Lecture 10:

Molecular Machines and Enzymes

Sources:

Nelson – Biological Physics (CH.10)
Julicher, Ajdari, Prost – Modeling molecular motors (RMP 1997)

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Outline

I. Survey of molecular devices in cells.

II. Mechanical machines.

III. Molecular implementation of mechanical principles.

IV. Kinetics of enzymes and molecular machines.

Biological Q: How do molecular machines convert ***scalar*** chemical energy into ***directed*** motion, a vector?

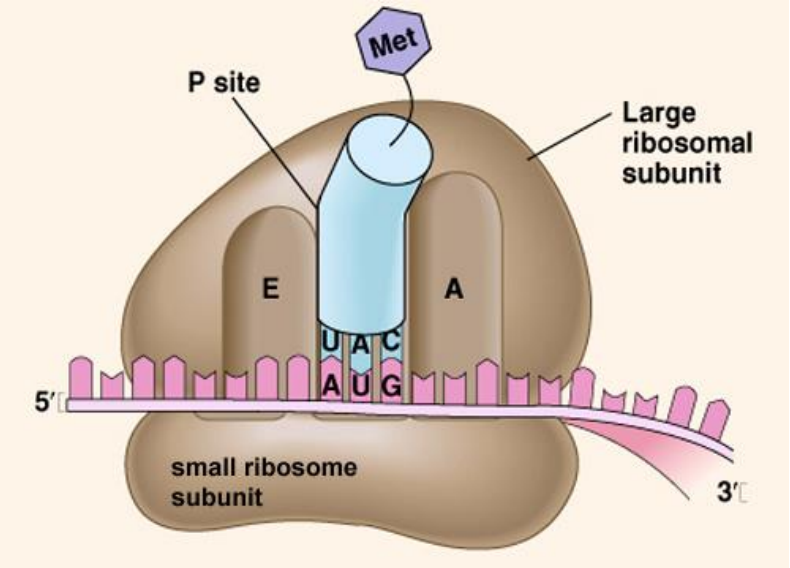
Physical idea: *Mechano-chemical coupling by random walk of the motor on a free energy landscape defined by the geometry of the motor.*

I. Survey of molecular devices in cells

Artificial and natural molecular machines

- A **molecular machine**, or a **nanomachine**: a molecule or a an assembly of a few molecules, that performs mechanical movements as a consequence of specific stimuli.

The most complex molecular machines are proteins found within cells.



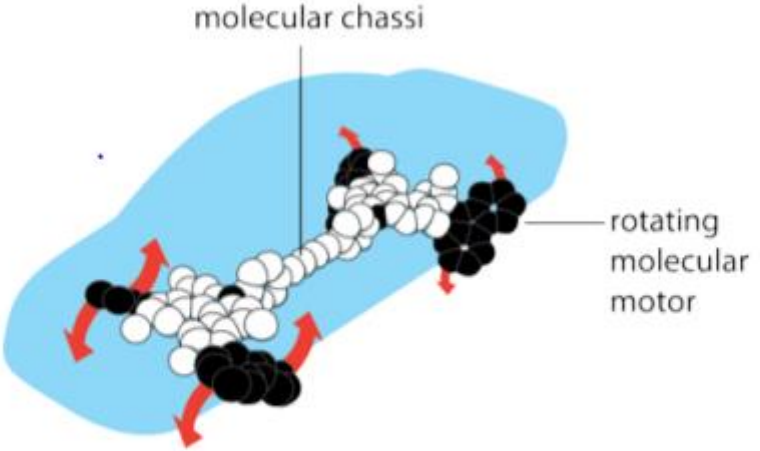


Figure 10: Feringa's proposed four-wheel-drive "nanocar".[55]

The Nobel Prize in Chemistry 2016:
Sauvage, Stoddart and Feringa
"for the design and synthesis of molecular machines".

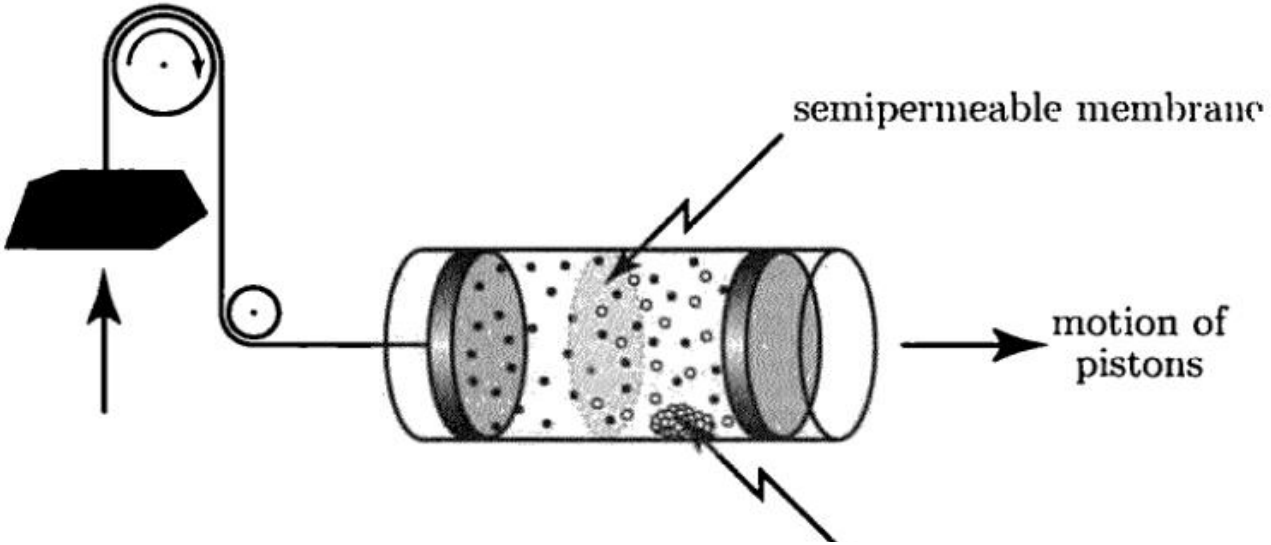
Classification of molecular devices in cells

(A) Enzymes: biological catalysts that enhance reaction rate.

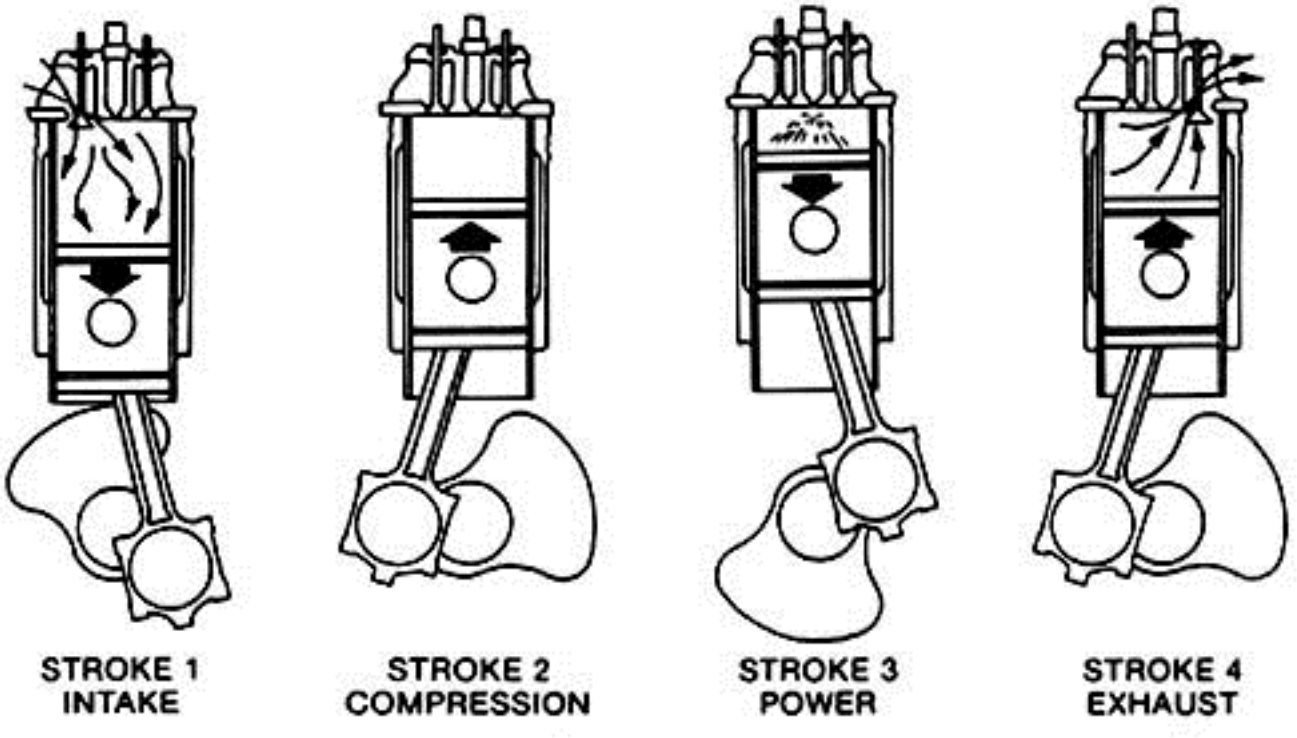
(B) Machines: reverse the natural flow of a chemical or mechanical process by coupling it to another process.

- **"One-shot" machines:** use free energy for a single motion.
- **Cyclic machines**: repeat motion cycles. Process some source of free energy such as food molecules, absorbed sunlight, concentration difference across a membrane, electrostatic potential difference.

- **“One-shot” machines**



- **Cyclic machines**



Cyclic machines are essential to Life

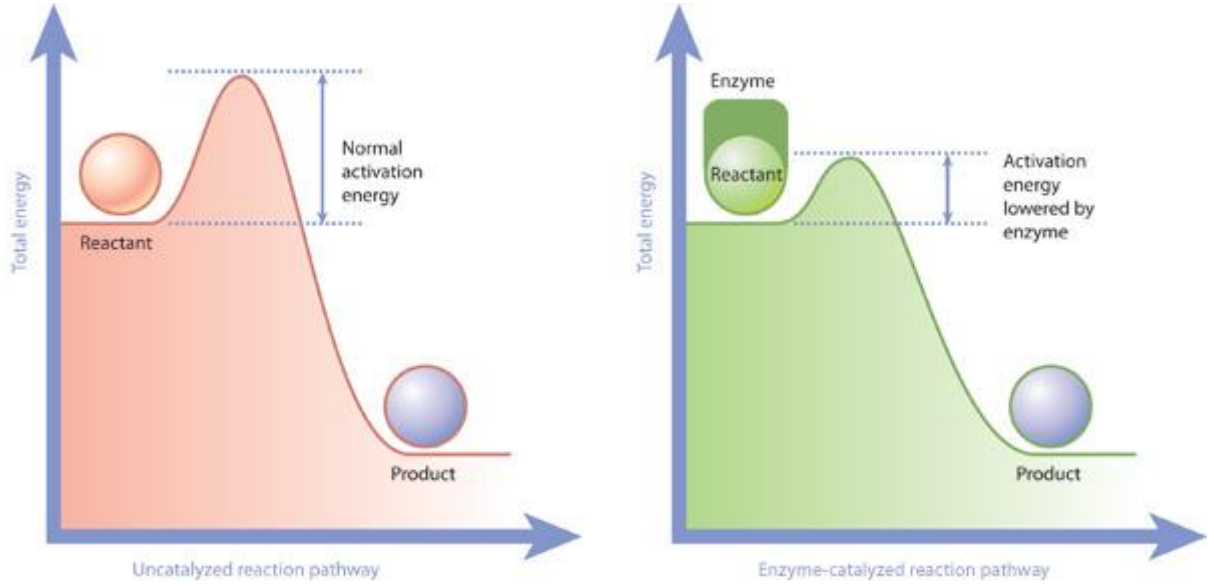
- **Motors:** transduce free energy into directional motion (case study: kinesin)
- **Pumps:** transduce free energy to create concentration gradients across membranes (ion channels).
- **Synthases:** transduce free energy to drive a chemical reaction and then synthesize some products (ATP synthase).

Let's look at a few representative classes of the molecular devices.

(A) Enzymes enhance chemical reactions

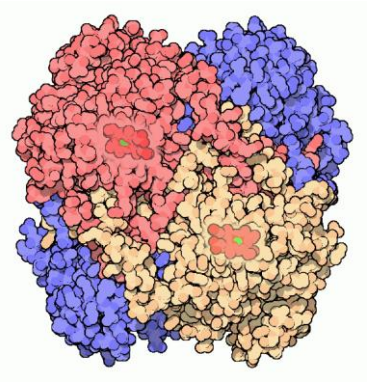
- Even if $\Delta G<0$, a reaction might still be very slow due energetic barriers.

- Good for energy storage, but how to release the energy when needed?



Enzymes display saturation kinetics

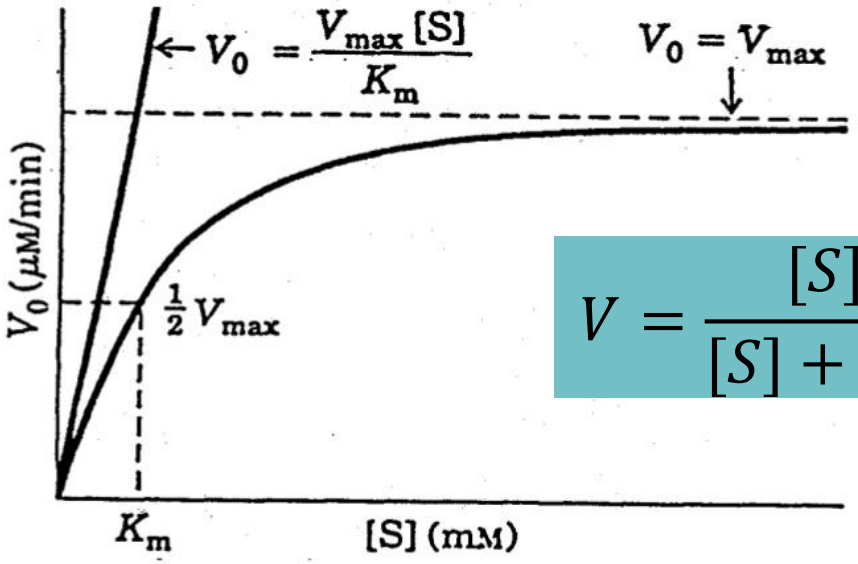
- Most enzymes are made of proteins.
- Ribozymes consist of RNA.
- Ribosome: complex of proteins with RNA.



speedup by 10^{12}

$V_{max}=10^{7}$ molec/sec

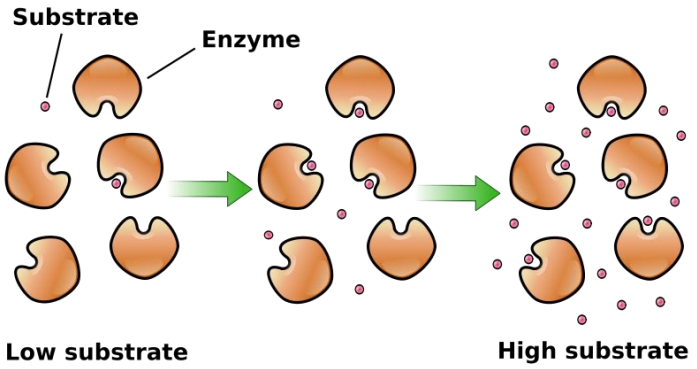
Michaelis-Menten Curve



$$V = \frac{[S]}{[S] + K_M} V_{\max}$$

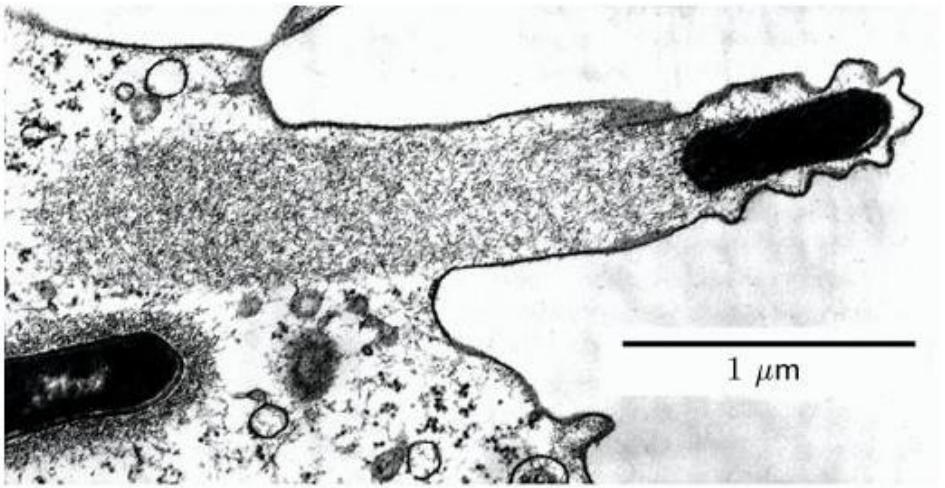
$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$

Hydrogen peroxide — Water — Oxygen



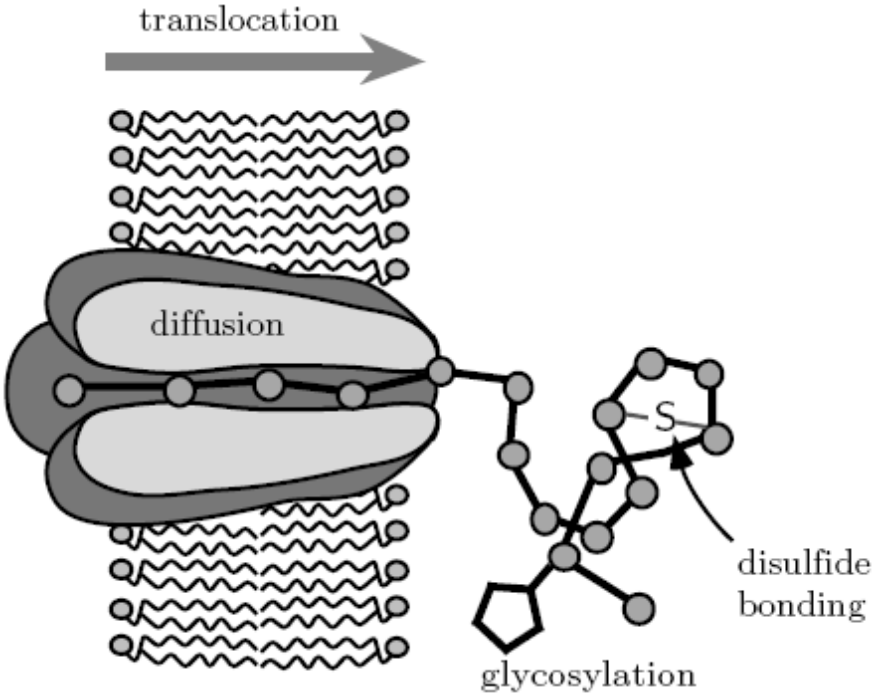
(B) One-shot motors assist in cell locomotion and spatial organization

- Polymerization



Actin polymerization from one end of an actin bundle provides force to propel a Listeria bacterium through the cell surface

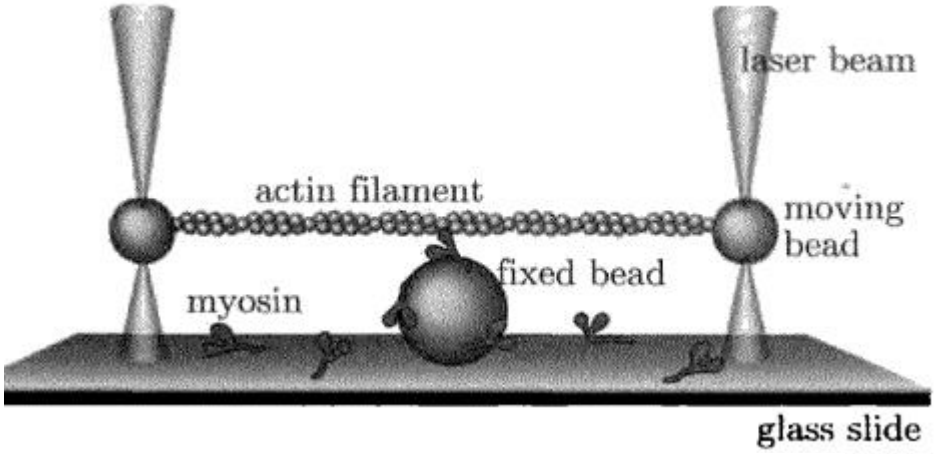
- Translocation

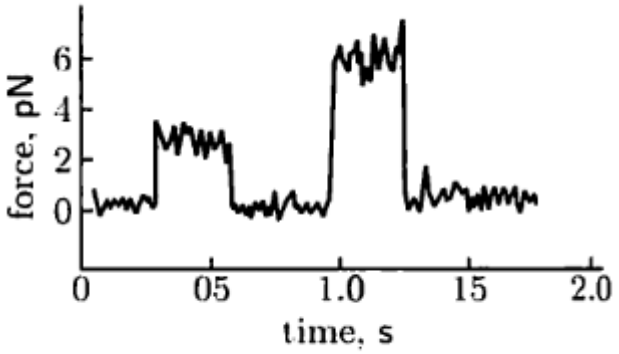


Several mechanisms can rectify (make unidirectional) the diffusive motion of protein through the pore.

All eukaryotic cells contain cyclic motors

- Molecular forces were measured by single molecule experiment.





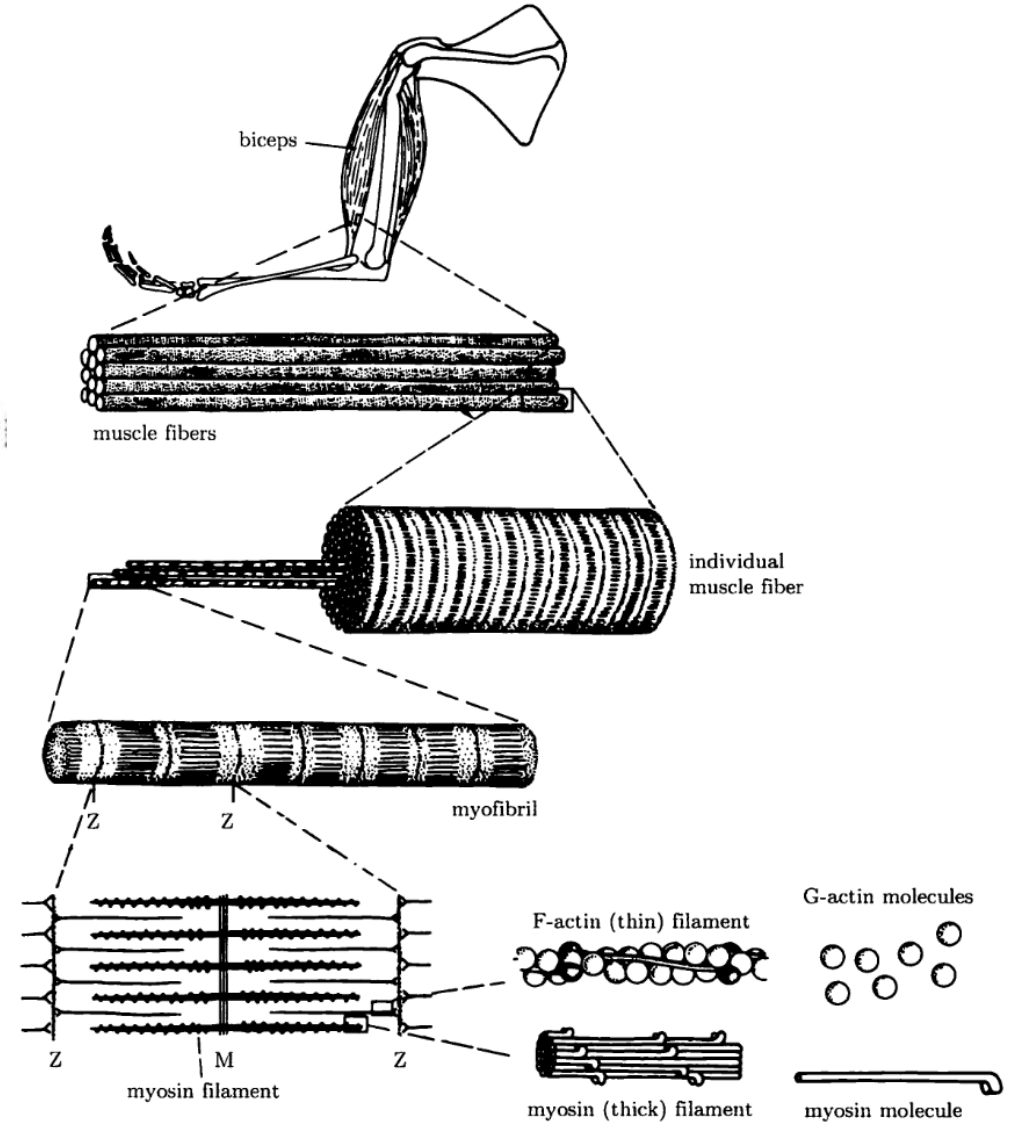


Figure 10.1: (Sketches.) Organization of skeletal muscle at successively higher magnifications. The ultimate generators of force in a myofibril (muscle cell) are bundles of myosin molecules, interleaved with actin filaments (also called F-actin). Upon activation, the myosins crawl along the actin fibers, pulling them toward the plane marked *M* and thus shortening the muscle fiber. [From McMahon, 1984.]

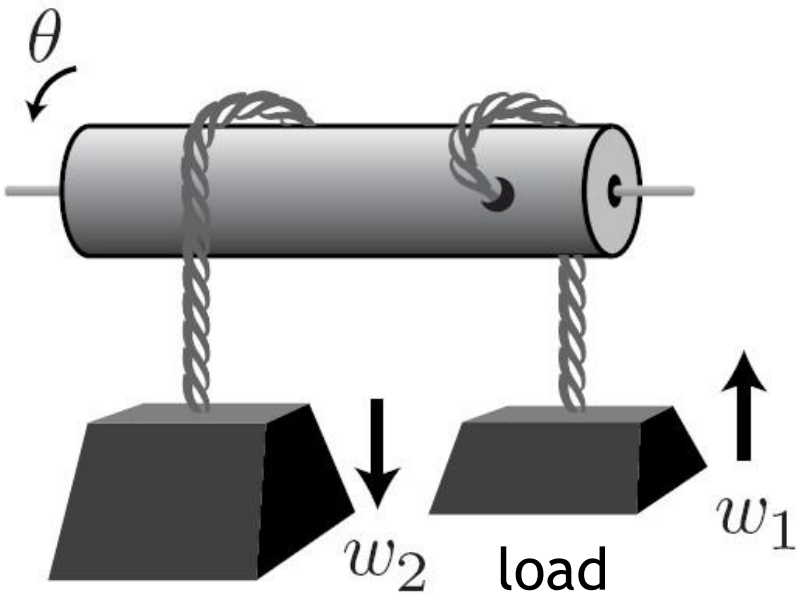
Some important cyclic machines

motor	pushes on	energy source	motion	role
Cytoskeletal motors:				
kinesin	microtubule	ATP	linear	mitosis, organelle transport
myosin	actin	ATP	linear	muscle contraction, organelle transport
dynein	microtubule	ATP	linear	ciliary beating, organelle transport, mitosis
Polymerization motors:				
actin	none	ATP	extend/shrink	cell motility
microtubule	none	GTP	extend/shrink	mitosis
dynamin	membranes	GTP	pinching	endocytosis, vesicle budding
G-proteins:				
EfG	ribosome	GTP	lever	movement of peptidyl-tRNA and mRNA in ribosomes
Rotary motors:				
F0 motor	F1 ATPase	$\Delta[H^+]$	rotary	ATP synthesis
bacterial flagellar	peptidoglycan	$\Delta[H^+]$	rotary	propulsion
Nucleic acid motors:				
polymerases	DNA/RNA	ATP	linear	template replication
helicases	DNA/RNA	ATP	linear	opening of DNA duplex
phage portal motor	DNA	ATP	linear	packing virus capsid

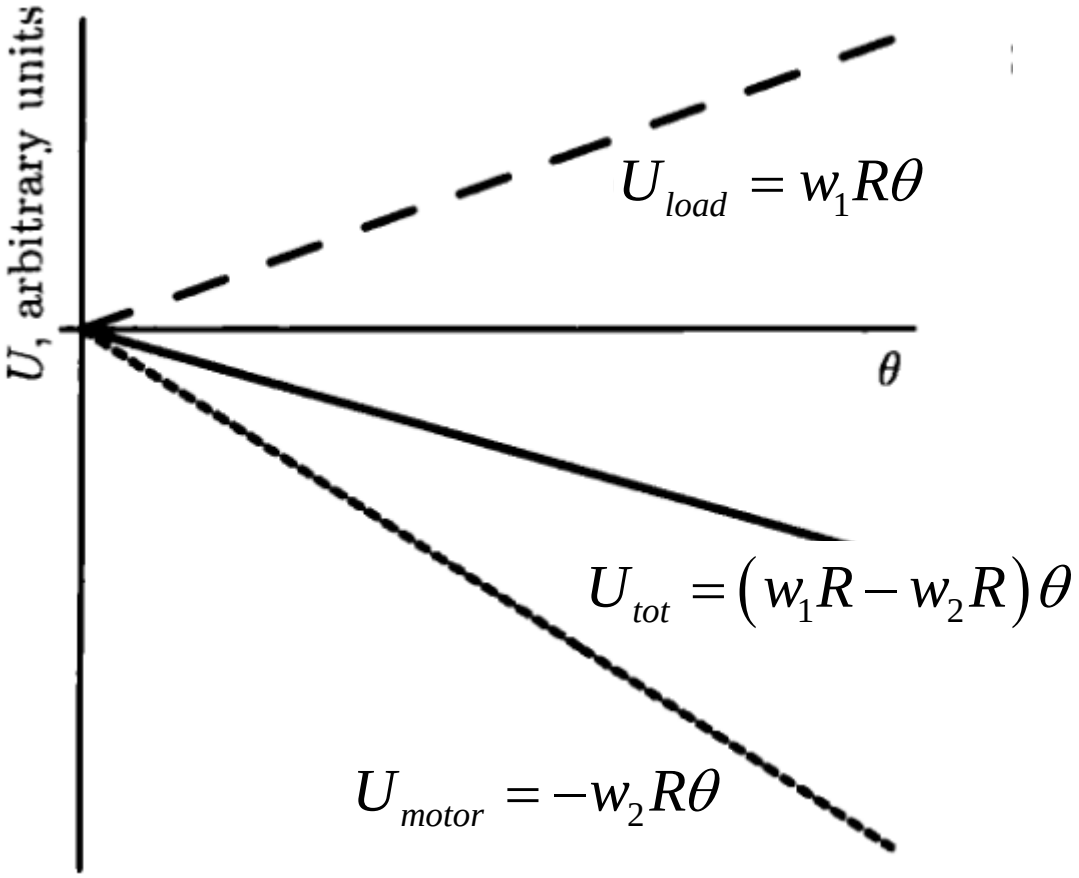
[See Vale, 1999.]

II. Mechanical machines

Macroscopic machines can be described by an energy landscape



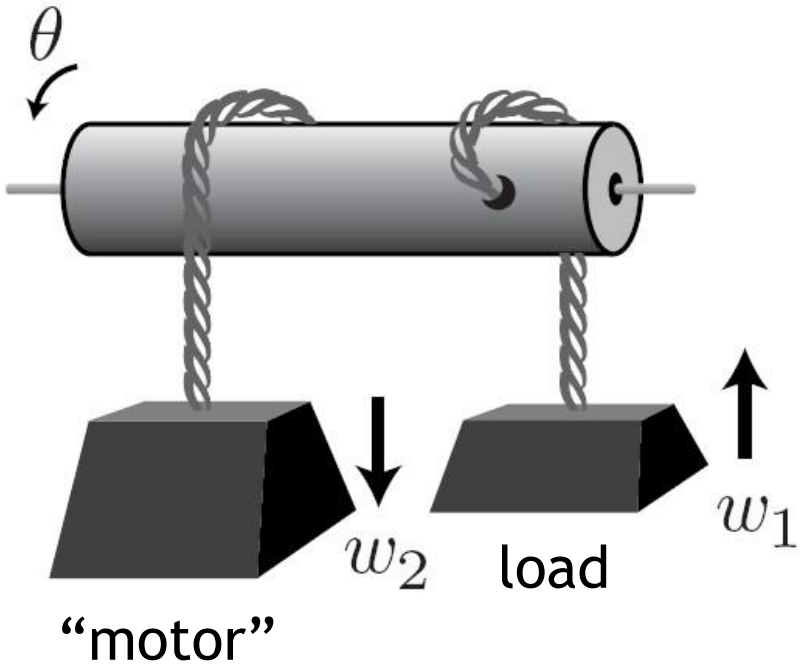
"motor"

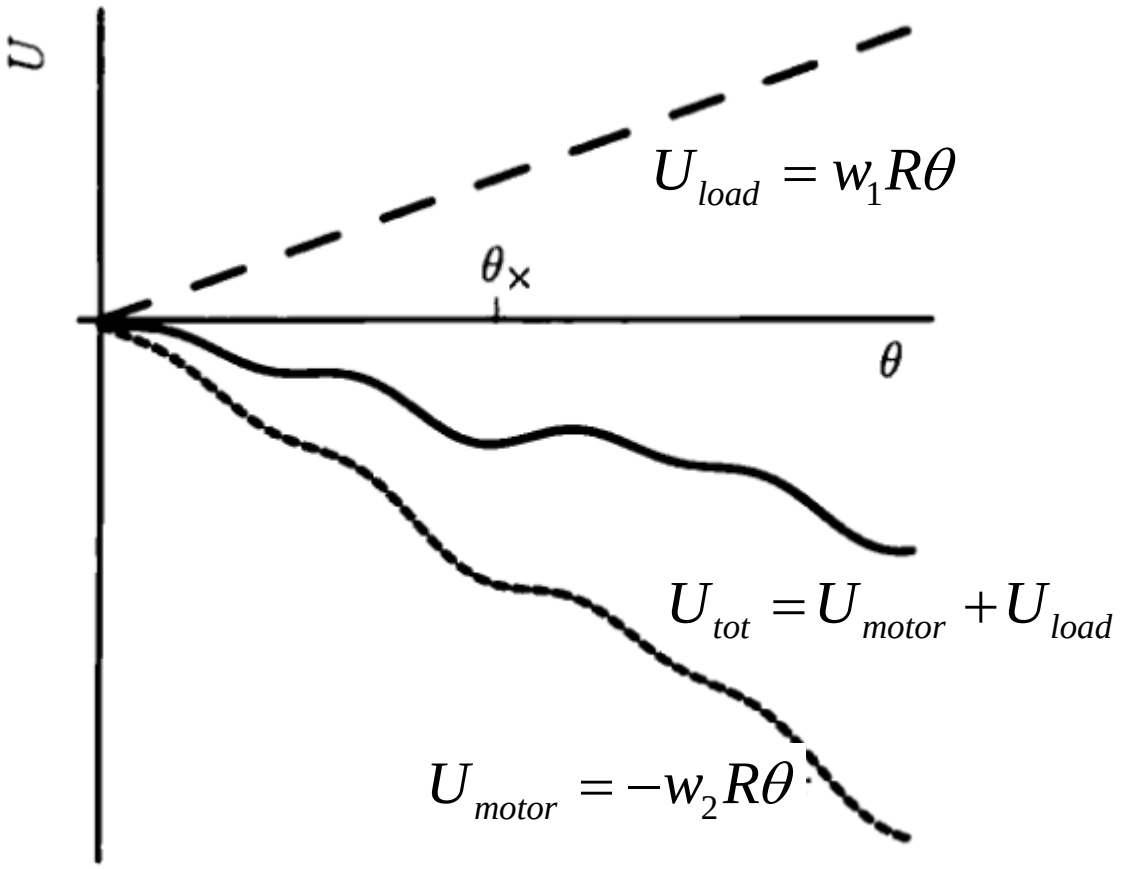


The movement of macroscopic machines is totally determined by the energy landscape.

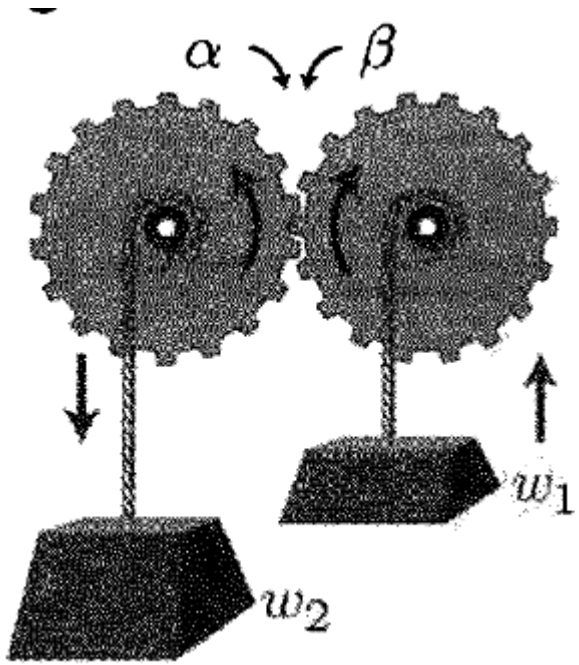
Overdamping macroscopic machines cannot step past energy barriers

imperfect axis
(e.g. friction)



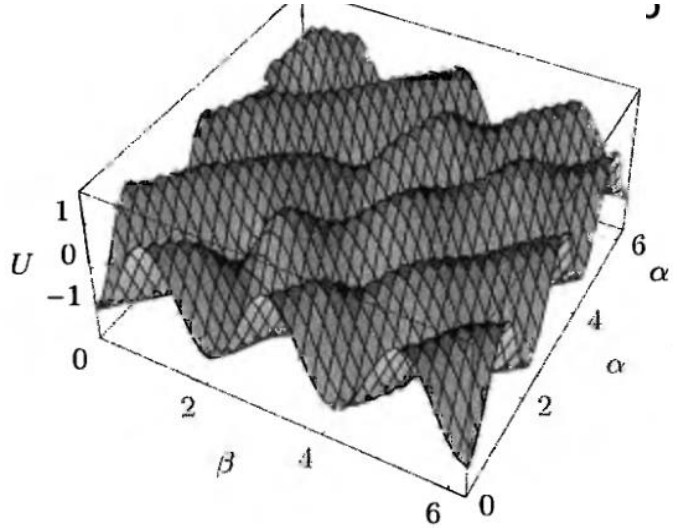


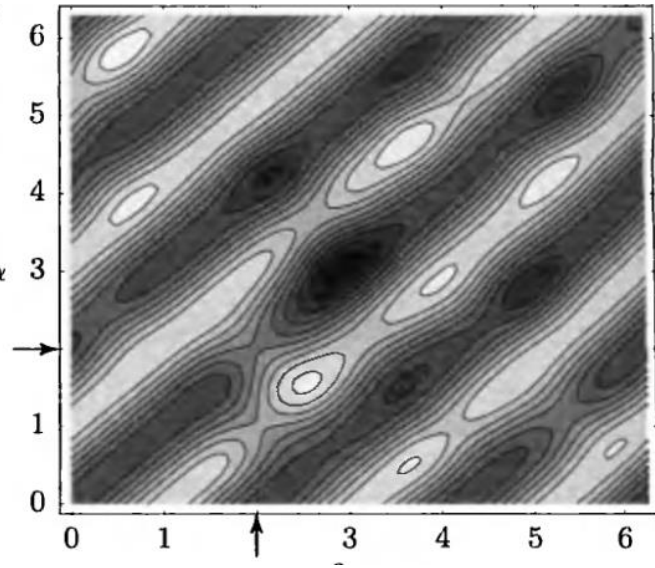
Some machines have more degrees of freedom



Gears: the angular variables both decrease as w_2 lifts w_1.

- The angles are constrained to “valleys.
- Slipping: imperfections (e.g. irregular tooth) may lead to hopping.

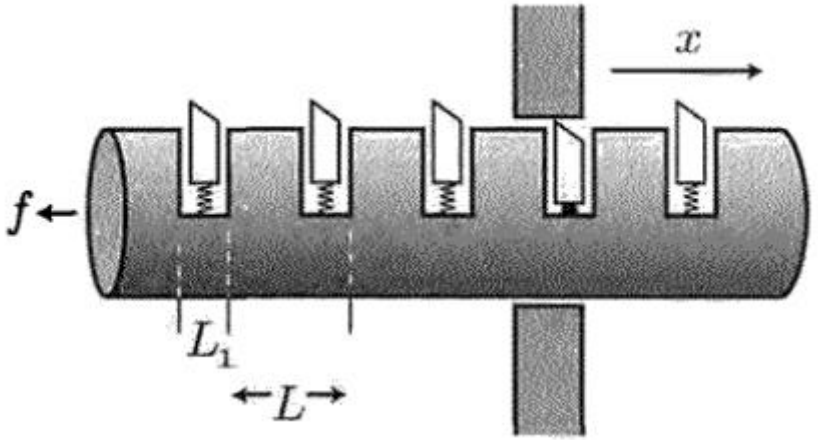




constraint: $\alpha = \beta + \frac{n}{N} \cdot 2\pi$

- The machine stops if:
  - $w_2 = w_1$.
  - Slipping rate too large.

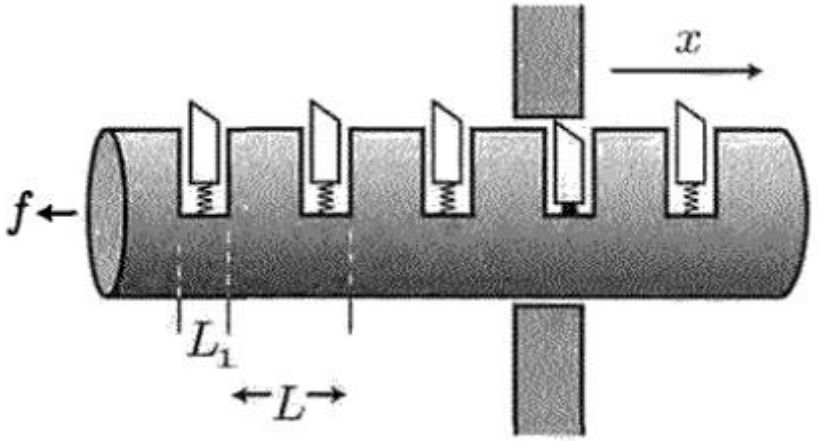
Microscopic machines can step past energy barriers



- A nanometric rod makes a one-way trip to the right driven by random thermal fluctuations.
- Moving to the left is impossible by sliding bolts, which can move down to allow rightward motion;
- It can move against external "load" **f.**

- Where does the work against **f** comes from?
- What happens if one makes the shaft into a circle?

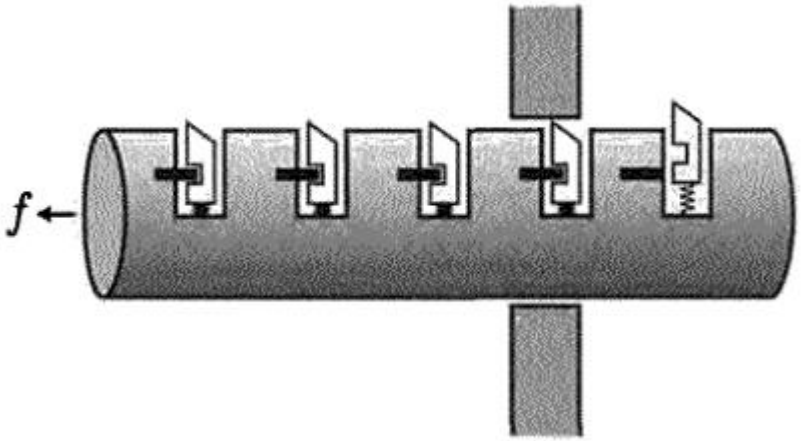
Can one extract work from thermal fluctuations?



- Rod cannot move at all unless $\varepsilon \sim k_B T$.
- But then bolts will spontaneously retract from time to time…
- Leftward thermal kick at just such a moment and rod steps leftwards.

- Where does the work against **f** comes from?
- What happens if one makes the shaft into a circle?

Can we save the second law of thermodynamics?

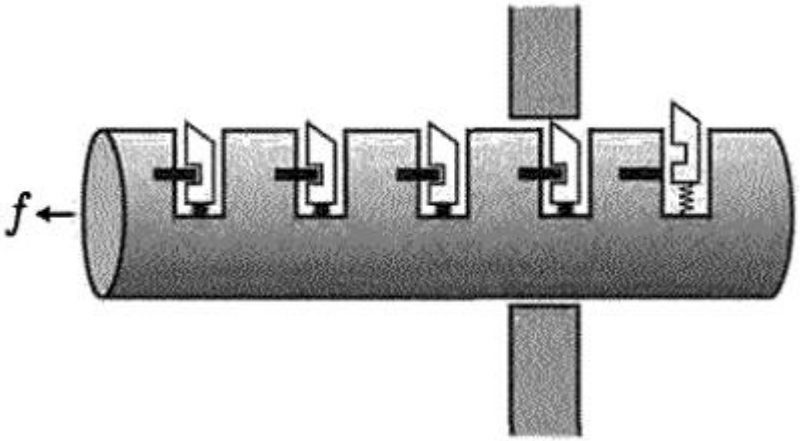


- Bolts are tied down on the left side,

then released as they emerge on the right.

- Where does the work against **f** comes from?
- What happens if one makes the shaft into a circle?

Can we save the second law of thermodynamics?



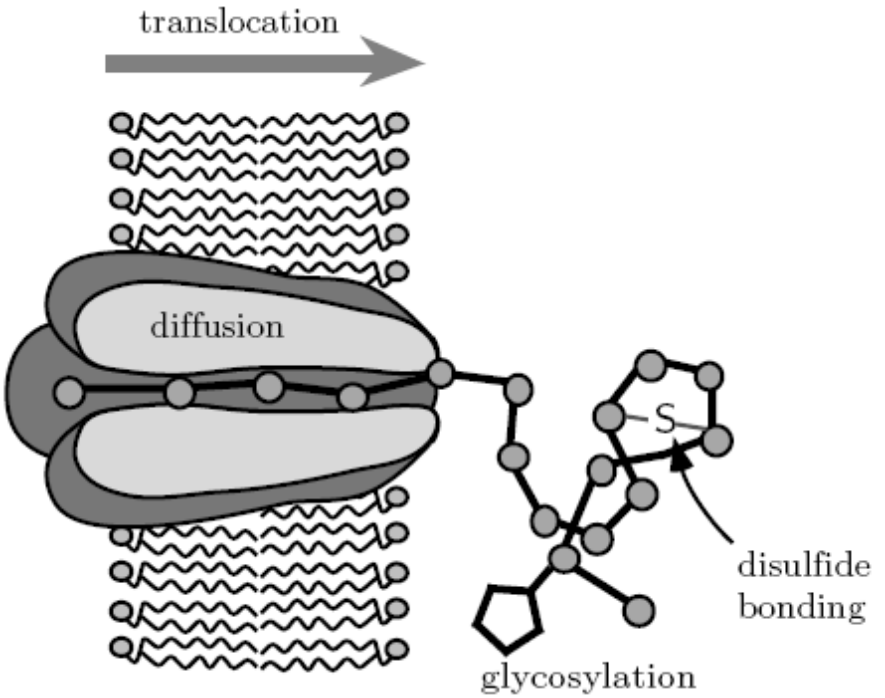
- Where does the work against **f** comes from?

  -- Potential energy stored in the compressed springs on the left.

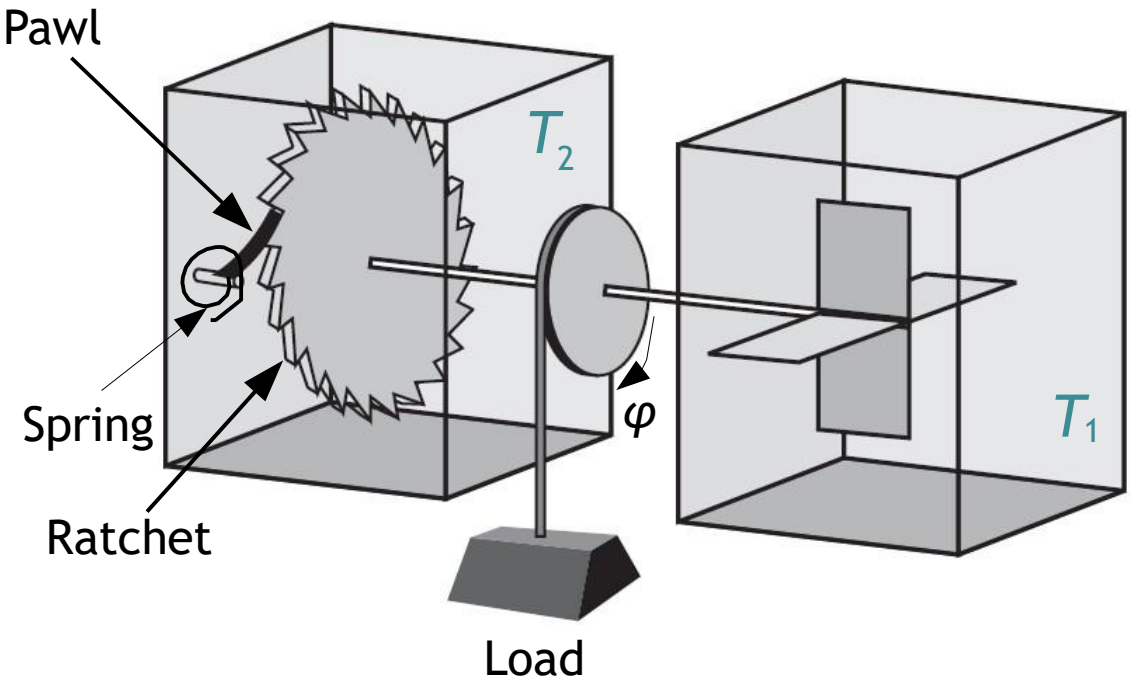
- What happens if one makes the shaft into a circle?

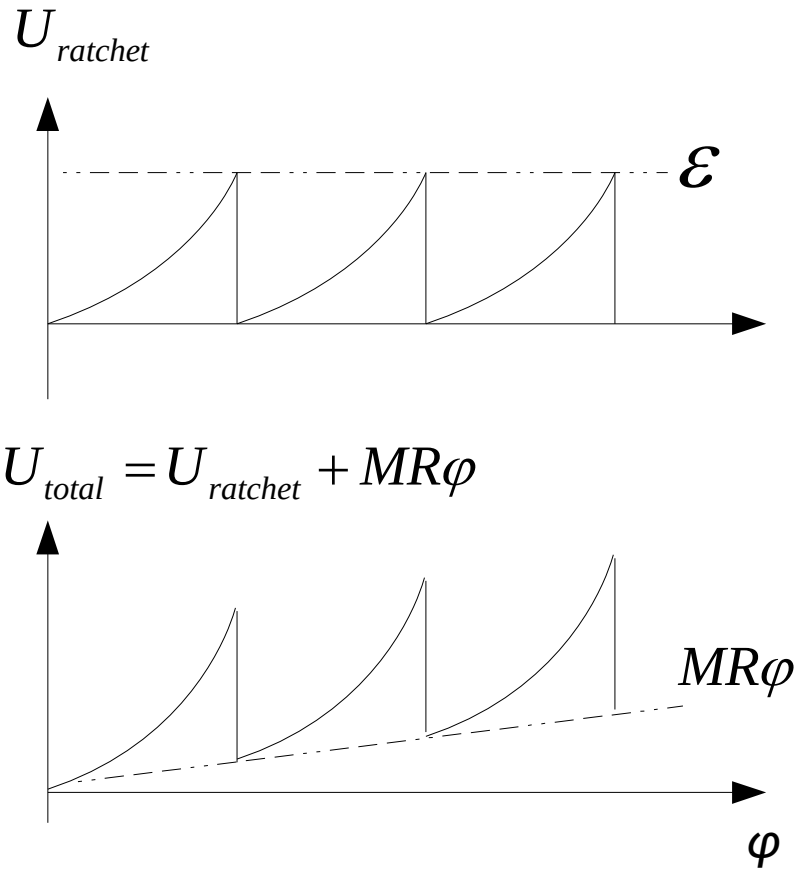
  -- All springs are released and the motion stops.

This is a toy model for model for molecular translocation



Feynman's ratchet and pawl





Q: Can the small load be lifted if $T_1 = T_2 = T$?

A: May look quite possible, but 2nd law of thermodynamics:

(Heat cannot be converted to work spontaneously) prohibits this.

- **load = 0:**

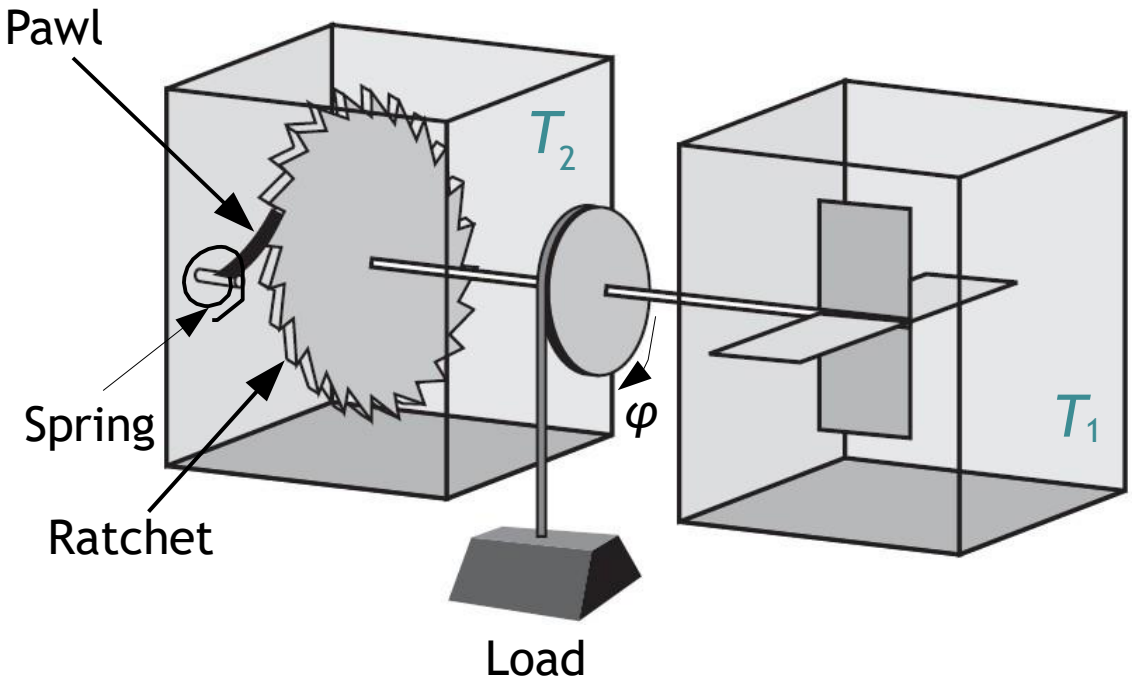
$$P\left(\begin{array}{l}\text{pawl pulled over tooth}\\ \text{by vanes}\end{array}\right)=\exp\left(-\frac{\varepsilon}{k_B T_1}\right)$$

- But also

$$P\left(\begin{array}{l}\text{pawl jumps}\\ \text{spontaneously}\end{array}\right)=\exp\left(-\frac{\varepsilon}{k_B T_2}\right)$$

$$\text{Rate}\left(\begin{array}{l}\text{pawl is up and wheel}\\ \text{can turn backwards freely}\end{array}\right)=\text{Rate}\left(\begin{array}{l}\text{enough energy}\\ \text{to turn wheel forward}\end{array}\right)$$

→ no net rotations.



- Q: what if load > 0?
- A: work → heat

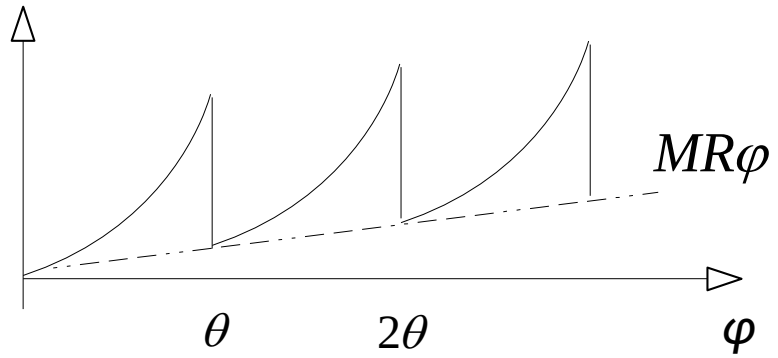
Ratchet can be made reversible if $T_1 > T_2$

- Forward rotation: $R_{\text{forward}} = \nu_0 \exp\left(-\frac{\varepsilon + MR\theta}{k_B T_1}\right)$
  - Heat from bath 1: $Q_1 = \varepsilon + MR\theta \rightarrow$ work $MR\theta +$ pawl ε
- Backward rotation: $R_{\text{backward}} = \nu_0 \exp\left(-\frac{\varepsilon}{k_B T_2}\right)$
  - Heat from bath 2: $Q_2 = \varepsilon \rightarrow$ work realeased $MR\theta,$ to the vanes $\varepsilon + MR\theta$
- Reversibility requires detailed balance $R_{\text{forward}} = R_{\text{backward}} \rightarrow \frac{Q_1}{T_1} = \frac{Q_2}{T_2}$
- The efficiency is

$$\eta = \frac{MR\theta}{Q_1} = \frac{Q_1 - Q_2}{Q_1}$$

$$\eta = 1 - \frac{T_2}{T_1} \rightarrow \text{maximal (Carnot)}$$

$$U_{total} = U_{ratchet} + MR\varphi$$

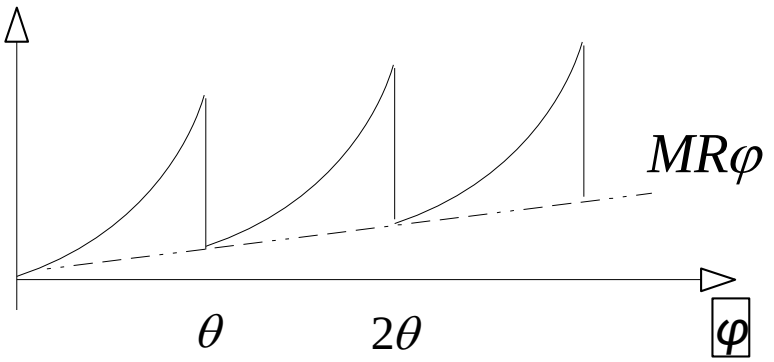


Ratchet is irreversible if $T_1 = T_2 = T$

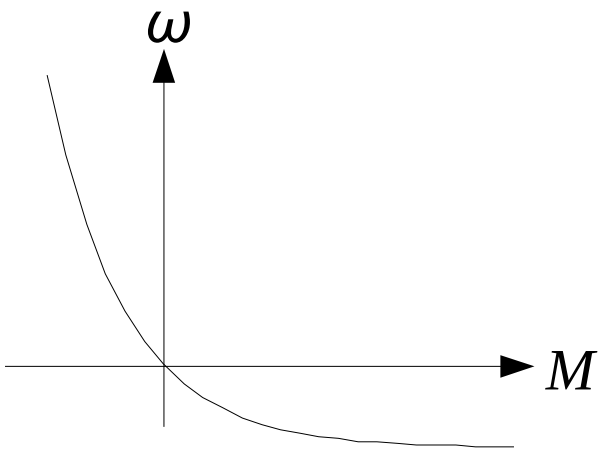
- Forward rotation: $R_{\text{forward}} = \nu_0 \exp\left(-\frac{\varepsilon + MR\theta}{k_B T_1}\right)$
- Backward rotation: $R_{\text{backward}} = \nu_0 \exp\left(-\frac{\varepsilon}{k_B T_2}\right)$

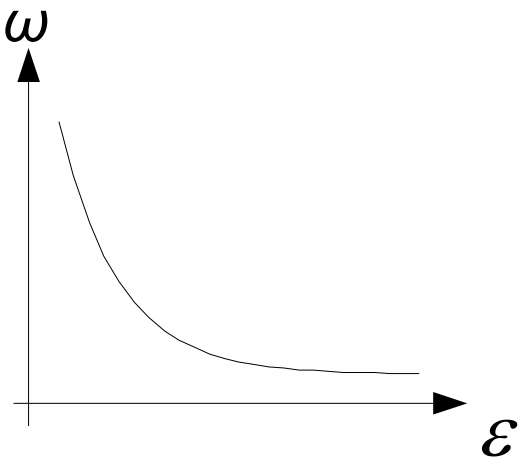
→ Ratchet moves backward

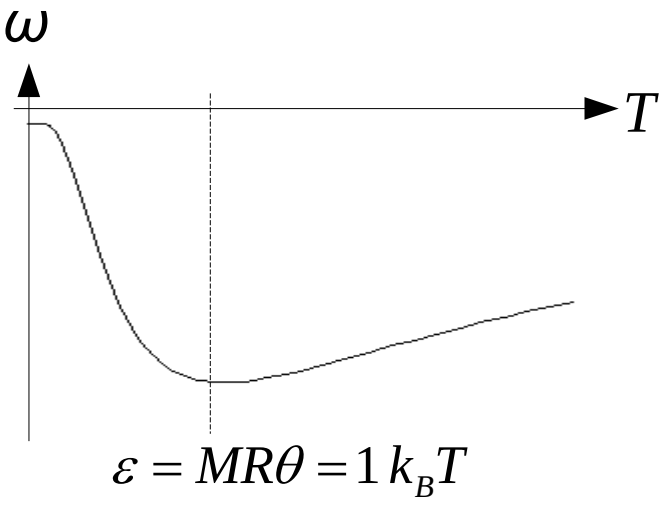
$$U_{total} = U_{ratchet} + MR\varphi$$



$$\omega = \left\langle \frac{d\varphi}{dt} \right\rangle = \theta\left(R_{\text{forward}} - R_{\text{backward}}\right) = \theta\nu_0 \exp\left(-\frac{\varepsilon}{k_B T}\right)\left[\exp\left(-\frac{MR\theta}{k_B T}\right) - 1\right]$$

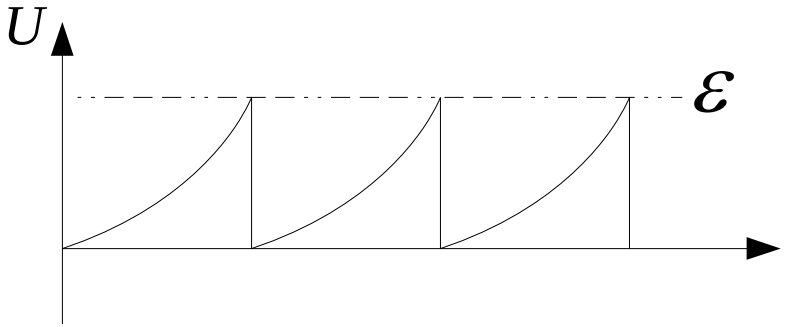




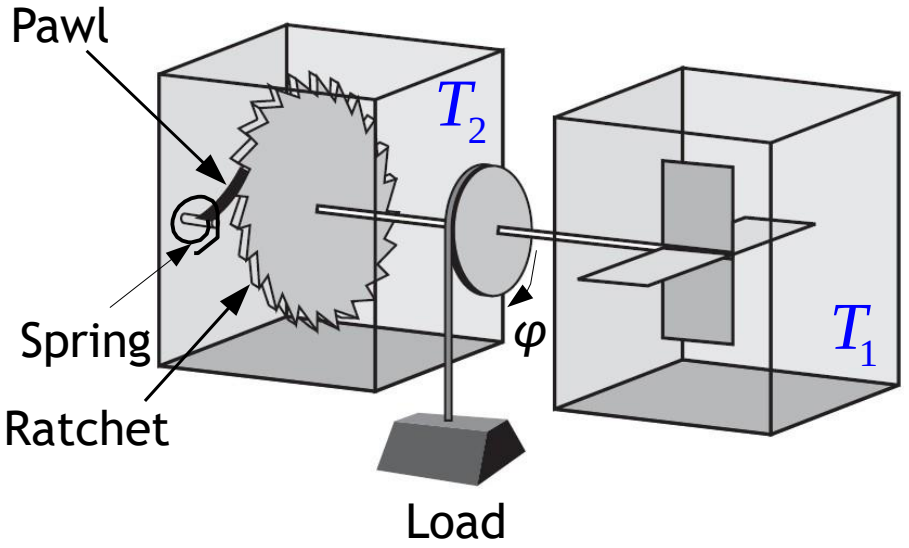


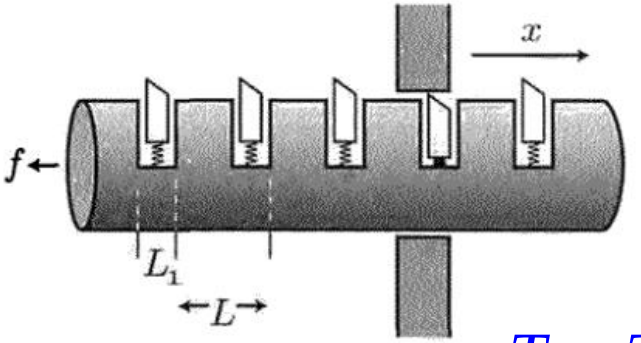
Summary: necessary conditions for directional motion

- Asymmetric potential:

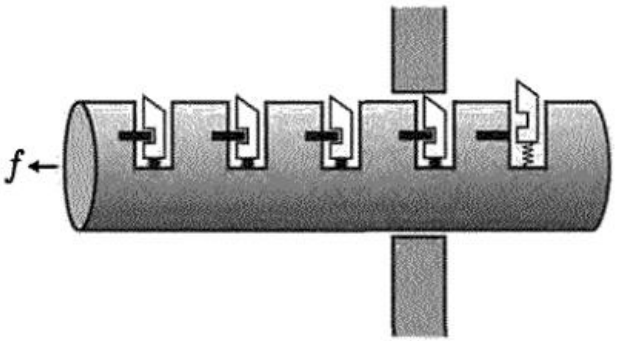


- Break detailed balance: $R_{\text{forward}} \neq R_{\text{backward}}$

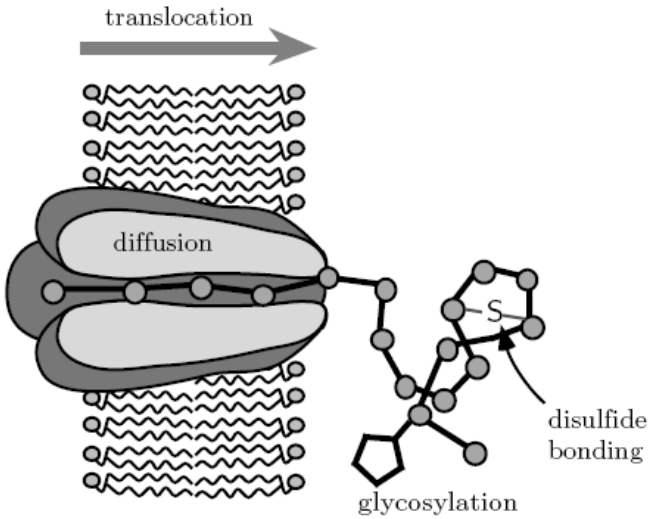


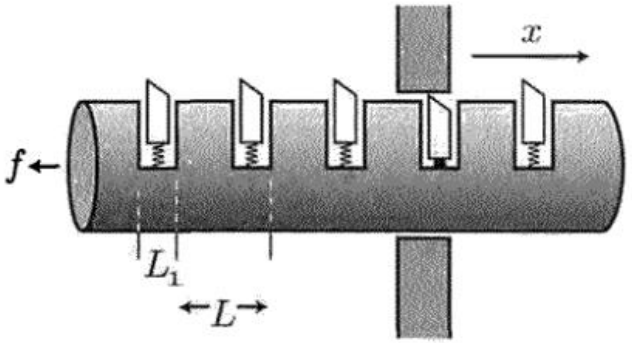


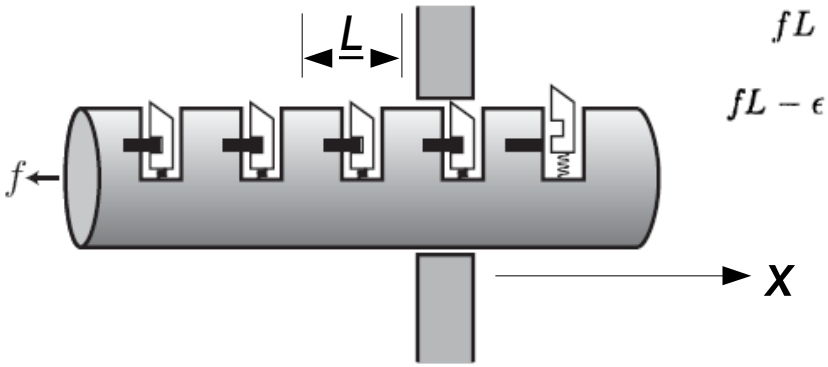
$T_1 = T_2$

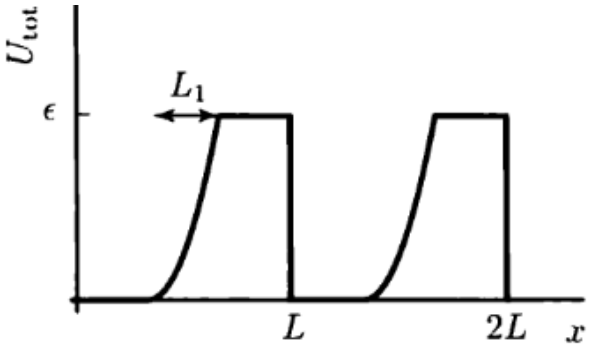


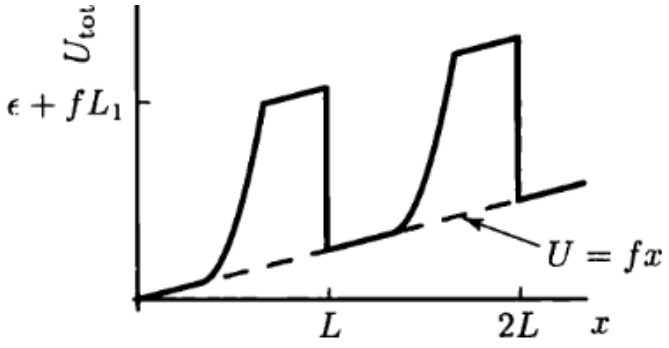
Modeling molecular machines: qualitative picture

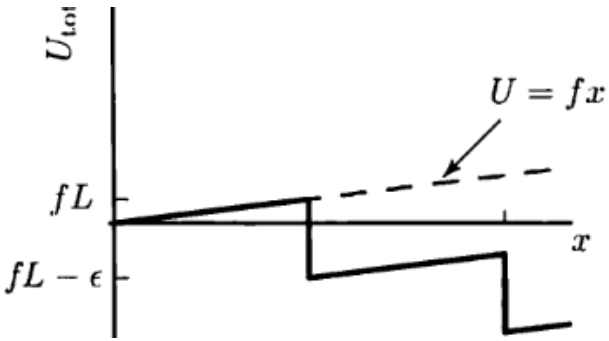


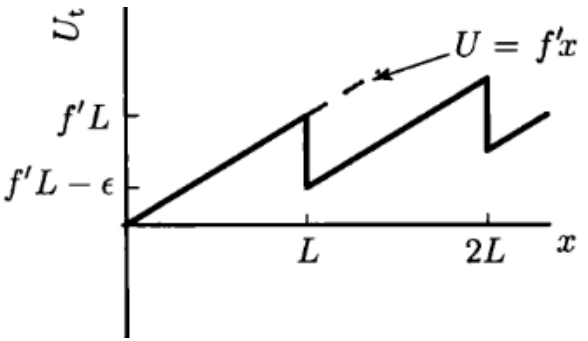








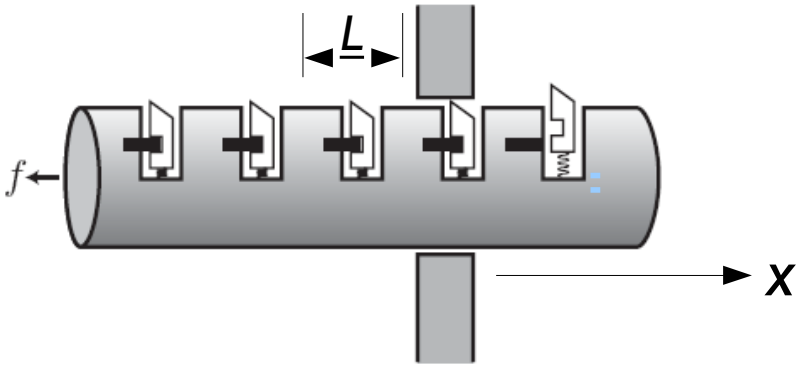


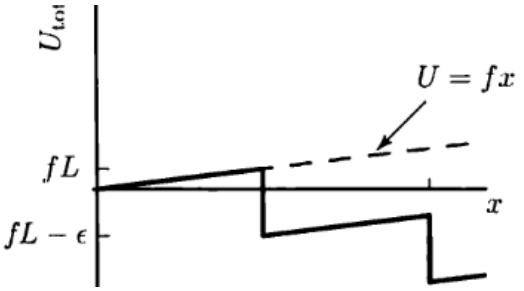


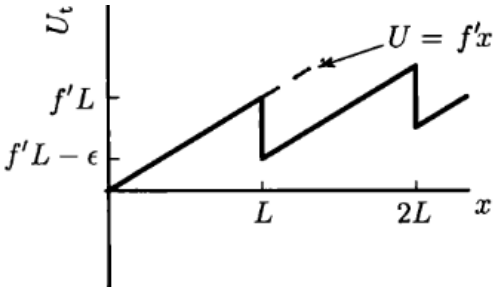
Low load: forward $f < \varepsilon / L$

High load: backward $f > \varepsilon / L$

Modeling molecular machines: qualitative picture







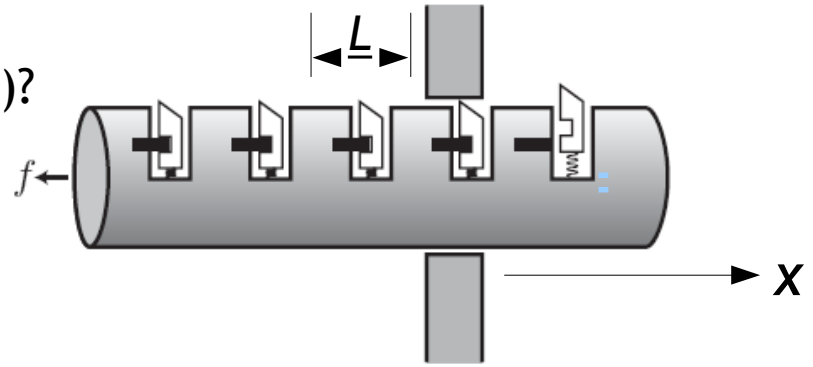
- To move right need a thermal fluctuation of $\delta E = f \cdot L$
- If $\varepsilon \gg k_B T$ then the leftwards motion is negligible.
- If $f = 0$ rod diffuses freely between steps with

$$t_{\text{step}} = \frac{L^2}{D} \quad \text{and velocity} \quad v = \frac{L}{t_{\text{step}}} = \frac{L}{D}$$

- What happens when $f \neq 0$?

Mathematical framework: Smoluchowski equation

- Think of large set of identical rods:
- What is the P(x,t) = prob. ratchet is in (x,x+dx)?
- Ratchet diffuse according to Fick's law:



$$j_D = -D\frac{\partial P}{\partial x}$$

- Rods drift in the force field:

$$j_v = -\left(\frac{D}{k_B T}\right) P \frac{\partial U}{\partial x}$$

$$\frac{D}{k_B T} = \mu$$

Einstein's relation

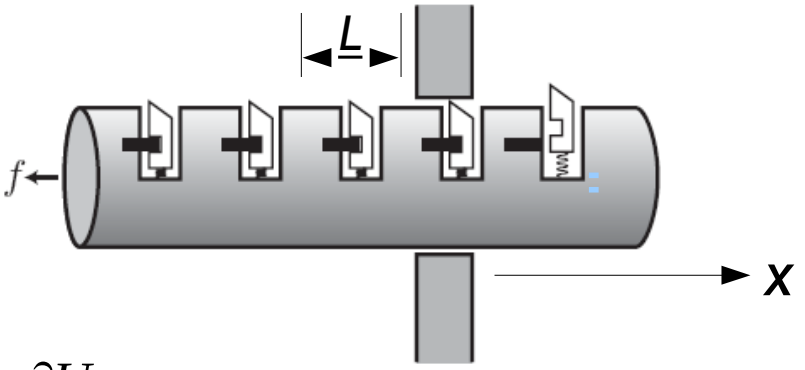
- The total current is:

$$j = j_D + j_v = -D\frac{\partial U}{\partial x} - \left(\frac{D}{k_B T}\right) P \frac{\partial U}{\partial x}$$

Fokker-Planck and Smoluchowski equations

- Number conservation (continuity equation):

$$\frac{\partial j}{\partial x} + \frac{\partial P}{\partial t} = 0$$



- With current :

$$j = j_D + j_v = -D\frac{\partial U}{\partial x} - \left(\frac{D}{k_B T}\right) P \frac{\partial U}{\partial x}$$

→ **Fokker-Planck equation**

$$\frac{\partial P}{\partial t} + D\frac{\partial}{\partial x}\left(\frac{\partial P}{\partial x} + \frac{P}{k_B T}\frac{\partial U}{\partial x}\right) = 0$$

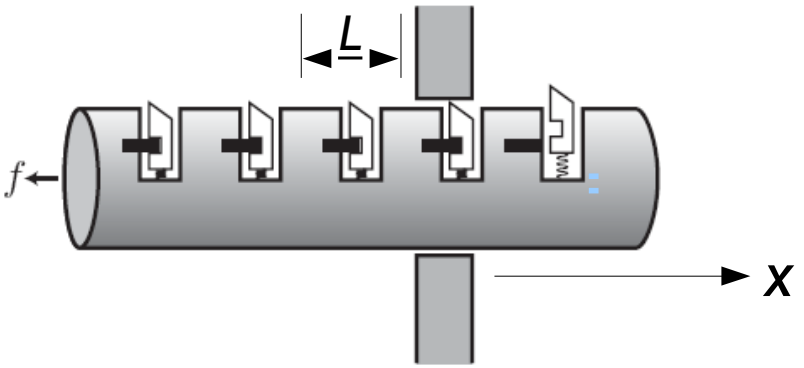
- At steady-state this is **Smoluchowski's equation**

$$\frac{\partial P}{\partial t} = 0 \quad \rightarrow j = -D\left(\frac{\partial P}{\partial x} + \frac{P}{k_B T}\frac{\partial U}{\partial x}\right) = \text{const.}$$

Steady-state is solution to Smoluchowski equation

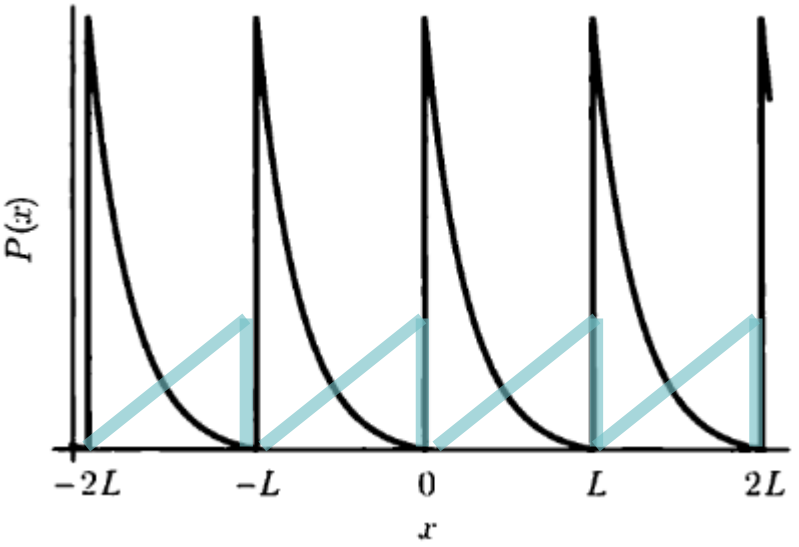
- No net current:

$$j = -D\left(\frac{\partial P}{\partial x} + \frac{P}{k_B T}\frac{\partial U}{\partial x}\right) = 0$$



- Gives equilibrium Boltzmann distribution:

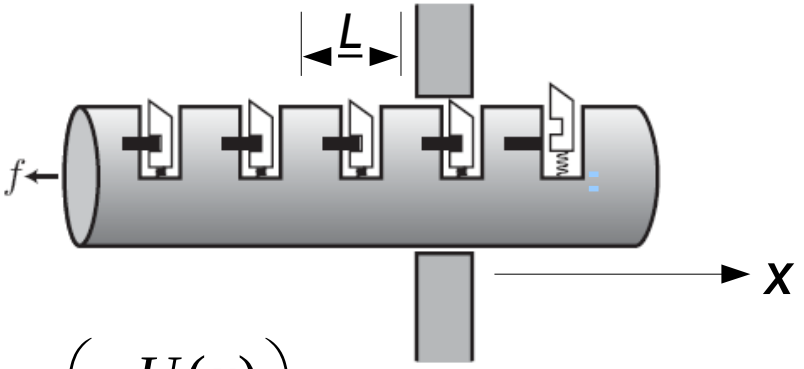
$$\frac{\partial P}{P} = -\frac{\partial U}{k_B T} \quad \Rightarrow \quad P(x,t) = \frac{1}{Z}\exp\left(-\frac{U(x)}{k_B T}\right).$$



The general steady state solution

- Steady-state

$$j = -D\left(\frac{\partial P}{\partial x} + \frac{P}{k_B T}\frac{\partial U}{\partial x}\right) = \text{const.}$$



Define $P(x) = P_0(x)\,p(x)$ where $P_0(x) \equiv \exp\left(-\frac{U(x)}{k_B T}\right)$

$$\frac{\partial P}{\partial x} + \frac{P}{k_B T}\frac{\partial U}{\partial x} = P_0\frac{\partial p}{\partial x} = -\frac{j}{D} \quad \Rightarrow \quad \frac{\partial p}{\partial x} = -\frac{j}{D}\exp\left(\frac{U(x)}{k_B T}\right)$$

$$p(x) = C - \frac{j}{D}\int_0^x \exp\left(\frac{U(y)}{k_B T}\right)dy$$

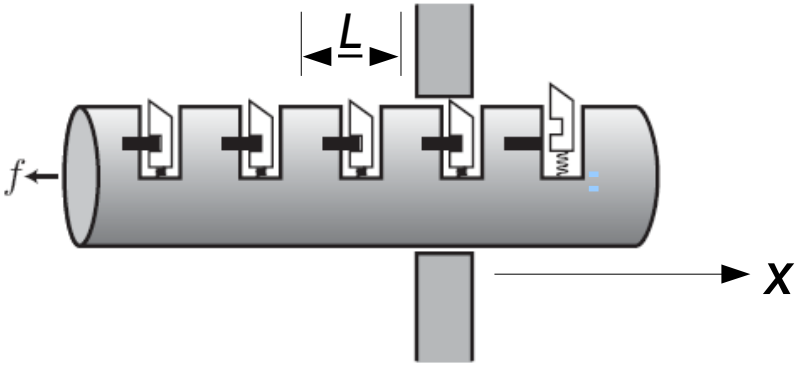
$$P(x) = \exp\left(-\frac{U(x)}{k_B T}\right)\left(C - \frac{j}{D}\int_0^x \exp\left(\frac{U(y)}{k_B T}\right)dy\right)$$

Solving the molecular ratchet

- Steady-state

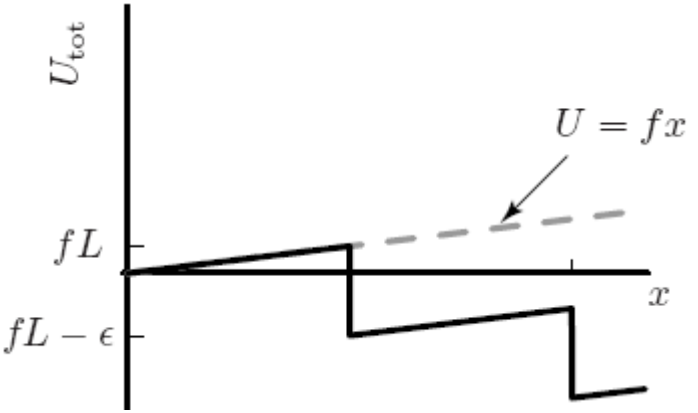
$$p(x) = C - \frac{j}{D}\int_0^x \exp\left(\frac{U(y)}{k_B T}\right) dy$$

$$P(x) = \exp\left(-\frac{U(x)}{k_B T}\right)\left(C - \frac{j}{D}\int_0^x \exp\left(\frac{U(y)}{k_B T}\right) dy\right)$$



- Sawtooth potential

$$U(x) = f \cdot x \quad \text{for} \quad 0 \le x \le L$$



- "Perfect" ratchet – because S-S bond strong

$$\varepsilon \gg k_B T$$

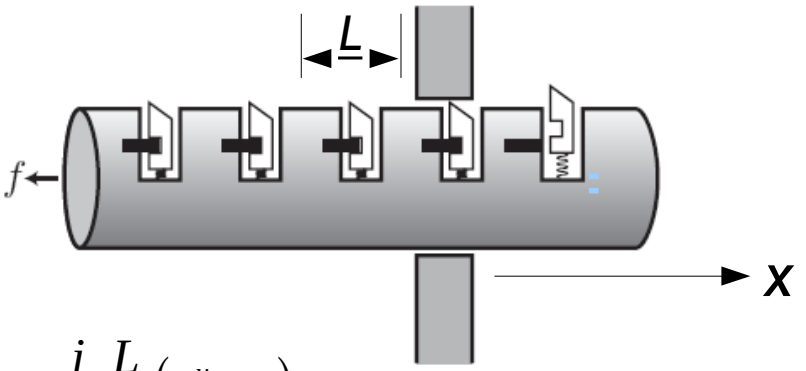
- Therefore: (1) $j > 0$ protein moves rightwards.

(2) $P(L) = 0$ because it cannot jump back.

Solving the molecular ratchet

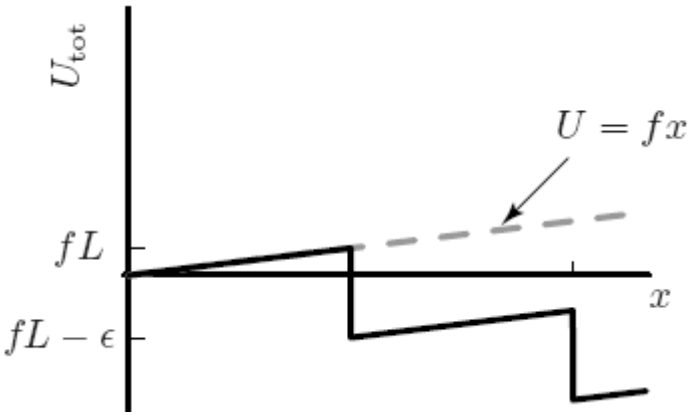
- Let's scale the variables:

$$u = \frac{f \cdot x}{k_B T}; \quad \bar{\varepsilon} = \frac{\varepsilon}{k_B T}; \quad F = \frac{f \cdot L}{k_B T};$$



$$p(x) = C - \frac{j}{D}\int_0^x \exp\left(\frac{U(y)}{k_B T}\right) dy = C - \frac{j}{D}\frac{L}{F}\int_0^u \mathrm{e}^{u'}\, du' = C - \frac{j}{D}\frac{L}{F}\left(e^u - 1\right)$$

$$P(x) = \mathrm{e}^{-u}\, p(x) = \left(C + B\right)e^{-u} - B \quad \text{where } \mathrm{B} \equiv \frac{j}{D}\frac{L}{F}$$

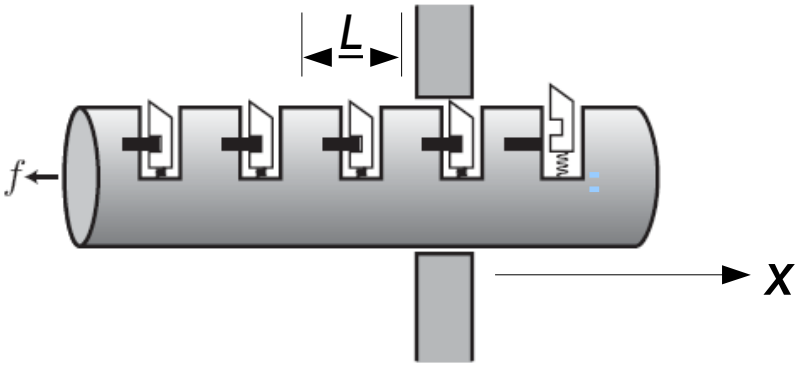


- Boundary condition

$$P(L) = \left(C + B\right)e^{-F} - B = 0 \quad \Rightarrow \quad C + B = Be^{F}$$

$$\Rightarrow \quad P(x) = B\left(e^{F-u} - 1\right)$$

$$u = \frac{f \cdot x}{k_B T}; \quad \bar{\varepsilon} = \frac{\varepsilon}{k_B T}; \quad F = \frac{f \cdot L}{k_B T};$$



- Normalization in [0,L]

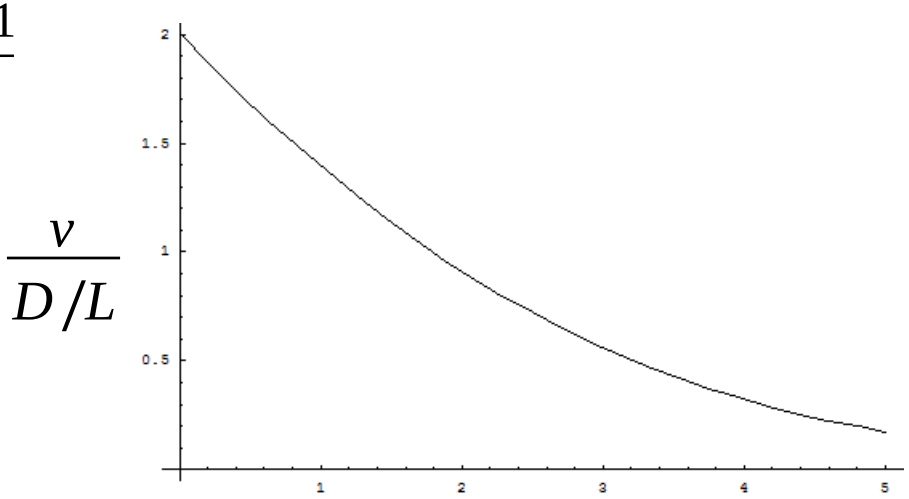
$$\int_0^L P(x)dx = 1 \quad \Rightarrow \quad \frac{j}{D}\left(\frac{L}{F}\right)^2 \int_0^F \left(e^{F-u} - 1\right) du = 1$$

$$\Rightarrow \quad \frac{j}{D}\left(\frac{L}{F}\right)^2 \left(e^F - F - 1\right) = 1 \quad \Rightarrow \quad j = \left(\frac{D}{L^2}\right) \frac{F^2}{e^F - F - 1}$$

- Time to move on bolt $\tau = \frac{1}{j} = \left(\frac{L^2}{D}\right) \frac{e^F - F - 1}{F^2}$

- Average speed

$$v = \frac{L}{\tau} = j \cdot L = \left(\frac{D}{L}\right) \frac{F^2}{e^F - F - 1}$$



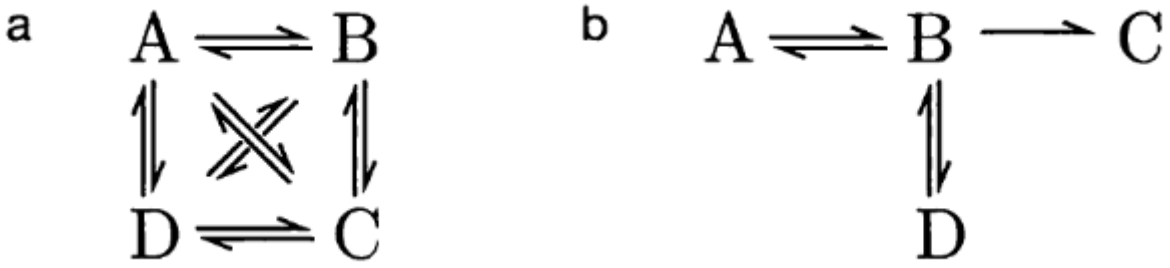
III. Molecular implementation of mechanical principles

What's missing for molecular machines?

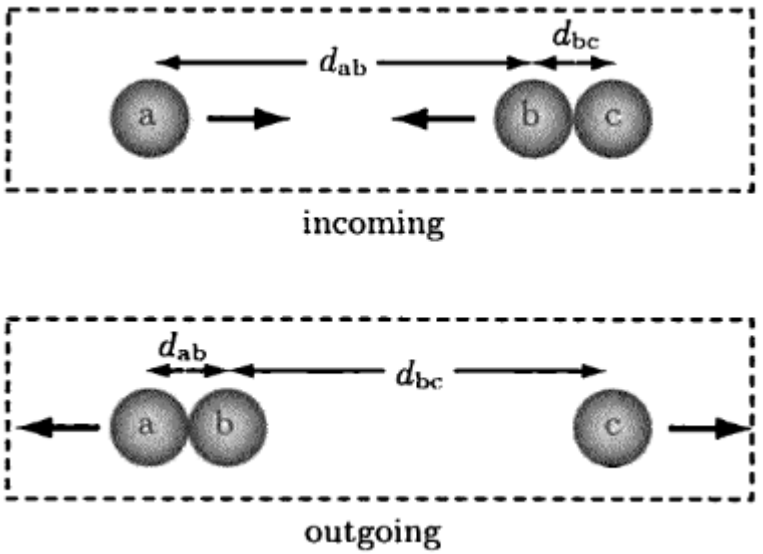
- How can we apply these macroscopic ideas to single molecules?
- What is the cyclic machine that eats chemical energy?
- Where are the experiments?

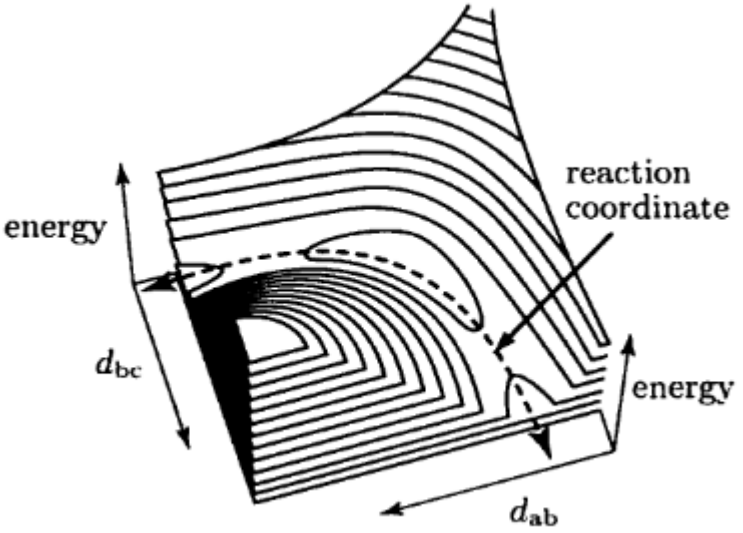
Reaction coordinate simplifies description of a chemical reactions

- Chemical reactions can be regarded as **transitions** between different molecular configurations. To move between configurations atoms **rearrange** their relative positions.
- The “states” are locally stable points in a multi-dimensional configuration space (local minima of free energy).
- Thermal motion can push molecules to transit between states.



Chemical reactions are random walks on free energy landscape in space of molecular configurations





$$H_2 + H \rightleftharpoons H + H_2$$

- There exists a path in configuration space that joins two minimums while climbing the free energy landscape *as little as possible*.
- Chemical reaction is approximately 1D walk along this path, which is called the **reaction coordinate.**

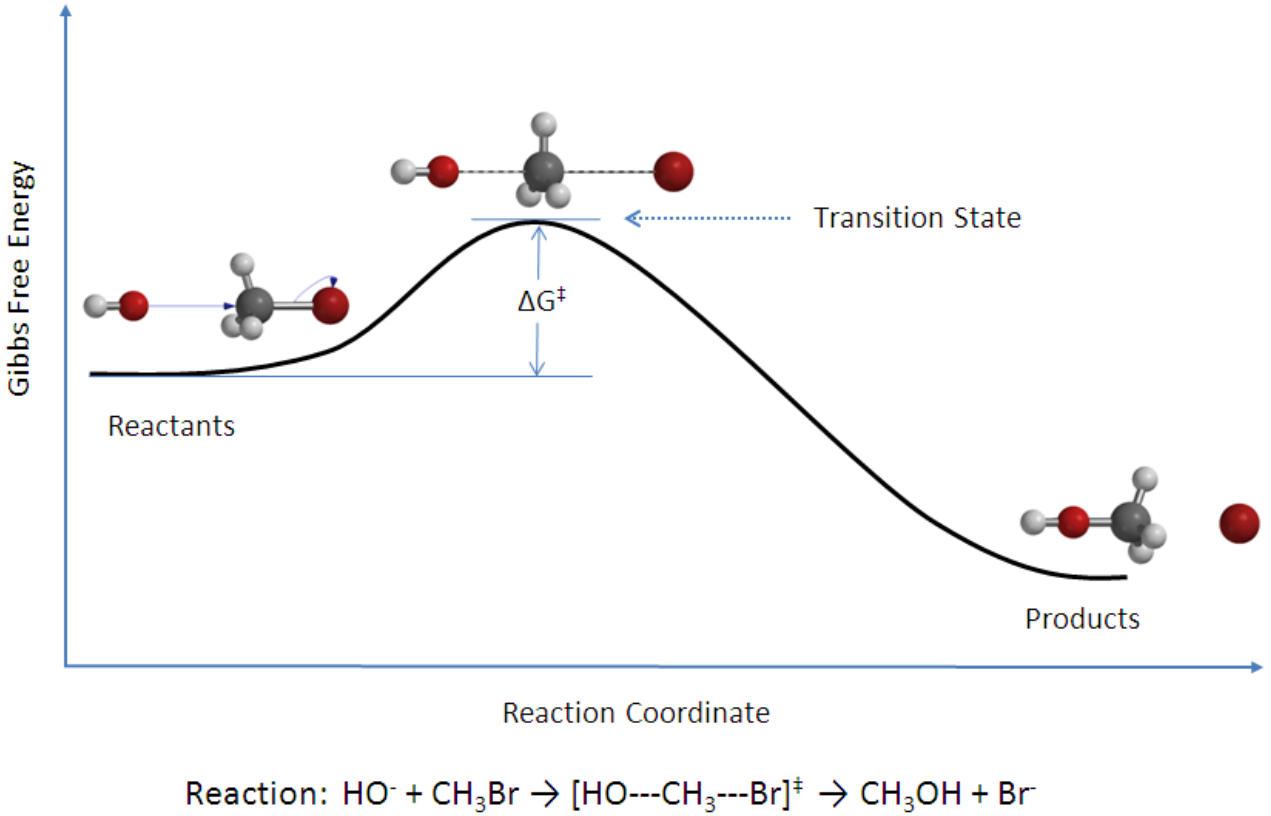
Even big macromolecules can be described by one or two reaction coordinates

- Rate of transition through a barrier

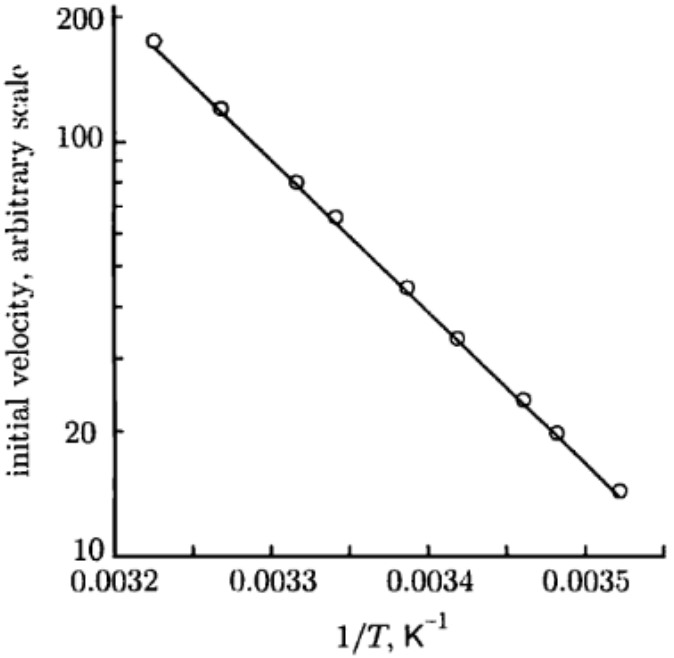
$$\Delta G^{\ddagger} = \Delta E^{\ddagger} - TS$$

$$\text{Rate} \propto \exp\left(-\frac{\Delta G^{\ddagger}}{k_B T}\right)$$

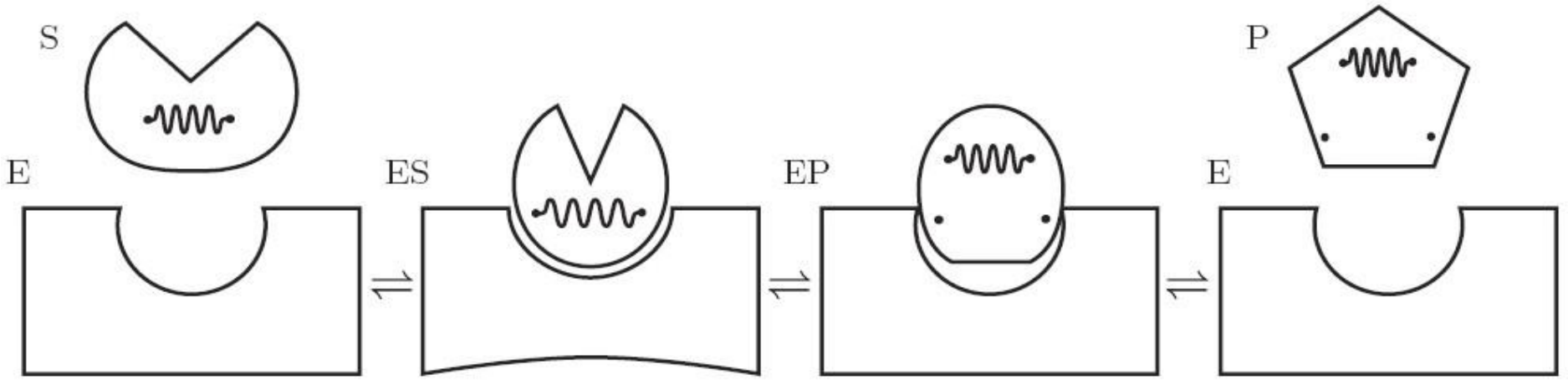
$$\text{Rate} \propto \exp\left(-\frac{\Delta E^{\ddagger}}{k_B T}\right)$$



Reaction: $HO^- + CH_3Br \rightarrow [HO{-}{-}{-}CH_3{-}{-}{-}Br]^{\ddagger} \rightarrow CH_3OH + Br^-$



Enzyme catalysis: Conceptual model of enzyme activity (Haldane 1930)



(1) Binding site of enzyme E matches substrate S.

(2) E and S deform to match perfectly and form ES state.

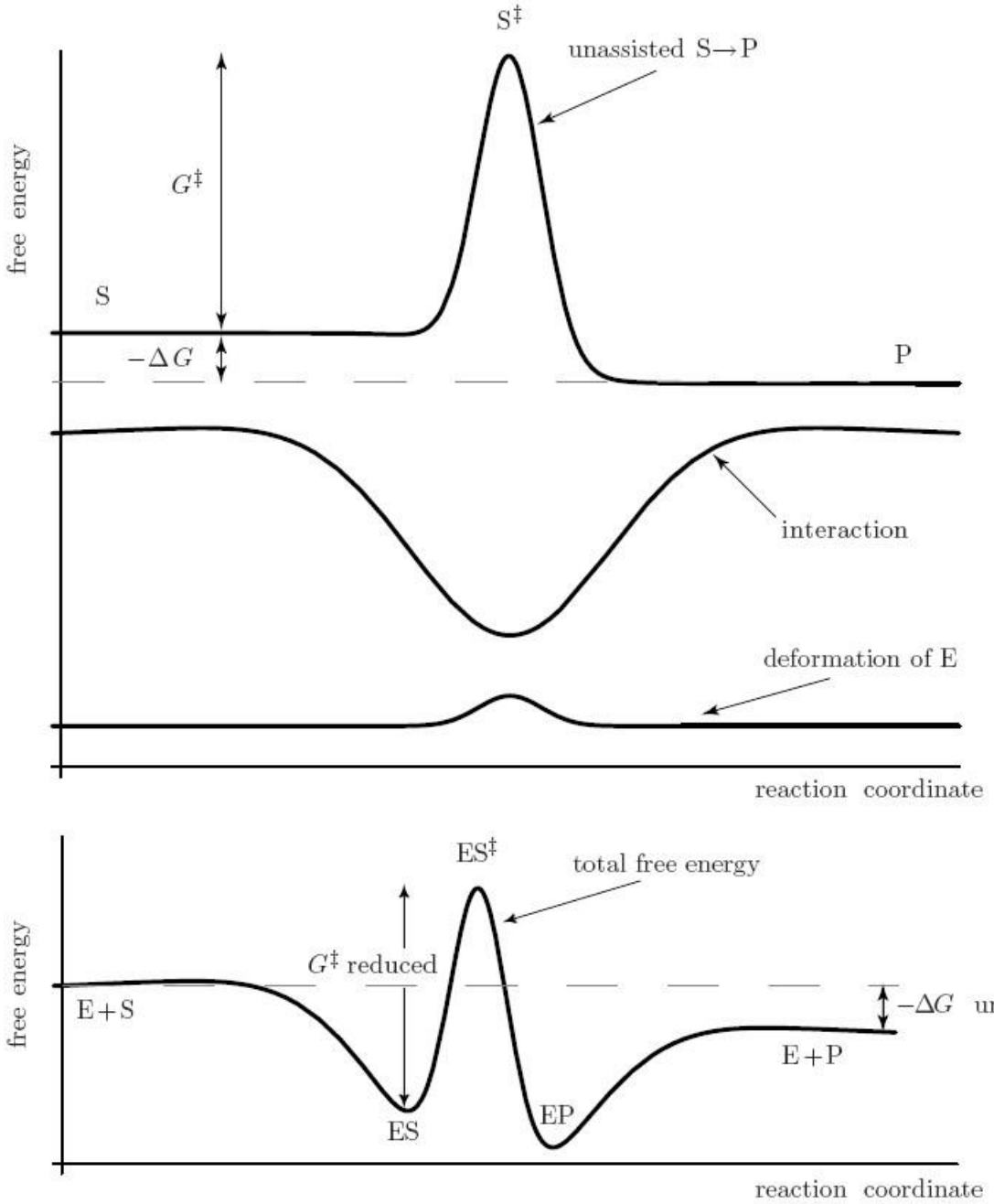
One bond in S ("spring" in S) is easily broken by deformation.

(3) Thermal fluctuation break bond and get EP state.

A new bond forms ("upper spring"), stabilizing product P.

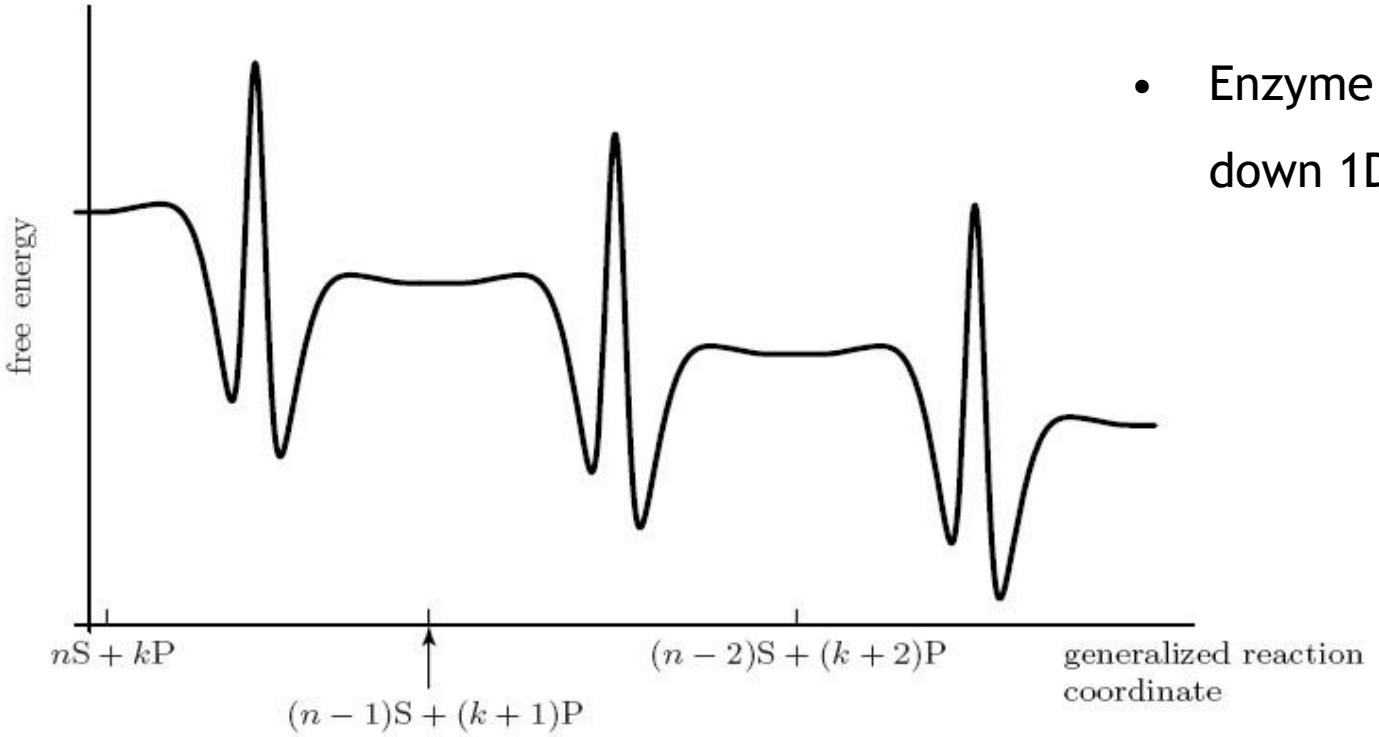
(4): P does not perfectly fit to binding site, so it readily unbinds, returning E to its original state.

Enzymes reduce activation energy by binding tightly to the substrate's transition state.

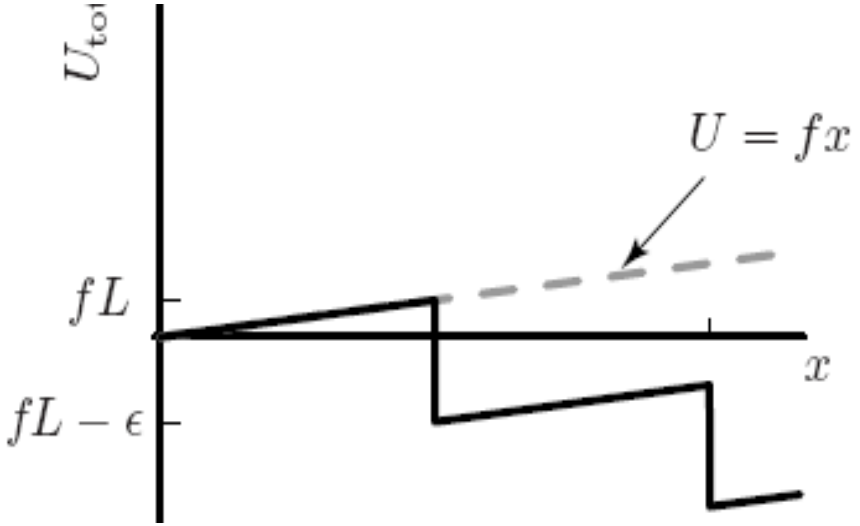


- Interaction free energy with large binding free energy (dip) with entropic cost aligning S and E.
- Binding free energy partly offset by deformation of E, but net effect is to reduce the barrier
- Net free energy landscape (sum of three curves): Enzyme reduced $\Delta G^{\ddagger}$ but not total ΔG

Enzymes are simple cyclic machines



- Enzyme work by random-walking down 1D free energy landscape.



Barrier:

$\Delta G^{\ddagger} = f \cdot L$

Net descent each work cycle:

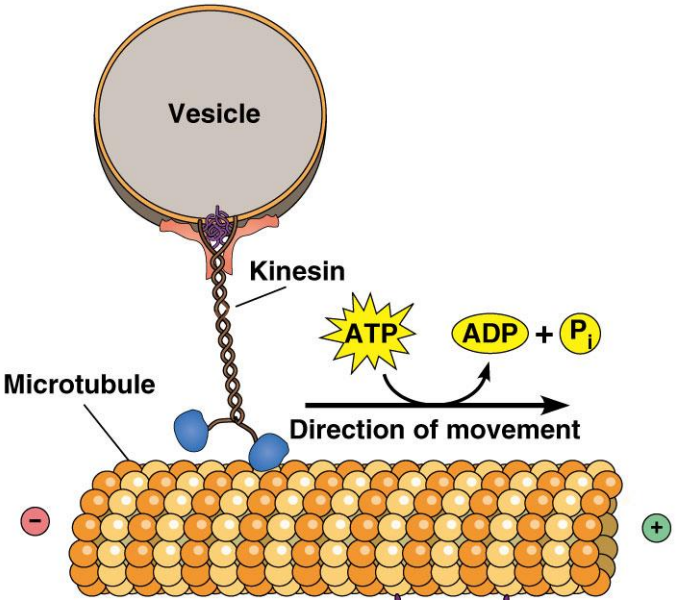
$\Delta G = f \cdot L - \varepsilon$

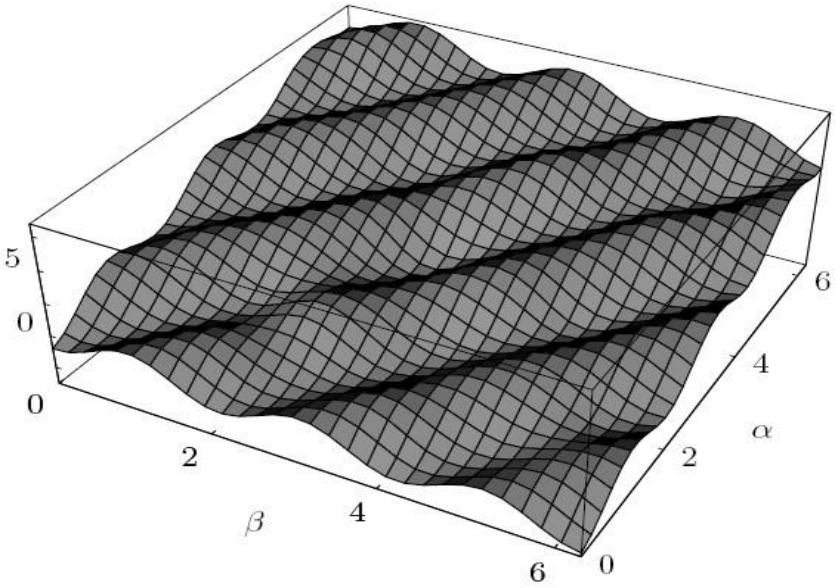
Mechanochemical motors move by random walking on a 2D landscape

- E catalyzes the reaction S at high chemical potential μ_S into P with low μ_P .

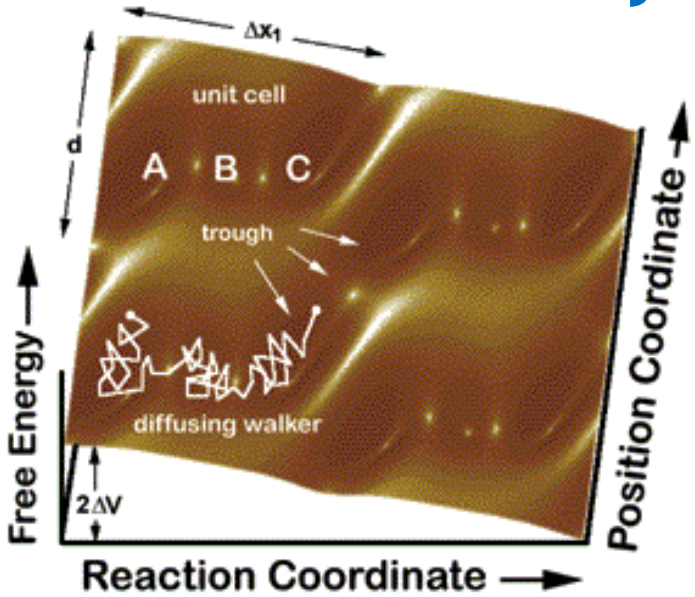
- E has another binding site which can attach to a periodic “track”. e.g., kinesin which converts ATP to ADP+Pi and can bind to a microtubule.

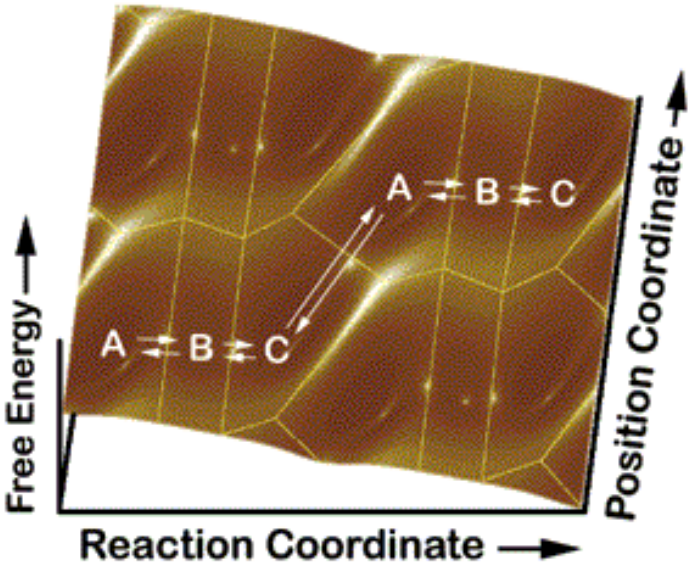
- 2D free energy landscape with 2 variables:
  - Reaction coordinate: # of remaining S.
  - location of machine on track.



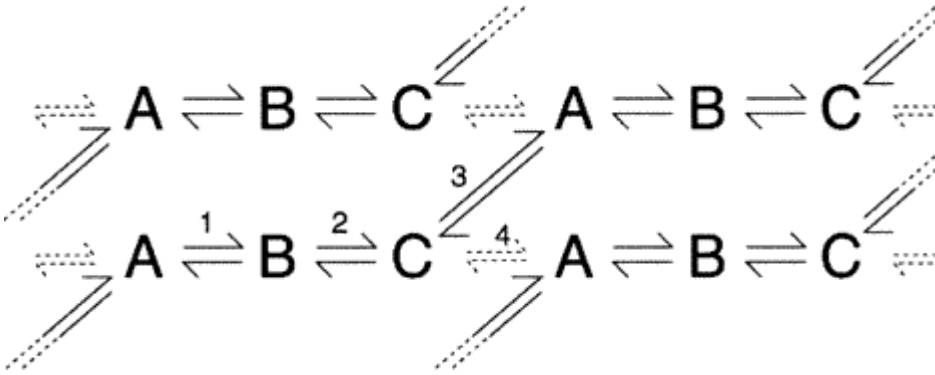


Potential energy is periodic in reaction coordinate and position, reflecting cyclic nature of enzymatic turnovers and motor cycles.





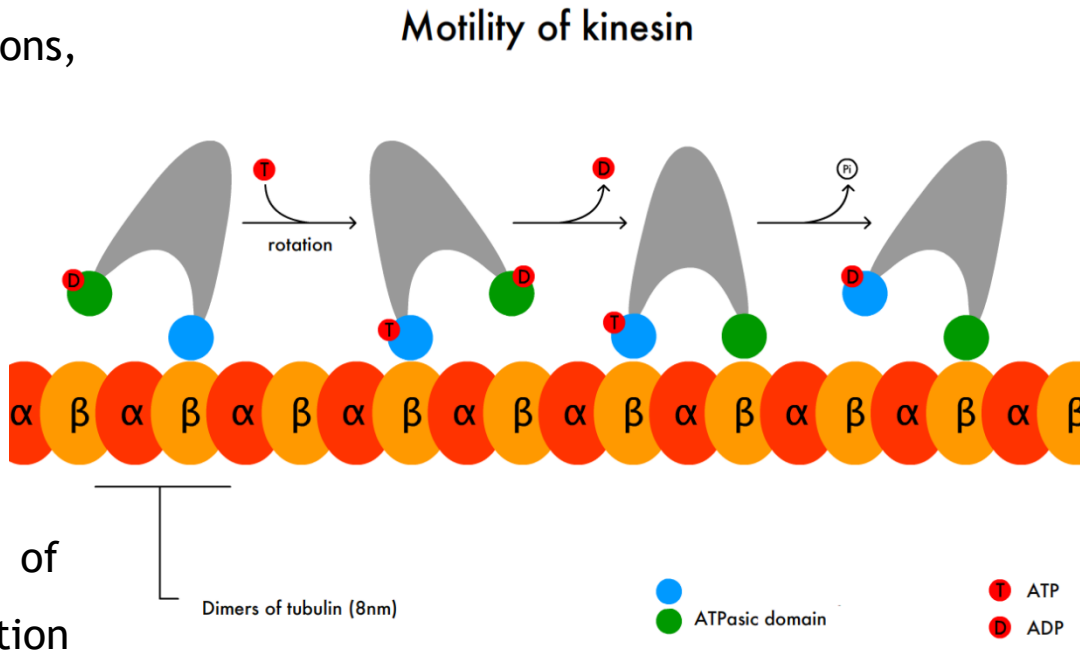
- tilted along chemical axis by free energy of reaction.
- load force tilts of the surface along position.
- Furrow couples chemical energy to mechanical motion. System random walks in furrow.

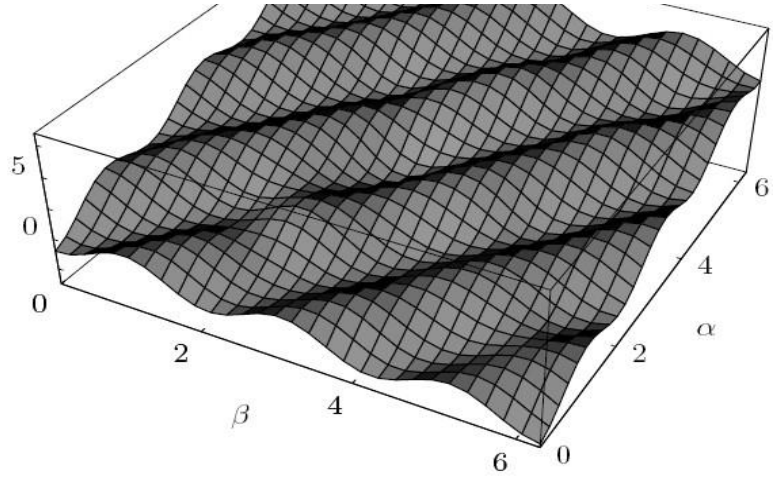


- Correspondence between potential energy surface and kinetic mechanism of motor.

Breaking the symmetry induces motion

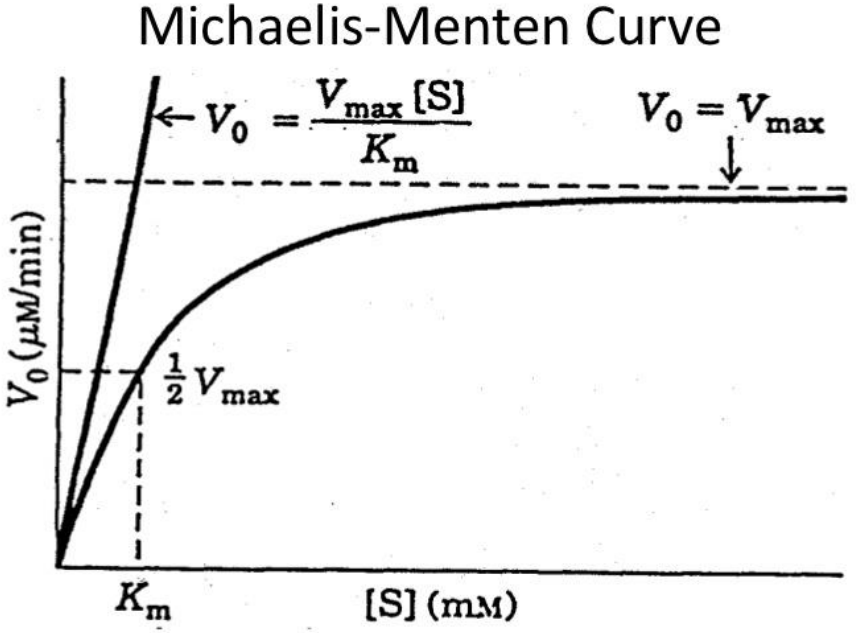
- Mechanochemical cycle: landscape with 2 directions, reaction coordinate and displacement.
- Landscape asymmetric in displacement + concentrations of S and P out of equilibrium = directed net motion.
- Deterministic free energy landscape: coupling of variables is tight and we can follow a single reaction coordinate one valley.





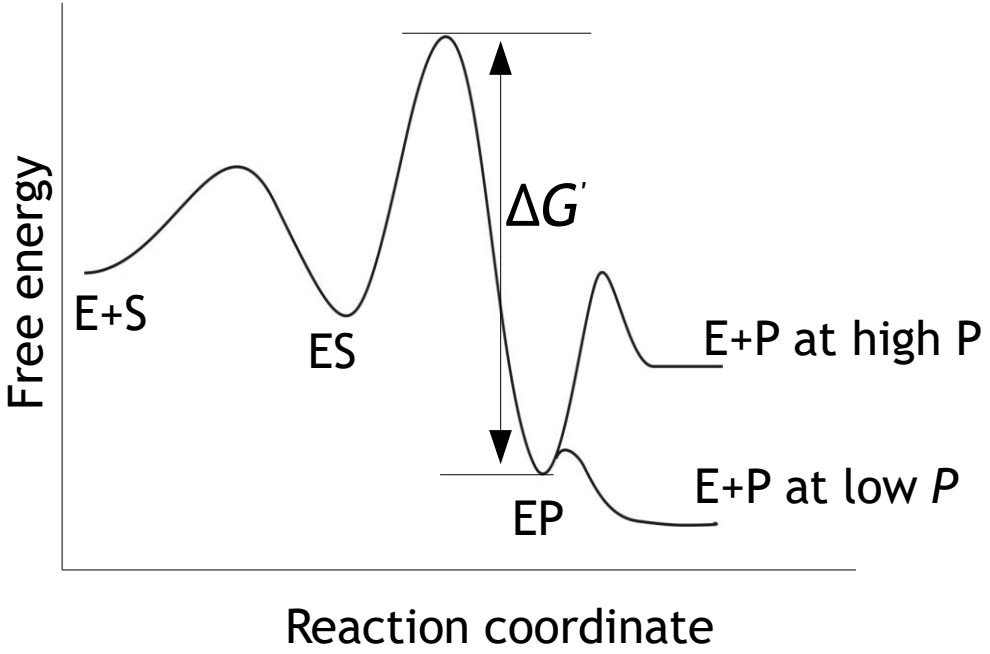
VI. Kinetics of enzymes and molecular machines

Michaelis-Menten rule describes the kinetics of simple enzymes



$$V = \frac{[S]}{[S] + K_M} V_{\max}$$

MM kinetics originate from the energy landscape of the enzyme



- low P: EP→E+P is quick process.

→ neglect shortly lived state EP.

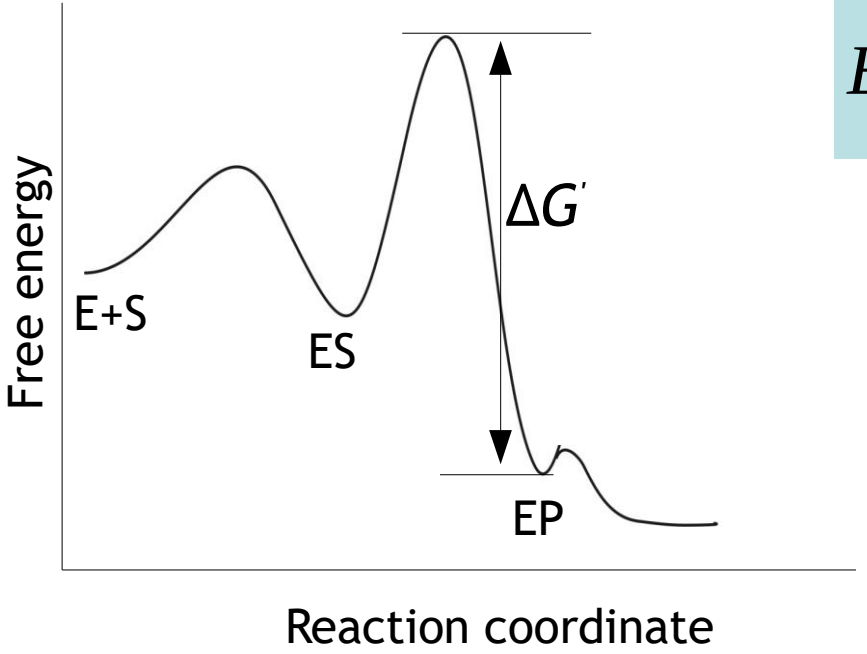
- $\Delta G'$ >> other barriers and $k_B T$

→ ES→EP is irreversible.

Simplified cyclic process: $E + S \underset{k_{-1}}{\overset{k_1 S}{\rightleftarrows}} ES \xrightarrow{k_2} E + P.$

- Different Initial and final states starting but enzyme returns to initial state.

MM kinetics originate from the energy landscape of the enzyme



$$E + S \underset{k_{-1}}{\overset{k_1 S}{\rightleftarrows}} ES \xrightarrow{k_2} E + P.$$

Steady state:

$$\frac{d[E]}{dt} = -k_1[S][E] + (k_{-1} + k_2)[ES] = 0$$

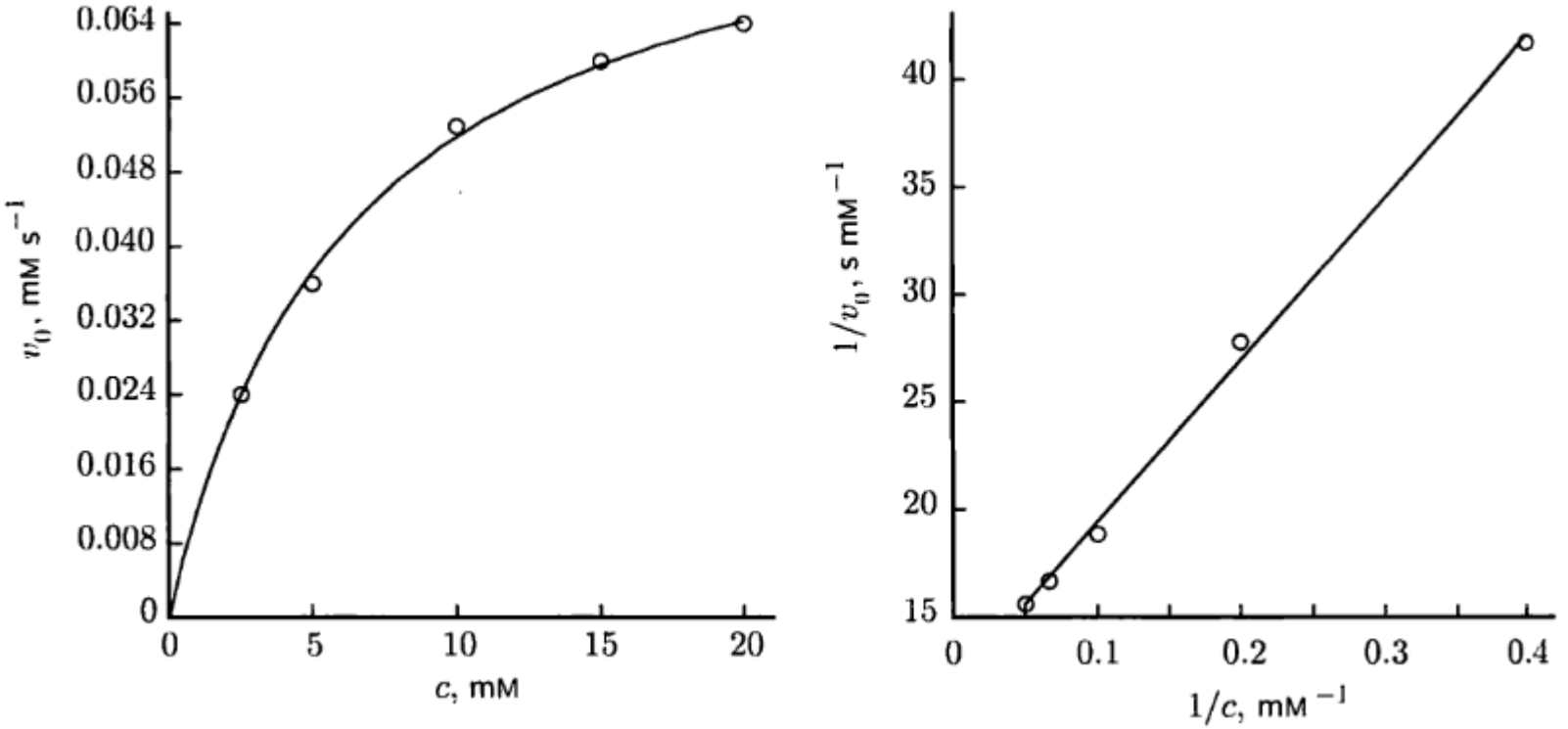
$$[ES] = \frac{[S][E]}{K_M + [S]},$$

$$K_M = \frac{k_{-1} + k_2}{k_1} \quad \text{MM constant}$$

$$v = \frac{d[P]}{dt} = k_2[ES] = \frac{k_2[S][E]}{K_M + [S]}$$

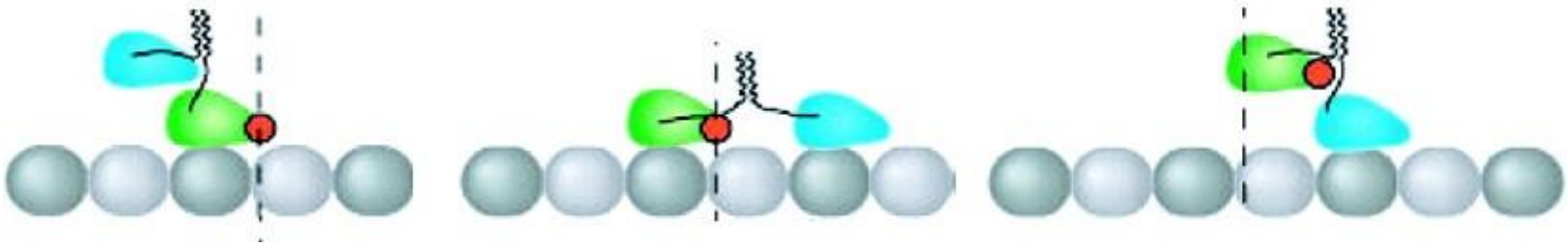
$$v = v_{\max} \frac{[S]}{K_M + [S]} \quad \text{with } v_{\max} = k_2[E]$$

Experimental test: Lineweaver-Burk graph



$$\frac{1}{v} = \frac{1}{v_{\max}}\left(1 + \frac{K_M}{[S]}\right)$$

Two-headed kinesin is a tightly coupled, perfect ratchet



- **tightly coupled molecular motor ~ enzyme:**

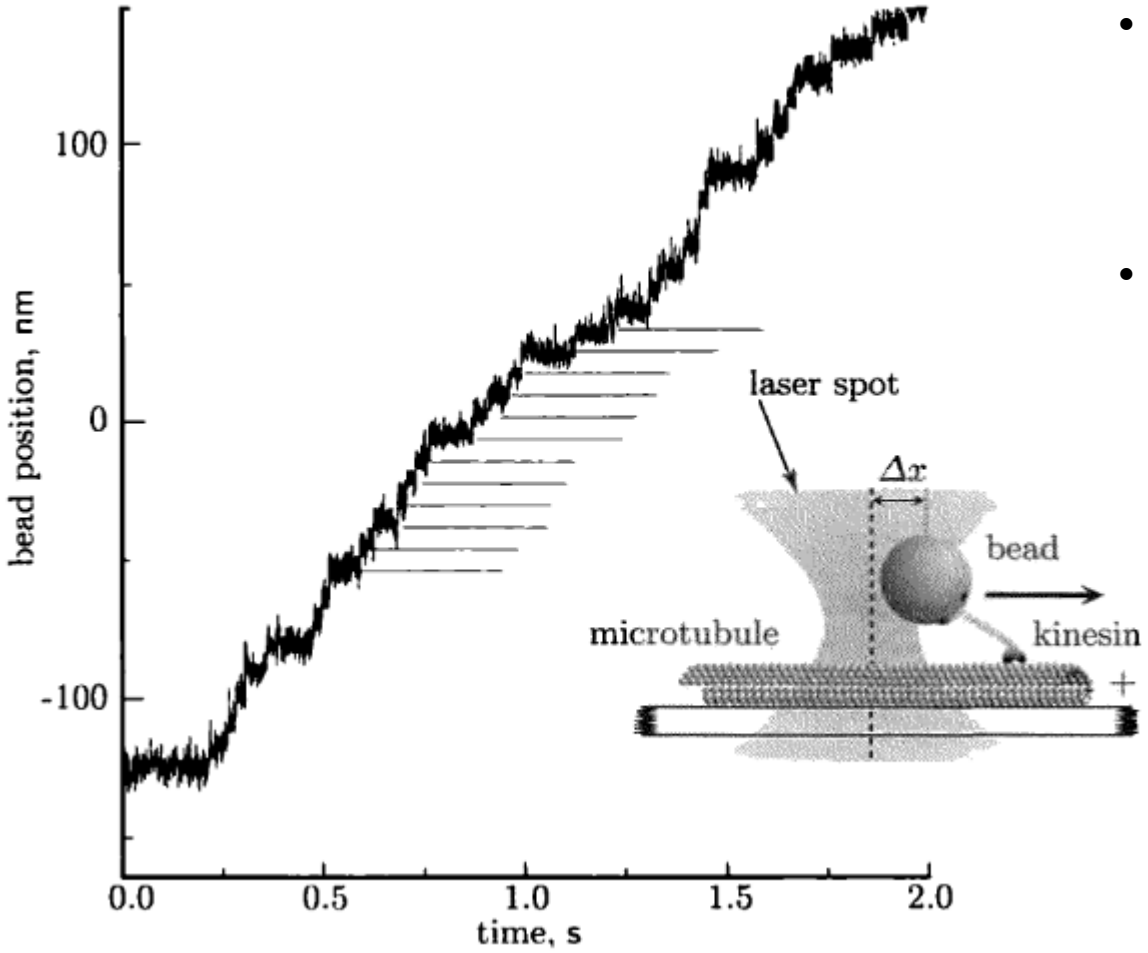
  1D random walk along a valley, a step per reaction.

- P kept low →motion is irreversible.
- A tightly coupled molecular motor with an irreversible step

move at a speed determined by MM kinetics.

- Kinesin is bound to microtubule ~ 100% **duty ratio.**
- Kinesin is highly **processive**: makes many steps before falling

Kinesin is highly processive, even with load



- kinesin motility assay: optical tweezers apparatus pulls a bead backwards.
- Feedback circuit continuously moves the trap following the kinesin at constant force 6.5 pN, with 2 mM ATP.

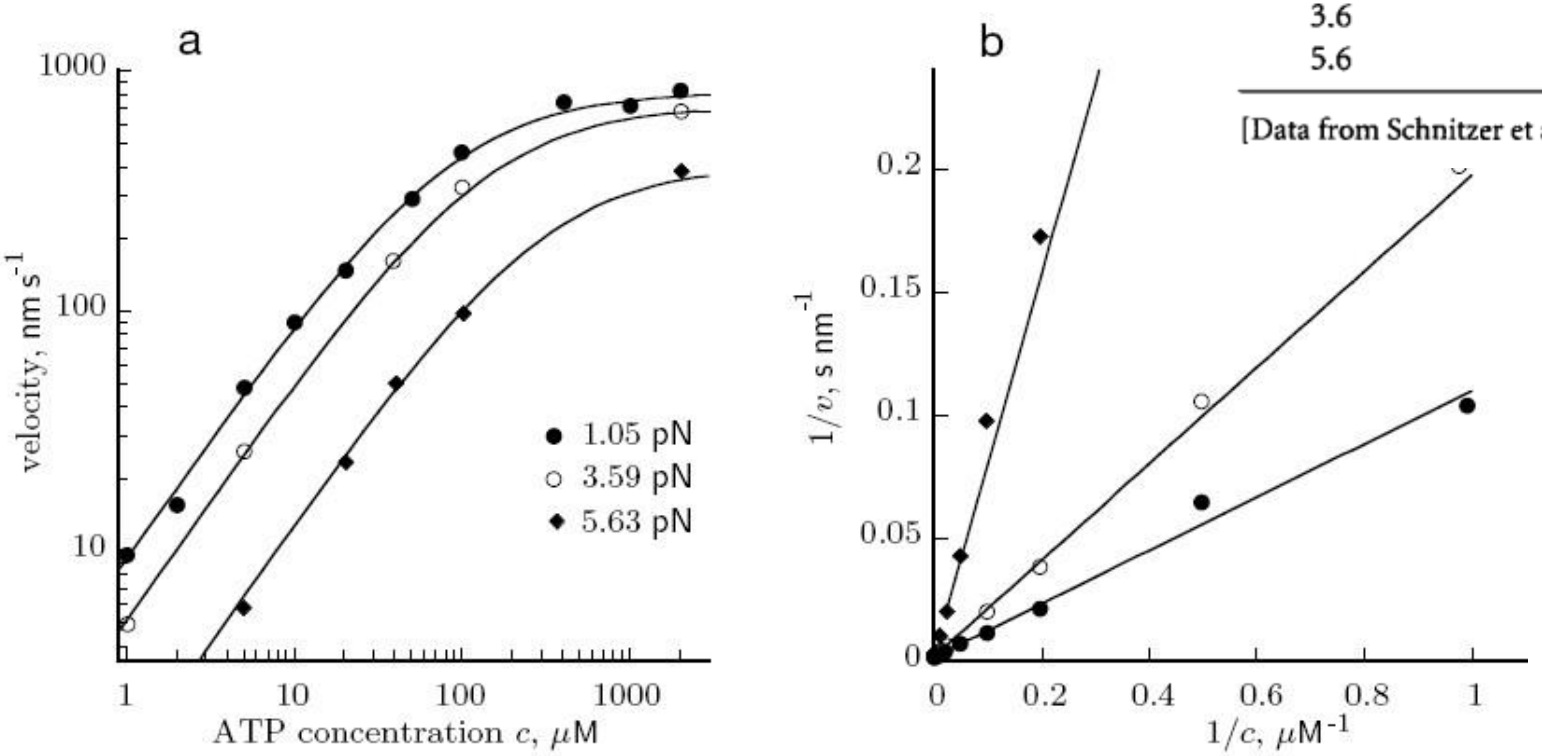
[Viscscher et al. 1999]

Kinesin obeys MM kinetics with load-dependent parameters

Table 10.1: Michaelis–Menten parameters for conventional kinesin stepping at fixed load force.

load force, pN	v_{max}, nm s^{-1}	K_M, μM
1.05	813 ± 28	88 ± 7
3.6	715 ± 19	140 ± 6
5.6	404 ± 32	312 ± 49

[Data from Schnitzer et al., 2000.]



[Data from Visscher et al. 1999]

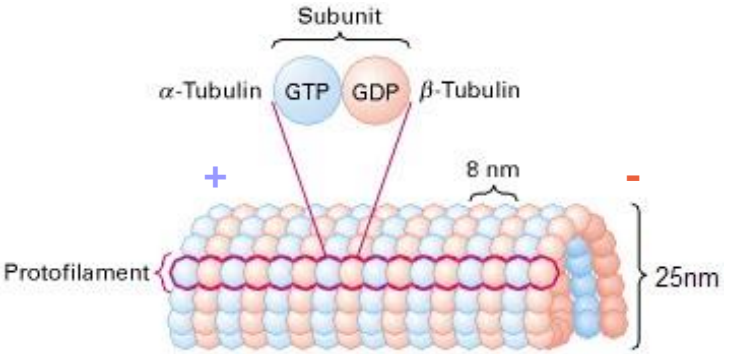
- Kinesin rarely moves backward → "perfect ratchet" limit.

- Kinesin is tightly coupled:
  exactly one step per one ATP molecule (even under load).

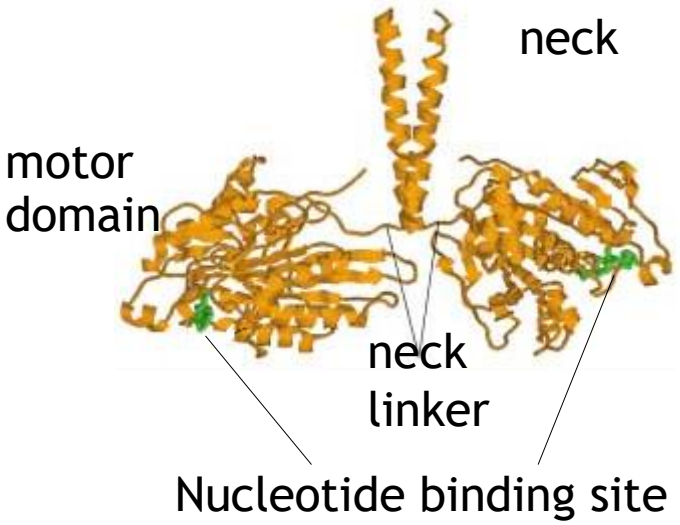
Structural clues for kinesin as perfect ratchet

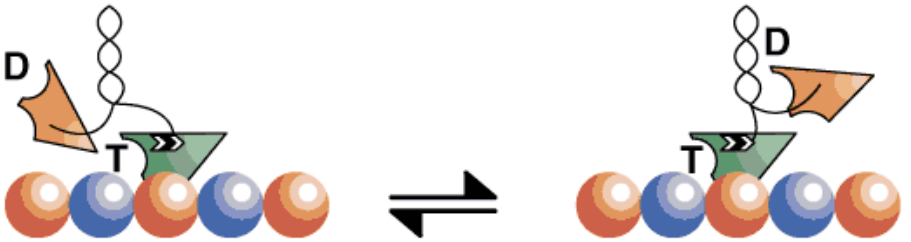
- Microtubule:
  - ß-subunit has a binding site for kinesin.
  - Regular space at 8 nm intervals.
  - Microtubule is polar (+/- ends).
- Kinesin binding is stereospecific:

  → bound kinesin points in one direction.



Kinesin heads:



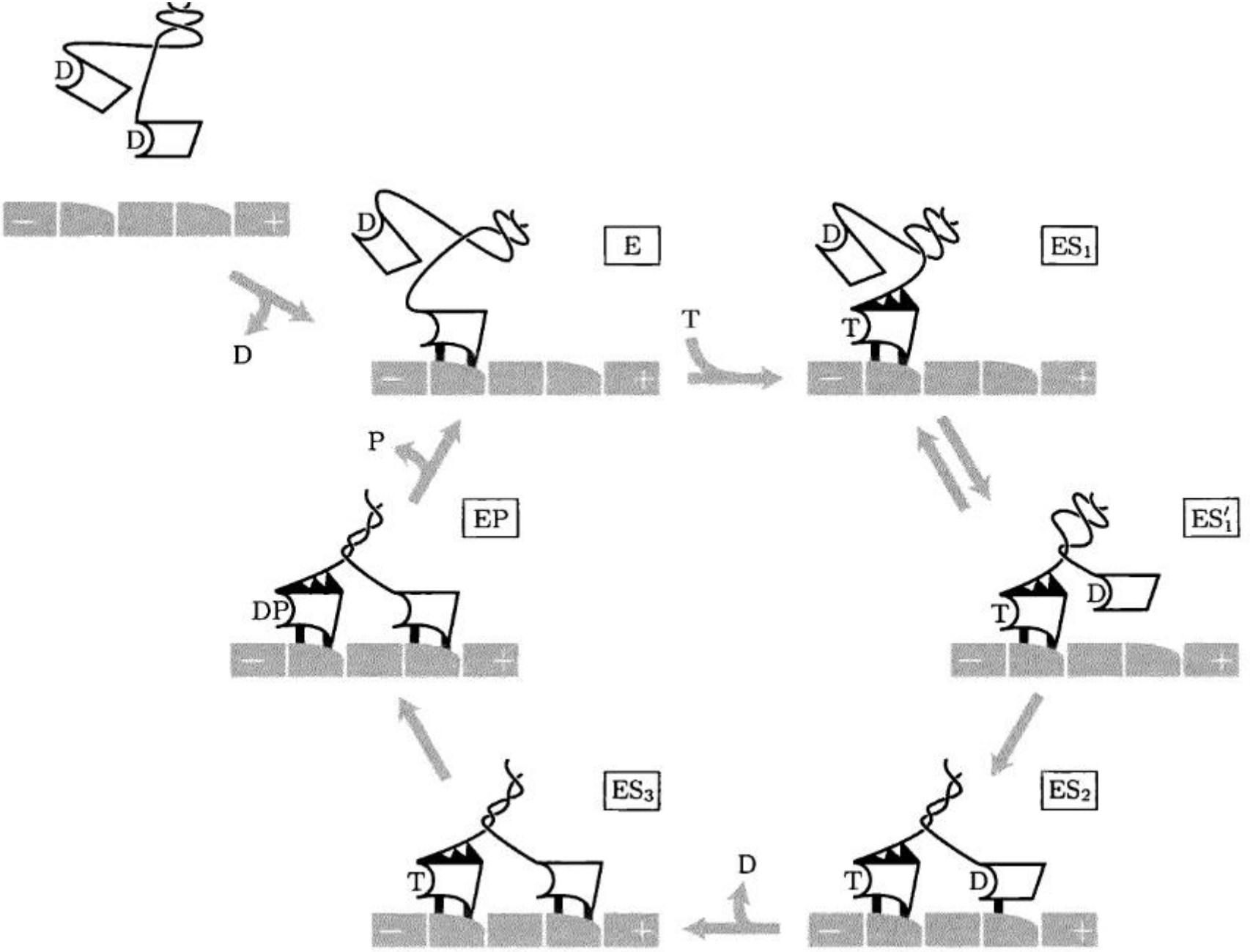


- ADP at nucleotide-binding site: neck linker flops between two different conformations.
- ATP at site: neck linker binds tightly to kinesin head and points to the + end.

Structural clues for kinesin as perfect ratchet

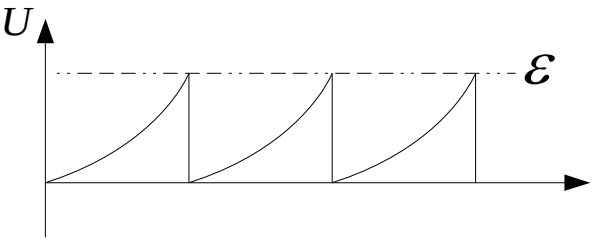
- Kinesin binds ADP strongly.
- Kinesin without bound nucleotide binds microtubules strongly.
- Complex **Microtubule·Kinesin·ADP** is only weakly bound.
- Complex **Microtubule·Kinesin·ATP** is strongly bound; Only one head could bind microtubule when only ADP exists;
- Binding ATP to one head stimulates another head to release its ADP

Cyclic model for kinesin stepping



Summary

- Necessary conditions for directional motion:
  - Break spatial inversion symmetry
  - Break thermal equilibrium.



$$R_{\text{forward}} \neq R_{\text{backward}}$$

- **Smoluchowski equation** for micro-machines:

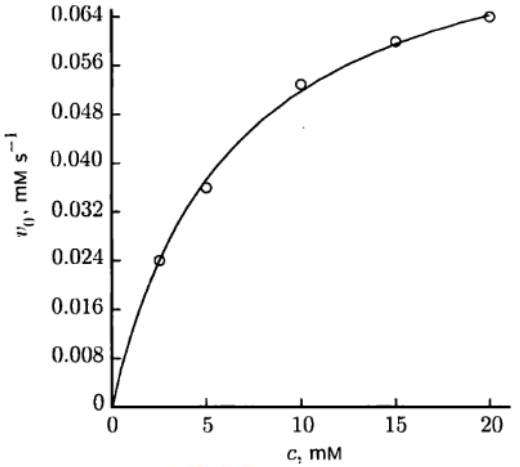
$$\frac{\partial P}{\partial t} = 0 \quad \rightarrow j = -D\left(\frac{\partial P}{\partial x} + \frac{P}{k_B T}\frac{\partial U}{\partial x}\right) = \text{const.}$$

- Enzymes are simple **cyclic machines**;

work by random walking on 1D free energy landscape.

- Saturation kinetics: Michaelis-Menten rule

$$v = v_{\max}\frac{[S]}{K_M + [S]}$$



- Mechanochemical motors move by **random-walking on 2D landscape** reaction coordinate vs. spatial displacement.
- **Two-headed kinesin** is a tightly coupled motor.