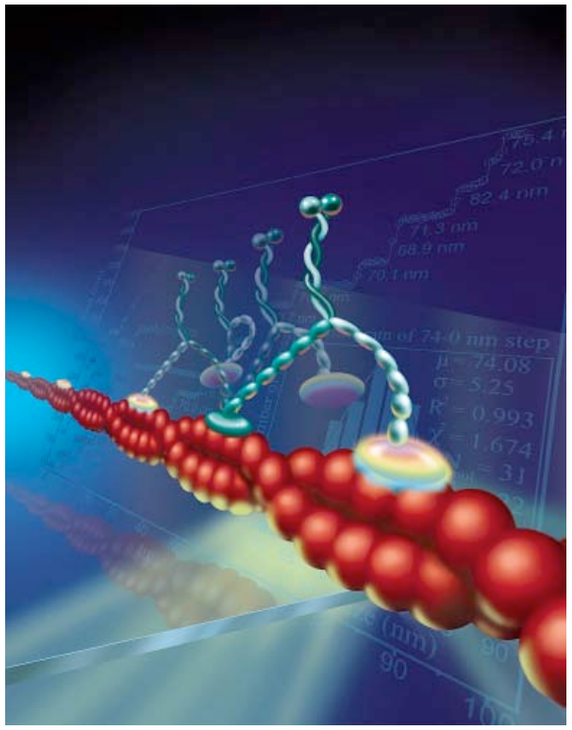
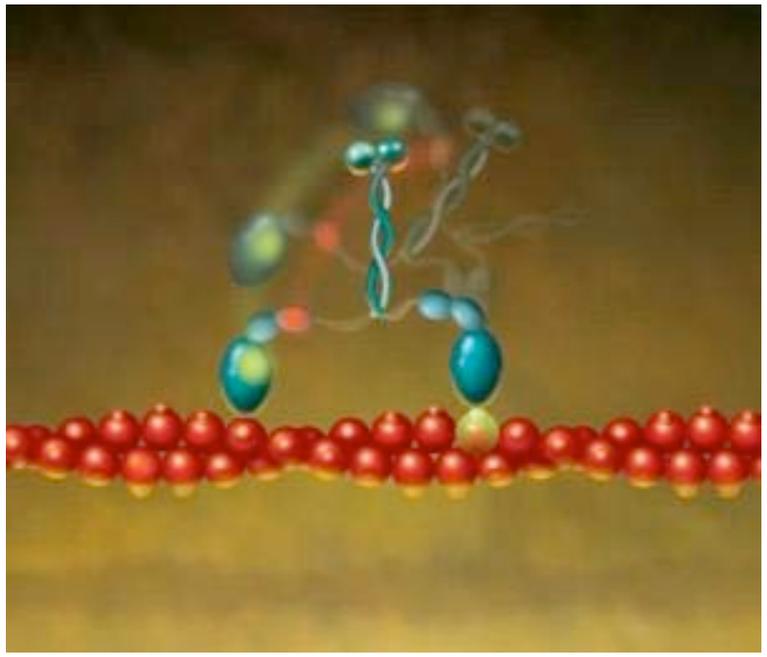
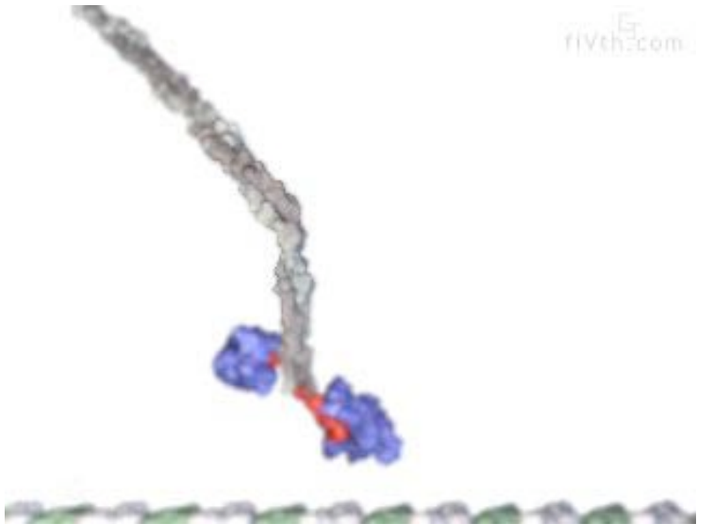
How Do Molecules Walk?







Sean Sun and Ganhui Lan

ME, ChemBE and Whitaker BME Institute
Johns Hopkins University

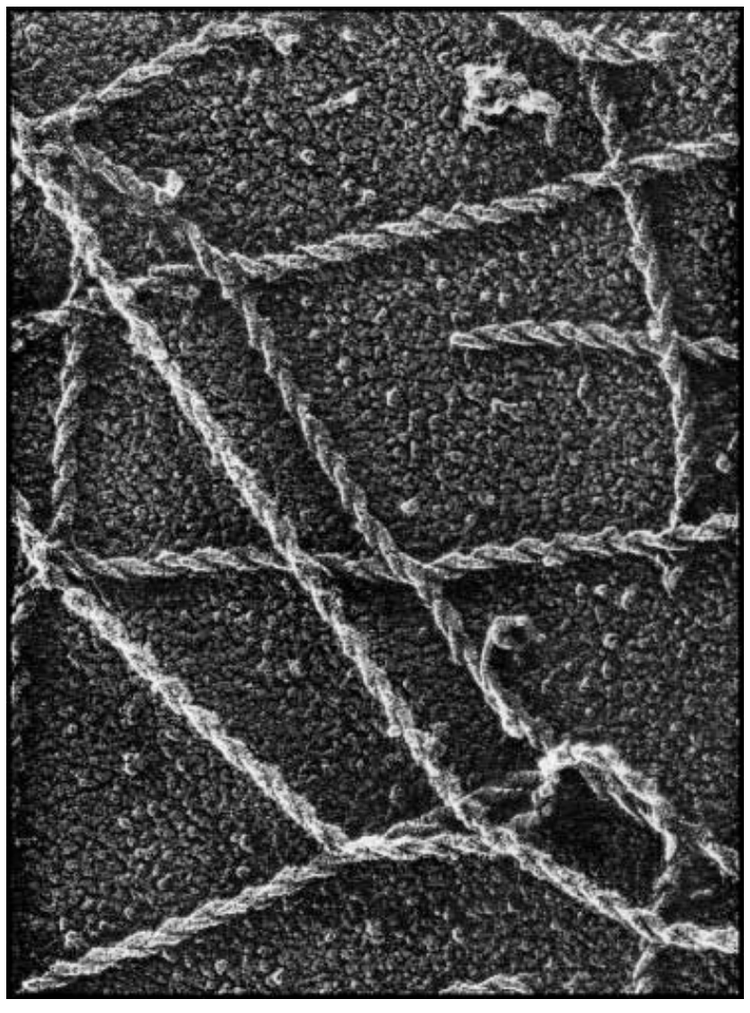
Lan and Sun, Biophys. J. 88, 999 (2005)
Lan and Sun, Biophys. J. submitted.

Why Walking is Needed: diffusion is too slow

$$d \sim \sqrt{Dt}$$

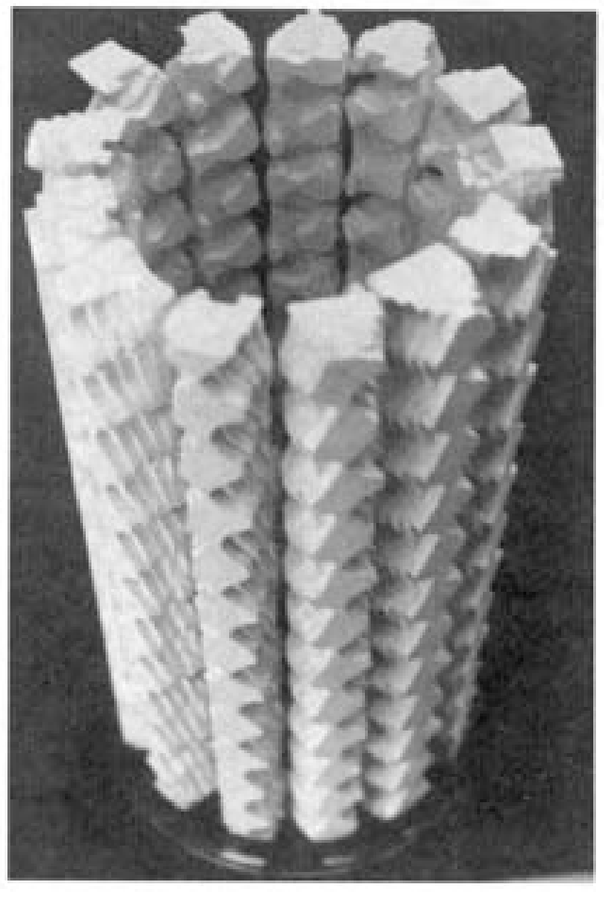
Square root is not really a function

Using cytoskeleton tracks (F-actin and microtubules).
Filaments are polar, therefore directionality can be established.



2 helical strands repeats 36nm

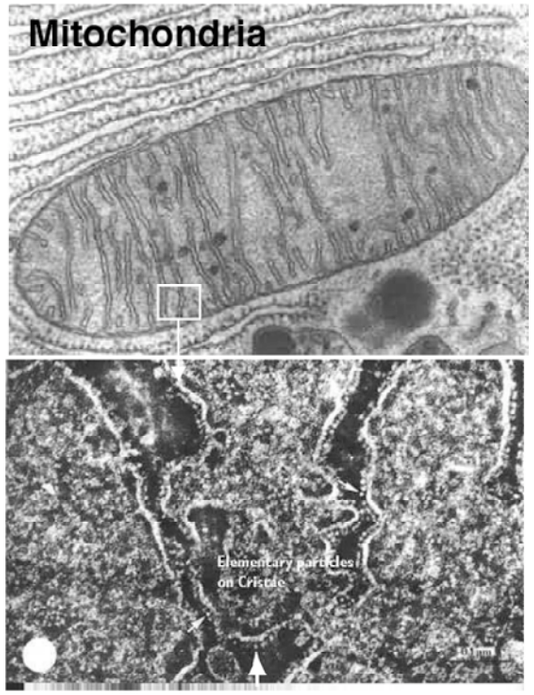
Each unit ~5nm

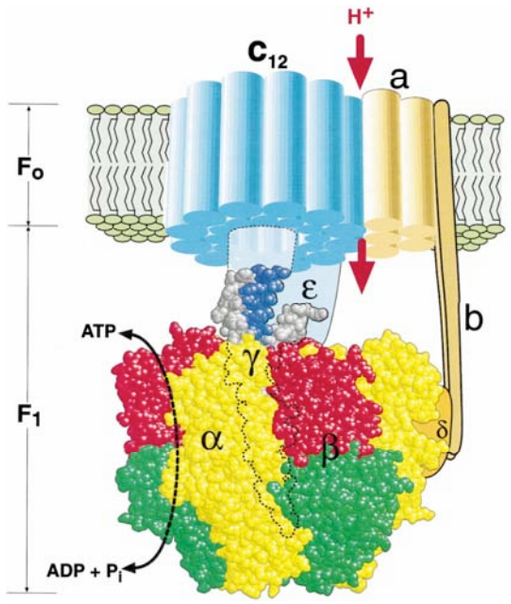


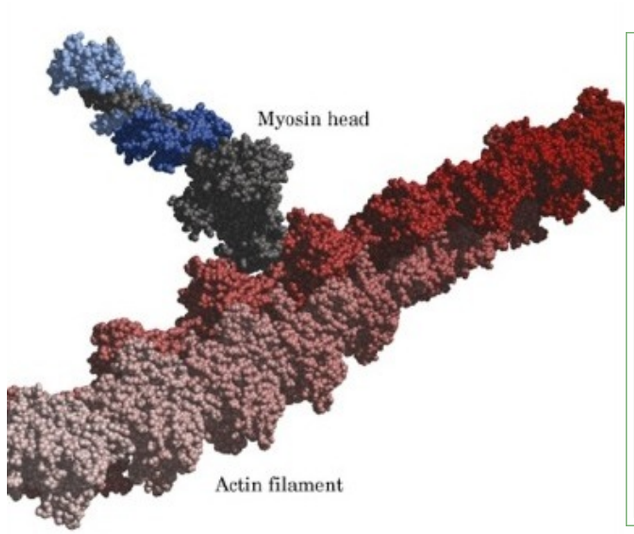
13 strands repeats 8nm

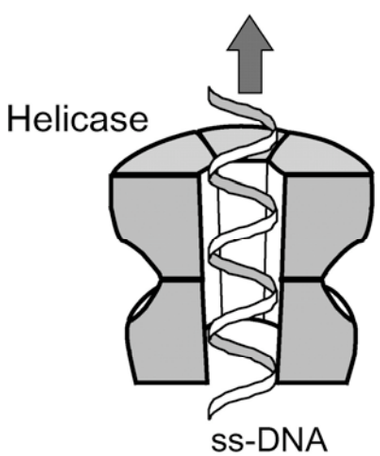
Each unit ~8nm

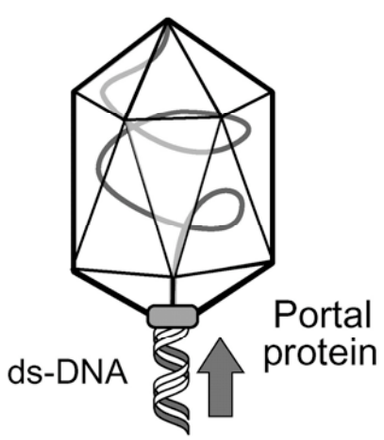
Molecular Motors











Molecular Motors are true nanomachines (<10nm).

Use single molecule chemical energy, or transmembrane ion gradients.

Typical force 10-50pN.

Operate in a viscous environment (Re~0).

Can borrow thermal energy from the surroundings (ratcheting mechanism).

Conformational change is dramatic, on the order of nanometers.

Mutations usually do not destroy motor function.

Motor Dynamics: Kinetics vs. Energy Landscape

Kinetic picture only describes the basins and transitions between them. Tight coupling between chemistry and conformation.

$$\text{ATP+MM} \longrightarrow \text{ADP+Pi+MM}$$

+Work

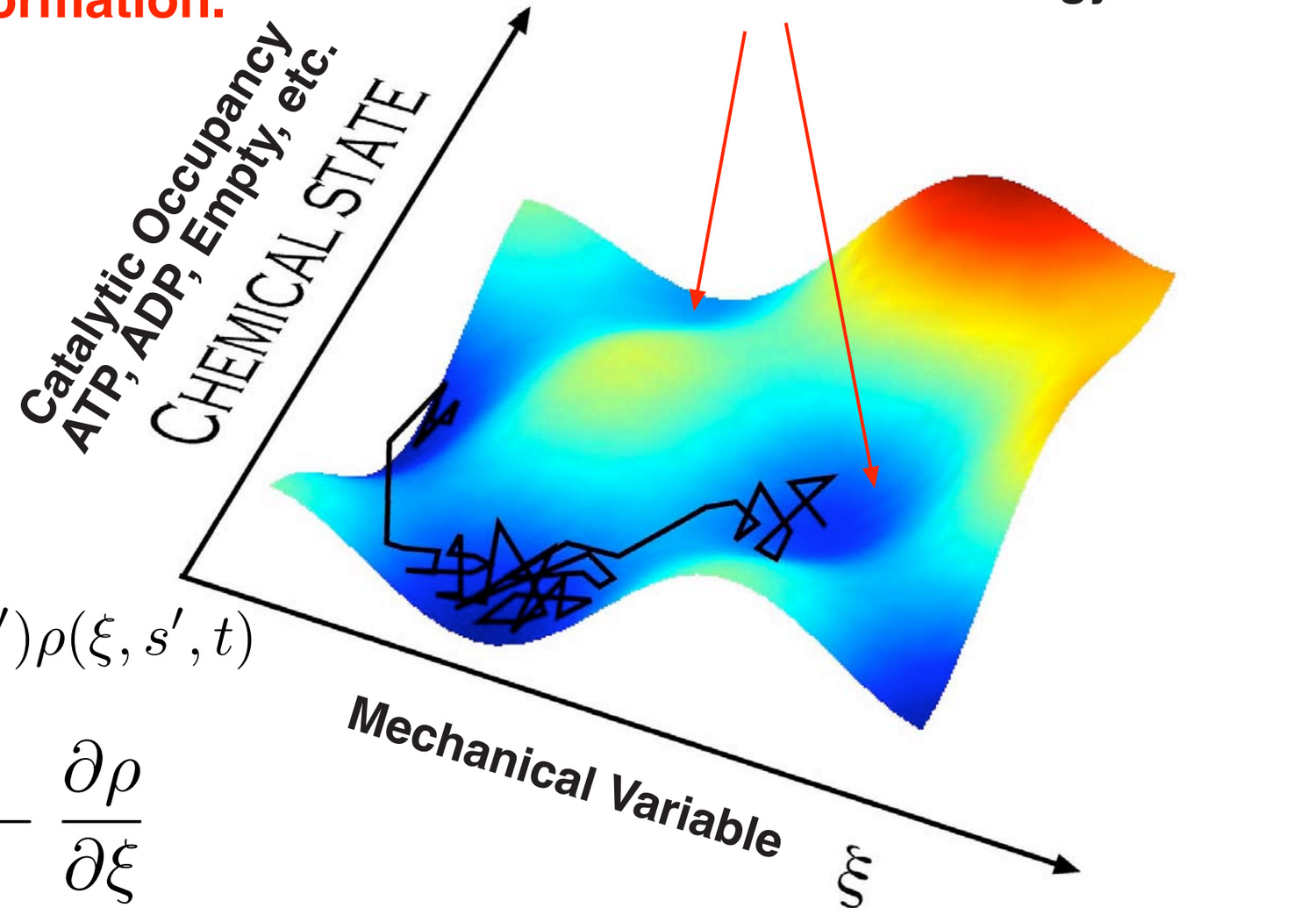
Energy landscape describes the response of the system to external forces. More relevant for single molecule experiments. No tight coupling. Fluctuations in conformation.

Slightly more difficult to compute.

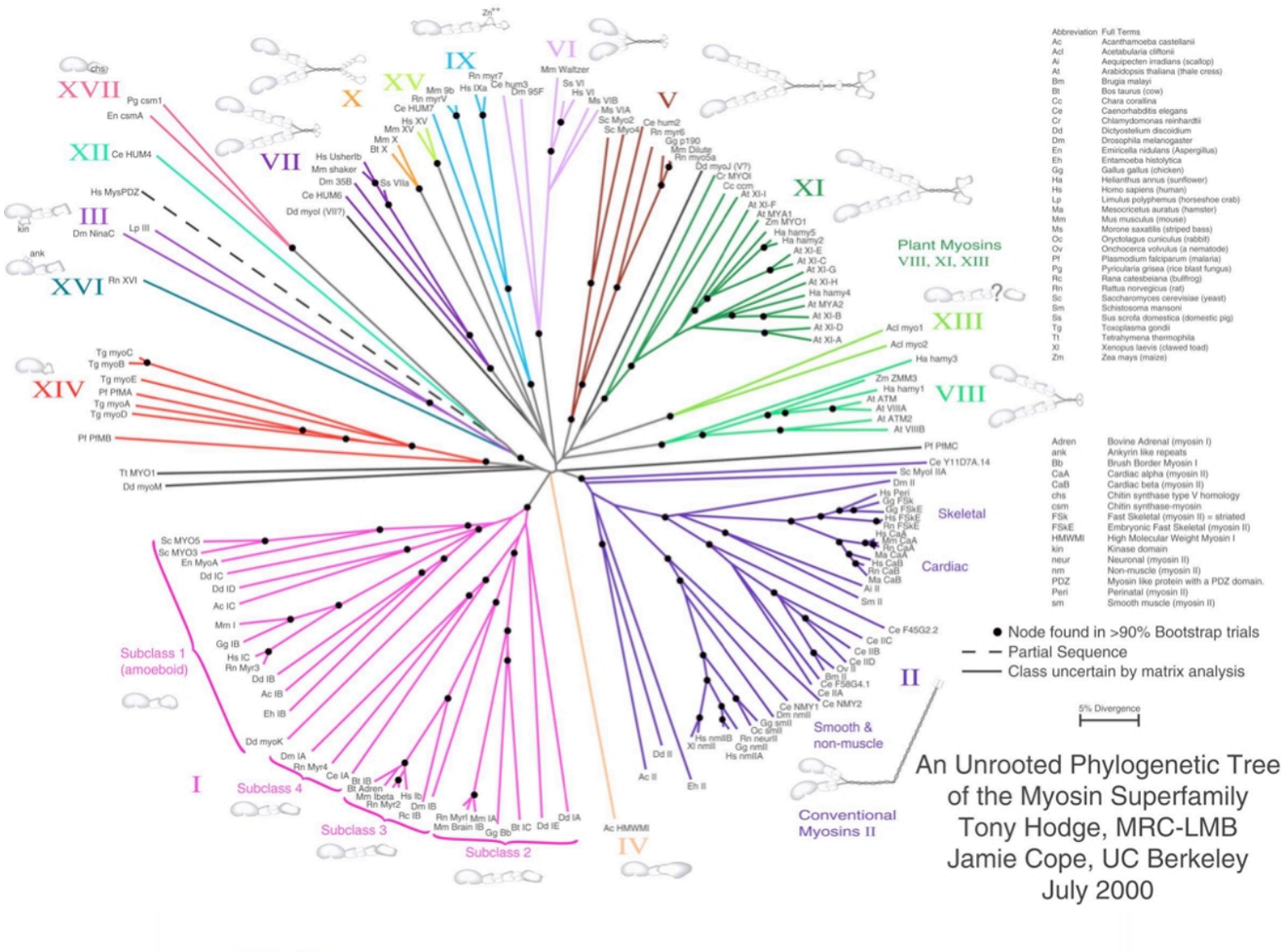
Requires a Fokker-Planck Eq. description.

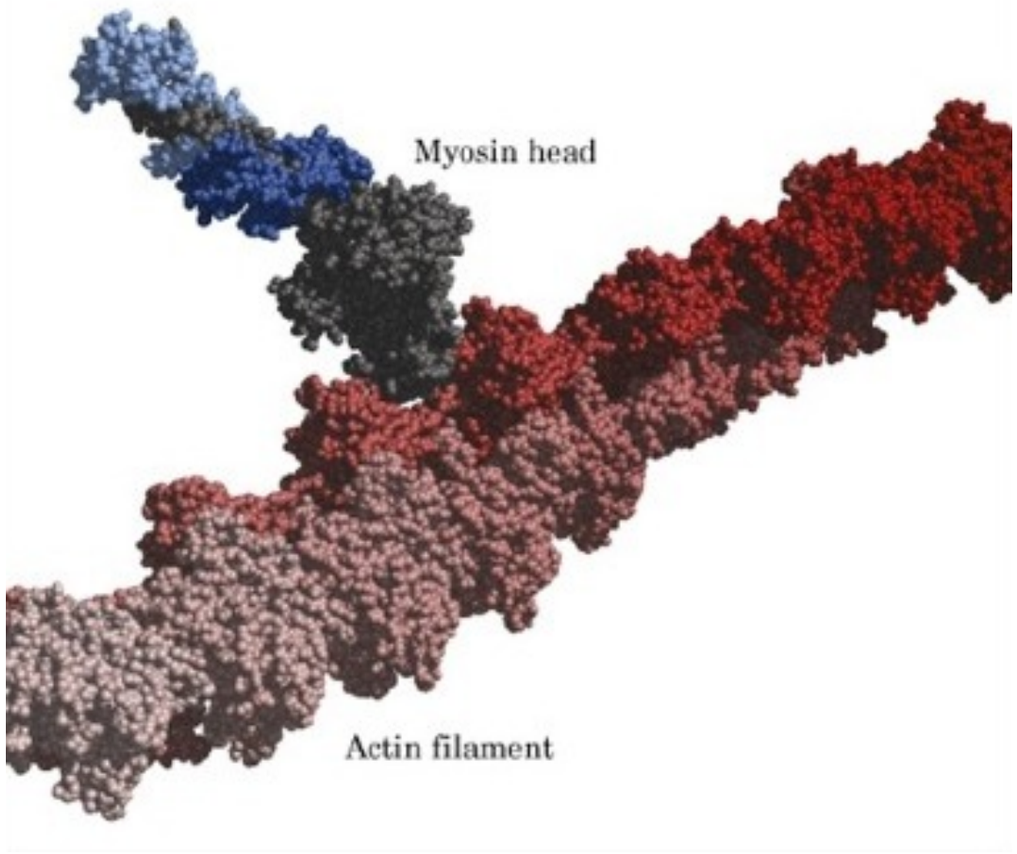
$$\frac{\partial \rho(\xi, s, t)}{\partial t} = -D\nabla \cdot \mathbf{J} + \sum_{s'} k(s, s')\rho(\xi, s', t)$$

$$\mathbf{J} = -\frac{\partial E(\xi, s)}{\partial \xi}\rho(\xi, s, t) - \frac{\partial \rho}{\partial \xi}$$



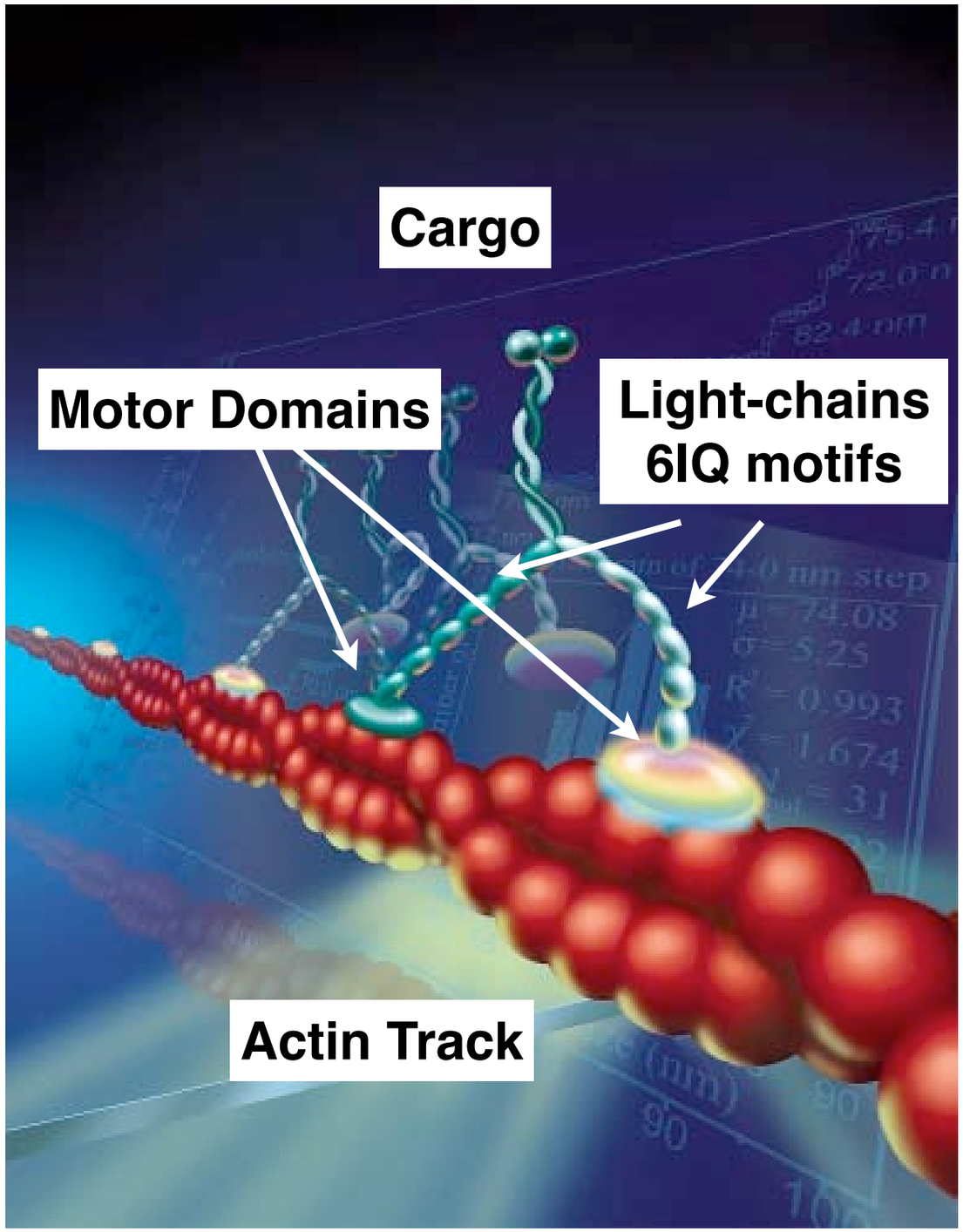
Myosin Motors





- **Muscle Contraction.**
  - Sliding Filament/ Swinging Cross-Bridge Mechanism.
- **Contractile Systems.**
  - Cytokinesis.
  - Membrane Ruffling.
- **Actin Based Transport.**
  - Vesicle Targeting and Cell Polarity.
  - Sensory Hair Cell Stereocillia Anchoring and Transport.
  - RNA Transport.

Myosin-V is a Processive Motor: Walks on F-actin

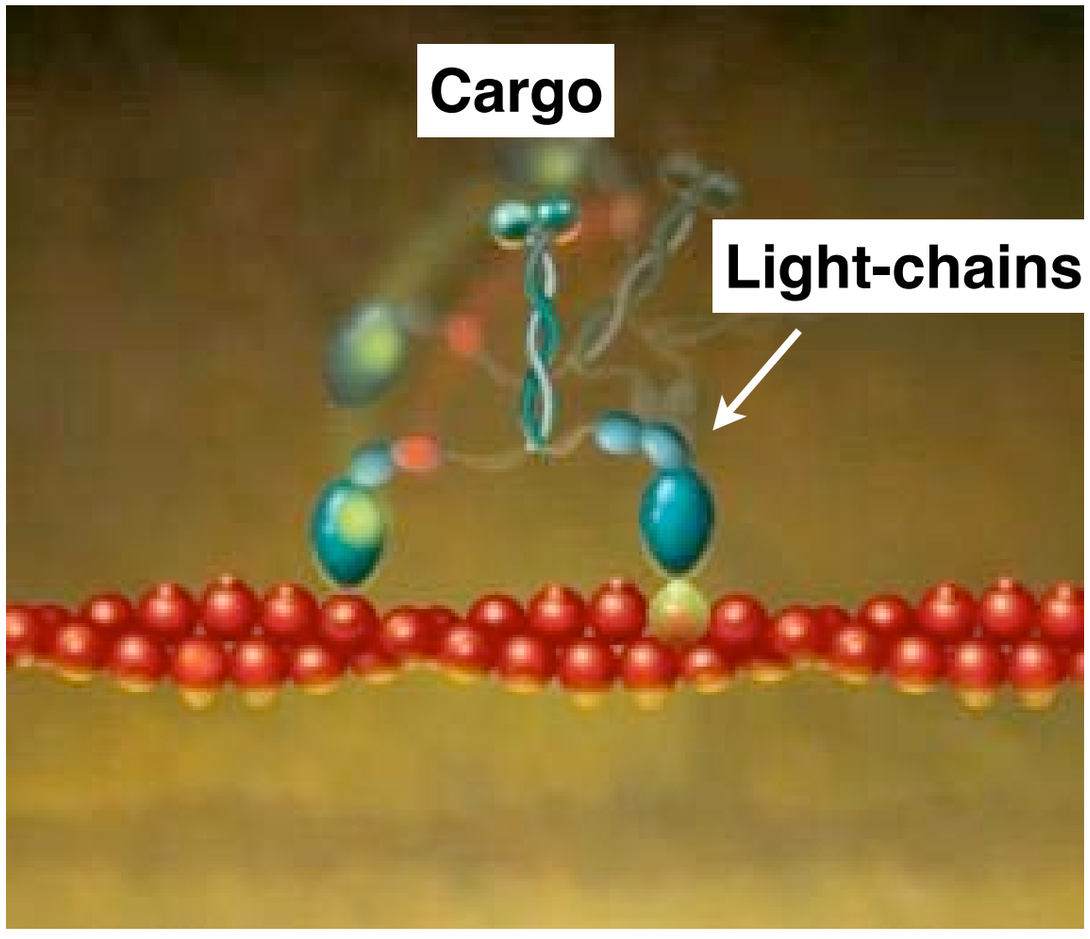


- Myosin V is involved in vesicle and organelle transport.
- Myosin V is a processive motor that walks toward the plus-end of F-actin.
- Myosin V has 2 myosin motor domains.
- Myosin V takes discrete steps (~36nm).
- Uses one ATP molecule per step.
- There are substeps.
- The step-size is independent of the load force!

Mehta et al. Nature, (1999).
Reif et al. PNAS, (2000).
Veigel et al. Nat. Cell. Biol., (2001).
Tanaka et al. Nature, (2002).
Yildiz et al. Science, (2003).

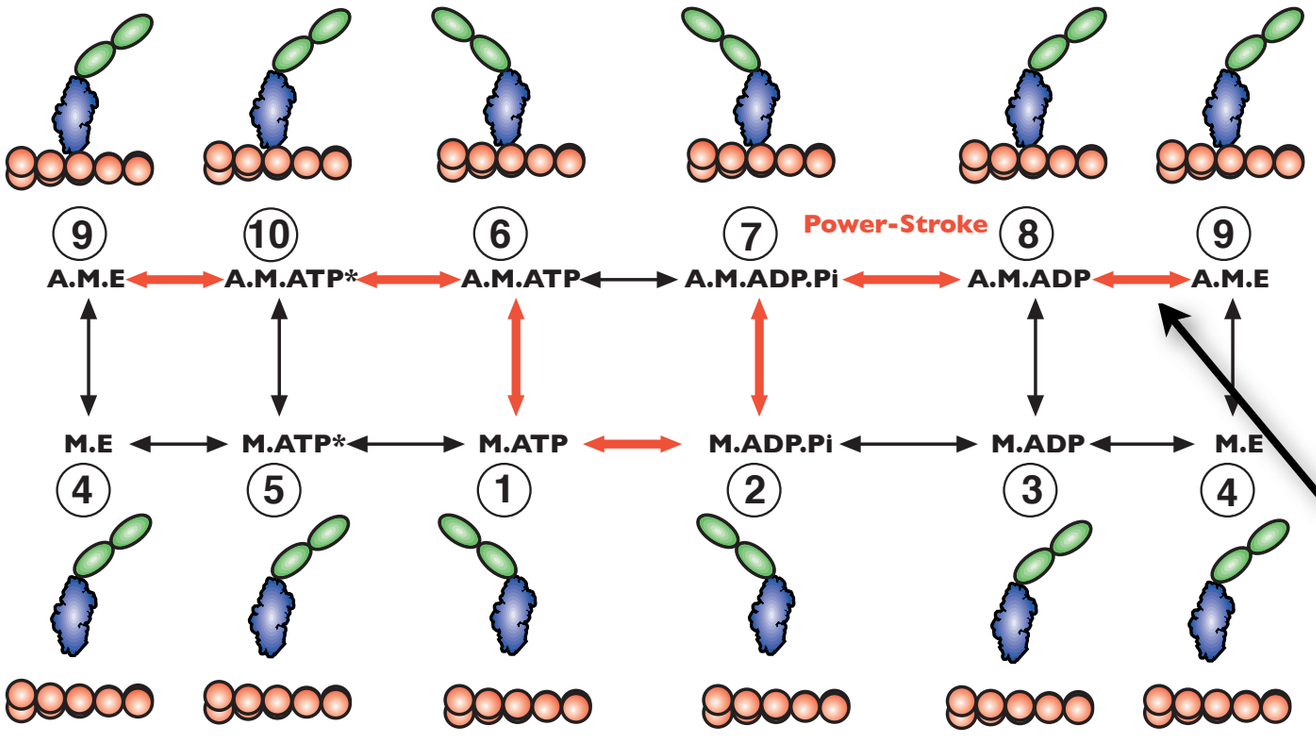
t-chains

Myosin-VI is also processive, but towards the minus end

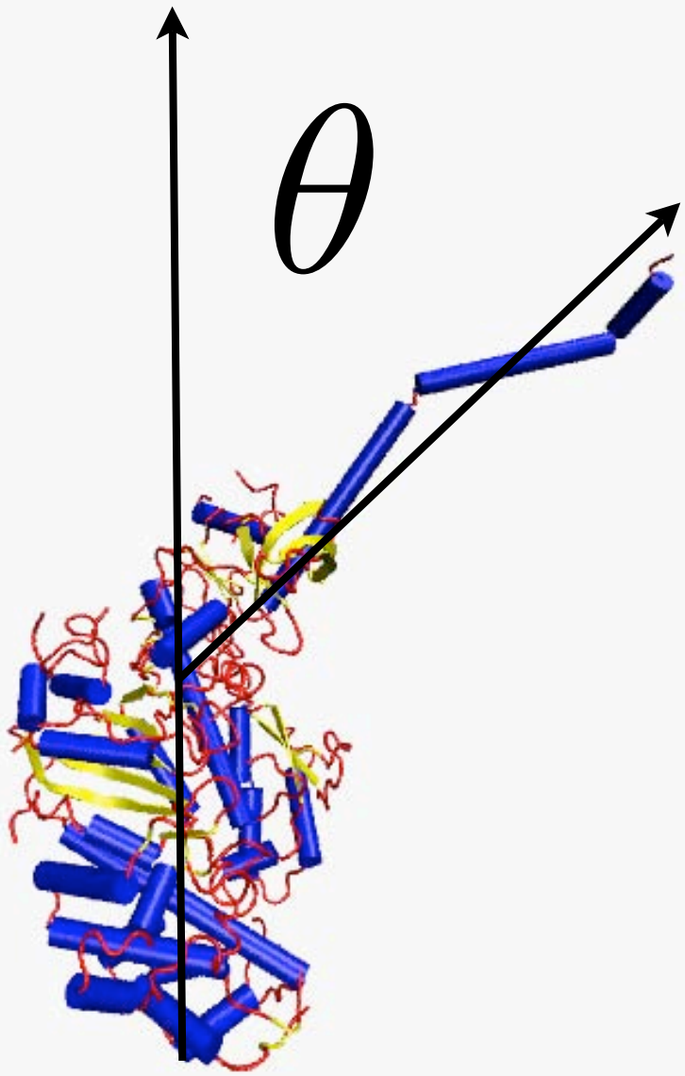


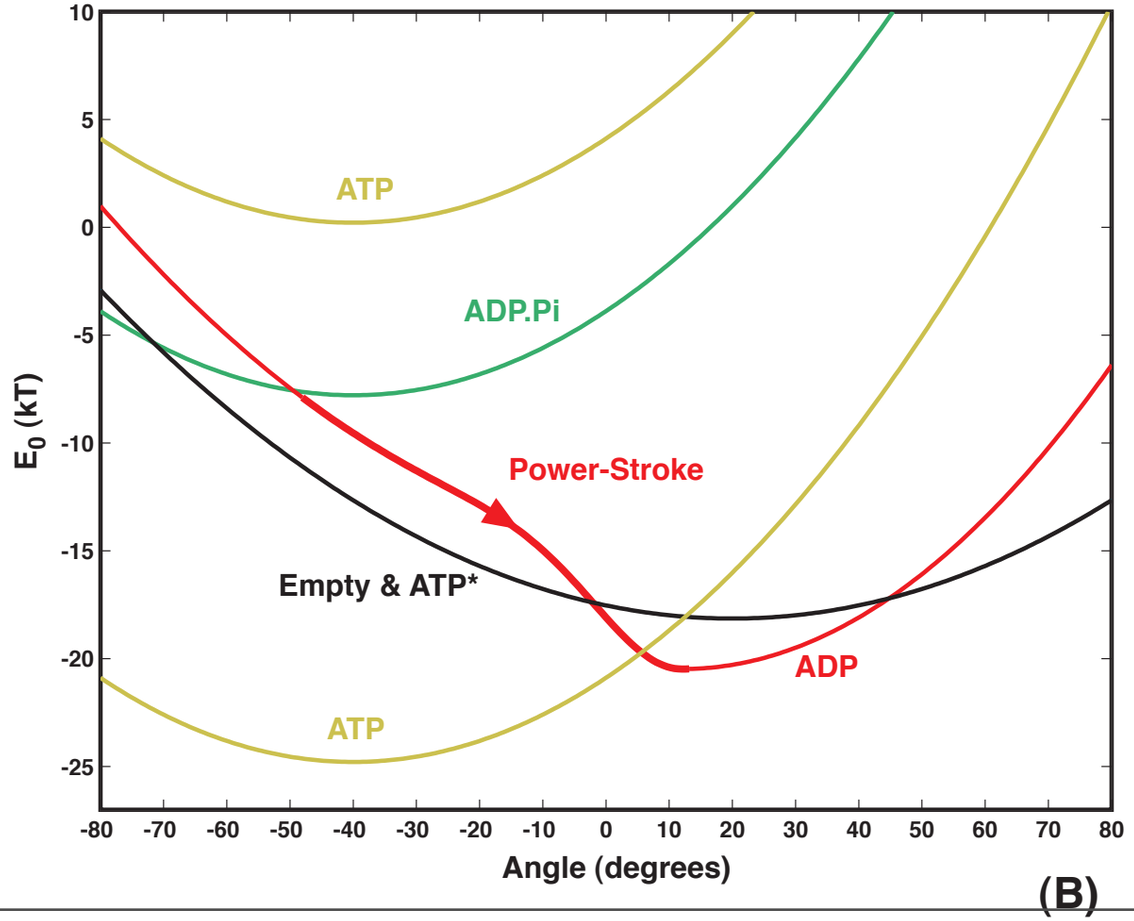
- Ligh-chain domain is much shorter (2 IQ motifs)
- Myosin VI still takes discrete steps (~36nm).
- Step-size distributions are much broader
- There are substeps.
- The step-size is independent of the load force!

Myosin Kinetics



rate limiting step





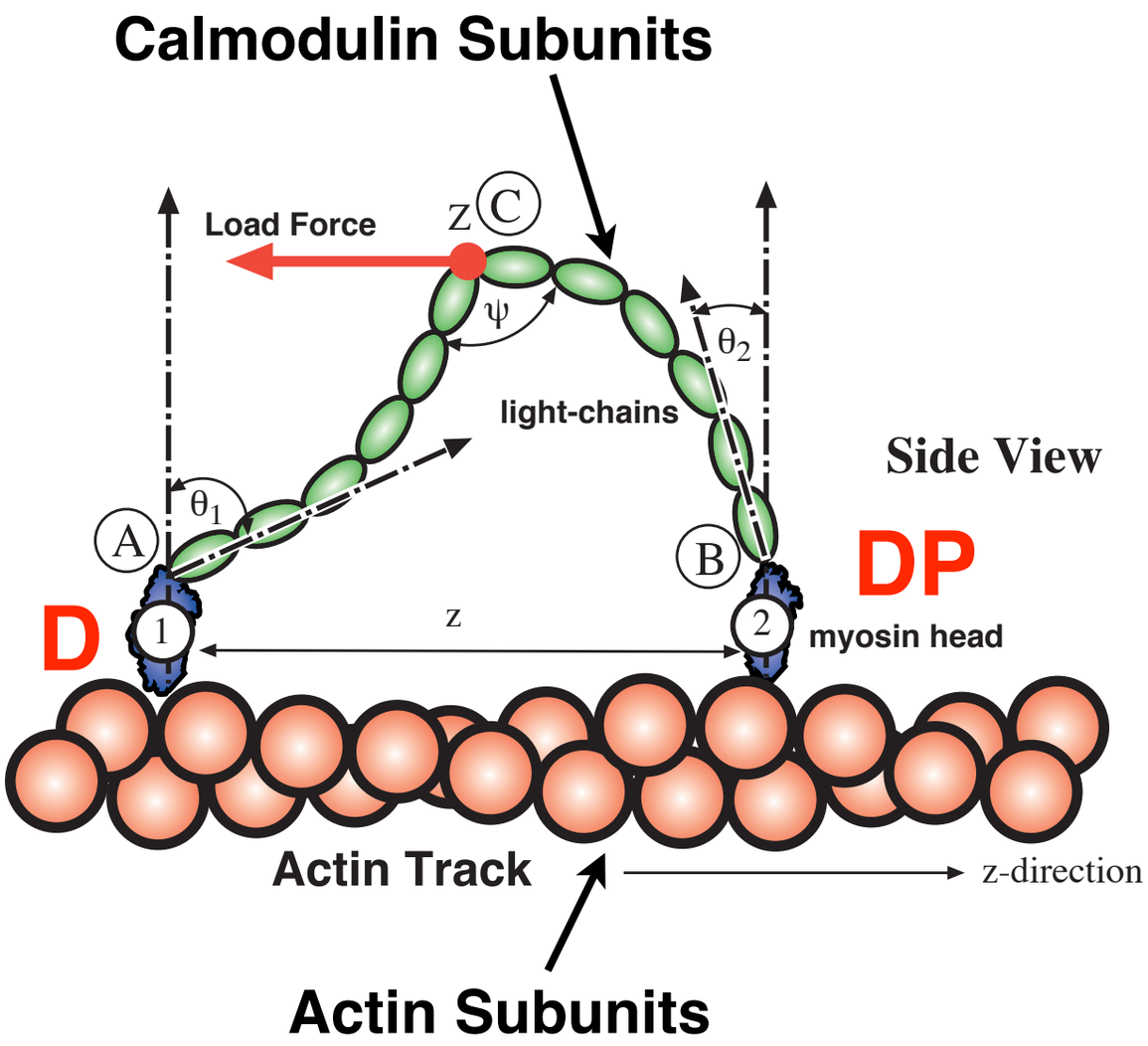
(B)

After Pi release, the lever-arm swings forward.

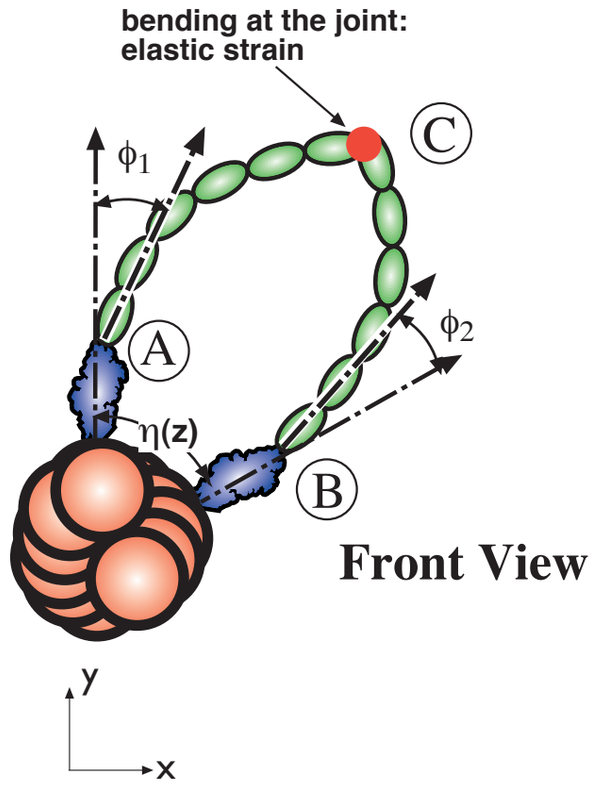
Binding of ATP releases myosin from actin and re-cocks the lever arm.

$$E_0(\theta, s)$$

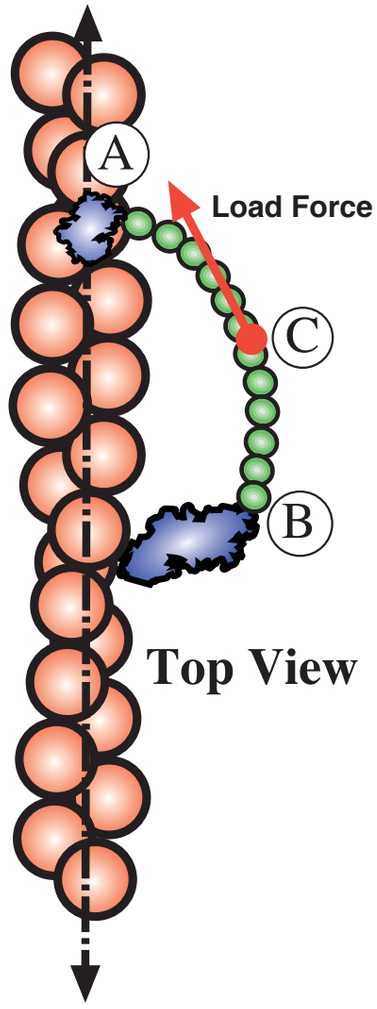
Myosin-V Docking Geometry



Side View



Front View



Top View

Myosin can bind to each actin.

$$E = E_0(\theta_1, \phi_1, s_1) + E_0(\theta_2, \phi_2, s_2) + E_l(\theta_1, \theta_2, \phi_1, \phi_2, z, \mathbf{F})$$

Bending energy of the light-chains

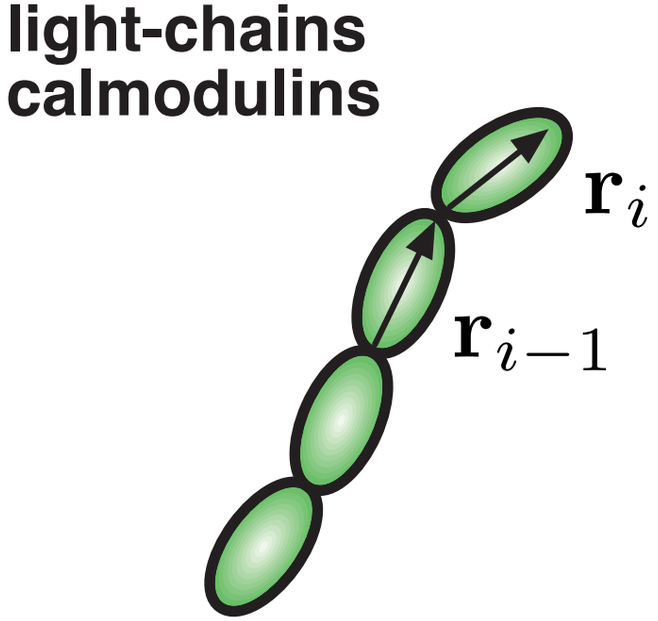
Light-chain Elasticity: Semiflexible Rods

$$E_l = -k_{\mathrm{B}}T \sum_{i=2}^{6} \frac{l_p}{a^3} [\mathbf{r}_i \cdot \mathbf{r}_{i-1} + \mathbf{r}'_i \cdot \mathbf{r}'_{i-1} - 2a^2] - \mathbf{F} \cdot \mathbf{r}_6 + C(\mathbf{r}_6, \mathbf{r}'_6)$$

l_p : persistance length $\sim 120nm$

a : IQ motif size $\sim 5nm$

$\mathbf{F}$: external Force



Bending energy depends on the boundary conditions: $(\theta_1, \phi_1, \theta_2, \phi_2)$

There are also fluctuations, free energy calculations are done

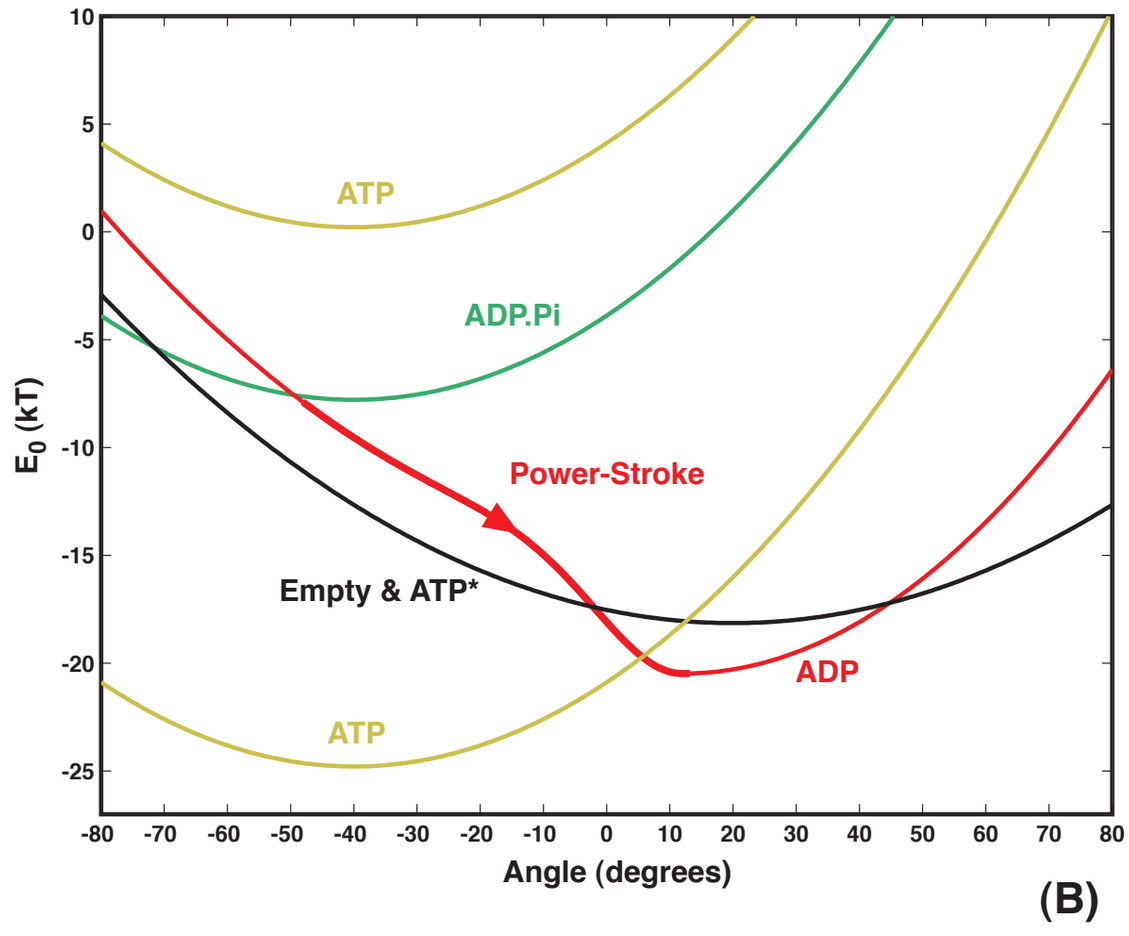
Motor domain energy

$$E_0(\theta_i, \phi_i, s_i) = \frac{1}{2}\kappa(s_i)(\theta - \theta_0(s_i)) + \frac{1}{2}\kappa'\phi_i^2 + c(s_i)$$

Preferred conformation for different motor states Can be obtained from structure

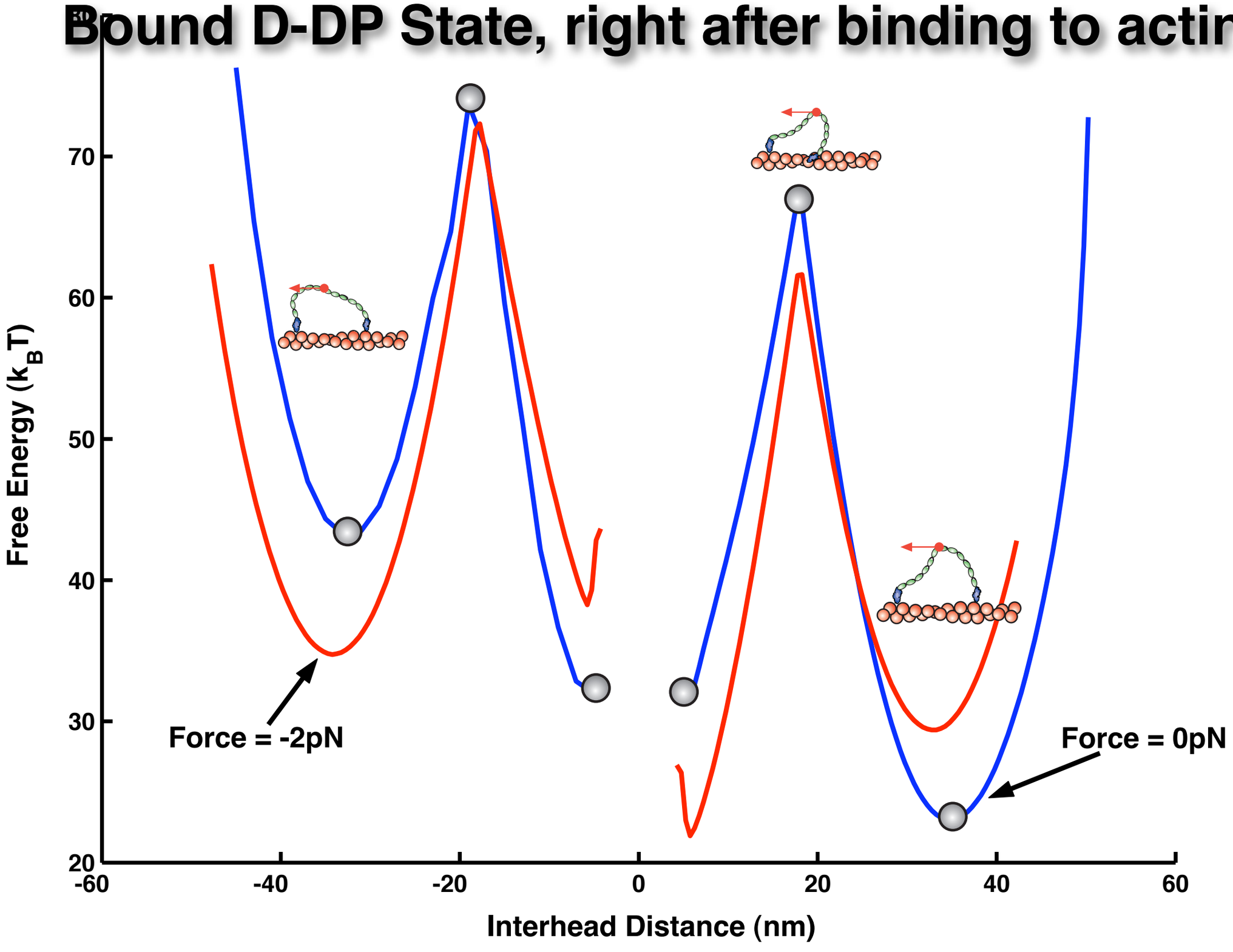
constant energy difference between states, obtained from monomer kinetic measurements

κ, κ' **Unknown, has to be guessed**



(B)

Bound D-DP State, right after binding to actin

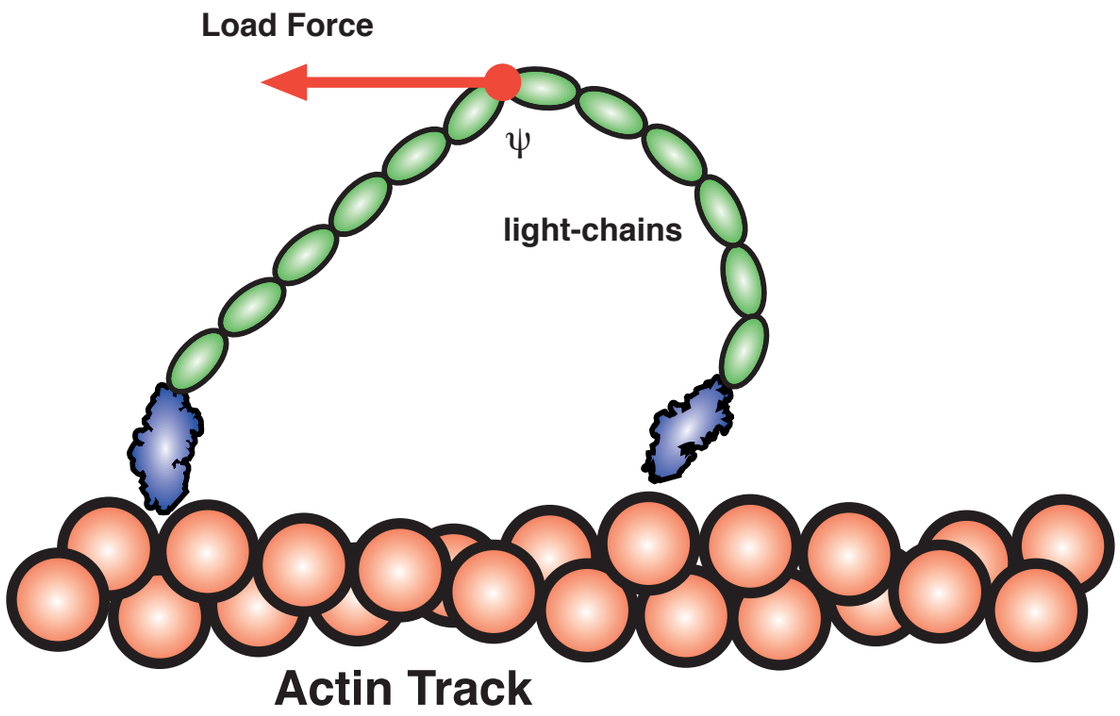


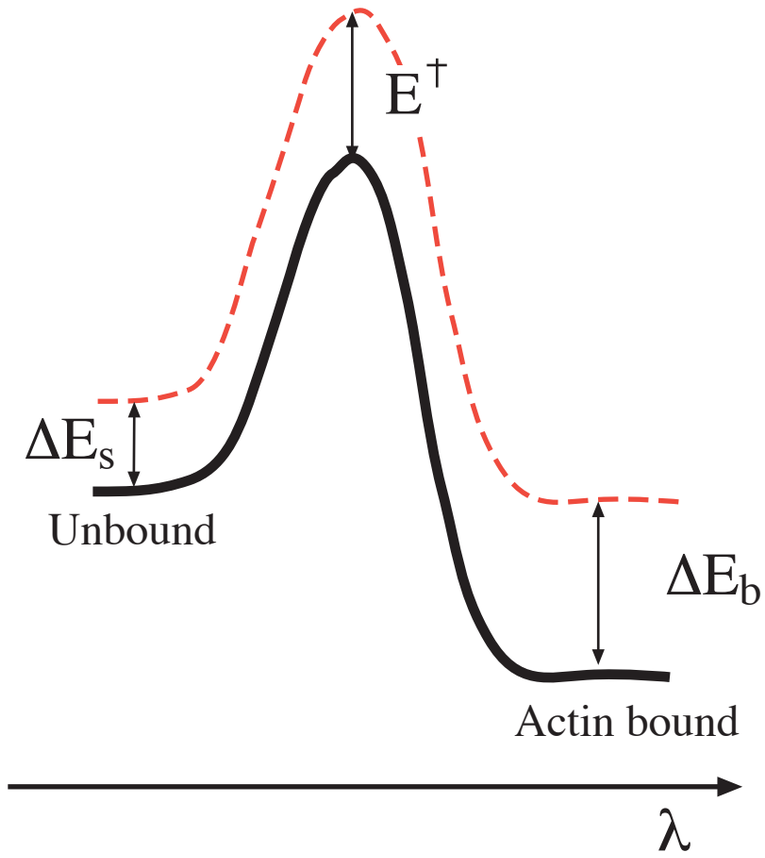
Binding to 36nm is lowest in energy, due to light-chain elasticity and helical nature of F-actin

at 2pN force, forward binding energy is the same as backward binding

Myosin-V Kinetics: Binding to Actin

The unbound myosin head is free to diffuse.
The diffusion constant is large, mean passage time to the binding sites are negligible.





$$\Delta E_s = E_0(\theta_1, \phi_1, s_1) + E_0(\theta_2, \phi_2, s_2) + E_l(\theta_1, \phi_1, \mathbf{F})$$

$$\Delta E_b = E_0(\theta_1, \phi_1, s_1) + E_0(\theta_2, \phi_2, s_2) + E_l(\theta_1, \phi_1, \theta_2, \phi_2, \mathbf{F}, z)$$

$$E^\dagger = \lambda(\Delta E_b - \Delta E_s) + \Delta E_s$$

Binding to actin slows down with F.

Transition state energy for binding to actin:
a function of the binding geometry and external force.

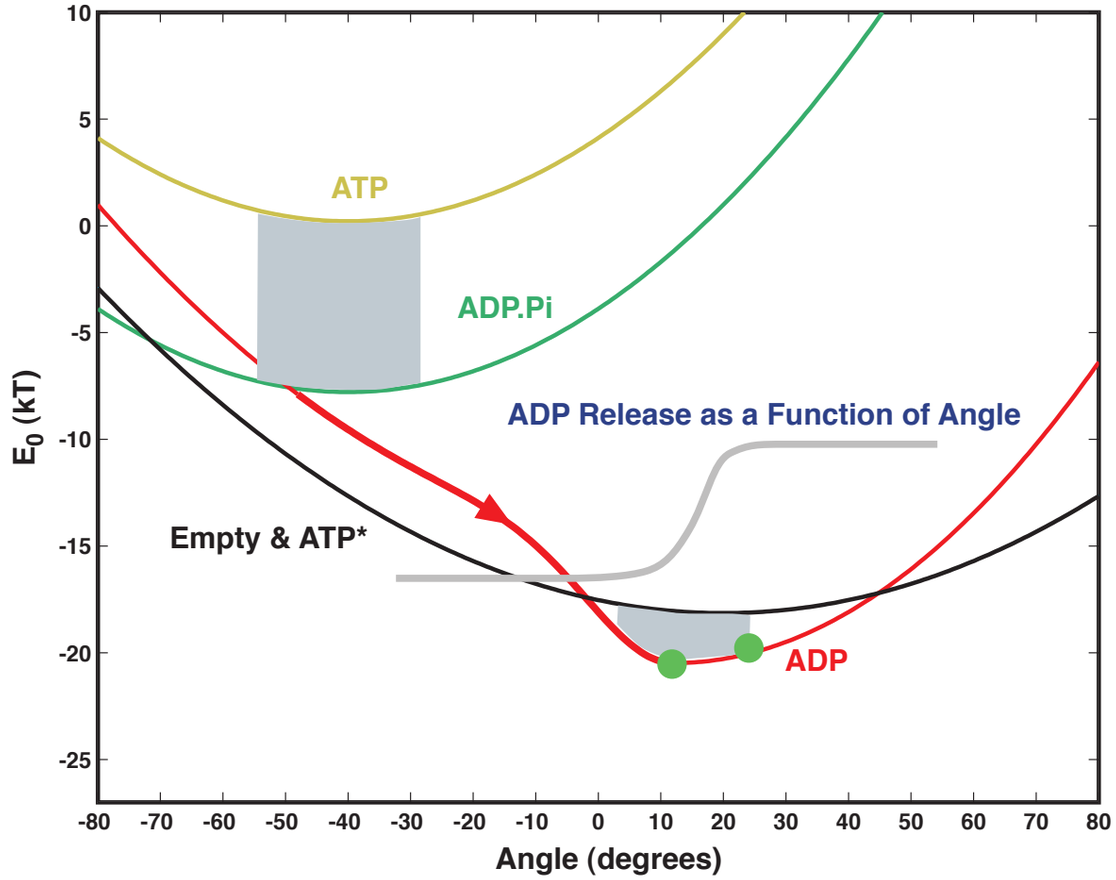
Gating: Which Head Detach First?

Rates are functions of conformation

$$k_{s \to s'}(\theta, \phi)$$

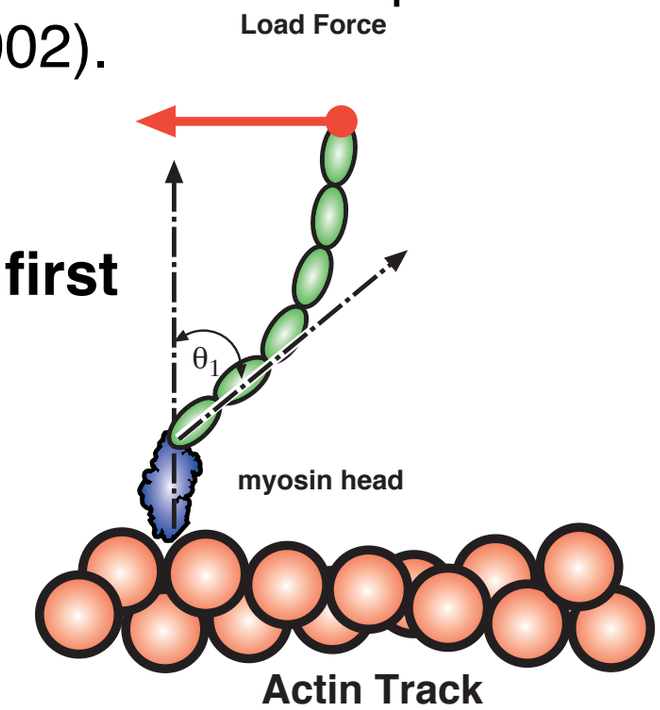
The angle of the lever-arm is correlated with the geometry of the binding pocket.

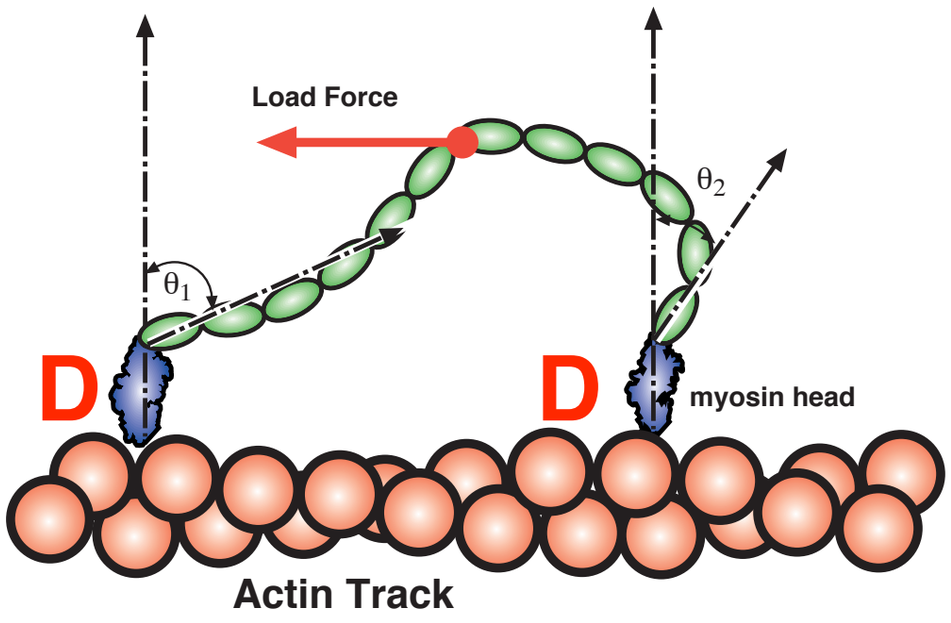
As the lever arm swings forward, the pocket becomes more open, ADP release is enhanced.



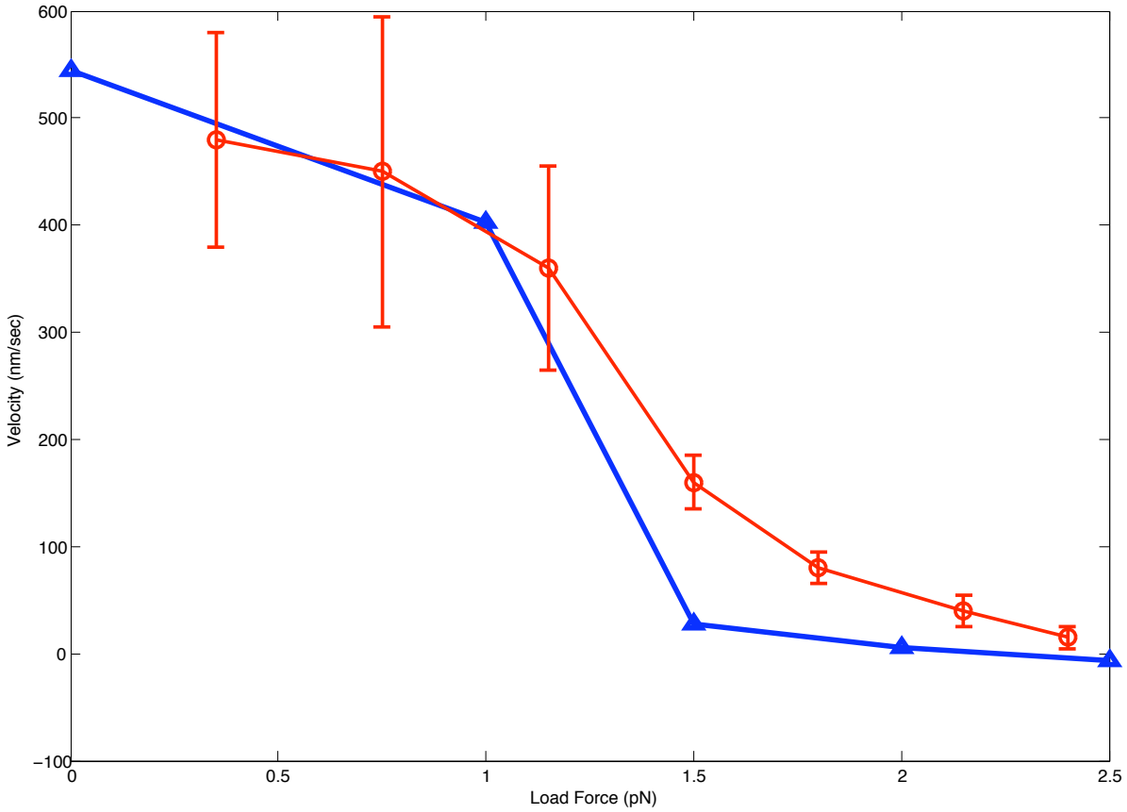
This conjecture has been confirmed in expt. (first due to Veigel et al, 2002).

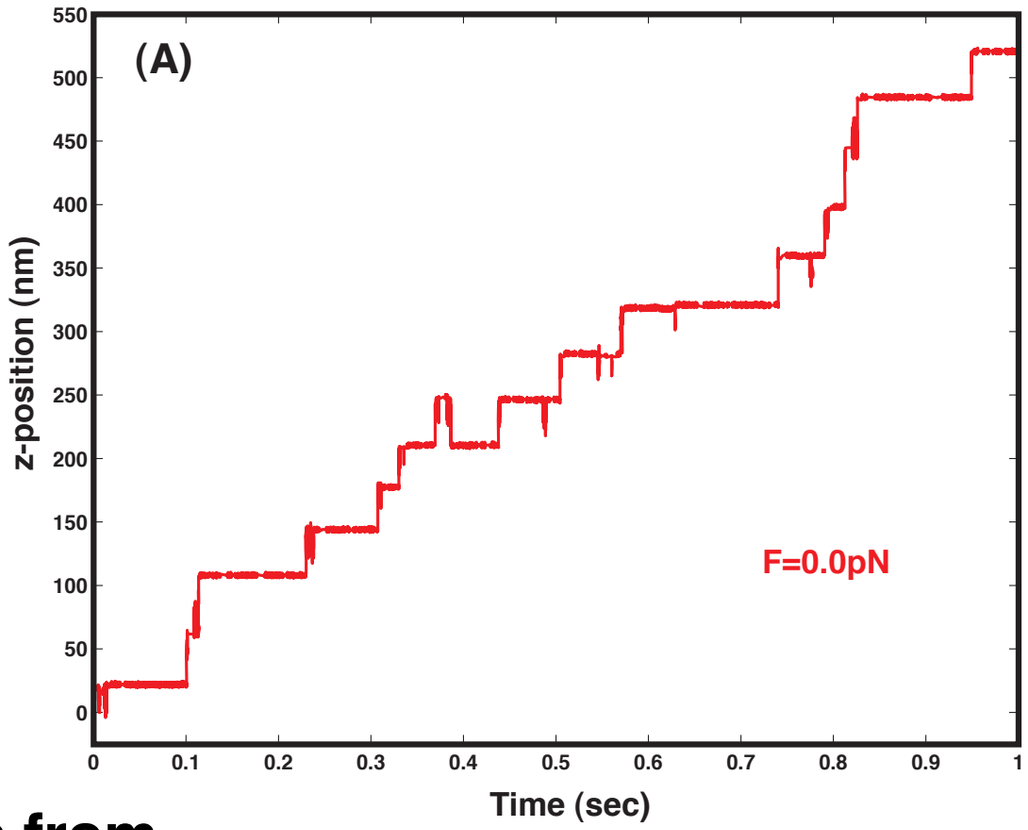
Trailing head release ADP first detach first from F-actin



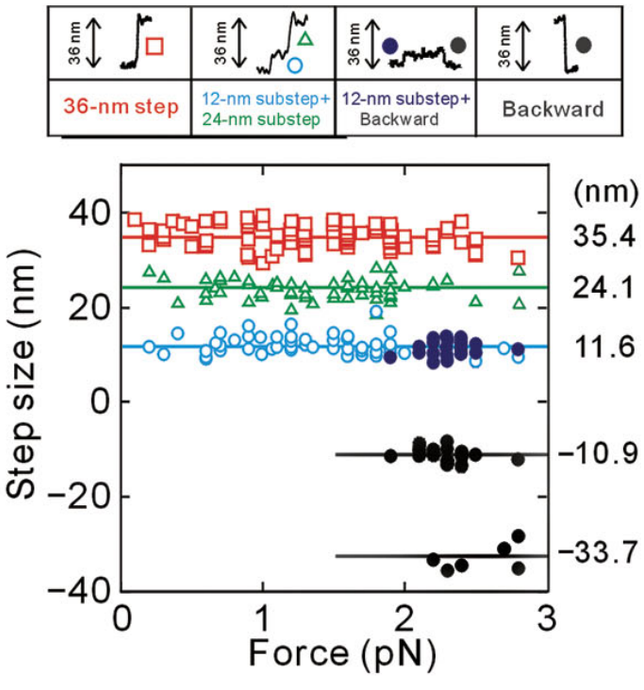


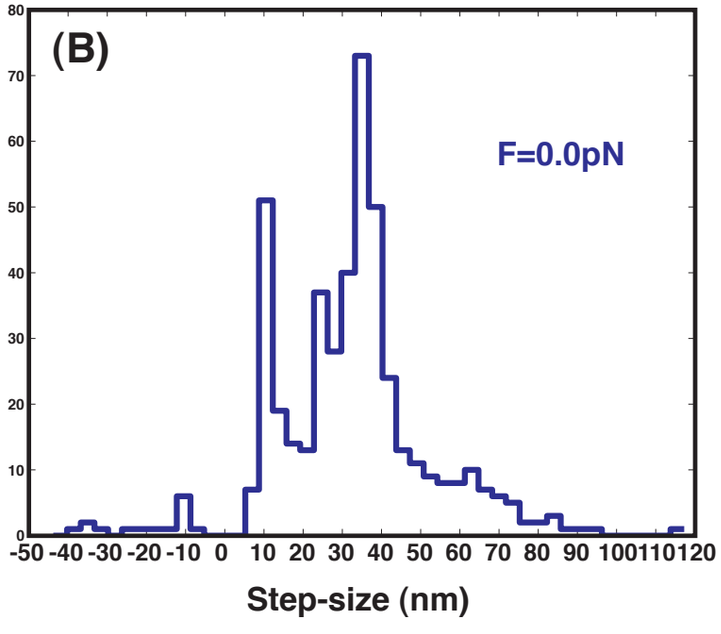
Results:

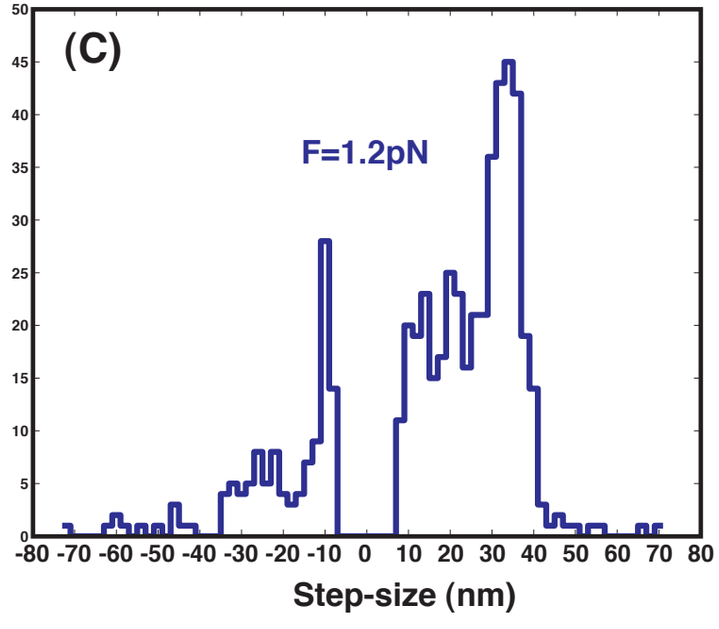




Measurements from Ishiwata group. 2005

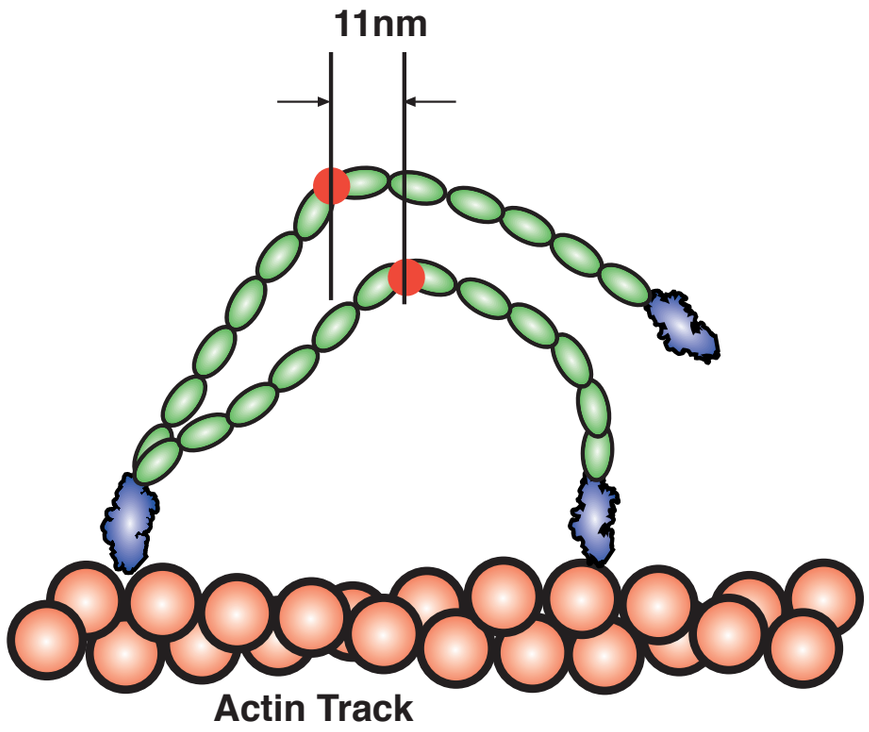




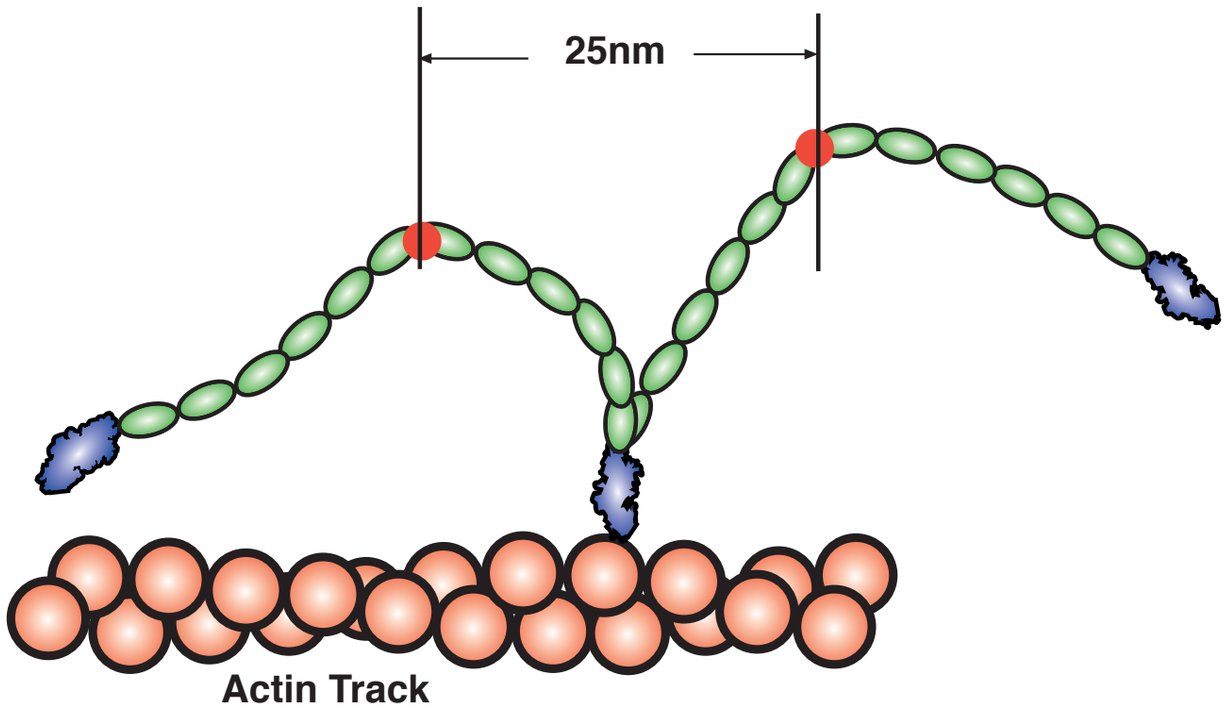


Substeps

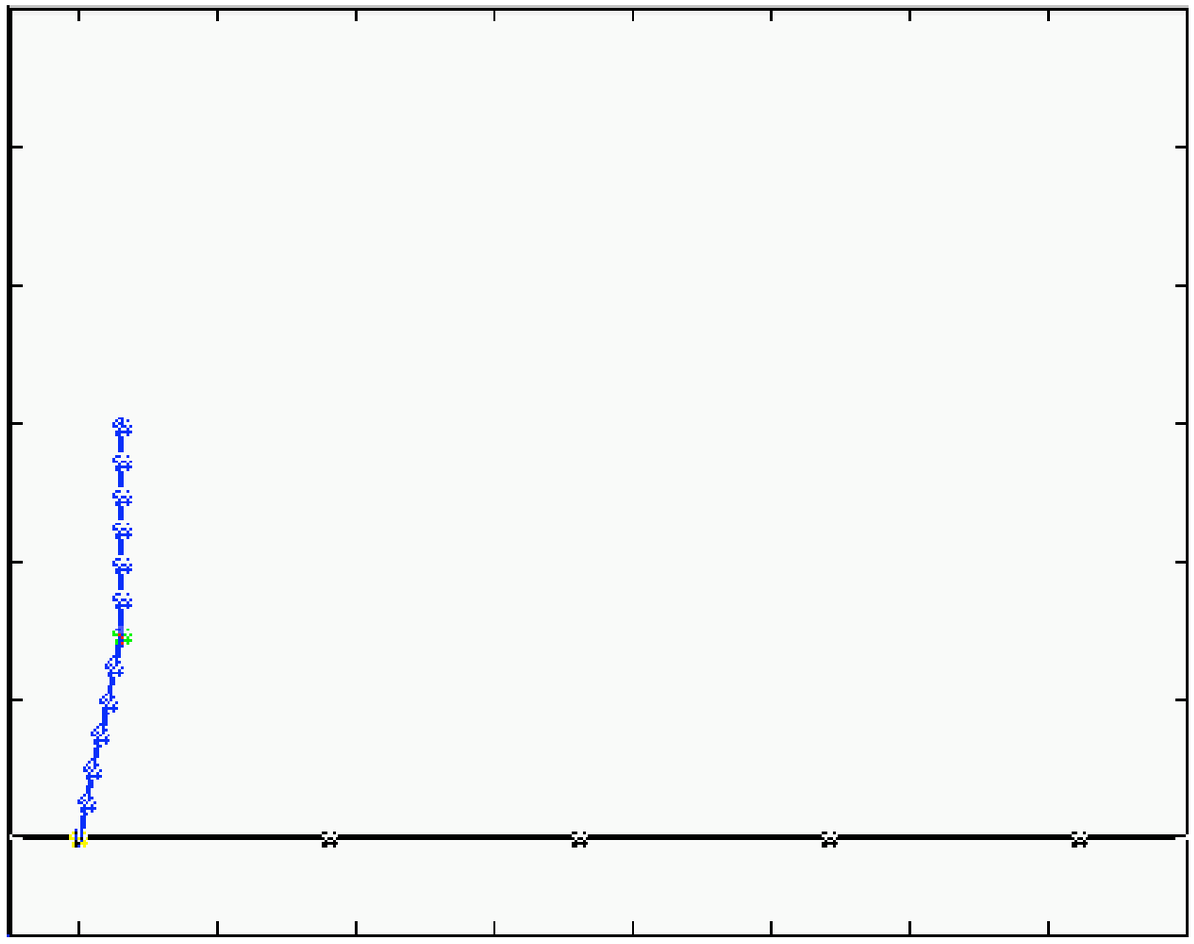
11nm substep is due to actin binding:



25 nm substep is due to power-stroke of the leading head.

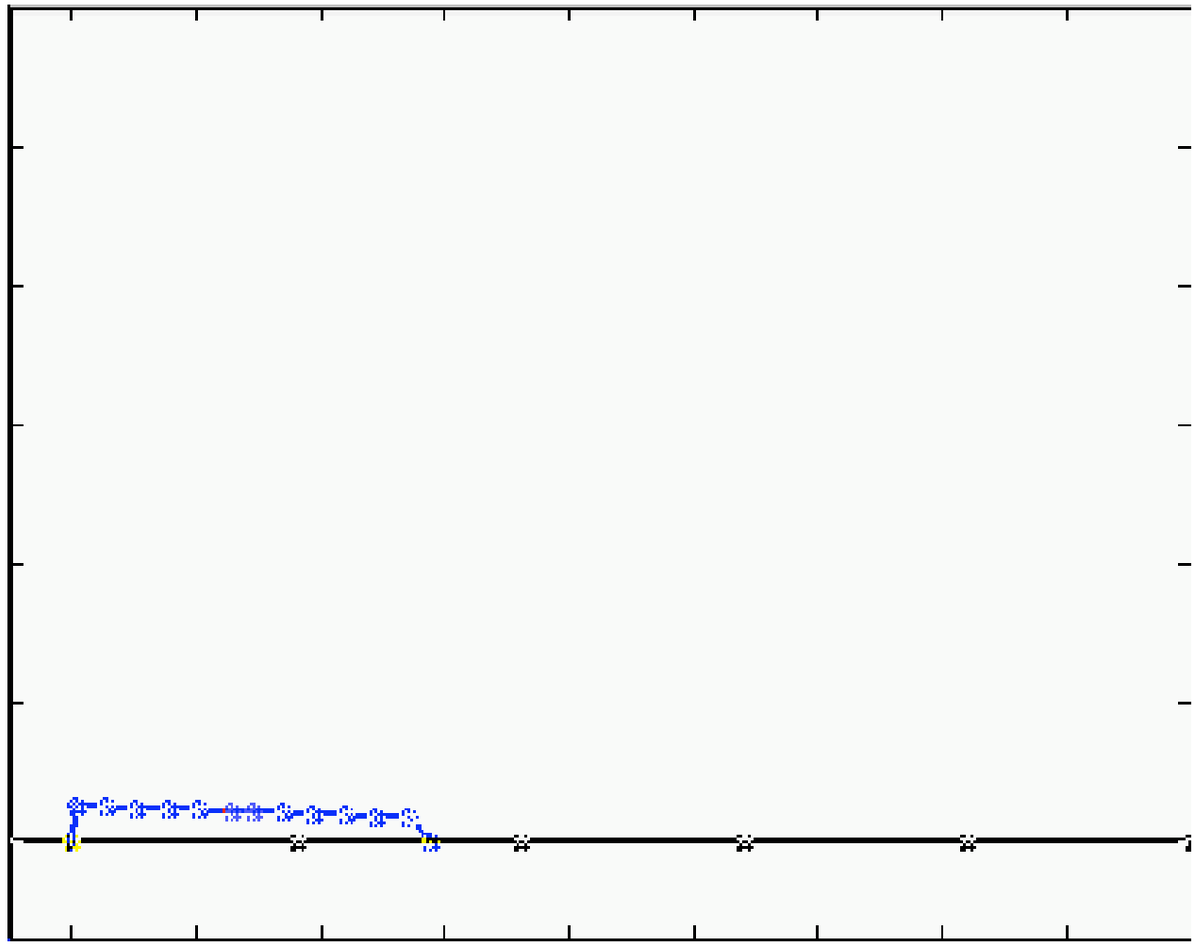


Movies of Myosin-V Movement

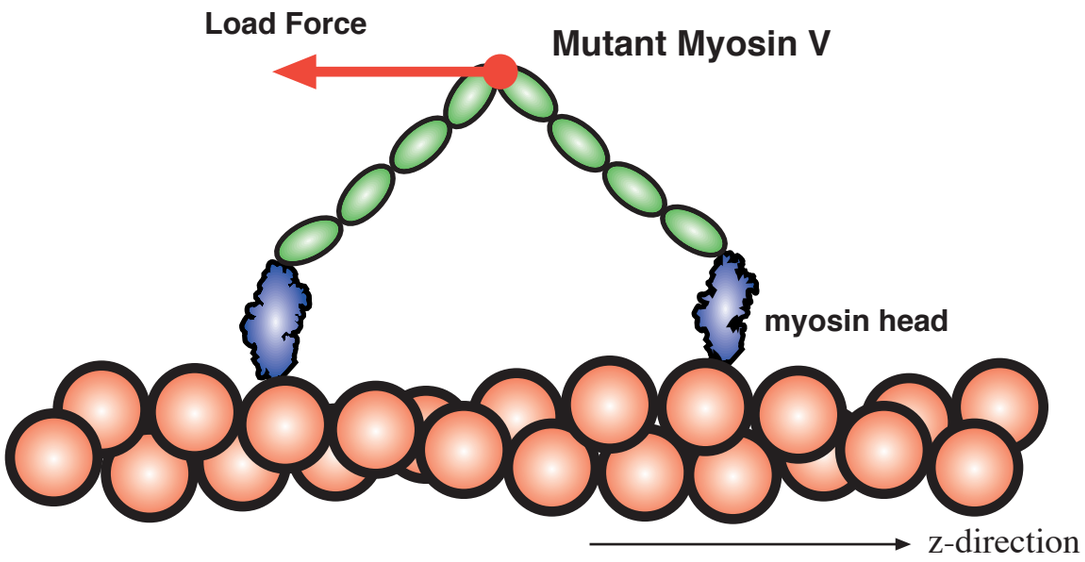


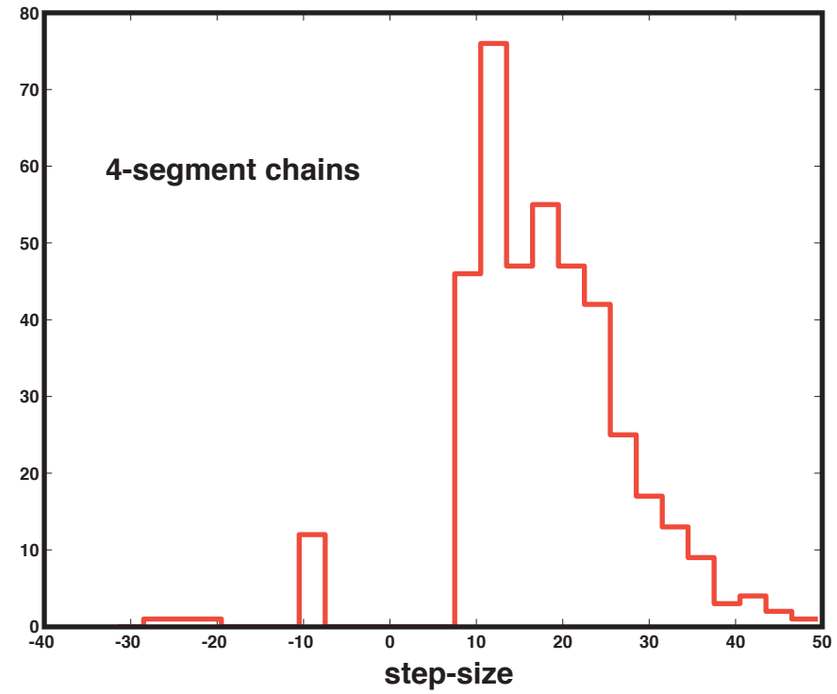
Load force = 0pN

Load force = 1.0pN



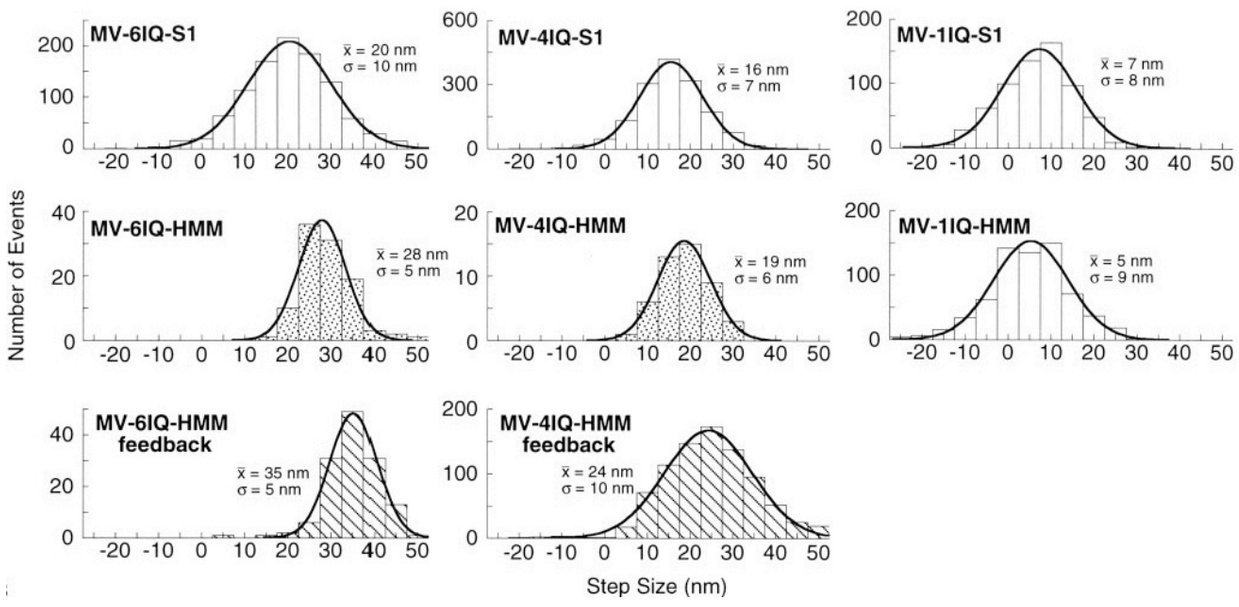
Mutants: Shorter Light-chains

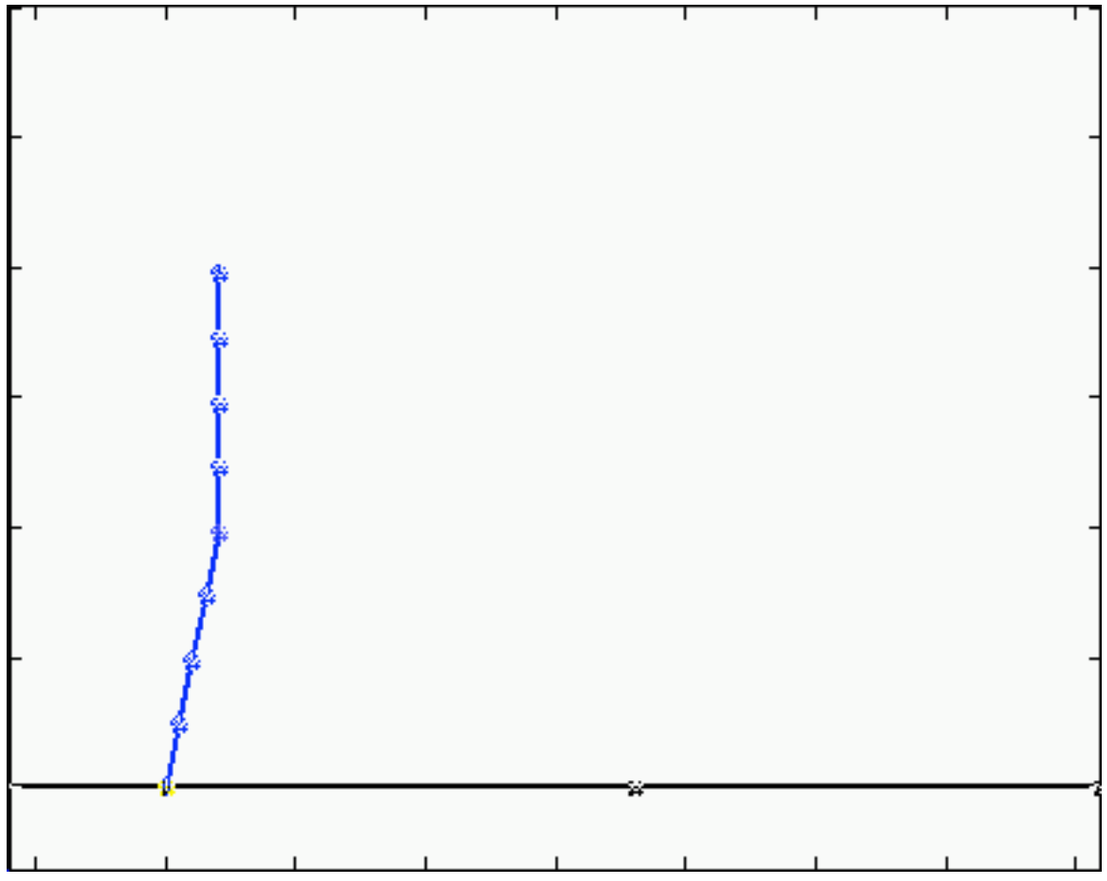




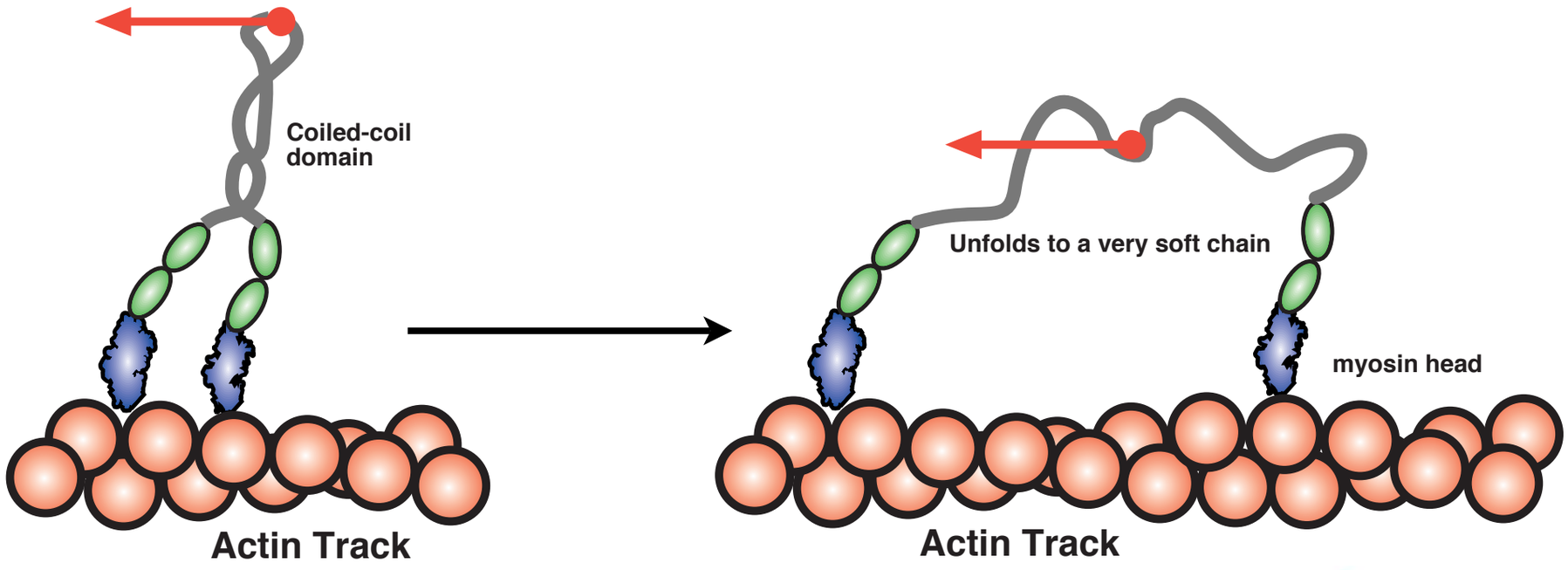
A Mutant with shorter light-chains still walks forward.

However, it takes smaller steps, but walks faster!





What about myosin-VI?

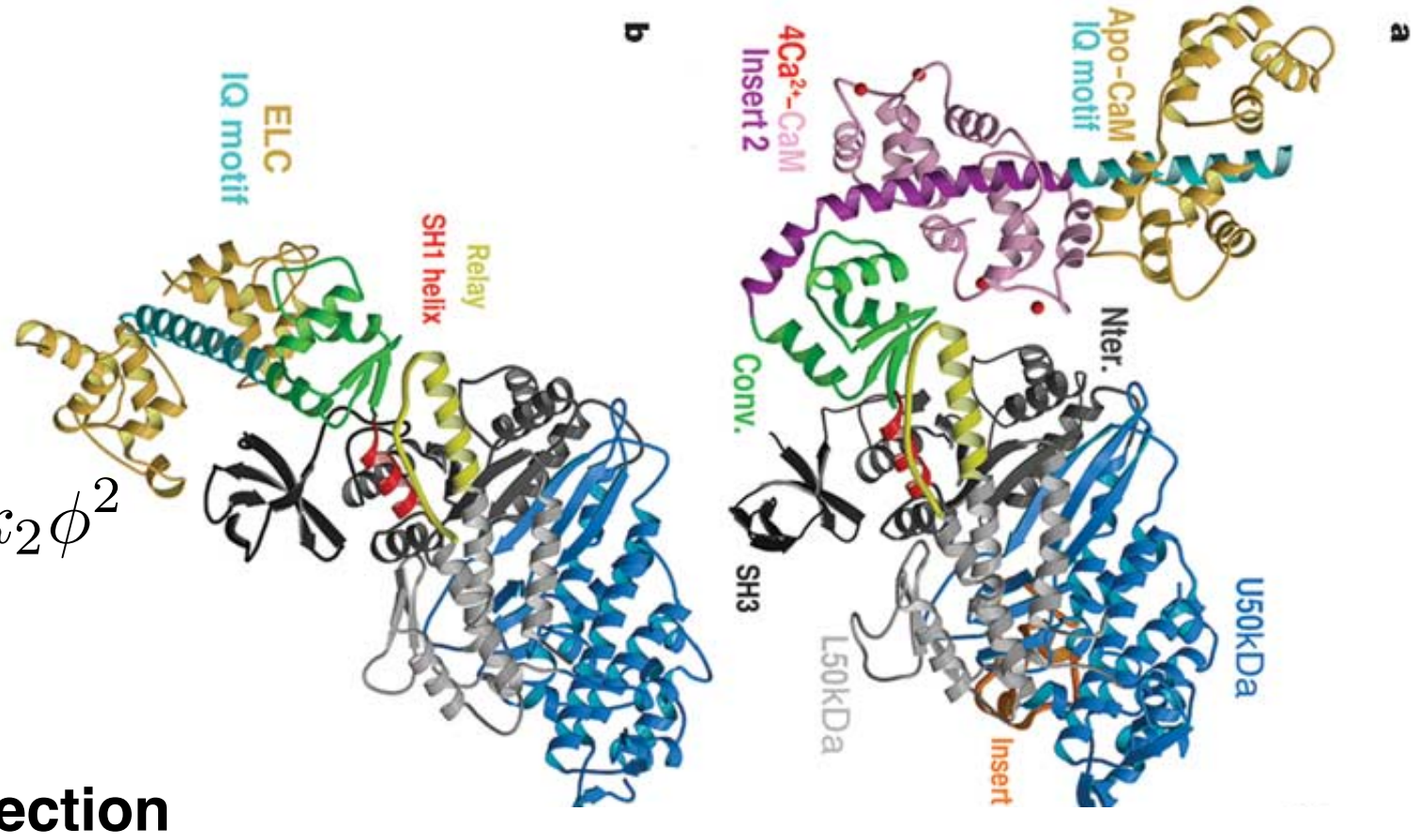


Rock, Spudich and Sweeney et al, 2005

myosin-VI light-chain unfolds, therefore can potentially reach 36nm binding site.

$$E_0(\theta, \phi, s) = \frac{1}{2} k_1 (\theta - \theta_0(s))^2 + \frac{1}{2} k_2 \phi^2$$

different equilibrium geometry



Structure from Sweeney and Houdusse. 2005

light-chains pointed in the other direction

Walk backwards !!

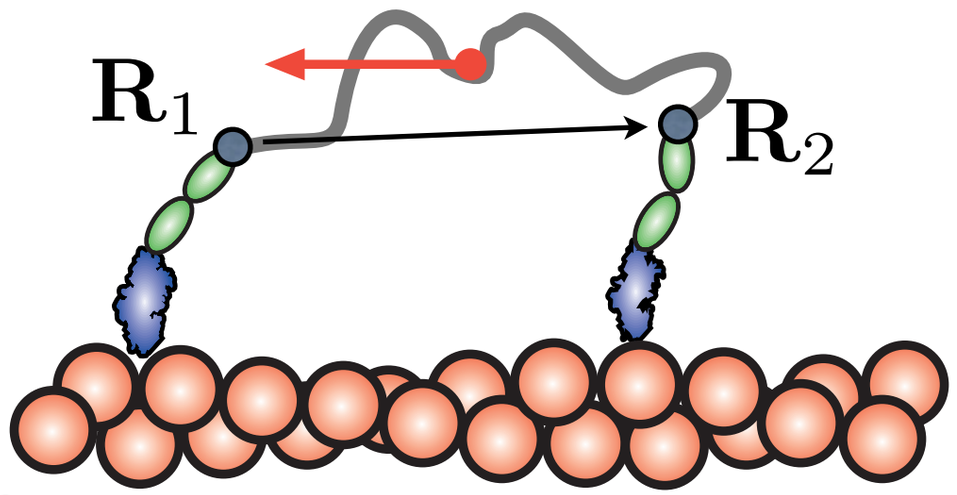
But how can it still walk?

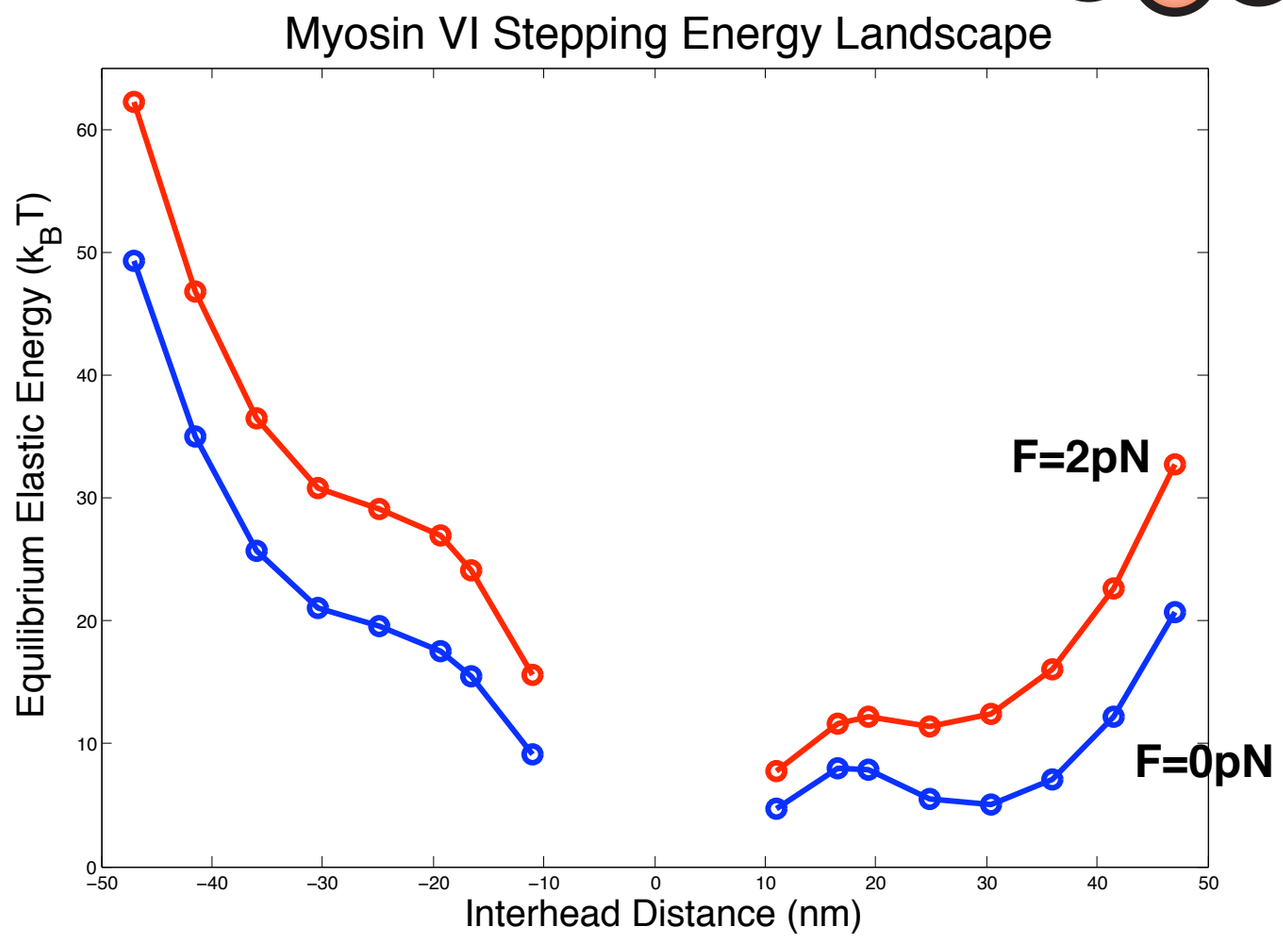
Light-chain very soft, entropic elasticity: worm like chain

$$F(x) = \frac{k_{\mathrm{B}}T}{l_p}\left[\frac{1}{4(1-x/L)^2} + \frac{x}{L} - \frac{1}{4}\right]$$

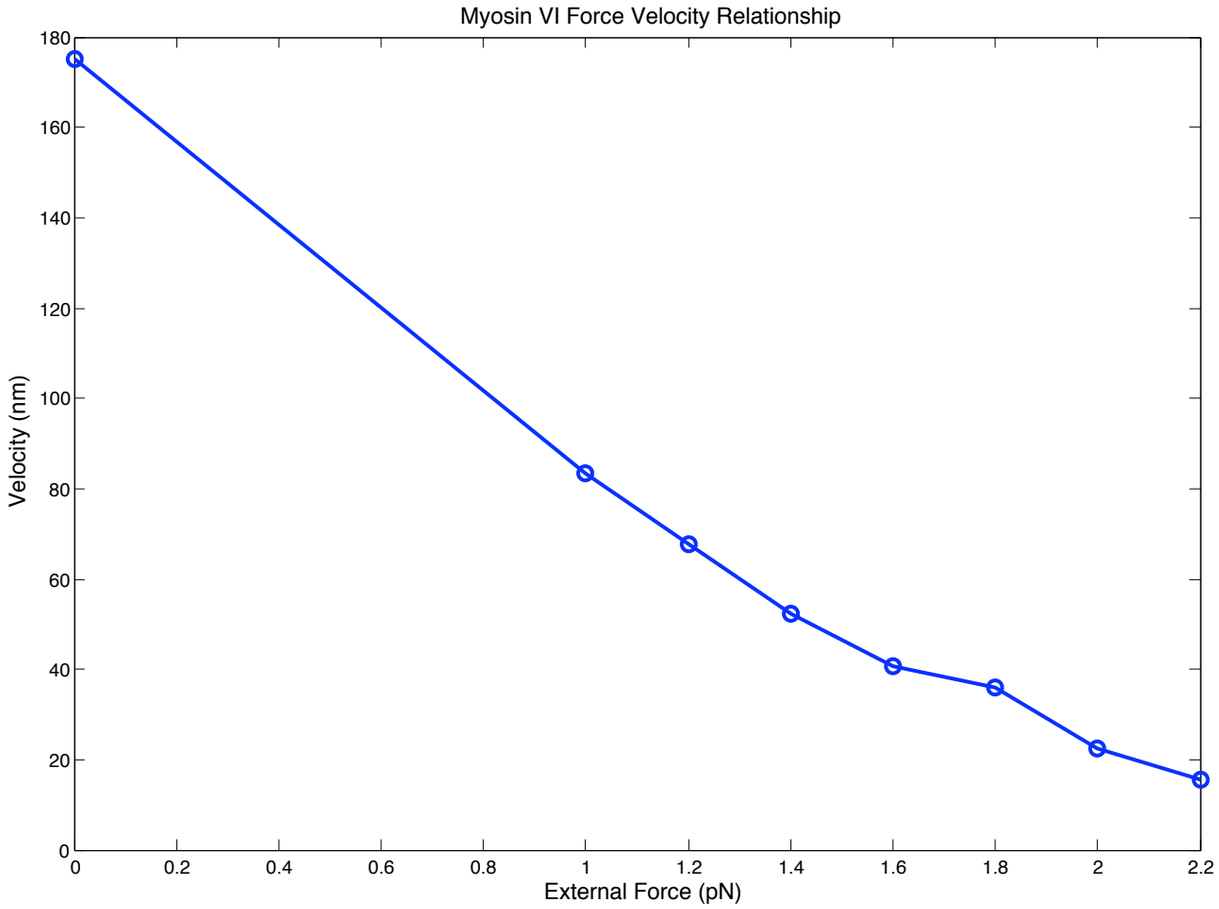
$$E_l(\mathbf{R}_1, \mathbf{R}_2) = E_l(|\mathbf{R}_1 - \mathbf{R}_2|) = \int_0^s dx F(x)$$

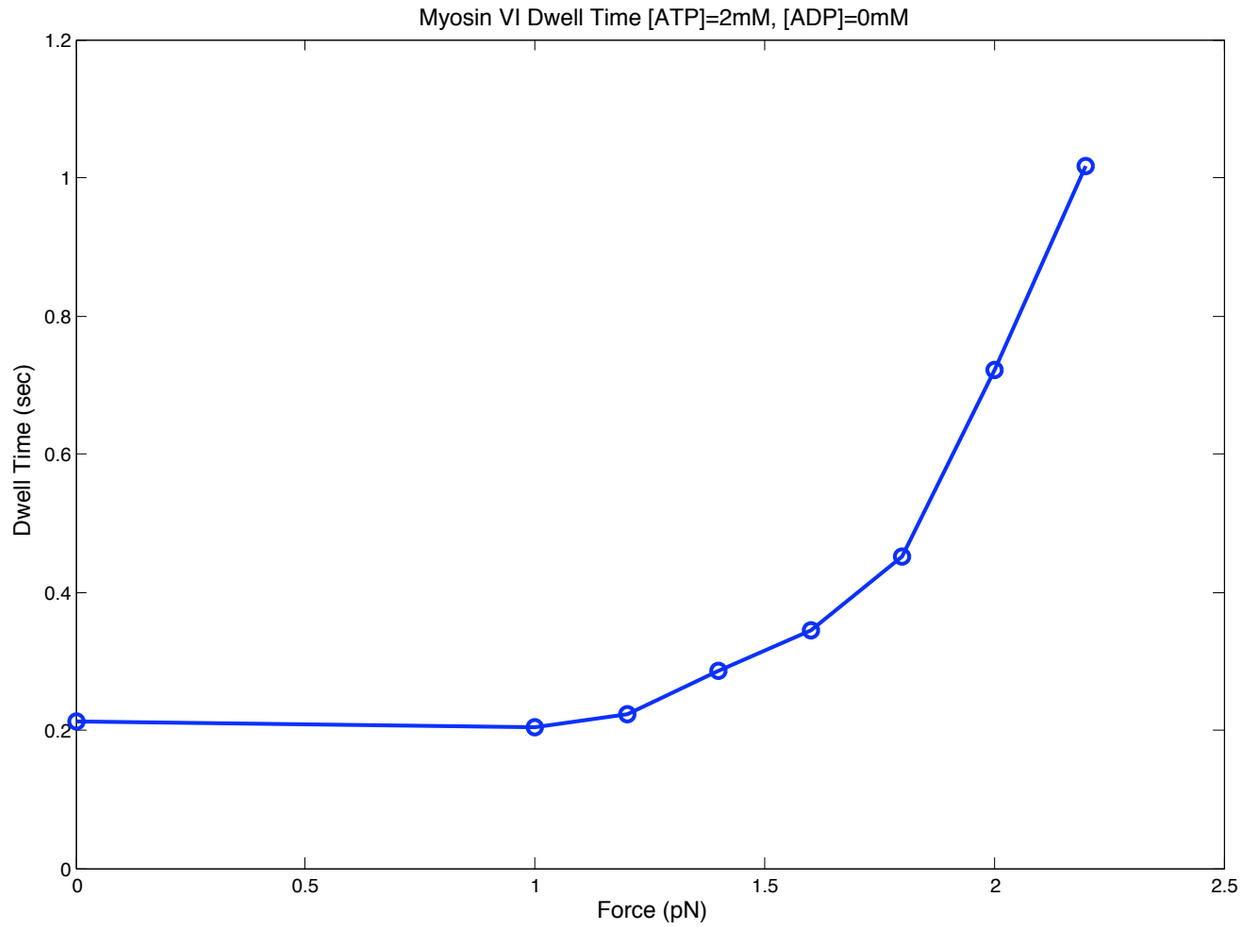
$$\mathbf{R}_1(\theta_1, \phi_1)$$





Myosin VI walks also

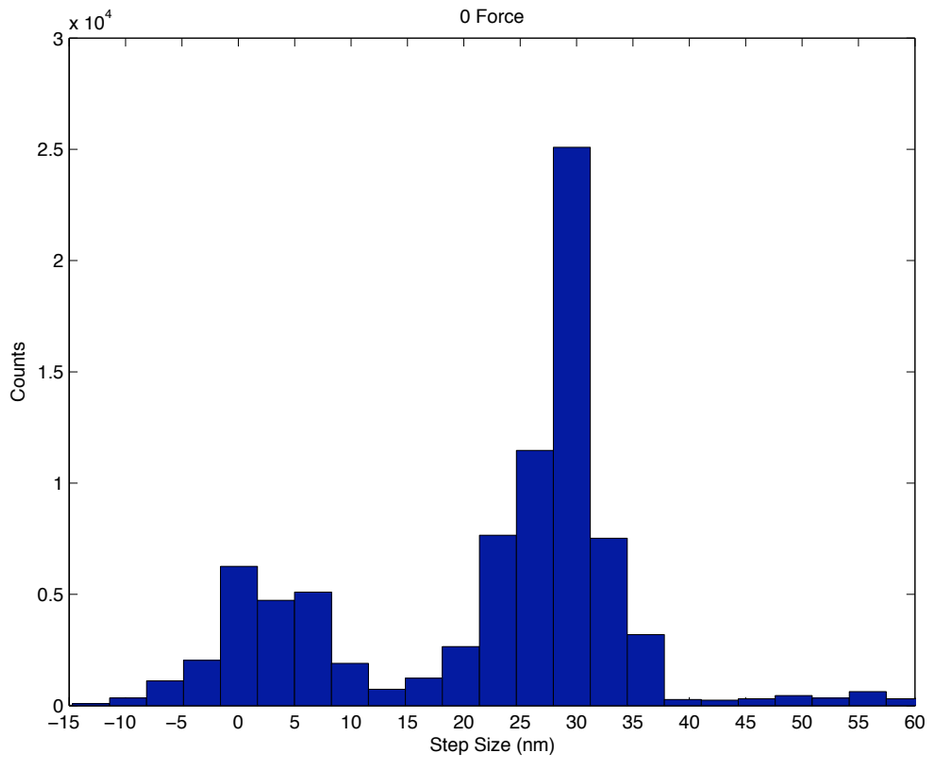


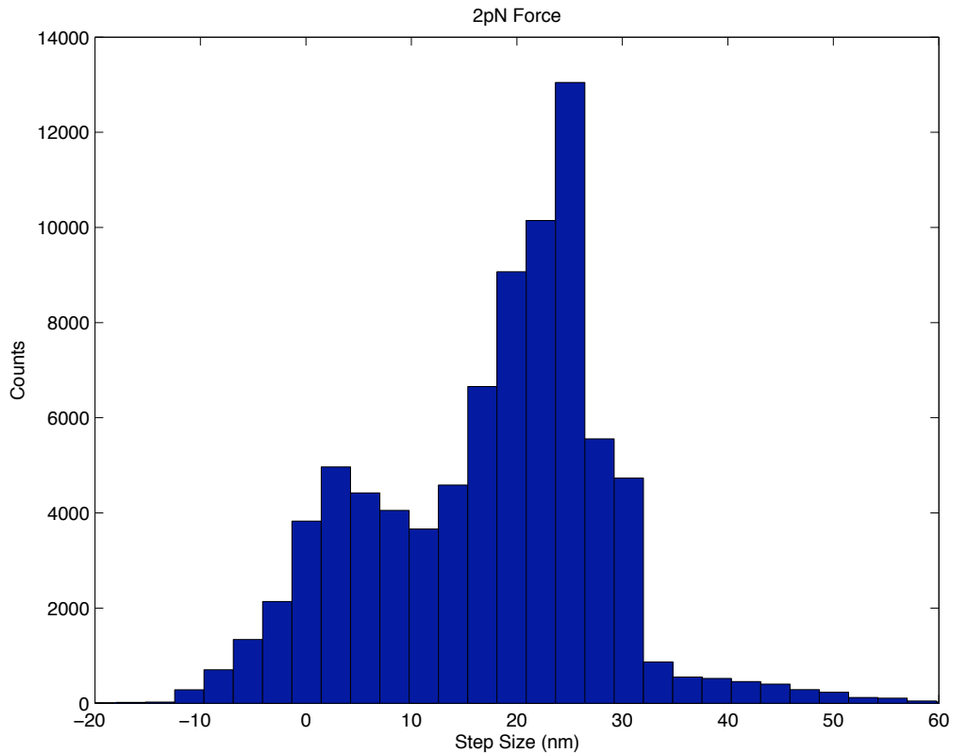


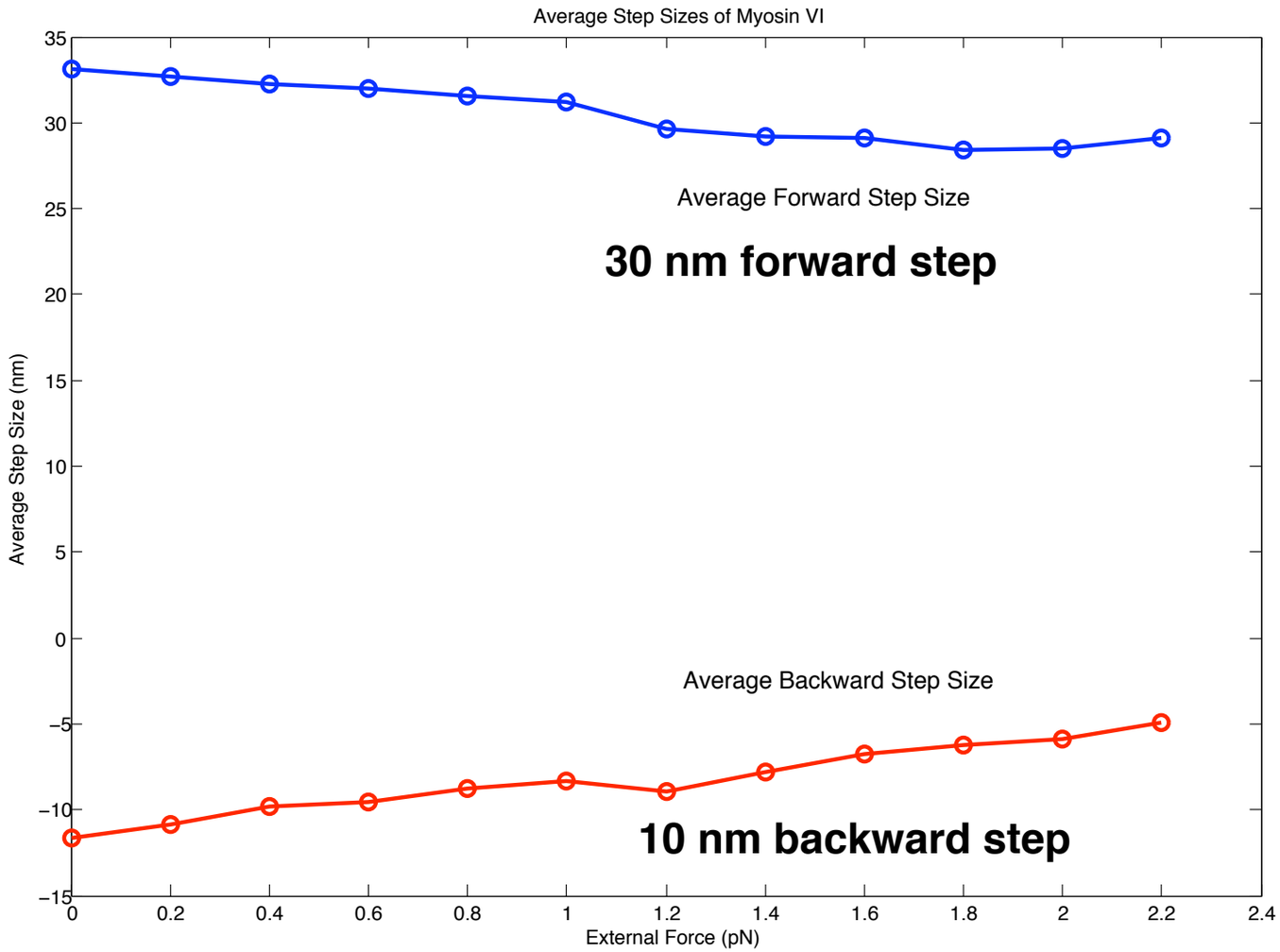
Force-velocity curve binding to actin is slower

Dwell time in between steps agrees with expt.

Step-size is random





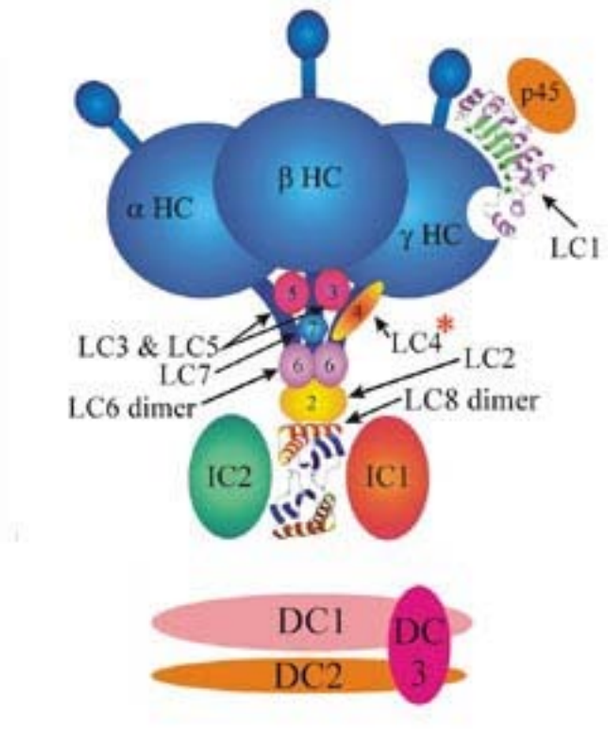


Floppy legs can walk too, but just more wobbly.

What can we say about kinesin and dynein?

The coordinated movement of 2 motor domains must follow the same principle as myosin-V and VI.

Kinesin and dynein seem to travel on a single protofilament. This has to do with the property of the connecting protein structures. (E_l)



Muscle can be thought of as many myosin-V's operating on F-actin.