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)
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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.**

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.

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

\[
J = -\frac{\partial E(\xi, s)}{\partial \xi} \rho(\xi, s, t) - \frac{\partial \rho}{\partial \xi}
\]
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 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!

Reif et al. PNAS, (2000).
Myosin-VI is also processive, but towards the minus end

- Light-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!
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

Calmodulin Subunits

Actin Subunits

Actin Track

Myosin can bind to each actin.

\[ E = E_0(\theta_1, \phi_1, s_1) + E_0(\theta_2, \phi_2, s_2) + E_l(\theta_1, \theta_2, \phi_1, \phi_2, z, F) \]

Bending energy of the light-chains
Light-chain Elasticity: Semiflexible Rods

\[ E_l = -k_B T \sum_{i=2}^{6} \frac{l_p}{a^3} [r_i \cdot r_{i-1} + r_i' \cdot r_{i-1}' - 2a^2] - F \cdot r_6 + C(r_6, r_6') \]

\( l_p \): persistence length \( \sim 120 \text{nm} \)

\( a \): IQ motif size \( \sim 5 \text{nm} \)

\( F \): 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

\( \kappa, \kappa' \) Unknown, has to be guessed

constant energy difference between states, obtained from monomer kinetic measurements

\[ E_0(\theta_i, \phi_i, s_i) = \begin{align*}
\frac{1}{2} \kappa(s_i)(\theta - \theta_0(s_i)) + \frac{1}{2} \kappa' \phi_i^2 + c(s_i) 
\end{align*} \]
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

Interhead Distance (nm)

Free Energy ($k_B T$)

Force = 0pN

Force = -2pN
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.

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

\[ \Delta E_s = E_0(\theta_1, \phi_1, s_1) + E_0(\theta_2, \phi_2, s_2) + E_l(\theta_1, \phi_1, F) \]

\[ \Delta E_b = E_0(\theta_1, \phi_1, s_1) + E_0(\theta_2, \phi_2, s_2) + E_l(\theta_1, \phi_1, \theta_2, \phi_2, F, z) \]

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

Binding to actin slows down with F.
Rates are functions of conformation

\[ k_{s \rightarrow s'}(\theta, \phi) \]

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

(A) Z-position (nm) with load force 0.0 pN

(B) Step-size (nm) distribution for 0.0 pN load force

(C) Step-size (nm) distribution for 1.2 pN load force

Graphs showing velocity vs. load force relationship.
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 = 0 pN

Load force = 1.0 pN
A Mutant with shorter light-chains still walks forward.

However, it takes smaller steps, but walks faster!
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

light-chains pointed in the other direction

Walk backwards !!
But how can it still walk?

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

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

\[ E_l(R_1, R_2) = E_l(|R_1 - R_2|) = \int_0^s dx F(x) \]

\[ R_1(\theta_1, \phi_1) \]
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.