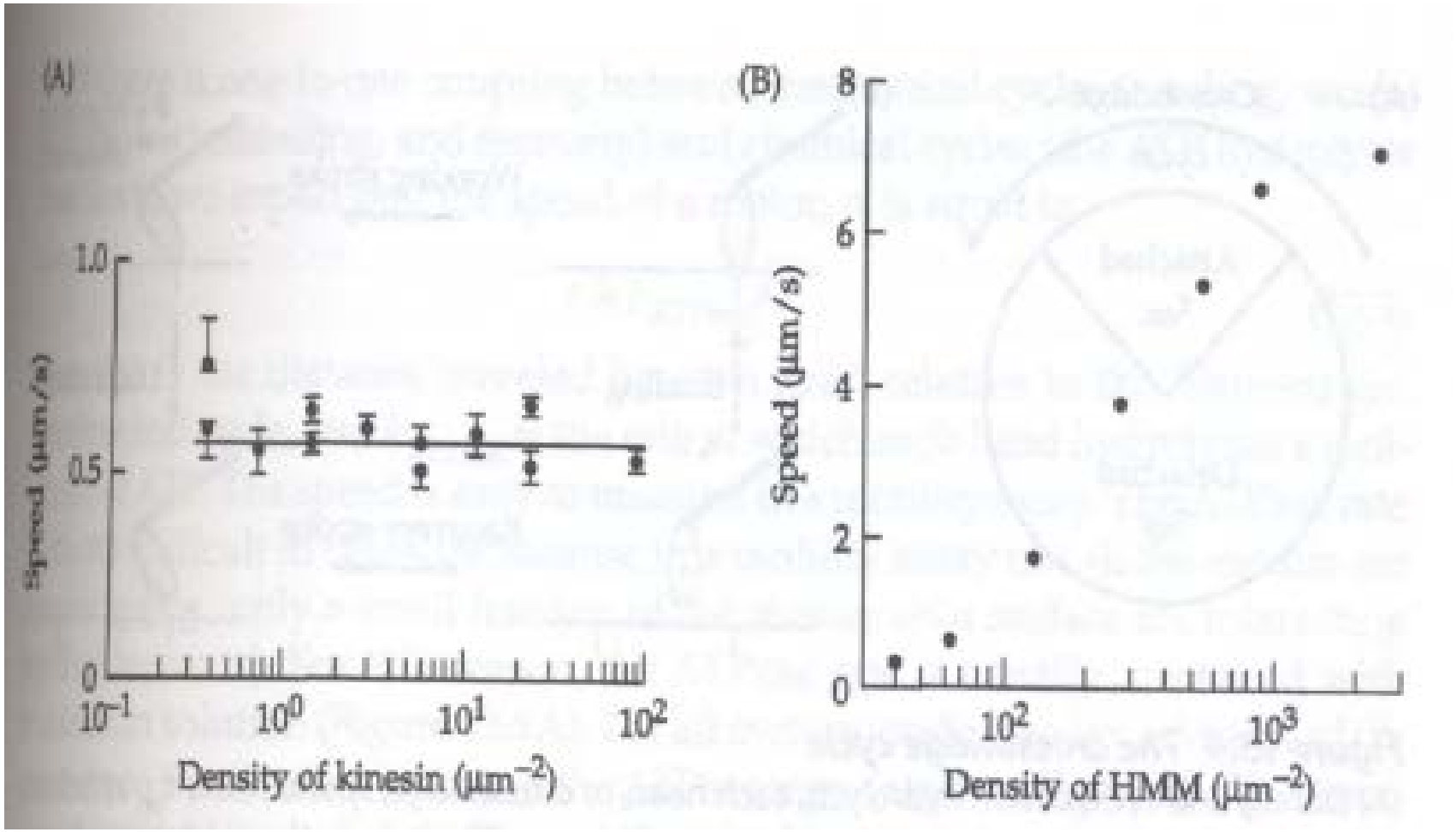
Processive and non-processive motors

- Muscle myosin II is non-processive
- Threshold density of myosin II is required for continuous motility of actin filaments → 4000 molecules /μm^2
- Myosin V and VI are processive
- Cytoplasmic dynein is processive, but outer-arm axonemal dynein is not.

Processive and non-processive motors

- Processivity/non-processivity -- dependence of speed of movement on the number of participating molecules
- Speed of kinesin – independent of no. of motors/filament length → co-ordination required
- Myosin – actin gliding occurs below threshold value when methyl cellulose added (prevents diffusion of filaments perpendicular to their long axes)

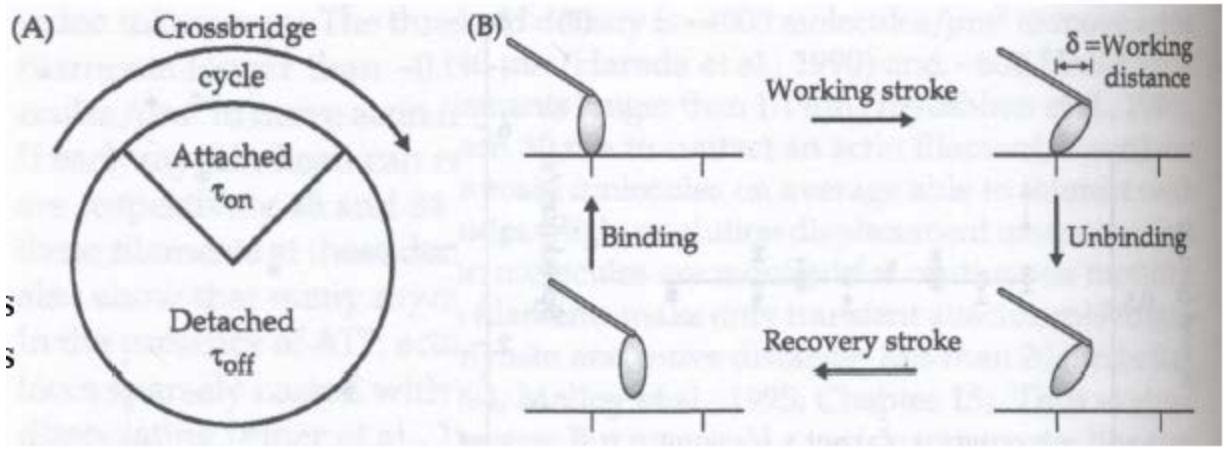
Processive and non-processive motors



Hydrolysis cycle and duty ratio

- Motors move along filaments through distances that are large compared to molecular dimensions → the motor reaction must be cyclic in which motor repeatedly bind and unbinds the filament

Hydrolysis cycle and duty ratio



- Average time motor domain spends attached to filament - τ_{on} (working stroke)
- Average time motor remains detached – τ_{off} (recovery stroke)
- Duty ratio – fraction of the time that each head spends in its attached phase:

$$r = \frac{\tau_{on}}{\tau_{on} + \tau_{off}} = \frac{\tau_{on}}{\tau_{total}}$$

Hydrolysis cycle and duty ratio

- Minimum no. Of heads required for continuous movement (N_{min})

$$r \cong \frac{1}{N_{min}}$$

- Guarantees that there will usually be at least one head bound to the filament
- Eg. Conventional 2-headed kinesin, dynein, myosin V - r must be at least 0.5 for each head.
- But for skeletal muscle myosin and outer arm dynein, large assemblies of 50-100 crossbridges, r is small ~0.01 to 0.02

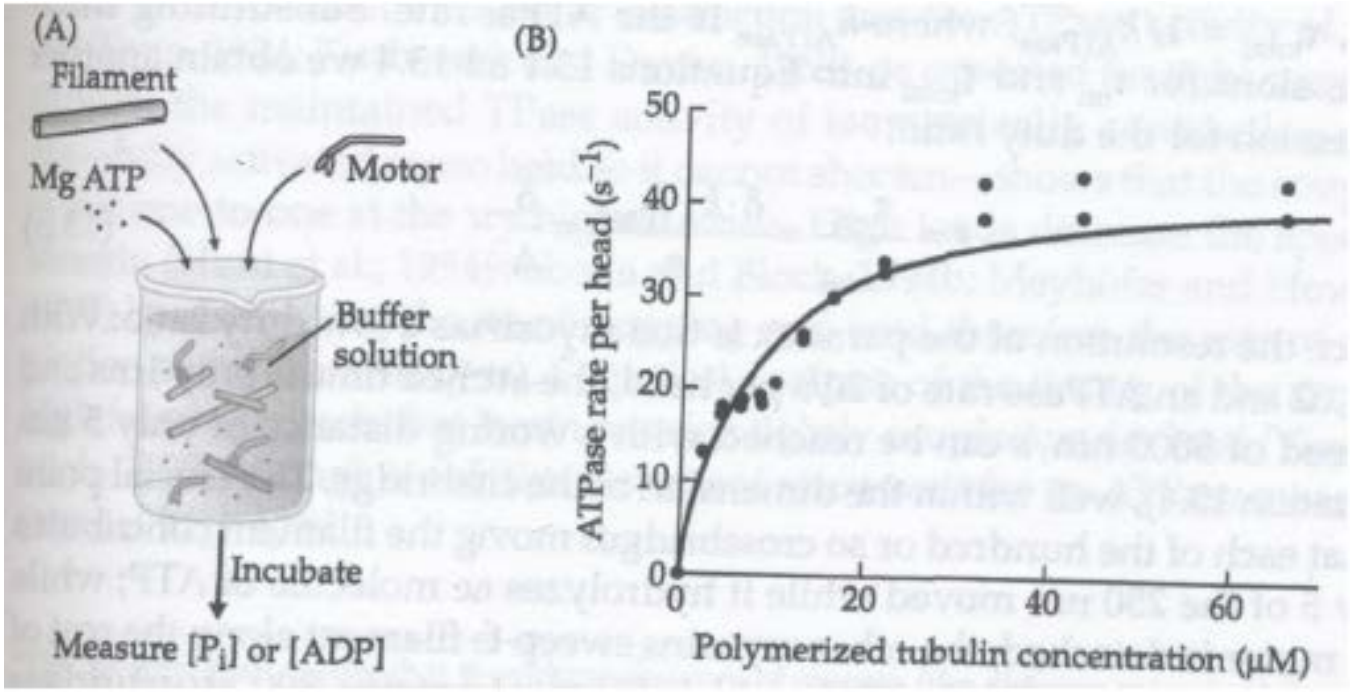
Hydrolysis cycle and duty ratio

- If there is one-to-one coupling between mechanical cycles and chemical cycles, then we expect speed of the motor:

$$v = k_{ATPase}\Delta$$

- k_{ATPase} – rate at which each head hydrolyzes a molecule of ATP, Δ – distance moved by each head per mechanical cycle.

Measurement of k_{ATPase}



- Maximum ATPase rate measured in solution (k_{cat}) is assumed to correspond to the ATPase during motility

Speed of motors and k_{cat}

Motor	Speed[a] in vivo (nm/s)	Speed[b] in vitro (nm/s)	In vitro ATPase[c] (s^{-1})	Function
Myosins				
1. Myosin IB	ND[d]	200	6	Amoeboid motility, hair cell adaptation
2. Myosin II	6000	8000	20	Fast skeletal muscle
3. Myosin II	200	250	1.2	Smooth muscle contraction
4. Myosin V	200	350	5	Vesicle transport
5. Myosin VI	ND	–58	0.8	Vesicle transport?
6. Myosin XI	60,000	60,000	ND	Cytoplasmic streaming in algae
Dyneins				
7. Axonemal	–7000	–4500	10	Sperm and cilial motility
8. Cytoplasmic	–1100	–1250	2	Retrograde axonal transport, mitosis, transport in flagella
Kinesins				
9. Conventional	1800	840	44	Anterograde axonal transport

No direct relation!

Step size vs. k_{cat}

$$\Delta = \frac{v}{k_{ATPase}} = \begin{cases} 400\ nm/ATP & \text{For skeletal muscle myosin} \\ 450\ nm/ATP & \text{For outer-arm dynein} \end{cases}$$

- For HMM, 190nm/ATP , order of magnitude more than the crossbridge➔ ‘step-size paradox’ leading to the suggestion that myosin might take multiple steps for each ATP hydrolyzed.
- Duty ratio provides a simple explanation for the step-size paradox.

Hydrolysis cycle and duty ratio

- Filament moving with constant speed *v,* over array of fixed motor proteins.
- Assuming enough motors to ensure continuous motility (1 kinesin/50-100 myosin heads)

$$v = \frac{\delta}{\tau_{on}}$$

δ – working distance

τ_{on} – time for which head is attached

Hydrolysis cycle and duty ratio

- Total cycle time $\tau_{total} = \frac{1}{k_{ATPase}}$

(Cycle driven by ATP hydrolysis)

$$\left.\begin{aligned} r &= \frac{\tau_{on}}{\tau_{total}} \\ \tau_{on} &= \delta / v \\ \tau_{total} &= 1/k_{ATPase} \end{aligned}\right\} \Rightarrow r = \frac{\delta k_{ATPase}}{v} \xrightarrow{v = k_{ATPase}\Delta} r = \frac{\delta}{\Delta}$$

Myosin:
- $r = 0.02$
- $k_{ATPase} = 20$/s per head
- $\tau_{on} = 1$ ms
- $v = 5000$ nm/s
- $\delta = 5$ nm ⟶ within the dimension of the crossbridge

Hydrolysis cycle and duty ratio

- Each of the 100 or so crossbridges that move the filament contribute only 5nm of the 250nm moved while it hydrolyzes one molecule of ATP
- Each thick filament contains 300 crossbridges, for duty ratio of 2% in a rapidly contracting muscle → at all times, 6 or so crossbridges maintain contact between thin and thick filaments

Processive motors

- Since kinesin has high *r*, high ATPase rate is needed to attain moderate speeds

kinesin: $k_{ATPase} = 50/s$ per head; $v = 800$ nm/s; $\delta = 8$ nm → $r = 0.5$

Consistent with the duty ratio required to account for kinesin's processivity.

Estimation of working distance

Myosin: $r = 0.01$ to 0.02, $\Delta = 200$ to 400 nm $\xrightarrow{r = \frac{\delta}{\Delta}}$ $\delta = 2$ to 8 nm

↓

not larger than the molecular dimension of the heads

↓

myosin just makes one mechanical cycle per chemical cycle of ATP hydrolysis

↓

Confirmed by experiments using single-molecule fluorescence to detect ATP hydrolysis and optical tweezers to detect steps.

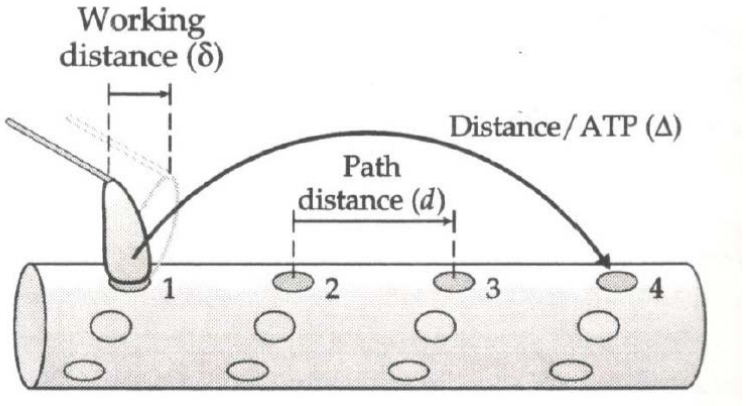
Analogy to walking and running

- Gait of a biped is walk if “the feet move alternatively with one foot not clear od the ground before the other touches”
- During run, times when both feet are off

Chapter 15
Steps and forces

Distances that characterize a motor reaction

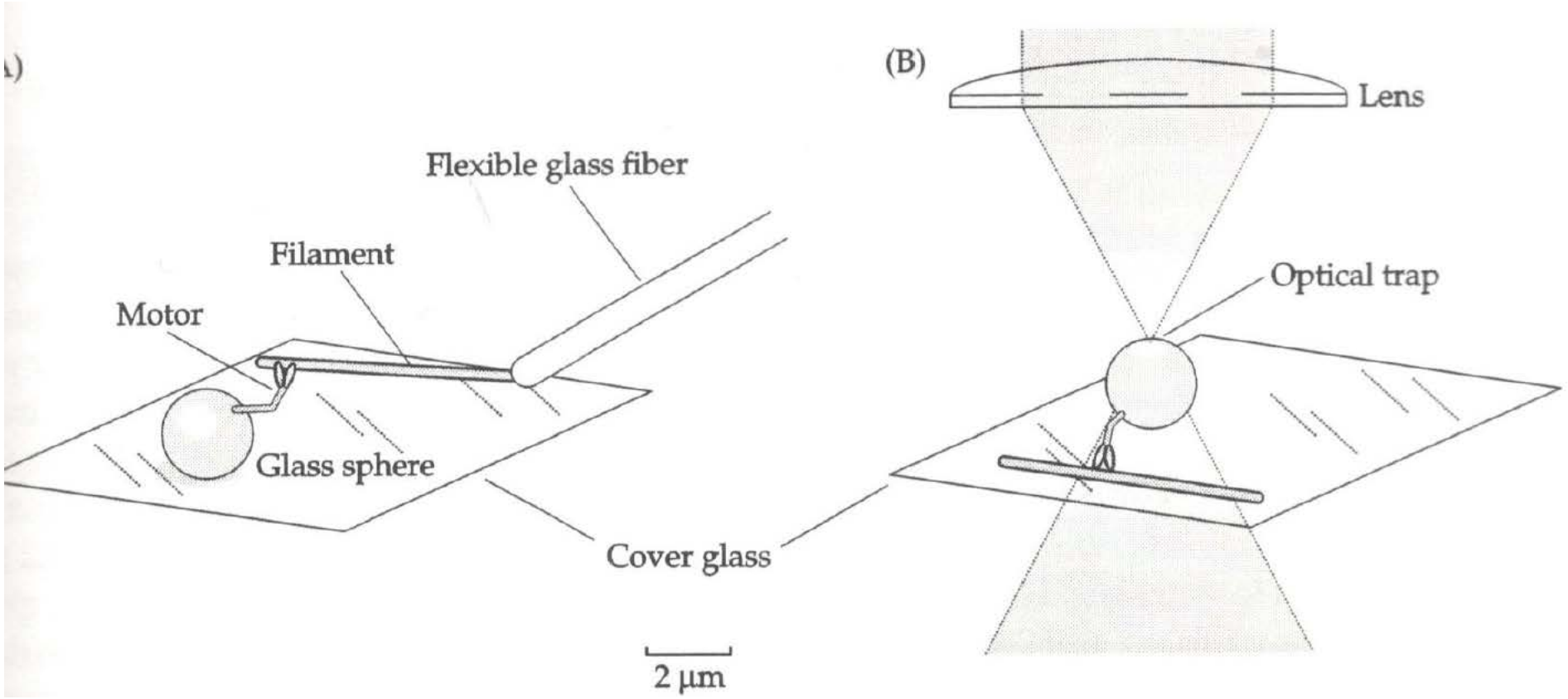
- Working distance δ (powerstroke distance)
- Distance per ATP for each motor domain Δ (Speed of movement/ATPase rate per head)
- Path distance d (Distance between the consecutive binding sites)



Single motor techniques

- Filament assay – MT held in force transducer and presented to a motor that is fixed to the surface
- Bead assay – Motor attached to a bead that is held in a force transducer and bead presented to a filament fixed on the surface
- Force transducers – cantilevered glass rods, AFMs or optical tweezers, must be able to produce and monitor forces in the piconewton range

Single motor techniques



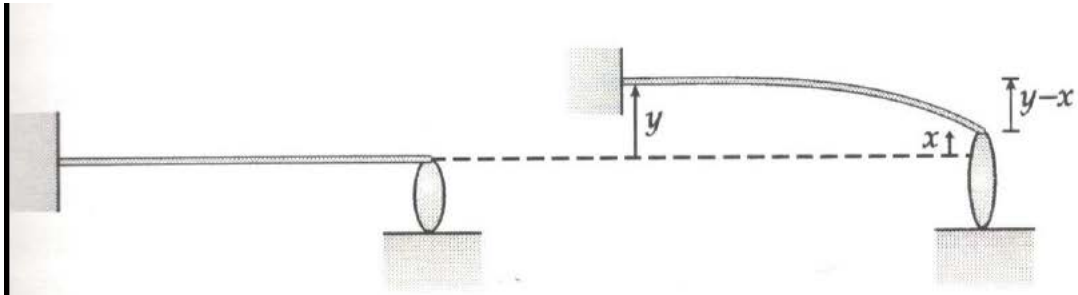
Cantilevered springs

- Slender glass fiber whose base is held in a micromanipulator – spring exerting known forces on the motor (principle of AFM). Stiffness of cantilevered fiber:

$$\kappa_f = \frac{3\pi}{4}\frac{Er^4}{L^3}$$ (Chapter 6)

E- Young's modulus, L-length of fiber, r-radius

- Stiffness of 0.001 to 1 pN/nm with glass rods (E ~ 70 GPa) of 100 to 200 μm and radius 100-250 nm



igure 15.3 Cantilever force transducer
he force equals $F = \kappa_f(y - x)$, where κ_f is the stiffness of the fiber, y is the displacement of he base of the fiber, and x is the displacement of the tip of the fiber, which is also equal to he displacement of the attached point of the protein. $y - x$ is the flexion in the fiber.

Cantilevered springs

- Time constant of a cantilevered spring

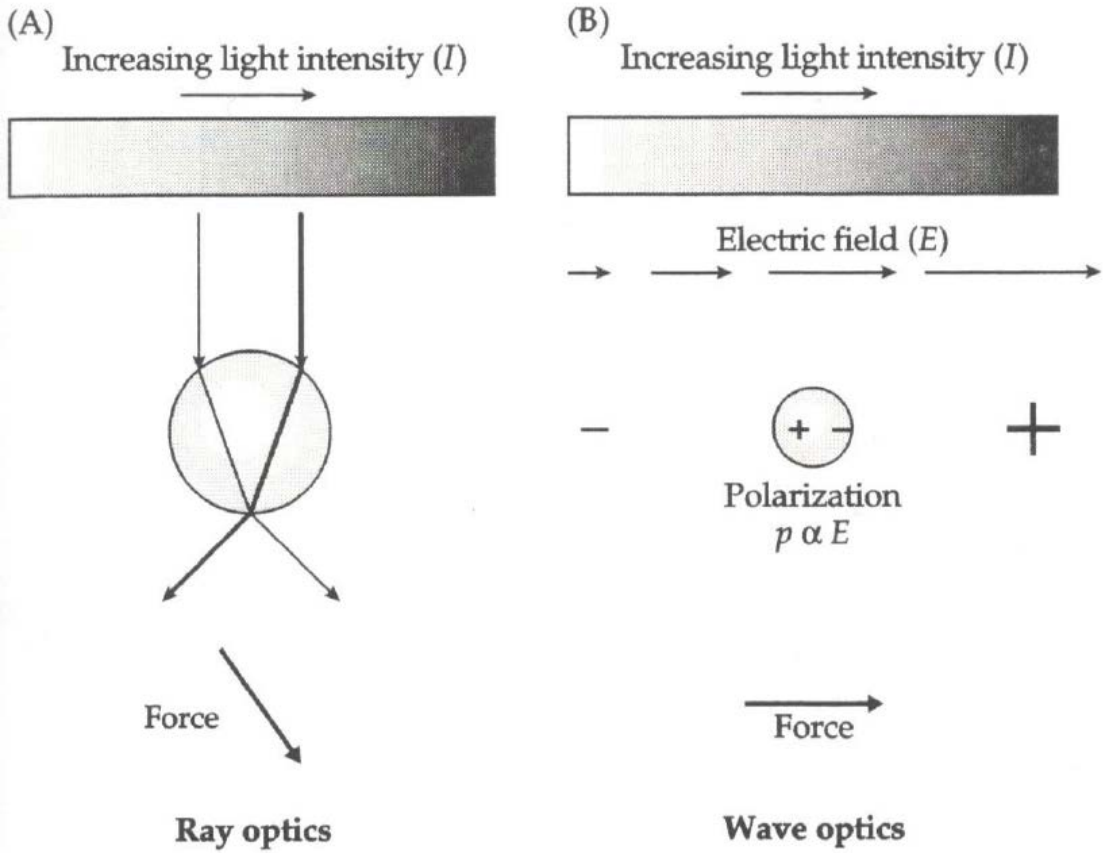
$$\tau \cong 0.2 \frac{\eta . L^4}{E . r^4} \text{ (Chapter 6)}$$

η – Viscosity of the solution

- Time constant for glass fibres is ~1ms → small enough to resolve individual transitions in hydrolysis cycle
- Modify radius and length to get desired time constants and stiffnesses

Optical tweezers

- Laser bean focused down to a diffraction-limited spot using high NA lens
- Dielectric non-absorbing glass or plastic bead experiences a force that will tend to move it into the region of highest intensity (focus of the laser)
- If size of particle >> λ, force due to change in momentum of the photons refracted by the particle.
- If size of particle << λ, force due to polarization of the particle induced by electric field component of the light wave



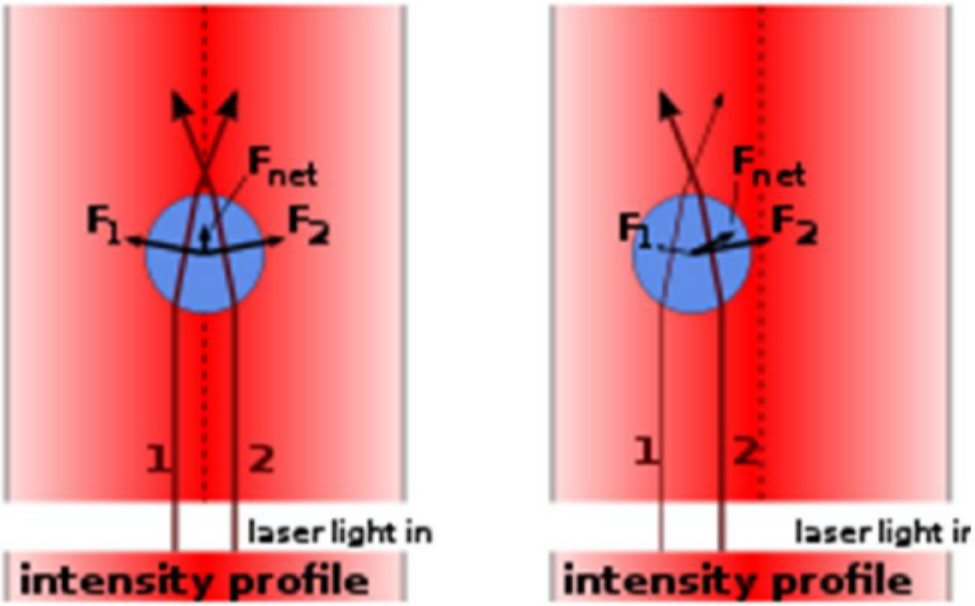


Figure 3: Lateral gradient forces on a particle in a Gaussian beam. The particle on the beam axis (left) has no net force in the lateral direction because the forces from Ray 1 and Ray 2 are equal from each side. The particle to the left of the beam axis (right) has a net force to the right since the higher intensity Ray 2 has a greater change in momentum than Ray 1.

Optical tweezers

- Force exerted by optical trap

$$F_{opt} = Q\frac{nP}{c}$$

- F_{opt} - force associated with the absorption of light (photon pressure)
- nP/c – force exerted by light on a perfect absorber (c/n – speed of light in the medium, P-power)
- Q- dimensionless constant, trapping efficiency

Optical tweezers

- If the particle does not move very far from the centre of the trap, the trap will behave like a spring with spring constant:

$$\kappa_{opt} \sim Q \frac{n^2 P}{\lambda c}$$

- Spring constants in the range – 0.01 to 0.1 pN/nm using laser powers of ~100mW on glass or plastic beads (n=1.50 or 1.57) of dia 1µm

Optical tweezers

- Physical size of the trap is determined by λ and size of the object. Usually λ of around 1 μm used since less photodamage
- Traps much softer, produce less force than cantilever beams and AFMs
- But shorter time constants – better temporal resolution

Displacements

- Photodiode detector
- Accurate measurement of centre of mass of fiber or bead → mean of distribution is can be measured very precisely even if the distribution itself is quite broad.
- Precision of the displacement measurement is limited only by the number of photons that are counted (Appendix 15)

Calibration

- Thermal fluctuations of force transducers are picked up by the detectors
- This is used for the calculation of the stiffness of the force transducer in situ

Single molecule fluorescence

- TIRF and laser scanning confocal
- Limitations – limited lifetime of fluorophores → limited spatial and temporal resolution

Steps, Paths and Forces

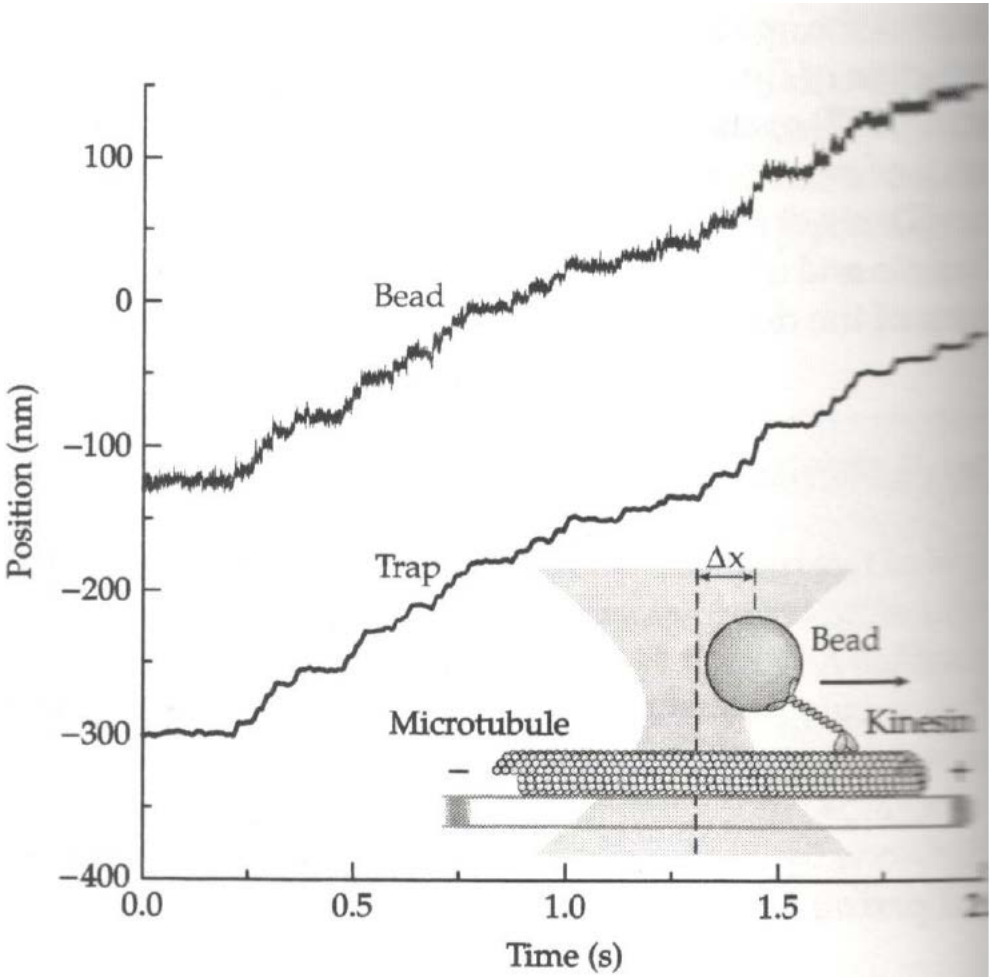
- Displacement sensitivity of optical tweezers, glass fibers and AFMs – 1nm sufficient for measuring step size of motor proteins
- Force sensitivity ~ 1pN, sufficient to resolve force of single motor.

Conventional kinesin

- Conventional kinesin is processive, pulling a 1µm glass bead 100s of nm on MT, even when under load of optical trap.
- At high force, motion is not smooth
- Steps have amplitude of 8 nm →kinesin moves from 1 tubulin dimer to the next along a protofilament (length of dimer – 8nm)
- No switching to neighbouring protofilament under low motor densities – no changing lanes by single motor

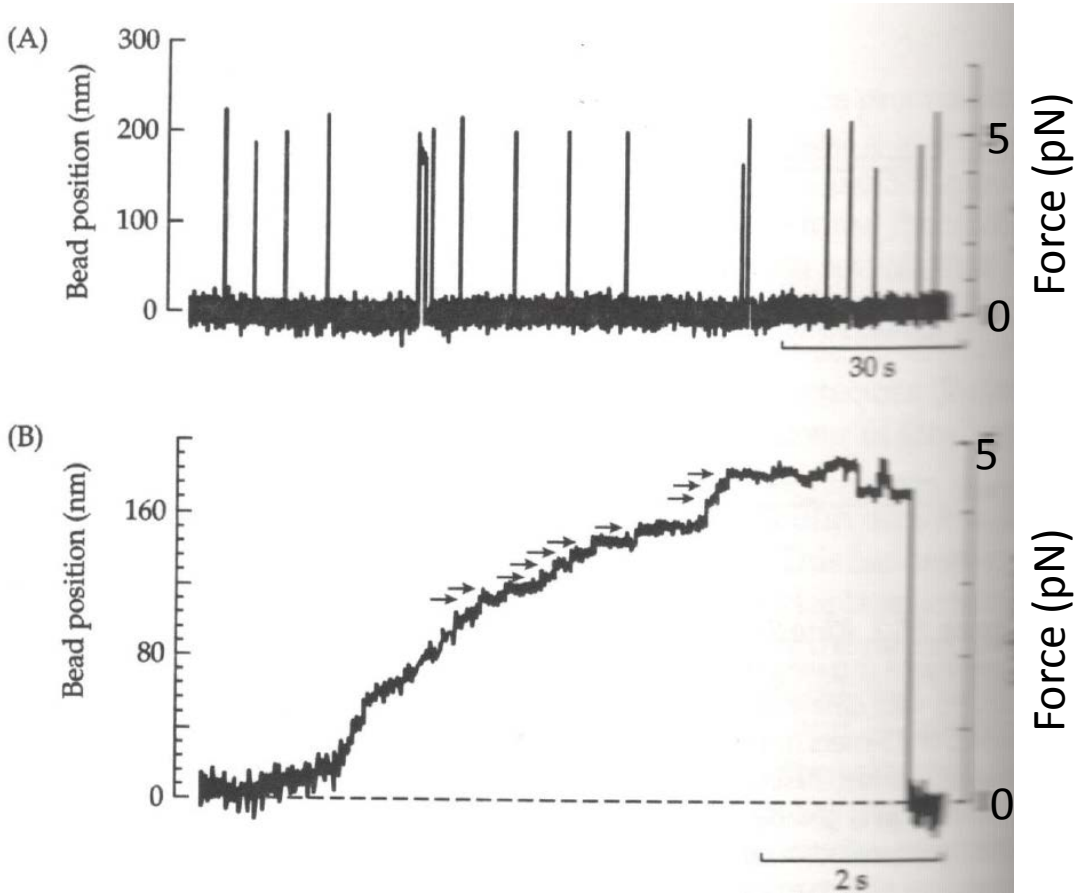
Conventional kinesin

- Maximum force that kinesin can work against ~ 6pN → Measured by making kinesin walk away from the centre of the trap
- As distance increases, load increases and speed decreases, eventually leading to stalling of the motor. Stall force – 4 to 8 pN have been measured
- Speed of movement decreases ~linearly with increase in opposing force and maximum force independent of ATP concentration and temperature



Conventional kinesin

- Maximum work done per step = 6pN x 8nm = 48pN.nm
- Energy derived from hydrolysis of ATP = 100 pN.nm
- Therefore, maximum efficiency of kinesin ~50%
- Maximum force is only 50% of the thermodynamic force (= chemical free energy/step size, for kinesin, thermodynamic force = 100pN.nm/8nm=12.5pN)
- Rate of forward stepping ~0 at high loads



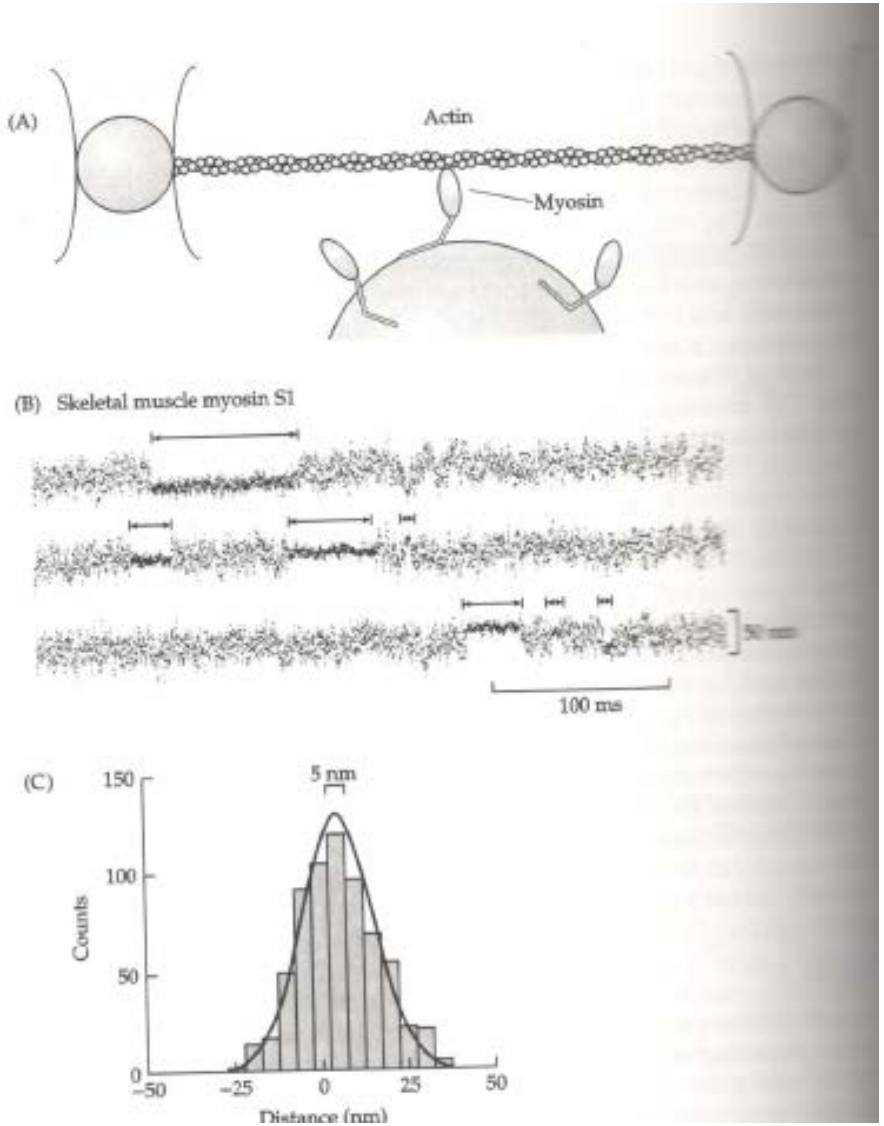
Myosin II

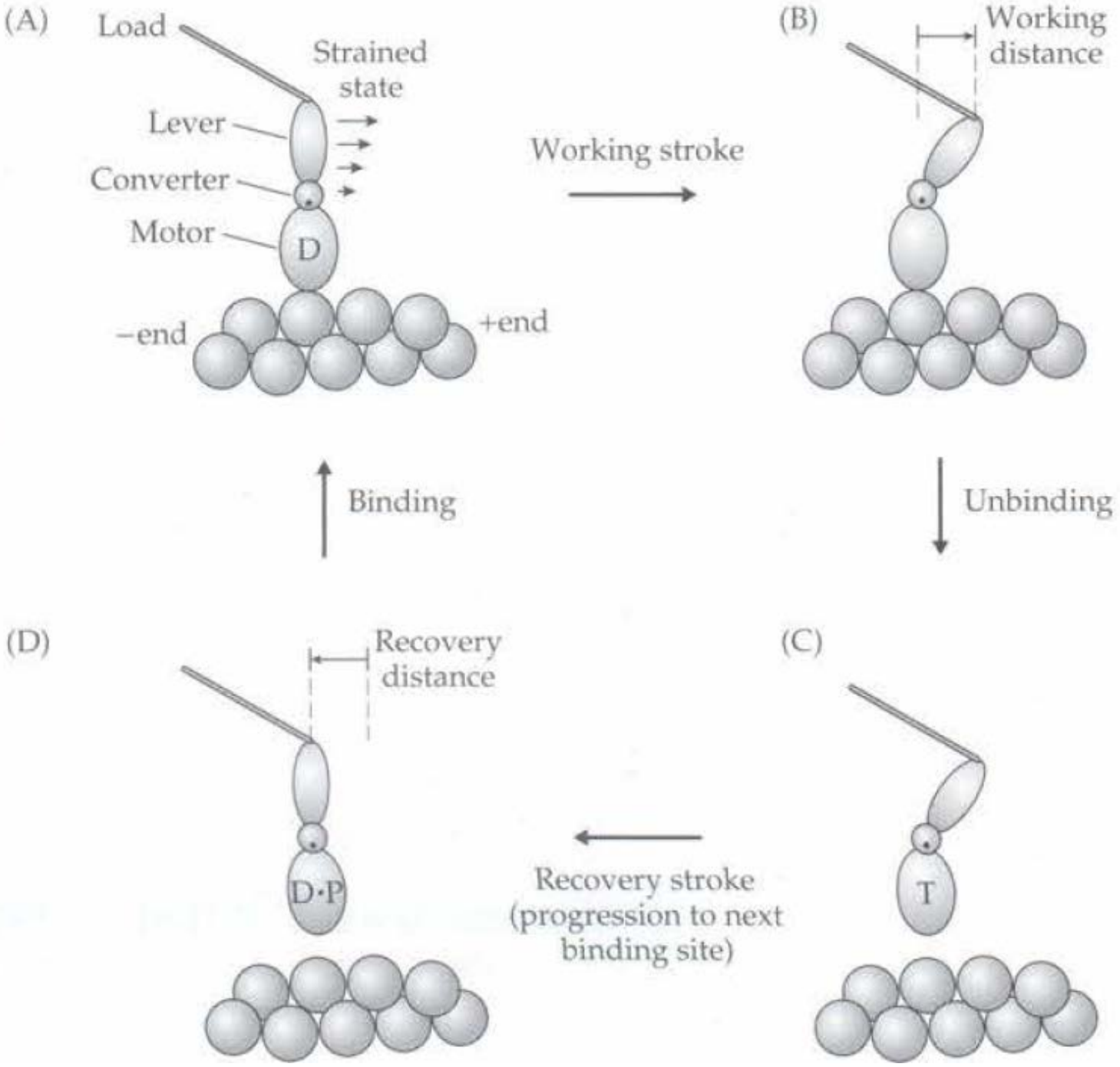
- Only interacts transiently with actin and does not step
- Discrete binding events observed → decrease in thermal fluctuations of the actin filament
- Not processive motor
- Binding of myosin displaces actin from average position, mean displacements + or –

→Large thermal fluctuations of the actin filament sweep binding sites past the fixed motors; motor binds to filament and holds it in place, displacements can be + or – because fluctuations are symmetric

Myosin

- Despite variability in displacement, directional bias for myosin → myosin undergoes a directed conformational change of 5nm (working distance)
- Can be confirmed by rotating filament by 180 degrees
- Length of the rotating lever altered by replacing the light chain binding domain with artificial levers of various lengths → changes amplitude of the step
- Maximum force exerted by myosin crossbridge >= 10pN




(A)
Load
Strained state
Lever
Converter
Motor
D
−end
+end
Working stroke
(B)
Working distance
Unbinding
(C)
T
Recovery stroke (progression to next binding site)
(D)
Recovery distance
D·P
Binding

Structural basis for duty ratio

- Relation between the distance per ATP and the path distance

$$\Delta = n.d$$

n- is an integer >=0.

- n=0 corresponds to futile ATP hydrolysis event, with no associated displacement (eg.: under high load)
- If n>1, the motor jumps over one or more of the stepping stones (myosin at low load)

Structural basis for duty ratio

- Duty ratio is working distance divided by distance per ATP, $r = \delta/\Delta$
- Structural expression for duty ratio:

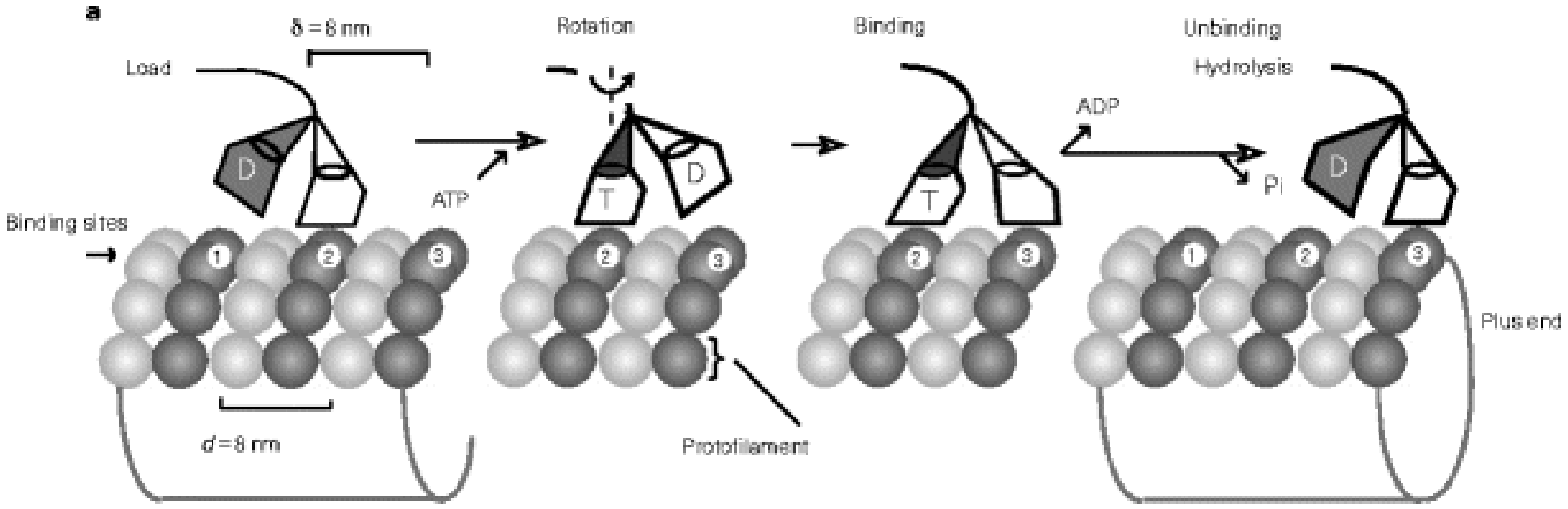
$$r = \frac{\delta}{n.d}$$

- Steric constraint that is present in moving motor-filament system
- If working distance δ is smaller than path distance d, each individual crossbridge must spend a significant time detached while other crossbridges move the filament: r<1 and motility will require an assembly of crossbridges

Table 15.1 Motor distances in vitro

Motor Distance	Parameter	Skeletal muscle myosin	Conventional kinesin
Working distance	δ	5 nm[a]	≥ 8 nm
Path distance	d	36 nm	8 nm
Distance/ATP per head[a]	Δ	200–400 nm	16 nm

[a]Low load


a
δ = 8 nm
Load
D
Binding sites
1
2
3
d = 8 nm
ATP
Rotation
T
D
Protofilament
Binding
ADP
Pi
Unbinding
Hydrolysis
Plus end