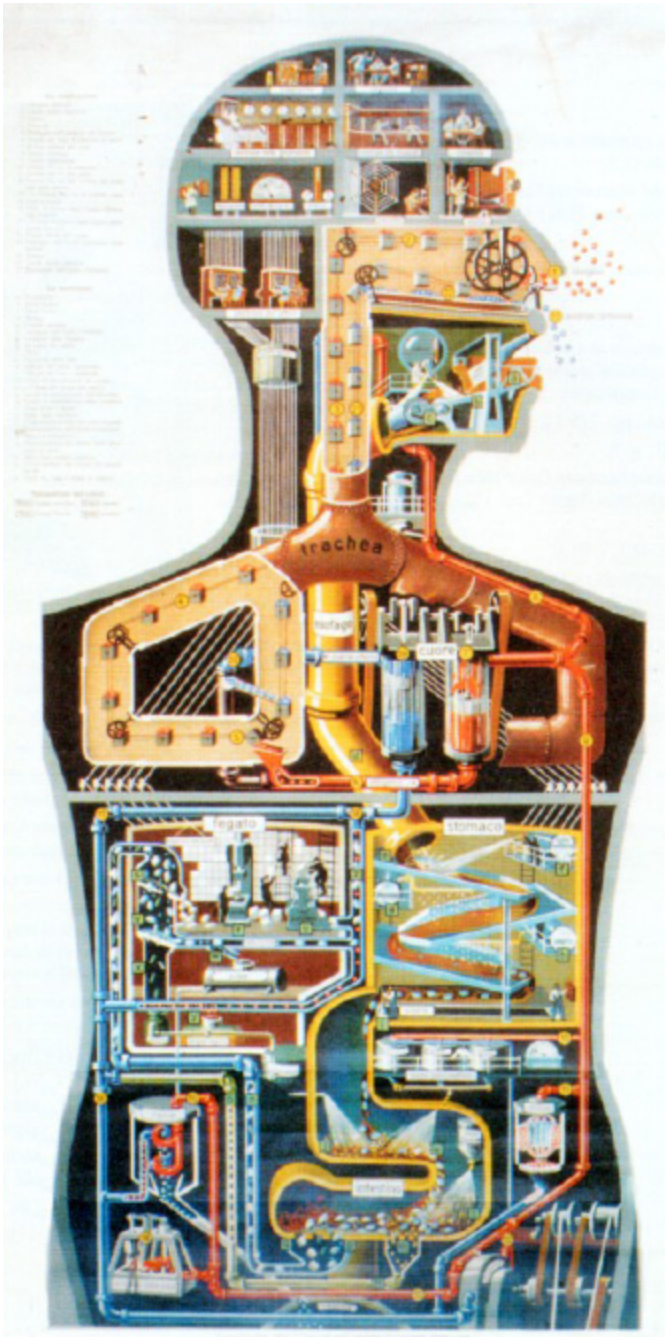
Exploring Protein Motors



*"The entire cell can be viewed as a **factory** that contains an elaborate network of interlocking assembly lines, each of which is composed of a set of large protein **machines**...*

Why do we call the large protein assemblies that underline cell function protein machines? Precisely because, like machines invented by humans to deal efficiently with the macroscopic world, these protein assemblies contain highly coordinated moving parts" - *Bruce Alberts, Cell 92, 291 (1998).*

President of the National Academy of Sciences USA (1993-2005)

Editor-in-chief, SCIENCE (March, 2008 -)

Molecular logic of the living state

Living systems create and maintain order by channeling energy to carry out a network of processes by molecular machines.

Molecular machines use 1 dimensional, linear, digital information to spontaneously create 3 dimensional, stable molecules capable of recognizing, binding, and altering other molecules.

Biological mechanisms share three characteristic features:

(i) Thermal noise to cause Brownian motion or activated transitions from one chemical state to another;

(ii) Anisotropy arising from the structure of the medium in which the particle diffuses;

(iii) Energy supplied either by an external variation of the constraints on the system or by a non-equilibrium chemical reaction.

Biological question

How does a molecular motor convert chemical energy, a scalar quantity, into directed motion, a vector?

Physical idea

Mechanochemical coupling arises from a free energy landscape with a direction set by the geometry of the motor and its track. The motor executes a biased random walk on this landscape.

„Active" transport system

The motor + the transport complex + the link between the two.

Molecular motors are complexes of proteins which use chemical energy to perform mechanical work.

Why do cells need motors?

Intracellular diffusion is inefficient over long distances or for large cargoes

Directionality of motion

Cell shape changes

Movement of whole cells relative to the environment

Molecular machines are encoded by a genetic material and have the potential to evolve.

Size: Nano-meters; Force: Pico-Newtons

Question I: Is the mechanism of molecular motors identical to those of their macroscopic counterparts (except for a difference of scale)?

NO.

(1) Far from equilibrium

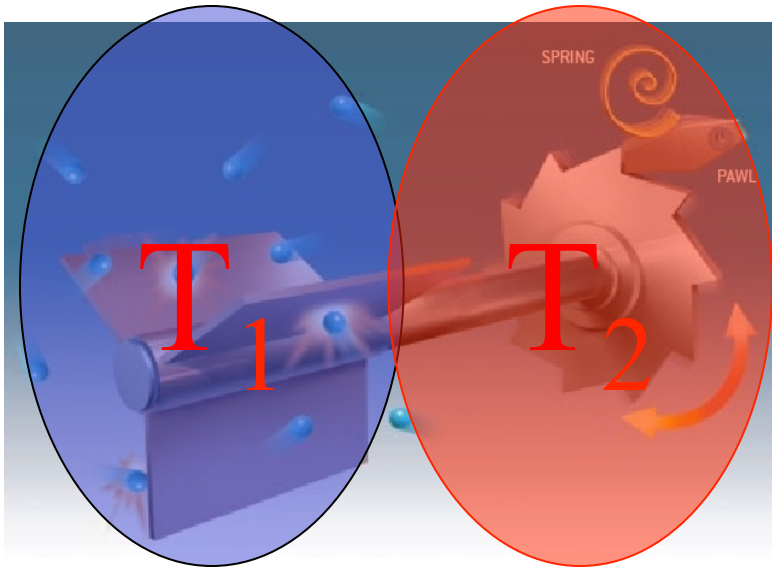
(2) Made of soft matter

(3) Dominant forces are non-inertial

"...gravitation is forgotten, and the viscosity of the liquid,...,the molecular shocks of the Brownian movement, ... Make up the physical environment....The predominant factor are no longer those of our scale; we have come to the edge of a world of which we have no experience, and where all our preconceptions must be recast".

- D'Arcy Thompson, "On Growth and Form" (1942).

The Feynman Thermal Ratchet



Works only if $T_1 > T_2$!

$$P_{forward} \sim \exp(-\Delta E/kT_1)$$
$$P_{backward} \sim \exp(-\Delta E/kT_2)$$

Motor protein conformational change take place within μs.

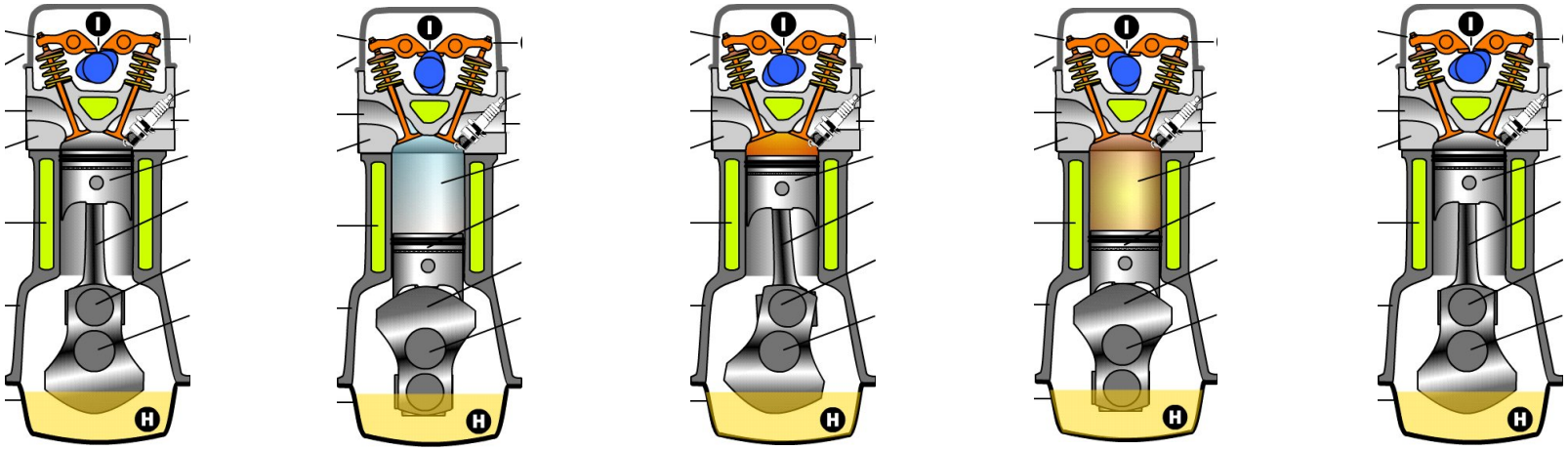
The decay of temperature gradient over 10 nm disappears within ns.

Wrong model !!

Molecular machine is an isothermal engine

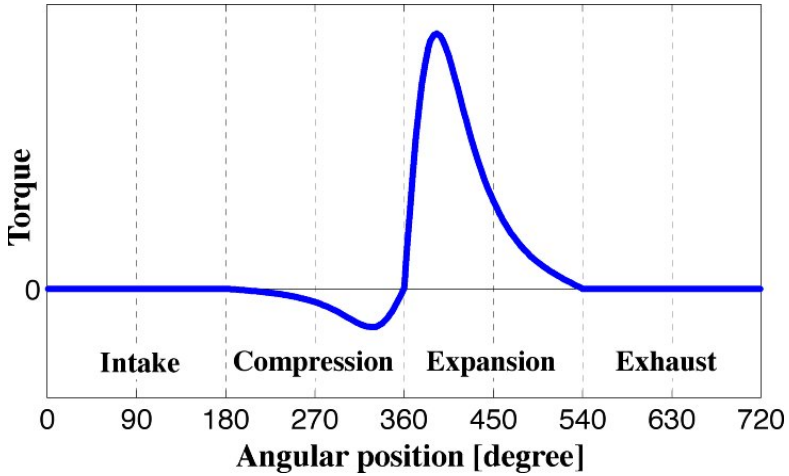
(not heat engine)

An example of macroscopic motor



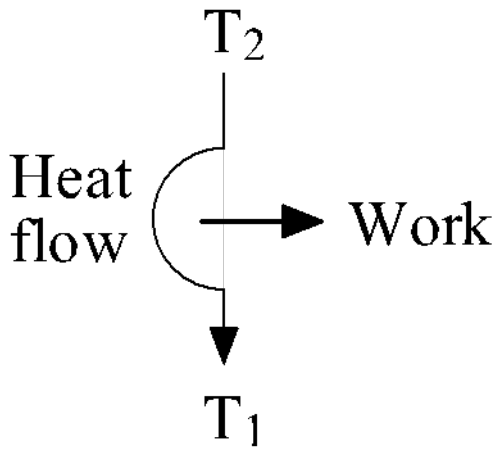
(1) Intake **(2) Compression** **(3) Expansion** **(4) Exhaust**

Torque of a single cylinder engine:

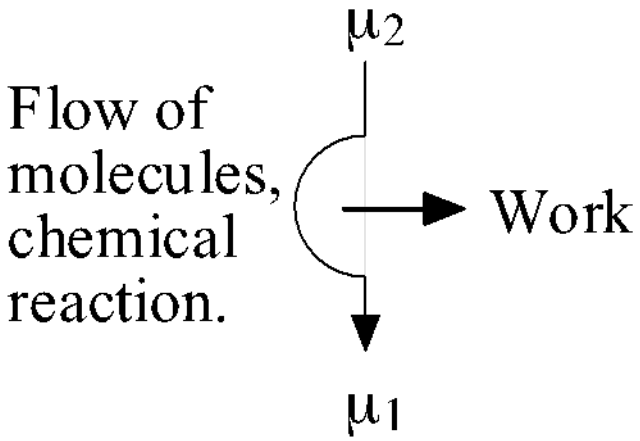


Isothermal engines

Nano-engines are *isothermal* - driven by differences in chemical potential.



Heat engine



Isothermal engine

General considerations

No inertia (diffusive behavior)

Reynolds number = inertial forces/viscous forces

Scale of nm ⇒ ***Only potential energy is stored***

Can assume local thermal equilibrium

motor time scales	~ 10^{-3} sec
local equilibrium time scale	~ 10^{-7} sec

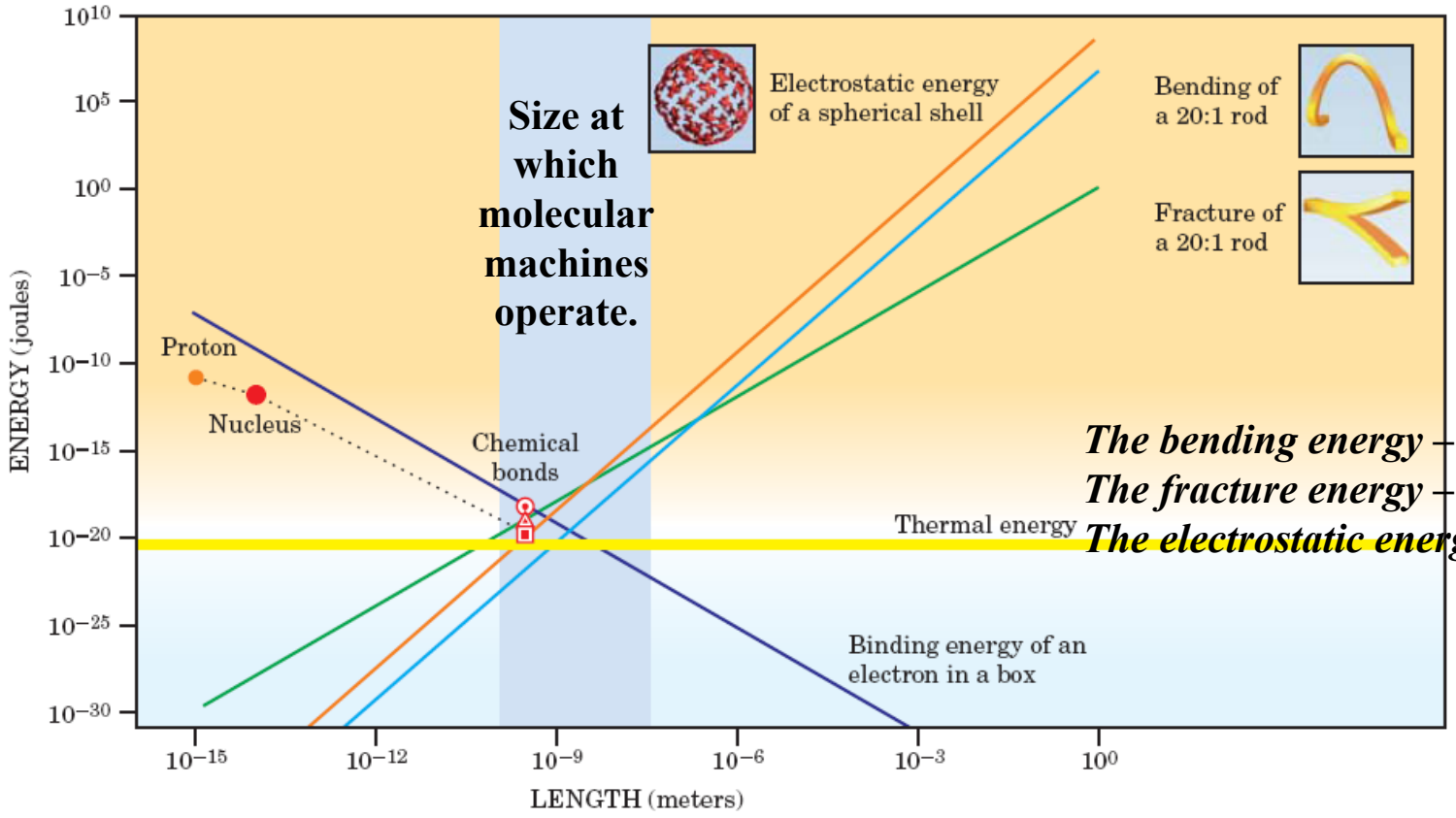
➢ Time scales of events are determined by depth of energy wells and height of barriers.

$$\langle waiting \quad time \rangle \propto e^{\frac{E_A}{k_B T}}$$

➢ They operate at energies close to kT and ***fluctuations*** play a central role.

The confluence of energy scale

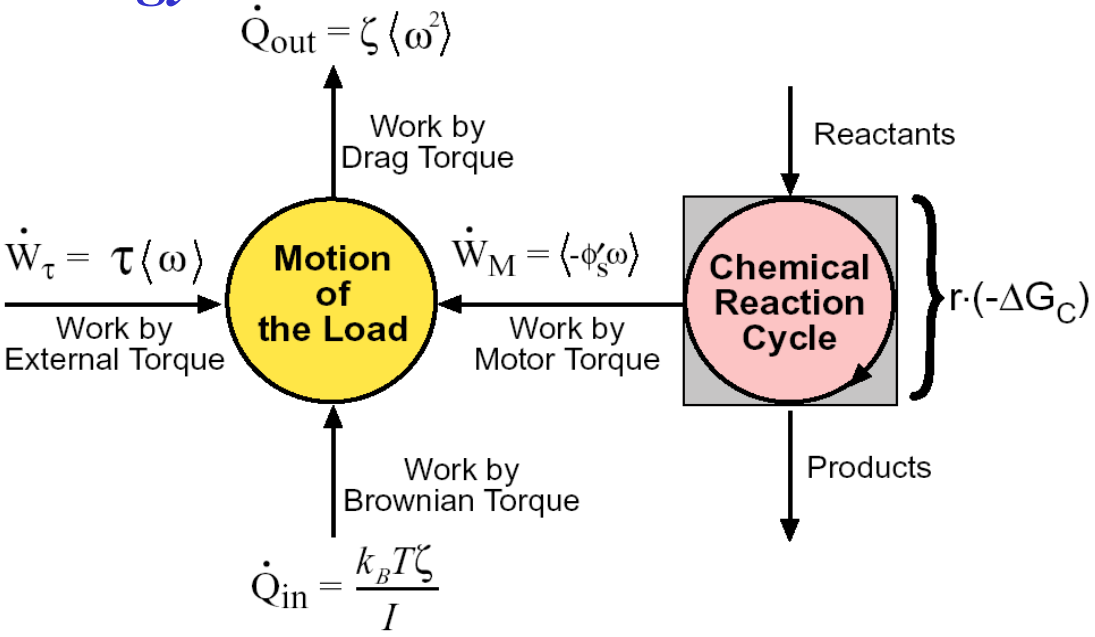
Thermal, chemical, mechanical, and electrostatic energies are associated with an object scale.



The bending energy – blue
The fracture energy – green
The electrostatic energy – orange.

Mechano-chemical models

Energy balance on a molecular motor



Mechanical motion:

$$\zeta \frac{dx}{dt} = \underbrace{\phi_S'(x)}_{\text{Force from potential}} + \underbrace{f_L}_{\text{Load force}} + \underbrace{\sqrt{2k_BT\zeta}\,\frac{dW(t)}{dt}}_{\text{Brownian force}}$$

Chemical reaction: $\frac{d\mathbf{S}}{dt} = \mathbf{K}(x) \cdot \mathbf{S}$

$$\begin{array}{ccc} [\mathbf{SITE}] & \Leftrightarrow & [\mathbf{SITE} * \mathrm{ATP}] \\ \Updownarrow & & \Updownarrow \\ [\mathbf{SITE} * \mathrm{ADP}] & \Leftrightarrow & [\mathbf{SITE} * \mathrm{ADP} * \mathrm{P_i}] \end{array}$$

Mechanical motion and chemical reaction are coupled.

Langevin formulation

(stochastic evolution of an individual motor):

$$\frac{dx}{dt} = D\frac{-\phi_{\mathbf{S}}'(x) + f_L}{k_B T} + \sqrt{2D}\frac{dW(t)}{dt}$$

(mechanical motion)

$$\frac{d\mathbf{S}}{dt} = \mathbf{K}(x)\cdot\mathbf{S}$$

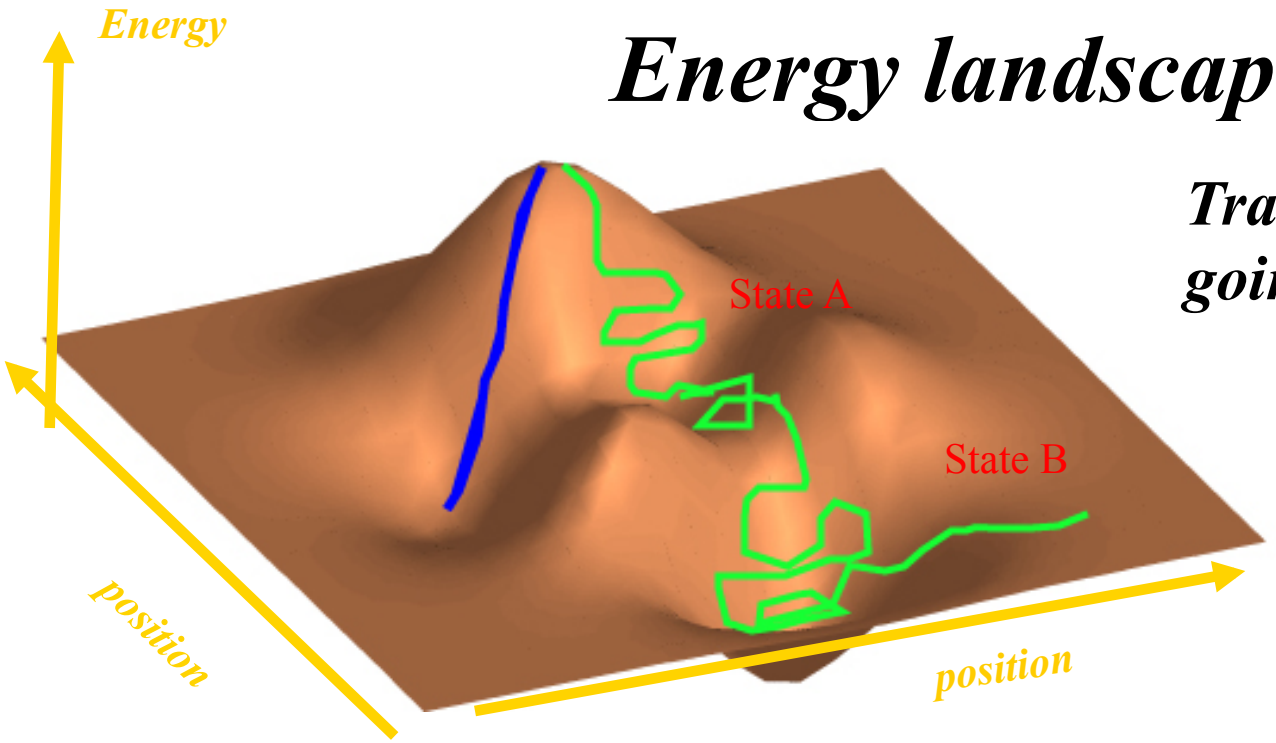
(chemical reaction)

Fokker-Planck formulation

(deterministic evolution of probability density):

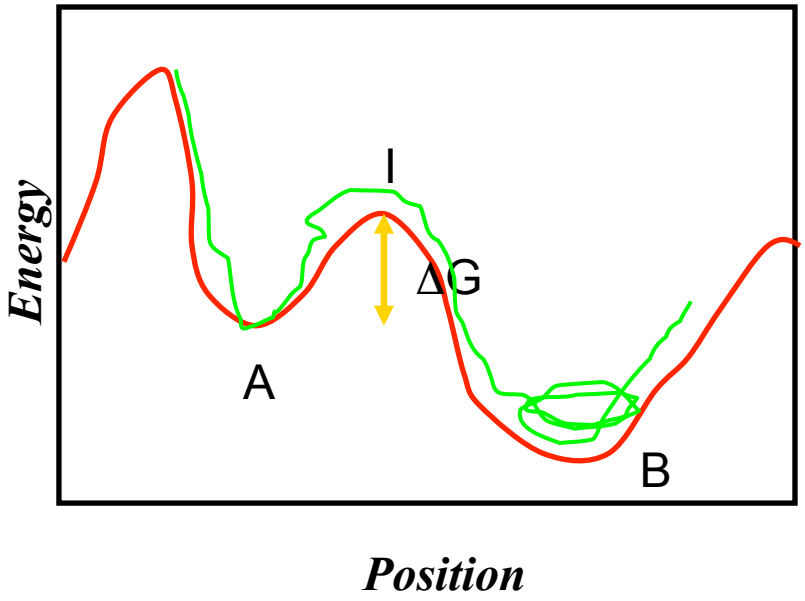
$$\frac{\partial \rho_S}{\partial t} = D\frac{\partial}{\partial x}\left(\underbrace{\frac{\phi_S'(x) - f_L}{k_B T}\rho_S}_{\text{Convection}} + \underbrace{\frac{\partial \rho_S}{\partial x}}_{\text{Diffusion}}\right) + \underbrace{\sum_{j=1}^{N} k_{Sj}(x)\rho_j}_{\text{Change of occupancy}}, \quad S = 1,2,\ldots,N$$

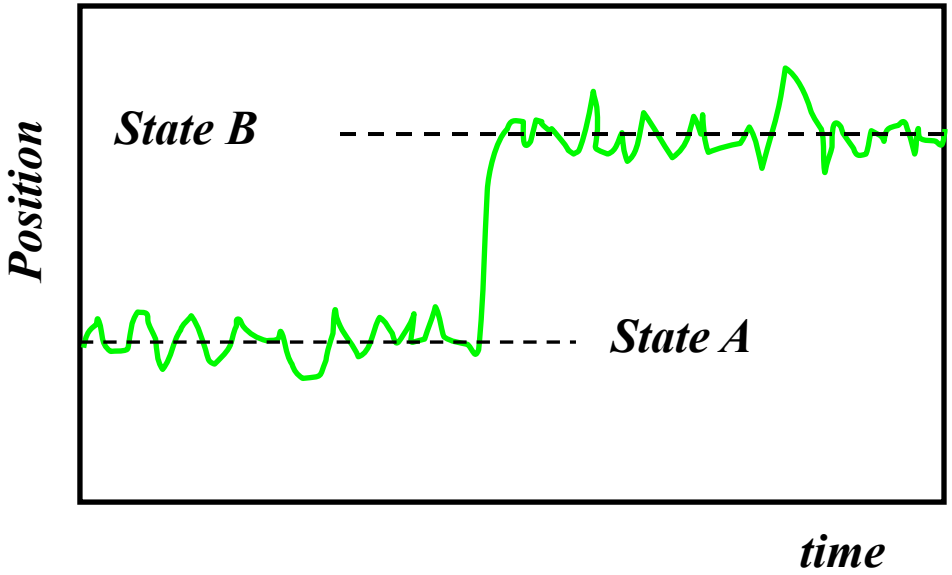
Energy landscape



Transition time for going from A to B

$$\tau_{A\to B} = Ce^{\frac{E_A}{kT}}$$



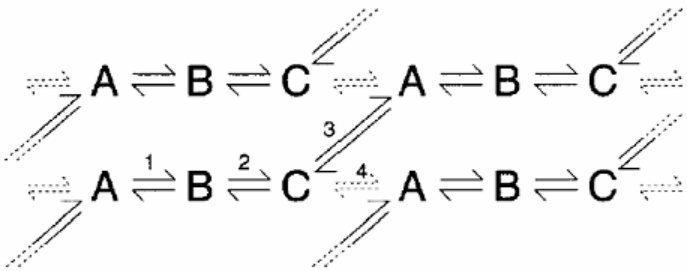


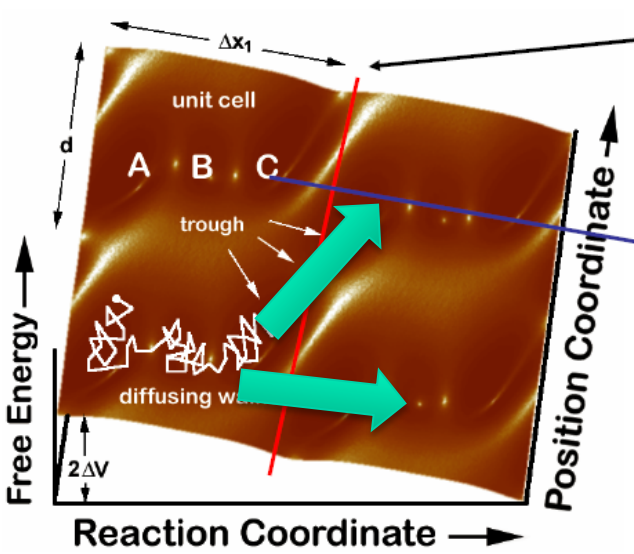
The potential energy surface is periodic.

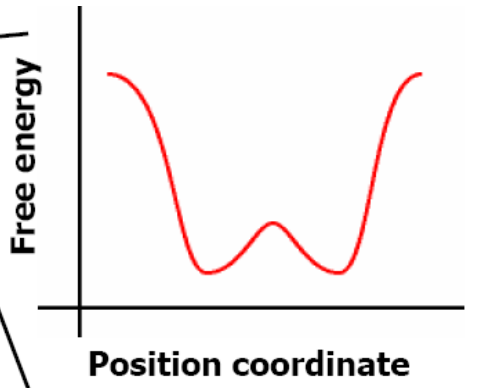
$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$$

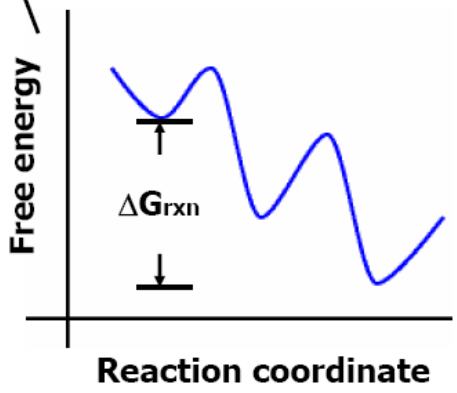
$$B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C$$

$$\left.\begin{array}{l} A \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C \\ A \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} C \end{array}\right\} A \underset{k_{-3}+k_{-4}}{\overset{k_3+k_4}{\rightleftharpoons}} C$$



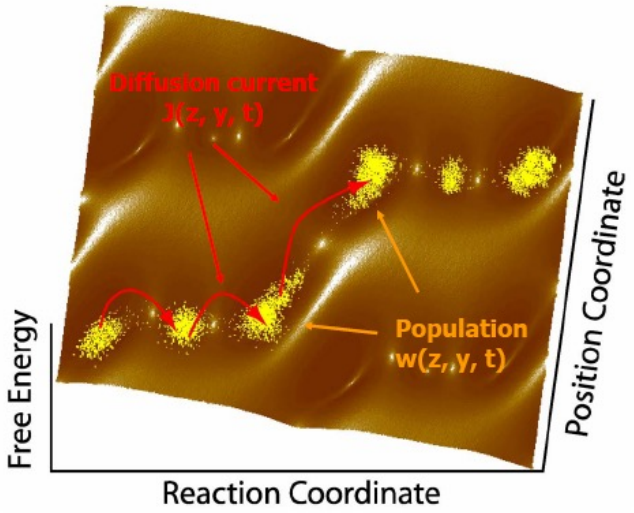


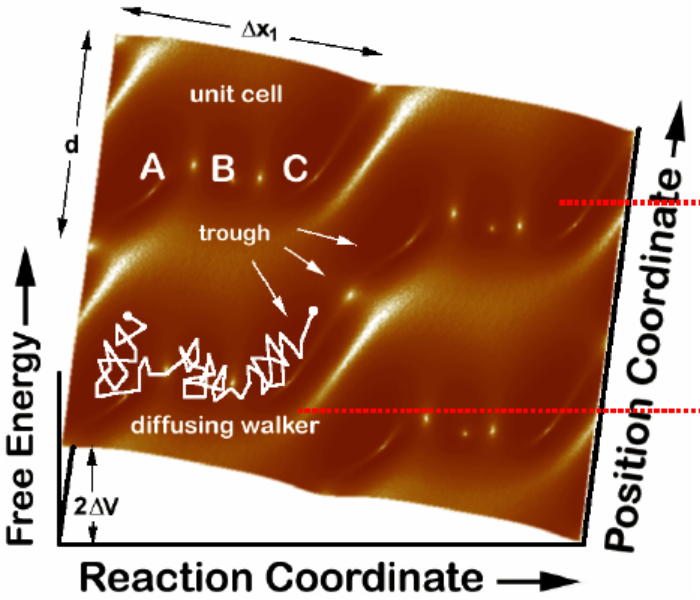


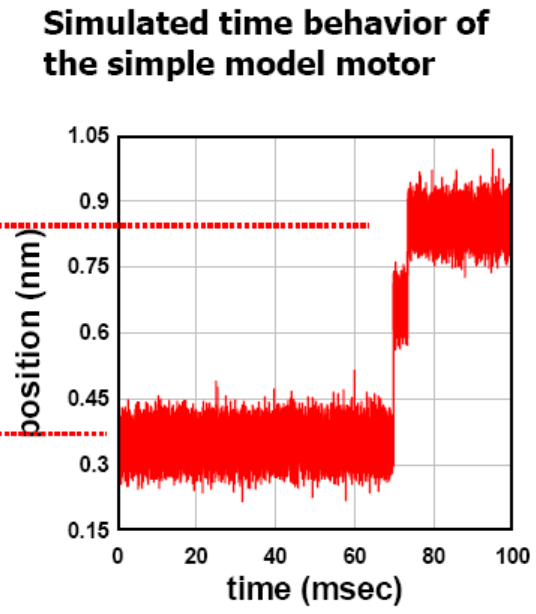


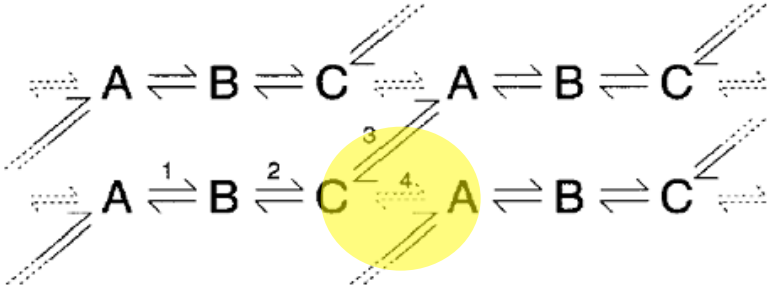
The motor description [chemical variable; mechanical variable].

Mechanochemical motors move by random-walking on a two-dimensiona¹ landscape [i.e. position, substrate molecules].



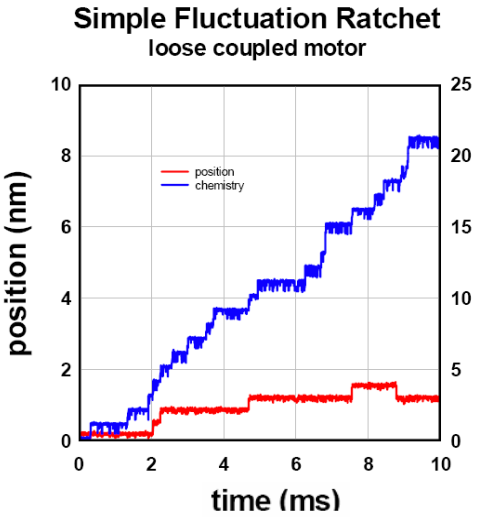




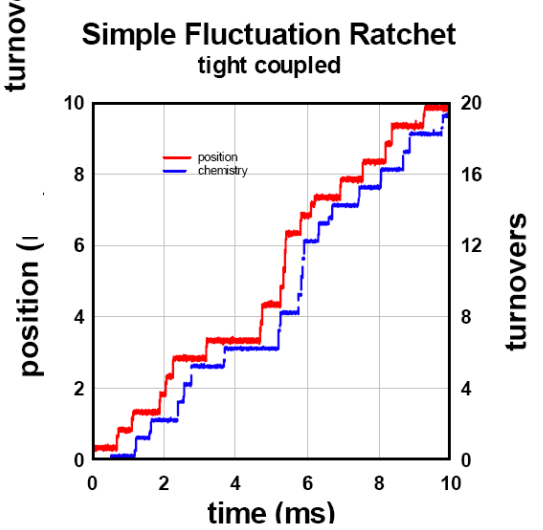


Coupling

*Path 4 – a "**leak**" in the coupling between chemistry and movement.*



A loose coupled – the rate constants along pathway 4 are significant compared to the productive path 3.



A tight coupled – path 4 is negligibly slow.

Mechanochemical coupling

$\langle v \rangle$ is the mean velocity,
$\langle r \rangle$ the mean reaction rate,
L the step size (i.e., the periodicity of the track).

$$\chi = \frac{\langle v \rangle}{L \langle r \rangle}$$

Tight coupled motor: $\chi \approx 1$

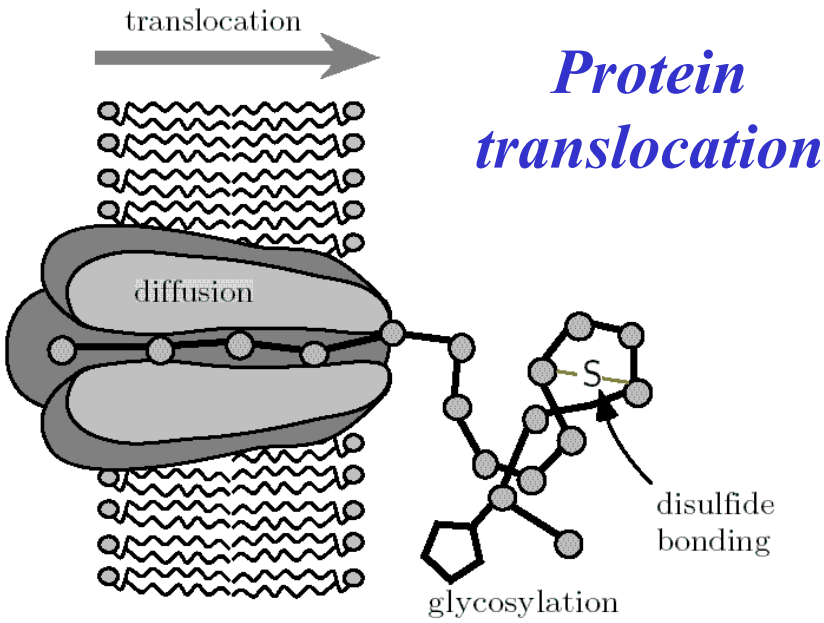
Molecular devices found in cells

Catalists – enhance the rate of a chemical reaction (enzymes)

Machines – actively reverse the natural flow of some chemical or mechanical process by coupling it to another one.

One-shot machines – exhaust some internal source of free energy

Cyclic machines – process some external source of free energy.



Protein translocation

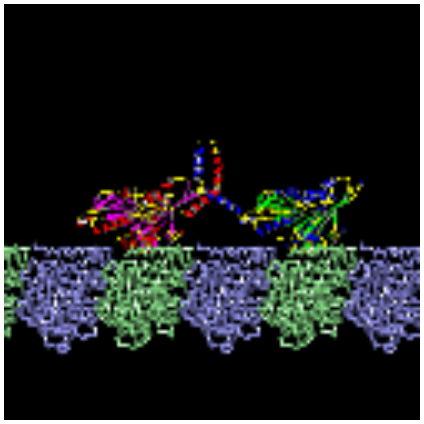
Motor Proteins

Enzymes that convert the chemical energy into mechanical work.

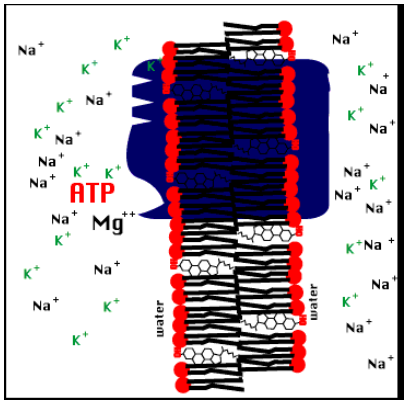
Properties:

- Non-equilibrium systems,
- Velocities: 0.01-100 μm/s,
- Step Sizes: 0.3-40 nm,
- Forces: 1-60 pN,
- Fuel: hydrolysis of ATP, or related compound, polymerization, membrane potential difference
- Efficiency: 50-100% (!!!)

Cyclic machines

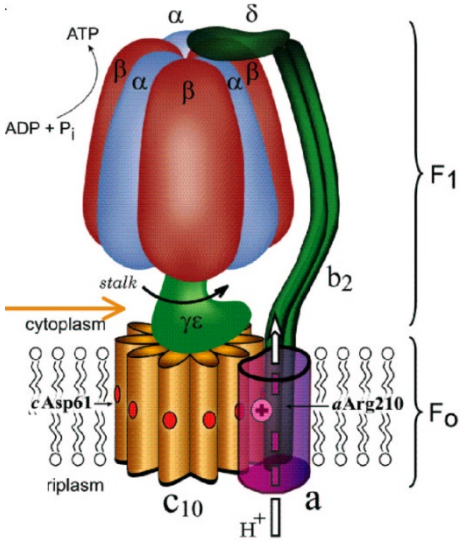


Motors – transduce some form of free energy into motion.

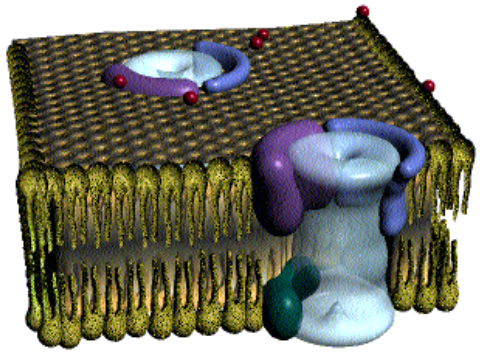


Pumps – transduce free energy to create concentration gradient

Synthases – transduce free energy to drive a chemical reaction.

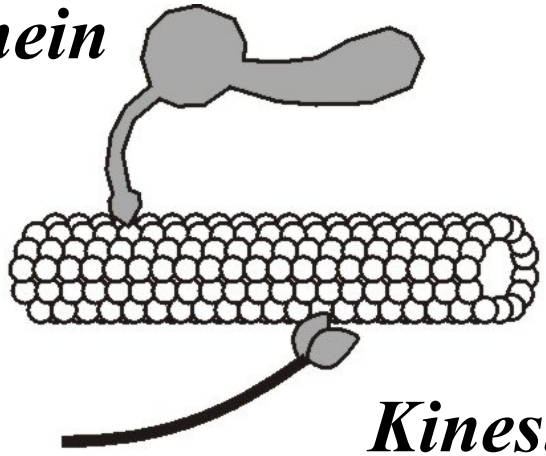


Gated channels

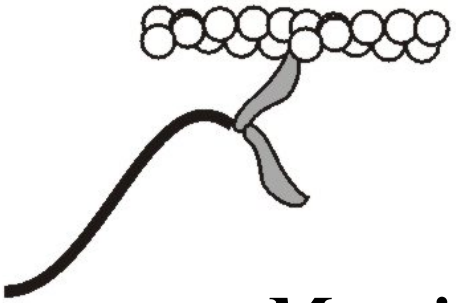


Molecular motors

Dynein

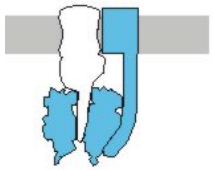


Kinesin

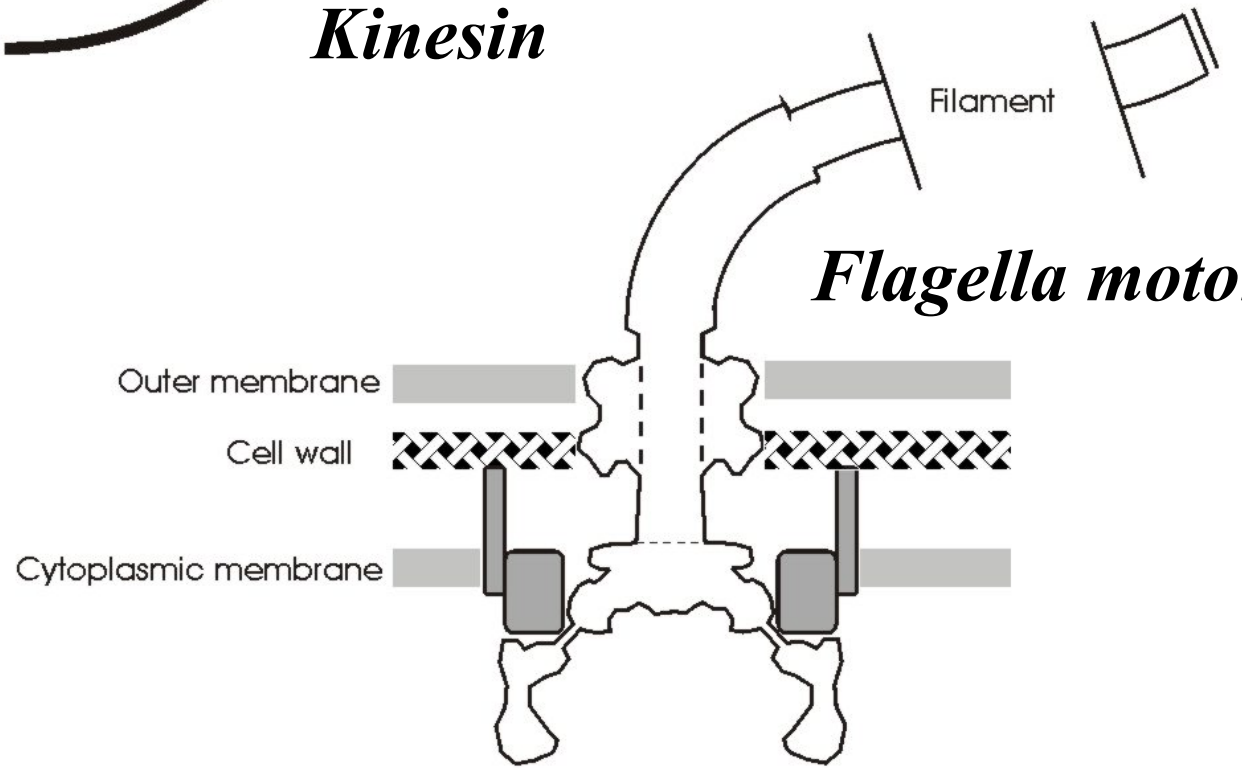


Myosin

F_0F_1-ATPase

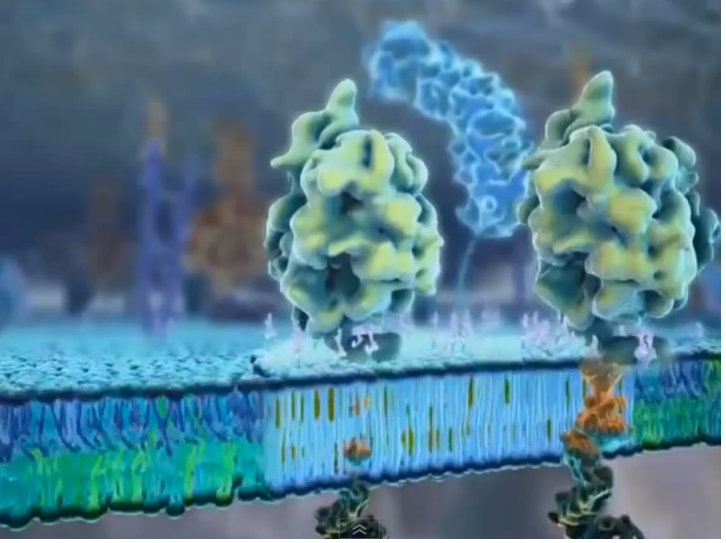


45 nm



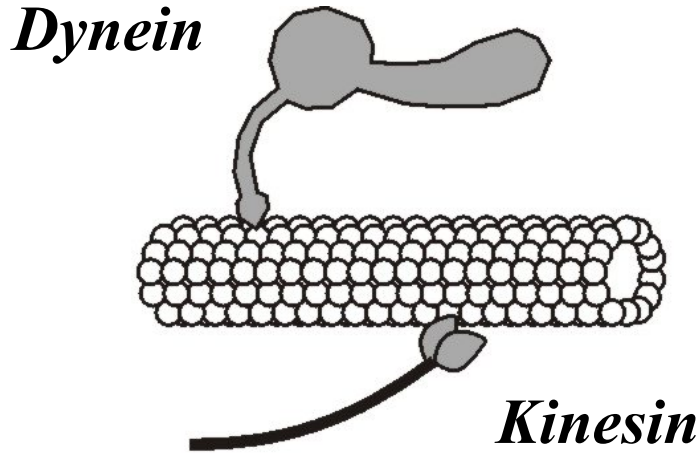
Flagella motor

	Motor	Max Force	Max Speed	Max Power
ROTARY	**Flagellar motor** (8 units, E. coli)	2400 pN nm **95 pN**	300 Hz **35 mm/s**	2000 pN nm @ 150 Hz **1.9×10^6 pN nm /s**
	(Vibrio)		1700 Hz **220 mm/s**	
	(single unit)	300 pN nm **12 pN**	300 Hz **35 mm/s**	250 pN nm @ 150 Hz **2.4×10^5 pN nm /s**
	F_1 -ATPase	40 pN nm **40 pN**	150 Hz **0.9 mm/s**	20 pN nm @ 75 Hz **9×10^3 pN nm /s**
LINEAR	**Myosin** (single molecule in muscle or *in vitro*)	**6 pN**	**10 mm/s** (speed of array, each molecule mostly detached)	2 pN @ 10 /s x 20 nm **400 pN nm /s**
	Kinesin	**5 pN**	**1 mm/s**	2.5 pN @ 0.5 mm /s **1.25×10^3 pN nm /s**
	RNA polymerase	**20 pN**	**0.01 mm/s**	**~200 pN nm /s**



An ion pump such as the Na,K ATPase can transport up to 1000 ions per sec ($r_{Na+} \approx 10^{-10}$ m) across the 10^{-8} m thick bilyer membrane, yielding an approximate velocity of 10^{-5} m/sec as a lower bound, resulting in a Reynolds number of 10^{-9}.

A motor molecule such as kinesin has a characteristic size of 10^{-8}m, and moves with a velocity approaching 10^{-6} m/sec, giving a Reynolds number of 10^{-8}.



Central dogma of Molecular Biology and assemblers

DNA

Transcription

(RNA polymerase)

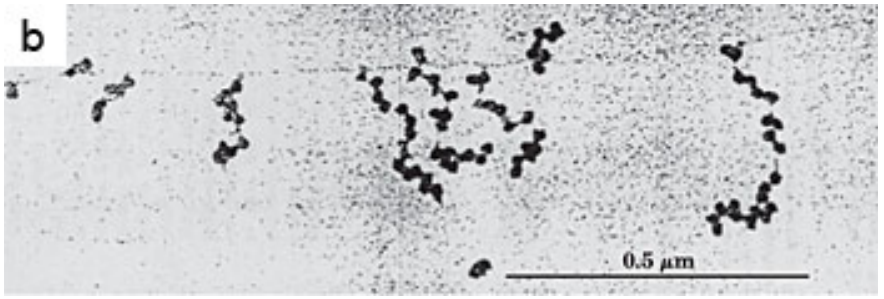
RNA

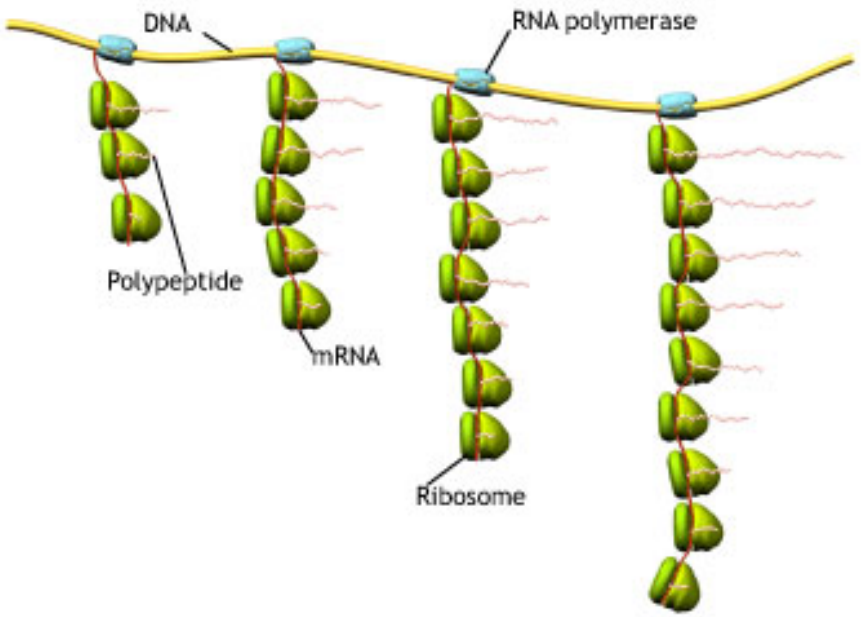
Translation

(Ribosome)

Protein

Simultaneous Transcription and Translation





Rob Phillips and Stephen R. Quake, Phys. Today, May 2006.

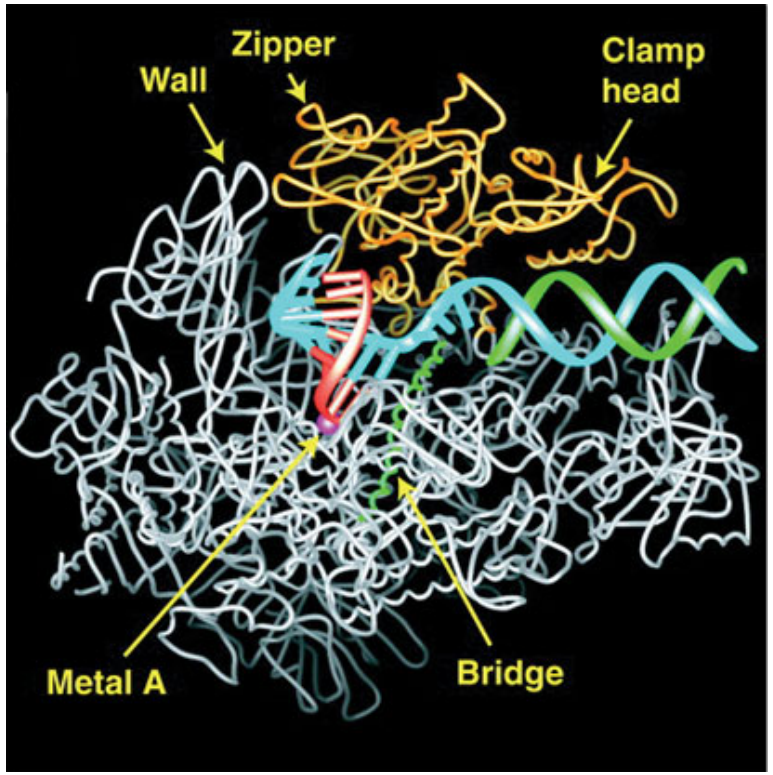
RNA polymerase: a mobile workshop

DNA → RNA polymerase → RNA

- a motor that moves along DNA track,

- decodes genetic message,

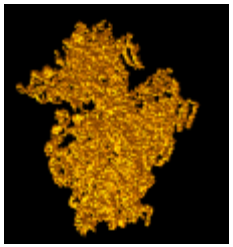
- polymerizes RNA using DNA as a template.



Roger Kornberg

Nobel prize in Chemistry (2006)

Ribosome: a mobile workshop



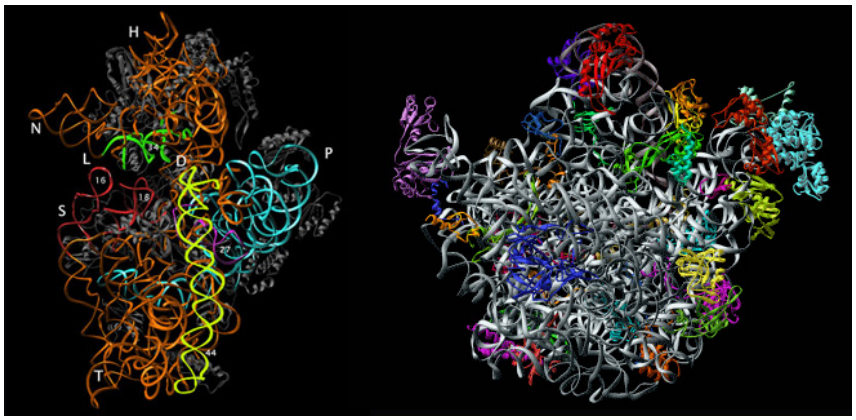
http://www.mpasmb-hamburg.mpg.de/

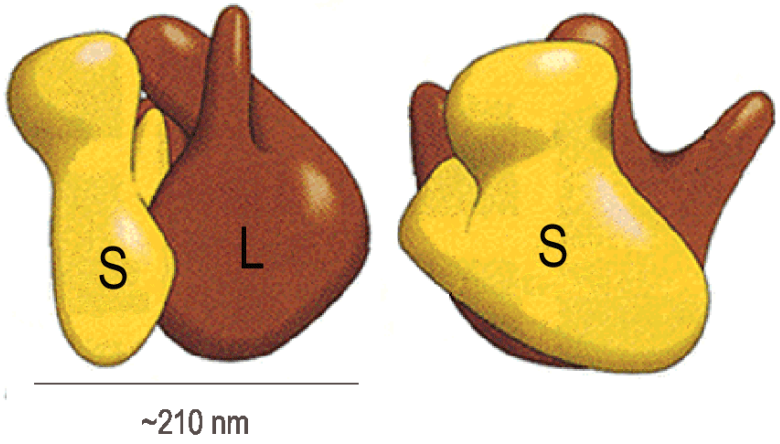
mRNA → Ribosome → Protein

- a motor that moves along mRNA track,

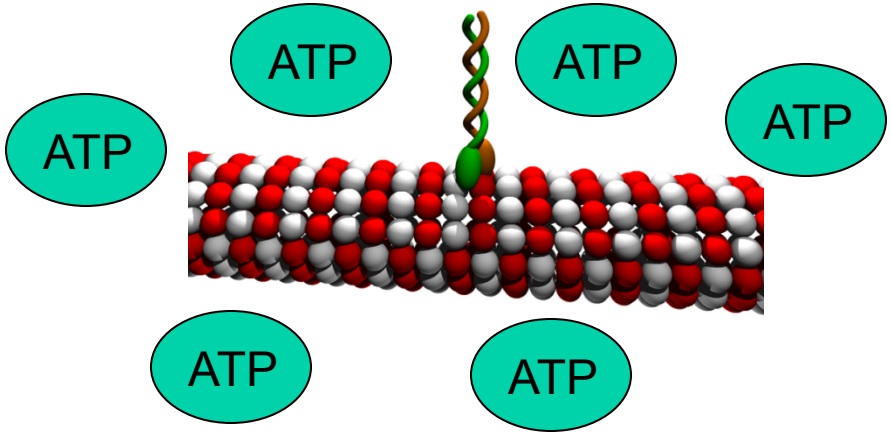
- decodes genetic message,

- polymerizes protein using mRNA as a template.





http://www.molgen.mpg.de/~ag_ribo/ag_franceschi/



Fuel

$$\Delta\mu = T\left[\ln\left(\frac{[ATP]}{[ADP][P]}\right) - \ln\left(\frac{[ATP]_{eq}}{[ADP]_{eq}[P]_{eq}}\right)\right]$$

created in cell

ATP hydrolysis ~ 25 $k_BT = 10^{-19}$ J

Generated force ~ 1 to 100 pN

Thermal energy $k_BT \sim 4.1 \times 10^{-21}$ J

Thermal force ~ 1 pN

ATP Energy and Conformational Changes in Proteins

Transducer proteins (F_1-ATPase, myosin) and allosteric proteins are both poised to undergo defined long-range conformational changes in response to ligands, at low energy cost.

(1) both show large changes in conformation in response to ligand binding,

(2) changes include domain and subunit rotations as well as plastic deformations,

(3) both sorts of proteins show only a few (usually 2) energetically accessible conformations.

Goldsmith, E. J., *FASEB Journal* (1996) 10: 702.

Motor mechano-chemical cycle:

- *binding*
- *conformational change*
- *release*
- *conformational relaxation*
- *rebinding*

Basic idea

The motor have

internal states

$$ATP + [M][\_\_] \rightarrow ADP + P + [\_\_][M]$$

Important characteristics of a motor

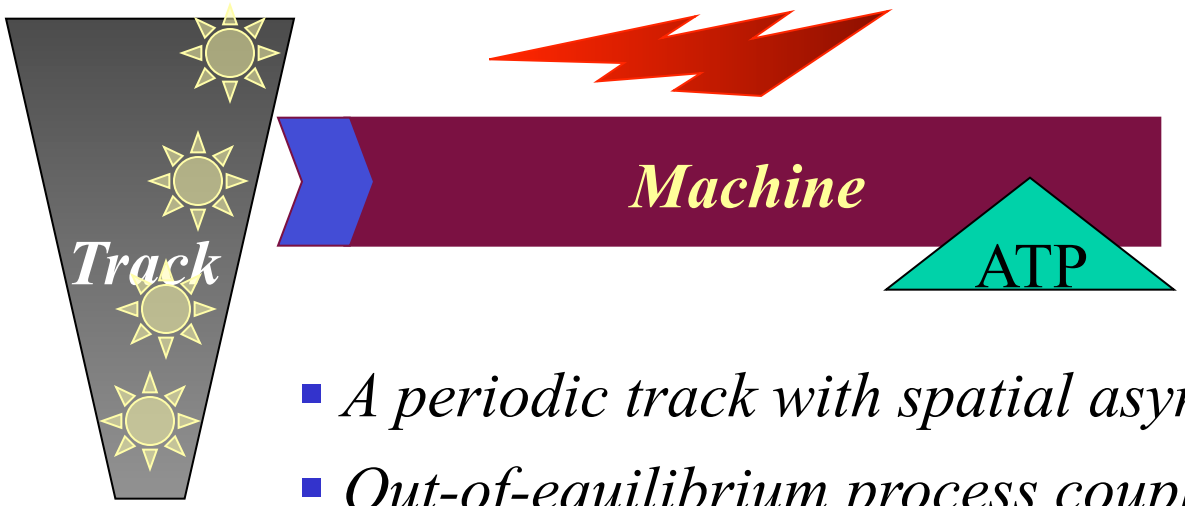
1. Efficiency of ATP hydrolysis (distance translocated as result of hydrolysis of 1 ATP molecule)

2. Rate (number of steps per unit of time)

3. Stall force

4. Processivity (probability of stepping forward)

The design of a molecular machine



- *A periodic track with spatial asymmetry.*
- *Out-of-equilibrium process coupled to a location.*
- *A catalitic site hydrolyzing ATP.*
- *A site which binds to the track.*
- *The allosteric interaction coupluing the ATPase cycle to the track binding.*

How do the motors use chemical energy to function?

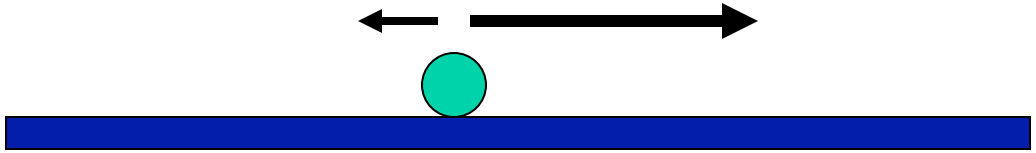
``two designs''

Brownian Ratchets *Power Stroke*

Powerstroke (Huxley, 1957)

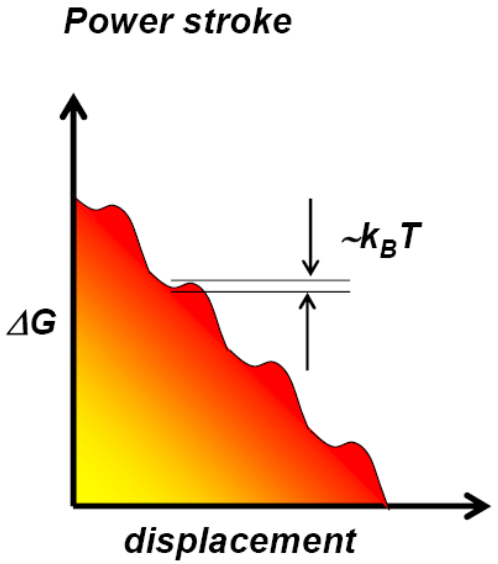
Idea: some internal ``spring'' is activated using chemical energy

description in terms of a biased random walker



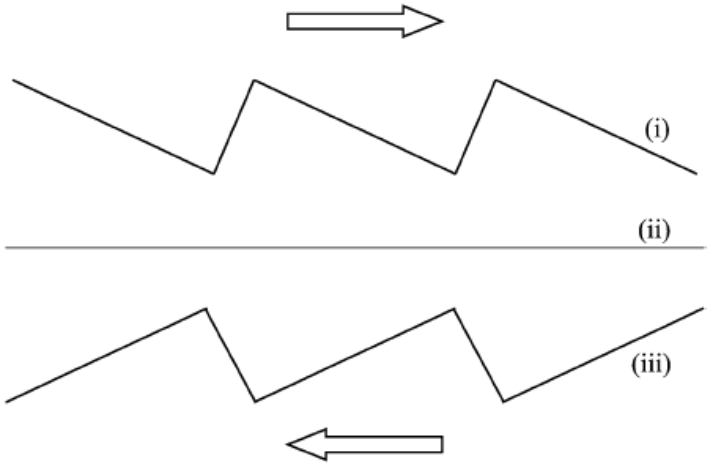
Can be complicated by introduction many internal states

The binding reaction is mechanically coupled to movement and generation of force.



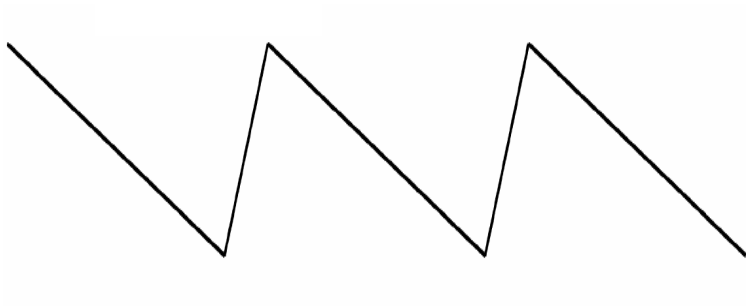
The mechanism of the Brownian ratchet

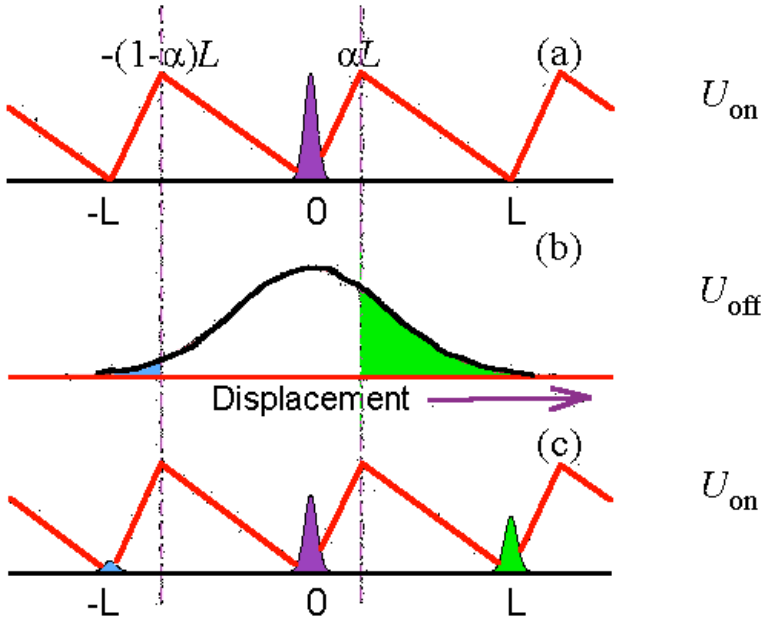
Changing directions



The ratchet potential exhibits broken spatial symmetry

$$V(x + L) = V(x)$$

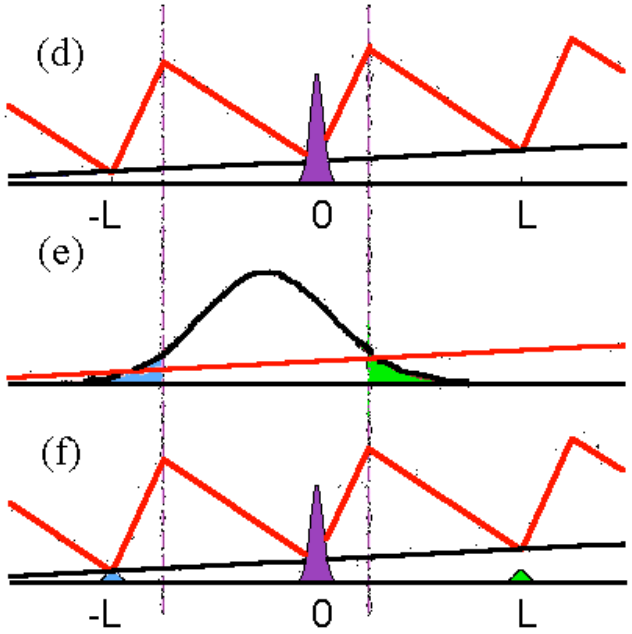




U_{on}

U_{off}

U_{on}

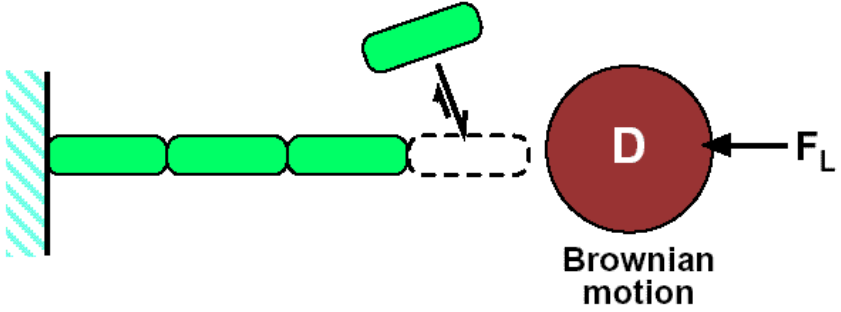


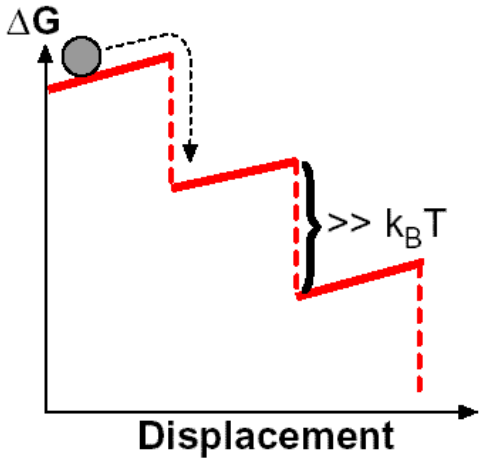
U_{on}

U_{off}

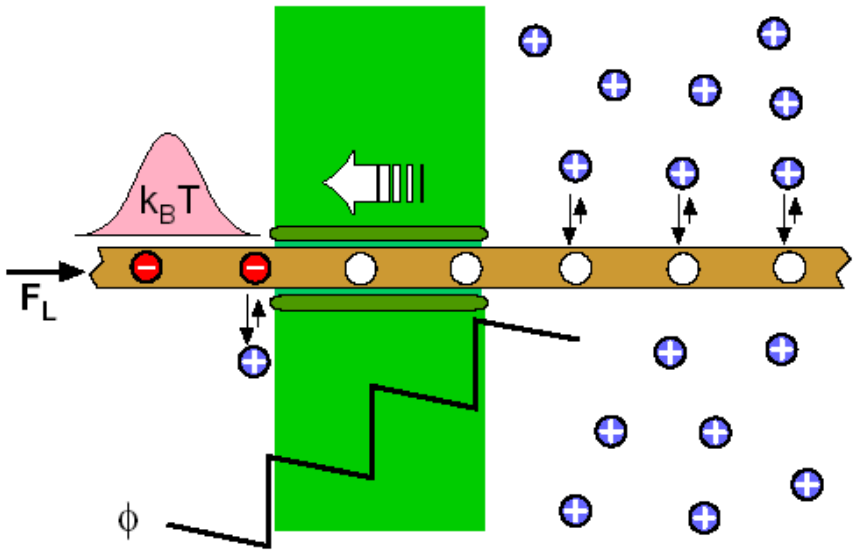
U_{on}

Brownian ratchet





- *Spatial periodicity.*
- *Spatial asymmetry.*
- *Random forces play a prominent role.*



Random diffusion coupled with energy-driven but non-directional binding and release events can lead to directional motion.

Energy of the ratched

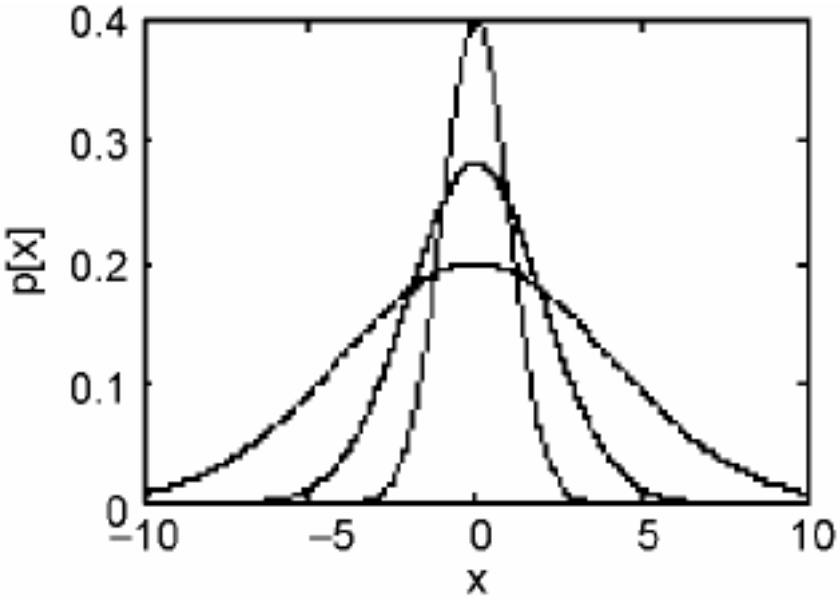
- The energy does not come from thermal noise but is provided when the potential is ***on***.
- The potential should remain ***on*** for long enough to allow the particle to reach the energy minimum.
- No energy is returned when the potential is turned ***off***.
- The energy is dissipated as heat resulting from viscous drag and thermal noise.

$$\Delta E = -\eta \dot{x}(t) + \xi(t)$$

When ratched flashing velocity is slow or fast the protein advances very slowly.

- The potential flashing is ***too fast*** – the protein does not have time to reach the local minimum, and the effect of asymmetry is lost.

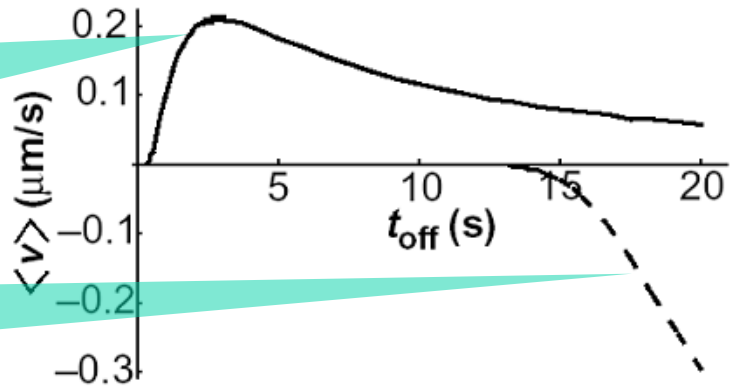
- The potential flashing is ***too slow*** – the freely diffusing protein moves too far from a local minimum – the average displacement becomes very small.

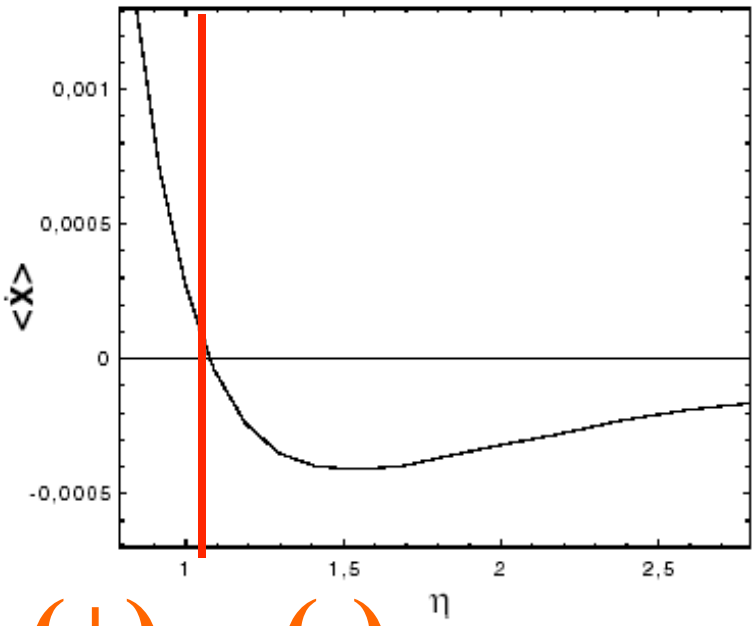


Velocity induced by cyclically turning an anisotropic sawtooth potential ($\alpha = 0.1$) versus the time spent in the off state t_{off} (with $t_{on} = t_{off}$).

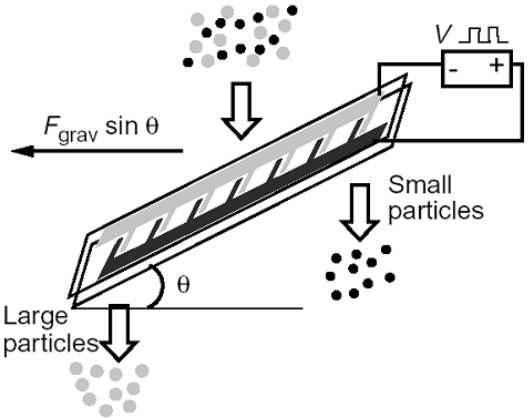
Average velocity of a spherical particle with radius $r_p = 2$ μm

Average velocity of a spherical particle with radius $r_p = 5$ μm



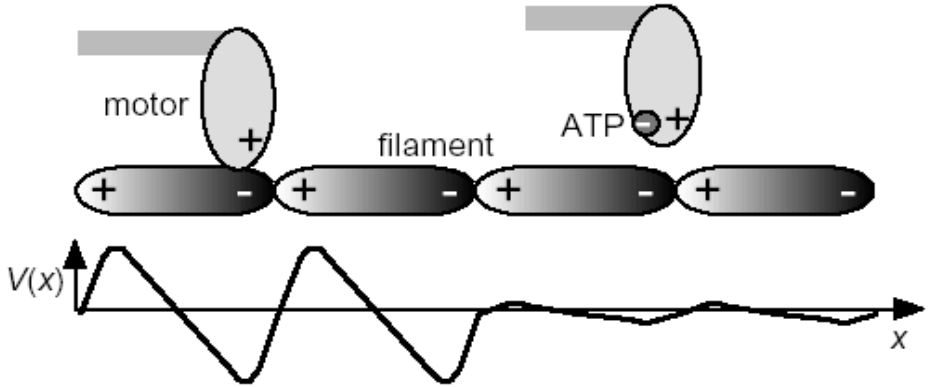


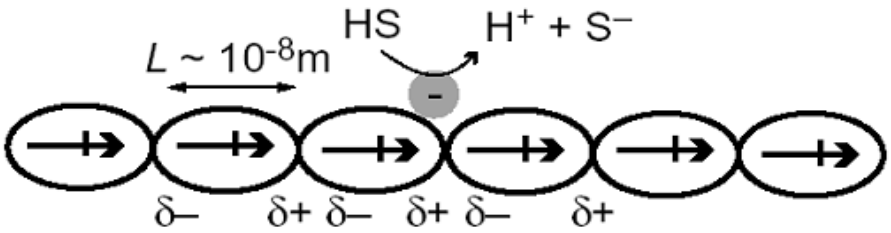
(+) (-)

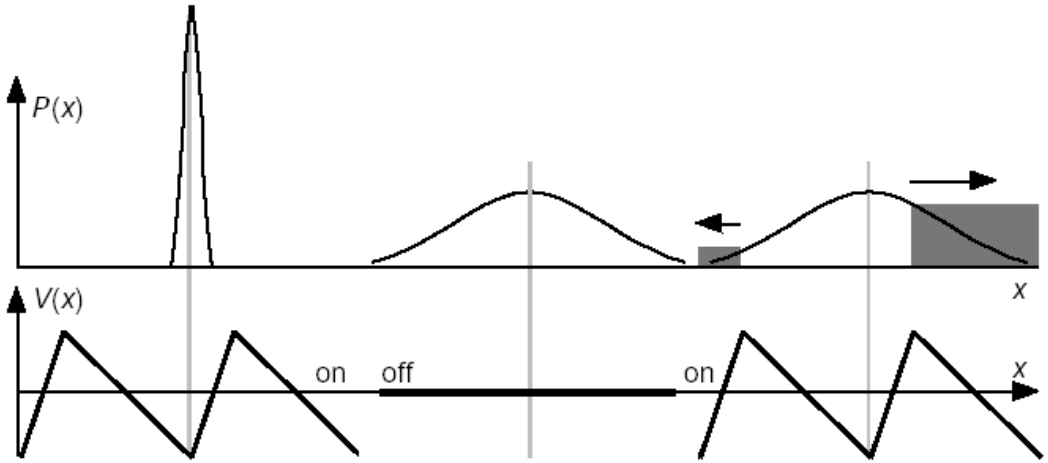


Brownian particles with different friction coefficients η move in opposite directions when exposed to the same ratchet conditions.

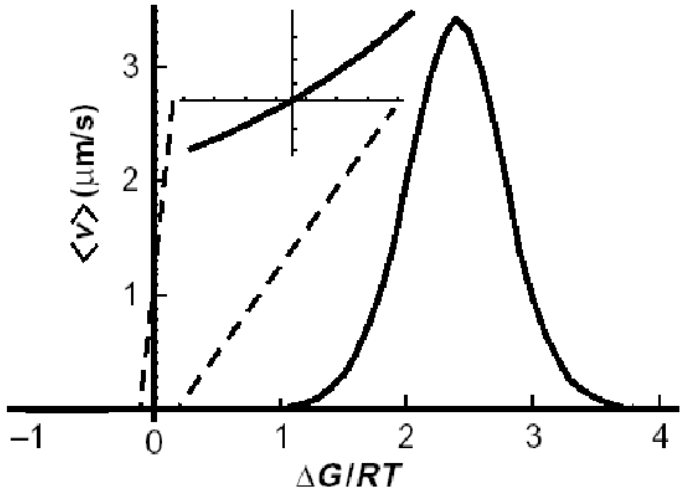
Example of the flashing ratchet



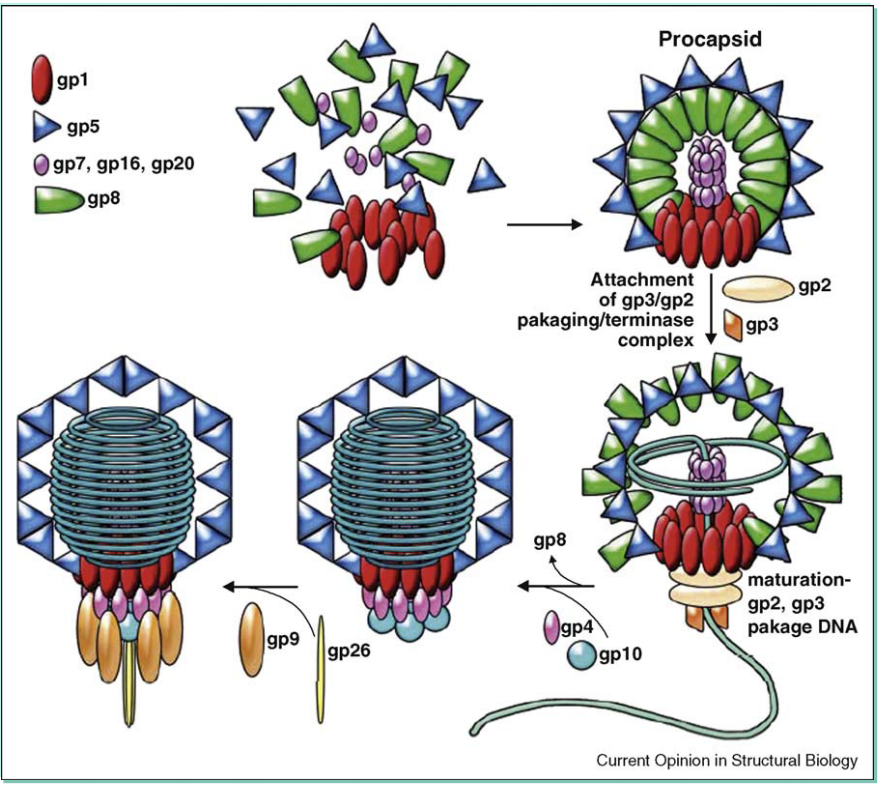


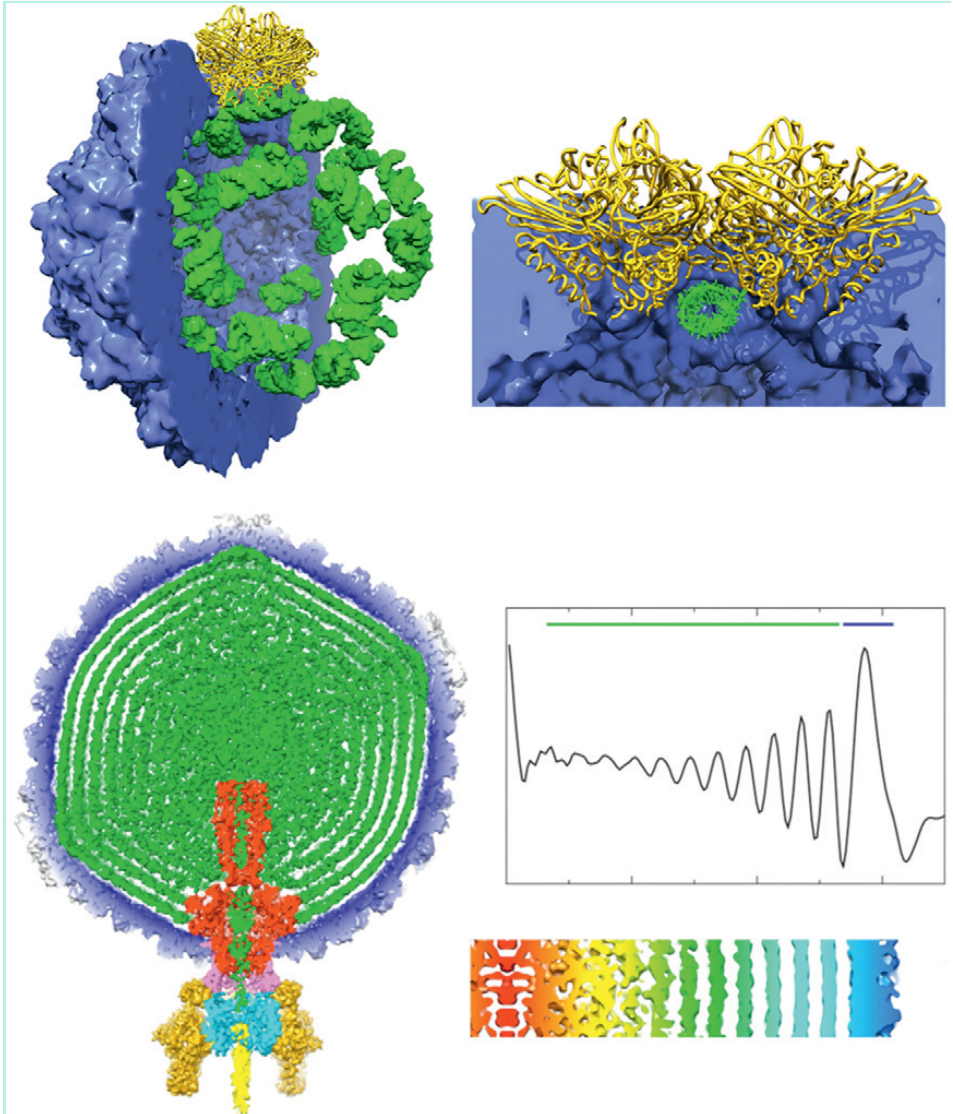


Average velocity as a function of the chemical reaction ΔG.

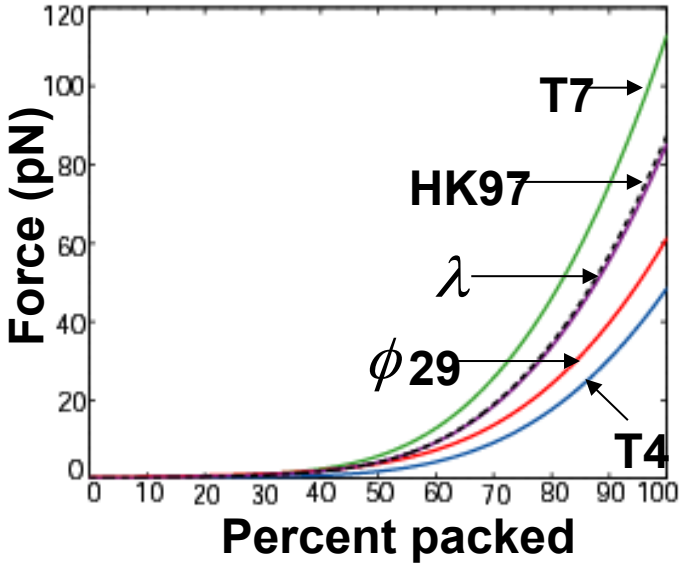


The bacteriophage P22 assembly pathway

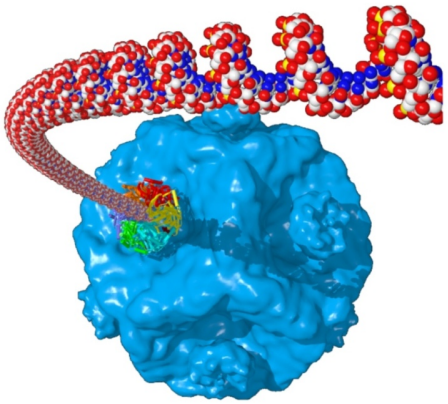




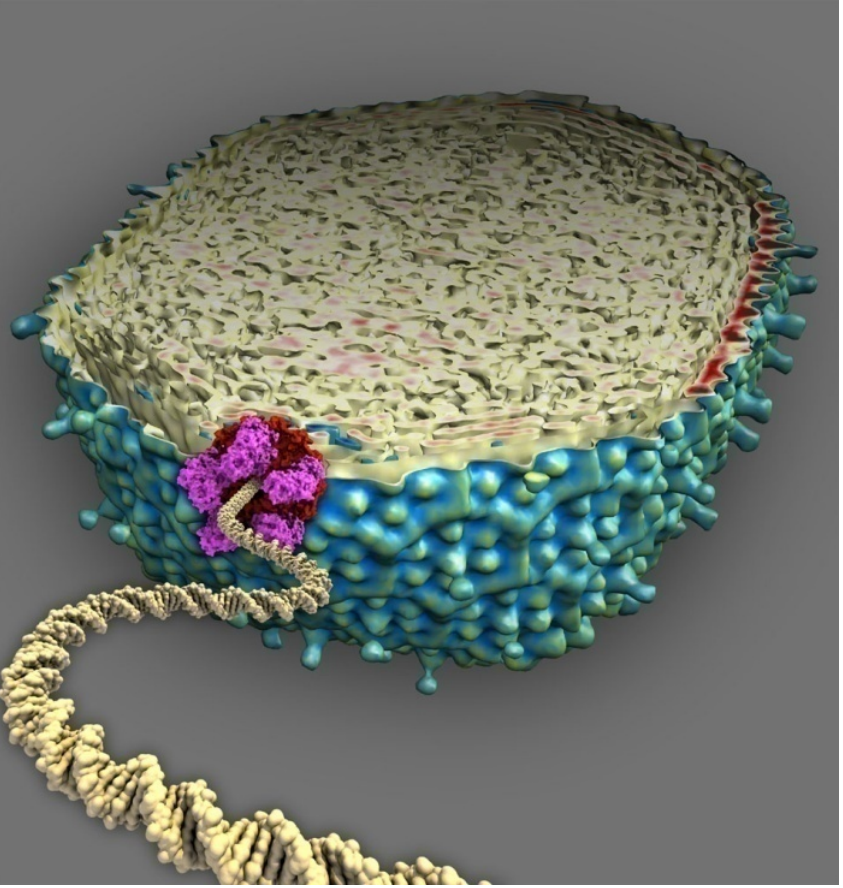
Structures adopted by encapsidated nucleic acid



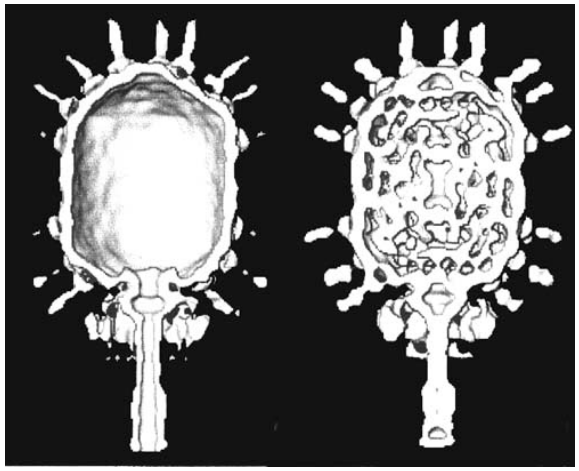
Rate of DNA Ejection

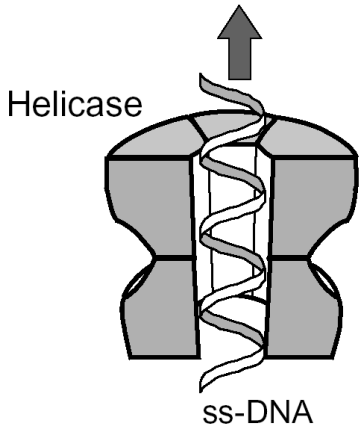


Phage	Hypothesized Mechanism	Genome Length (kbp)	Ejection time (sec)	Av. Ejection rate (kbp/sec)
lambda	Pressure	48.5	60	0.8
T4	Pressure	169	30	5.6
T7	Enzyme	40	600	0.06
T5	Pressure+ Enzyme	121	360	0.3
phi29	Pressure+ Enzyme	19	1800	0.05

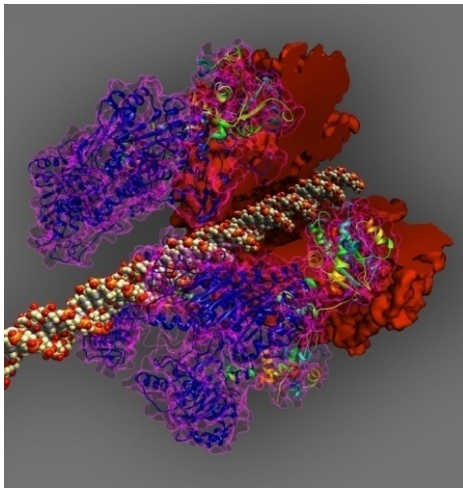


Capsid of a phage





The packaging motor can generate a force large enough to withstand this pressure!!



The packaging motor can generate a force large enough to withstand this pressure in a Phi-29 viral capsid ~ 60 atmospheric pressure ~10 times the pressure in a champagne bottle.

Measurements on Rate of DNA Ejection

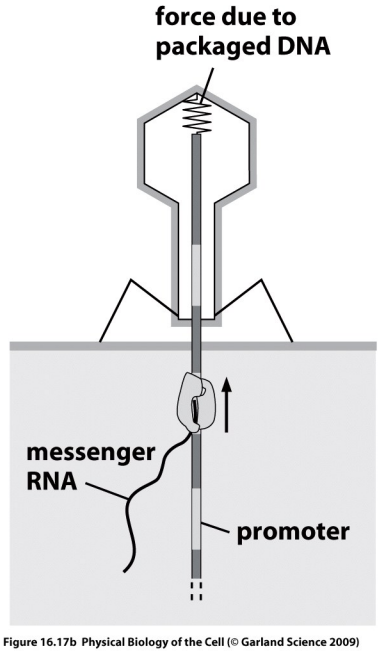
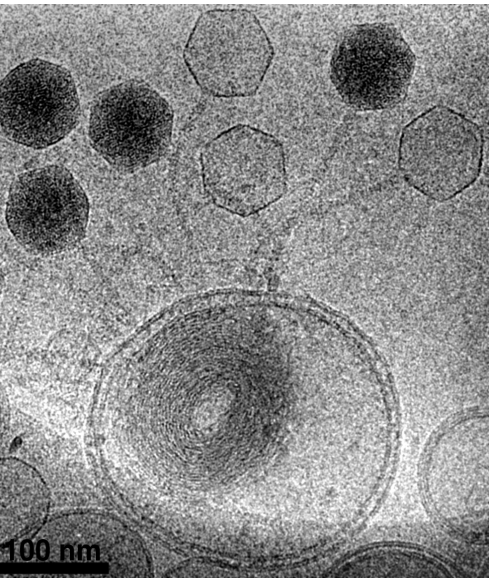


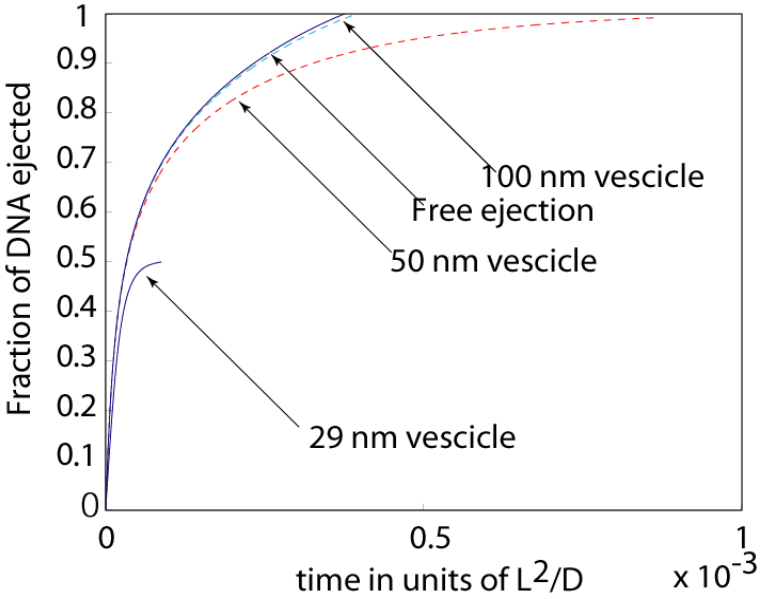
Figure 16.17b Physical Biology of the Cell (© Garland Science 2009)

(Bohm *et al.*)

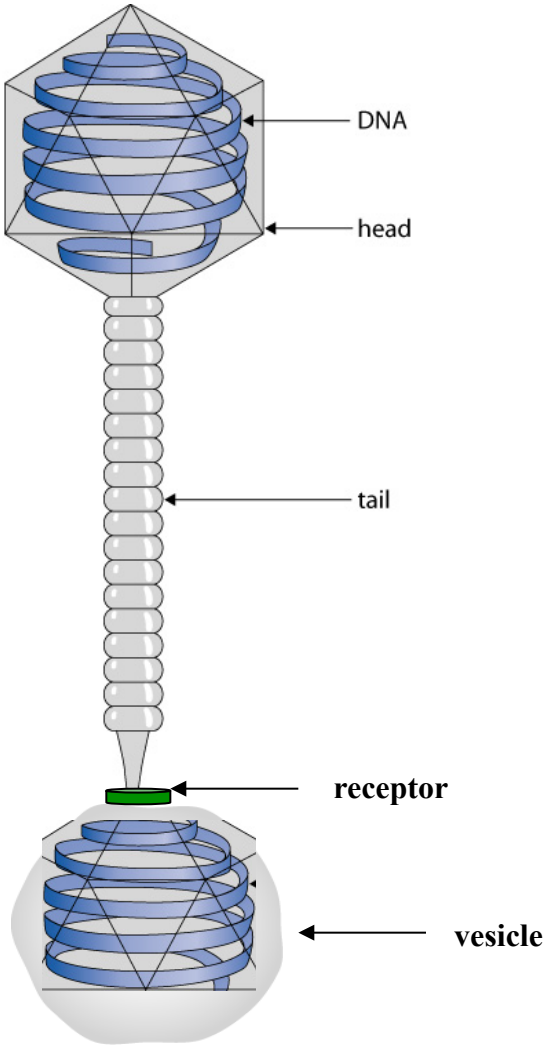


DNA injection from T5 into vesicle

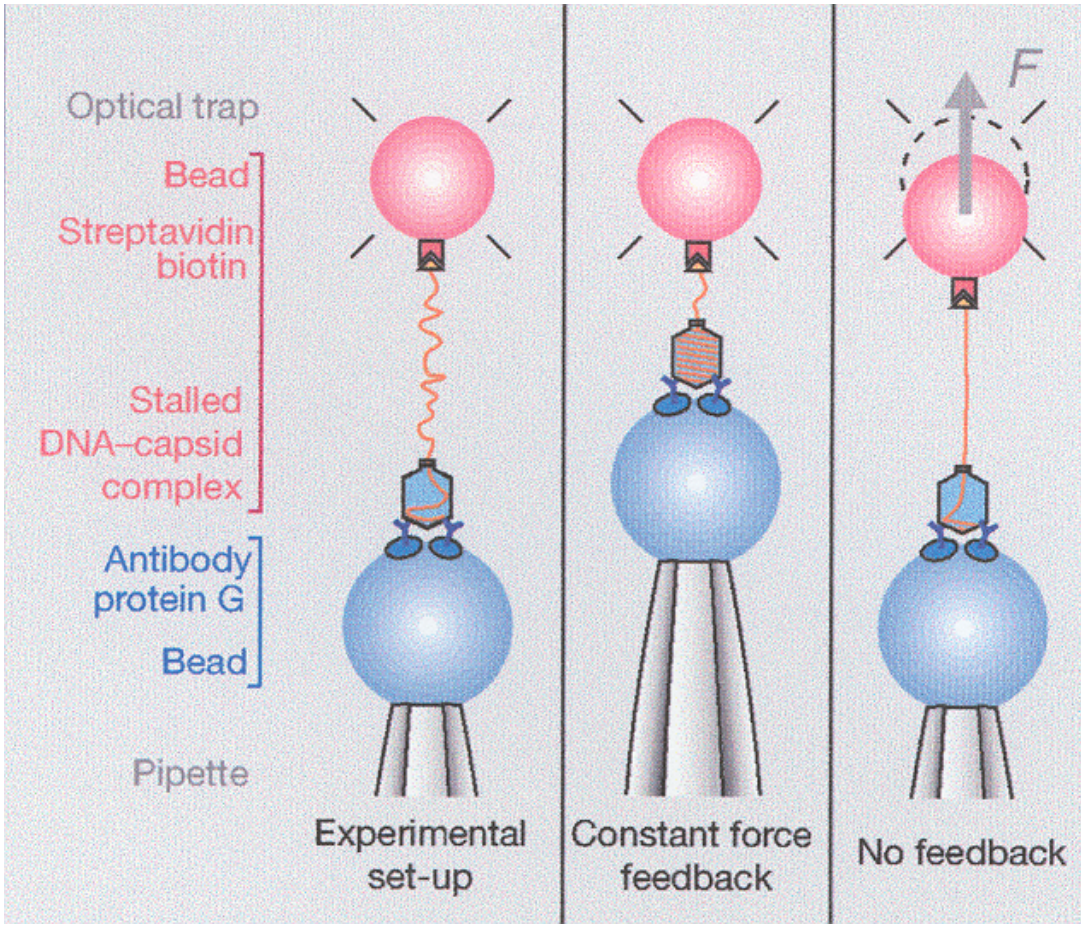
No protein interactions



The Time of Ejection

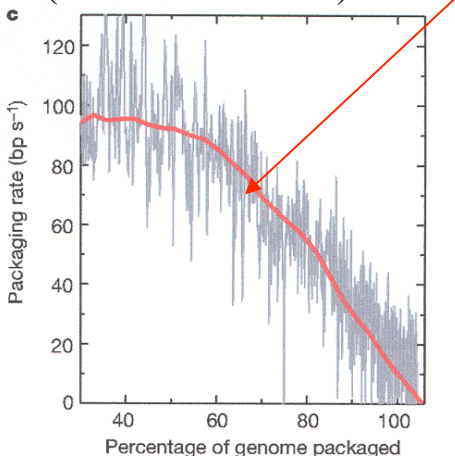


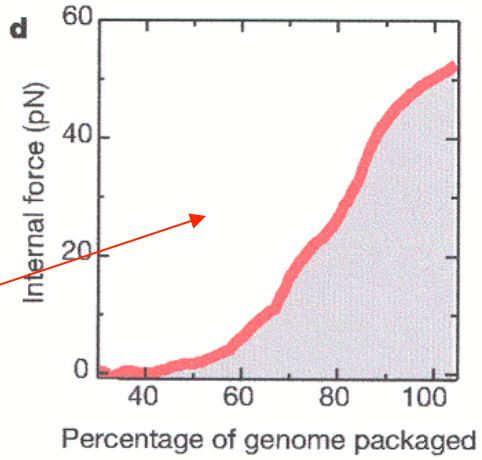
Forces and packing rates as a function of fraction packed



Rate of packing

(Bustamante *et al.*)





Force resisting further packaging

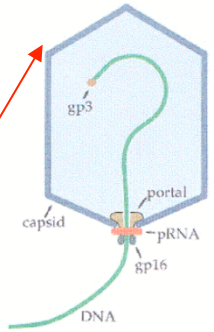
Viral packing – relevant scales

Dimensionless parameter

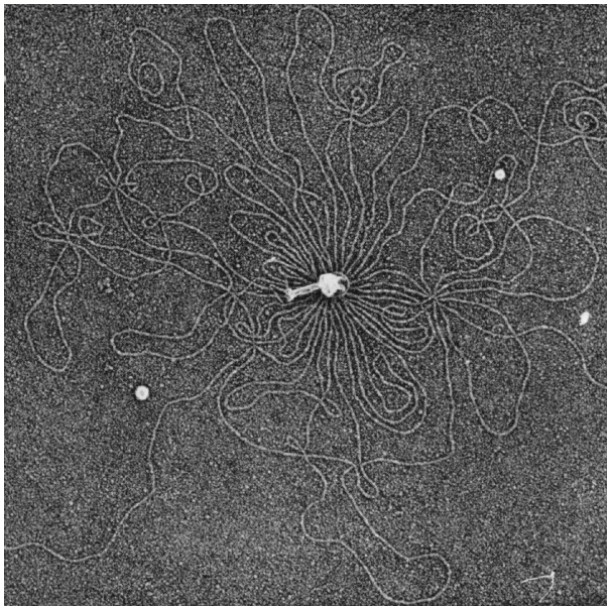
$$\frac{\Omega_{genome}}{\Omega_{capsid}} = \frac{N_{bp}\Omega_{bp}}{\Omega_{capsid}}$$

$$\Omega_{bp} \approx 1000 \mathring{A}^3$$

Capsid size = 40nm



(Hendrix)



Persistence length of DNA, length over which DNA can be thought of as being stiff.

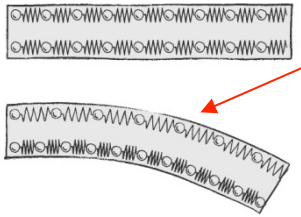
$$\xi_p = \frac{EI}{k_B T} \approx 50nm$$

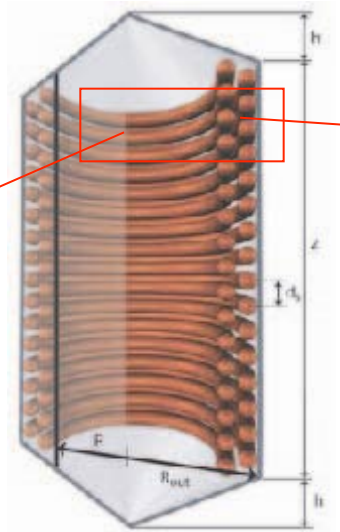
There is a negative charge every 0.17 nm of length along DNA – electrostatic energy is crucial.

Note: kT = 4.1 pN nm – coexistence of thermal and deterministic forces

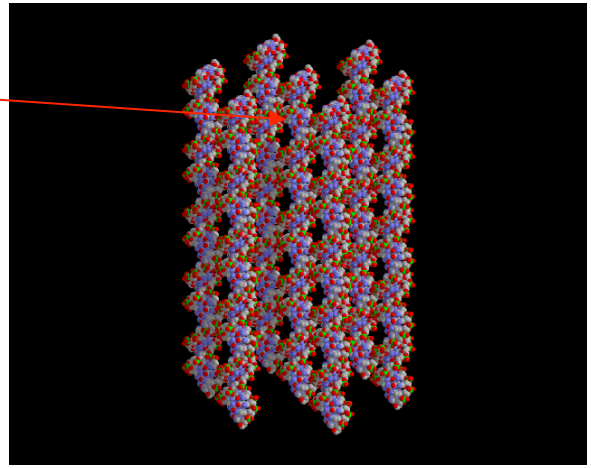
Viral packing: free energy of confinement

DNA has elasticity





DNA is charged



$$G_{hoop} = \frac{\xi_p \pi k_B T}{R}$$

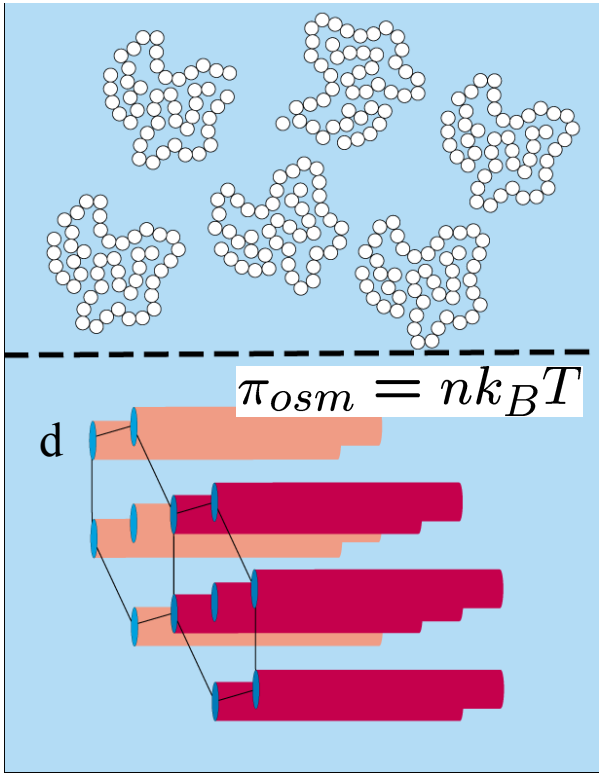
Hoop model of packed DNA

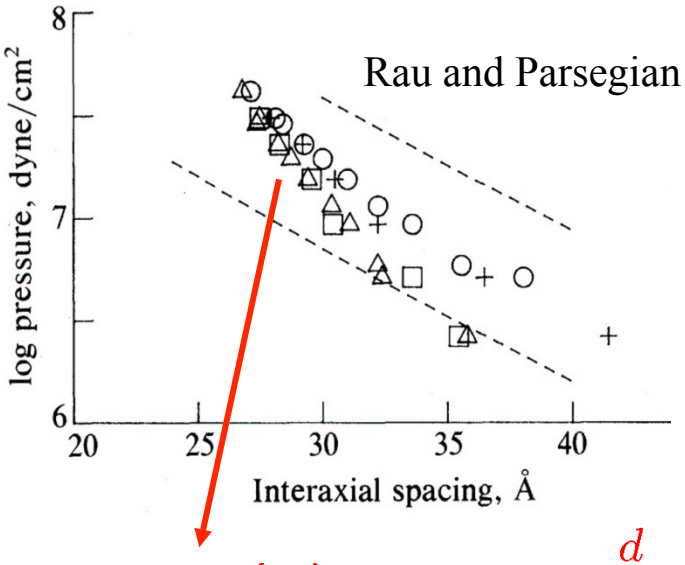
$$G_{interact} = \sqrt{3} F_0 (c^2 + dc) e^{-d/c}$$

The idea: set up a free energy function that reflects the competition between these two effects (Riemer et al.,Odijk, Gelbart et al.)

$$G_{tot}(d, L) = G_{bend}(d, L) + G_{interact}(d, L)$$

DNA-DNA Interaction: osmotic stress measurements





$$\pi_{osm}(d) = F_0 e^{-\frac{d}{c}}$$

where F_0 = 55000 pN/nm^2 and c = 0.27nm.

The measured pressure can be turned into an interaction energy between pairs of DNA strands. This energy, per strand length is:

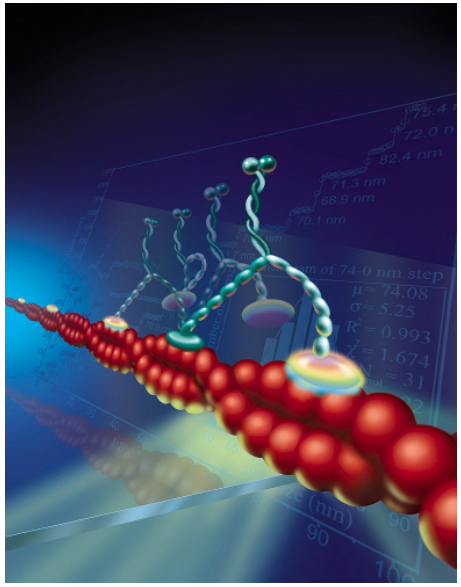
$$e(d) = \sqrt{3}\int_{\infty}^{d} x\pi_{osm}(x)dx = \sqrt{3}F_0(c^2 + cd)e^{-\frac{d}{c}}$$

Cytoskeletal Motors

Porters

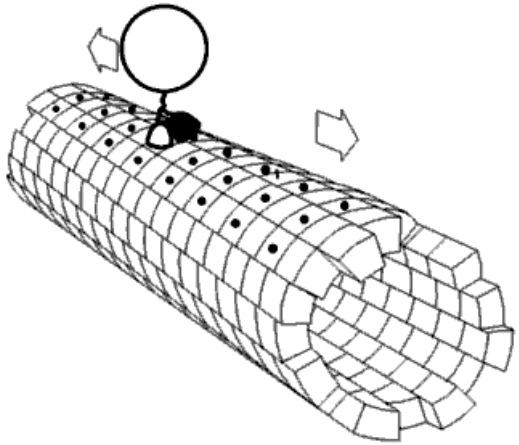
Rowers

Myosin-V



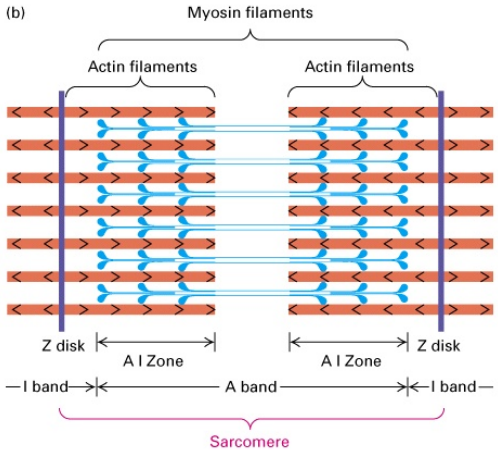
Science, 27 June (2003)

Kinesin-1



Animated cartoon: MCRI, U.K.

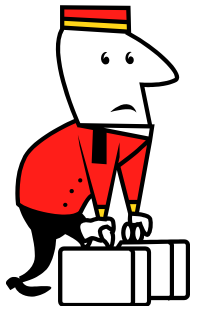
Myosin-II



Processive machines:

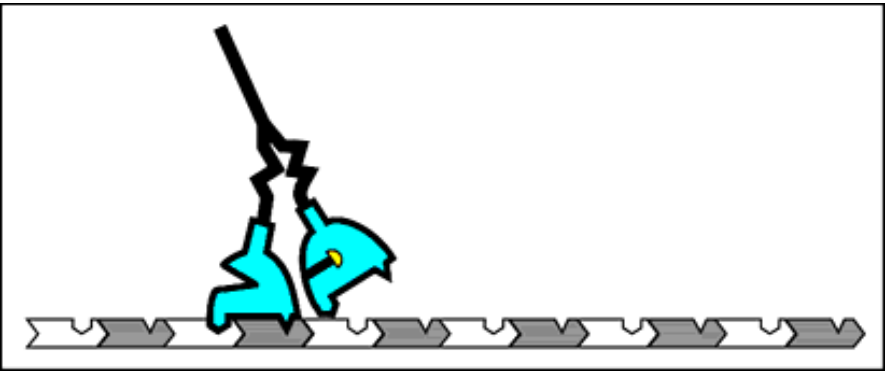
Porters

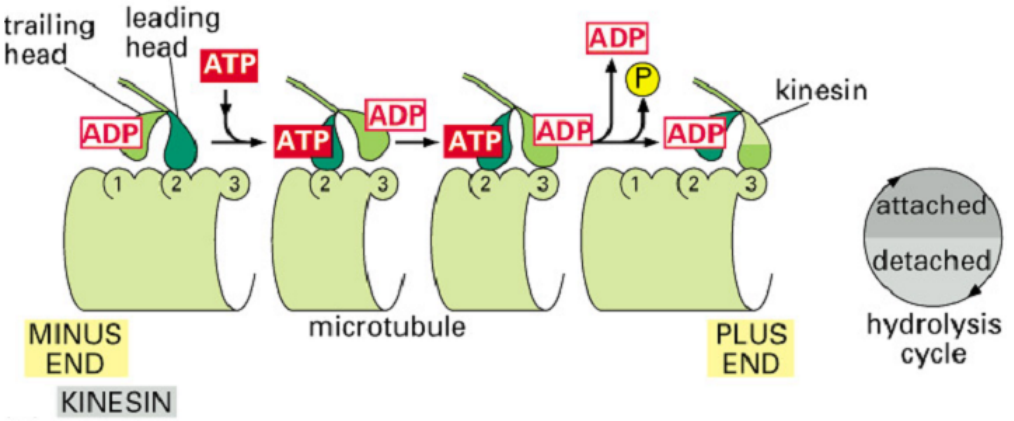
It goes through repeated complete enzymatic cycles, while remaining bound to the substrate (in this case the MT or AF) so the cargo doesn't diffuse away.



$$\text{The duty ratio} = \frac{\text{time bound to substrate}}{\text{complete time for enzymatic cycle}}$$

Duty ratio = 1 for processive motor





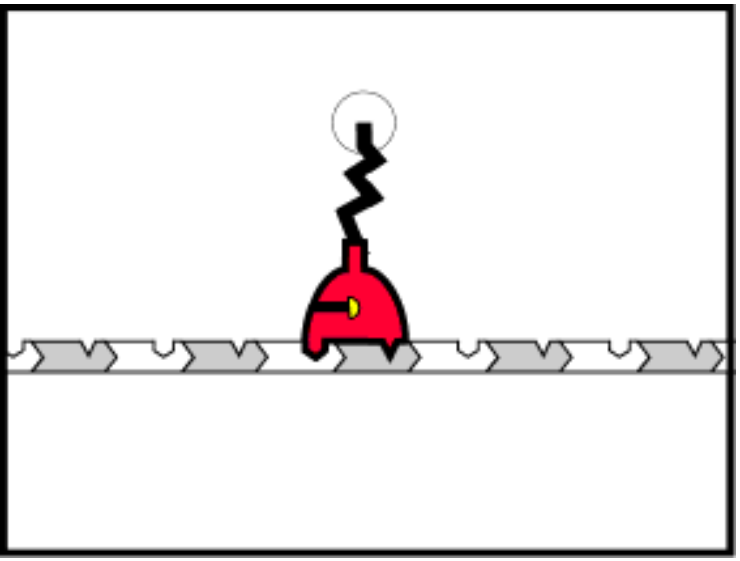
Rowers

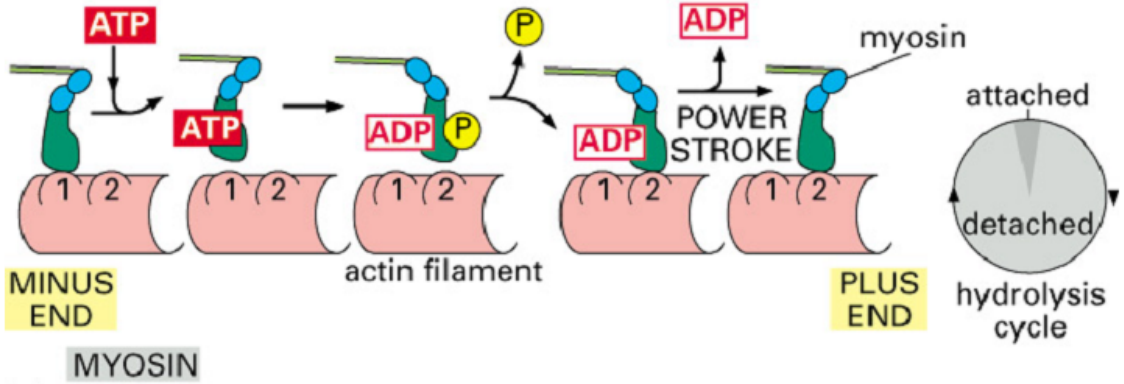
Non - processive machines

A non-processive motor lets go of the filament at some point in its enzymatic cycle.

$$The\ \ duty\ \ ratio = \frac{time\ \ bound\ \ to\ \ substrate}{complete\ \ time\ \ for\ \ enzymatic\ \ cycle}$$

Duty ratio << 1 for non-processive motor

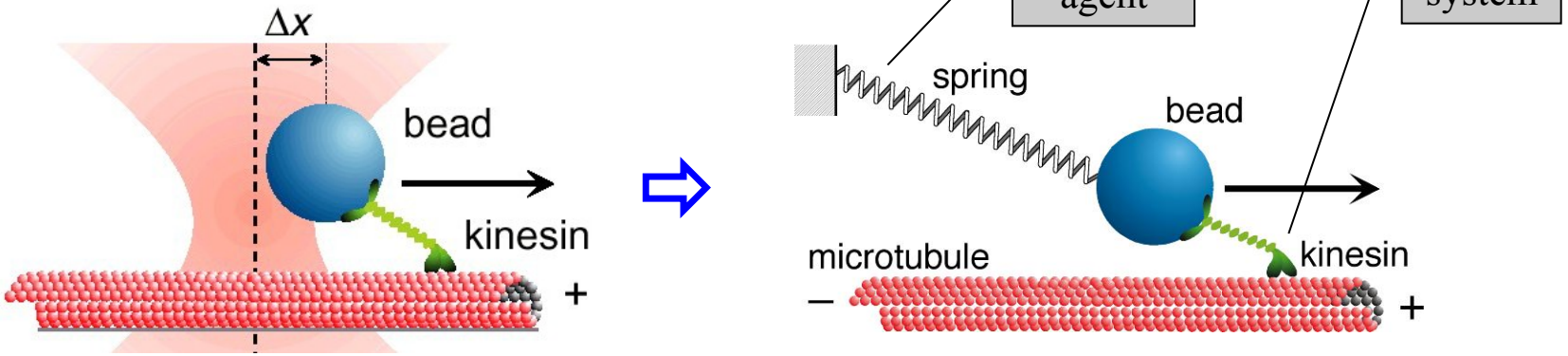




Efficiencies of a molecular motor

Thermodynamic efficiency of a motor working against a conservative force ($f = -\delta\psi/\delta x$)

Visscher et al (1999). *Nature*



$$\text{Thermodynamic efficiency} = \frac{\text{Energy output}}{\text{Energy input}}$$

$$\eta_{TD} \equiv \frac{fL}{(-\Delta G)}$$

L is the "step size" of the motor, and ΔG is the free energy drop per cycle.

The thermodynamic efficiency

- For a tightly coupled motor the average motor velocity $\langle v \rangle = L\langle r \rangle$, where $\langle r \rangle$ is the average reaction rate.

$$\eta_{\mathrm{TD}} = \frac{f \cdot \langle v \rangle}{(-\Delta G) \cdot \langle r \rangle},$$

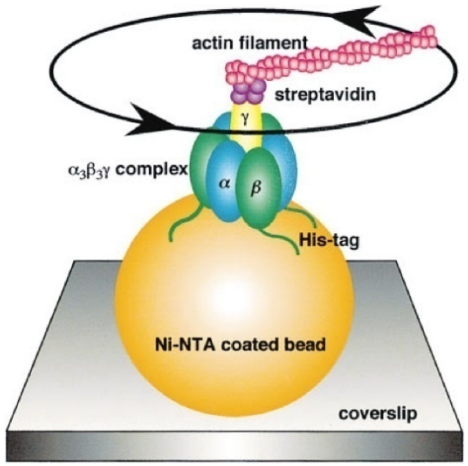
$\langle v \rangle$ = average velocity,

$\langle r \rangle$ = reaction rate,

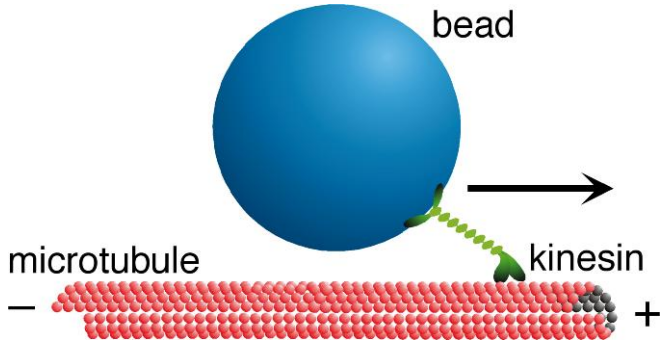
$(-\Delta G)$ = free energy drop of each cycle

Many motors are tightly coupled when they are loaded to near their stall force. This means that, near stall, they have a very high thermodynamic efficiency.

Stokes efficiency of a motor working against a viscous drag



Yasuda, et al (1998). *Cell*.



Hunt et al (1994). *Biophys. J.*

- **Viscous drag is not a conservative force:**
  - **Energy output = 0**

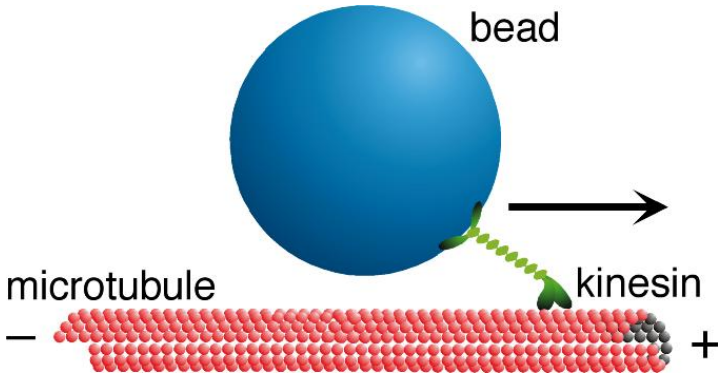
The Stokes efficiency:

$$\eta_{\text{Stokes}} = \frac{\zeta \cdot \langle v \rangle^2}{(-\Delta G) \cdot \langle r \rangle}$$

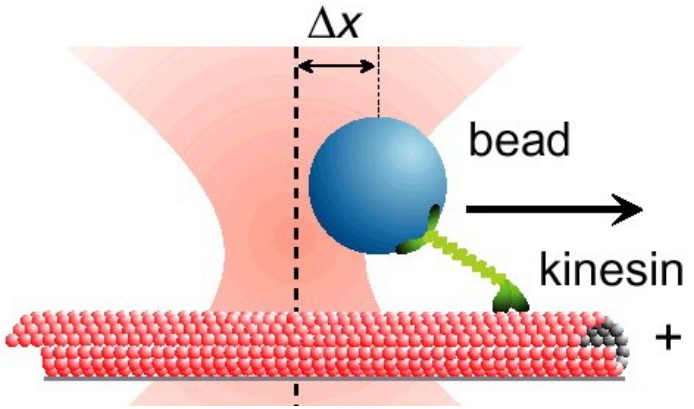
Stokes efficiency ≠ thermodynamic efficiency (experimental observations)

Viscous stall load < Thermodynamic stall load

$$\lim_{\zeta \to \infty} \zeta \cdot \langle v \rangle < f_{\text{Stall}}$$



Hunt et al (1994). *Biophys. J.*



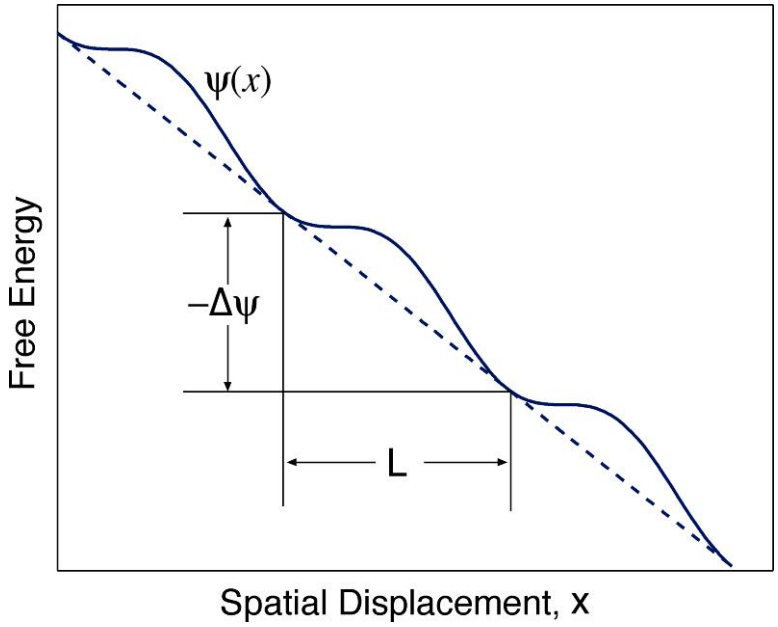
Visscher et al (1999). *Nature*

Which measurement is correct?

Stokes efficiency ≠ thermodynamic efficiency (theory)

Viscous stall load:

$$\lim_{\zeta \to \infty} \zeta \cdot \langle v \rangle = \frac{\int_0^1 \exp\left(\frac{\Delta\psi}{k_B T} s\right) ds}{\int_0^1 \left\{ \int_0^1 \exp\left(\frac{\phi(sL + \tilde{s}L) - \phi(\tilde{s}L)}{k_B T}\right) d\tilde{s} \cdot \exp\left(\frac{\Delta\psi}{k_B T} s\right) \right\} ds} < f_{\text{Stall}}$$



… implies that the motor force is not uniform.

"Natural" Nano-machines within a living cell

Designs of molecular machines have been perfected by Nature over millions or billions of years on the principles of evolutionary biology.

↓

Understanding mechanisms through experiments and theoretical modeling

Design using natural components extracted from living cells

Design using artificial components synthesized in the laboratory

↓ ↓

"Artificial" Nano-machines for practical applications

All the design and manufacturing completed so far have succeeded only in establishing "***proof-of-principle***", but still far from commercial prototypes.