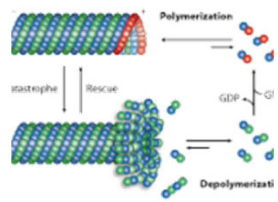
Directed & purposeful motion

- nonrandom movement -> cost energy
- require a mechanism that converts chemical energy into mechanical energy → **a motor**
- e.g. phosphoanhydride bond of ATP or ion gradients across membranes



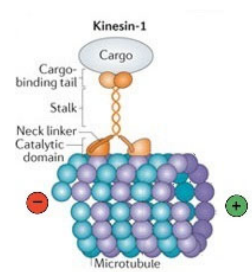
types of motors:

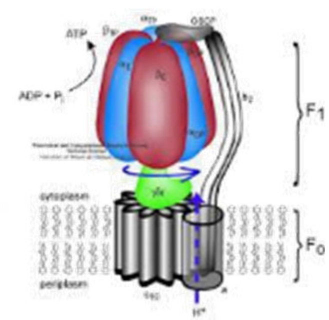
i. **polymerization motors**

force gen. by polymerization

ii. **translational motors**

move along a track



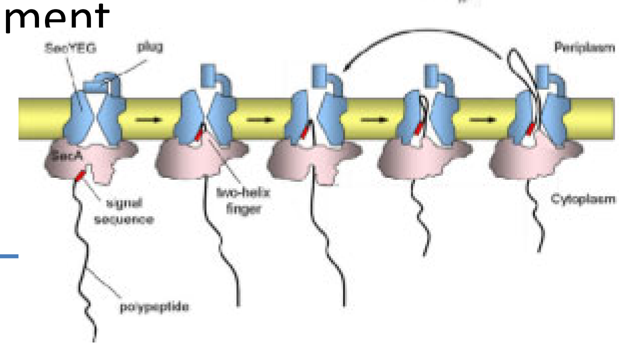


iii. **rotary motors**

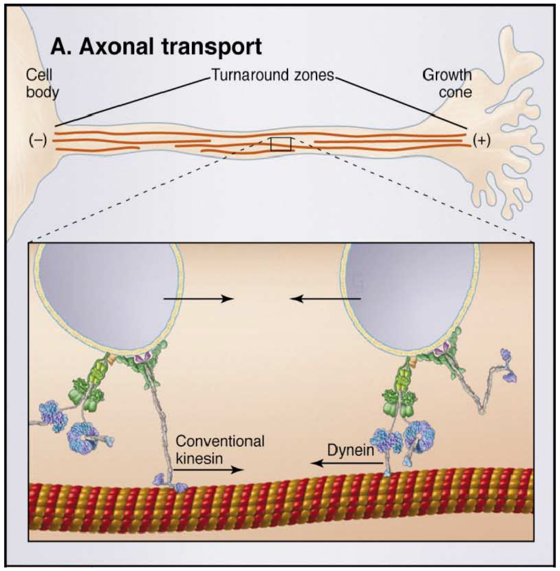
convert chemical energy in rotational movement

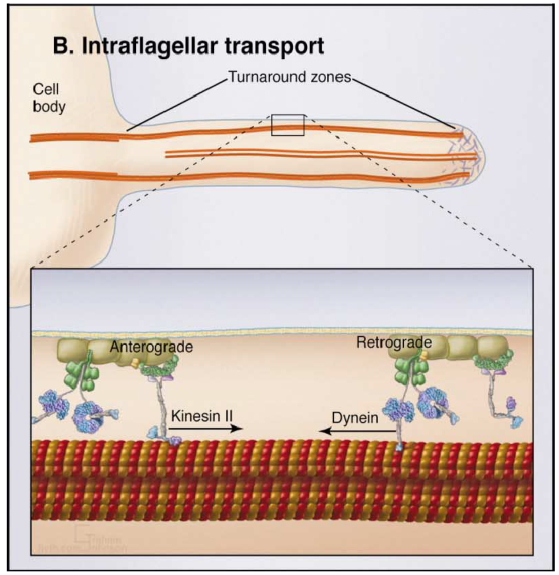
iv. **translocation motors**

threading something through a pore



Transport processes in cells

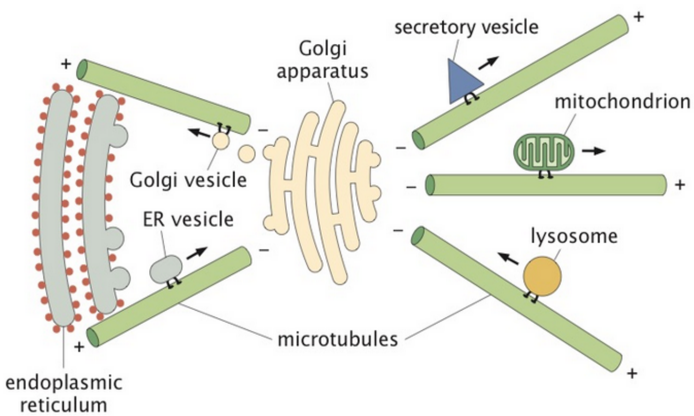


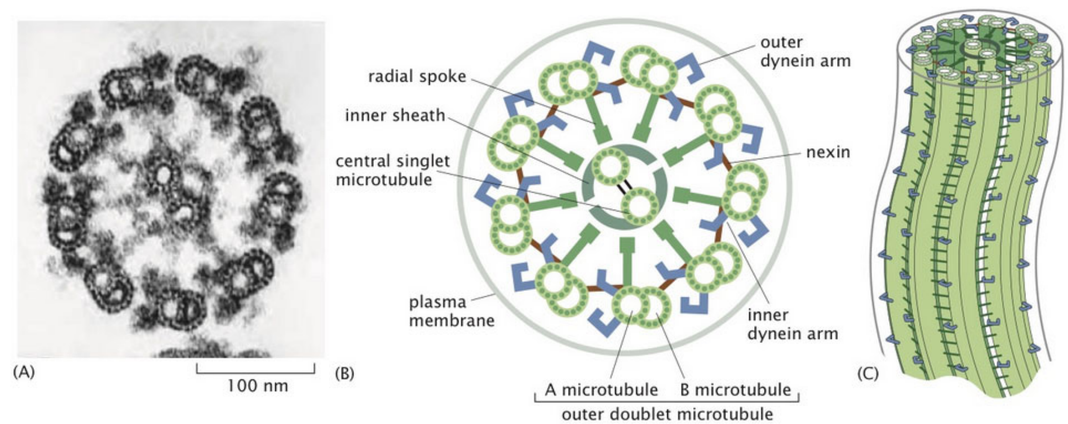


Different motor proteins have distinct roles in the cell

movement of cargo along the cystoskeleton (often in defined direction)

Generation of cell movement (cilia, muscles)



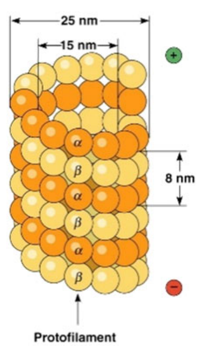


Translational motor systems

Main actors

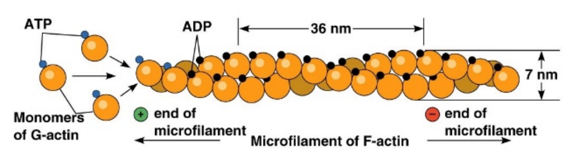
Microtubules

- Formed by polymerization
- of dimers of α and β tubulin
- Dynamics controlled by GTP
- hydrolysis



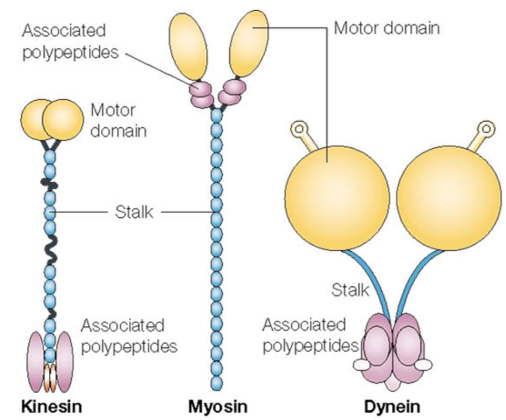
Actin filaments

- Formed by polymerization
- of actin subunits
- Dynamics controlled by ATP
- hydrolysis

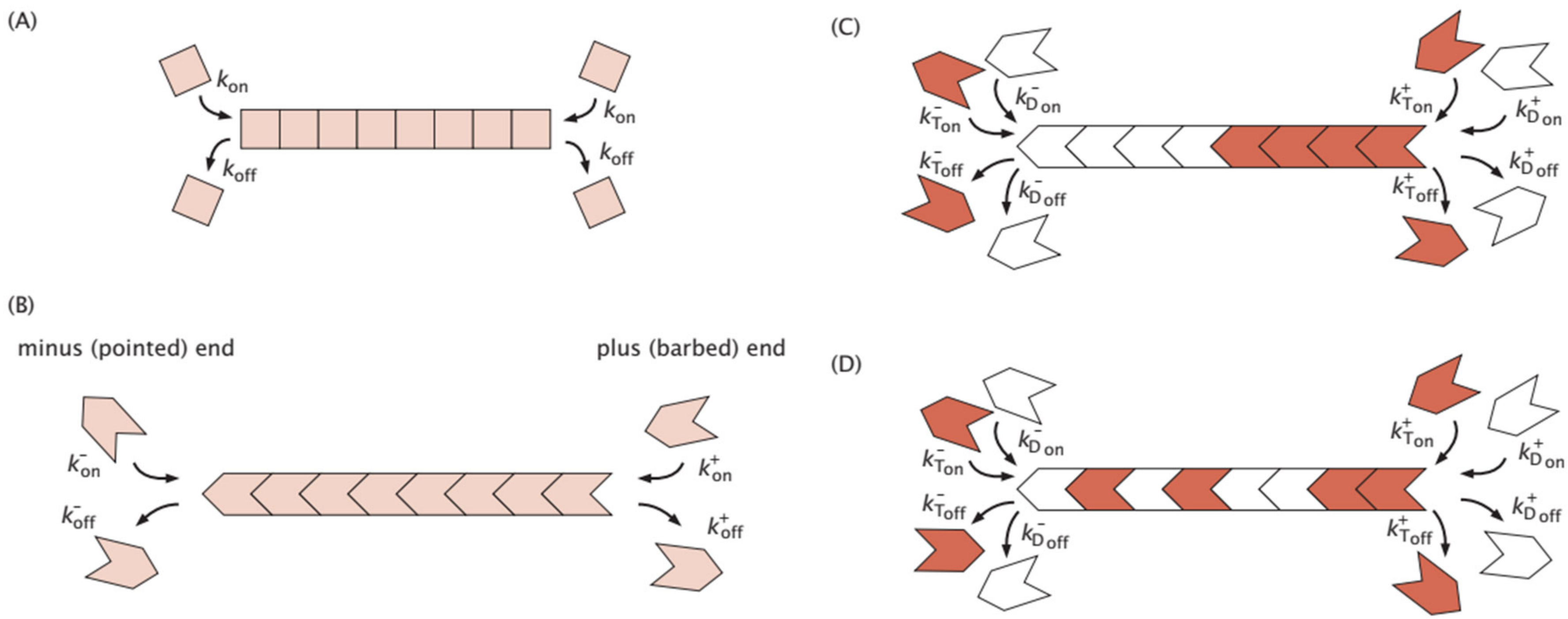


Molecular motors

- Utilizes energy of ATP to
- perform mechanical work
- (→ motion,cargo transport)

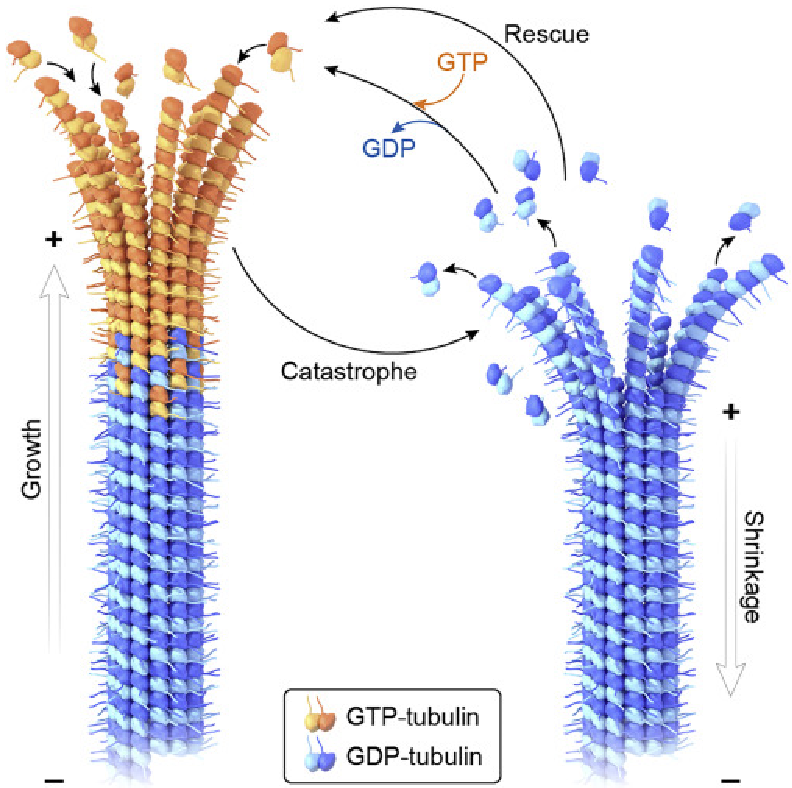


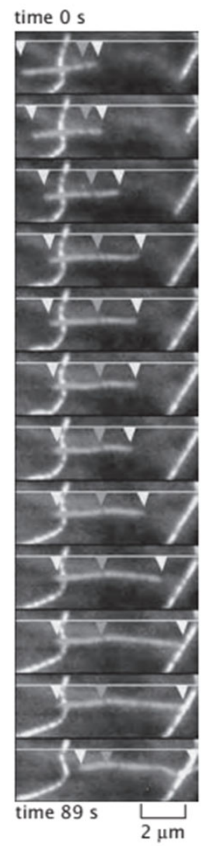
Dynamics of cytoskeletal polymers

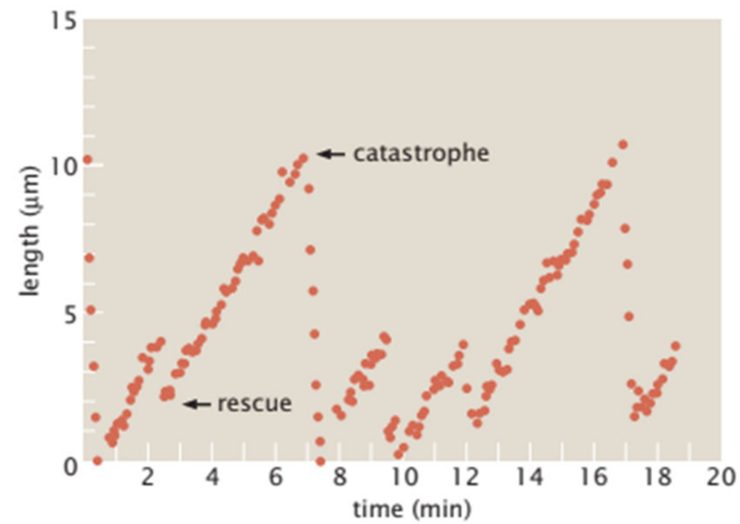


Models of e.g. Actin, with increasing complexity

Dynamic instability in microtubules







Growth phases are interspersed with '**catastrophe'** events, followed by **'rescue**'

Directed & purposeful motion

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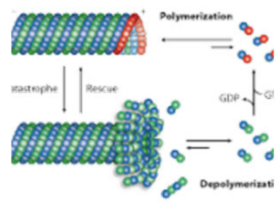
- e.g. phosphoanhydride bond of ATP or ion gradients across membranes



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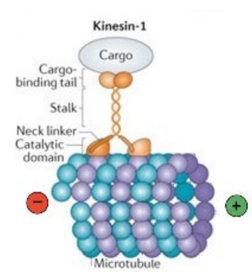

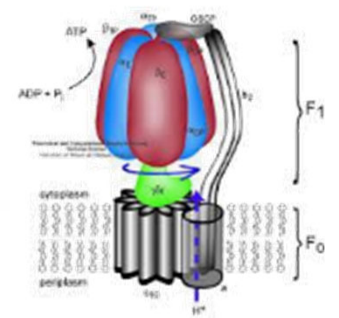

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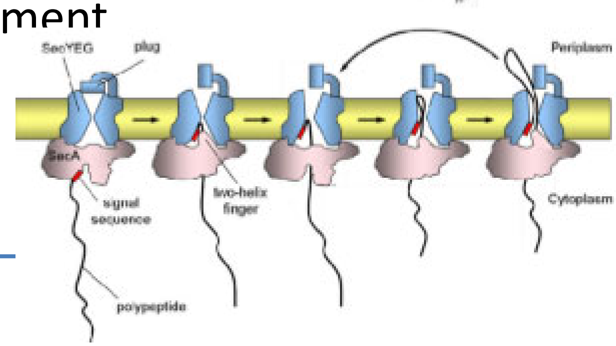


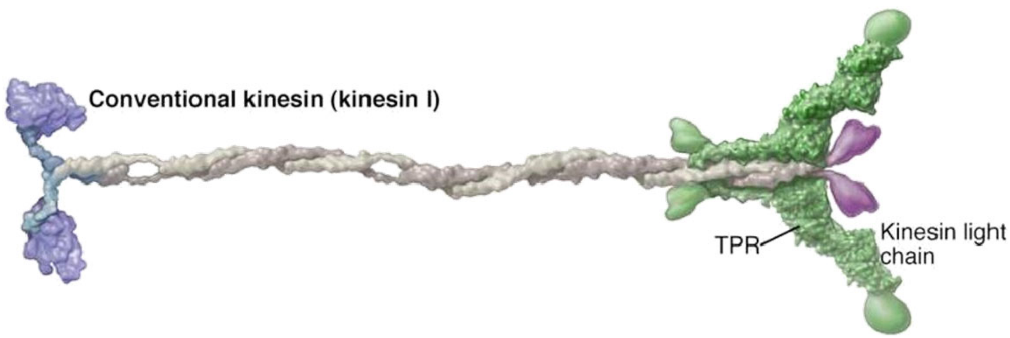
Basic « Tool Box » of molecular motors

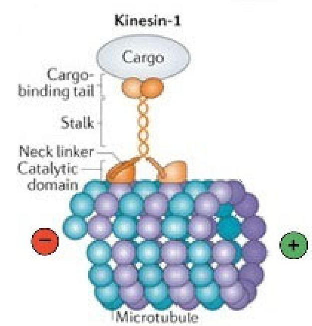
Microtubules plus-end-directed kinesins

Kinesins (family) :

- Motor at the N-terminal of the heavy chain
- Long coiled-coil stalk
- Globular tail domain
- Tetratricopeptide repeat domain (TPR) of the light chain
- allows an expanded repertoire of cargoes

→ transport of numerous cargoes (organelles, vesicles in axons, mRNA, intermediate filaments…)



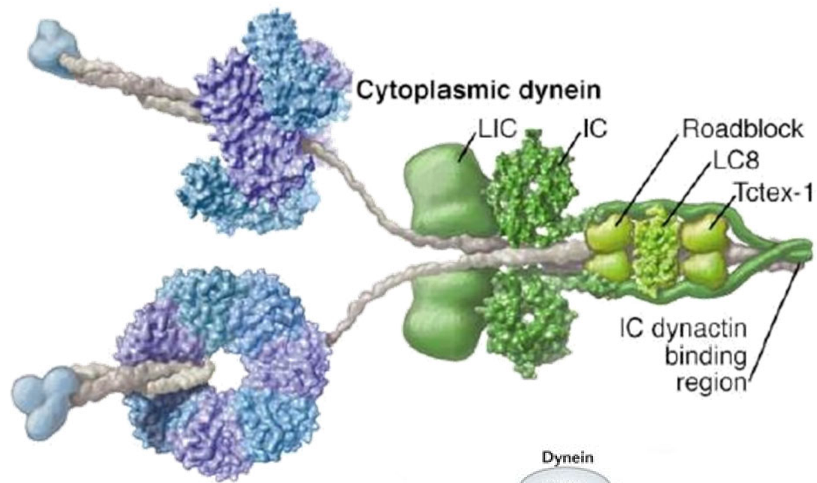


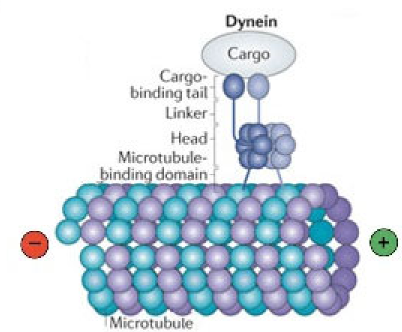
Basic « Tool Box » of molecular motors

Microtubules minus-end-directed dyneins

Cytoplasmic dynein:

- Large motor domain (7 ATPase-like domains in a ring)
- Several light chain subunits are bound to the intermediate chain
- The dynactin complex has a regulatory role in the transport



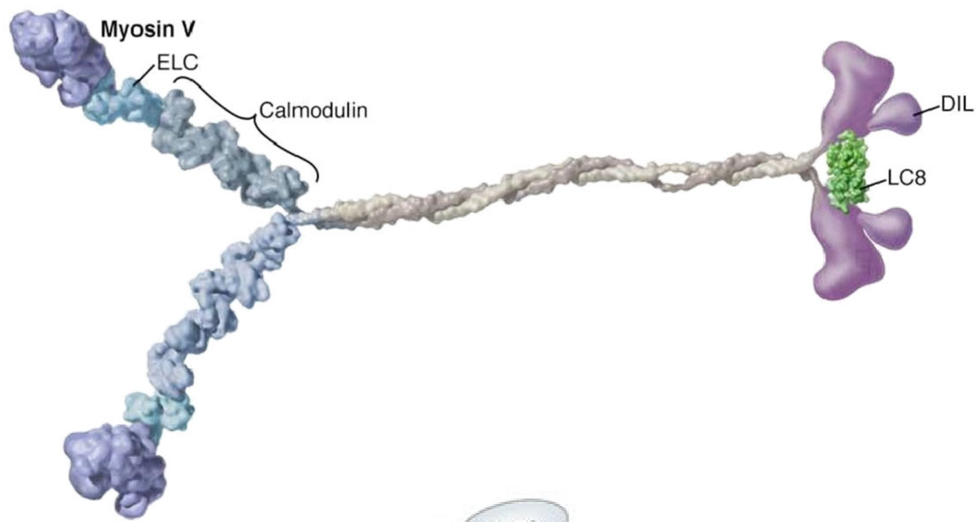


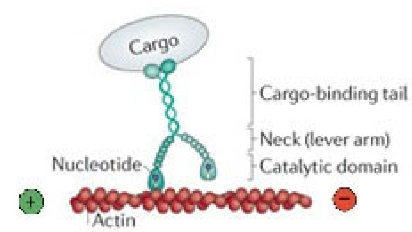
Basic « Tool Box » of molecular motors

Actin minus-end-directed myosin V

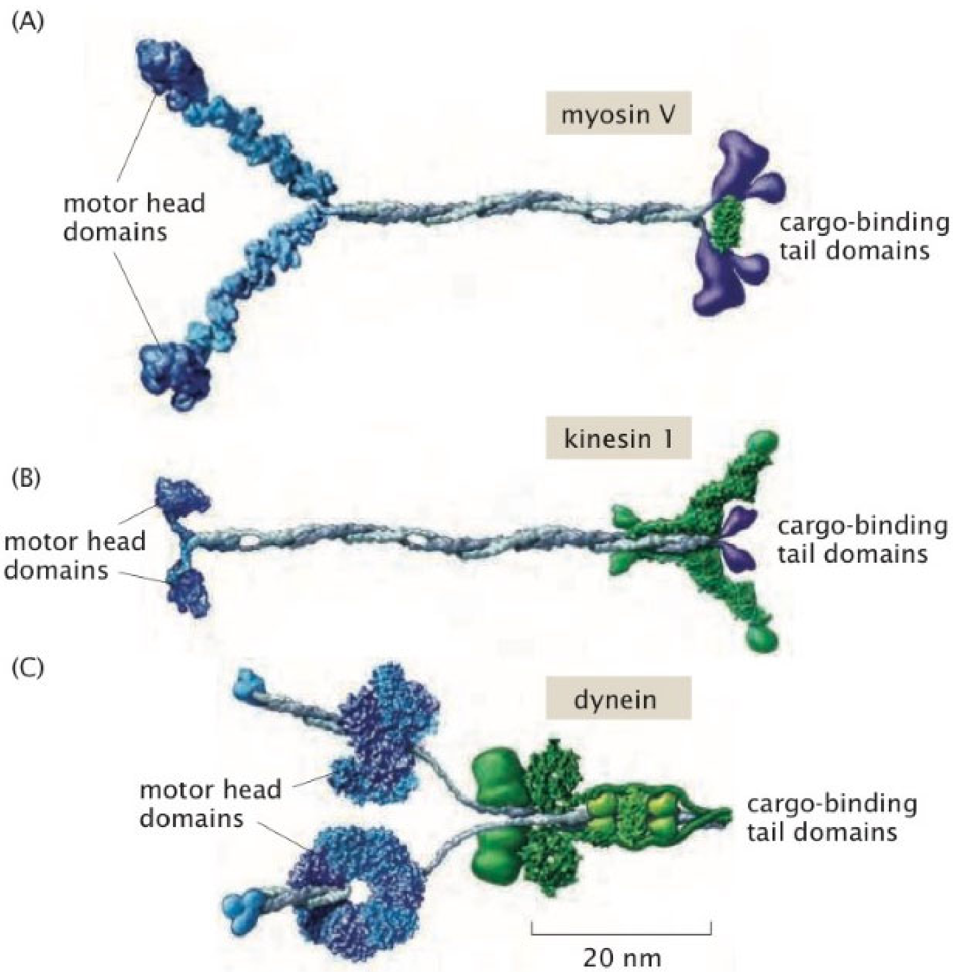
- Motor head
- Long lever arm helix (neck domain) stabilized by binding one essential light chain (ELC) and five calmodulins
- Long coiled-coil stalk
- ~ 100 a.a. C-terminal domain (DIL, dilute domain)
- Same LC8 light chain as cytoplasmic dynein

→ Organelle transport, melanosome and other diverse cargo transport activities





Translational motors – beating the diffusion speed limit



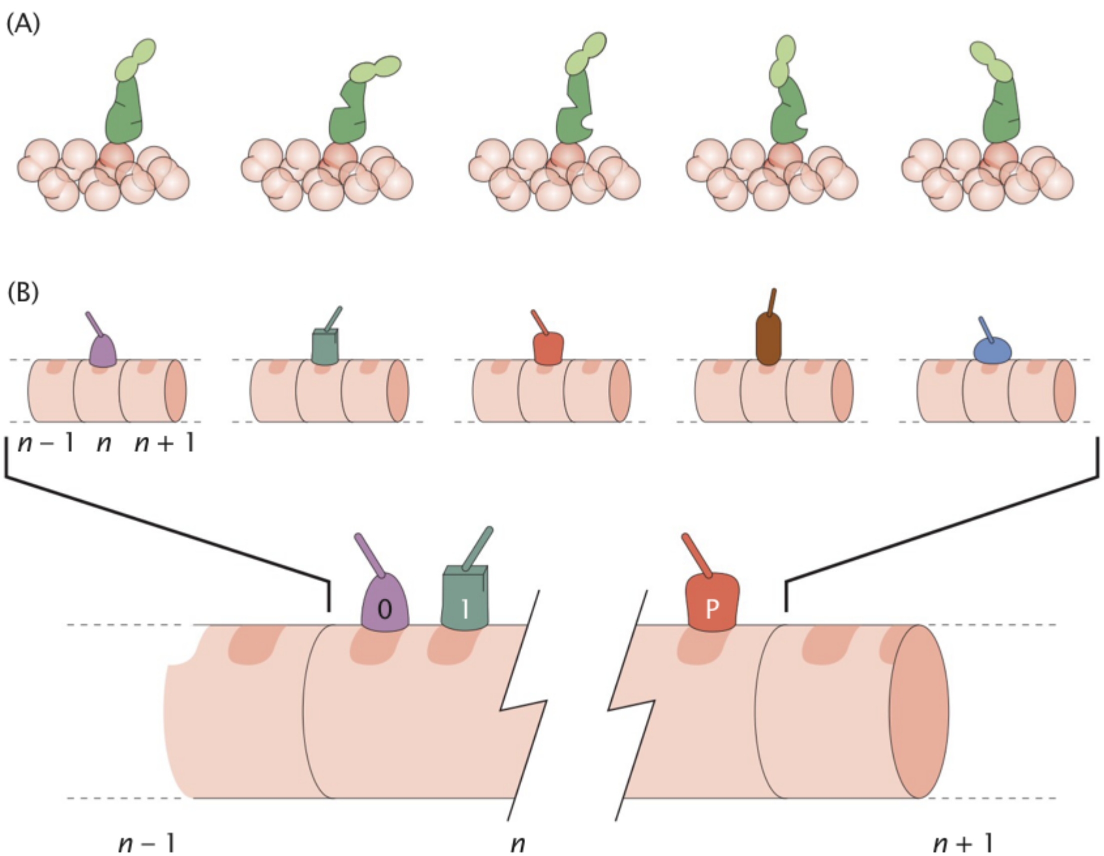
walking on cytoskeleton

kinesin takes steps of approx. 8nm per ATP hydrolysis event

energy per step:
1 ATP -> 20 kT

force generated:
20kT/8nm = 10 pN

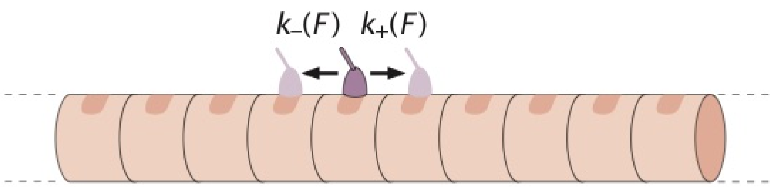
Position – State models



Myosin II head:
exists in a variety of conformational states ATP, ADP bound, no nucleotide etc.

sequence of bins n
where the motor translate along

One-state model



quasi-one-state

- motor can only be in one state in each box
- different forward and backward rates (k_+, k_-)
- force F acting on the motor:
  - probability of moving forward: $k_+(F)$
  - probability of moving backward: $k_-(F)$

Question:
why would k+ and k- be different?

- Probability of finding the motor at position n at time t is given by $p(n,t)$

Master-equation description

Linear differential equation to describe the time-evolution of a system, using a probabilistic description

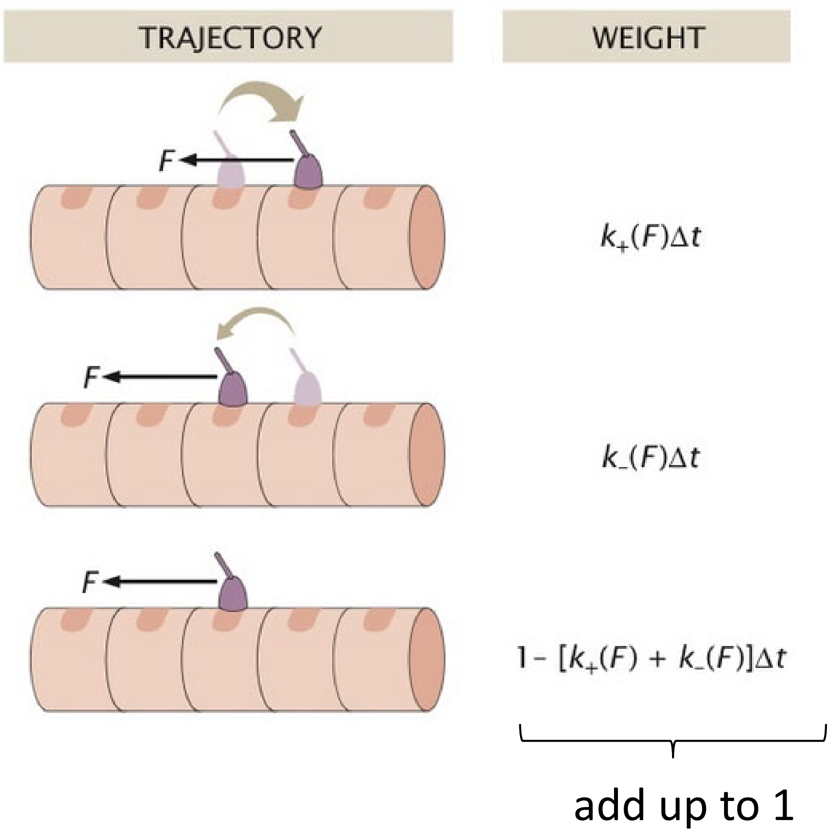
Composed of:

- a set of possible states the system can be in
- a rate-matrix governing the state transitions

or similar:

- a set of possible trajectories that the system can take
- associated statistical weights for each trajectory

Master equation approach



$$p(n,t+\Delta t) = \underbrace{k_+\Delta t\, p(n-1,t)}_{\text{from left to site}} + \underbrace{k_-\Delta t\, p(n+1,t)}_{\text{from right to site}}$$

$$+\underbrace{(1-k_-\Delta t - k_+\Delta t)\,p(n,t)}_{\text{no change}}$$

differential form:

$$\frac{p(n,t+\Delta t)-p(n,t)}{\Delta t} = k_+\left[p(n-1,t)-p(n,t)\right] + k_-\left[p(n+1,t)-p(n,t)\right]$$

Solving the equation

$$\frac{p(n,t+\Delta t)-p(n,t)}{\Delta t}=k_+\left[p(n-1,t)-p(n,t)\right]+k_-\left[p(n+1,t)-p(n,t)\right]$$

we need a **continuous function** for *p(n,t), p(n-1,t)* and *p(n+1,t)*

motor with step size a

change of variables: $x=n\cdot a$ and bin size $x\pm a$:

then: $$p(x\pm a,t)\approx p(x,t)\pm\frac{\partial p}{\partial x}a+\frac{1}{2}\frac{\partial^2 p}{\partial x^2}a^2$$

Taylor expansion, assuming that we can approximate p in the vicinity of $x\pm a$ by a quadratic expansion

insert into differential equation from above…

$$\frac{\partial p}{\partial t}=-(\mathrm{k}_+-k_-)\frac{\partial p}{\partial x}a+\frac{1}{2}(\mathrm{k}_++k_-)\frac{\partial^2 p}{\partial x^2}a^2$$

with
$$\frac{p(n,t+\Delta t)-p(n,t)}{\Delta t}=\frac{\partial p(x,t)}{\partial t}$$

$$\frac{\partial p}{\partial t}=\boxed{-V\frac{\partial p}{\partial x}}+D\frac{\partial^2 p}{\partial x^2}^{2}$$

<- Diffusion equation

(in the presence of force)

$$V=a\left[\mathrm{k}_+(F)-k_-(\mathrm{F})\right]$$ (Velocity)

$$D=\frac{a^2}{2}\left[\mathrm{k}_+(F)+k_-(\mathrm{F})\right]$$ (Diff. coeff.)

Biased diffusion equation

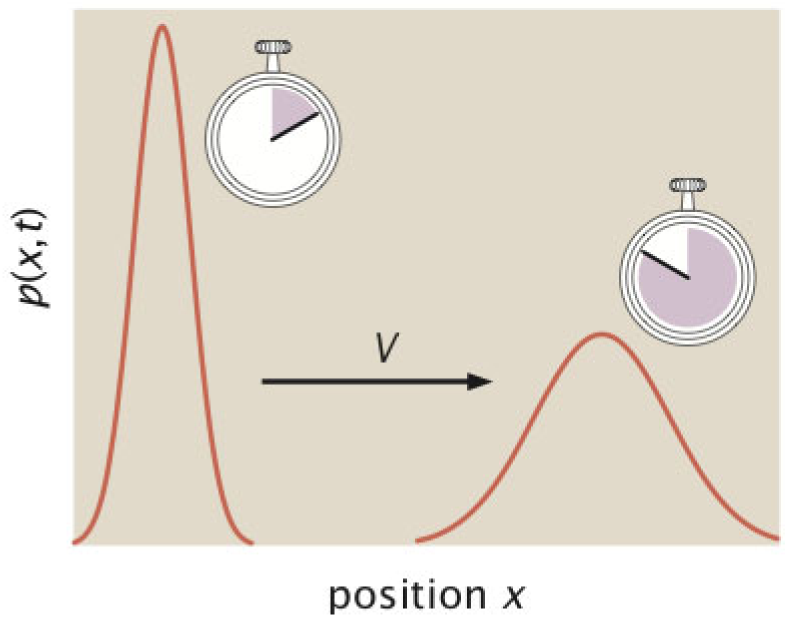
$$\frac{\partial p}{\partial t} = \boxed{-V\frac{\partial p}{\partial x}} + D\frac{\partial^2 p}{\partial x^2}$$ biased / driven diffusion equation

solution: using changes of variables $\bar{t} = t$, $\bar{x} = x - Vt$ resulting in $\frac{\partial p}{\partial \bar{t}} = D\frac{\partial^2 p}{\partial \bar{x}^2}$

$$p(\bar{x},\bar{t}) = \frac{1}{\sqrt{4\pi Dt}} e^{-\bar{x}^2/4D\bar{t}}$$

change back

$$p(x,t) = \frac{1}{\sqrt{4\pi Dt}} e^{-(x-Vt)^2/4Dt}$$



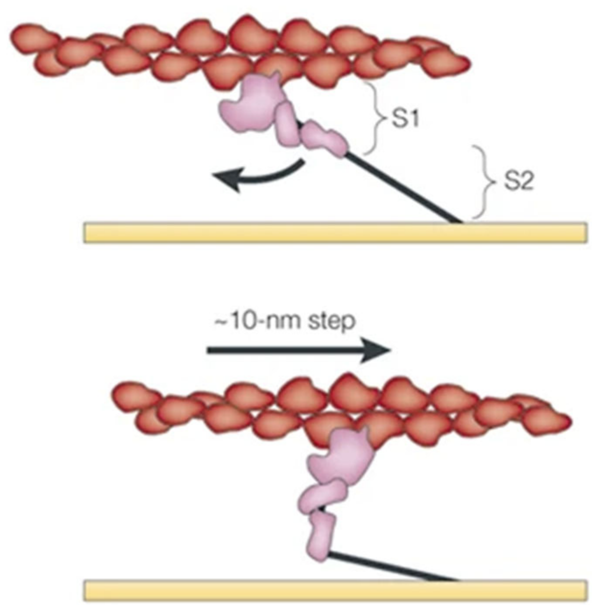
Biased diffusion equation

biased / driven
diffusion equation

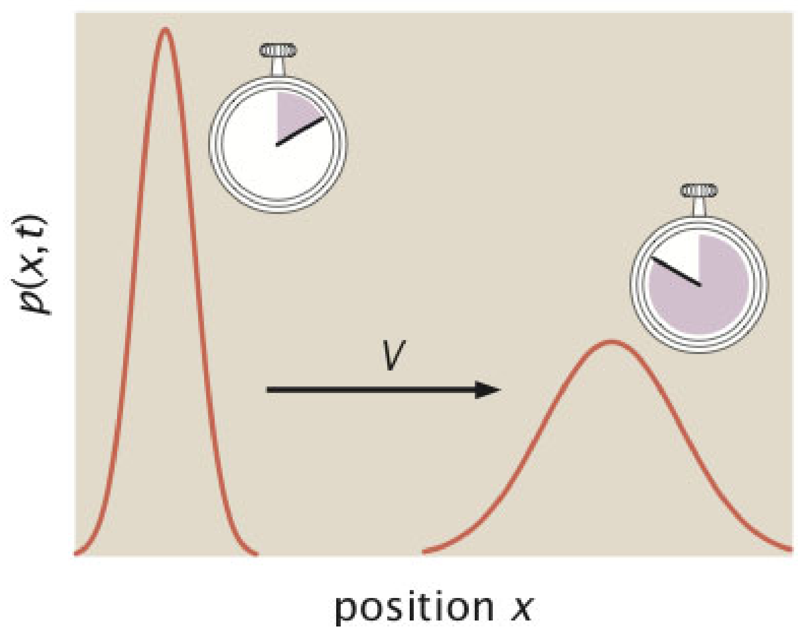
$$\frac{\partial p}{\partial t} = -V\frac{\partial p}{\partial x} + D\frac{\partial^2 p}{\partial x^2}$$

solution:

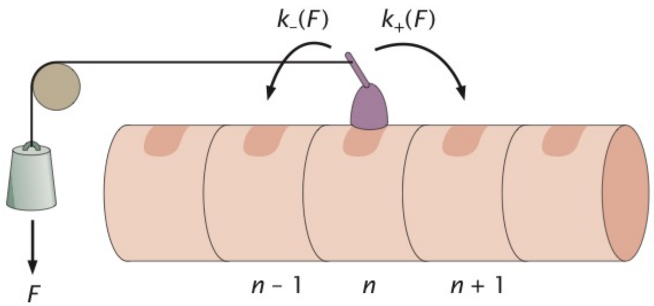
$$p(x,t) = \frac{1}{\sqrt{4\pi D t}}\, e^{-(x-Vt)^2/4Dt}$$



Nature Reviews | Molecular Cell Biology



Effect of force on transport



What are the actual values of k_+ and k_- ?

- they are different dependent on direction
- they are dependent on the applied force

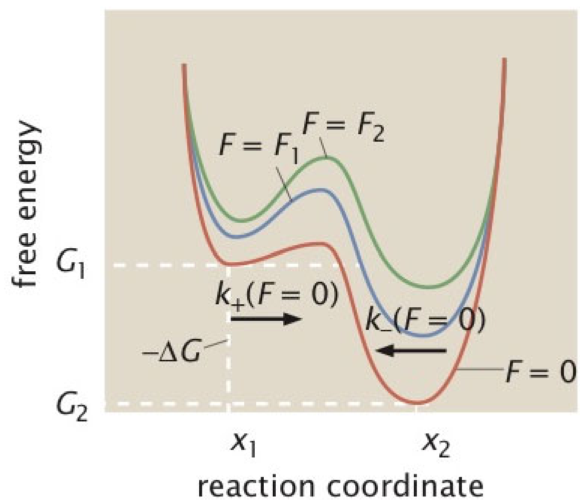
Moreover, they are determined by a free energy landscape

$$k_+ p_n = k_- p_{n+1}$$

$$\frac{k_+}{k_-} = e^{-\beta \Delta G} \quad \text{with} \quad \Delta G = G_{n+1} - G_n$$

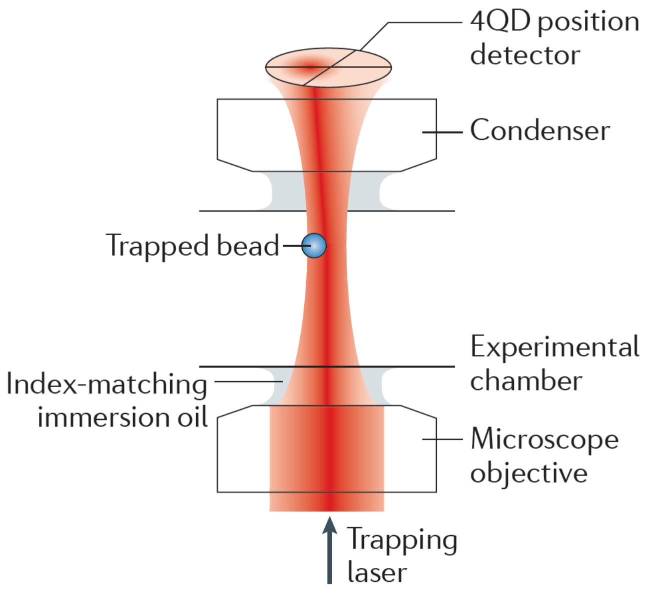
with applied force Fna

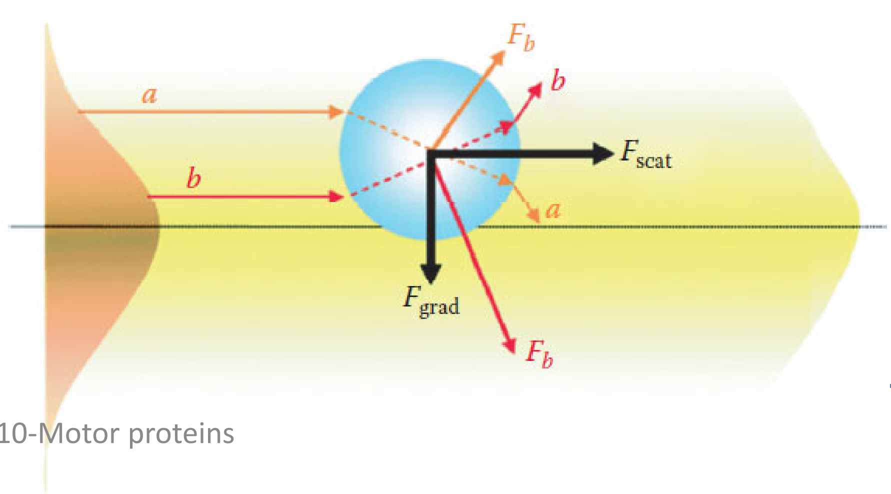
$$\frac{k_+(F)}{k_-(F)} = e^{-\beta \Delta G + Fa}$$



Question:
what are the consequences of tilting the energy surface?

Using Light to Trap Objects





Particle with diameter d in laser beam with gaussian profile

Gradient force
momentum transfer upon bead due to light refraction

Scattering force
Light scattering induces force along light propagation direction

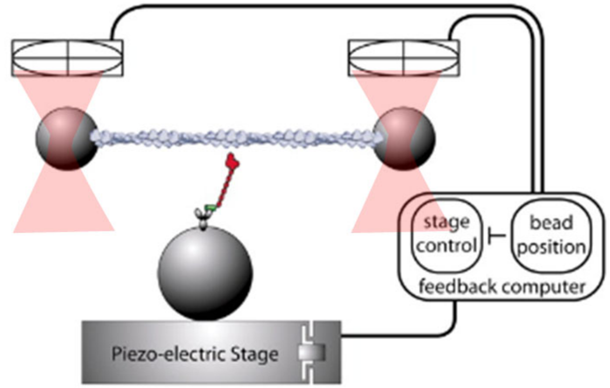
Equilibrium trapping position: just behind focal point of the sytem

Nanobiotechnology Handbook, Y. Xie, CRC press

Step frequency as a function of force

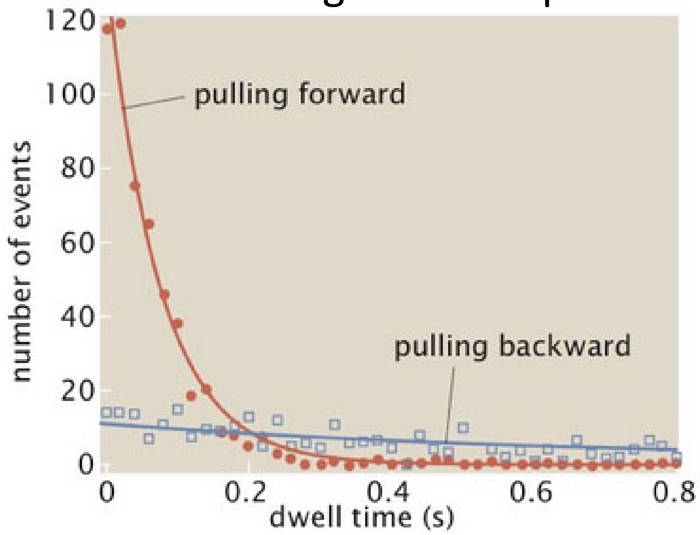
$$\frac{k_+(F)}{k_-(F)} = e^{-\beta\Delta G + Fa}$$

can be measured with force spectroscopy (e.g. optical tweezers)



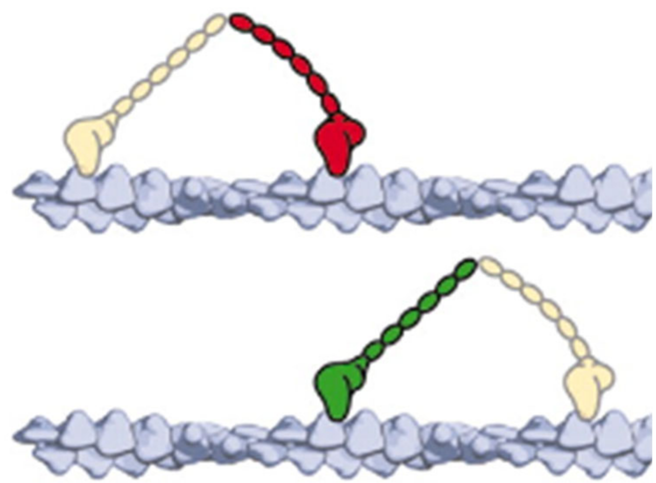
Purcell et al. Nature 2005

histogram of steps



Purcell et al. PNAS 2005

Effect of force on stepping behavior of myosin V



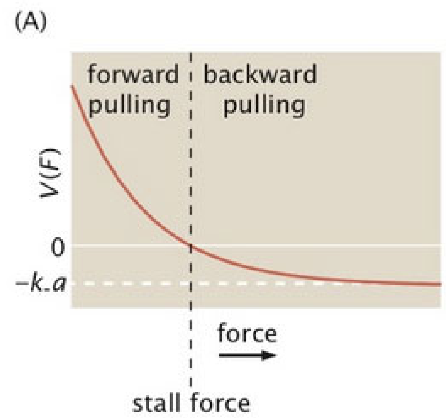
Velocity as a function of force

assuming all the dependence is on k_+

$$k_+(F) = k\_e^{-\beta(\Delta G + Fa)}$$

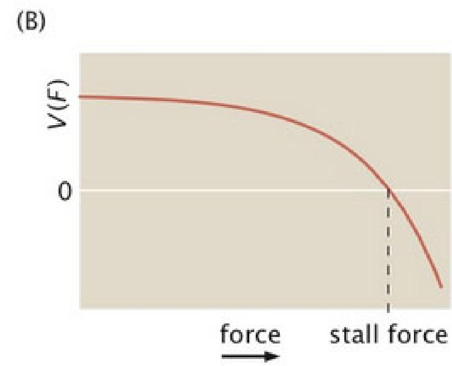
then using $V = a[\mathrm{k}_+(F) - k\_(\mathrm{F})]$

the motor velocity becomes $V(F) = ak\_\left(e^{-\beta(\Delta G+Fa)} - 1\right)$

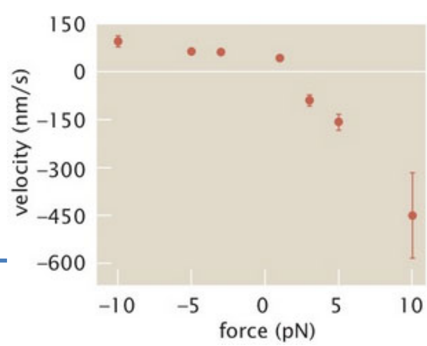


all the dependence is on $k\_$ $\quad k\_(F) = k_+ e^{\beta(\Delta G+Fa)}$

the motor velocity becomes $V(F) = ak_+\left(1 - e^{\beta(\Delta G+Fa)}\right)$

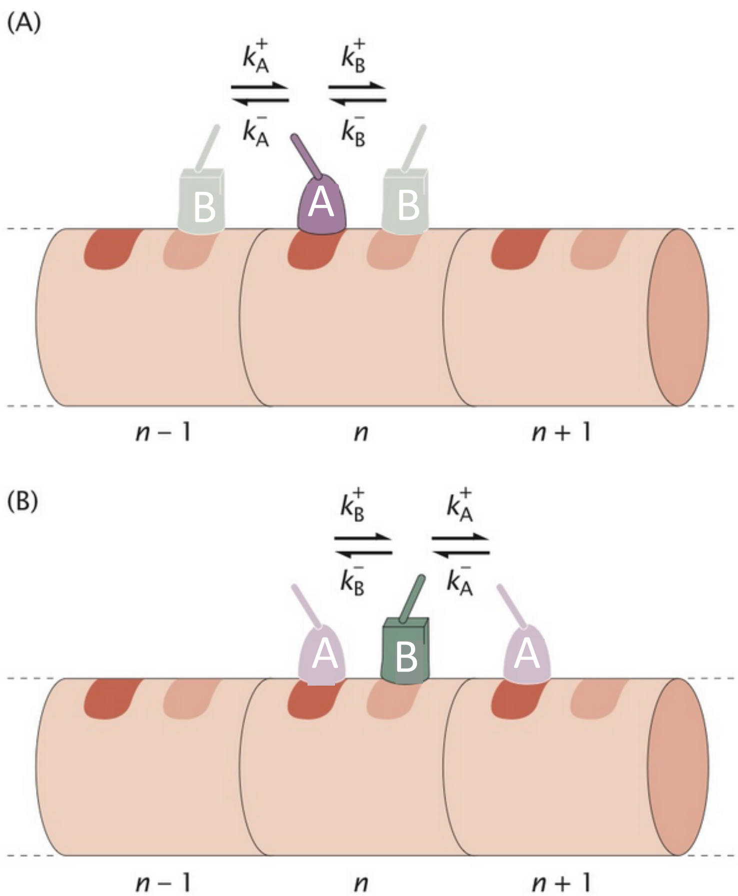


myosin V



Question:
Could you come up with an explanation for this behavior?

Theoretical description: 2-State Motor



Rate equation, $p_i(n,t)$

i: state, either A or B
→ index 0, 1
always switches

n: position on track

time evolution of p_0

$$\frac{\mathrm{d}\,p_0(n,t)}{\mathrm{d}\,t} = k_A^+ p_1(n-1,t) + k_B^- p_1(n,t) - \underbrace{p_0(n,t)\left[k_A^- + k_B^+\right]}_{\text{no motion}}$$

time evolution of p_1

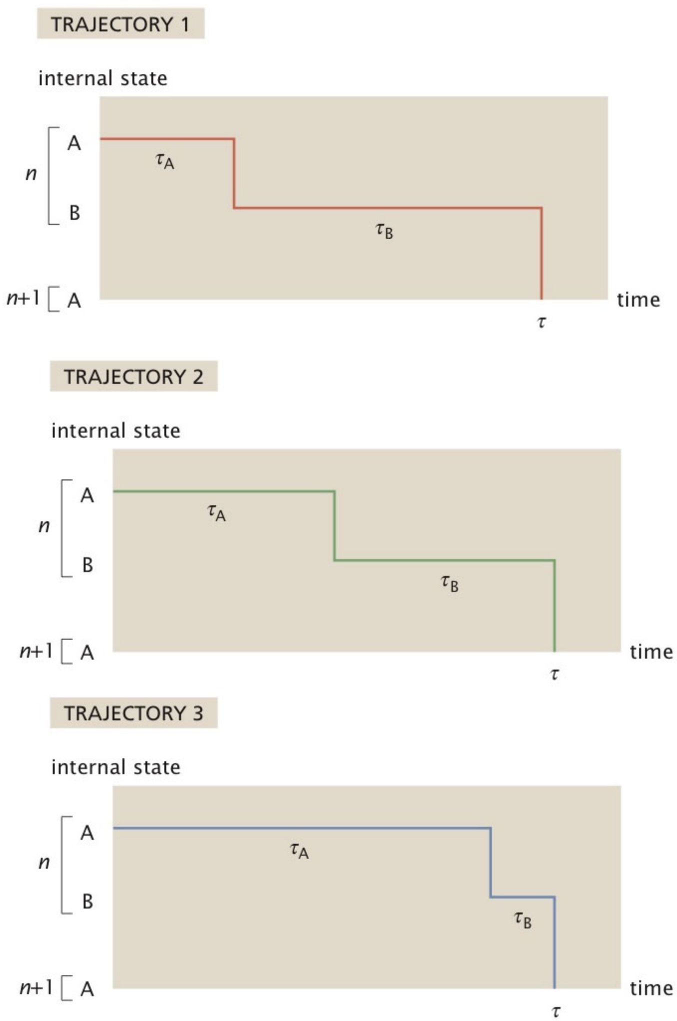
$$\frac{\mathrm{d}\,p_1(n,t)}{\mathrm{d}\,t} = k_A^- p_0(n+1,t) + k_B^+ p_0(n,t) - p_1(n,t)\left[k_A^+ + k_B^-\right]$$

Ignore motion

$$\frac{\mathrm{d}\,P_0}{\mathrm{d}\,t} = k_A^+ P_1 + k_B^- P_1 - P_0\left[k_A^- + k_B^+\right]$$

3-state model
step only between B and A

Internal states reveal themselves in the form of waiting time distributions



distribution of waiting times t
for an average waiting time τ

$A \xrightarrow{k} B$ p(t): probability for a step to occur

$$\tau = \int_0^\infty t\, p(t)\, dt \longrightarrow p(t) = \frac{1}{\tau} e^{-t/\tau} \qquad \tau = 1/k$$

if there are several steps involved

$A \xrightarrow{k_A} B \xrightarrow{k_B} A'$

$$p(t) = \int_0^t p_A(\tau) p_B(t-\tau)\, d\tau$$

with

$$p_A(t) = \tau_A^{-1} e^{-t/\tau_A} \qquad \tau_A = 1/k_A$$

$$p_B(t) = \tau_B^{-1} e^{-t/\tau_B}$$

Internal states reveal themselves in the form of waiting time distributions

plug into probability distribution

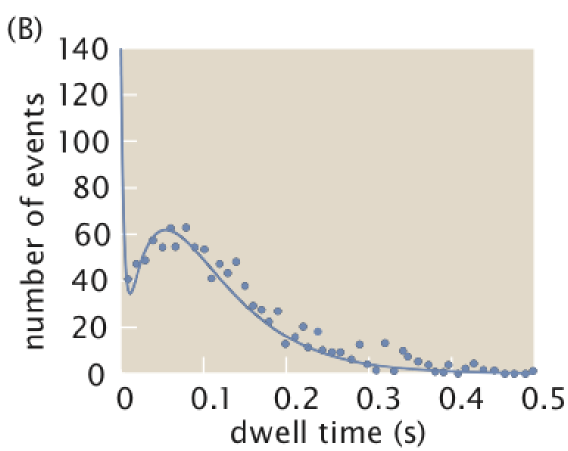
$$p(t) = \int_0^t e^{-\tau/\tau_A} e^{-(t-\tau)/\tau_B} d\tau \frac{1}{\tau_A \tau_B} \longrightarrow$$

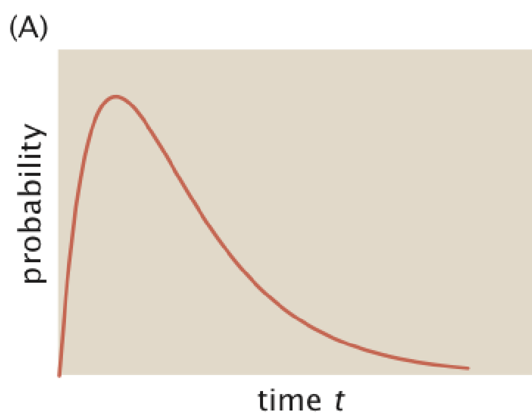
dual exponential distribution

$$p(t) = \frac{1}{\tau_B - \tau_A}\left(e^{-t/\tau_B} - e^{-t/\tau_A}\right)$$

observed: a maximum in the waiting (dwell) times

motors have many internal states, which might not be visible, but kinetically manifest themselves





Question:
How could this be directly observed?

Purcell et al. PNAS 2005

Conclusions

- Motors perform a directed diffusion motion
- single-molecule imaging allows to identify the mechanisms of directional motion
- single-exponential waiting time distributions reveal a one-step process
- dual-exponential waiting time distributions → identify a hidden step!